NOVA SCHOOL OF
SCIENCE & TECHNOLOGY

DEPARTAMENT OF
EARTH SCIENCES

FROM MAIZE FLOUR TO BREAD:

ASSESSING THE IMPACT OF PROCESSING ON PHENOLIC AND VOLATILE COMPOSITION

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Master in Pharmaceutical Sciences

DOCTORATE IN AGRO-INDUSTRIAL TECHNOLOGIES

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"Life is crazy and meaningfully at once. And when we do not laugh over the one aspect and speculate about the other, life is exceedingly drab, and everything is reduced to the littlest scale. There is then little sense and little nonsense either. When you come to think about it, nothing has any meaning, for when there was nobody to think, there was nobody to interpret what happened. Interpretations are only for those who don't understand; it is only the things we don't understand that have any meaning. Man woke up in a world he did not understand, and that is why he tries to interpret it."

– Jung, C. G., in *The Archetypes and The Collective Unconscious*

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Abstract

Broa is a Portuguese ethnic bread traditionally prepared from maize open pollinated varieties (OPVs). These traditional varieties have been threatened by the progressive introduction of hybrid varieties, which do not possess the ability to produce high-quality *broas* and are less resilient to adverse environmental conditions. Scarce scientific studies have been reported regarding the nutritional and sensorial quality of *broas* and traditional Portuguese maize varieties. This work aimed to address this gap by providing information on the phenolic and volatile composition of *broas* and maize OPVs, important quality parameters in foods, related to health benefits and sensory attributes.

Results showed that Portuguese maize OPVs and *broas* are a valuable source of phenolic compounds, in particular hydroxycinnamic acids and hydroxycinnamic acid amides (HCAAs). Several HCAAs were tentatively identified for the first time. Phenolic compounds not only resisted to the bread making process, but their free content also increased, suggesting that their bioaccessibility was improved. The traditional OPVs and corresponding *broas* showed higher phenolic contents when compared with a commercial maize flour and *broa*.

The major volatiles detected in maize flours and *broas* were aldehydes derived from lipid oxidation reactions. Other compounds, derived from bread making, as pyranones, were also detected in *broas*. According to the results from a sensory panel evaluation, *broas* with higher contents in pyranones and lower in aldehydes were preferred by the consumers. Phenolic compounds appear to inhibit lipid oxidation reactions and increase browning reactions during bread making and, consequently, origin *broas* with better sensory characteristics. In addition, results suggested that a more advanced technique (comprehensive two-dimensional gas chromatography) is able to unveil relevant volatile compounds, since it allowed the detection of minor volatiles which may significantly influence *broas* overall aroma, as sulphur compounds. Overall, the results obtained in this work reinforce the importance of preserving these valuable genetic resources.

Keywords: maize, *broa*, food processing, phenolic compounds, hydroxycinnamic acids, volatile compounds.

Resumo

A broa é um pão tradicional português preparado originalmente a partir de variedades de milho de polinização aberta. Estas variedades tradicionais têm sido ameaçadas pela introdução progressiva de variedades híbridas, menos resistentes a condições ambientais adversas e que originam broas de qualidade inferior.

Existem poucos estudos científicos sobre a qualidade nutricional e sensorial das broas e das variedades de milho portuguesas. Este trabalho teve como objetivo colmatar esta lacuna, reunindo dados sobre a composição fenólica e volátil das variedades de milho tradicionais e broas, importantes parâmetros de qualidade alimentar, relacionados com características sensoriais e possíveis benefícios para a saúde.

Os resultados demonstraram que as variedades de milho tradicionais portuguesas e respetivas broas são uma fonte importante de compostos fenólicos, em particular de ácidos hidroxicinâmicos e amidas de ácidos hidroxicinâmicos (HCAAs). Foram putativamente identificadas novas HCAAs que não tinham sido previamente reportadas. Os compostos fenólicos não apenas resistiram ao processo de panificação, como o seu teor livre aumentou, indicando uma melhoria na sua bioacessibilidade. As variedades tradicionais de milho e respetivas broas apresentaram teores superiores de compostos fenólicos, quando comparadas com uma farinha de milho e broa comercial.

Os principais compostos voláteis detetados nas farinhas de milho e broas foram aldeídos derivados de reações de oxidação lipídica. Também foram identificados compostos resultantes do processo de panificação das broas, como piranonas. De acordo com os resultados de uma avaliação sensorial, as broas preferidas pelos consumidores apresentaram teores superiores em piranonas e inferiores em aldeídos. Os compostos fenólicos aparentam inibir as reações de oxidação lipídica e aumentar as reações que ocorrem durante o processo de cozedura e, consequentemente, originam broas com melhores características sensoriais. Adicionalmente, os resultados mostraram que a utilização de uma técnica mais avançada (cromatografia gasosa bidimensional abrangente) permite a deteção de voláteis minoritários, tais como compostos com enxofre, que podem influenciar significativamente o aroma das broas. De modo geral, os resultados obtidos neste trabalho reforçam a importância da preservação destes preciosos recursos genéticos.

Palavras-chave: milho, broa, processamento alimentar, compostos fenólicos, ácidos hidroxicinâmicos, compostos voláteis.

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Acronyms

AA	Antioxidant activity
AAi	Insoluble antioxidant activity
AAs	Soluble antioxidant activity
ANOVA	Analysis of variance
B213	Broa 213
*bis*DFP	*bis*-Diferuloyl putrescine
CAS	Chemical Abstracts Service registry number
CFP	Coumaroyl feruloyl putrescine
Com	Commercial
CV	Castro Verde
DAD	Diode array detector
DCS	Dicoumaroyl spermidine
DFA	Dehydrodiferulic acid
DFP	Diferuloyl putrescine
dw	Dry weight
ED	Electrochemical detector
EI	Electron impact
ESI	Electrospray ionisation
EtOAc	Ethyl acetate
EtOH	Ethanol
FA	Ferulic acid
FAc	Soluble-conjugated ferulic acid
FAE	Ferulic acid equivalents
FAf	Soluble-free ferulic acid
FAi	Insoluble ferulic acid
Fan	Fandango
GAE	Gallic acid equivalents
GC	Gas chromatography
GC×GC	Comprehensive two-dimensional gas chromatography
GI	Gastrointestinal
HCAAs	Hydroxycinnamic acid amides
HPLC	High performance liquid chromatography
HS	Headspace
IF	Insoluble fraction
LAB	Lactic acid bacteria
LOD	Limit of detection
LOQ	Limit of quantitation
LRI	Linear retention index
MM	Monoisotopic mass
MS	Mass spectrometry
MRM	Multiple reaction monitoring

MS/MS	Tandem mass spectrometry
m/z	Mass-to-charge ratio
n/a	Not applicable
n/f	Not found
n/i	Not identified
OAV	Odour activity value
OPV	Open pollinated variety
ORAC	Oxygen radical absorbance capacity
OT	Odour threshold
PC	Phenolic content
PCi	Insoluble phenolic content
PCs	Soluble phenolic content
pCA	*p*-Coumaric acid
pCAc	Soluble-conjugated *p*-coumaric acid
pCAE	*p*-Coumaric acid equivalents
pCAf	Soluble-free *p*-coumaric acid
pCAi	Insoluble *p*-coumaric acid
PCA	Principal component analysis
Pig	Pigarro
RSD	Relative standard deviation
RT	Retention time
SF	Soluble fraction
SHF	Soluble-hydrolysed fraction
SI	Similarity index
S/N	Signal to noise ratio
SPME	Solid phase microextraction
TE	Trolox equivalents
TeFA	Dehydrotetraferulic acid
TFA	Dehydrotriferulic acid
TI	Tentatively identified
ToF	Time-of-flight
TQ	Triple quadrupole
UV	Ultraviolet
VA	Verdial de Aperrela
XIC	Extracted-ion chromatogram

CHAPTER 1

Introduction

1. Brief overview

The importance of cereals to the nutrition of millions of people around the world is widely recognized, once they make up of a very significant part of the diet in developing countries [1]. Maize (*Zea mays* L. ssp. *mays*) is one of the three main cereal crops in the world, together with rice and wheat [1–4]. *Zea* is an ancient Greek word meaning “sustaining life” and *mays* is a word from Taíno language that means "life giver" [2]. Maize is a staple food for more than 200 million people. Since this number is expected to increase as the world population approaches 9 billion by 2050 [5], maize is considered a prime crop in the context of global nutrition [3]. In the last years, it has registered the highest production growth rate among all cereal grains [6]. Maize popularity is largely due to its diversified functionality as a food source for humans and animals [1–3], as well as raw material for the industry [1]. Although the exact origin of maize is not clear [3,4], it probably resulted from teosinte (*Zea mays* ssp. *parviglumis* and *mexicana*), due to its genetic proximity [4,7]. Most experts agree that it arose in Central America, particularly in Mexico, before 5000 BC [1,3,8]. In Mayan and Aztec civilizations, maize played an important role in their religious beliefs, festivities and nutrition [4].

Maize is a member of the Gramineae family, characterized by an erect green stem, which can reach up to 2.5 m in height [1,3,4]. This cereal is reproduced by cross-pollination and the female flower (ear) and male (tassel) are in different parts of the plant. The leaves assume an elongated form, closely wounded on the stem, from which the ear (female inflorescence) is born. This is the structure where the grain develops [4]. Depending on the environment and genetics, maize grains may vary in colour (white, yellow, orange, red, blue and black), according to the variety of the chemical compounds present [1,3,4]. The grains are arranged in the ear, attached to it by a lower appendage, "tip cap" or peduncle [1].

The maize grain is constituted by three distinct parts: pericarp, endosperm and germ. The main component is the endosperm (79-85% of the weight), followed by the germ (10-13%) and finally the pericarp (5-8%) [4,6]. The pericarp is the transparent outer layer of the grain and the endosperm is the source of food for the embryo [9]. Genetic and environmental factors, as well as plant age, may affect grain composition among maize varieties [3].

Although there are hundreds of maize cultivars, five general classes of maize grown specifically for human consumption can be considered, based on the characteristics of the grain: dent, flint, pop, floury and sweet maize [3,4]. Dent maize (*Zea mays* var. *indentata*) has a vitreous and corneous endosperm on the sides and a soft and floury endosperm on the inside [1,4]. As the upper part of the grain is floury, the dehydration of this area causes a slight collapse during maturation, producing a depression in the crown and a characteristic toothed appearance [4]. It is the maize of major commercial importance, representing almost 73% of world production, being widely used as food in Mexico, Guatemala and Andean Countries [1]. Flint maize (*Zea mays* var. *indurata*) has a thick, hard and vitreous endosperm around a small, granular and starchy centre [1,4] and represents approximately 14% of world production [4], being the most common in the northern Europe [10]. Pop maize (*Zea mays* var. *everta*) has a very hard grain endosperm with only a small fraction of starch in a dense, strong pericarp. When the grain is heated, the moisture trapped in the floured part of the endosperm expands and explodes, originating a popcorn [4]. Floury maize (*Zea mays* var. *amylacea*) has a rounded or smooth crown and its endosperm is similar to an opaque grain and predominates in the high Andean region and Mexico, where it is widely used for human consumption [4]. Finally, sweet maize (*Zea mays* var. *saccharata*) has an endosperm constituted mainly by sugar, with low starch content [4]. Yellow dent maize currently predominates in the USA, Canada, and much of Europe, while in Portugal and developing countries, flint and dent white varieties predominate mainly due to cultural traditions [3].

2. Maize-based foods

Maize is consumed in many ways in different parts of the world, either in the form of cooked, fried, or roasted grains, or in the form of other products such as polenta, tortillas, bread, popcorn, breakfast cereals and alcoholic beverages [1–3]. It is also used to produce food thickeners, sweeteners and non-consumable oils [3]. Most maize end products start by grain milling processes, particularly dry and wet milling, and maize flour is then used in the preparation of different products [1].

In developed countries, maize is generally processed by wet milling, especially the dent variety [1,4]. The basic characteristic of this milling process is to achieve separation of the main maize components using large amounts of water, which differs from dry milling [1,4]. The wet milling of maize involves chemical, biochemical and mechanical operations to separate the grain into its main components: starch, a rich endosperm protein fraction, germ and fibre [1,4]. The starch obtained through this technique has different industrial applications and it is also used to produce alcohol and food sweeteners [1]. Maize oil is obtained from the germ and is constituted by 56% polyunsaturated, 30% monounsaturated and 14% saturated fatty acids and it can be considered a valuable source of phytosterols and tocopherols [2].

Maize flour or semolina (larger particle size) are the primary products obtained through dry milling of the maize grain, especially from flint varieties [1,4]. In order to obtain whole or complete maize flour, the whole grain is milled, obtaining a granulated flour composed of heterogeneous particles [4]. In turn, refined maize flour is obtained from the milling of the endosperm of maize grain previously separated from the germ and pericarp [4]. The germ can be used to obtain maize oil, while the pericarp is mainly used as animal feed, although in recent years it has been gaining attention as a source of dietary fibre [4]. Flours obtained by dry milling have a large number of applications in a wide variety of foods, especially in breakfast cereals, cereal bars and for the brewing industry as malt substitutes [1,2,4]. Breakfast cereals are made from semolina or flours, subject to a process of humidification, steam cooking and subsequent extrusion [3,4].

Maize flour is also the main ingredient of many traditional food products [1,2,4,11]. Maize porridges can be part of the basic diet of populations in developing countries, as *ogi* in Nigeria and other African countries, *uji* in Kenya and *kenkey* in Ghana [1]. Maize flour is also used to produce *polenta* in Italy, *talo* and *gofio* in Spain [11], *arepas* in Colombia and Venezuela, beer in Benin [1,4], flat buns denominated *chapatis*, in India [2], *markouk* in Lebanon, *aish merahra* in Egypt [1], and *broa* in Portugal [4]. Since maize flour does not have the proteins that make up wheat gluten, it has more recently been introduced in the formulation of special breads, which can be consumed by celiac patients [4]. Being devoid of gluten, which gives viscoelastic properties to the matrix when it is mechanically mixed with water, the resulting breads have a low volume and a compact texture and, as a result, they do not reach the volume and texture of wheat breads [7,12].

Maize can also be submitted to a thermal-alkaline process known as nixtamalization, in order to obtain a flour that can be used to make tortillas, considered the main food of Mexican diet, and other products, as traditional Mexican drinks, tacos, and tamales [4]. For the production of nixtamalized flour, maize is cooked in a lime solution for 1 to 3 hours. The action of calcium hydroxide generates two products: nixtamal, which is the soft grain used for making *masa* or derived products, and *nejayote*, which is the wastewater whose physicochemical properties result from the components in the maize [4,13]. After standing overnight, the *nejayote* is removed and the floating material is eliminated, such as impurities, part of the grain pericarp, the pedicel and a part of the germ [1,4]. Cooked grains are milled in stone mills to obtain the wet mass with which the tortillas are made [4].

Broa is a traditional maize bread in the north and centre of Portugal and the Spanish Galicia [4], which was considered a hearty peasant bread and one of the 50 world's best breads by CNN Travel, in October 2019 [14]. *Broa* (Portugal), *borona* (Asturias, Spain) or *brona* (Galicia, Spain) are words that derive from the Celtic "bron" or the German "broth" meaning "bread". This type of bread was introduced in the fifth century by the Suevi in the northwest of the Iberian Peninsula [4]. In the middle of 19th century, maize was the major cereal for bread making [7]. Today, *broa* is produced in Portugal following traditional methods of baking [4,15], and it is still widely consumed on Central and Northern Portuguese rural communities, having an important economic and social role, as it helps to settle people down in these regions, preventing desertification toward urban areas [15,16].

The process of *broa* production is fundamentally empirical [4,7,15]. The quality of this bread is closely related not only to the kernel processing and fermentation and baking procedures, but also to the maize quality [7]. The dry milling of the whole grain is processed in stone mills, traditionally driven by water or wind and more recently by electricity [4]. The most traditional process for the production of *broa* is the addition of maize flour (50 to 80%), boiling water (blanching), wheat or rye flour (50 to 20%), and sourdough [12,15,17]. Given the reduced amount of gluten, the dough consistency is guaranteed by the partial gelatinization of maize starch [4,12,17], which involves the diffusion of water into the starch granules, hydration and swelling, during heating [18].

The sourdough is a portion of the mass of the previous day, where yeasts (*Saccharomyces cerevisiae*) and lactic bacteria, mainly Lactobacillus (*L. brevis, L. bulgaris plantarum*) are present [12,15]. Most chemical reactions that occur therein depend on this microflora, which is empirically passed from batch to batch [15]. After mixing, the dough is fermented for 2 hours and then baked in a wood oven at about 250 °C for 40 minutes [12]. This empirical process leads to an ethnic product highly accepted for its distinctive sensory characteristics [12]. This type of bread, if produced only from maize, can be consumed by celiac patients [12]. Baking assays showed that *broa* bread making technology could be used to obtain a gluten-free bread with satisfactory sensory characteristics and similar appearance with the traditional *broa* [7,12].

3. Nutritional value of maize

3.1. Macronutrients

Similar to other seeds, maize grains are storage organs that contain essential components for the growth and reproduction of plants. Many of these grain constituents are also essential for human health [3]. Epidemiological studies have shown an inverse correlation between the intake of whole-grain cereals, as maize, and the risk of developing certain chronic diseases, such as type

II diabetes, cardiovascular diseases, obesity and cancer [19–21]. Maize is considered a good source of energy (365 kcal 100 g^{-1} dry weight [3]), mainly because of its high carbohydrate content, especially starch (72-74% of the grain), followed by proteins (6-18%), lipids (3-18%) and dietary fibre (7-12%) [1,3,4].

There are significant differences in the chemical composition of the main parts of maize grain. The pericarp is characterized by a high fibre content (about 87%), mainly composed of hemicellulose, cellulose and lignin. The endosperm is characterized by a high proportion of starch (87.6%) and about 8% protein, and the germ has a high fat content (33%), proteins and minerals. There is a great variability in the chemical composition of maize grain, mainly due to environmental factors, genetic diversity, and to the different proportion of the components of the grain (pericarp, endosperm and germ) [1,4]. After carbohydrates, the second largest chemical component of maize grain is protein. Generally, its content can vary between 6 to 18%, being mostly found in the endosperm [1,3,4], although the germ has a higher protein concentration (18.4%) [3]. Maize is deficient in certain amino acids, especially tryptophan and lysine; however, more recently fortified varieties (quality protein maize) can enhance these levels, thus showing a superior nutritional value [22]. The lipid content of maize grain is mainly derived from the germ (almost 85%). Maize flour lipids are low in saturated fatty acids and relatively high in polyunsaturated fatty acids, including essential fatty acids that are involved in the prevention of cardiovascular diseases such as linoleic (60%) and oleic (24%) acids, as well as small amounts of arachidonic and linolenic acid [1,3].

Dietary fibre is defined as the portion of food which is resistant to hydrolysis or digestion by the elementary enzyme system of humans, as resistant starch [22]. Insoluble fibre is indigestible and insoluble in water, while soluble fibres are soluble in water [22]. The dietary fibre represents about 7-12% of the total maize grain composition, being mostly insoluble [3,22], and consists of polysaccharides, oligosaccharides and lignin [4], mainly from the cell walls of the pericarp and peduncle and, to a less extent, from the endosperm and germ cell walls [1,4,22]. Dietary fibre is considered an important ingredient for the human diet due to its effect on the prevention of chronic diseases [4,22,23]. The insoluble fibre present in maize can also help preventing constipation and diverticulitis [22].

3.2. Micronutrients

3.2.1. Vitamins and minerals

Maize is a valuable source of vitamins and other micronutrients. Ash content in maize grains is approximately 1.5% and it is mostly present in germ [4,22]. Examples of minerals present in maize are phosphorus, potassium, magnesium, iron, copper and chromium, which have a vital role in bone development, tooth and haemoglobin formation, growth regulation, absorption and transport of nutrients, and metabolism of carbohydrates, proteins and fats [4,22]. Environmental factors strongly influence the quality and quantity of mineral content present in maize [4,22].

B-complex vitamins, as thiamine (vitamin B1) and riboflavin (vitamin B2), are water-soluble vitamins found mainly in the aleurone layer, and to a lesser extent in the germ and endosperm [4,22]. B-complex vitamins play a vital role in growth, contribute to a healthy skin, hair and nails, improve digestion and help preventing cardiovascular and neurological diseases, as dementia [22].

Maize grains also contain fat-soluble vitamins, mainly provitamin A and vitamin E [1,3,4,22]. Fat-soluble vitamins act as antioxidants and are able to scavenge free radicals, thus protecting against different types of cancer [22]. In particular, tocopherols (vitamin E), present almost exclusively in the germ [3,4], help in the prevention of different types of skin cancer, due to their antioxidant properties [22]. Provitamin A can be further metabolized to vitamin A, which plays a vital role in human's eye and prevents night blindness [22,24]. Maize also contains ergocalciferol (vitamin D2), which helps in the bone formation, and phylloquinone (vitamin K1), essential for blood coagulation [22].

3.2.2. Carotenoids

The phytochemical carotenoids belong to the isoprenoids family and their basic structure is made up of eight isoprene units, having a C_{40} backbone. They can be divided in two types: (i) carotenes, constituted by a single long hydrocarbon chain, such as α- and β-carotenes and lycopene, and (ii) xanthophylls, derivatives that contain one or more oxygen functions, as lutein, cryptoxanthin and zeaxanthin [24]. In plants, these compounds protect the cells from extra UV light not useful for photosynthesis and can be cleaved into apocarotenoids, which are responsible for aroma, colour and phytohormone production [24]. The main carotenoids present in maize are lutein and zeaxanthin [3], mostly found in the endosperm in varying amounts, depending on the genotype [1,2,4,22]. They are present mainly in yellow maize, while the white maize contains a very low amount, or is devoid of carotenoids [1,4,22].

Consumption of carotenoids has been associated with various health benefits [24,25]. They primarily exert antioxidant effects, but individual carotenoids may also act through other mechanisms; for example, β-carotene and cryptoxanthin have a pro-vitamin A activity, while lutein and zeaxanthin are present in the macular region of retina responsible for sharp and detailed vision [24,25]. Other health benefits include a reduced risk of developing age-related macular degeneration, cataracts, coronary heart diseases, type II diabetes, neurodegenerative diseases and certain types of cancer [24,25].

3.2.3. Phenolic compounds[1]

Phenolic compounds are the most widely distributed phytochemical category in the plant kingdom and are abundantly present in maize [2]. These compounds are derived from the secondary metabolism of plants, and have at least one aromatic ring to which one or more hydroxyl groups are bonded to aromatic or aliphatic structures [26]. There is a wide variety of phenolic compounds, which can be divided in two main groups: (i) phenolic acids, which contain an aromatic ring that can be attached to different functional groups or esterified to organic acids, as benzoic (e.g. hydroxybenzoic acid) and cinnamic acids (e.g. ferulic acid, *p*-coumaric acid), and (ii) flavonoids, composed of two aromatic rings linked through an oxygen heterocycle and which can be sub-classified as flavanols, flavones, isoflavones, anthocyanins, proanthocyanins and flavanones, depending on the degree of hydrogenation and the replacement of the heterocycle. Some other phenolic compounds are stilbenes, tannins, lignins, and lignans [26]. The antioxidant activity of phenolic compounds, such as ferulic acid, has been studied and associated with several positive health effects, mainly anti-carcinogenic, neuro-protective and cardiovascular effects [27,28].

Maize is of the cereals with the highest content of phenolic compounds. Maize phenolic composition depends on several factors, such as genetics, environmental conditions, maturation stages, UV light exposure and insect and pathogens attack [26]. The main group of phenolic compounds are hydroxycinnamic acids, namely phenolic acids, as ferulic, *p*-coumaric, *o*-coumaric, caffeic, sinapic, syringic, *p*-hydroxybenzoic, vanillic and protocatechuic acids [9,19,21,29]. Other phenolic compounds families described in maize are flavonoids, such as kaempferol and quercetin [29,30], and anthocyanins, such as cyanidin 3-glucoside, cyanidin disuccinyl-glucosides, cyanidin malonyl-glucosides, cyanidin succinyl glucoside, pelargonidin 3-glucoside and pelargonidin malonyl-glucoside [31]. Anthocyanins are mostly present in pigmented maize (red, blue and purple maize), and their total content range from 53.1 to 105.2 mg cyanidin 3-glucoside equivalents 100 g^{-1} of maize [31,32]. Cyanidin-3-O-glucoside, kaempferol and quercetin have also been detected in trace amounts in yellow maize [29].

The most abundant phenolic compound in whole maize grain is, by far, ferulic acid (4-hydroxy-3-methoxycinnamic acid). Its content in maize is around 216–3400 mg 100 g^{-1} maize [33–35], representing about 3.1–4% of maize grain dry weight [27,36–38]. Ferulic acid content in this cereal is at least 10 times higher than in other cereal grains [35]. Therefore, maize is one

[1] **This topic is based on a few segments of the following publication:**
Bento-Silva A, Vaz Patto MC, Bronze MR. Relevance, structure and analysis of ferulic acid in maize cell walls. *Food Chemistry*. **2018**, *246*, 360-378.
DOI: 10.1016/j.foodchem.2017.11.012

of the most interesting sources of ferulic acid in human diet [36]. Besides ferulic acid, *p*-coumaric acid (4-hydroxycinnamic acid) is also present in high concentrations, and it may represent up to 3% of the dry weight of maize cell walls [37]. Ferulic acid was first isolated in 1866 from *Ferula foetid* [27]. It is a metabolite of lignin biosynthesis from phenylalanine and tyrosine [33], thus being derived from the phenylpropanoid metabolism [27]. It can be found in the free or conjugated form (usually linked to cell wall components) and the sum of these forms corresponds to the total ferulic acid content [33]. Both forms have substantial antioxidant properties [20]. The percentage of free ferulic acid in maize is very low (1–6% of total ferulic acid) [33,39,40] and some differences can be observed in the distribution of free and bound phenolic acids in maize grains. A significantly higher free phenolic content was found in the germ in comparison to the maize pericarp [29]. In grains, ferulic acid is largely found in the cell walls [27,33], occurring in hemicellulosic polysaccharides, esterified with arabinose residues [33]. Indeed, hydroxycinnamic acids, specially ferulic acid, are important for the cell wall's function and structure, because they can cross-link polysaccharide chains [27].

3.2.3.1. Cross-linking of cell wall components by phenolic acids

Cell walls are complex multifunctional structures [27] considered biphasic, as microfibrils of semi-crystalline cellulose are embedded in the wall matrix [9,27]. Besides cellulose, plant cell walls are also rich in other types of polysaccharides, usually called noncellulosic polysaccharides (hemicellulose and pectin) [9]. Unlike cellulose, which is a homopolymer consisting entirely of β-(1,4)-linked chains of *D*-glucopyranose residues [9,27,41], noncellulosic polysaccharides are mainly heteropolymers. Hemicelluloses, in particular, are diverse structures and usually branched-chained [9]. Hemicelluloses based on a main chain of β-(1,4)-linked *D*-xylopyranose residues are the most commonly found in nature [9,42]. These xylopyranose units form a xylan backbone, with side chains connected to it, typically arabinose and glucuronic acid, referred to as glucuronoarabinoxylans or just arabinoxylans, when they contain much more arabinose than glucuronic acid residues. Therefore, arabinoxylans consist of a xylan backbone within which the xylose residues are highly substituted, mainly with monomeric side-chains of arabinofuranose, and sometimes glucuronic acid and oligomeric side-chains containing arabinose, xylose and galactose, linked to the O-2, O-3 or both positions of the xylosyl units [***Figure 1.1***] [36,41–44]. Xylan units can also be acetylated on the C2 and/or C3. Arabinosyl units may be substituted with xylosyl units on C2 [45,46]. Maize is particularly rich in arabinoxylans (4.7% dry matter in whole grain) [41].

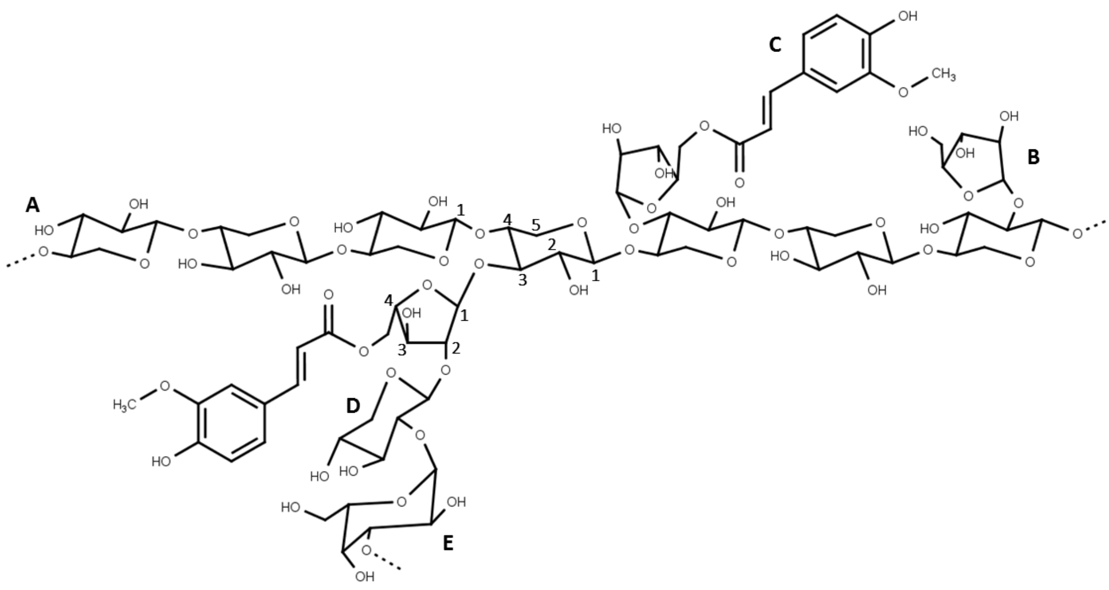


Figure 1.1: Molecular representation of arabinoxylans. **A**: Xylan backbone; **B**: Arabinosyl group; **C**: Feruloyl group; **D**: Xylosyl group; **E**: Galactosyl group.

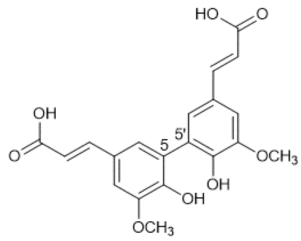
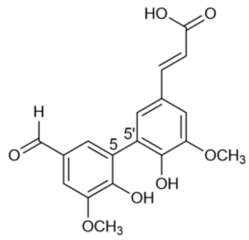
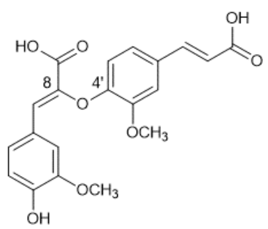
In addition to arabinoxylans and cellulose, other cell walls polymers, namely β-glucan and lignin, are considered the predominant polymers in cereals' cell walls. They occur in different proportions depending on the species and tissue type [41]. For instance, the pericarp (that constitutes the maize bran) is composed of approximately 50% heteroxylans, it has a very low lignin content (around 1%), and approximately 5% ferulic acid, the highest level of ferulic acid reported in plant tissue [38,47]. Phenolic compounds are also present in high concentrations in the germ, with traces in the endosperm [29,48]. Usually, the phenolic composition among flint and dent maize does not exhibit great variations, but their distribution is distinct. The pericarp and endosperm of yellow flint maize has shown a higher total phenolic content when compared to the yellow dent maize. However, the germ of yellow dent maize showed a higher total phenolic content [29].

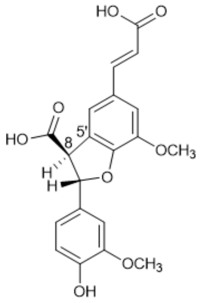
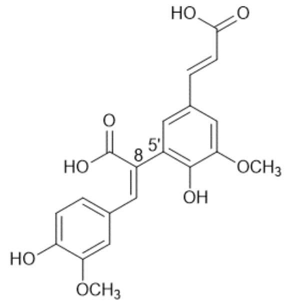
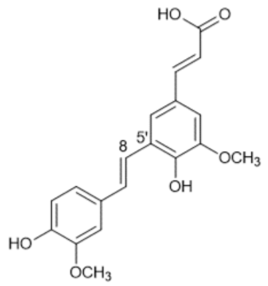
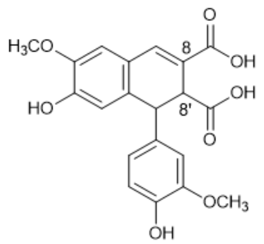
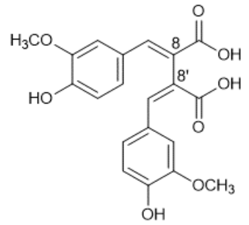
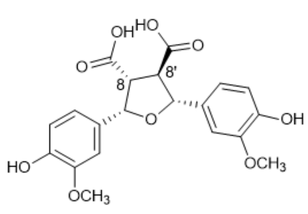
As a result of the high molecular weight of arabinoxylans and the high ferulic acid content, covalent and non-covalent linkages are formed between arabinoxylan chains and other cell wall components, such as proteins, β-glucans, lignin and cellulose [37,44,47]. Ferulic acid is usually attached to the O-5 of some arabinopyranose residues (primary alcohol on the C5 carbon) of the arabinoxylans, through an ester linkage [27,34,36,37,44], but can also be covalently linked to lignin [27,37,49] and proteins, enhancing the rigidity of plant cell walls [49].

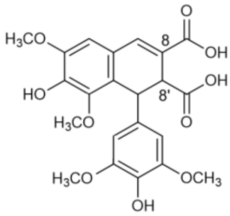
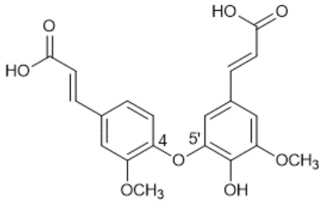
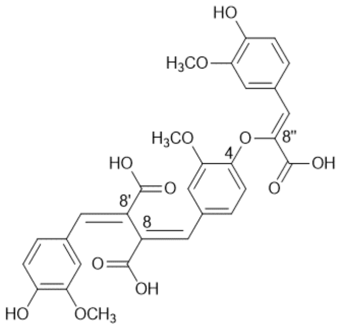
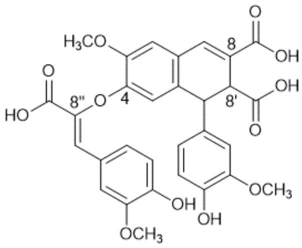
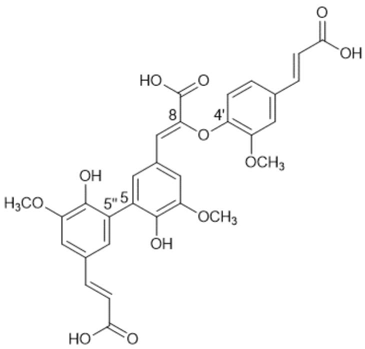
Several dehydrodimers, trimers and tetramers of ferulic acid have been identified. Their extraction from cell walls has been possible using different hydrolysis procedures, particularly saponification [24,38–42]. Dehydrodimers of ferulic acid represent about 2.5% (w/w) of maize bran [34], and its concentration is around 1.8–2.5 mg 100 g^{-1} whole maize grain [32,36]. The most abundant dehydrodimers in maize cell walls are 8-5′-linkages (around 35–45% of total dehydrodimers), followed by 5-5′ linkages (19–25%), 8-8′ (17–21%), 8-O-4′ (17–21%) and 4-O-5′ (0.3%) [***Table 1.1***] [36,50–52]. Molecular modelling experiments have demonstrated that 5-5′-diferulate could be formed intramolecularly (between two ferulic acid residues of the same polysaccharide chain, particularly when the ferulates are separated by two xylose residues) [50] [***Figure 1.2a***]. Therefore, they do not necessarily cross-link two polysaccharides. However, other dehydrodiferulic acids have been identified, namely 8-O-4′-diferulic acid, a strong indicator of intermolecular coupling of two different arabinoxylans [***Figure 1.2b***] [50]. Compounds containing an 8-5(non-cyclic)-coupled dimer unit probably do not exist in plants, but are formed from their phenylcoumaran precursors containing an 8-5-(cyclic)-coupled dimeric unit, during saponification [53–56]. In contrast, all the 8-8′-coupled dehydrodimers seem to be naturally present in plants [55].

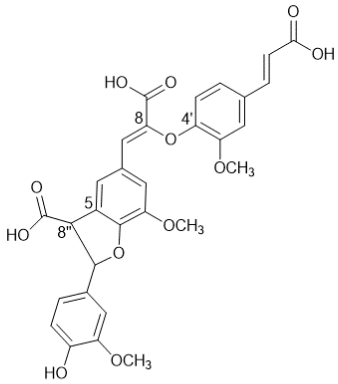
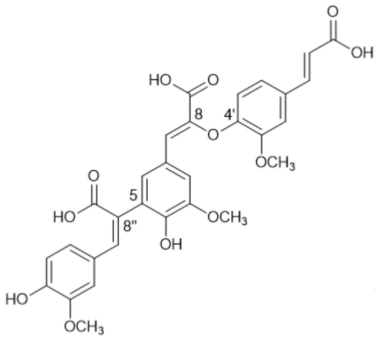
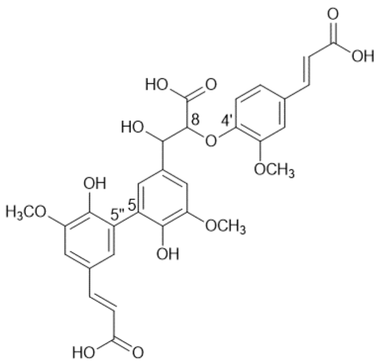
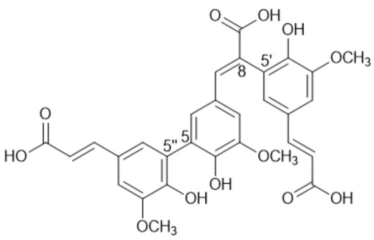
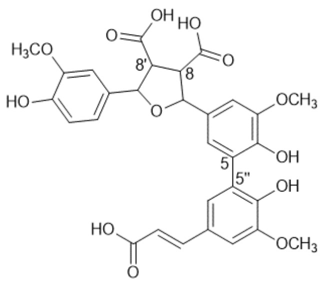
Table 1.1: Dehydrodimers, trimers and tetramers of ferulic acid and related compounds identified in maize bran after saponification.

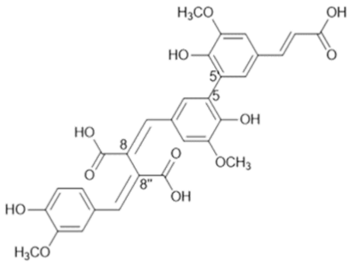
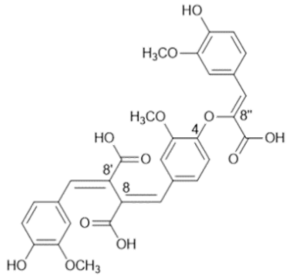
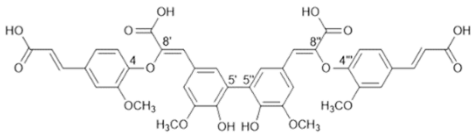
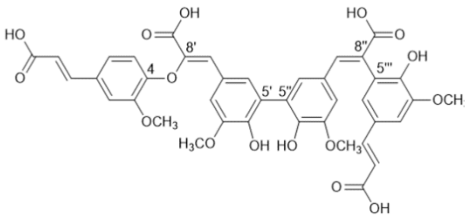
MM: Monoisotopic Mass; **MF**: Molecular Formula; **Ref**: References; *Probably formed during the saponification process.

Compound	Molecular structure	MM	MF	Ref
	Dehydrodimers			
5-5′-DFA (5-5′-dehydroferulic acid) (*E*,*E*)-4-4′-dehydroxy-5-5′-dimethoxy-3′-bicinnamic acid		386	$C_{20}H_{18}O_8$	[27,36,37,43,46,50–52,54,57–60]
5-5′-VFA (5-5′-vanillin-ferulic acid cross-product) (oxidative degradation from ferulic acid to vanillin)		344	$C_{18}H_{16}O_7$	[53]
8-O-4′-DFA (8-O-4′-dehydroferulic acid) (*Z*)-β-4-hydroxy-3-methoxy-cinnamic acid; (*Z*)-β-4-[(*E*)-2-carboxyvinyl]-2-methoxyphenoxy-4-hydroxy-3-methoxycinnamic acid		386	$C_{20}H_{18}O_8$	[27,36,37,43,46,50–52,57–61]

8-5′-DFA*f* (8-5′-dehydroferulic acid, benzofuran form) *trans*-5-((*E*)-2-carboxyvinyl)-2-(4-hydroxy-3-methoxyphenyl)-7-methoxy-2,3-dihydrobenzofuran-3-carboxylic acid		386	$C_{20}H_{18}O_8$	[27,36,37,43,46,50,52,57,59,60]
8-5′-DFA (8-5′-dehydrodiferulic acid)* (*E*,*E*)-4-4′-dihydroxy-3,5′-dimethoxy-β-3’-bicinnamic acid		386	$C_{20}H_{18}O_8$	[27,36,37,43,46,50,52,57,59]
8-5′-DFA*dc* (decarboxylated 8-5′-dehydrodiferulic acid)*		342	$C_{19}H_{18}O_6$	[50–52,57]
8-8′-DFA*c* (8-8′-aryldehydrodiferulic acid) *trans*-7-hydroxy-1-(4-hydroxy-3-methoxyphenyl)-6-methoxy-1,2-dihydronaphthalene-2,3-dicarboxylic acid		386	$C_{20}H_{18}O_8$	[36,43,46,50–52,57,61]
8-8′-DFA (8-8′-dehydrodiferulic acid) 4,4′-dihydroxy-3,3′-dimethoxy-β,β′-bicinnamic acid		386	$C_{20}H_{18}O_8$	[27,43,46,50–52]
8-8′-DFA*f* (8-8′-dehydrodiferulic acid, tetrahydrofuran) 2,5-*bis*-(4-hydroxy-3-methoxyphenyl)-tetrahydrofuran-3,4-dicarboxylic acid		404	$C_{20}H_{20}O_9$	[52,54,55,57,62]

8-8′-DSA*c* (8-8′cyclic-dehydrodisinapic acid) (traces) *trans*-7-hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-6,8-dimethoxy-1,2-dihydronaphthalene-2,3-dicarboxylic acid		446	$C_{22}H_{22}O_{10}$	[57,58]
4-O-5′-DFA (4-O-5′-dehydrodiferulic acid)		386	$C_{20}H_{18}O_{8}$	[37,46,51,57]
	Dehydrotrimers			
8-O-4′/4-O-8′′-TFA (8-O-4′/4-O-8′′-dehydrotriferulic acid)		578	$C_{30}H_{26}O_{12}$	[52,57,61]
8-8′*c*/4-O-8′′-TFA (8-8′cyclic/4-O-8′′-dehydrotriferulic acid)		578	$C_{30}H_{26}O_{12}$	[52,57,61]
8-O-4′/5-5′′-TFA (8-O-4′/5-5′′-dehydrotriferulic acid) (*Z*)-3-{5′-[(*E*)-2-carboxyvinyl]-6,2′-dihydroxy-5,3′-dimethoxybiphenyl-3-yl}-2-{4-[(*E*)-2-carboxyvinyl]-2-methoxyphenoxy}-acrylic acid		578	$C_{30}H_{26}O_{12}$	[50,52,57,59,63,64]

8-O-4′/5-8′′$_c$-TFA (8-O-4′/5-8′′cyclic-dehydrotriferulic acid)		578	$C_{30}H_{26}O_{12}$	[53]
8-O-4′/5-8′′-TFA (8-O-4′/5-8′′-dehydrotriferulic acid)*		578	$C_{30}H_{26}O_{12}$	[53]
8-O-4′/5-5′′(H$_2$O)-TFA (8-O-4/5-5′′(H$_2$O)-dehydrotriferulic acid) (probably derived from the 5-5/8-O-4-trimer)		596	$C_{30}H_{28}O_{13}$	[53]
8-5′/5-5′′-TFA (8-5′/5-5′′-dehydrotriferulic acid)*		578	$C_{30}H_{26}O_{12}$	[54]
8-8′$_f$/5-5′′-TFA (8-8′$_f$ tetrahydrofuran/5-5′′-dehydrotriferulic acid)		596	$C_{30}H_{28}O_{13}$	[54]

5-5′/8-8′′-TFA (5-5′/8-8′′-dehydrotriferulic acid)		578	$C_{30}H_{26}O_{12}$	[65]
8-8′/4-O-8′′-TFA (8-8′/4-O-8′′-dehydrotriferulic acid)		578	$C_{30}H_{26}O_{12}$	[49,52]
Dehydrotetramers				
4-O-8′/5′-5′′/8′-O-4′′′-TeFA (4-O-8′/5′-5′′/8′-O-4′′′-dehydrotetraferulic acid)		770	$C_{40}H_{34}O_{16}$	[54]
4-O-8′/5′-5′′/8′′-5′′′-TeFA (4-O-8′/5′-5′′/8′′-5′′′-dehydrotetraferulic acid)*		770	$C_{40}H_{34}O_{16}$	[54]

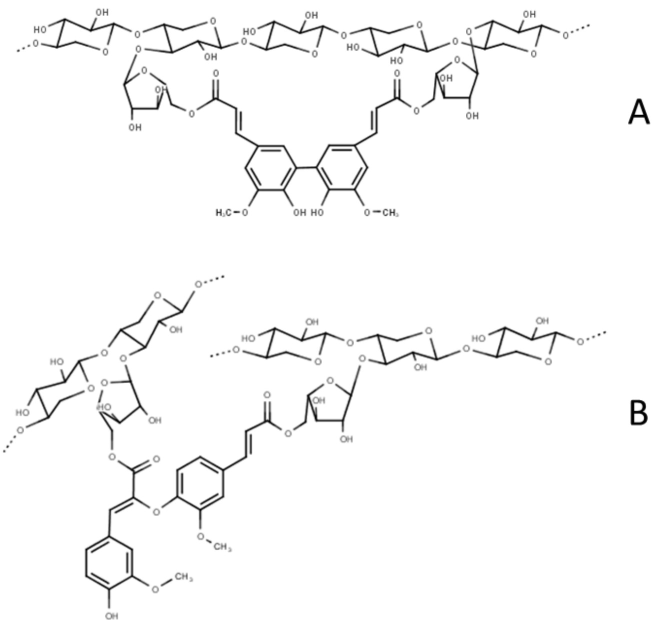


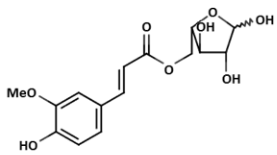
Figure 1.2: Representation of **(A)**: 5-5′-diferulate formed intramolecularly and **(B)**: 8-O-4′-dehydrodiferulic acid coupling of two different arabinoxylan chains.

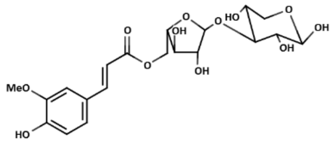
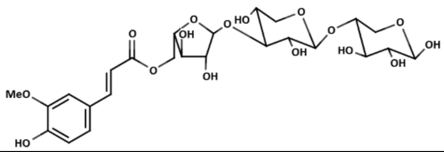
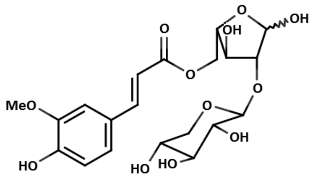
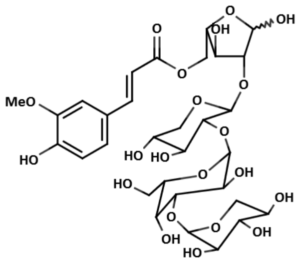
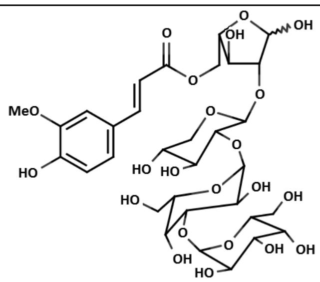
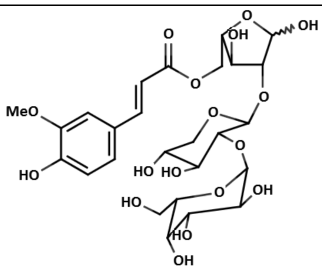
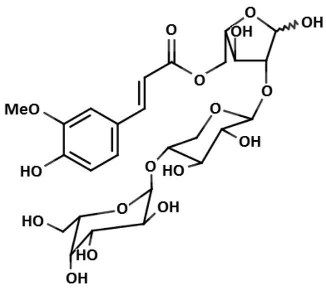
In addition to dimers, more complex linkages have been observed in maize bran [66], and some trimers and tetramers of ferulic acid have also been identified [***Table 1.1***] [37,52,59,66], providing additional strength to the plant cell wall polysaccharides [57]. In maize bran, the ferulate trimers content is 1.8 mg g^{-1}, which represents 12% (w/w) of the ferulic acid oligomers [59]. However, it is not known whether they actually cross-link three polysaccharide chains [57,59,62]. Ferulates on two polysaccharide chains may couple, and another ferulate on one chain can cross-couple with either of the two ferulate moieties already involved in a diferulate unit (back-crossing) [57,59,61,62]. Since 5-5-dehydrodiferulate is likely the only dimer that can be formed intramolecularly [57], the finding of 8-O-4′/4-O-8″, 8-8′-/4-O-8″, and 8-O-4′/5-8″ coupled trimers shows that coupling of three polysaccharide chains via higher oligomers is still a possibility [50,52,53,61]. The most abundant dehydrotrimer found in maize is 8-O-4′/5-5″, which represents 86% of total dehydrotrimers, followed by 8-O-4′/4-O-8″ (8%) and 8-8′-/4-O-8″ (6%) [52]. Ferulate dehydrotetramers (4-O-8′/5′-5″/8′-O-4‴-TeFA and 4-O-8′/5′-5″/8″-5‴-TeFA) [***Table 1.1***] were described for the first time in maize by Bunzel *et al.* (2006) [54]. They may also cross-link polysaccharides in the plant but it is not possible to deduce whether tetramers are formed by coupling of a fourth ferulate unit to a dehydrotriferulate, or by 5-5′-coupling of an already present 8-O-4′- and 8-5′-dehydrotriferulates (coupling of two dimers) [54].

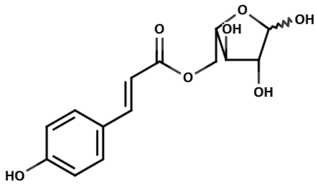
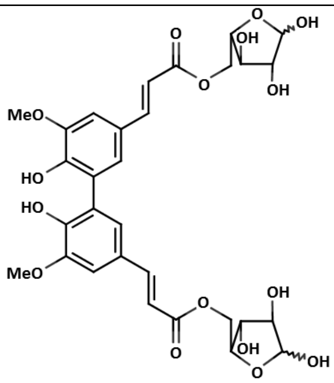
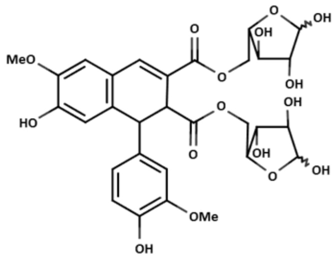
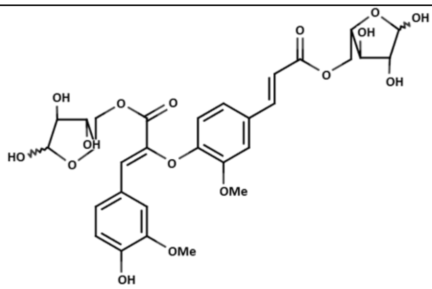
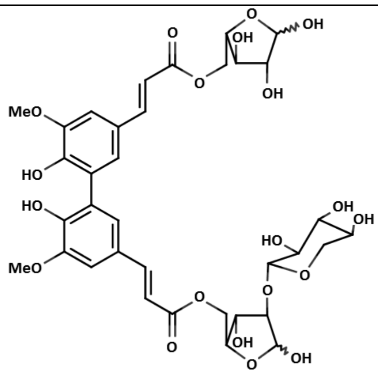
Despite the identification of ferulic acid dimers in the cell wall of maize, they do not necessarily provide structural evidence that they are, in fact, attached to cell wall polysaccharides. However, some oligomers of dehydrodiferulic acid have been identified [***Table 1.2***], providing evidence that they do actually cross-link polysaccharide chains [34,36]. *In vitro*, ferulic acid can dimerize and isomerize from the *E*-form (*trans*) to the *Z*-form (*cis*), and possibly in the reverse direction, due to daylight, where the *E*-form predominates. *In vivo*, it is possible that light is responsible for the presence of (*Z*)-dimers in plant [36].

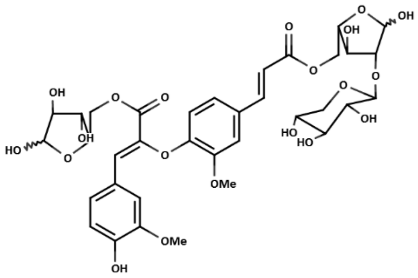
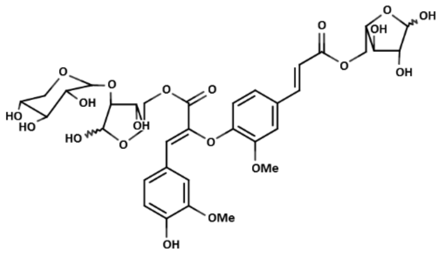
Table 1.2: Hydroxycinnamic acids oligomers identified in maize bran.

FA: Ferulic acid, **Ara**: Arabinose, **Xyl**: Xylose, Gal: Galactose, **pCA**: *p*-Coumaric acid, **MM**: Monoisotopic Mass; **MF**: Molecular Formula; **Ref**: References. [b]Released via enzymatic hydrolysis of the xylan backbone. All the other compounds are substituents of xylopyranosyl moieties obtained after acidic hydrolysis.

Compound	Molecular structure	MM	MF	Ref
FA-Ara 5-O-*trans*-feruloyl-*L*-arabinofuranose		326	$C_{15}H_{18}O_8$	[38,43,67–69]

FA-Ara-Xyl[b] 3-O-(5-O-*trans*-feruloyl-α-*L*-arabinofuranosyl)-*D*-xylose		458	$C_{20}H_{26}O_{12}$	[27,69,70]
FA-Ara-Xyl-Xyl[b] O-(5-O-*trans*-feruloyl-α-*L*-arabinofuranosyl)-(1→3)-O-β-*D*-xylopyranosyl-(1→4)-*D*-xylopyranose		590	$C_{25}H_{34}O_{16}$	[27,36]
FA-Ara-Xyl O-β-*D*-xylopyranosyl-(1→2)-(5-O-*trans*-feruloyl-L-arabinofuranose)		458	$C_{20}H_{26}O_{12}$	[27,38,43,67,68]
FA-Ara-Xyl-Gal-Xyl α-*D*-xylopyranosyl-(1→3)-α-L-galactopyranosyl-(1→2)-β-*D*-xylopyranosyl-(1→2)-5-O-*trans*-feruloyl-L-arabinofuranose		752	$C_{31}H_{44}O_{21}$	[68]
FA-Ara-Xyl-Gal-Gal α-*D*-galactopyranosyl-(1→3)-α-*L*-galactopyranosyl-(1→2)-β-*D*-xylopyranosyl-(1→2)-5-O-*trans*-feruloyl-L-arabinofuranose; O-β-Dxylopyranosyl-(1-4)-O-[5-O-(*trans*-feruloyl-α-*L*-arabinofuranosyl-(1-3)]-O-β-*D*-xylopyranosyl-(1-4)-*D*-xylopyranose		782	$C_{32}H_{46}O_{22}$	[68]
FA-Ara-Xyl-Gal(1→2) α-*L*-galactopyranosyl-(1→2)-β-*D*-xylopyranosyl-(1→2)-5-O-*trans*-feruloyl-*L*-arabinofuranose		620	$C_{26}H_{36}O_{17}$	[68]
FA-Ara-Xyl-Gal(1→4) O-*L*-Galp-(1→4)-O-*D*-Xylp-(1→2)-[5-O-(*trans*-feruloyl)-*L*-Araf]		620	$C_{26}H_{36}O_{17}$	[38,43]

pCA-Ara 5-O-*trans*-*p*-coumaroyl-*L*-arabinofuranose		296	$C_{14}H_{16}O_7$	[68]
Ara-FA-(5-5)-FA-Ara		650	$C_{30}H_{34}O_{16}$	[43,57]
Ara-FA-(8-8)-FA-Ara		650	$C_{30}H_{34}O_{16}$	[63,66]
Ara-FA-(8-O-4)-FA-Ara di-5-O-*L*-arabinosyl ester of 8-O-4-dehydroferulate		650	$C_{30}H_{34}O_{16}$	[63,66,67]
Ara-FA-(5-5)-FA-Ara-Xyl		782	$C_{35}H_{42}O_{20}$	[43,53,57,67]

Ara-FA-(8-O-4)-FA-Ara-Xyl		782	$C_{35}H_{42}O_{20}$	[63,66]
Xyl-Ara-FA-(8-O-4)-FA-Ara		782	$C_{35}H_{42}O_{20}$	[63,66]

Although ester-linked ferulic acid dimers are the most important phenolic compounds to cross-link plant cell wall polysaccharides, *p*-coumarate dimers can also be potential cross-linking compounds [57], but only very small quantities of *p*-coumarate are esterified to arabinoxylans [37,62]. Sinapic acid may also be found ester-linked to polysaccharides in maize (traces were found in the insoluble dietary fibre fraction) and other cereals, mainly by 8-8′-coupling [57,58].

Ferulic acid also participates with lignin monomers in oxidative coupling pathways, to generate ferulate-polysaccharide-lignin complexes that cross-link the cell wall [27,51]. Lignin is the generic term for a large group of aromatic polymers resulting from the oxidative coupling of 4-hydroxyphenylpropanoids. It is formed by monolignols, namely coniferyl and sinapyl alcohols, with typically minor amounts of *p*-coumaryl alcohol. The units resulting from the monolignols, when incorporated into the lignin polymer, are called guaiacyl (coniferyl alcohol), syringyl (sinapyl alcohol), and *p*-hydroxyphenyl (*p*-coumaryl alcohol) units [71]. Extensive ferulate dehydrodimer formation occurs at the onset of lignification, when generation of hydrogen peroxide is initiated [56]. Ferulic acid is laid down in ester linkages to primary cell wall polysaccharides and provides ether linkage initiation sites for lignin, while *p*-coumaric acid does not become involved in this bridge, and is more extensively esterified to lignin during the later wall development. Thus, *p*-coumaric acid is linked to lignin by ester bonds, whereas ferulic acid is linked to lignin by both ether and ester bonds [50] [***Figure 1.3***].

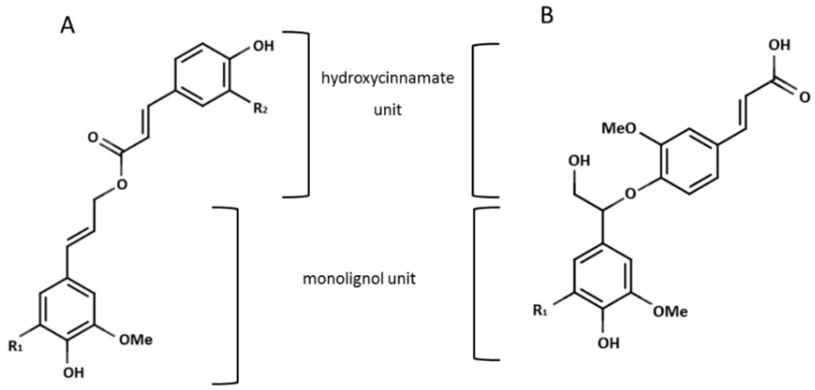
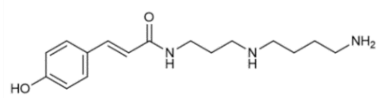
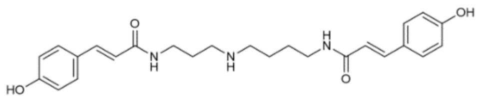
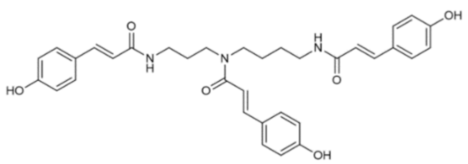


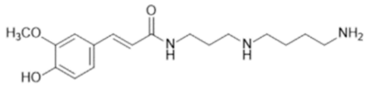
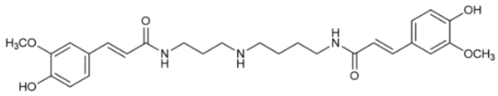
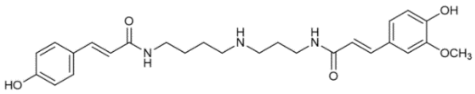
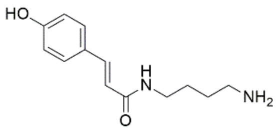
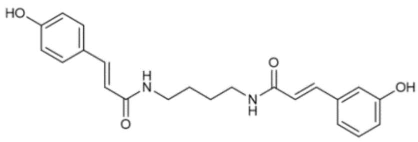
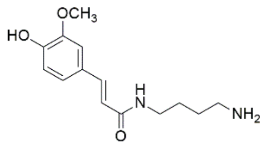
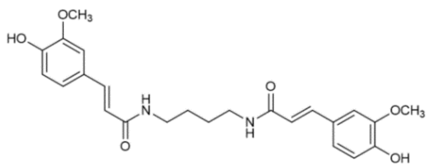
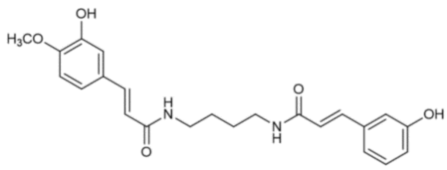
Figure 1.3: Ester (**A**) and ether (**B**) linkages of monolignols and hydroxycinnamates in grass lignins [72,73]. R_1 = H for coniferyl alcohol; $R_1 = OCH_3$ for sinapyl alcohol; R_2 = H for *p*-coumarate; $R_2 = OCH_3$ for ferulate.

3.2.3.2. Hydroxycinnamic acid amides

Ferulic and *p*-coumaric acids can also be conjugated with polyamines, known as hydroxycinnamic acid amides (HCAAs), phenolamides, or phenylamides, as putrescines and spermidines derivatives [74,75]. HCAAs consist of two hydroxycinnamic acids linked through their carboxylic acid moiety by a polyamine chain [60]. Some examples of hydroxycinnamic acid amides found in maize are described in ***Table 1.3*** and include feruloyl and *p*-coumaroyl putrescines and spermidines [60,76]. HCAAs are mainly located in subcellular compartments such as vacuole, cell wall, or within the cytosol of the pericarp and aleurone-layer of maize grains [75,77], but are also present in lower concentrations in the embryo and endosperm [76].

Table 1.3: Hydroxycinnamic acid amides described in maize grains [76,78–80].
MM: Monoisotopic Mass; **MF**: Molecular Formula.

Compound	Molecular structure	MM	MF
	Spermidine derivatives		
***N*-Coumaroyl spermidine**		291	$C_{16}H_{25}N_3O_2$
***N,N′*-Dicoumaroyl spermidine**		437	$C_{25}H_{31}N_3O_4$
***N,N′,N′′*-Tricoumaroyl spermidine**		583	$C_{34}H_{37}N_3O_6$

***N*-Feruloyl spermidine**		321	$C_{17}H_{27}N_3O_3$
***N,N′*-Diferuloyl spermidine**		497	$C_{27}H_{35}N_3O_6$
***N,N′*-Coumaroyl feruloyl spermidine**		467	$C_{26}H_{33}N_3O_5$
Putrescine derivatives			
***N*-Coumaroyl putrescine**		234	$C_{13}H_{18}N_2O_2$
***N,N′*-Dicoumaroyl putrescine**		380	$C_{22}H_{24}N_2O_4$
***N*-Feruloyl putrescine**		264	$C_{14}H_{20}N_2O_3$
***N,N′*-Diferuloyl putrescine**		440	$C_{24}H_{28}N_2O_6$
***N,N′*-Coumaroyl feruloyl putrescine**		410	$C_{23}H_{26}N_2O_5$

3.2.3.3. Biological role of phenolic compounds in maize

More than 50% of the bound ferulic acid of cell walls can undergo dimerization, and it has been suggested that the degree of dimerization may be controlled independently, rather than by the levels of ferulic acid available for cross-linking [81]. Cross-linking has been implied in different processes in plants, such as the control of the cell wall extensibility (cell wall stiffening and growth deceleration) [27,36,37] and cell wall adhesion [37]. Ferulic acid dimerization may also be involved in the growth suppression of maize shoots in the first phase of salt stress [82]. Furthermore, increased cross-linking of feruloylated arabinoxylans probably results in thinner and firmer cell walls [37,81], and therefore may decrease larvae penetration into plant tissues [37]. Consequently, cross-linking has been postulated to protect against pathogens [27,37,83], such as maize borers [37,83,84], maize weevil and fungal diseases as Gibberella ear and stalk rot [37].

The role of HCAAs in maize grains is not known for sure [75,85] and these compounds are still poorly studied [77]. They may be associated with several processes, such as plant growth and development, floral induction and reproduction, cell division, control of intracellular polyamine concentrations, cell wall reinforcement, and plant adaptation to stress, such as resistance to cold or pathogen attack [74,75,78,86,87]. Similar to cross-linking, HCAAs have also been suggested to protect against pathogens, since their increase results in thinner and firmer cell walls, therefore decreasing pathogen penetration into plant tissues [75,78]. A recent study has shown that they protect against *A. flavus* infection and aflatoxin accumulation [87]. In addition, they are also involved in the adaptation to drought, saline conditions and low temperatures [88].

Dehydrodiferulic acid deposition and cross-linkages in maize cell walls is a highly heritable trait that is genetically regulated [81–83]. Similarly, some studies suggest that the level of HCAAs in maize grain is genetically regulated, and that some maize lines landraces[1] are more resistant to biotic and abiotic stresses possibly due to their higher HCAAs content [77,87,88]. Thus, maize breeding for improved hydroxycinnamic acid concentration can increase the natural resistance of maize plants [81,83,89].

3.2.3.4. Bioavailability of ferulic acid in maize products

Feruloylated arabinoxylans mono and oligosaccharides are a subject of growing interest because they possess the physiological functions of both ferulic acid and dietary fibre [69,90]. The oral administration of feruloylated oligosaccharides showed higher prebiotic activity compared with the xylooligosaccharides (similar structure without bound ferulic acid). Feruloylated oligosaccharides significantly increased bacterial richness and diversity in the faeces of rats, particularly diabetes-resistant bacteria, whereas decreased diabetes-prone bacteria [90].

The bioavailability of ferulic acid is greatly influenced by the food matrix and arabinoxylans' structure [26,91]. Having higher concentration of feruloyl mono or oligosaccharides may be advantageous on ferulic acid bioaccessibility, due to an increase in their solubility [69]. In addition, after consumption of maize fibre, the increase in ferulic acid concentration in plasma and urine is quite low, but maintained for longer [69]. After ingestion, the low gastric pH may partially release feruloyl arabinose from arabinoxylans, however, data on release of feruloylated arabinoxylans during gastric digestion is limited [69]. The ferulic acid

[1] **Landrance**: Dynamic population of a locally adapted cultivated plant that has historical origin, distinct identity, and shows genetic diversity [7].

covalently bound to indigestible polysaccharides can be absorbed after being released by intestinal esterases from intestinal tissues and from the colon microbiota [20,92]. After the action of esterases, aglycones, such as free ferulic acid, and some simple ferulic acid oligosaccharides become available for absorption by a diffusion mechanism [26,69].

The colon is also an active site of ferulic acid metabolism. Ferulic acid is rapidly metabolized by the intestinal microbiota into phenylpropionic acids, that can be absorbed [91,92]. The bound phenolic acids have very low bioavailability because the bran matrix severely reduces the interactions between esterases and other hydrolysing enzymes and the polymers of ferulic acid [26,33]. However, mechanical treatments leading to particle size reduction and structural breakdown of cereal matrices, such as in the production of maize flour, may also affect the bioaccessibility and bioavailability of phenolic compounds [26,93].

3.2.3.5. Analysis of phenolic acids and phenolic acid derivatives

Hydroxycinnamic acids and their derivatives can be identified and quantitated using different methods of extraction and analysis. Usually, the maize sample is finely grounded to a particle size of 0.5–1 mm [49,63,94–96]. The soluble-free phenolics and small feruloylated oligosaccharides that are only loosely bound at the cell wall surface can then be extracted by microwave irradiation [97], or ultrasonication with 60–80% methanol [30,83,98] or ethanol [32,69,94,99]. Bound phenolics are not easily extracted because of the linkages between arabinoxylan chains and other cell wall components [44], thus it is necessary to apply a hydrolysis treatment to the residue obtained after the centrifugation of the soluble extract [100]. This residue is sometimes described as alcohol insoluble residue (AIR) [45,49,81].

Different hydrolysis procedures (mainly alkaline, acid and enzymatic) have been developed to remove arabinoxylans from the cell walls. The macromolecular characteristics of extracted arabinoxylans and the extraction yields achieved exhibit huge differences, depending on the methods used for extraction and hydrolysis [44].

Alkaline hydrolysis (saponification) is the main procedure for extracting phenolic acids, since acid hydrolysis may degrade them [44,100]. Hydroxyl ions disrupt the hydrogen bonds between cellulose and hemicellulose, and also break ester linkages, particularly between phenolic acids linked to the cell wall, and then solubilise part of the hemicellulose material. Ether linkages can also be broken in stronger hydrolysis conditions, and insoluble arabinoxylans can be extracted [36,44]. However, an appreciable amount of phenolics bound to the cell walls constituents, or trapped in cores within the food matrix, may remain insoluble [100]. Alkaline hydrolysis can be performed at different NaOH or KOH concentrations (0.1–10 M), and at different temperatures (room temperature to 170 °C), and for different incubation times (1–24 h), generally under slow agitation [46,94,96,101]. To avoid oxidative processes, saponification is usually performed under nitrogen or argon and protected from light, to prevent *cis/trans* isomerization [32,83,94].

Hydroxycinnamic acids esterified to the wall lignin can be released by a dilute alkali treatment at room temperature with 1 M NaOH [99]. The ester-linkages of ferulic acid and *p*-coumaric acid monomers are normally broken performing a hydrolysis using 2 M NaOH, which releases hydroxycinnamic acids and their dehydrodimers, trimers and tetramers [56,102]. Different conditions result in different released compounds, for instance, some reported results show that a hydrolysis for 4 h, at room temperature, releases 8-5′-DFA, 8-O-4′-DFA, and 5-5′-DFA [81,103], whereas hydrolysis for 18 h, at room temperature, also releases 8-8′-DFA, tri and tetraferulic acids [52,81]. When increasing the temperature to 39 °C and hydrolysis time to 24 h, ester-linked ferulic acid and *p*-coumaric acid monomers are obtained [81,103]. Therefore, esterified phenolic acids are determined by the difference between the ferulic acid content of the saponified (soluble FA) and unsaponified (soluble-free phenolic acid content) samples [94,97]. Total (ester- and ether-linked) ferulate in the cell wall, as well as esterified *p*-coumaric acids, are liberated with 3–4 M NaOH at 120–170 °C for 2–3 h [69,81,95]. Thus, ether-linked hydroxycinnamic acids are calculated as the differences between total and ester-linked hydroxycinnamic acids [99,103].

The hydrolysis reaction is stopped by lowering the pH, usually below 3.0 with HCl, trifluoroacetic or phosphoric acid [49,69,94–96]. The acidification of the extract increases the solubility and extractability of phenolic compounds [60]. The resulting aqueous solution is submitted to extractions with diethyl ether, ethyl acetate, or chloroform, usually performed successively (3–5 times) [32,49,69,94,96]. The extracts are usually evaporated to dryness and dissolved in aqueous solvents, as methanol/water (50:50) before analysis [32,49,83,84,94,98].

In order to obtain ferulic or dehydrodiferulic acids and polysaccharides linked together by ester bonds, cell walls materials have been submitted to enzymatic degradation or to controlled acid hydrolysis [34]. Thus, acid hydrolysis is performed when the aim is to analyse hydroxycinnamic acids linked to arabinoxylans. Molecules of hemicelluloses obtained by acid hydrolysis are more linear, mainly because this treatment cleaves the side chains of xylans from the backbone [44]. Acid hydrolysis conditions have been developed and improved, for the esterified ferulic acid and feruloylated side-chains analysis, with minimal release of free phenolics [34,38,67,96]. An alternative hydrolysis method for feruloylated oligosaccharides is the enzymatic process, usually using endoxylanases combined with cellulases, arabinofuranosidases and feruloyl esterases, releasing arabinoxylans [36,44,104].

Usually, phenolic acids [29,105], including ferulic acid dehydrodimers [34,43], trimers and tetramers [54], esterified hydroxycinnamic acids [69,99], and hydroxycinnamic acid amides [77,78,87] are analysed by HPLC, using a reversed phase C18 column [29,69,82,87]. Several methods of HPLC analysis have been developed and optimised. The mobile phases used are mainly methanol [29,98] or acetonitrile [83,94,96] and acidified water, using, for instance, phosphoric [97] or formic [54,96,106] acids. A diode array detector is often used after HPLC separation. The detection and quantification of phenolic acids is usually performed at 280 and 320 or 325 nm [82,94,98,107], and, when standards of authentic phenolics are available, the retention time and UV are compared [49,84,94]. Frequently, HPLC coupled to mass spectrometry is used in order to confirm or identify hydroxycinnamic acids and their oligosaccharides derivatives by their molecular masses and characteristic fragmentation patterns, using different mass analysers, as quadrupole-time of flight (Q-TOF), ion trap and triple quadrupole (TQ) mass spectrometers [19,30,46,49,69,96].

3.3. The effect of maize processing on its nutritional quality

The nutritional quality of maize grains is greatly influenced by their processing mode [3]. During the processing of maize until its final product, and depending on the techniques employed, significant losses in nutrients can occur, as well as changes on their bioaccessibility[1] and bioavailability[2] [1,108]. Only the compounds that are released from the matrix or absorbed in the small intestine will become bioavailable [26].

For instance, refined maize flour obtained by mechanical separation of its constituents (endosperm, bran, and germ) by dry milling process has a different composition than whole maize flour, due to the removal of pericarp and germ [1]. Generally, after conventional dry milling, there is a decline in the nutritional quality of the flour, namely a decrease in the total amounts of fibre, fatty acid, water-soluble vitamins, carotenoids, and minerals [1,3,4]. Refined maize flour has a very low concentration of antioxidants and bioactive compounds [48], because the majority of phenolic compounds is located in the external layers of the seed [26].

Similarly, during the nixtamalization processing, there is also a physical loss of the pericarp and peduncle, resulting in amino acids, lipid, starch, vitamins, carotenoids, sugar and fibre losses [1,3]. Fermentation processes can also decrease the amount of crude fibre, minerals and protein contents and change the amino acid profile of fermented maize products [1]. In addition, factors as temperature, humidity and storage time largely influence the integrity of maize grain carotenoids, xanthophylls and phenolic compounds [1,3,26].

On opposite, the bioaccessibility of some nutrients can be improved by some processing techniques. For instance, there is a slight increase in the protein quality of tortillas due to the

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[1] **Bioaccessibility**: The amount of an ingested compound that is released from the food matrix in the gut and that may be able to pass through the intestinal barrier [26].

[2] **Bioavailability**: The amount of a compound that reaches the systemic circulation and the specific sites where it can exert its biological action [26].

improved availability of essential amino acids [1,3,4] and an improved bioaccessibility of thiamine, riboflavin, niacin and carotene, despite the decrease in their total contents [1,3]. The nixtamalization process causes an 400% increase in the calcium content, due to the use of calcium hydroxide [1,3,4]. Fermentation and cooking under heat and pressure can also improve the bioaccessibility of water-soluble vitamins [1,3].

Thermal processing techniques widely used in cereal processing, such as steaming, autoclaving, drying, roasting, bread making and microwave heating, have the potential to increase the extractability of phenolic compounds, due to the increase in the amounts of free phenolic acids [26,42,48]. However, high temperatures during cooking may lead to polymerization of some phenolic compounds, decreasing the extractable phenolic content [93]. Extrusion cooking is known to cause a decomposition of heat-labile phenolic compounds [26]. The leaching of phenolic compounds in the boiling water can occur during cooking, decreasing free phenolic acids content [60,109]. However, in contrast to cooked pasta prepared with traditional milling process, cooked pasta prepared with micronized kernels preserved the total phenolic content. This suggests a protective role of the kernel external layers, preventing the leaching of phenolic compounds in the boiling water [110].

Nutrient bioavailability is also affected by the presence of endogenously produced antinutrients, as phytic acid [14]. Although this compound is essential for seed germination and phosphate storage, it can negatively affect the bioavailability of essential minerals for human health, since it chelates cation minerals, such as calcium, iron and zinc [1]. In addition, dietary fibre also contributes to the low bioavailability of iron in maize [3]. Milling, soaking or heating the grain can degrade or remove about 40% of the phytic acid, but also remove binding minerals, making these processes only partially successful [3].

Some maize compounds, as arabinoxylans, can also affect the quality of the resulting food products. For instance, in breads, free arabinoxylans result in better retention of gases and enhanced elasticity and strength, increasing bread quality, whereas bound arabinoxylans reduce gas retention, having a negative impact on baking quality [42].

4. Aroma[1] and food quality

The sensory[2] properties are still one of the first reasons for consumer choices among different food products. In the particular case of breads, the sensory quality is defined by their

[1] **Aroma**: sensory attribute perceptible by the olfactory organ via the back of the nose when tasting.
[2] **Sensory**: relating to the use of the senses, i.e., to the experience of a person [152].

appearance (colour, volume), texture, flavour[1] and aroma [12,111]. Aroma and taste[2] (sweet, sour, salty, bitter and umami) play an important role in flavour perception, which can also be influenced by other factors, as food texture and temperature. In turn, aroma and taste can also influence each other [112]. The aroma strongly influences bread quality and is considered one of the main requirements for its acceptance by consumers [7,113]. A huge number of descriptors are used to distinguish different odours[3], which contribute to different olfactory responses [7]. Therefore, the contribution of a compound to the final bread aroma depends not only on its concentration, but also on its odorant power, which is determined by its odour threshold (OT), or odour activity value (OAV) [114]. Indeed, the main volatile compounds responsible for foods' aroma are often present in trace amounts, which difficult the task of aroma analysis [7]. The most relevant volatiles are alcohols, aldehydes, ketones, hydrocarbons and terpenes, generally from the plant or originated during food processing [7]. About 500 compounds have been detected in the complex aroma fraction of wheat bread [115] and the main chemical classes of volatile compounds are aldehydes, alcohols, ketones, esters acids, pyrazines, pyrrolines, hydrocarbons, furans and lactones [114].

4.1. Origin of volatile compounds in breads

In spite of the contribution of the flour to the final bread flavour has been estimated to be minor [114,116], some studies have indicated that variations in wheat flour odour directly affect bread flavour and odour [117,118]. The type of volatile compounds present in plants depends on several factors, as their species, genotype and environmental conditions [7]. Thus, organoleptic[4] and rheological quality of *broa* may depend on the variety of maize used. In fact, as mentioned previously, sensory analysis[5] in baking assays have demonstrated a preference for traditional in detriment of hybrid maize varieties for *broa* production, due to a better mouth feel flavour, texture and aroma [12]. However, until now, information about aroma volatile compounds in Portuguese maize and *broa* is scarce [7].

Due to the multiple origin of bread volatile compounds, their origin is difficult to determine [119], and depends on several factors, namely on the (i) recipe used (ingredients and processing techniques), (ii) dough fermentation [120], (iii) enzymatic activity during kneading [113,114], (iv) lipid oxidation, (v) thermal reactions during baking, such as Maillard and caramelization reactions [114] and (vi) storage [113]. However, it is at the baking stage that some

[1] **Flavour**: complex combination of the olfactory, gustatory and trigeminal sensations perceived during tasting [152].
[2] **Taste**: sensations perceived by the taste organ when stimulated by certain soluble substances [152].
[3] **Odour**: sensation perceived by means of the olfactory organ in sniffing certain volatile substances [152].
[4] **Organoleptic**: relating to an attribute perceptible by the senses, i.e., to an attribute of a product [152].
[5] **Sensory analysis**: science involved with the assessment of the organoleptic attributes of a product by the senses [152].

of the most valuable aroma impact compounds are generated [114,121–123]. These reactions are often quoted as "nonenzymatic browning reactions" [111] and include the Maillard reaction and, to a lower extent, caramelization reactions [114,116]. Caramelization occurs mainly when the bread surface temperature is above 120 °C [111,124]. Around 2 to 3% of sugars that are present in the dough undergo caramelization [125] and give rise to carbonyl compounds, furans, and brown-coloured complex polymers [111,114,116,123–125]. The much more relevant Maillard reaction [***Figure 1.4***] occurs between carbonyls (most often reducing sugars) and free amino groups of amino acids, peptides, or proteins [116,121,124,126–128]. Firstly, sugars and an amino compound condense, following by a rearrangement, originating 1-amino-1-deoxy-2-ketose (Amadori product), when an aldose sugar is involved [111,114,119,122,127,128], or 2-amino-2-deoxyaldose (Heyns product), when a ketose is involved [114,128]. Amadori and Heyns rearrangement products are unstable above ambient temperature [128] and are degraded by different pathways depending on the conditions [111,114,123]. When the pH is below 7, furfural (when pentoses are involved) or hydroxymethylfurfural (HMF) (when hexoses are involved) are formed [111,114,123,124,128]. Other furans, furanones, pyrans and pyranones are also formed at this stage [128,129]. Alternatively, when the pH is above 7, fragmentation of the Amadori and Heyns products or direct sugar dehydration and fragmentation [111,127] are favoured, giving rise to furan derivatives [114]. At this stage, some amino acids can also be degraded by Strecker degradation and originate volatile compounds, without the need of sugars [130]. Ultimately, condensation and polymerization occur, leading to the formation of highly coloured melanoidins [111,119,122,127,128] and several flavour compounds, including pyrazines, pyrroles, pyridines, furans, oxazoles, thiazoles and thiophenes [111,114,116,122,128,131].

The Maillard reaction is known to be promoted by low moisture, high temperature [132,133] and sugary ingredients [114]. Therefore, increasing the cooking temperature, reducing the moisture level, or prolonging the residence times generally increase the quantity of Maillard-derived products [133]. This reaction can also be inhibited by hydroxycinnamic acids, particularly ferulic acid, abundant in maize [35,134]. The presence of ferulic acid in whole wheat breads has been reported as the main reason for the difference in the aroma of breads prepared from whole and refined wheat flours [134].

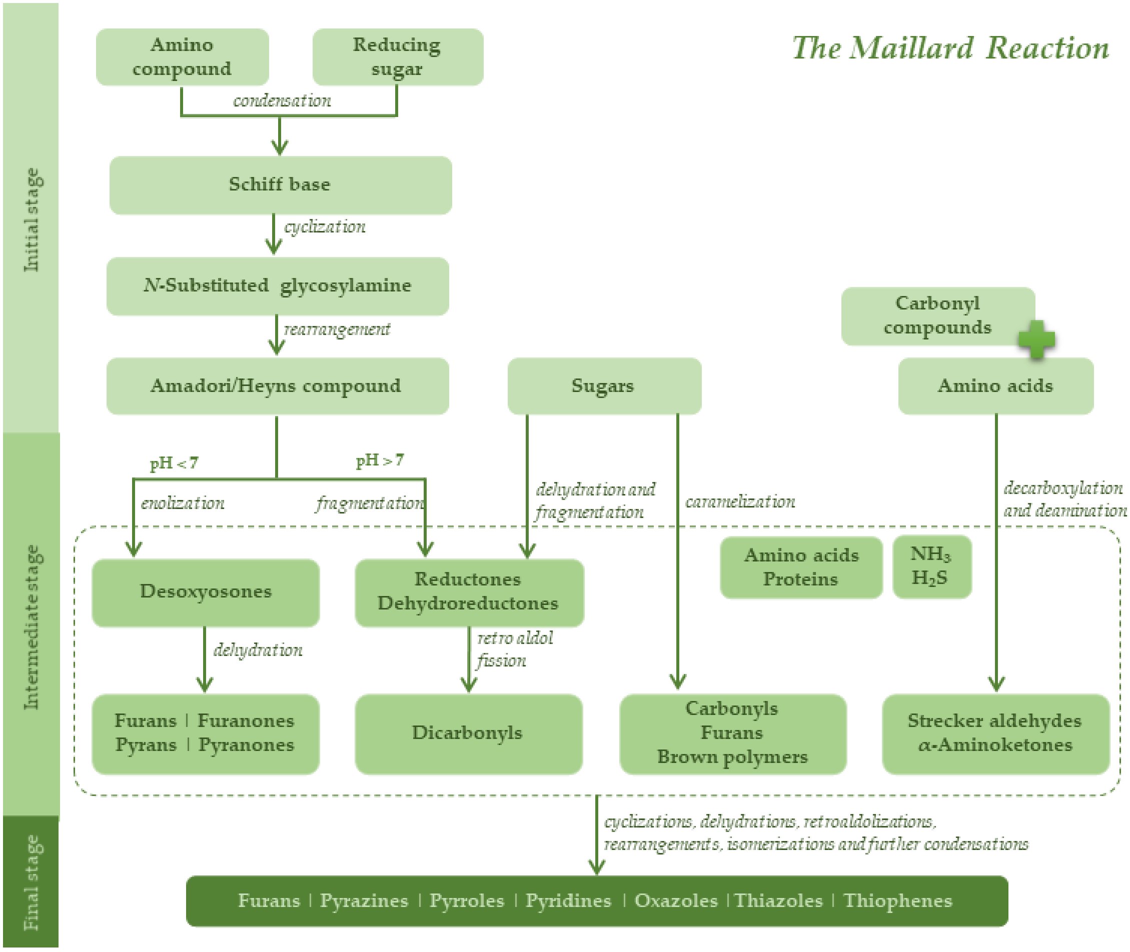

Figure 1.4: Main stages of the Maillard reaction [119,122].

Another abundant volatile compounds in breads which are usually considered off-flavours[1], contributing negatively for their aroma [126,135], are aldehydes, alcohols, acids, esters and hydrocarbons, mainly derived from lipid oxidation reactions [113,114,123,124,126,136]. These volatiles are produced by the plants through the action of lipoxygenases in response to wounding [122,137], and can also be produced during breadmaking, by the action of both the flour lipoxygenase [114] and enzymes associated with the metabolic activity of yeasts and LAB, during fermentation [125]. Antioxidant compounds, as phenolic acids, may contribute indirectly to flavour quality trough inhibition of lipid oxidation [7].

4.2. Analysis of volatile compounds

Nowadays, HS-SPME (headspace-solid phase microextraction) combined with gas chromatography – mass spectrometry (GC-MS) has become the preferred option for the analysis

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[1] **Off-flavour**: atypical flavour often associated with deterioration or transformation of the product [152].

of volatile flavour compounds from bakery products, including several breads [113]. Classic methodologies usually involve the extraction of volatile compounds by organic solvents, as dichloromethane and diethyl ether, and the use of a Soxhlet apparatus [113]. The volatiles must then be isolated from the non-volatile fraction, in order to avoid interferences in the gas chromatographic separation and GC column contamination, usually by distillation or sublimation processes [113]. Finally, the sample volume must be reduced to around 100 – 400 µL, in order to increase the intensity of the peak areas [113]. Therefore, HS-SPME has been widely used in bread volatiles analyses, as they are solvent free, environmentally friendly, less time consuming (they only require a minimum manipulation of the sample), and show high sensitivity and sensibility, with no interferences of non-volatile compounds [113,138–140]. The reduced sample handling prevents the production of artifacts, when comparing to conventional solvent extraction procedures [140].

SPME has been used for qualitative rather than quantitative purposes, mainly due to the difficulties in quantification arising from the complex interactions between volatile compounds and other compounds from the food matrix. However, HS-SPME-GC-MS methods have been improved and can give semi-quantitative information with a satisfactory precision [141]. Internal standards can be used [115,141], but the volatile fraction of bread includes quite different chemical groups and one or two internal standards are likely poorly able to mimic the extraction and chromatographic behaviour of compounds from all these structurally heterogeneous chemical groups [141]. Thus, the use of internal standards is not mandatory in the analysis of breads aromas [135,141–143]. The most accurate method of quantification of volatiles involves the use of isotopically labelled standards, but these are commercially available only for a small number of compounds, are expensive, and sometimes unstable, whereas their in-house preparation is, generally, labour intensive and complex [113,141].

Although the volatile composition of breads and maize-based foods has been usually characterized by HS-SPMS-GC-MS techniques (1D-GC), the volatile composition of breads is usually very complex, and there are some volatiles which are present in very low concentrations that are essential for breads aroma [114,116,124,138,141]. Thus, an analysis with a highly sensitive technique can detect important volatiles for the overall food aroma, which have not been previously detected. Comprehensive two-dimensional gas chromatography (GC×GC) has emerged as a powerful analytical technique which is an excellent choice when the composition of complex samples has to be unravelled [144,145]. This technique shows an enhanced separation capacity, presenting a very high resolving power [140,145]. Theoretically, GC×GC employs two orthogonal mechanisms to separate the constituents of the sample within a single analysis, based on the application of two GC columns coated with different stationary phases, which increases peak capacity [140,146]. The entire first-dimension eluate is divided into small adjacent portions and subjected to further analysis on the second (second-dimension) column [144,146]. This means that each ^{1}D peak is modulated several times, largely preserving the ^{1}D separation [144,146]. Compounds co-eluting from ^{1}D undergo additional separation on the ^{2}D [144] and therefore, the separation potential is greatly enhanced when compared to one-dimensional GC (1D-GC). Sensitivity and limits of detection are improved due to focusing of the peak in the modulator and separation of analytes from chemical background [140,146]. Signal-to-noise ratio is enhanced for GC×GC, compared to 1D-GC [140]. Advantages to this technique are that it considerably shortens the identification process and avoids enrichment steps that might result in artifact formation, for instance in the case of sulphur compounds [145]. If structural information has to be provided to enable unambiguous identification – and ensure high selectivity throughout the chromatogram– a mass spectrometer has to be used as detection method [144]. Peaks are usually recorded by using high data acquisition speed of ToFMS (time-of-flight mass spectrometry), which provides sufficient data density required for GC×GC separations [140,144,146]. Other advantage is that it allows a full mass spectra acquisition at trace level sensitivity and mass spectral continuity, which allows for deconvolution of spectra of co-eluted peaks [140,146].

5. Portuguese traditional maize varieties

Due to its privileged historical and geographical position as an enter point of new species into Europe, Portugal was among the first European nations to adopt maize in its agricultural systems, at the end of the fifteenth century [1,7,17]. This species rapidly spread throughout the country [7,17], resulting on a very diverse germplasm with an important role on animal feed and human food, as in the production of *broa* [17]. This led to an agricultural revolution, enhancing the rural population’s standard of living [7]. Natural and human selection adapted varieties to the different pedoclimatic environments existing in the country, and numerous landraces, mainly white kernel flint type open pollinated varieties (OPV), have been developed during centuries of cultivation, adapted to specific regional growing conditions and farmer’s needs, creating a diverse maize germplasm [7,16,17]. The Iberian maize germplasm displays no close relationship with any American types, although it shares alleles with both Caribbean and North American flints [7].

Maize breeding started with open pollinated varieties, but today commercial hybrid varieties are the most distributed throughout the world [4]. After World War II, Portugal was one of the first European countries to test the American maize hybrids. Although initially they were not well accepted by the Portuguese farmers, due to their unsuitability for food or polycropping systems, a vast number of cultivated landraces were replaced by modern hybrid varieties, especially in more favourable environments [7]. Consequently, a growing concern that several maize landraces may had been lost forever started to emerge in the late 1970’s, which led, in

1975, Silas Pego to initiate collection missions for maize [7]. A few years later, in 1986, substantial changes in our agriculture policy took place, after Portugal and Spain entered the European Community. According to European Union regulations on food safety, only varieties that are officially registered and listed, after meeting several requirements, can be commercialized [7]. However, these rules are not realistically applied to endogenous food products and have been pointed out as one of the major threats for their extinction [11]. In fact, the traditional small farming was severely affected and, in two decades, farmers were pushed to bankruptcy [7]. These changes have contributed to a major loss of genetic diversity [16], and the risk of losing precious sources of interesting agronomic traits selected over centuries of cultivation can soon become a reality [17].

Despite all of these challenges, landrace cultivation has persisted in Portugal, in particular in less favourable environments, mainly due to their increased stability to biotic and abiotic stresses, such as drought or aluminium toxicity, accomplished through generations of natural and deliberate selection [7]. Another main reason for the preservation of these landraces in particular Portuguese regions is the high-quality of the resulting *broa*, which seems to have compensated their lower yields [7]. These varieties show technological capacity and aroma characteristics highly valued for bread production and origin *broas* with better flavour and aromas, which are not found on the available commercial hybrid varieties [7,17]. Depending on the region of the country, white or yellow maize varieties may be used to produce *broa* [4]. Due to cultural and historical reasons, the white maize is the preferred choice by northwest rural populations, once it resembles white wheat bread, which was a symbol of wealth and prestige around the 18th century [7].

In the last few years, the world has shown us its unpredictability. From the challenges posed by climate changes and major conflicts, to the sudden onset of pandemic crises, the world has been advising us that we need to redefine our priorities. Building resilience is essential to face the unexpected. Currently, Portugal does not produce enough food to all the population, and imports around 75% of the maize it needs [147]. The cultivation of local foods, as Portuguese maize traditional varieties, can help decreasing food reliance from other countries. In addition, the total global food demand is expected to increase by 30% to 62% between 2010 and 2050 [5]. The world population growth requires raising overall food production and therefore, developing agriculture in more marginal and stress environments is of foremost importance [7]. Portuguese OPVs are crucial for achieving this goal, since they are more resilient to biotic and abiotic stresses, and the erosion of these resources can result in a severe risk to food security [7]. In this regard, participatory research approaches have emerged world-wide, as a relevant and necessary response to the problem of preserving genetic diversity in industrialized countries [7]. Contrary to conventional plant breeding, which focuses on developing modern varieties with high yield and wide adaptation to favourable environments, participatory plant breeding (PPB) programs intend to fit a multicrop agricultural system [7,148]. In PPB, biodiversity can be increased due to the use of heterogeneous populations, and different varieties are selected at different locations [7]. A participatory maize breeding was initiated in Portugal in 1984 by Silas Pêgo at Sousa Valley (VASO project), in the Northwest of Portugal, in order to provide an incentive for *in-situ* conservation of traditional maize landraces and to improve the social well-being of rural communities by increasing small farmers' income [7,148]. In a first survey, two regional varieties with local adapted germplasm were selected: "Pigarro" and "Amarelo miúdo" ("Amiúdo"). This project has been extended to other regions of the country, and additional landraces were also conserved, such as "Basto", "Aljezudo", "Castro Verde", and "Verdial de Aperrela" [7]. Besides the already considered technological ability for *broas* production, a special attention has been also given to quality traits such as health-promoting effects [7]. A study in rats revealed that *broa* showed a lower glycaemic index than wheat bread, due to its greater resistant starch content, which may reduce the risk of prevalent chronic diseases, as obesity and diabetes [149]. In addition, *broas* prepared from Portuguese traditional maize varieties may show additional beneficial health effects than hybrids, since traditional flours have shown significant higher protein and fibre contents than commercial varieties [150].

However, Portuguese maize varieties are still poorly studied, especially in regards to their phenolic composition. The study of phytochemicals is important to identify the specific compounds that simultaneously offer protection against biotic and abiotic stresses and have health-promoting properties [8]. This knowledge could be used for the development of marketing and information campaigns that would result in the valorisation of the landraces cultivated by traditional farmers [8]. Indeed, consumers are increasingly aware of the importance of diet in human health, which has influenced consumers' choice of food products and led to a growing demand for functional foods products [151].

In addition, a label for traditional *broa* could support local development, not only by enhancing the maize value chain, but also due to positive externalities in other sectors, as in gastronomy and tourism [11]. In order to give recognition and protection to traditional varieties, the European Union has approved, in 2008, a special treatment for the so called "Conservation Varieties" by which landraces threatened by genetic erosion, as traditional Portuguese maize varieties, can be registered for commercialization under certain conditions (Directive 2008/62/EC from 20 June 2008) [7]. Thus, maize Portuguese varieties should be studied and characterized in order to encourage the cultivation of the most promising ones.

6. Objectives and layout of the thesis

The aim of this work was to contribute to the valorisation of a traditional Portuguese product, *broa*, and promote the cultivation of the most promising traditional maize varieties. Traditional Portuguese maize varieties are not well characterized, in fact, scarce scientific studies on *broa* have been reported. This work focused on the study of relevant quality attributes which have a major impact on consumers' preferences, specially related to health benefits and sensory properties. Phenolic compounds can contribute to the prevention of non-transmissible diseases, whereas volatile compounds contribute to odour and aroma characteristics.

In order to achieve these goals, the work developed in this PhD project is presented in the next fourth chapters, comprising the characterization of the phenolic (chapters 2 and 3) and volatile (chapters 4 and 5) composition of both maize flours and *broas*. These chapters were written in the form of research articles. The general state of the art was presented in the current chapter (chapter 1, introduction). The samples studied in the present work are also described in this chapter.

In more detail, the identification of phenolic compounds of maize and *broas*, mainly hydroxycinnamic acids and derivatives, is presented in chapter 2. The total (soluble and insoluble) phenolic composition of *broas* was described for the first time, and several hydroxycinnamic acid amides, which had not been previously described, were tentatively identified in both maize flours and *broas*.

In chapter 3, the main soluble and insoluble phenolic compounds present in maize flour and *broas* were quantitated and the impact of processing to *broas* was evaluated and discussed. The knowledge about the phenolic acids' derivatives present in *broas* is extremely important, in order to understand their bioaccessibility in this final product and, consequently, their possible health effects. This information is particularly relevant for future bioavailability studies.

The main volatile compounds present in traditional Portuguese maize flours and *broas* are described in chapter 4. The influence of the volatiles, phenolic compounds and carotenoids, on the volatile composition of *broas* was also analysed. These results were correlated with a sensory evaluation of this traditional bread, in order to identify the volatiles which may have a more important impact on *broas* flavour, contributing to consumers' acceptance. A deeper look into the volatiles associated with the baking process and mostly characterized by favourable sensory characteristics is presented in chapter 5.

Finally, a general discussion is presented in chapter 6, aiming at articulating the previous chapters. Future perspectives are also discussed, taking into account the results obtained and described in this thesis.

7. Cereal flours and *broas* studied in this work

In this work, the whole flour from eleven traditional or participatory improved maize Portuguese OPVs (F1 to F11, from now on referred to as traditional varieties, since the participatory improved were also derived from traditional varieties) and a commercial hybrid maize flour (Nacional Type 175, F12), were studied. Their general characteristics are summarized in ***Table 1.4***. The selected traditional varieties are representative of the national maize germplasm variability, taking into account their agronomic performance in field trials, basic nutritional quality and genetic diversity evaluated under the scope of the FP7 SOLIBAM European project. Nine of these varieties were obtained from the VASO participatory maize breeding program [7], and the remaining two (Broa-213, F5 and Broa-57, F11) were collected from a field expedition to the Central Northern region of Portugal (Beira Interior and Beira Litoral), in 2005 [16]. The maize landraces collected in this expedition were known for the good quality of the resulting *broas*, with the idea of establishing an on-farm conservation project through participatory breeding approaches [16].

All maize samples were obtained from field trials conducted at ESAC (Escola Superior Agrária de Coimbra, Coimbra, Portugal). Flours were obtained after milling the whole maize grain in an artisan water mill with millstones (Moinhos do Inferno, Viseu, Portugal), with the exception of the commercial hybrid sample (F12), which was acquired already milled. The commercial rye (Concordia type 70, Portugal) and wheat (National type 65, Portugal) flours used for *broas* preparation were also acquired already milled.

Table 1.4: Maize flours and *broas* identification and description.

Flour	Broa	Variety	Kernel Colour	Description	Origin
F1	**B1**	SinPre	white	Synthetic OPV: from the cross of 12 divergent original maize populations developed as an experimental higher-quality cultivar with increased precocity.	VASO participatory maize breeding program [7]
F2	**B2**	Aljezudo	yellow	Flint FAO 300 improved hybrid OPV: from the cross made around the years 2000–2005 between two historical populations, Aljezur × Amiúdo.	VASO participatory maize breeding program [7]
F3	**B3**	Bastos	white	Early flint OPV.	VASO participatory maize breeding program [7]
F4	**B4**	Amiúdo	yellow	Also known as “Amarelo miúdo”. Early flint FAO 200 OPV adapted to stress conditions (soils with low pH, water stress and aluminium toxicity), but also with quality for bread production.	VASO participatory maize breeding program [7,17]

F5	**B5**	Broa-213	yellow	Early intermediate, traditional farmer OPV.	Collected from the farmer in the 2005 expedition to the Central Northern region of Portugal [16]
F6	**B6**	Pigarro	white	Flint FAO 300 OPV with strong fasciation expression, used in the best soils for human consumption. Pigarro is the type of seed that better fits the high-quality standards of the most famous bread (*broa* de Avintes).	VASO participatory maize breeding program [7,17]
F7	**B7**	Algarro	yellow	Early flint hybrid OPV: from the cross made around the years 2000–2005 between two historical populations, Aljezur × Pigarro.	VASO participatory maize breeding program [7]
F8	**B8**	Castro Verde	yellow	Late flint FAO 600 improved OPV, with big kernel row number and large ear size.	VASO participatory maize breeding program [7]
F9	**B9**	Verdial de Aperrela	white	Late flint FAO 600 OPV selected for higher bread making quality, which was almost extinct at the time it entered the breeding project.	VASO participatory maize breeding program [7,17]
F10	**B10**	Fandango	yellow	Synthetic dent FAO 600 (originally FAO 700) OPV with big kernel row number and large ear size, competing yield over 10 t/ha.	VASO participatory maize breeding program [7]
F11	**B11**	Broa-57	white	Early flint traditional farmer OPV.	Collected from the farmer in the 2005 expedition to the Central Northern region of Portugal [16]
F12	**B12**	Commercial	white	Nacional Type 175, wholegrain flour (from a commercial hybrid maize variety).	Obtained already milled from a bakery

Broas were prepared in a bakery following a traditional recipe [12,149]. The ingredients included 70% maize flour, 20% commercial rye flour and 10% commercial wheat flour, as well as 95% (v/w, flour basis) of water, 3.6% (w/w, flour basis) sugar, 2.2% (w/w, flour basis) salt, 0.5% (w/w, flour basis) of improver (S500 Acti-plus, Puratos) and 0.8% (w/w, flour basis) dry yeast (Fermipan, DSM, Holland). Sourdough was prepared using the same recipe of *broa* and adding enough bacteria suspension (*Lactobacillus brevis* and *plantarum* previously isolated) to yield 107 CFU/g mass concentration and was kept at 25 °C during 12 h before its use.

Flours were mixed with 80% boiling water (vol/wt, flour basis) containing 1.76% salt and kneaded for 5 min (Ferneto AEF035). The dough was allowed to rest and cool to 27 °C, and the remaining ingredients (sugar, salt, dry yeast, sourdough) and 20% water were added. The dough was again kneaded for 8 min and left to rest for bulk fermentation at 25 °C for 90 min. After fermentation, the dough was manually moulded into 400 g balls and baked in the oven (Matador, Werner & Pfleiderer Lebensmitteltechnik GmbH, Dinkelsbühl, Germany) at 270 °C for 40 min. Before extraction of phenolic compounds, *broas* were milled using a grinding mill (IKA MF 10.2, Königswinter, Germany) with a 1.5 mm sieve. For the analysis of the volatile compounds, *broas* were smashed manually.

References

1. Food and Agriculture Organization of the United Nations (FAO) *Maize in Human Nutrition*; FAO: Rome, Italy, 1992; ISBN 9789251030134.
2. Rouf Shah, T.; Prasad, K.; Kumar, P. Maize: A Potential Source of Human Nutrition and Health: A Review. *Cogent Food Agric.* **2016**, *2*, 1–9.
3. Nuss, E.T.; Tanumihardjo, S.A. Maize: A Paramount Staple Crop in the Context of Global Nutrition. *Compr. Rev. Food Sci. Food Saf.* **2010**, *9*, 417–436.
4. León, A.E.; Rosell, C.M. *De Tales Harinas, Tales Panes: Granos, Harinas y Productos de Panificación En Iberoamérica*; Hugo Báez: Córdoba, Argentina, **2007**; ISBN 9789871311071
5. van Dijk, M.; Morley, T.; Rau, M.L.; Saghai, Y. A Meta-Analysis of Projected Global Food Demand and Population at Risk of Hunger for the Period 2010–2050. *Nat. Food* **2021**, *2*, 494–501.
6. Bento-Silva, A.; Patto, M.C.V.; do Rosário Bronze, M.; Vaz Patto, M.C.; do Rosário Bronze, M.; Patto, M.C.V.; do Rosário Bronze, M. Relevance, Structure and Analysis of Ferulic Acid in Maize Cell Walls. *Food Chem.* **2018**, *246*, 360–378.
7. Vaz Patto, M.C.; Mendes-Moreira, P.M.; Alves, M.L.; Mecha, E.; Brites, C.; Bronze, R.; Pego, S. Participatory Plant Quality Breeding : An Ancient Art Revisited by Knowledge Sharing. The Portuguese Experience. In *Plant breeding from laboratories to fields*; InTechOpen, 2013; pp. 255–288.
8. Elisa, D.H.; Marcela, G.M.; Janet Alejandra, G.U.; Martha Elena, D.H. The Nutraceutical Value of Maize (*Zea Mays* L.) Landraces and the Determinants of Its Variability: A Review. *J. Cereal Sci.* **2022**, *103*.
9. Roberts K *Handbook of Plant Science Vol. 2.*; John Wiley & Sons, Ltd.: New Jersey, USA, 2007; ISBN: 9780470057230.
10. Tenaillon, M.I.; Charcosset, A. A European Perspective on Maize History. *Comptes Rendus - Biol.* **2011**, *334*, 221–228.
11. Revilla, P.; Alves, M.L.; Andelković, V.; Balconi, C.; Dinis, I.; Mendes-Moreira, P.; Redaelli, R.; Ruiz de Galarreta, J.I.; Vaz Patto, M.C.; Žilić, S.; et al. Traditional Foods From Maize (*Zea Mays* L.) in Europe. *Front. Nutr.* **2022**, *8*.
12. Brites, C.; Trigo, M.J.; Santos, C.; Collar, C.; Rosell, C.M. Maize-Based Gluten-Free Bread: Influence of Processing Parameters on Sensory and Instrumental Quality. *Food Bioprocess Technol.* **2010**, *3*, 707–715.
13. Díaz-Montes, E.; Castro-Muñoz, R.; Yáñez-Fernández, J. An Overview of Nejayote, a Nixtamalization by Product. *Ing. Agrícola y Biosist.* **2016**, *8*, 41–60.
14. Smith, J.R. 50 of the World's Best Breads. Available online: https://edition.cnn.com/travel/article/world-50-best-breads/index.html. (Accessed on 20 March 2022).
15. Rocha, J.M.; Malcata, F.X. On the Microbiological Profile of Traditional Portuguese Sourdough. *J. Food Prot.* **1999**, *62*, 1416–1429.
16. Patto, M.C.V.; Moreira, P.M.; Carvalho, V.; Pego, S. Collecting Maize (*Zea Mays* L. Convar. Mays) with Potential Technological Ability for Bread Making in Portugal. *Genet. Resour. Crop Evol.* **2007**, *54*, 1555–1563.
17. Vaz Patto, M.C.; Alves, M.L.; Almeida, N.F.; Santos, C.; Moreira, P.M.; Satovic, Z.; Brites, C.; Patto, V.; Alves, N.F.; Almeida, C.S.; et al. Is the Bread Making Technological Ability of Portuguese Traditional Maize Landraces Associated with Their Genetic Diversity? *Maydica* **2009**, *54*, 297–311.

18. Correia, P.M.R.; Soares, A.M.; Brites, C. Quality Characteristics of Maize Flours and Breads. *ETP Int. J. Food Eng.* **2016**, *2*, 113–118.

19. Guo, W.; Beta, T. Phenolic Acid Composition and Antioxidant Potential of Insoluble and Soluble Dietary Fibre Extracts Derived from Select Whole-Grain Cereals. *Food Res. Int.* **2013**, *51*, 518–525.

20. Lappi, J.; Aura, A.M.; Katina, K.; Nordlund, E.; Kolehmainen, M.; Mykkänen, H.; Poutanen, K. Comparison of Postprandial Phenolic Acid Excretions and Glucose Responses after Ingestion of Breads with Bioprocessed or Native Rye Bran. *Food Funct.* **2013**, *4*, 972–981.

21. Van Hung, P. Phenolic Compounds of Cereals and Their Antioxidant Capacity. *Crit. Rev. Food Sci. Nutr.* **2016**, *56*, 25–35.

22. Bathla, S.; Jaidka, M.; Kaur, R. Nutritive Value. In *Maize - Production and use*; Hossain, A., Ed.; IntechOpen, 2019; p. 13.

23. Lattimer, J.M.; Haub, M.D. Effects of Dietary Fiber and Its Components on Metabolic Health. *Nutrients* **2010**, *2*, 1266–1289.

24. Bhatt, T.; Patel, K. Carotenoids: Potent to Prevent Diseases Review. *Nat. Products Bioprospect.* **2020**, *10*, 109–117.

25. Eggersdorfer, M.; Wyss, A. Carotenoids in Human Nutrition and Health. *Arch. Biochem. Biophys.* **2018**, *652*, 18–26.

26. Gutiérrez-Grijalva, E.P.; Ambriz-Pére, D.L.; Leyva-López, N.; Castillo-López, R.I.; Heredia, J.B. Review: Dietary Phenolic Compounds, Health Benefits and Bioaccessibility. *Arch. Latinoam. Nutr.* **2016**, *66*, 87–100.

27. Buanafina, M.M. de O. Feruloylation in Grasses: Current and Future Perspectives. *Mol. Plant* **2009**, *2*, 861–872.

28. Mancuso, C.; Santangelo, R. Ferulic Acid: Pharmacological and Toxicological Aspects. *Food Chem. Toxicol.* **2014**, *65*, 185–195.

29. Das, A.K.; Singh, V. Antioxidative Free and Bound Phenolic Constituents in Pericarp, Germ and Endosperm of Indian Dent (*Zea Mays* Var. *Indentata*) and Flint (*Zea Mays* Var. *Indurata*) Maize. *J. Funct. Foods* **2015**, *13*, 363–374.

30. Das, A.K.; Singh, V. Antioxidative Free and Bound Phenolic Constituents in Botanical Fractions of Indian Specialty Maize (*Zea Mays* L.) Genotypes. *Food Chem.* **2016**, *201*, 298–306.

31. Urias-Lugo, D.A.; Heredia, J.B.; Muy-Rangel, M.D.; Valdez-Torres, J.B.; Serna-Saldívar, S.O.; Gutiérrez-Uribe, J.A. Anthocyanins and Phenolic Acids of Hybrid and Native Blue Maize (*Zea Mays* L.) Extracts and Their Antiproliferative Activity in Mammary (MCF7), Liver (HepG2), Colon (Caco2 and HT29) and Prostate (PC3) Cancer Cells. *Plant Foods Hum. Nutr.* **2015**, *70*, 193–199.

32. Urias-Lugo, D.A.; Heredia, J.B.; Serna-Saldivar, S.O.; Muy-Rangel, M.D.; Valdez-Torres, J.B. Total Phenolics, Total Anthocyanins and Antioxidant Capacity of Native and Elite Blue Maize Hybrids (*Zea Mays* L.). *CyTA-Journal Food* **2015**, *13*, 336–339.

33. Zhao, Z.; Moghadasian, M.H. Chemistry, Natural Sources, Dietary Intake and Pharmacokinetic Properties of Ferulic Acid: A Review. *Food Chem.* **2008**, *109*, 691–702.

34. Saulnier, L.; Thibault, J.-F. Ferulic Acid and Diferulic Acids as Components of Sugar-Beet Pectins and Maize Bran Heteroxylans. *J. Sci. Food Agric.* **1999**, *79*, 396–402.

35. Vitaglione, P.; Napolitano, A.; Fogliano, V. Cereal Dietary Fibre: A Natural Functional Ingredient to Deliver Phenolic Compounds into the Gut. *Trends Food Sci. Technol.* **2008**, *19*, 451–463.

36. Mathew, S.; Abraham, T.E. Ferulic Acid: An Antioxidant Found Naturally in Plant Cell Walls and Feruloyl Esterases Involved in Its Release and Their Applications. *Crit. Rev. Biotechnol.* **2004**, *24*, 59–83.

37. Santiago, R.; Malvar, R.A. Role of Dehydrodiferulates in Maize Resistance to Pests and Diseases. *Int. J. Mol. Sci.* **2010**, *11*, 691–703.

38. Saulnier, L.; Vigouroux, J.; Thibault, J.F. Isolation and Partial Characterization of Feruloylated Oligosaccharides from Maize Bran. *Carbohydr. Res.* **1995**, *272*, 241–253.

39. Lopez-Martinez, L.X.; Oliart-Ros, R.M.; Valerio-Alfaro, G.; Lee, C.-H.; Parkin, K.L.; Garcia, H.S. Antioxidant Activity, Phenolic Compounds and Anthocyanins Content of Eighteen Strains of Mexican Maize. *LWT-Food Sci. Technol.* **2009**, *42*, 1187–1192.

40. Mora-Rochin, S.; Gutiérrez-Uribe, J.A.; Serna-Saldivar, S.O.; Sánchez-Peña, P.; Reyes-Moreno, C.; Milán-Carrillo, J. Phenolic Content and Antioxidant Activity of Tortillas Produced from Pigmented Maize Processed by Conventional Nixtamalization or Extrusion Cooking. *J. Cereal Sci.* **2010**, *52*, 502–508.

41. Knudsen, K.E.B. Fiber and Nonstarch Polysaccharide Content and Variation in Common Crops Used in Broiler Diets. *Poult. Sci.* **2014**, *93*, 2380–2393.

42. Saeed, F.; Pasha, I.; Anjum, F.M.; Sultan, M.T. Arabinoxylans and Arabinogalactans: A Comprehensive Treatise. *Crit. Rev. Food Sci. Nutr.* **2011**, *51*, 467–476.

43. Saulnier, L.; Crépeau, M.J.; Lahaye, M.; Thibault, J.F.; Garcia-Conesa, M.T.; Kroon, P.A.; Williamson, G. Isolation and Structural Determination of Two 5,5'-Diferuloyl Oligosaccharides Indicate That Maize Heteroxylans Are Covalently Cross-Linked by Oxidatively Coupled Ferulates. *Carbohydr. Res.* **1999**, *320*, 82–92.

44. Zhang, Z.; Smith, C.; Li, W. Extraction and Modification Technology of Arabinoxylans from Cereal By-Products: A Critical Review. *Food Res. Int.* **2014**, *65*, 423–436.

45. Kim, H.; Ralph, J. Solution-State 2D NMR of Ball-Milled Plant Cell Wall Gels in DMSO-d 6/Pyridine-D5. *Org. Biomol. Chem.* **2010**, *8*, 576–591.

46. Vismeh, R.; Lu, F.; Chundawat, S.P.S.S.; Humpula, J.F.; Azarpira, A.; Balan, V.; Dale, B.E.; Ralph, J.; Jones, A.D. Profiling of Diferulates (Plant Cell Wall Cross-Linkers) Using Ultrahigh-Performance Liquid Chromatography-Tandem Mass Spectrometry. *Analyst* **2013**, *138*.

47. Chateigner-Boutin, A.-L.; Ordaz-Ortiz, J.J.; Alvarado, C.; Bouchet, B.; Durand, S.; Verhertbruggen, Y.; Barrière, Y.; Saulnier, L. Developing Pericarp of Maize: A Model to Study Arabinoxylan Synthesis and Feruloylation. *Front. Plant Sci.* **2016**, *7*, 1–20.

48. Ragaee, S.; Seetharaman, K.; Abdel-Aal, E.-S.M. The Impact of Milling and Thermal Processing on Phenolic Compounds in Cereal Grains. *Crit. Rev. Food Sci. Nutr.* **2014**, *54*, 837–849.

49. Pedersen, M.B.; Bunzel, M.; Schäfer, J.; Knudsen, K.E.B.; Sørensen, J.F.; Yu, S.; Lærke, H.N.; Schäfer, J.; Knudsen, K.E.B.; Sørensen, J.F.; et al. Ferulic Acid Dehydrodimer and Dehydrotrimer Profiles of Distiller's Dried Grains with Solubles from Different Cereal Species. *J. Agric. Food Chem.* **2015**, *63*, 2006–2012.

50. Bunzel, M.; Funk, C.; Steinhart, H. Semipreparative Isolation of Dehydrodiferulic and Dehydrotriferulic Acids as Standard Substances from Maize Bran. *J. Sep. Sci.* **2004**, *27*, 1080–1086.

51. Bunzel, M.; Ralph, J.; Marita, J.M.; Hatfield, R.D.; Steinhart, H. Diferulates as Structural Components in Soluble and Insoluble Cereal Dietary Fibre. *J. Sci. Food Agric.* **2001**, *81*, 653–660.

52. Dobberstein, D.; Bunzel, M. Separation and Detection of Cell Wall-Bound Ferulic Acid Dehydrodimers and Dehydrotrimers in Cereals and Other Plant Materials by Reversed Phase High-Performance Liquid Chromatography with Ultraviolet Detection. *J. Agric. Food Chem.* **2010**, *58*, 8927–8935.

53. Bunzel, M.; Ralph, J.; Funk, C.; Steinhart, H. Structural Elucidation of New Ferulic Acid-Containing Phenolic Dimers and Trimers Isolated from Maize Bran. *Tetrahedron Lett.* **2005**, *46*, 5845–5850.

54. Bunzel, M.; Ralph, J.; Brüning, P.; Steinhart, H. Structural Identification of Dehydrotriferulic and Dehydrotetraferulic Acids Isolated from Insoluble Maize Bran Fiber. *J. Agric. Food Chem.* **2006**, *54*, 6409–6418.

55. Schatz, P.F.; Ralph, J.; Lu, F.; Guzei, I.A.; Bunzel, M. Synthesis and Identification of 2,5-Bis-(4-Hydroxy-3-Methoxyphenyl)- Tetrahydrofuran-3,4-Dicarboxylic Acid, an Unanticipated Ferulate 8-8-Coupling Product Acylating Cereal Plant Cell Walls. *Org. Biomol. Chem.* **2006**, *4*, 2801–2806.

56. Ralph, J.; Quideau, S.; Grabber, J.H.; Hatfieida, R.D. Ldentification and Synthesis of New Ferulic Acid Dehydrodimers Present in Grass Cell Walls. *J. Chem. Soc. Perkin Trans. 1* **1994**, *602*, 3485–3498.

57. Bunzel, M.; Ralph, J.; Steinhart, H. Phenolic Compounds as Cross-Links of Plant Derived Polysaccharides. *Czech J. Food Sci.* **2004**, *22*, S64–S67.

58. Bunzel, M.; Ralph, J.; Kim, H.; Lu, F.; Ralph, S.A.; Marita, J.M.; Hatfield, R.D.; Steinhart, H. Sinapate Dehydrodimers and Sinapate-Ferulate Heterodimers in Cereal Dietary Fiber. *J. Agric. Food Chem.* **2003**, *51*, 1427–1434.

59. Rouau, X.; Cheynier, V.; Surget, A.; Gloux, D.; Barron, C.; Meudec, E.; Louis-Montero, J.; Criton, M. A Dehydrotrimer of Ferulic Acid from Maize Bran. *Phytochemistry* **2003**, *63*, 899–903.

60. Acosta-Estrada, B.A.; Serna-Saldívar, S.O.; Gutiérrez-Uribe, J.A. Chemopreventive Effects of Feruloyl Putrescines from Wastewater (Nejayote) of Lime-Cooked White Maize (*Zea Mays*). *J. Cereal Sci.* **2015**, *64*, 23–28.

61. Funk, C.; Ralph, J.; Steinhart, H.; Bunzel, M. Isolation and Structural Characterisation of 8-O-4/8-O-4- and 8-8/8-O-4-Coupled Dehydrotriferulic Acids from Maize Bran. *Phytochemistry* **2005**, *66*, 363–371.

62. Ralph, J.; Bunzel, M.; Marita, J.M.; Hatfield, R.D.; Lu, F.; Kim, H.; Schatz, P.F.; Grabber, J.H.; Steinhart, H. Peroxidase-Dependent Cross-Linking Reactions of p-Hydroxycinnamates in Plant Cell Walls. *Phytochem. Rev.* **2004**, *3*, 79–96.

63. Bunzel, M.; Allerdings, E.; Ralph, J.; Steinhart, H. Cross-Linking of Arabinoxylans via 8-8-Coupled Diferulates as Demonstrated by Isolation and Identification of Diarabinosyl 8-8(Cyclic)-Dehydrodiferulate from Maize Bran. *J. Cereal Sci.* **2008**, *47*, 29–40.

64. Bunzel, M.; Ralph, J.; Funk, C.; Steinhart, H. Isolation and Identification of a Ferulic Acid Dehydrotrimer from Saponified Maize Bran Insoluble Fiber. *Eur. Food Res. Technol.* **2003**, *217*, 128–133.

65. Waterstraat, M.; Bunzel, M. A Multi-Step Chromatographic Approach to Purify Radically Generated Ferulate Oligomers Reveals Naturally Occurring 5-5/8-8(Cyclic)-, 8-8(Noncyclic)/8-O-4-, and 5-5/8-8(Noncyclic)-Coupled Dehydrotriferulic Acids. *Front. Chem.* **2018**, *6*, 1–10.

66. Bunzel, M. Chemistry and Occurrence of Hydroxycinnamate Oligomers. *Phytochem. Rev.* **2010**, *9*, 47–64.

67. Allerdings, E.; Ralph, J.; Schatz, P.F.; Gniechwitz, D.; Steinhart, H.; Bunzel, M. Isolation and Structural Identification of Diarabinosyl 8-O-4-Dehydrodiferulate from Maize Bran Insoluble Fibre. *Phytochemistry* **2005**, *66*, 113–124.

68. Allerdings, E.; Ralph, J.; Steinhart, H.; Bunzel, M. Isolation and Structural Identification of Complex Feruloylated Heteroxylan Side-Chains from Maize Bran. *Phytochemistry* **2006**, *67*, 1276–1286.

69. Malunga, L.N.; Beta, T. Isolation and Identification of Feruloylated Arabinoxylan Mono- and Oligosaccharides from Undigested and Digested Maize and Wheat. *Heliyon* **2016**, *2*.

70. Smith, M.M.; Hartley, R.D. Occurrence and Nature of Ferulic Acid Substitution of Cell-Wall Polysaccharides in Graminaceous Plants. *Carbohydr. Res.* **1983**, *118*, 65–80.

71. Vanholme, R.; Demedts, B.; Morreel, K.; Ralph, J.; Boerjan, W. Lignin Biosynthesis and Structure. *Plant Physiol.* **2010**, *153*, 895–905.

72. Hatfield, R.D.; Chaptman, A.K. Comparing Corn Types for Differences in Cell Wall Characteristics and P-Coumaroylation of Lignin. *J. Agric. Food Chem.* **2009**, *57*, 4243–4249.

73. Lam, T.B.T.; Kadoya, K.; Iiyama, K. Bonding of Hydroxycinnamic Acids to Lignin: Ferulic and p-Coumaric Acids Are Predominantly Linked at the Benzyl Position of Lignin, Not the β-Position, in Grass Cell Walls. *Phytochemistry* **2001**, *57*, 987–992.

74. Collison, A.; Yang, L.; Dykes, L.; Murray, S.; Awika, J.M. Influence of Genetic Background on Anthocyanin and Copigment Composition and Behavior during Thermoalkaline Processing of Maize. *J. Agric. Food Chem.* **2015**, *63*, 5528–5538.

75. Macoy, D.M.; Kim, W.-Y.; Lee, S.Y.; Kim, M.G. Biosynthesis, Physiology, and Functions of Hydroxycinnamic Acid Amides in Plants. *Plant Biotechnol. Rep.* **2015**, *9*, 269–278.

76. Sen, A.; Bergvinson, D.; Miller, S.S.; Atkinson, J.; Fulcher, R.G.; Arnason, J.T. Distribution and Microchemical Detection of Phenolic Acids, Flavonoids, and Phenolic Acid Amides in Maize Kernels. *J. Agric. Food Chem.* **1994**, *42*, 1879–1883.

77. Burt, A.J.; Arnason, J.T.; García-Lara, S. Natural Variation of Hydroxycinnamic Acid Amides in Maize Landraces. *J. Cereal Sci.* **2019**.

78. Li, Z.; Zhao, C.; Zhao, X.; Xia, Y.; Sun, X.; Xie, W.; Ye, Y.; Lu, X.; Xu, G. Deep Annotation of Hydroxycinnamic Acid Amides in Plants Based on Ultra-High-Performance Liquid Chromatography–High-Resolution Mass Spectrometry and Its In Silico Database. *Anal. Chem.* **2018**, *90*, 14321–14330.

79. Buitimea-Cantúa, N.E.; Gutiérrez-Uribe, J.A.; Serna-Saldivar, S.O. Phenolic–Protein Interactions: Effects on Food Properties and Health Benefits. *J. Med. Food* **2018**, *21*, 188–198.

80. Choi, S.W.; Lee, S.K.; Kim, E.O.; Oh, J.H.; Yoon, K.S.; Parris, N.; Hicks, K.B.; Moreau, R.A. Antioxidant and Antimelanogenic Activities of Polyamine Conjugates from Corn Bran and Related Hydroxycinnamic Acids. *J. Agric. Food Chem.* **2007**, *55*, 3920–3925.

81. Barros-Rios, J.; Malvar, R.A.; Jung, H.J.G.; Bunzel, M.; Santiago, R. Divergent Selection for Ester-Linked Diferulates in Maize Pith Stalk Tissues. Effects on Cell Wall Composition and Degradability. *Phytochemistry* **2012**, *83*, 43–50.

82. Uddin, M.N.; Hanstein, S.; Faust, F.; Eitenmüller, P.T.; Pitann, B.; Schubert, S. Diferulic Acids in the Cell Wall May Contribute to the Suppression of Shoot Growth in the First Phase of Salt Stress in Maize. *Phytochemistry* **2014**, *102*, 126–136.

83. Barros-Rios, J.; Santiago, R.; Jung, H.J.G.; Malvar, R.A. Covalent Cross-Linking of Cell-Wall Polysaccharides through Esterified Diferulates as a Maize Resistance Mechanism against Corn Borers. *J. Agric. Food Chem.* **2015**, *63*, 2206–2214.

84. Ayala-Soto, F.E.; Serna-Saldívar, S.O.; García-Lara, S.; Pérez-Carrillo, E. Hydroxycinnamic Acids, Sugar Composition and Antioxidant Capacity of Arabinoxylans Extracted from Different Maize Fiber Sources. *Food Hydrocoll.* **2014**, *35*, 471–475.

85. Salinas-Moreno, Y.; García-Salinas, C.; Ramírez-Díaz, J.L.; Alemán-de la Torre, I. Phenolic Compounds in Maize Grains and Its Nixtamalized Products. In *Phenolic Compounds-Natural Sources, Importance and Applications*; InTech, 2017.

86. Gupta, K.; Dey, A.; Gupta, B. Plant Polyamines in Abiotic Stress Responses. *Acta Physiol. Plant.* **2013**, *35*, 2015–2036.

87. Majumdar, R.; Minocha, R.; Lebar, M.D.; Rajasekaran, K.; Long, S.; Carter-Wientjes, C.; Minocha, S.C.; Cary, J.W. Contribution of Maize Polyamine and Amino Acid Metabolism towards Resistance against Aspergillus Flavus Infection and Aflatoxin Production. *Front. Plant Sci.* **2019**, *10*, 692.

88. Tiburcio, A.F.; Alcázar, R. *Polyamines - Methods and Protocols*; Springer: New York, U.S.A., 2018; ISBN 9781493973972.

89. Butts-Wilmsmeyer, C.J.; Mumm, R.H.; Bohn, M.O. Quantitative Genetic Analysis of Hydroxycinnamic Acids in Maize (*Zea Mays* L.) for Plant Improvement and Production of Health-Promoting Compounds. *J. Agric. Food Chem.* **2020**, *68*, 9585–9593.

90. Ou, J. ying; Huang, J. qing; Song, Y.; Yao, S. wen; Peng, X. chun; Wang, M. fu; Ou, S. yi Feruloylated Oligosaccharides from Maize Bran Modulated the Gut Microbiota in Rats. *Plant Foods Hum. Nutr.* **2016**, *71*, 123–128.

91. Snelders, J.; Olaerts, H.; Dornez, E.; Van de Wiele, T.; Aura, A.M.; Vanhaecke, L.; Delcour, J.A.; Courtin, C.M. Structural Features and Feruloylation Modulate the Fermentability and Evolution of Antioxidant Properties of Arabinoxylanoligosaccharides during in Vitro Fermentation by Human Gut Derived Microbiota. *J. Funct. Foods* **2014**, *10*, 1–12.

92. Aura, A.M. Microbial Metabolism of Dietary Phenolic Compounds in the Colon. *Phytochem. Rev.* **2008**, *7*, 407–429.

93. Wang, T.; He, F.; Chen, G. Improving Bioaccessibility and Bioavailability of Phenolic Compounds in Cereal Grains through Processing Technologies: A Concise Review. *J. Funct. Foods* **2014**, *7*, 101–111.

94. Dynkowska, W.M.; Cyran, M.R.; Ceglińska, A. Soluble and Cell Wall-bound Phenolic Acids and Ferulic Acid Dehydrodimers in Rye Flour and Five Bread Model Systems: Insight into Mechanisms of Improved Availability. *J. Sci. Food Agric.* **2015**, *95*, 1103–1115.

95. Li, M.; Heckwolf, M.; Crowe, J.D.; Williams, D.L.; Magee, T.D.; Kaeppler, S.M.; De Leon, N.; Hodge, D.B. Cell-Wall Properties Contributing to Improved Deconstruction by Alkaline Pre-Treatment and Enzymatic Hydrolysis in Diverse Maize (*Zea Mays* L.) Lines. *J. Exp. Bot.* **2015**, *66*, 4305–4315.

96. Schendel, R.R.; Meyer, M.R.; Bunzel, M. Quantitative Profiling of Feruloylated Arabinoxylan Side-Chains from Graminaceous Cell Walls. *Front. Plant Sci.* **2016**, *6*.

97. Rose, D.J.; Inglett, G.E. Production of Feruloylated Arabinoxylo-Oligosaccharides from Maize (*Zea Mays*) Bran by Microwave-Assisted Autohydrolysis. *Food Chem.* **2010**, *119*, 1613–1618.

98. Irakli, M.N.; Samanidou, V.F.; Biliaderis, C.G.; Papadoyannis, I.N. Development and Validation of an HPLC-Method for Determination of Free and Bound Phenolic Acids in Cereals after Solid-Phase Extraction. *Food Chem.* **2012**, *134*, 1624–1632.

99. Sun, R.C.; Sun, X.F.; Wang, S.Q.; Zhu, W.; Wang, X.Y. Ester and Ether Linkages between Hydroxycinnamic Acids and Lignins from Wheat, Rice, Rye, and Barley Straws, Maize Stems, and Fast-Growing Poplar Wood. *Ind. Crops Prod.* **2002**, *15*, 179–188.

100. Arranz, S.; Calixto, F.S. Analysis of Polyphenols in Cereals May Be Improved Performing Acidic Hydrolysis: A Study in Wheat Flour and Wheat Bran and Cereals of the Diet. *J. Cereal Sci.* **2010**, *51*, 313–318.

101. De Castro, M.; Largo-Gosens, A.; Alvarez, J.M.; García-Angulo, P.; Acebes, J.L. Early Cell-Wall Modifications of Maize Cell Cultures during Habituation to Dichlobenil. *J. Plant Physiol.* **2014**, *171*, 127–135.

102. Ralph, J.; HatTield, R.D.; Quideau, S.; Helm, R.F.; Grabber, J.H.; Jung, H.-J.G. Pathway of P-Coumaric Acid Incorporation into Maize Lignin As Revealed by NMR. *J. Am. Chem. Soc. Soc.* **1994**, *116*, 9448–9456.

103. Barros-Rios, J.; Malvar, R.A.; Jung, H.J.G.; Santiago, R. Cell Wall Composition as a Maize Defense Mechanism against Corn Borers. *Phytochemistry* **2011**, *72*, 365–371.

104. Lovegrove, A.; Wilkinson, M.D.; Freeman, J.; Pellny, T.K.; Tosi, P.; Saulnier, L.; Shewry, P.R.; Mitchell, R.A.C. RNA Interference Suppression of Genes in Glycosyl Transferase Families 43 and 47 in Wheat Starchy Endosperm Causes Large Decreases in Arabinoxylan Content. *Plant Physiol.* **2013**, *163*, 95–107.

105. Andreasen, M.F.; Christensen, L.P.; Meyer, A.S.; Hansen, Å. Content of Phenolic Acids and Ferulic Acid Dehydrodimers in 17 Rye (Secale Cereale L.) Varieties. *J. Agric. Food Chem.* **2000**, *48*, 2837–2842.

106. Levigne, S. V.; Ralet, M.C.J.; Quéméner, B.C.; Pollet, B.N.L.; Lapierre, C.; Thibault, J.F.J. Isolation from Sugar Beet Cell Walls of Arabinan Oligosaccharides Esterified by Two Ferulic Acid Monomers. *Plant Physiol.* **2004**, *134*, 1173–1180.

107. Agger, J.; Viksø-Nielsen, A.; Meyer, A.S. Enzymatic Xylose Release from Pretreated Corn Bran Arabinoxylan: Differential Effects of Deacetylation and Deferuloylation on Insoluble and Soluble Substrate Fractions. *J. Agric. Food Chem.* **2010**, *58*, 6141–6148.

108. La Frano, M.R.; de Moura, F.F.; Boy, E.; Lönnerdal, B.; Burri, B.J. Bioavailability of Iron, Zinc, and Provitamin A Carotenoids in Biofortified Staple Crops. *Nutr. Rev.* **2014**, *72*, 289–307.

109. Martini, D.; Ciccoritti, R.; Nicoletti, I.; Nocente, F.; Corradini, D.; D'Egidio, M.G.; Taddei, F. From Seed to Cooked Pasta: Influence of Traditional and Non-Conventional Transformation Processes on Total Antioxidant Capacity and Phenolic Acid Content. *Int. J. Food Sci. Nutr.* **2018**, *69*, 24–32.

110. Angelino, D.; Cossu, M.; Marti, A.; Zanoletti, M.; Chiavaroli, L.; Brighenti, F.; Del Rio, D.; Martini, D. Bioaccessibility and Bioavailability of Phenolic Compounds in Bread: A Review. *Food Funct.* **2017**, *8*, 2368–2393.

111. Purlis, E. Browning Development in Bakery Products–a Review. *J. Food Eng.* **2010**, *99*, 239–249.

112. Tournier, C.; Sulmont-rossé, C.; Guichard, E.; Tournier, C.; Sulmont-rossé, C.; Guichard, E.; Tournier, C.; Elisabeth, C.S. Flavour Perception : Aroma , Taste and Texture Interactions To Cite This Version : HAL Id : Hal-02823959 Flavour Perception : Aroma, Taste and Texture Interactions. **2020**.

113. Pico, J.; Gómez, M.; Bernal, J.; Bernal, J.L. Analytical Methods for Volatile Compounds in Wheat Bread. *J. Chromatogr. A* **2016**, *1428*, 55–71.

114. Pozo-Bayón, M.A.; Guichard, E.; Cayot, N. Flavor Control in Baked Cereal Products. Food Reviews International. **2006**, *22*, 335–379.

115. Paraskevopoulou, A.; Chrysanthou, A.; Koutidou, M. Characterisation of Volatile Compounds of Lupin Protein Isolate-enriched Wheat Flour Bread. *Food Res. Int.* **2012**, *48*, 568–577.

116. Cho, I.H.; Peterson, D.G. Chemistry of Bread Aroma: A Review. *Food Sci. Biotechnol.* **2010**, *19*, 575–582.

117. Starr, G.; Hansen, T.S.; Petersen, M.A.; Bredie, W.L.P.P.; Hansen, Å.S.; Petersen, M.A.; Bredie, W.L.P.P.; Hansen, T.S.; Petersen, M.A.; Bredie, W.L.P.P. Aroma of Wheat Porridge and Bread-Crumb Is Influenced by the Wheat Variety. *LWT - Food Sci. Technol.* **2015**, *63*, 590–598.

118. Makhoul, S.; Romano, A.; Capozzi, V.; Spano, G.; Aprea, E.; Cappellin, L.; Benozzi, E.; Scampicchio, M.; Märk, T.D.; Gasperi, F. Volatile Compound Production During the Bread-Making Process: Effect of Flour, Yeast and Their Interaction. *Food Bioprocess Technol.* **2015**, *8*, 1925–1937.

119. Martins, S.I.F.S.; Jongen, W.M.F.; Van Boekel, M.A.J.S. A Review of Maillard Reaction in Food and Implications to Kinetic Modelling. *Trends Food Sci. Technol.* **2000**, *11*, 364–373.

120. Sigma-Aldrich Co.; Sigma-Aldrich; Sigma-Aldrich Co. Flavors & Fragrances Available online: http://www.sigmaaldrich.com/industries/flavors-and-fragrances.html.

121. Starowicz, M.; Zieliński, H. How Maillard Reaction Influences Sensorial Properties (Color, Flavor and Texture) of Food Products? *Food Rev. Int.* **2019**, *35*, 707–725.

122. Bastos, D.M.; Monaro, É.; Siguemoto, É.; Séfora, M.; Markowicz, D.; Monaro, E.; Siguemoto, E.; Sefor, M. Maillard Reaction Products in Processed Food: Pros and Cons. *Food Ind. Process. - Methods Equip.* **2012**.

123. Belitz, H.-D.; Grosch, W.; Schieberle, P. Cereals and Cereal Products. *Food Chem.* **2009**, 670–745.

124. Pico, J.; Bernal, J.; Gómez, M. Wheat Bread Aroma Compounds in Crumb and Crust: A Review. *Food Res. Int.* **2015**, *75*, 200–215.

125. Cornell, H.; Cauvain, S.P. Bread Making: Improving Quality. *Woodhead Publ. Cambridge Dalmo, RA, Bogwald, J Fish Shellfish Immunol.*–**2003**, 384.

126. Birch, A.N.; Petersen, M.A.; Hansen, Å.S. Aroma of Wheat Bread Crumb. *Cereal Chem.* **2014**, *91*, 105–114.

127. Tamanna, N.; Mahmood, N. Food Processing and Maillard Reaction Products: Effect on Human Health and Nutrition. *Int. J. Food Sci.* **2015**, *2015*.

128. Berger, R.G. *Flavours and Fragrances: Chemistry, Bioprocessing and Sustainability*; Springer Science & Business Media: Berlin, Germany, 2007; ISBN 3540493395

129. Bailey, M.E.; Gutheil, R.A.; Hsieh, F.-H.; Cheng, C.-W.; Gerhardt, K.O. Maillard reaction volatile compounds and color quality of a whey protein concentrate—Corn meal extruded product. In *Thermally Generated Flavors*; Thomas, H.P., Michael, J.M., Robert, J.M., Eds.; ACS Publications: Washington, DC, USA, **1993**; Chapter 25; pp. 315–327.

130. Seok, Y.-J.; Kim, Y.-G.; Jeong, S.Y.; Lee, J.; Yoon, H.-J. Furan in Thermally Processed Foods-a Review. *Toxicol. Res.* **2015**, *31*, 241.

131. Rizzi, G.P. The Strecker Degradation of Amino Acids: Newer Avenues for Flavor Formation. *Food Rev. Int.* **2008**, *24*, 416–435.

132. Bredie, W.L.P.; Mottram, D.S.; Hassell, G.M.; Guy, R.C.E. Sensory Characterisation of the Aromas Generated in Extruded Maize and Wheat Flour. *J. Cereal Sci.* **1998**, *28*, 97–106.

133. Bredie, W.L.P.; Mottram, D.S.; Guy, R.C.E. Aroma Volatiles Generated during Extrusion Cooking of Maize Flour. *J. Agric. Food Chem.* **1998**, *46*, 1479–1487.

134. Moskowitz, M.R.; Bin, Q.; Elias, R.J.; Peterson, D.G. Influence of Endogenous Ferulic Acid in Whole Wheat Flour on Bread Crust Aroma. *J. Agric. Food Chem.* **2012**, *60*, 11245–11252.

135. Jensen, S.; Oestdal, H.; Skibsted, L.H.; Larsen, E.; Thybo, A.K. Chemical Changes in Wheat Pan Bread during Storage and How It Affects the Sensory Perception of Aroma, Flavour, and Taste. *J. Cereal Sci.* **2011**, *53*, 259–268.

136. Hansen, A.; Schieberle, P. Generation of Aroma Compounds during Sourdough Fermentation: Applied and Fundamental Aspects. *Trends Food Sci. Technol.* **2005**, *16*, 85–94.

137. Schwab, W.; Davidovich-Rikanati, R.; Lewinsohn, E. Biosynthesis of Plant-derived Flavor Compounds. *plant J.* **2008**, *54*, 712–732.

138. Pétel, C.; Onno, B.; Prost, C. Sourdough Volatile Compounds and Their Contribution to Bread: A Review. *Trends Food Sci. Technol.* **2017**, *59*, 105–123.

139. Merkle, S.; Kleeberg, K.; Fritsche, J. Recent Developments and Applications of Solid Phase Microextraction (SPME) in Food and Environmental Analysis—A Review. *Chromatography* **2015**, *2*, 293–381.

140. Rocha, S.M.; Freitas, R.; Cardoso, P.; Santos, M.; Martins, R.; Figueira, E. Exploring the Potentialities of Comprehensive Two-Dimensional Gas Chromatography Coupled to Time of Flight Mass Spectrometry to Distinguish Bivalve Species: Comparison of Two Clam Species (Venerupis Decussata and Venerupis Philippinarum). *J. Chromatogr. A* **2013**, *1315*, 152–161.

141. Raffo, A.; Carcea, M.; Castagna, C.; Magrì, A. Improvement of a Headspace Solid Phase Microextraction-Gas Chromatography/Mass Spectrometry Method for the Analysis of Wheat Bread Volatile Compounds. *J. Chromatogr. A* **2015**, *1406*, 266–278.

142. Galoburda, R.; Straumite, E.; Sabovics, M.; Kruma, Z. Dynamics of Volatile Compounds in Triticale Bread with Sourdough: From Flour to Bread. *Foods* **2020**, *9*.

143. Birch, A.N.; Petersen, M.A.; Hansen, Å.S. The Aroma Profile of Wheat Bread Crumb Influenced by Yeast Concentration and Fermentation Temperature. *LWT-Food Sci. Technol.* **2013**, *50*, 480–488.

144. Adahchour, M.; Beens, J.; Brinkman, U.A.T. Recent Developments in the Application of Comprehensive Two-Dimensional Gas Chromatography. *J. Chromatogr. A* **2008**, *1186*, 67–108.

145. McGorrin, R.J. The Significance of Volatile Sulfur Compounds in Food Flavors. *ACS Symp. Ser.* **2011**, *1068*, 3–31.

146. Costa, C.P.; Bezerra, A.R.; Almeida, A.; Rocha, S.M. Candida Species (Volatile) Metabotyping through Advanced Comprehensive Two-Dimensional Gas Chromatography. *Microorganisms* **2020**, *8*, 1–23.

147. Agroportal Segurança Alimentar. Portugal Em Risco Máximo de Abastecimento de Cereais. Available online: https://www.agroportal.pt/portugal-em-risco-maximo-de-abastecimento-de-cereais. (Accessed on 8 March 2022).

148. Moreira, P.M.R.M. Participatory Maize Breeding in Portugal. A Case Study. *Acta Agron. Hungarica* **2006**, *54*, 431–439.

149. Brites, C.M.; Trigo, M.J.; Carrapiço, B.; Alviña, M.; Bessa, R.J. Maize and Resistant Starch Enriched Breads Reduce Postprandial Glycemic Responses in Rats. *Nutr. Res.* **2011**, *31*, 302–308.

150. Alves, M.L.; Brites, C.; Paulo, M.; Carbas, B.; Belo, M.; Mendes-Moreira, P.M.R.; Brites, C.; Bronze, M. do R.; Gunjača, J.; Šatović, Z. Setting up Decision-Making Tools toward a Quality-Oriented Participatory Maize Breeding Program. *Front. Plant Sci.* **2017**, *8*, 2203.

151. Domínguez Díaz, L.; Fernández-Ruiz, V.; Cámara, M. An International Regulatory Review of Food Health-Related Claims in Functional Food Products Labeling. *J. Funct. Foods* **2020**, *68*, 103896.

152. ISO (International Organization for Standardization). ISO Sensory Analysis – Vocabulary 2008. ISO: Geneva, Switzerland. Available online: https://www.iso.org/obp/ui/#iso:std:iso:5492:ed-2:v1:en. (Accessed on 20 March 2022).

CHAPTER 2

Hydroxycinnamic acids and their derivatives in broa*, a traditional ethnic maize bread*[1]

Abstract

Maize is one of the most interesting dietary sources of hydroxycinnamic acids, widely known for their beneficial health effects, namely antioxidant properties. This work aims to identify hydroxycinnamic acids and their derivatives in *broa*, a Portuguese traditional ethnic maize bread, and corresponding maize flours. Soluble and insoluble phenolic fractions of diverse maize flours and corresponding *broas* were prepared and analysed by HPLC-DAD-MS/MS (high-performance liquid chromatography coupled with diode array detector and tandem mass spectrometry). Besides free hydroxycinnamic acids, mainly ferulic and *p*-coumaric acids, several structural isomers and stereoisomers of insoluble ferulic acid dehydrodimers (n = 18) and trimers (n = 11), were also identified. Hydroxycinnamic acid amides consisting of coumaroyl and feruloyl conjugates (n = 22) were present in both soluble and insoluble fractions of maize flours and breads, in different isomeric forms. A new compound was putatively identified as *bis-N,N′*-diferuloyl putrescine. Additionally, more complex and insoluble hydroxycinnamic acid amides, derived from ferulic acid dehydrodimers (n = 47) and trimers (n = 18), were also putatively identified for the first time, suggesting that hydroxycinnamic acid amides are also linked to maize cell walls. Since hydroxycinnamic derivatives were not only identified in maize flours, but also in *broas*, they can contribute to the antioxidant properties and beneficial health effects of maize-based foods.

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[1]**This chapter is based on the following publication:**
Bento-Silva A, Duarte N, Mecha E, Belo M, Vaz Patto MC, Bronze MR. Hydroxycinnamic Acids and Their Derivatives in *Broa*, a Traditional Ethnic Maize Bread. *Foods*. **2020**, *9*, 1471.
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1. Introduction

Maize (*Zea mays*) is widely grown throughout the world, being considered a staple cereal in many countries, where it can be used to produce different food products [1]. In Portugal, maize is used to produce *broa*, a traditional ethnic bread prepared with whole grain maize (50–100%), and rye and/or wheat (0–50%) flours [2]. *Broa* was considered a hearty peasant bread and one of the 50 world's best breads by CNN Travel, in October 2019 [3].

Among the phytochemicals present in cereals, phenolic compounds, as hydroxycinnamic acids, contribute positively for human health [4,5] due to their antibacterial, anti-ageing, anti-carcinogenic, neuroprotective, cardiovascular and anti-diabetic properties [4]. In comparison to other cereals, whole maize grains contain higher levels of hydroxycinnamic acids [4].

Generally, phenolic compounds can be found in their soluble or insoluble forms. Soluble forms, also known as "extractable phenols" [6], can be present in their free form or conjugated with smaller molecules, such as simple sugars and amines [5,7–9]. Particularly abundant free hydroxycinnamic acids present in maize grains are ferulic (FA) and *p*-coumaric (pCA) acids. These acids can be conjugated with polyamines, yielding hydroxycinnamic acid amides (HCAAs), such as *N,N′*-feruloyl putrescine (DFP) [7–9].

The role of HCAAs in maize grains is not known for sure [5]. They may be associated with several processes, such as plant growth and development, floral induction and reproduction, cell division, control of intracellular polyamine concentrations, cell wall reinforcement, and plant adaptation to stress, such as resistance to cold or pathogen attack [9–12]. To the best of our knowledge, HCAAs have not been detected in maize-based foods.

The "insoluble phenolic compounds", also known as "non-extractable polyphenols", are mainly high molecular weight compounds mostly (>94%) bound to arabinoxylans [5,6]. They include, among others, dehydrodiferulic (DFAs), dehydrotriferulic (TFAs) and dehydrotetraferulic (TeFAs) acids [4,5]. These compounds are responsible for the cross-linking of cell wall polysaccharides, which is implicated in different processes in plants, such as the control of cellular expansion associated with growth [4]. Similar to HCAAs, cross-linking has been suggested to protect against pathogens, since its increase results in thinner and firmer cell walls, therefore decreasing pathogen penetration into plant tissues [4].

It has been reported that HCAAs exert interesting antioxidant, anti-inflammatory, and chemopreventive properties [8,9,13–16]. Similarly, most DFAs have shown higher radical-scavenging efficacies than free FA [17]. Despite their possible benefits for human health, the presence of HCAAs, DFAs, TFAs, and TeFAs in maize-based foods has been poorly studied. The bioavailability of hydroxycinnamic acids depends on their presence in food matrices which is in turn affected by food processing [18]. In particular, the majority of bound compounds, such as

DFAs, reaches the colon and need to be liberated from the food matrix by the action of enzymes during small intestinal digestion or colonic fermentation in order to be absorbed [18,19]. Conversely, soluble compounds are generally readily available for absorption [4].

In order to evaluate the importance and possible health effects associated with the consumption of maize products, hydroxycinnamic acids and their derivatives should be characterized and their bioaccessibility evaluated. The present study aimed at identifying the main phenolic compounds present in whole grain maize flours and *broas*, focusing on the identification of FA dimers and trimers, as well as hydroxycinnamic acid derivatives, including new insoluble dehydrodiferulic and dehydrotriferulic acid putrescines.

2. Materials and methods

2.1. Cereal flours and *broas* samples

Five traditional maize flours (Broa-213, Pigarro, Castro Verde, Verdial de Aperrela and Fandango), one commercial maize flour, and the corresponding *broas* were studied. These five traditional varieties were randomly chosen after an initial screening of the soluble phenolic composition and total phenolic content of all twelve maize flours and *broas*. The commercial wheat and rye flours used in the preparation of *broas* were also studied. All cereal samples and *broas* are described in more detail in ***Section 7, Chapter 1***.

2.2. Reagents

Absolute ethanol (EtOH), sodium hydroxide (NaOH), formic acid ≥ 95%, and all the standards used in the present work (ferulic, *p*-coumaric, *o*-coumaric, *m*-coumaric, *p*-hydroxybenzoic, caffeic, syringic, citric, vanillic, and protocatechuic acids, syringaldehyde, vanillin, quercetin, and kaempferol) were obtained from Sigma-Aldrich, St. Louis, MO, USA. Acetonitrile HPLC Plus Gradient grade, hexane, and ethyl acetate (EtOAc) were from Carlo Erba, Val de Reuil, France. Phosphoric acid 85% p.a. was from Panreac, Barcelona, Spain. Water was purified by a Milli-Q water purification system from Millipore, Burlington, MA, USA.

2.3. Extraction of phenolic compounds

A conventional extraction procedure [22] for raw maize, wheat, and rye flours and *broas* (4 g) was performed. Briefly, 4 g of maize flour was extracted with 20 mL of EtOH/H_2O (50%, v/v) for 15 min, using an Ultra Turrax T25 (Janke & Kunkel, IKA Labortechnik, Burlington, Germany), at room temperature, yielding an ethanolic solution that contained the soluble phenolic compounds (SF) and a solid residue comprising the insoluble compounds (IF), as described in ***Figure 2.1***.

In order to obtain the insoluble fraction (IF), the solid residue was defatted with hexane (3 × 20 mL), centrifuged (7000 × g, 10 min), and hydrolysed with NaOH 4 M (60 mL, pH 14 ± 0.5), for 15 h at room temperature, in the presence of N_2 [23,24]. After hydrolysis, the pH was set to 1.5 ± 0.5 with concentrated HCl and the solution was extracted with EtOAc (3 × 30 mL), evaporated until dryness through a SpeedVac (Labconco, Kansas City, MO, USA) and reconstituted in 20 mL of EtOH 50%. Both fractions (SF and IF) from maize flours and *broas* were prepared in duplicate, filtered through 0.20 µm polytetrafluoroethylen (PTFE) syringe filters (Chromafil® Macherey-Nagel, Düren, Germany) and analysed by HPLC-DAD (high-performance liquid chromatography coupled with diode array detector). Extracts (10 mL) obtained from commercial rye and wheat flours and Verdial de Aperrela maize flour and *broa* were concentrated until dryness and reconstituted in 500 µL of EtOH/H_2O (50%, *v/v*) before HPLC-DAD-MS/MS (HPLC coupled with DAD and tandem mass spectrometry) analysis.

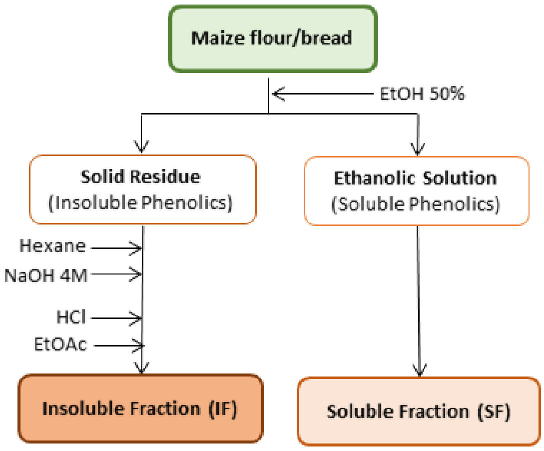


Figure 2.1: Representative scheme of the extraction procedure of the soluble and insoluble phenolic fractions.

2.4. Analysis of phenolic compounds by liquid chromatography

In order to compare the phenolic composition, extracts from all samples, maize flour and corresponding *broa*, were analysed in a Thermo Fisher Scientific Surveyor HPLC system, equipped with a DAD (Waltham, MA, USA). The eluents used were A: 0.1% phosphoric acid in Milli-Q® water and B: 0.1% phosphoric acid in acetonitrile and Milli-Q® water (0.1/40/59.9), at a flow rate of 0.7 mL min^{-1}. The following gradient of eluents was used: 0–20% B over 15 min, held isocratically at 20% B for 10 min, 20–70% B from 25 to 70 min, 70% B for 5 min, 70–100% B from 75 to 85 min, and finally, 100% B for 15 min, followed by an equilibration step of 10 min. DAD was programmed for scanning between 192 and 798 nm at a speed of 1 Hz with a bandwidth of 5 nm. The detection was monitored using three individual channels, 280, 320 and 360 nm, at a speed of 10 Hz with a bandwidth of 11 nm.

Extracts of the SF and IF of Verdial de Aperrela sample, as well as the commercial wheat rye flours, were analysed on an Alliance 2695 separation module HPLC system (Waters, Dublin, Ireland) coupled to a 2996 Photodiode Array Detector and a Micromass® Quattro Micro triple quadrupole (TQ) (Waters, Dublin, Ireland). The mobile phase consisted of water with 0.5% formic acid as eluent A and acetonitrile as eluent B at a flow rate of 0.30 mL min^{-1}. The system was run with the following gradient program: 0–15 min from 1 to 10% B; 15–20 min from 10 to 11% B; 20–30 min at 11% B; 30–45 min from 11 to 15% B; 45–55 min at 15% B; 55–95 min from 15 to 30% B; 95–150 min at 30% B; 150–160 min from 30 to 50% B; 160–180 min at 50% B; finally returning to the initial conditions for 20 min. DAD was used to scan wavelength absorption from 210 to 600 nm. Tandem mass spectrometry (MS/MS) detection was performed using an electrospray ionisation (ESI) source operating at 120 °C, applying a capillary voltage of 3.0 kV, cone voltage of 30 V, and collision energies of 10, 20, and 30 eV. The compounds were ionised in both negative and positive ion modes. High purity nitrogen (N_2) was used both as drying gas and as a nebulising gas. Ultra-high purity Argon (Ar) was used as the collision gas. MS/MS experiments were performed in order to identify the major phenolic compounds. Additionally, when standards were commercially available, MS/MS conditions were optimised [***Table A.1, Appendix A***] and extracts were analysed in multiple reaction monitoring (MRM) mode in order to increase selectivity and sensitivity.

In both types of equipment, the injection volume was 20 µL, and the chromatographic separation procedure was carried out using a Lichrocart® RP-18 column (250 × 4 mm, 5 µm) and a Manu-cart® RP-18 pre-column (Merck, Darmstadt, Germany) in a thermostated oven at 35 °C.

2.5. Data analysis

ChromQuest (Thermo Fisher Scientific, Waltham, MA, USA) and MassLynx (Waters, Dublin, Ireland) software were used to control analytical conditions and collect data from HPLC-DAD and HPLC-DAD-MS/MS, respectively. For compounds identification purposes, mass and UV spectra were compared with spectra already published in the literature. When standards were commercially available, the identification was based on the comparison of their fragmentation patterns and retention times.

3. Results and discussion

After a preliminary analysis by HPLC-DAD at 280 and 320 nm (maximum absorption of phenolic compounds and hydroxycinnamic acids) [24] it was possible to conclude that SF and IF fractions from all maize flours showed identical chromatographic profiles, but with differences in peaks' intensity [***Figure A.1, Appendix A***]. Similar results were obtained from the comparison of *broas* [***Figure A.1, Appendix A***]. Therefore, aiming at characterising their phenolic composition, both fractions of a randomly chosen maize flour (Verdial de Aperrela) and corresponding bread were analysed by HPLC-DAD-MS/MS. Rye and wheat flours used for *broa* production were also analysed. The putatively identified compounds are described in ***Table 2.1***.

3.1. Small phenolic compounds

The main free phenolic compounds detected in both SF and IF of all raw flours (maize, rye, wheat) and *broas* were the *trans* isomeric forms of FA (16) and pCA (13) [***Table 2.1***]. Their presence was confirmed by the comparison with the chromatograms and UV spectra of the respective commercial standards.

An additional peak at *m/z* 193 $[M-H]^-$ was identified as *cis*-FA (17), especially evident in the IF of maize flours and *broas*. Previously, Guo *et al*. [34] identified isoferulic acid (*m/z* 193 $[M-H]^-$) as one of the major components of cereal alkaline extracts, due to its unique fragmentation behaviour observed by HPLC-DAD-MS/MS analysis. However, in the present work, isoferulic acid was not detected in any of the samples analysed, since the retention time, UV and MS/MS spectra were not coincident with the reported data. Similarly, *cis*-pCA (15) was also identified in the SF and IF of all samples (raw flours and breads) studied.

Vanillic (6) and syringic (9) acids were also identified by commercial standards in the SF of all raw flours (Verdial de Aperrela and commercial wheat and rye) and *broa*. Caffeic acid (8) was detected in the IF but not in the SF of all raw flours, suggesting it was linked to insoluble cereal components. Conversely, it was detected in the SF of *broas*, possibly due to its release from cellular vacuoles during processing, as previously suggested [35]. Other phenolic acids, such as *p*-hydroxybenzoic, *m*-, and *o*-coumaric acids, as well as some flavonoids, such as quercetin and kaempferol, have been reported in maize grains in low or trace amounts [5,29,36]. However, these compounds were not detected in any of the analysed samples. Protocatechuic acid (3) was detected in the IF of all raw flours and *broa*, but as caffeic acid, it was not extracted using the conventional ethanolic extraction procedure (SF), suggesting it was linked to insoluble cereals components. On the other hand, it was not detected in the SF of *broa*, meaning that it was degraded or not released during its processing.

Furthermore, it was possible to identify *p*-hydroxybenzaldehyde (10) and vanillin (11) in the SF and IF of all raw flours and *broa*. Syringaldehyde (14) was also detected in the SF of *broa*, maize, and rye, and in the IF of all samples. It has been described that vanillin and *p*-hydroxybenzaldehyde can be produced from FA and pCA, respectively [37].

Table 2.1: Compounds identified by HPLC-MS/MS in maize (Verdial de Aperrela), wheat, and rye flours and *broa* prepared from Verdial de Aperrela maize flour, in the soluble (SF) and insoluble (IF) fractions.

Peak number; **(+)** Compounds detected in positive ion mode; **(-)** Compounds detected in negative ion mode, **¥** Compounds identified by commercial standards; **RT**: Retention Time (minutes); **SF**: Soluble fraction; **IF**: Insoluble fraction. **B, M, R, W**: Compounds detected in Bread, Maize, Rye, and Wheat. **Bold**: characteristic fragment ions described by other authors [7,9,10,12,13,25-33]; main MS/MS ions are ordered according to their decreasing intensities.

#	RT	Putative Identification	*m/z*	MS/MS ions	SF	IF
		Small Phenolic Compounds				
3	30.96	Protocatechuic acid (-) ¥	153	109, 108	-	B, M, R, W
4	39.65	Ferulic acid hexoside 1 (+)	357	**195**	B	-
5	46.16	Ferulic acid hexoside 2 (+)	357	**195**, 149, 185	B	-
6	46.93	Vanillic acid (-) ¥	169	93, 123, 65	B, M, R, W	B, M, R, W
7	47.83	Ferulic acid hexoside 3 (+)	357	**195**, 149, 185	B, W	-
8	51.99	Caffeic acid (-) ¥	179	135	B	B, M, R, W
9	53.60	Syringic acid (+) ¥	199	140, 155, 123	B, M, R, W	B, M, R, W
10	55.08	*p*-Hydroxybenzaldehyde (-)	121	39, 92	B, M, R, W	B, M, R, W
11	65.88	Vanillin (+) ¥	153	93, 125, 65	B, M, R, W	B, M, R, W
12	69.60	Coumaroyl glycerol (-)	237	**145**, **119**, **163**	B, M, R	-
13	70.91	*p*-Coumaric acid (*trans*) (-) ¥	163	119, 93	B, M, R, W	B, M, R, W
14	71.31	Syringaldehyde (+) ¥	183	123, 95, 155, 140	B, M, R	B, M, R, W
15	73.00	*p*-Coumaric acid (*cis*) (-)	163	119, 93	B, M, R, W	B, M, R, W
16	78.97	Ferulic acid (*trans*) (-) ¥	193	134, 149, 178	B, M, R, W	B, M, R, W
17	81.11	Ferulic acid (*cis*) (-)	193	134, 149, 178	B, M, R, W	B, M, R, W
		Ferulic Acid Dehydrodimers				
18	82.86	8-8′-DFA_c (-)	385	267, **158**, **173**	-	B, M, R, W
20	85.29	DFA, hydrated 1 (-)	403	178, 148, 193, 134	-	B, M, R, W
22	86.84	8-8′-DFA (-)	385	282, **173**, **123**	-	B, M, R
23	87.23	DFA, hydrated 2 (-)	403	239, 279, 265, 134, 148	-	B, M
26	88.91	8-8′-DFA_f (-)	403	**151**, 148, **233**, **163**	-	B, M, R
28	89.64	8-5′-DFA (-)	385	**282**, **267**, **326**, **297**, 323, **341**	-	B, M, R, W
29	90.88	DFA, hydrated 3 (-)	403	193, 308, 149, 164	-	B, M, R
49	97.33	DFA 1 (-)	385	173, 123, 282	-	B, M
52	98.64	4-O-5′-DFA (-)	385	139, **193**, 267, 329	-	B, M
62	101.78	DFA 2 (-)	385	267, 382	-	B, M, R, W
70	104.50	5-5′-DFA (-)	385	**282**, **326**, **341**, **267**	-	B, M, R, W
84	109.19	8-5′-DFA_f (-)	385	**282**, **326**, **341**, **267**	-	B, M, R, W
89	110.85	8-O-4′-DFA (*trans/trans*) (-)	385	**134**, **178**, **149**, **193**	-	B, M, R, W
93	113.35	8-O-4′-DFA (*trans/cis*) (-)	385	**134**, **178**, **149**, **193**	-	B, M

98	115.83	DFA 3 $^{(-)}$	385	-	-	B, M, R
107	121.94	DFA 4 $^{(-)}$	385	-	-	B, M
122	129.81	8-5′-DFA$_{dc}$ (*trans*) $^{(-)}$	341	-	-	B, M
123	131.10	8-5′-DFA$_{dc}$ (*cis*) $^{(-)}$	341	-	-	B, M
		Ferulic Acid Dehydrotrimers and Tetramers				
31	91.54	TFA 1 $^{(-)}$	577	435, 508, 178	-	B, M, R
48	96.94	TFA, hydrated 1 $^{(-)}$	595	-	-	B, M
53	98.91	8-O-4′/5-8″-TFA $^{(-)}$	577	193, 355	-	B, M, R
56	100.12	TFA, hydrated 2 $^{(-)}$	595	317, 545, 367	-	B, M
61	101.70	TFA 2 $^{(-)}$	577	146	-	B, M
64	102.67	TFA, hydrated 3 $^{(-)}$	595	-	-	B, M
71	104.91	8-8′$_c$-/4-O-8″-TFA $^{(-)}$	577	341, **533**, **489**	-	B, M
94	113.54	4-O-8′/5′-5″/8″-5‴-TeFA $^{(-)}$	769	274	-	B, M
101	117.17	8-O-4′-5-5″-TFA $^{(-)}$	577	355, 533, **193**, **489**	-	B, M, R, W
109	122.74	TFA 3 $^{(-)}$	577	355	-	B, M
113	124.73	TFA 4 $^{(-)}$	577	355	-	B, M
126	131.98	8-O-4′/4-O-8″-TFA $^{(-)}$	577	**193**	-	B, M, R
130	135.24	4-O-8′/5′-5″/8’’-O-4-TeFA $^{(-)}$	769	**193**	-	B, M
		Soluble Hydroxycinnamic Acid Amides				
21	85.88	*N*-Coumaroyl spermidine $^{(+)}$	292	**147**, 204	B	-
25	88.74	*N,N′*-Dicoumaroyl spermidine (*cis/cis*) $^{(+)}$	438	**147**, 204, **292**, 275, 72	B, M	B, M
32	91.75	*N,N′*-Coumaroyl feruloyl spermidine (*cis/cis*) $^{(+)}$	468	**177**, 234, 204, **292**, **147**, **322**, 145	B, M	-
36	93.05	*N,N′*-Dicoumaroyl spermidine (*cis/trans*) $^{(+)}$	438	**147**, 204, **292**, 275, 72	B, M	B, M
39	94.40	*N,N′*-Diferuloyl spermidine (*cis/cis*) $^{(+)}$	498	**177**, 234, 322, 145	B, M, W	-
40	94.60	*N,N′*-Coumaroyl feruloyl spermidine (*cis/trans*) 1 $^{(+)}$	468	**177**, 204, **292**, **147**, **322**, 234, 275, 145	B, M, W, R	-
44	96.13	*N,N′*-Dicoumaroyl putrescine (*cis/cis*) $^{(+)}$	381	189, 145, **147**, 101, 277, 177, 321	B, M	-
45	96.42	*N,N′*-Diferuloyl spermidine (*cis/trans*) $^{(+)}$	498	**177**, 234, 322, 305, 145	B, M	-
46	96.59	*N,N′*-Coumaroyl feruloyl spermidine (*cis/trans*) 2 $^{(+)}$	468	**177**, 204, **292**, **147**, **322**, 234, 275, 145	B, M, W, R	-
47	96.62	*N,N′*-Dicoumaroyl spermidine (*trans/trans*) $^{(+)}$	438	147, 204, **292**, 275, 72, 221	B, M	B, M
51	98.52	*N,N′*-Coumaroyl feruloyl putrescine (*cis/cis*) $^{(+)}$	411	**177**, **147**, **235**	B, M	B, M
54	99.44	*N,N′*-Coumaroyl feruloyl spermidine (*trans/trans*) $^{(+)}$	468	**177**, 234, 204, **292**, **322**, **147**, 145, 305, 275	B, M	-
57	100.61	*N,N′*-Diferuloyl spermidine (*trans/trans*)	498	**177**, **322**, 234, 145	B, M, W	-
58	100.83	*N,N′*-Diferuloyl putrescine (*cis/cis*) $^{(+)}$	441	**177**, 265, 145, 89, 117, 248	B, M	B, M
60	101.43	*N,N′*-Dicoumaroyl putrescine (*cis/trans*) $^{(+)}$	381	**147**, **235**, 218	B, M	-
67	103.47	*N,N′*-Coumaroyl feruloyl putrescine (*cis/trans*) 1 $^{(+)}$	411	**177**, **147**, 145, 265, **235**, 218	B, M	B, M
69	104.09	*N,N′*-Coumaroyl feruloyl putrescine (*cis/trans*) 2 $^{(+)}$	411	**177**, **147**, 145, 265, **235**, 218	B, M	B, M
73	105.30	*N,N′*-Dicoumaroyl putrescine (*trans/trans*) $^{(+)}$	381	**147**, **235**, 218, 89, 72	B, M	-
74	105.88	*N,N′*-Diferuloyl putrescine (*cis/trans*) $^{(+)}$	441	**177**, 145, 265, 248	B, M	B, M
78	107.56	*N,N′*-Coumaroyl feruloyl putrescine (*trans/trans*) $^{(+)}$	411	**177**, **147**, 145, 235, 265, 89, 218	B, M	B, M
82	108.65	*bis-N,N′*-Diferuloyl putrescine $^{(-)}$	877	439	B, M	-

86	109.65	*N,N′*-Diferuloyl putrescine (*trans/trans*) $^{(+)}$	441	**177**, 145, 265, 248, 89, 117, 72	B, M	B, M
Insoluble Hydroxycinnamic Acid Amides						
19	84.03	*N,N′*-Coumaroyl dehydrotriferuloyl putrescine 1 $^{(+)}$	795	409, 519, 719	-	M
24	87.48	*N,N′*-Coumaroyl dehydrodiferuloyl putrescine 1 $^{(+)}$	603	177, 427, 265, 195	-	B, M
27	89.40	*N,N′*-Coumaroyl dehydrodiferuloyl putrescine 2 $^{(+)}$	603	177, 265	-	B, M
30	91.20	*N,N′*-Coumaroyl dehydrotriferuloyl putrescine 2 $^{(+)}$	795	409	-	B, M
33	92.22	*N*-Dehydrodiferuloyl putrescine 1 $^{(+)}$	457	89, 72, 115	-	B, M
34	92.24	*N,N′*-Coumaroyl dehydrotriferuloyl putrescine 3 $^{(+)}$	795	409, 533	-	B, M
35	92.44	*N,N′*-Coumaroyl dehydrodiferuloyl putrescine 3 $^{(+)}$	603	177, 265, 72	-	B, M
37	93.35	*N*-Dehydrodiferuloyl putrescine 2 $^{(+)}$	457	115, 265, 72	-	B, M
38	93.59	*N,N′*-Coumaroyl dehydrodiferuloyl putrescine 4 $^{(+)}$	603	415, 148	-	B, M
41	95.02	*N*-Dehydrodiferuloyl putrescine 3 $^{(+)}$	457	115, 298, 72	-	B, M
42	95.61	*N,N′*-Coumaroyl dehydrotriferuloyl putrescine 4 $^{(+)}$	795	539, 148	-	B, M
43	95.68	*N*-Dehydrodiferuloyl putrescine 4 $^{(+)}$	457	351, 319, 277, 115	-	B, M
50	97.44	*N,N′*-Coumaroyl dehydrotriferuloyl putrescine 5 $^{(+)}$	795	539, 135, 195	-	B, M
55	99.70	*N*-Dehydrodiferuloyl putrescine 5 $^{(+)}$	457	115, 98, 177, 244, 365	-	B, M
59	101.10	*N*-Dehydrodiferuloyl putrescine 6 $^{(+)}$	457	351, 440, 115, 72, 369	-	B, M
63	101.80	*N*-Dehydrotriferuloyl putrescine 1 $^{(+)}$	649	89, 177, 631, 265, 72	-	B, M
65	103.11	*N*-Dehydrodiferuloyl putrescine 7 $^{(+)}$	457	351, 177, 175, 440, 72, 115, 263	-	B, M
66	103.33	*N,N′*-Coumaroyl dehydrotriferuloyl putrescine 6 $^{(+)}$	795	539, 394, 435		B, M
68	103.87	*N,N′*-Feruloyl dehydrodiferuloyl putrescine 1 $^{(+)}$	633	177, 432, 465, 387	-	B, M
72	105.27	*N,N′*-Feruloyl dehydrodiferuloyl putrescine 2 $^{(+)}$	633	177, 457, 369, 341, 72	-	B, M
75	106.33	*N,N′*-Coumaroyl dehydrodiferuloyl putrescine 5 $^{(+)}$	603	457, 369, 72, 83, 369, 411	-	B, M
76	106.82	*N,N′*-Feruloyl dehydrodiferuloyl putrescine 3 $^{(+)}$	633	177, 265, 439, 457	-	B, M
77	107.45	*N,N′*-Feruloyl dehydrodiferuloyl putrescine 4 $^{(+)}$	633	177, 341, 439, 589	-	B, M
79	107.97	*N*-Dehydrotriferuloyl putrescine 2 $^{(+)}$	649	265, 89, 177, 440	-	B, M
80	108.18	*N,N′*-Feruloyl dehydrodiferuloyl putrescine 5 $^{(+)}$	633	177, 369, 457, 439, 574, 291, 145, 89	-	B, M
81	108.58	*N,N′*-Coumaroyl dehydrodiferuloyl putrescine 6 $^{(+)}$	603	457, 385	-	B, M
83	109.16	*N*-Dehydrotriferuloyl putrescine 3 $^{(+)}$	649	89, 72, 148, 265	-	B, M
85	109.34	*N,N′*-Feruloyl dehydrodiferuloyl putrescine 6 $^{(+)}$	633	177, 351, 245, 439	-	B, M
87	110.28	*N,N′*-Coumaroyl dehydrodiferuloyl putrescine 7 $^{(+)}$	603	365, 439, 351	-	B, M
88	110.34	*N,N′*-Feruloyl dehydrodiferuloyl putrescine 7 $^{(+)}$	633	457, 177, 439, 369, 265	-	B, M
90	110.90	*N,N′*-Coumaroyl dehydrotriferuloyl putrescine 7 $^{(+)}$	795	409	-	B, M
91	111.80	*N,N′*-Feruloyl dehydrodiferuloyl putrescine 8 $^{(+)}$	633	177, 439, 457, 589	-	B, M
92	112.32	*N,N′*-Feruloyl dehydrodiferuloyl putrescine 9 $^{(+)}$	633	265, 177, 351, 369, 439	-	B, M
95	113.60	*N*-Dehydrotriferuloyl putrescine 4 $^{(+)}$	649	72, 89, 177, 631	-	B, M
96	114.02	*N,N′*-Feruloyl dehydrodiferuloyl putrescine 10 $^{(+)}$	633	439, 457, 369, 277	-	B, M
97	115.02	*N,N′*-Feruloyl dehydrodiferuloyl putrescine 11 $^{(+)}$	633	519, 439, 351, 145, 175, 177	-	B, M
99	115.88	*N*-Dehydrotriferuloyl putrescine 5 $^{(+)}$	649	177, 115	-	B, M
100	116.69	*N,N′*-Feruloyl dehydrodiferuloyl putrescine 12 $^{(+)}$	633	245, 151, 291, 351, 177, 439	-	B, M

102	117.21	*N*-Dehydrotriferuloyl putrescine 6 $^{(+)}$	649	245, 177, 323	-	B, M
103	118.49	*N,N′*-Feruloyl dehydrodiferuloyl putrescine 13 $^{(+)}$	633	439, 457, 177	-	B, M
104	118.97	*N,N′*-Didehydrodiferuloyl putrescine 1 $^{(+)}$	825	367, 631	-	B, M
105	119.43	*N,N′*-Feruloyl dehydrodiferuloyl putrescine 14 $^{(+)}$	633	177, 439, 351, 457, 115, 89	-	B, M
106	120.04	*N,N′*-Feruloyl dehydrotriferuloyl putrescine 1 $^{(+)}$	825	631, 177, 265, 649	-	B, M
108	122.23	*N*-Dehydrotriferuloyl putrescine 7 $^{(+)}$	649	382, 265, 72, 89, 439	-	B, M
110	122.80	*N,N′*-Feruloyl dehydrodiferuloyl putrescine 15 $^{(+)}$	633	177, 439, 265, 369, 351, 457	-	B, M
111	123.55	*N*-Dehydrotriferuloyl putrescine 8 $^{(+)}$	649	473, 145, 177	-	B, M
112	124.17	*N,N′*-Feruloyl dehydrodiferuloyl putrescine 16 $^{(+)}$	633	351, 177, 265, 72	-	B, M
114	124.93	*N,N′*-Coumaroyl dehydrodiferuloyl putrescine 8 $^{(+)}$	603	457, 235, 147	-	B, M
115	125.38	*N,N′*-Didehydrodiferuloyl putrescine 2 $^{(+)}$	825	177, 650, 483, 369, 631	-	B, M
116	126.08	*N,N′*-Coumaroyl dehydrodiferuloyl putrescine 9 $^{(+)}$	603	439, 369, 147, 457	-	B, M
117	126.54	*N,N′*-Feruloyl dehydrodiferuloyl putrescine 17 $^{(+)}$	633	177, 457, 439	-	B, M
118	127.85	*N,N′*-Feruloyl dehydrodiferuloyl putrescine 18 $^{(+)}$	633	177, 369, 265, 115, 439	-	B, M
119	127.97	*N,N′*-Feruloyl dehydrotriferuloyl putrescine 2 $^{(+)}$	825	177, 369, 244, 115, 649	-	B, M
120	129.06	*N,N′*-Coumaroyl dehydrodiferuloyl putrescine 10 $^{(+)}$	603	457, 369, 86, 175, 219, 147	-	B, M
121	129.76	*N,N′*-Feruloyl dehydrodiferuloyl putrescine 19 $^{(+)}$	633	177, 439, 457, 589, 115, 145	-	B, M
124	131.12	*N*-Dehydrotriferuloyl putrescine 9 $^{(+)}$	649	351, 177	-	B, M
125	131.19	*N,N′*-Coumaroyl dehydrodiferuloyl putrescine 11 $^{(+)}$	603	147, 439, 457, 89	-	B, M
127	132.59	*N,N′*-Didehydrodiferuloyl putrescine 3 $^{(+)}$	825	177, 631	-	B, M
128	133.20	*N,N′*-Feruloyl dehydrodiferuloyl putrescine 20 $^{(+)}$	633	177, 369, 457, 265, 291, 439, 145, 72, 89	-	B, M
129	134.97	*N,N′*-Feruloyl dehydrodiferuloyl putrescine 21 $^{(+)}$	633	177, 457, 439, 291, 145, 351, 589	-	B, M
131	139.79	*N,N′*-Feruloyl dehydrodiferuloyl putrescine 22 $^{(+)}$	633	177, 457, 439	-	B, M
132	144.90	*N,N′*-Didehydrodiferuloyl putrescine 4 $^{(+)}$	825	177, 439	-	B, M
133	145.78	*N,N′*-Feruloyl dehydrodiferuloyl putrescine 23 $^{(+)}$	633	177, 457, 439, 135	-	B, M
134	150.15	*N,N′*-Feruloyl dehydrodiferuloyl putrescine 24 $^{(+)}$	633	177, 457	-	B, M
135	153.38	*N,N′*-Feruloyl dehydrodiferuloyl putrescine 25 $^{(+)}$	633	177	-	B, M
			Other Compounds			
1	11.50	Citric acid $^{(-)}$ ¥	191	87, 111, 85, 67	B, M, R, W	B, M
2	28.45	Tyrosyl-tryptophan $^{(-)}$	366	160	B, M	-

Coumaroyl glycerol (12) was detected in the SF of maize and rye flours and *broa*, but not detected in the IF of any sample, meaning that, if present, it was possibly hydrolysed to free pCA during the IF extraction procedure.

Three FA hexosides (peaks 4, 5, 7: FA hexoside 1, 2, 3) were also detected in the SF of *broa*. In addition, FA hexoside 3 was also detected in the SF of wheat flour. A FA hexoside has been previously identified in wheat [26]. The detection of FA hexosides 1 and 2 in *broa* SF suggests that they should be present in at least one of the raw flours used for *broa* production (maize, wheat, or rye). However, since they were not detected in the SF of any raw flours, they were probably associated with insoluble compounds and hydrolysed to FA during the IF extraction procedure.

It is widely known that soluble phenolic acids, such as free FA and some small FA oligosaccharides, are readily available for absorption by the human gastrointestinal (GI) tract. Results obtained confirm that several small phenolic acids can be found in *broa* SF, being bioaccessible and able to reach the specific sites where they can exert their biological actions [4].

3.2. Ferulic acid dehydrodimers

In cereals, FA dimerises mainly by free phenoxyl radicals coupling reactions, at their O-4, C-5 or C-8 positions, yielding diferulate esters connected via 8-5′, 8-O-4′, 5-5′, 8-8′, and 4-O-5′ linkages [***Table 1.1***].

Due to the lack of commercial standards, the identification of DFAs in the samples analysed (maize, wheat, rye, and *broa*) was performed comparing the data obtained with the information described in the literature, when similar analytical conditions were applied, namely: (i) the presence of characteristic precursor ions at *m/z* 385, 403, and 341 $[M - H]^-$ [***Table 2.1***, ***Figure 2.2***]; (ii) MS/MS spectra; (iii) characteristic UV absorption spectra with maximum wavelengths at 280 and 320 nm; (iv) relative intensities of UV chromatogram peaks at 280 nm; and (v) relative retention times (RTs).

Since DFAs are bound to arabinoxylans [5,6], they were not detected in the SF of any sample (raw flours and *broa*). However, in maize and *broa* IF, 12 peaks at *m/z* 385 were detected and associated with DFAs, according to their characteristic MS/MS spectra. These peaks presented one or more nonspecific product ions at *m/z* 341, 326, 311, 297, 282, and 267, which can be related to the loss of CO_2 (×2), $CH_3^{\cdot}$ and CH_2O, as reported by Callipo *et al*. [26]. Although only six DFAs with a molecular mass of 386 Da have been commonly described in maize grains, it is known that they can be present as *trans*- or *cis*- isomeric forms, as well as in *anti*- or *syn*-cyclic forms, therefore increasing the number of compounds that can be identified [26].

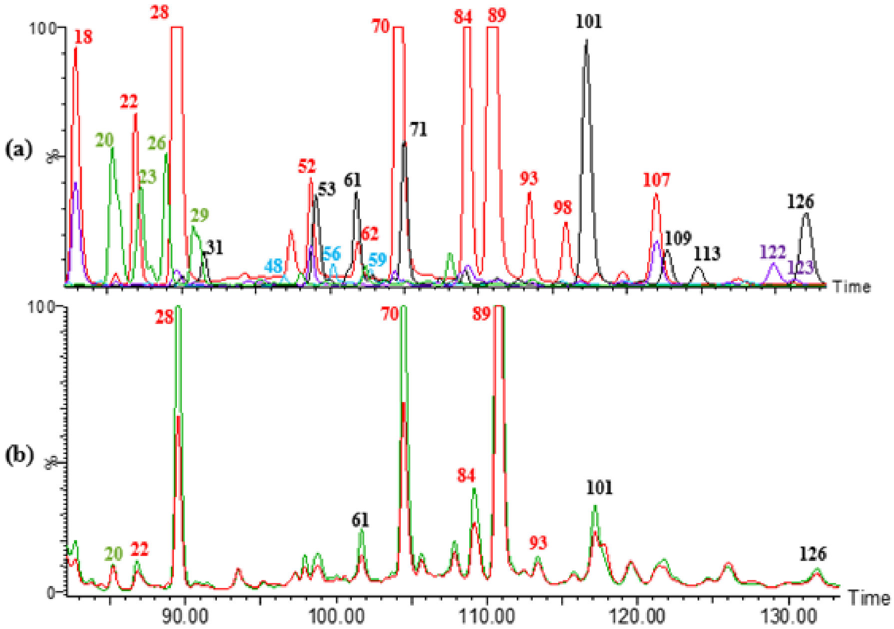


Figure 2.2: **(a)** Extracted-ion chromatogram (XIC) from 82 to 133 min of dehydrodiferulic acids (DFAs) and dehydrotriferulic acids (TFAs) of maize flour (Verdial de Aperrela) IF at *m/z* 385 (red), 403 (green), 341 (purple), 577 (black) and 595 (blue). **(b)** Chromatogram of maize flour IF, at 280 nm (red) and 320 nm (green). Peaks are labelled as described in ***Table 2.1***.

Dobberstein and Bunzel [24] presented the UV chromatogram at 280 nm of an insoluble fibre extract from whole maize grains and the UV spectra of each isolated DFA [24]. The chromatographic profile obtained in the present work for maize and *broas* IF [***Figure A.1, Appendix A***] was similar to the one presented by these authors [24]. The main peaks observed at 280 nm and at *m/z* 385 were peaks 28, 70, 84 and 89. According to their MS/MS [26,28,33,38] and UV [24] spectra, relative peaks intensity [24] and RTs [24], they were putatively identified as 8-5′-DFA, 5-5′-DFA, 8-5′-DFA$_f$, and 8-O-4′-DFA, respectively. These four main DFAs were also detected in rye and wheat IF. Compounds containing an 8-5′(noncyclic)-coupled dimer unit, probably do not exist in plants [4] but are formed from their phenylcoumaran precursors, containing an 8-5′-(cyclic)-coupled dimeric unit, during saponification [4].

The identification of other minor DFAs became a challenge since they have not been so commonly characterised. Peaks 18, 22, and 52 (*m/z* 385) were putatively identified as 8-8′-DFA$_c$, 8-8′-DFA, and 4-O-5′-DFA, respectively, according to their UV [24] and MS/MS [24,26,33,39] spectra, and elution order [24,33,39].

Additionally, the MS/MS spectra of compounds 93 and 89 (*m/z* 385, 8-O-4′-DFA) were identical. Therefore, compound 89 could correspond to the more common *trans/trans* isomeric form of 8-O-4′-DFA and compound 93 to its *trans/cis* isomeric form [26,33]. Similar to *trans*-8-O-4′-DFA (89), peak 93 showed a characteristic product ion at *m/z* 193, which corresponds to the cleavage of the ether link between the two monomeric units and elimination of a neutral FA, suggesting the presence of a C-O bond, less stable than C-C linkages [33,40]. Furthermore, the characteristic fragments of FA at *m/z* 134, 149 and 178 were also present.

It was possible to detect four additional peaks (49, 62, 98, and 107: DFA 1, 2, 3, and 4) at *m/z* 385 with characteristic DFAs product ions. These compounds may correspond to different isomeric forms of the DFAs already described above, particularly to the *cis* and *syn* configurations.

Another common DFA in maize grains is 8-8′-DFA_f (*m/z* 403). Taking into account its elution order [24,33], and UV [24] and MS/MS [26,33] spectra, this compound was putatively identified as peak 26. Additionally, it was possible to detect three peaks at *m/z* 403 (peaks 20, 23, and 29), with MS/MS spectra related to hydrated forms of DFAs. Another structure related to DFAs is the 8-5′ decarboxylated form (8-5′-DFA_{dc}) [24,39], with a molecular mass of 342 Da. It was possible to detect peaks 122 and 123 which, according to their elution order [24,26,39], may correspond to *trans*-8-5′-DFA_{dc} and *cis*-8-5′-DFA_{dc}, respectively. However, due to their low intensities, it was not possible to compare their UV and MS/MS spectra with data from the literature. It has been described that both compounds are not present in the plant, but instead, they may be formed during the saponification process [4], as previously mentioned for compounds containing an 8-5′(noncyclic)-coupled dimer unit.

Although it has been described that DFAs exhibit higher antioxidant activity than free FA [17], they are not readily absorbed by the human gastrointestinal (GI) system, since they are covalently bound to indigestible polysaccharides [4,18]. However, DFAs can be released by digestive enzymes or microorganisms in the intestinal lumen and be further absorbed [4,41], or exhibit their beneficial action directly in the GI system [4].

3.3. Ferulic acid dehydrotrimers and tetramers

Although MS/MS spectra of dehydrotriferulic acids (TFAs) [***Table 1.1***] have not been so commonly characterised, it was possible to putatively identify eight signals at *m/z* 577 $[M - H]^-$ and three at *m/z* 595 $[M - H]^-$ (hydrated forms of TFAs) [4] in raw flours and breads IF [***Figure 2.2***], using the same criteria described for DFAs. In both maize flour and breads IF, peak 101 was the most intense TFA at *m/z* 577, followed by peaks 126 and 71. According to their MS/MS spectra, relative intensities [24,39], and elution order [24,39], they were tentatively identified as 8-O-4′/5-5″-, 8-O-4′/4-O-8″-, and 8-8′$_c$/4-O-8″-TFA, respectively.

The MS/MS spectrum of peak 101 (8-O-4′/5-5″-TFA) showed product ions at *m/z* 533 $[M – H – CO_2]^–$, 489 $[M – H – 2CO_2]^–$, 355, 311, and 193. The detected ion at *m/z* 193 confirmed the presence of a C-O bond, as previously mentioned for DFAs. The signals at *m/z* 355 and 311 could be originated by the fragmentation of the C-O bond and the loss of CH_2O (–30 Da, *m/z* 355) and CH_2O and CO_2 (–74 Da, *m/z* 311) of the 5-5″-diferuloyl moiety. The MS/MS spectrum of peak 126 (8-O-4′/4-O-8″-TFA) showed the presence of the characteristic product ion at *m/z* 193. Fragments with higher *m/z* values, characteristic of C-C linkages, were not detected. Peak 71 (8-8′$_c$/4-O-8″-TFA) showed a product ion at *m/z* 341 (loss of one feruloyl moiety and CO_2) and 297 (loss of another CO_2). The product ion at *m/z* 297 has been described as characteristic of 8-8′-DFA$_c$ [26].

Peak 53 was tentatively identified as 8-O-4′/5-8″-TFA, according to its elution order [39] and MS/MS spectrum, which showed a very intense peak at *m/z* 193, and smaller peaks at *m/z* 355 and 311, similarly to those described for peak 101 (8-O-4′/5-5″-TFA).

Compounds 31, 61, 109, and 113 (TFA 1, 2, 3, and 4) also presented a precursor ion at *m/z* 577. Other minor TFAs that have been described in maize are 8-O-4′/5-8″*c* -, 8-8′/4-O-8″- and, possibly, 5-5′/8-8-TFA [4,42]. Additionally, TFAs may also exhibit *cis* or *trans* configurations, therefore increasing their structural diversity.

A precursor ion at *m/z* 595 was detected in peaks 48, 56, and 64 (TFA, hydrated 1, 2, and 3), which may correspond to isomers of 8-8′$_f$/5-5″-TFA or 8-O-4′/5-5″(H_2O)-TFA.

Dehydrotetraferulic acids (TeFA) have also been reported in maize bran [4]. The extracted ion chromatogram (XIC) at *m/z* 769 showed a chromatogram with several low-intensity peaks. According to their elution order, two of them, peak 94 and peak 130, may correspond to 4-O-8′/5′-5″/8″-5‴-TeFA and 4-O-8′/5′-5‴/8″-O-4‴-TeFA, respectively [***Table 1.1***].

Similar to FA dehydrodimers, FA trimers and tetramers are not readily absorbed by the human GI system and need to be released by digestive enzymes or microorganisms in the intestinal lumen before absorption [4]. However, since they were also detected in *broa*, they should be considered when studying the phenolic composition of maize-based food products and in bioavailability studies.

3.4. Soluble hydroxycinnamic acid amides

The major peaks observed in the SF of maize flours and *broas* [***Figure 2.2***] were peaks 51, 67, 69, and 78 at *m/z* 409 or 411, peaks 25, 36, and 47 at *m/z* 436 or 438, and peaks 58, 74, and 86 at *m/z* 439 or 441 ($[M - H]^-$ or $[M + H]^+$, respectively), corresponding to the monoisotopic masses (MM) of 410, 437 and 440. These compounds were tentatively identified as HCAAs, namely *N,N′*-coumaroyl feruloyl putrescine (CFP), *N,N′*-dicoumaroyl spermidine (DCS), and *N,N′*-diferuloyl putrescine (DFP) [***Tables 1.3*** and ***2.2***], which have not been described in maize-based foods before. HCAAs are formed by hydroxycinnamic moieties in which double bonds can assume either a *cis* or *trans* configuration [11], giving rise to the formation of several isomeric amides with the same precursor and product ions.

The different isomers observed in the present work correspond to the different possibilities of double bonds configuration on FA and pCA moieties. In plants, the *cis* isomers are less common [4] and, in reversed-phase chromatography, they elute earlier than *trans* isomers [31], presenting lower peak areas as well [***Figure 2.3***]. These compounds were not detected in neither the analysed wheat nor rye flours. Indeed, it is known that maize contains large amounts of conjugated putrescine and spermidine, when comparing to other cereals, such as rice and wheat [31].

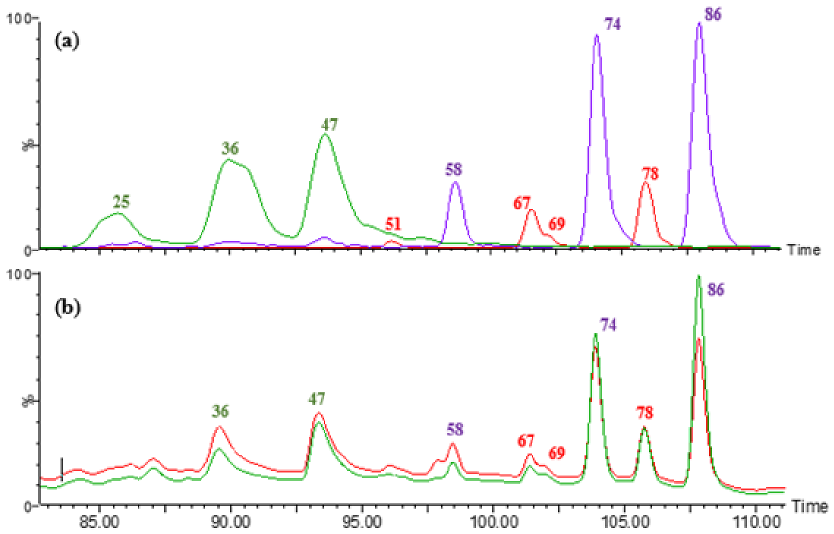
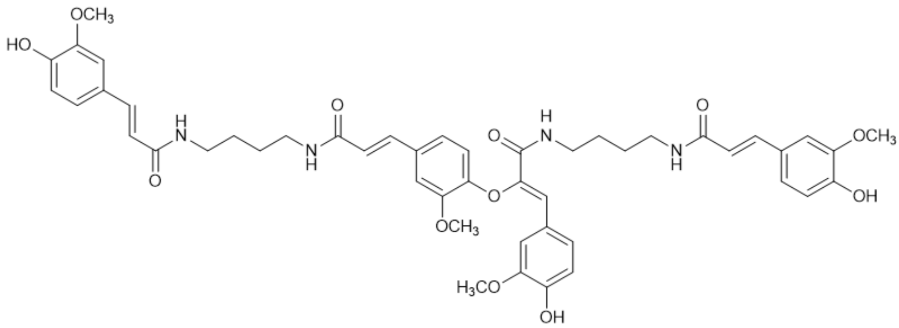


Figure 2.3: **(a)** Extracted-ion chromatogram (XIC) of hydroxycinnamic acid amides (HCAAs) of *broa* Verdial de Aperrela SF, at *m/z* 411 (red), 438 (green), and 441 (purple). **(b)** Chromatogram of *broa* SF, at 280 nm (red) and 320 nm (green). Peaks are labelled as described in ***Table 2.1***.

The monoconjugate *N*-coumaroyl spermidine (21) was identified in *broas*, probably formed from the hydrolysis of DCS during processing. The monoconjugates feruloyl and *p*-coumaroyl putrescine were not detected either in maize flours or *broas*, although they have been described in maize grains [31,43]. A possible explanation could be that the extraction procedures used by other authors (80% of methanol with 1% of HCl and methanol/isopropanol/water, 8/1/1), could have led to the hydrolysis of more complex HCAAs, liberating the described monoconjugates.

Other minor HCAAs were also identified in maize flours and *broas* extracts, namely compounds 32, 40, 46, 54 (*N,N′*-coumaroyl feruloyl spermidine isomers, CFS), compounds 44, 60, 73 (*N,N′*-dicoumaroyl putrescine isomers, DCP), and compounds 39, 45, 57 (*N,N′*-diferuloyl spermidine isomers, DFS). Compounds 40 and 46 (CFS) were also detected in the commercial wheat and rye flours SF and compounds 39 and 57 (DFS) were also detected in wheat flour SF. These compounds have been recently described in maize grains [31].

Peak 82 showed a precursor ion at *m/z* 877 $[M-H]^-$, with a UV spectrum similar to other HCAAs (λmax: 316, 293) and was detected in maize flours and *broas* extracts. The MS/MS experiments showed a product ion at *m/z* 439, which may correspond to a DFP molecule. This compound was putatively identified as *bis-N,N′*-diferuloyl putrescine, a molecule with two DFP moieties [***Figure 2.4***]. To the best of our knowledge, this compound has never been previously described.



***bis-N,N′*-Diferuloyl putrescine**
m/z 877 $[M-H]^-$

Figure 2.4: Molecular structure of the tentatively identified *bis-N,N′*-Diferuloyl putrescine.

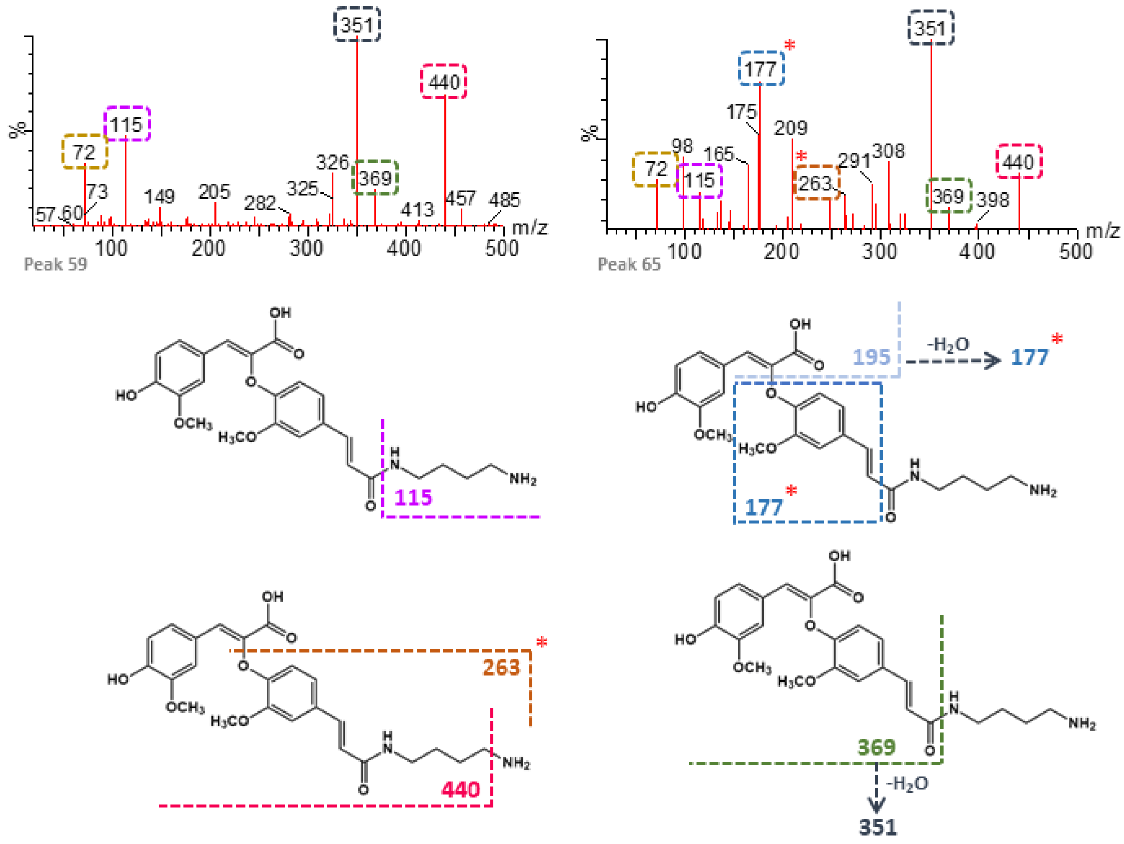
Recently, some studies have pointed out that HCAAs, specially feruloyl putrescines, exhibit antioxidant [7,8], anti-inflammatory [44] and chemopreventive [13] properties, capable of inducing apoptosis in human leukemia U937 cells [14]. However, these compounds have not been studied in maize-based foods and thus there is no information about their bioavailability. HCAAs were detected in the SF of *broas*, which suggests that they can be readily absorbed or easily exposed to the action of digestive enzymes [4].

3.5. Insoluble hydroxycinnamic acid amides

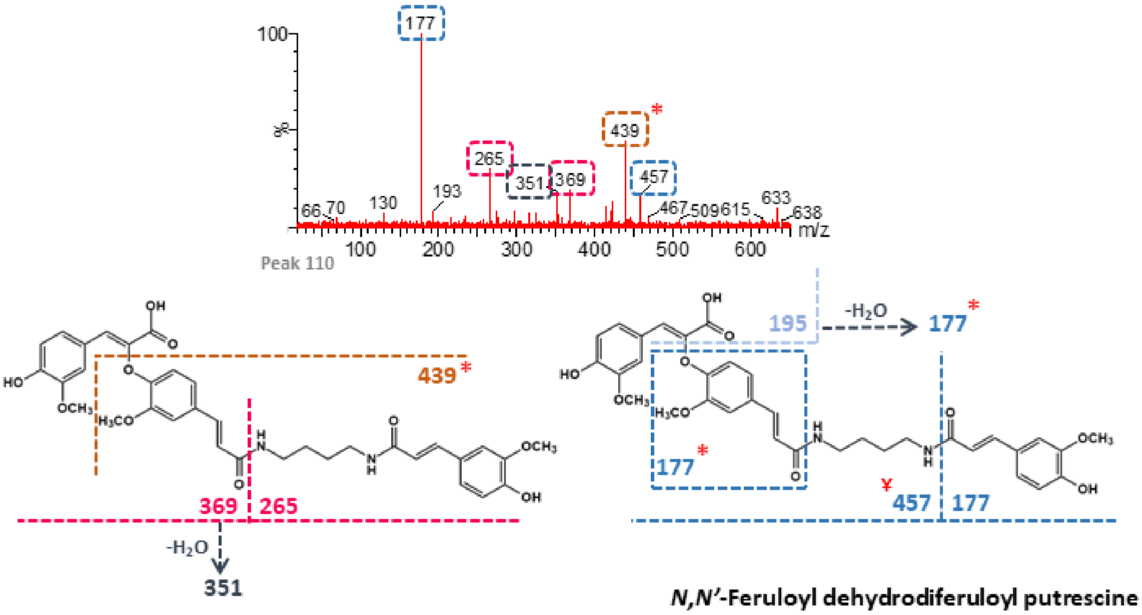
Some of the HCAAs described above were also identified in the IF of maize flours and *broas* [***Table 2.1***]. Therefore, they were probably linked to the maize grain matrix, such as cell walls, as previously suggested [11]. Analyses by XIC in positive ion mode were performed in order to search for the presence of dehydrodiferulic and dehydrotriferulic acid amides. Results suggest that these compounds were present in maize flours and *broas* IF, but not in wheat or rye flours. To the best of our knowledge, these compounds have not been previously described, and these results provide evidence that HCAAs are also constituents of maize cell walls.

Since there are several isomeric forms of DFAs, numerous dehydrodiferuloyl and dehydrotriferuloyl putrescine isomers can also be formed. Seven peaks were tentatively identified as *N*-dehydrodiferuloyl (*m/z* 457, $[M + H]^+$), twenty-five as *N,N′*-feruloyl dehydrodiferuloyl (*m/z* 633), four as *N,N′*-didehydrodiferuloyl (*m/z* 825), and eleven as *N,N′*-coumaroyl dehydrodiferuloyl (*m/z* 603) putrescines [***Table 1.3***]. Additionally, nine peaks were tentatively identified as *N*-dehydrotriferuloyl (*m/z* 649), two as *N,N′*-feruloyl dehydrotriferuloyl (*m/z* 825), and seven as *N,N′*-coumaroyl dehydrotriferuloyl (*m/z* 795) putrescines [***Table 1.3***]. ***Figures 2.5*** and ***2.6*** show the fragmentation patterns proposed for several dehydrodiferuloyl and dehydrotriferuloyl putrescines, respectively. These compounds were identified mainly based on the characteristic cleavages between amide bonds that have been described for HCAAs [31], which originate, among others, the product ions coumaroyl (*m/z* 147), feruloyl (*m/z* 177), and dehydrodiferuloyl (*m/z* 369). Feruloyl dehydrotriferuloyl putrescines were distinguished from didehydrodiferuloyl putrescines (*m/z* 825) by the presence of the product ion at *m/z* 649 (dehydrotriferuloyl putrescine) [***Figure 2.5***]. According to the chemical structures proposed for each dehydrodiferulic and dehydrotriferulic acid putrescines, specific product ions suggest the presence of C-O linkages between feruloyl moieties, probably 8-O-4′ linkages. In contrast, the absence of these ions suggests the presence of C-C linkages. However, for simplification purposes, only 8-O-4′ linkages are represented in ***Figures 2.4*** and ***2.5***. It was not possible to detect either HCAAs derived from hydrated forms of DFAs or TFAs, nor spermidine-linked DFAs or TFAs.

Since it has been described that HCAAs and FA dehydrodimers exhibit higher antioxidant and anti-inflammatory activities than free FA [8,17,44], insoluble HCAAs constituted by FA dehydrodimers and trimers may also exhibit interesting beneficial health effects. Future studies on the bioactivity of these compounds should be performed. As FA dehydrodimers, trimers and tetramers, insoluble HCAAs were only detected after hydrolysis, which suggests they were bound to indigestible polysaccharides, and therefore are not easily absorbed by the human GI system, but can eventually exhibit their action in this system.



N-Dehydrodiferuloyl putrescine
m/z 457 [M+H]$^+$



N,N′-Feruloyl dehydrodiferuloyl putrescine
m/z 633 [M+H]$^+$

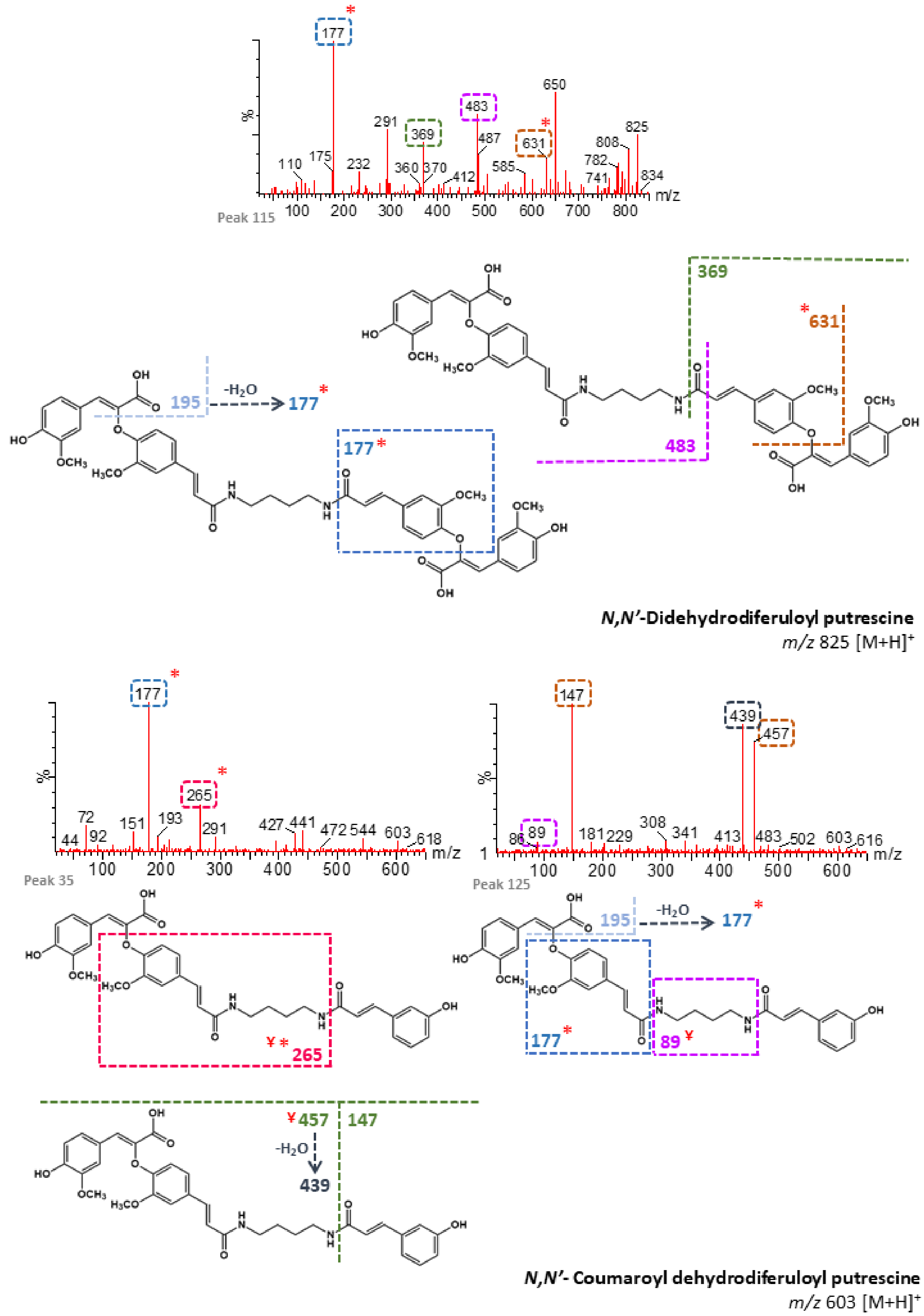
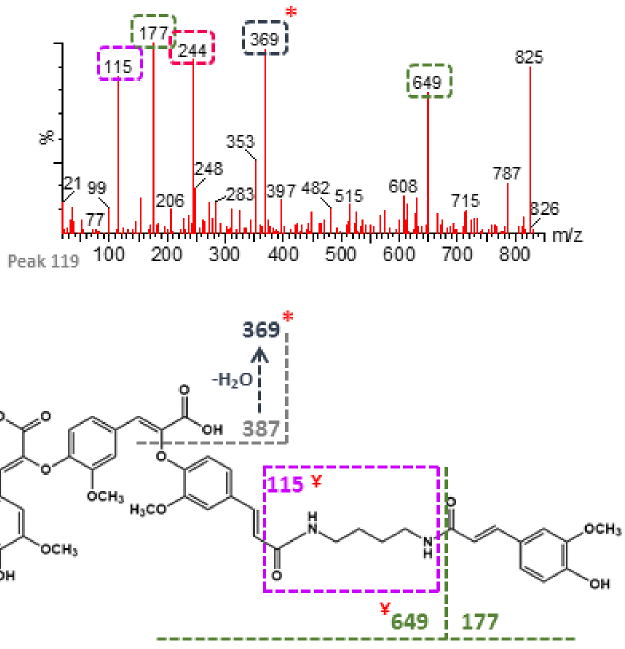
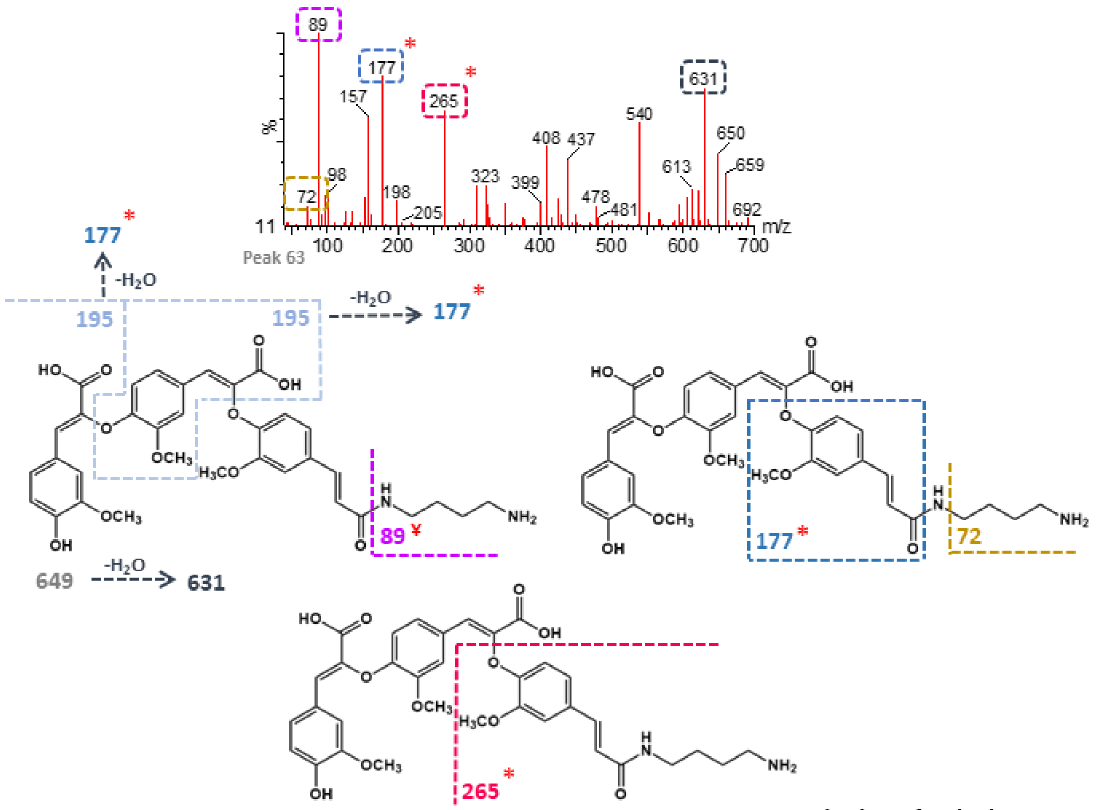


Figure 2.5: Fragmentation patterns proposed for dehydrodiferuloyl (8-O-4′-DFA) putrescines.

*****: Characteristic product ions of C-O linkages between feruloyl moieties. ¥: Product ions formed after amine protonation.



***N,N'*-Feruloyl dehydrotriferuloyl putrescine**
m/z 825 $[M+H]^+$



***N*-Dehydrotriferuloyl putrescine**
m/z 649 $[M+H]^+$

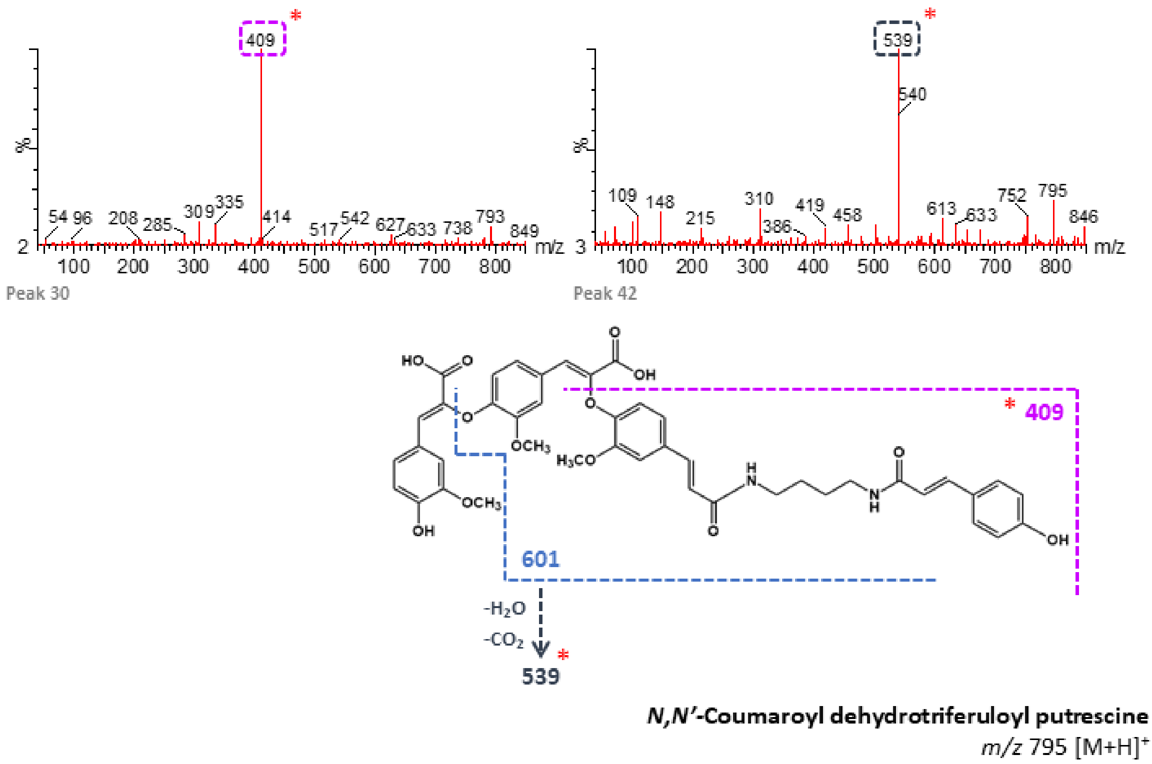


Figure 2.6: Fragmentation patterns proposed for dehydrotriferuloyl (8-O-4′/4-O-8″-TFA) putrescines.

*****: Characteristic product ions of C-O linkages between feruloyl moieties. [¥]: Product ions formed after amine protonation.

4. Conclusions

This study sheds light on the identification of different isomers of FA dimers, FA trimers and HCAAs in maize flour and *broas*, by HPLC-DAD-MS/MS analysis. Complex HCAAs were identified for the first time, consisting of putrescine-linked DFAs and TFAs, suggesting that HCAAs are associated with maize cell walls. The presence of these compounds in *broas* shows that they are resistant to processing. Thus, they can also contribute to the total phenolic content and antioxidant properties of this maize-based bread and associated health benefits. Therefore, in addition to FA and pCA, hydroxycinnamic acid derivatives should be considered when studying the phenolic composition of maize and maize-based food products. Future work is needed in order to characterise more isomeric forms of DFAs, TFAs, and their respective putrescine derivatives using mass spectrometry tools. Additionally, the intestinal release and uptake of the studied compounds, especially soluble HCAAs, should be evaluated. The differences among the phenolics content of raw flours and *broas* are currently being studied, to understand the possible contribution of *broa* as a health-promoting bread.

Funding and acknowledgments

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References

1. Singh, N.; Singh, S.; Shevkani, K. Maize: Composition, bioactive constituents, and unleavened bread. In *Flour and Breads and Their Fortification in Health and Disease Prevention*; Elsevier: Amsterdam, The Netherlands, **2019**; pp. 111–121.
2. Vaz Patto, M.; Alves, M.; Almeida, N.; Santos, C.; Mendes Moreira, P.; Satovic, Z.; Brites, C. Is the bread making technological ability of Portuguese traditional maize landraces associated with their genetic diversity? *Maydica* **2009**, *54*, 297–311.
3. Smith, J.R. 50 of the World's Best Breads. Available online: https://edition.cnn.com/travel/article/world-50-best-breads/index.html. (Accessed on 30 April 2020).
4. Bento-Silva, A.; Patto, M.C.V.; do Rosário Bronze, M. Relevance, structure and analysis of ferulic acid in maize cell walls. *Food Chem.* **2018**, *246*, 360–378.
5. Salinas-Moreno, Y.; García-Salinas, C.; Ramírez-Díaz, J.L.; Alemán-de la Torre, I. Phenolic Compounds in Maize Grains and Its Nixtamalized Products. In Phenolic Compounds-Natural Sources, Importance and Applications; InTech: London, UK, **2017**.
6. Gutiérrez-Grijalva, E.P.; Ambriz-Pére, D.L.; Leyva-López, N.; Castillo-López, R.I.; Heredia, J.B. *Dietary Phenolic Compounds, Health Benefits and Bioaccessibility*; Archivos Latinoamericanos de Nutrición: Caracas, Venezuela, **2016**; Volume 66.
7. Buitimea-Cantúa, N.E.; Gutiérrez-Uribe, J.A.; Serna-Saldivar, S.O. Phenolic–protein interactions: Effects on food properties and health benefits. *J. Med. Food* **2018**, *21*, 188–198.
8. Choi, S.W.; Lee, S.K.; Kim, E.O.; Oh, J.H.; Yoon, K.S.; Parris, N.; Hicks, K.B.; Moreau, R.A. Antioxidant and antimelanogenic activities of polyamine conjugates from corn bran and related hydroxycinnamic acids. *J. Agric. Food Chem.* **2007**, *55*, 3920–3925.
9. Collison, A.; Yang, L.; Dykes, L.; Murray, S.; Awika, J.M. Influence of genetic background on anthocyanin and copigment composition and behavior during thermoalkaline processing of maize. *J. Agric. Food Chem.* **2015**, *63*, 5528–5538.
10. Gupta, K.; Dey, A.; Gupta, B. Plant polyamines in abiotic stress responses. *Acta Physiol. Plant* **2013**, *35*, 2015–2036.
11. Macoy, D.M.; Kim,W.-Y.; Lee, S.Y.; Kim, M.G. Biosynthesis, physiology, and functions of hydroxycinnamic acid amides in plants. *Plant Biotechnol. Rep.* **2015**, *9*, 269–278.
12. Majumdar, R.; Minocha, R.; Lebar, M.D.; Rajasekaran, K.; Long, S.; Carter-Wientjes, C.; Minocha, S.C.; Cary, J.W. Contribution of maize polyamine and amino acid metabolism towards resistance against Aspergillus flavus infection and aflatoxin production. *Front. Plant Sci.* **2019**, *10*, 692.
13. Acosta-Estrada, B.A.; Serna-Saldívar, S.O.; Gutiérrez-Uribe, J.A. Chemopreventive effects of feruloyl putrescines from wastewater (*Nejayote*) of lime-cooked white maize (*Zea mays*). *J. Cereal Sci.* **2015**, *64*, 23–28.
14. Kim, E.-O.; Kwon, T.-K.; Choi, S.-W. Diferuloylputrescine, a predominant phenolic amide in corn bran, potently induces apoptosis in human leukemia U937 cells. *J. Med. Food* **2014**, *17*, 519–526.
15. Pei, K.; Ou, J.; Huang, C.; Ou, S. Derivatives of ferulic acid: Structure, preparation and biological activities. *Annu. Res. Rev. Biol.* **2015**, 512–528.

16. Wang, S.; Suh, J.H.; Zheng, X.; Wang, Y.; Ho, C.-T. Identification and quantification of potential anti-inflammatory hydroxycinnamic acid amides from wolfberry. *J. Agric. Food Chem.* **2017**, *65*, 364–372.

17. Jia, Y.; He, Y.; Lu, F. The structure-antioxidant activity relationship of dehydrodiferulates. *Food Chem.* **2018**, *269*, 480–485.

18. Bento-Silva, A.; Koistinen, V.M.; Mena, P.; Bronze, M.R.; Hanhineva, K.; Sahlstrøm, S.; Kitryt ˙ e, V.; Moco, S.; Aura, A.-M. Factors affecting intake, metabolism and health benefits of phenolic acids: Do we understand individual variability? *Eur. J. Nutr.* **2020**, *59*, 1275–1293.

19. Ribas-Agustí, A.; Martín-Belloso, O.; Soliva-Fortuny, R.; Elez-Martínez, P. Food processing strategies to enhance phenolic compounds bioaccessibility and bioavailability in plant-based foods. *Crit. Rev. Food Sci. Nutr.* **2018**, *58*, 2531–2548.

20. 20. Vaz Patto, M.; Moreira, P.; Carvalho, V.; Pego, S. Collecting maize (*Zea mays* L. convar. *mays*) with potential technological ability for bread making in Portugal. *Genet. Resour. Crop Evol.* **2007**, *54*, 1555–1563.

21. Brites, C.M.; Trigo, M.J.; Carrapiço, B.; Alviña, M.; Bessa, R.J. Maize and resistant starch enriched breads reduce postprandial glycemic responses in rats. *Nutr. Res.* **2011**, *31*, 302–308.

22. Alves, M.L.; Belo, M.; Carbas, B.; Brites, C.; Paulo, M.; Mendes-Moreira, P.; Brites, C.; Bronze, M.d.R.; Šatovi´c, Z.; Vaz Patto, M.C. Long-term on-farm participatory maize breeding by stratified mass selection retains molecular diversity while improving agronomic performance. *Evol. Appl.* **2018**, *11*, 254–270.

23. Mussi de Mira, N.V.; Cerdeira Barros, R.M.; Schiocchet, M.A.; Noldin, J.A.; Lanfer-Marquez, U.M. Extração, análise e distribuição dos ácidos fenólicos em genótipos pigmentados e não pigmentados de arroz (Oryza sativa L.). *Cienc. Tecnol. Aliment.* **2008**, *28*, 994–1002.

24. Dobberstein, D.; Bunzel, M. Separation and detection of cell wall-bound ferulic acid dehydrodimers and dehydrotrimers in cereals and other plant materials by reversed phase high-performance liquid chromatography with ultraviolet detection. *J. Agric. Food Chem.* **2010**, *58*, 8927–8935.

25. Zaupa, M.; Calani, L.; Del Rio, D.; Brighenti, F.; Pellegrini, N. Characterization of total antioxidant capacity and (poly) phenolic compounds of differently pigmented rice varieties and their changes during domestic cooking. *Food Chem.* **2015**, *187*, 338–347.

26. Callipo, L.; Cavaliere, C.; Fuscoletti, V.; Gubbiotti, R.; Samperi, R.; Laganà, A. Phenilpropanoate identification in young wheat plants by liquid chromatography/tandem mass spectrometry: Monomeric and dimeric compounds. *J. Mass Spectrom.* **2010**, *45*, 1026–1040.

27. Arranz, S.; Calixto, F.S. Analysis of polyphenols in cereals may be improved performing acidic hydrolysis: A study in wheat flour and wheat bran and cereals of the diet. *J. Cereal Sci.* **2010**, *51*, 313–318.

28. Chiremba, C.; Taylor, J.R.; Rooney, L.W.; Beta, T. Phenolic acid content of sorghum and maize cultivars varying in hardness. *Food Chem.* **2012**, *134*, 81–88.

29. Das, A.K.; Singh, V. Antioxidative free and bound phenolic constituents in botanical fractions of Indian specialty maize (*Zea mays* L.) genotypes. *Food Chem.* **2016**, *201*, 298–306.

30. Ndolo, V.U.; Beta, T. Comparative studies on composition and distribution of phenolic acids in cereal grain botanical fractions. *Cereal Chem.* **2014**, *91*, 522–530.

31. Li, Z.; Zhao, C.; Zhao, X.; Xia, Y.; Sun, X.; Xie, W.; Ye, Y.; Lu, X.; Xu, G. Deep Annotation of Hydroxycinnamic Acid Amides in Plants Based on Ultra-High-Performance Liquid Chromatography–High-Resolution Mass Spectrometry and Its In Silico Database. *Anal. Chem.* **2018**, *90*, 14321–14330.

32. Burt, A.J.; Arnason, J.T.; García-Lara, S. Natural variation of hydroxycinnamic acid amides in maize landraces. *J. Cereal Sci.* **2019**, *88*, 145–149.

33. Xiang, J.; Zhang, M.; Apea-Bah, F.B.; Beta, T. Hydroxycinnamic acid amide (HCAA) derivatives, flavonoid C-glycosides, phenolic acids and antioxidant properties of foxtail millet. *Food Chem.* **2019**, *295*, 214–223.

34. Guo, W.; Beta, T. Phenolic acid composition and antioxidant potential of insoluble and soluble dietary fibre extracts derived from select whole-grain cereals. *Food Res. Int.* **2013**, *51*, 518–525.

35. Ragaee, S.; Seetharaman, K.; Abdel-Aal, E.-S.M. The impact of milling and thermal processing on phenolic compounds in cereal grains. *Crit. Rev. Food Sci. Nutr.* **2014**, *54*, 837–849.

36. Van Hung, P. Phenolic compounds of cereals and their antioxidant capacity. *Crit. Rev. Food Sci. Nutr.* **2016**, *56*, 25–35.

37. Takada, M.; Niu, R.; Minami, E.; Saka, S. Characterization of three tissue fractions in corn (*Zea mays*) cob. *Biomass Bioenergy* **2018**, *115*, 130–135.

38. Qiu, Y.; Liu, Q.; Beta, T. Antioxidant properties of commercial wild rice and analysis of soluble and insoluble phenolic acids. *Food Chem.* **2010**, *121*, 140–147.

39. Pedersen, M.B.; Bunzel, M.; Schäfer, J.; Knudsen, K.E.B.; Sørensen, J.F.; Yu, S.; Lærke, H.N. Ferulic acid dehydrodimer and dehydrotrimer profiles of distiller's dried grains with solubles from different cereal species. *J. Agric. Food Chem.* **2015**, *63*, 2006–2012.

40. Vismeh, R.; Lu, F.; Chundawat, S.P.; Humpula, J.F.; Azarpira, A.; Balan, V.; Dale, B.E.; Ralph, J.; Jones, A.D. Profiling of diferulates (plant cell wall cross-linkers) using ultrahigh-performance liquid chromatography-tandem mass spectrometry. *Analyst* **2013**, *138*, 6683–6692.

41. Andreasen, M.F.; Kroon, P.A.; Williamson, G.; Garcia-Conesa, M.-T. Intestinal release and uptake of phenolic antioxidant diferulic acids. *Free Radic. Biol. Med.* **2001**, *31*, 304–314.

42. Waterstraat, M.; Bunzel, M. A multi-step chromatographic approach to purify radically generated ferulate oligomers reveals naturally occurring 5-5/8-8 (cyclic)-, 8-8 (noncyclic)/8-O-4-, and 5-5/8-8 (noncyclic)-coupled dehydrotriferulic acids. *Front. Chem.* **2018**, *6*, 190.

43. Sen, A.; Bergvinson, D.; Miller, S.S.; Atkinson, J.; Fulcher, R.G.; Arnason, J.T. Distribution and microchemical detection of phenolic acids, flavonoids, and phenolic acid amides in maize kernels. *J. Agric. Food Chem.* **1994**, *42*, 1879–1883.

44. Kim, E.O.; Min, K.J.; Kwon, T.K.; Um, B.H.; Moreau, R.A.; Choi, S.W. Anti-inflammatory activity of hydroxycinnamic acid derivatives isolated from corn bran in lipopolysaccharide-stimulated Raw 264.7 macrophages. *Food Chem. Toxicol.* **2012**, *50*, 1309–1316.

CHAPTER 3

Broa, *an ethnic maize bread, as a source of phenolic compounds*[1]

Abstract

Maize is an important source of phenolic compounds, specially hydroxycinnamic acids, which are widely known for their antioxidant activity and associated health benefits. However, these effects depend on their bioaccessibility, which is influenced by the different techniques used for food processing. Several traditional products can be obtained from maize and, in Portugal, it is used for the production of an ethnic bread called *broa*. In order to evaluate the effect of processing on maize phenolic composition, one commercial hybrid and five open pollinated maize flours and *broas* were studied. The total phenolic content and antioxidant activity were evaluated by the *Folin-Ciocalteu* and ORAC assays, respectively. The major phenolics, namely ferulic and *p*-coumaric acids (in their soluble-free, soluble-conjugated and insoluble forms), insoluble ferulic acid dimers and soluble hydroxycinnamic acid amides were quantitated. Results show that the total phenolic content, antioxidant activity and hydroxycinnamic acids resisted traditional processing conditions used in the production of *broas*. The content in soluble-free phenolics increased after processing, meaning that their bioaccessibility improved. Portuguese traditional *broas*, produced with open pollinated maize varieties, can be considered an interesting dietary source of antioxidant compounds due to the higher content in hydroxycinnamic acids and derivatives.

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[1] **This chapter is based on the following publication:**

Bento-Silva, A.; Duarte, N.; Mecha, E.; Belo, M.; Serra, A.T.; Vaz Patto, M.C.; Bronze, M.R. *Broa*, an Ethnic Maize Bread, as a Source of Phenolic Compounds. *Antioxidants*. **2021**, *10*, 672.
DOI: 10.3390/antiox10050672

1. Introduction

Maize (*Zea mays*) is considered a staple cereal in many countries, where it is used to prepare different types of food products [1]. In Portugal, maize is the main ingredient (50–100%) of an ethnic bread known as *broa*. Rye, wheat, (0–50%) or a combination of the flours are also commonly added to the recipe [2]. *Broas* are traditionally prepared using open-pollinated maize varieties. However, Portuguese maize landraces are at risk of disappearing, due to the progressive adoption of more productive hybrid varieties, which originate *broas* less appreciated by consumers [2].

Epidemiological studies have demonstrated that the consumption of phenol-rich foods, as wholegrain cereals, in a regular diet is inversely associated with the risk of developing chronic diseases, such as oncologic and cardiovascular diseases, and metabolic syndrome [3]. Compared to other cereals, maize contains a high level of phenolic compounds, specially hydroxycinnamic acids, which can be found in their soluble or insoluble forms [4]. In particular, the ferulic acid (FA) content is at least ten-fold higher than in other cereal grains, which makes maize one of the most interesting sources of FA in the human diet [4]. Soluble phenolic compounds can be present in their free form or conjugated with smaller molecules, such as simple sugars and amines, as hydroxycinnamic acid amides [5–9]. Insoluble phenolic compounds are mostly (>94%) bound to arabinoxylans [4,5,10], and include dehydrodiferulic, dehydrotriferulic and dehydrotetraferulic acids [4,5] and their derivatives, such as the recently described insoluble hydroxycinnamic acid amides [11].

Evidence from human intervention trials on the protective effects of phenol-rich foods, such as wholegrains, has been inconsistent, mainly due to differences in the food composition and in the bioaccessibility and bioavailability of phenolic compounds [3]. Their bioaccessibility is influenced by the processing techniques involved in the preparation of food products [4,12]. Soluble compounds are usually available for absorption by a simple diffusion mechanism [4,13]. Conversely, insoluble compounds have a very low bioaccessibility [4,13] and need to be liberated from the food matrix during small intestinal digestion or colonic fermentation, in order to be absorbed and become bioavailable [12]. In a previous study, several phenolic compounds, mainly hydroxycinnamic acids and hydroxycinnamic acid amides, were identified in maize flours and *broas* [11].

Since the total content and bioaccessibility of phenolic compounds is influenced by the techniques used for food processing [3], it is mandatory to evaluate the phenolic composition of the final food product. The understanding of the influence of processing also allows the selection of processing conditions that are capable of preserving and increasing the bioaccessibility of phenolic compounds. The study reported herein aimed at elucidating the changes occurring in the soluble-free, soluble-conjugated and insoluble phenolic compositions caused by the processing of raw flours to *broas*, and the potential implications on their bioaccessibility, bioavailability and bioactivity.

2. Materials and methods

2.1. Cereal flours and *broas* samples

Five traditional maize flours (Broa-213, Pigarro, Castro Verde, Verdial de Aperrela and Fandango), one commercial maize flour, and the corresponding *broas* were studied. These five traditional varieties were randomly chosen after an initial screening of the soluble phenolic composition and total phenolic content of all twelve maize flours and *broas*. The commercial wheat and rye flours used in the preparation of *broas* were also studied. All cereal samples and *broas* are described in more detail in ***Section 7, Chapter 1***.

2.2. Reagents

The reagents and commercial standards used for chromatographic analysis were described elsewhere [11]. Additionally, Folin-Ciocalteu reagent, sodium carbonate, 2’,2’-azobis-(2-amidinopropane) dihydrochloride (AAPH), Trolox and fluorescein sodium salt were obtained from Sigma-Aldrich, St. Louis, MO, USA.

2.3. Preparation of phenolic fractions

Cereal flours (maize, rye and wheat) and *broas* (4 g) were submitted to a conventional extraction procedure [11] with 20 mL of EtOH/H_2O (50%, *v/v*), to obtain an ethanolic solution containing the soluble phenolic compounds and a solid residue comprising the insoluble compounds. Alkaline hydrolyses were applied to both the ethanolic solution and residue. Three phenolic fractions were obtained: the soluble (SF), the soluble-hydrolysed (SHF) and the insoluble (IF) fractions [***Figure 3.1***]. The abbreviations used throughout the paper, including tables and figures, are listed in the ***Acronyms*** section.

Briefly, in order to obtain the soluble fraction (SF), the pH of the ethanolic solution (5 mL) was adjusted to 1.5 ± 0.5 with concentrated HCl, followed by a liquid-liquid extraction with ethyl acetate (EtOAc, 3 × 7.5 mL). The combined EtOAc fractions were evaporated until dry and reconstituted in 5 mL of EtOH 50%. To obtain the soluble-hydrolysed fraction (SHF), the ethanolic solution (5 mL) was hydrolysed with NaOH 4 M (40 mL, pH 14 ± 0.5), under N_2, for 15 h, at room temperature [15,16]. After hydrolysis, the pH was set to 1.5 ± 0.5 with concentrated HCl, and the phenolic compounds were extracted with EtOAc (3 × 30 mL). The combined EtOAc fractions were evaporated until dry and reconstituted in 5 mL of EtOH 50%.

The insoluble fraction (IF) was prepared from the solid residue, which was defatted with hexane (3 × 20 mL) and centrifuged (7000 × g, 10 min). The defatted residue was hydrolysed with NaOH 4 M (60 mL, pH 14 ± 0.5), for 15 h at room temperature, in the presence of N_2. The obtained solution was extracted with EtOAc (3 × 30 mL), evaporated until dry and reconstituted in 20 mL of EtOH 50%. All fractions (SF, SHF and IF) of cereal flours and *broas* were prepared in duplicate and kept at –20 °C until analysis.

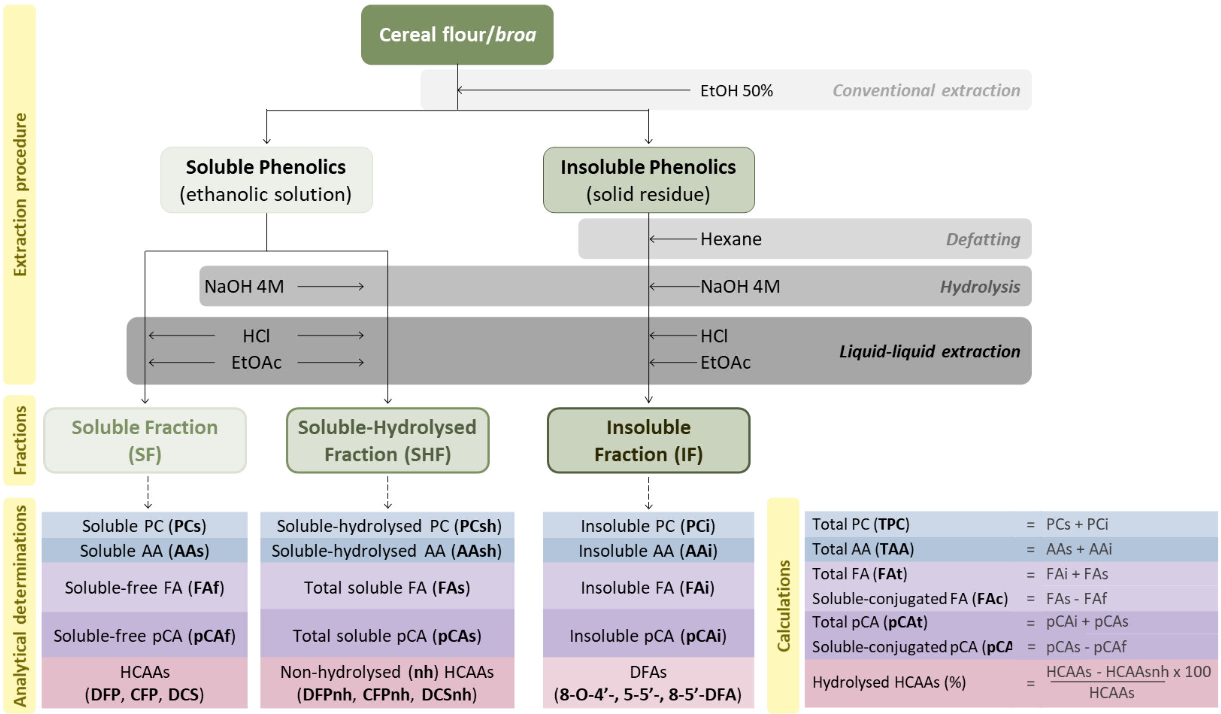

Figure 3.1: Representative scheme of the soluble (**SF**), soluble-hydrolysed (**SHF**) and insoluble (**IF**) phenolic fractions and respective determinations.

2.4. Phenolic content and antioxidant activity

The phenolic content (PC) and antioxidant activity (AA) were determined in each fraction [***Figure 3.1***], using *Folin-Ciocalteu* and ORAC (oxygen radical absorbance capacity) assays, respectively, as previously reported [17]. Determinations were performed in triplicate and reported as mg GAE (gallic acid equivalents) and mmol of Trolox equivalents (TE) per 100 g of sample's dry weight (dw), respectively. The total PC and the total AA were calculated as described in ***Figure 3.1***.

2.5. HPLC-DAD-ED analysis

Phenolic compounds were analysed in a Thermo Fisher Scientific (Waltham, MS, USA) Surveyor high-performance liquid chromatography (HPLC) system, equipped with a diode array detector (DAD) programmed for scanning between 192 and 798 nm and an electrochemical detector, ED 40 Dionex (Sunnyvale, CA, USA). Analytical conditions for HPLC-DAD were previously reported [11]. In order to detect the phenolic compounds that could exhibit AA, electrochemical detection was programmed for a linear variation from –1.0 to 1.0 V in 1.00 s (detection by integrated voltammetry using a cyclic variation of the potential). The measurements were taken with a 50 Hz frequency with an analogic/digital converter.

Hydroxycinnamic acids (FA and pCA) were quantitated in all fractions (SF, SHF and IF) using standard ethanolic solutions prepared from the corresponding commercial standards, at 320 nm, in a range from 0.15 to 100 mg L^{-1} ($y = 443378x + 1415.4$, $R^2 = 0.9989$) and from 0.3 to 50 mg L^{-1} ($y = 624015x - 6094.7$, $R^2 = 0.9998$), respectively. The major phenolic compounds (dehydrodiferulic acids and hydroxycinnamic acid amides) were previously identified by HPLC-DAD-MS/MS [11]. Due to the absence of commercially available standards, dehydrodiferulic acids, feruloyl putrescine and coumaroyl feruloyl putrescine were quantitated as FA equivalents (FAE) and dicoumaroyl spermidine was quantitated as pCA equivalents (pCAE). Concentrations were expressed as mg 100 g^{-1} dw. Limits of quantitation and detection (LOQ and LOD, signal to noise ratio (S/N) of 10 and 3, respectively) of FA and pCA were determined and confirmed by analysing five independent solutions corresponding to these concentrations. The LOQ for both compounds corresponded to 0.05 mg L^{-1} (approximately 0.03 mg 100 g^{-1} dw) and the LOD to 0.02 mg L^{-1} (0.01 mg 100 g^{-1} dw). In order to control the signal of both detectors (DAD and ED), standard mixtures of FA and pCA at 20 mg L^{-1} were analysed after every fifteen injections. The total and soluble-conjugated pCA and FA contents were calculated as described in ***Figure 3.1***.

2.6. Data analysis

ChromQuest (version 3.1.6) software, Thermo Fisher Scientific, Waltham, MA, USA and 4880 software (Unicam, Lisbon, Portugal) were used for data acquisition and treatment of HPLC-DAD and ED analyses, respectively. The identification of pCA and FA was performed by comparison with standard solutions using commercially available standards and the identification of the main dehydrodiferulic acids and hydroxycinnamic acid amides was based on results previously reported [11]. For quantitative data analyses, the limit of significance was set at $p < 0.05$. Paired-samples *t*-tests, independent-samples *t*-tests, ANOVA followed by post hoc Tukey tests, principal component analyses (PCA) and Pearson’s coefficient correlations were obtained using the software SPSS version 21 (IBM, NY, USA).

3. Results and discussion

Maize-based foods can be considered important sources of phenolic compounds in a balanced diet. *Broas* are usually prepared using open-pollinated traditional maize varieties but, more recently, hybrid maize varieties have allowed their production on a larger scale. This work intended to study the total (soluble and insoluble) phenolic composition of five Portuguese traditional maize varieties, which were cultivated in the same environment and in the same period of time, and the corresponding *broas*, contributing to a better understanding of the effect of processing on their bioaccessibility. A commercial maize flour and *broa* were also studied for comparison. As rye and wheat flours were used in *broas* recipes, these flours were also characterized. To achieve these goals, for each cereal flour and *broa* sample, three phenolic fractions were prepared according to ***Figure 3.1***, namely, the soluble (SF), the soluble-hydrolysed (SHF) and the insoluble (IF) fractions. The abbreviations used throughout the paper, including tables and figures, are listed in the ***Acronyms*** section.

3.1. Phenolic content and antioxidant activity of cereal flours

The results from the phenolic characterization, phenolic content (PC) and antioxidant activity (AA) of the different cereal flours used in the production of *broas* are presented in ***Table 3.1***.

The total PC of maize flours ranged from 150 to 276 mg GAE 100 g^{-1} dw. These values are according to those already described for maize [18–22] but, as expected, are lower than the values reported for pigmented (red, purple, blue or black) varieties (up to 3400 mg GAE 100 g^{-1} dw in purple maize), due to the absence of anthocyanins and other flavonoids [19,23]. For the total AA, values ranged from 3.39 to 6.39 mmol TE 100 g^{-1} dw, which are within the range of the values described in the literature [20,21,23]. The insoluble compounds were responsible for the majority of maize flours' PC and AA (82.5 ± 3.9% and 85.3 ± 3.3%, respectively) [***Table 3.1***].

A strong and positive correlation was observed between insoluble AA and PC (R = 0.902, $p < 0.05$) and between soluble AA and PC (R = 0.863, $p < 0.05$) [***Table 3.2***]. These results were expected, as the PC has been described as one of the most important contributors to the AA of cereal grains [23].

Table 3.1: Cereal flours mean values for: phenolic content, antioxidant activity and individual phenolic contents (ferulic acid, *p*-coumaric acid, diferuloyl putrescine, coumaroyl feruloyl putrescine, dicoumaroyl spermidine and dehydrodiferulic acids) in the different fractions, as described in ***Figure 3.1***.

Mean values within rows with no letters (a-d–c) in common are significantly different ($p < 0.05$).

Description	**Phenolic Fraction**	**Maize**							**Wheat**	**Rye**
		Broa-213	Pigarro	Castro Verde	Verdial de Aperrela	Fandango	Commercial	Average		
Phenolic Content (PC) (mg GAE 100 g^{-1} dw)										
Soluble	SF	47.9 ± 2.3^{a}	37.0 ± 0.9^{ab}	37.9 ± 5.1^{ab}	41.7 ± 2.0^{ab}	37.1 ± 2.5^{ab}	32.2 ± 3.1^{b}	39.0 ± 5.3	5.73 ± 0.1	15.9 ± 0.9
Soluble-hydrolysed	SHF	52.2 ± 1.9^{ab}	42.6 ± 1.4^{ab}	42.6 ± 4.9^{ab}	53.0 ± 3.3^{a}	41.0 ± 2.2^{ab}	39.2 ± 3.0^{b}	45.1 ± 6.0	6.87 ± 0.5	17.1 ± 1.0
Insoluble	IF	157 ± 8.4^{ab}	200 ± 19.4^{ac}	203 ± 9.25^{ac}	226 ± 22.7^{ac}	239 ± 17.6^{c}	118 ± 9.0^{b}	190 ± 45.3	12.3 ± 1.0	27.6 ± 0.7
Total	SF+IF	205 ± 10.7^{ab}	237 ± 20.3^{ac}	240 ± 14.4^{ac}	268 ± 24.7^{ac}	276 ± 20.1^{c}	150 ± 12.1^{b}	229 ± 46.4	18.0 ± 1.1	43.5 ± 1.6
% Insoluble	n/a	76.6	84.4	84.3	84.5	86.6	78.5	82.5 ± 3.9	68.1	63.4
Antioxidant Activity (AA) (mmol TE 100 g^{-1} dw)										
Soluble	SF	0.87 ± 0.05^{a}	0.75 ± 0.11^{a}	0.75 ± 0.18^{a}	0.72 ± 0.08^{a}	0.66 ± 0.07^{a}	0.46 ± 0.04^{a}	0.70 ± 0.14	0.17 ± 0.02	0.42 ± 0.03
Soluble-hydrolysed	SHF	1.65 ± 0.08^{a}	1.59 ± 0.20^{a}	1.28 ± 0.20^{a}	1.39 ± 0.33^{a}	1.51 ± 0.15^{a}	1.38 ± 0.11^{a}	1.47 ± 0.14	0.13 ± 0.02	0.47 ± 0.07
Insoluble	IF	3.40 ± 0.24^{ab}	4.66 ± 0.71^{ab}	4.02 ± 0.44^{ab}	4.38 ± 0.73^{ab}	5.73 ± 0.80^{a}	2.93 ± 0.48^{b}	4.19 ± 0.99	0.30 ± 0.02	0.62 ± 0.07
Total	SF+IF	4.27 ± 0.29^{ab}	5.41 ± 0.83^{ab}	4.76 ± 0.61^{ab}	5.10 ± 0.81^{ab}	6.39 ± 0.88^{a}	3.39 ± 0.52^{b}	4.89 ± 1.02	0.47 ± 0.04	1.03 ± 0.10
% Insoluble	n/a	79.6	86.2	84.3	85.8	89.7	86.3	85.3 ± 3.3	63.7	59.6
Ferulic acid (FA) (mg 100 g^{-1} dw) and contribution (%) for the total FA (**bold**)										
Soluble-free	SF	0.35 ± 0.01^{a} **(0.3)**	0.24 ± 0.01^{ab} **(0.2)**	0.25 ± 0.05^{ab} **(0.2)**	0.29 ± 0.06^{ab} **(0.2)**	0.23 ± 0.02^{ab} **(0.1)**	0.18 ± 0.04^{b} **(0.2)**	0.26 ± 0.06 **(0.2)**	0.10 ± 0.01 **(3.0)**	0.26 ± 0.01 **(3.6)**
Soluble-conjugated	SHF-SF	6.49 ± 0.00^{a} **(6.3)**	5.80 ± 1.44^{a} **(4.5)**	4.57 ± 2.33^{a} **(3.6)**	6.66 ± 0.43^{a} **(4.5)**	5.21 ± 0.51^{a} **(3.3)**	5.32 ± 0.13^{a} **(7.1)**	5.67 ± 0.80 **(4.9)**	0.25 ± 0.02 **(7.3)**	1.08 ± 0.00 **(14.9)**
Total soluble	SHF	6.84 ± 0.02^{a} **(6.6)**	6.04 ± 1.43^{a} **(4.7)**	4.81 ± 2.38^{a} **(3.8)**	6.95 ± 0.49^{a} **(4.7)**	5.44 ± 0.53^{a} **(3.4)**	5.49 ± 0.10^{a} **(7.3)**	5.93 ± 0.84 **(5.1)**	0.35 ± 0.03 **(10.3)**	1.34 ± 0.01 **(18.5)**
Insoluble	IF	95.8 ± 14.0^{ab} **(93.3)**	122 ± 18.0^{abc} **(95.3)**	122 ± 10.0^{abc} **(96.2)**	143 ± 21.6^{bc} **(95.4)**	152 ± 7.2^{c} **(96.5)**	69.6 ± 2.32^{a} **(92.7)**	117 ± 30.4 **(94.9)**	3.04 ± 0.24 **(89.7)**	5.92 ± 0.49 **(81.6)**
Total	SHF+IF	103 ± 14.0^{ab}	128 ± 19.4^{ab}	127 ± 12.3^{ab}	150 ± 21.1^{a}	157 ± 7.8^{a}	75.1 ± 2.2^{b}	123 ± 31.5	3.39 ± 0.20	7.26 ± 0.50

***p*-Coumaric acid (pCA)** (mg 100 g^{-1} dw) and contribution (%) for the total pCA (**bold**)										
Soluble-free	SF	0.16 ± 0.03^{a} **(1.5)**	0.09 ± 0.00^{a} **(0.6)**	0.15 ± 0.05^{a} **(1.2)**	0.11 ± 0.01^{a} **(0.9)**	0.12 ± 0.01^{a} **(1.1)**	0.12 ± 0.01^{a} **(2.5)**	0.13 ± 0.03 **(1.3)**	0.004 ± 0.00 **(4.3)**	0.06 ± 0.02 **(8.7)**
Soluble-conjugated	SHF-SF	1.84 ± 0.10^{a} **(16.8)**	1.63 ± 0.32^{a} **(11.5)**	1.56 ± 0.63^{a} **(12.1)**	1.46 ± 0.06^{a} **(12.1)**	1.24 ± 0.04^{a} **(10.9)**	1.11 ± 0.04^{a} **(22.8)**	1.48 ± 0.27 **(14.4)**	0.01 ± 0.00 **(7.4)**	0.21 ± 0.02 **(27.9)**
Total soluble	SHF	2.00 ± 0.13^{a} **(18.3)**	1.72 ± 0.32^{a} **(12.1)**	1.72 ± 0.68^{a} **(13.3)**	1.58 ± 0.05^{a} **(13.0)**	1.37 ± 0.04^{a} **(12.0)**	1.23 ± 0.03^{a} **(25.3)**	1.60 ± 0.28 **(15.7)**	0.01 ± 0.00 **(11.7)**	0.27 ± 0.00 **(36.6)**
Insoluble	IF	8.96 ± 2.01^{a} **(81.7)**	12.41 ± 5.80^{a} **(87.8)**	11.2 ± 4.16^{a} **(86.7)**	10.5 ± 1.82^{a} **(87.0)**	10.0 ± 0.72^{a} **(88.0)**	3.63 ± 0.94^{a} **(74.7)**	9.45 ± 3.08 **(84.3)**	0.08 ± 0.01 **(88.2)**	0.47 ± 0.03 **(63.4)**
Total	SHF+IF	10.96 ± 2.1^{a}	14.14 ± 6.1^{a}	12.90 ± 4.8^{a}	12.08 ± 1.8^{a}	11.38 ± 0.8^{a}	4.86 ± 0.97^{a}	11.05 ± 3.24	0.09 ± 0.01	0.74 ± 0.02
Diferuloyl putrescine (DFP) (mg FAE 100 g^{-1} dw)										
DFP	SF	2.61 ± 0.15^{ab}	1.24 ± 0.27^{ac}	2.73 ± 1.02^{ab}	3.37 ± 0.13^{b}	1.95 ± 0.03^{abc}	0.47 ± 0.02^{c}	2.06 ± 1.07	<0.03	<0.03
Non-hydrolysed DFP	SHF	1.76 ± 0.14^{ab}	0.84 ± 0.12^{ac}	1.62 ± 0.38^{ab}	2.74 ± 0.56^{b}	1.36 ± 0.05^{ac}	0.39 ± 0.01^{c}	1.45 ± 0.81	<0.03	<0.03
% Hydrolysed	n/a	32	32	41	19	30	17	28 ± 9	n/a	n/a
Coumaroyl feruloyl putrescine (CFP) (mg FAE 100 g^{-1} dw)										
CFP	SF	1.40 ± 0.15^{a}	0.59 ± 0.02^{bc}	1.11 ± 0.25^{a}	1.56 ± 0.08^{a}	1.08 ± 0.03^{ab}	0.26 ± 0.03^{c}	1.00 ± 0.49	<0.03	<0.03
Non-hydrolysed CFP	SHF	0.76 ± 0.07^{abc}	0.28 ± 0.03^{ad}	0.51 ± 0.18^{abd}	1.20 ± 0.15^{c}	0.87 ± 0.21^{bc}	0.17 ± 0.00^{d}	0.63 ± 0.39	<0.03	<0.03
% Hydrolysed	n/a	46	53	54	23	19	34	38 ± 15	n/a	n/a
Dicoumaroyl spermidine (DCS) (mg pCAE 100 g^{-1} dw)										
DCS	SF	1.01 ± 0.06^{a}	0.54 ± 0.05^{ab}	0.93 ± 0.30^{a}	0.71 ± 0.07^{ab}	0.79 ± 0.03^{a}	0.25 ± 0.03^{b}	0.71 ± 0.28	<0.03	<0.03
Non-hydrolysed DCS	SHF	0.21 ± 0.00^{a}	0.14 ± 0.00^{ab}	0.13 ± 0.01^{ab}	0.29 ± 0.05^{a}	0.22 ± 0.08^{a}	0.04 ± 0.02^{b}	0.17 ± 0.08	<0.03	<0.03
% Hydrolysed	n/a	80	74	86	59	73	83	76 ± 9	n/a	n/a
Dehydrodiferulic acids (DFA) (mg FAE 100 g^{-1} dw)										
8-O-4′-DFA	IF	6.81 ± 0.16^{a}	10.0 ± 5.29^{a}	10.9 ± 4.63^{a}	12.89 ± 3.21^{a}	14.48 ± 0.02^{a}	4.04 ± 0.99^{a}	9.85 ± 3.87	0.14 ± 0.01	0.32 ± 0.02
5-5′-DFA	IF	2.43 ± 0.10^{a}	4.03 ± 2.60^{a}	4.24 ± 2.07^{a}	4.59 ± 1.05^{a}	6.27 ± 0.41^{a}	1.92 ± 0.45^{a}	3.92 ± 1.57	0.04 ± 0.01	0.11 ± 0.00
8-5′-DFA	IF	2.39 ± 0.24^{a}	3.78 ± 1.56^{a}	3.13 ± 1.37^{a}	3.66 ± 1.19^{a}	3.77 ± 0.63^{a}	1.44 ± 0.45^{a}	3.03 ± 0.94	0.07 ± 0.00	0.21 ± 0.04

Table 3.2: Correlation coefficients among maize variables.

Very strong correlations (|*R*| > 0.8) are highlighted in bold. *p*-Value corresponds to the significance level of Pearson correlation coefficient indicated as *: significant at $p < 0.05$; **: significant at $p < 0.01$.

		SF							SHF-SF		IF						
		PCs	**AAs**	**FAf**	**pCAf**	**DFP**	**CFP**	**DCS**	**FAc**	**pCAc**	**PCi**	**AAi**	**FAi**	**pCAi**	**8-O-4′-DFA**	**5-5′-DFA**	**8-5′-DFA**
SF	**PCs**		0.863*	0.996**	0.521	0.715	0.813*	0.776	0.665	0.810	0.137	−0.065	0.146	0.352	0.097	−0.073	0.147
	AAs			0.887*	0.368	0.687	0.716	0.846*	0.403	0.939**	0.375	0.185	0.356	0.731	0.306	0.154	0.460
	FAf				0.494	0.743	0.821*	0.779	0.663	0.841*	0.162	−0.065	0.166	0.404	0.116	−0.066	0.184
	pCAf					0.388	0.409	0.664	−0.136	0.369	−0.275	−0.413	−0.283	−0.168	−0.253	−0.295	−0.420
	DFP						0.966**	0.794	0.354	0.506	0.578	0.219	0.572	0.551	0.562	0.371	0.472
	CFP							0.822*	0.472	0.512	0.556	0.262	0.565	0.492	0.547	0.373	0.447
	DCS								0.096	0.680	0.451	0.254	0.439	0.575	0.434	0.327	0.376
SHF-SF	**FAc**									0.420	−0.004	−0.120	0.029	0.072	−0.044	−0.203	0.080
	pCAc										0.082	−0.102	0.055	0.588	−0.002	−0.159	0.230
IF	**PCi**											0.902*	0.998**	0.794	0.994**	0.950**	0.952**
	AAi												0.912*	0.652	0.909*	0.961**	0.882*
	FAi													0.762	0.996**	0.955**	0.942**
	pCAi														0.729	0.633	0.898*
	8-O-4′-DFA															0.970**	0.918**
	5-5′-DFA																0.866*
	8-5′-DFA																

The hydrolysis of the SF is usually performed to quantitate the total soluble and conjugated major phenolic acids of maize [18,24,25]. In the present work, the AA and PC were also measured in the SHF [***Table 3.1***], in order to evaluate the effect of the hydrolysis procedure on the soluble phenolics and associated AA. The hydrolysis of maize soluble phenolics (from SF to SHF) caused an increase (115 ± 45%) in their AA (t = 16.5, p < 0.001) [***Table B.1, Appendix B***], suggesting that the hydrolysed phenolic compounds exhibited a higher AA. Similarly, the PC increased by around 16 ± 7% (t = 6.96, p < 0.001) after hydrolysis [***Table B.1, Appendix B***], suggesting that this procedure influenced the content of interfering compounds, such as sugars, tyrosine or ascorbic acid, which can influence the PC determined by the Folin-Ciocalteu assay [26]. These findings show the importance of choosing other methods of analysis which can complement the global methods and give more detailed information, enabling the identification and quantitation of individual phenolics. Therefore, HPLC-DAD-ED analyses were performed in all fractions, allowing the quantitation of soluble-free, soluble-conjugated and insoluble ferulic (FA) and *p*-coumaric (pCA) acids and insoluble dehydrodiferulic acids [***Figure 3.1***], as previously described for other cereal-based foods [18,24,25]. Additionally, since free and conjugated phenolics may exhibit different antioxidant activities and health effects [7,10], the major soluble-conjugated hydroxycinnamic acids derivatives (hydroxycinnamic acid amides), which are readily available for absorption after consumption, were also quantitated [***Figure 3.1***]. Moreover, the HPLC-ED analysis allowed the detection of compounds that can have antioxidant potential due to their free radical scavenger ability [27,28]. These results were compared with the AA obtained by the ORAC assay, which evaluates the AA against peroxyl radicals [17].

The results obtained for maize flours [***Table 3.1***] show that the total FA and pCA contents ranged from 75.1 to 157.4 and from 4.86 to 14.14 mg 100 g^{-1} dw, respectively. On average, 94.9% of total FA and 84.3% of total pCA of maize flours were present in their insoluble form. Only around 0.2% of the total FA and 1.3% of total pCA were in their soluble-free forms, while 4.9% of total FA and 14.4% of total pCA were soluble, but conjugated with other compounds (soluble-conjugated) [***Table 3.1***]. These results are also according to the literature [4,18–23,29–31].

FA and pCA are known to have antioxidant characteristics [27,28] and were detected, as expected, by HPLC-ED analysis. Additionally, maize FA content strongly influenced the AA determined by the ORAC assay, since strong positive correlations were found between AA and FA in both SF and IF (R > 0.8, p < 0.05) [***Table 3.2***]. Insoluble FA was responsible for 43.6 ± 4.9% of maize insoluble AA [***Table B.2, Appendix B***]. Contrastingly, only around 0.6 ± 0.1% of soluble-free FA and 0.4 ± 0.1% of soluble-free pCA were responsible for maize soluble AA [***Table B.2, Appendix B***]. Thus, soluble-free phenolics were not the main contributors of maize soluble AA. Instead, hydroxycinnamic acid amides, which include diferuloyl putrescine, coumaroyl feruloyl putrescine and dicoumaroyl spermidine, the major soluble phenolic compounds present in maize flours [***Table 3.1***], can be the main contributors to maize AA.

The *trans*-unsaturated compounds can isomerize into the *cis* form by daylight or during the extraction procedure [4]. As previously reported [11], all the isomeric forms (*cis/cis*, *cis/trans* and *trans/trans*) of diferuloyl putrescine, coumaroyl feruloyl putrescine and dicoumaroyl spermidine were detected in the SF of maize, but the *cis* isomers were only present in trace amounts, below the LOQ of the analytical method (0.03 mg FA/pCA 100 g^{-1} dw). Therefore, in this work, hydroxycinnamic acid amides were quantitated considering the sum of all corresponding isomeric forms which were above the LOQ.

The SHF showed higher ($p < 0.01$) amounts of FA and pCA and lower ($p < 0.01$) amounts of hydroxycinnamic acid amides than the SF [***Table 3.1***], which indicated that FA and pCA were released from hydroxycinnamic acid amides during the hydrolysis procedure of the soluble phenolics [***Figure 3.1***]. The higher amounts in free phenolic acids can also explain the higher AA values obtained for the SHF, suggesting that free FA and pCA are more efficient than hydroxycinnamic acid amides in inhibiting the oxidation induced by peroxyl radicals [17]. Nevertheless, dicoumaroyl spermidine, the most abundant conjugated form of pCA, also contributed for the soluble AA, since very strong and positive correlations were found between the soluble AA with both conjugated pCA (R = 0.939, $p < 0.01$) and dicoumaroyl spermidine (R = 0.846, $p < 0.05$) [***Table 3.2***]. Additionally, the ED analysis showed that diferuloyl putrescine exhibited an antioxidant radical-scavenging activity linked to their hydrogen- or electron-donating ability [27,28]. Indeed, it has been reported that hydroxycinnamic acid amides are also potent antioxidants and that feruloyl derivatives exhibited higher radical scavenging activities linked to their hydrogen- or electron-donating ability than soluble-free FA [7].

Maize flours' SHF still presented considerable amounts of hydroxycinnamic acid amides [***Table 3.1***], since only around 28 ± 9% of diferuloyl putrescine, 38 ± 15% of coumaroyl feruloyl putrescine and 76 ± 9% of dicoumaroyl spermidine were hydrolysed [***Table 3.1***]. As it has been reported [32], hydroxycinnamic acid amides are difficult to extract quantitatively. Therefore, it is possible that the contents of maize soluble-conjugated FA and pCA have been underestimated.

Other abundant phenolic compounds detected in maize were dehydrodiferulic acids, such as 8-O-4′-dehydrodiferulic acid, followed by 5-5′- and 8-5′-dehydrodiferulic acids [***Table 3.1***], which were only detected in maize IF, since they are bound to arabinoxylans [4,5,10]. The most abundant dehydrodiferulic acid (8-O-4′-) was electrochemically active and therefore showed AA linked to their hydrogen- or electron-donating ability. Additionally, all three main dehydrodiferulic acids showed very strong and positive correlations with the insoluble AA determined by the ORAC assay (R > 0.88, $p < 0.05$) and insoluble PC (R > 0.95, $p < 0.01$) [***Table 3.2***], which suggests they can be important contributors for maize AA against peroxyl radicals and PC. It has also been reported that dehydrodiferulic acids show higher radical-scavenging efficacies than FA [33].

Very strong and positive correlations were also found among maize insoluble FA and the three main dehydrodiferulic acids (R > 0.87, $p < 0.05$), between dicoumaroyl spermidine and coumaroyl feruloyl putrescine (R = 0.822, $p < 0.05$) and coumaroyl feruloyl putrescine and diferuloyl putrescine (R = 0.966, $p < 0.01$) [***Table 3.2***]. A possible explanation for these correlations could be the different levels of biotic and abiotic stresses that the plants had been exposed, such as drought or salt stress, which are known to increase the content in phenolic acids [4,34]. However, the commercial maize was the only variety which had been exposed to a different environment, and therefore differences in maize genotypes may be the main responsible for the results observed. Indeed, when considering only the traditional samples (n = 5, data not shown), which had been submitted to the same edaphoclimatic and agronomic conditions, similar correlations were found, namely, between diferuloyl putrescine and coumaroyl feruloyl putrescine (R = 0.927, $p < 0.05$), 5-5′-dehydrodiferulic acid and dicoumaroyl spermidine (R = 0.982, $p < 0.01$), 8-O-4′- and 5-5′-dehydrodiferulic acids (R = 0.966, $p < 0.01$), insoluble FA and 8-O-4′-dehydrodiferulic acid (R = 0.994, $p < 0.01$) and insoluble FA and 5-5′-dehydrodiferulic acid (R = 0.953, $p < 0.05$). Therefore, maize samples with higher hydroxycinnamic acid amides, ferulic acid and dehydrodiferulic acids contents may indicate a higher genetic resistance or tolerance of the variety to biotic and abiotic stresses. Recently, Butts-Wilmsmeyer *et al.* (2020) [35] showed that, in maize, hydroxycinnamic acids contents are quantitative traits and may be influenced by the environment in which the plants have grown. Furthermore, dehydrodiferulic acids and hydroxycinnamic acid amides can both decrease pathogen penetration into plant tissues [4,36].

As expected, [4] when compared to maize, the rye and wheat flours used in *broa* recipes presented lower total PC, total AA and individual phenolics ($p < 0.01$). Moreover, the contribution of the IF for the total PC and total AA was also smaller ($p < 0.01$), with values between 59.6 and 68.1% [***Table 3.1***].

3.2. Phenolic content and antioxidant activity of *broas*

The characterization of *broas* is presented in ***Table 3.3***. Their total PC (159–223 mg GAE 100 g^{-1} dw) was similar to that reported for other maize-based food products, such as tortillas and tortilla chips (60.7–207 mg 100 g^{-1} dw) [18,21]. In particular, the FA and pCA contents obtained for *broas* (81.6 ± 10.1 and 7.4 ± 2.8 mg 100 g^{-1} dw, respectively) were higher than the contents described for rye (54.0 and 2.8 mg 100 g^{-1}) and wheat (8.2 and 0.28 mg 100 g^{-1}) breads [37], possibly due to the higher phenolic contents of maize [4].

In *broas*, the IF was responsible for 71.6 ± 3.0% of total PC and 77.9 ± 1.9% of the total AA [***Table 3.3***]. On average, 95.9% of total FA and 81.3% of total pCA of *broas* were present in their insoluble form. Only around 0.99% and 8.2% of the total FA and pCA, respectively, were in their soluble-free forms, whilst 3.7% of total FA and 10.9% of total pCA were soluble, but conjugated with other compounds [***Table 3.3***].

As for maize flours, hydroxycinnamic acid amides and dehydrodiferulic acids were detected as major phenolic compounds present in *broas* SF and IF, respectively [***Table 3.3***]. Differences in the maize varieties used for *broas* production can explain the strong and positive correlations among the insoluble FA and dehydrodiferulic acids (R > 0.85, $p < 0.05$), as well as between dicoumaroyl spermidine and dehydrodiferulic acids (R > 0.92, $p < 0.05$) [***Table 3.4***].

Contrary to the observations for maize flours, the hydrolysis of *broa* soluble phenolics (SF) [***Figure 3.1***] did not influence its soluble PC ($p > 0.05$) [***Table B.3, Appendix B***], which may indicate the presence of less interfering compounds. Nevertheless, the hydrolysis procedure also caused an average increase of 45 ± 28% in *broas* soluble AA [***Table B.3, Appendix B***] ($t = 6.3$, $p < 0.001$), which was lower than the increase observed for maize flours (115 ± 45%). As previously discussed, this increase can be explained by higher free FA and pCA contents in the SHF. These findings imply that, in *broas*, the amount of FA and pCA released after hydrolysis was lower than in maize flours, due to lower contents in soluble-conjugated phenolics. Indeed, the contribution of compounds other than FA and pCA for the soluble AA of maize flours was around 99.0% [***Table B.2, Appendix B***], while in *broas* it was around 96.9% [***Table B.4, Appendix B***] ($t = 14.4$, $p < 0.01$).

Soluble-free FA and pCA and dicoumaroyl spermidine contents strongly influenced *broas* soluble AA (R > 0.86, $p < 0.05$), while insoluble pCA and 8-5′-dehydrodiferulic acid strongly influenced *broas* insoluble AA (R > 0.81, $p < 0.05$) [***Table 3.3***]. Since hydroxycinnamic acid amides and dehydrodiferulic acids were detected in *broas* as abundant phenolic compounds with antioxidant properties, they may contribute to *broas* health promoting effects. After consumption, dehydrodiferulic acids can be released from the matrix during digestion and further absorbed [38]. To the best of our knowledge, there is no data on hydroxycinnamic acid amides bioavailability, but it is known that other FA conjugates, such as feruloylated oligosaccharides, can be absorbed by a diffusion mechanism [4]. Additionally, some insoluble phenolics may also exhibit their beneficial action directly in the gastrointestinal system [4].

Table 3.3: *Broas* mean values for: phenolic content, antioxidant activity and individual phenolic contents (ferulic acid, *p*-coumaric acid, diferuloyl putrescine, coumaroyl feruloyl putrescine, dicoumaroyl spermidine and dehydrodiferulic acids) obtained in the different fractions, as described in ***Figure 3.1***.

Mean values within rows with no letters in common (a-d) are significantly different ($p < 0.05$).

Description	Phenolic Fraction	Broa-213	Pigarro	Castro Verde	Verdial de Aperrela	Fandango	Commercial	*Average*
Phenolic Content (PC) (mg GAE 100 g^{-1} dw)								
Soluble	SF	56.9 ± 3.0^{a}	53.5 ± 2.7^{a}	53.2 ± 3.7^{a}	58.1 ± 2.3^{a}	59.8 ± 1.2^{a}	52.1 ± 5.6^{a}	55.6 ± 3.1
Soluble-hydrolysed	SHF	55.8 ± 1.7^{a}	62.4 ± 1.9^{ab}	67.8 ± 2.0^{b}	69.9 ± 4.8^{b}	55.6 ± 1.0^{a}	52.1 ± 1.7^{a}	60.6 ± 7.2
Insoluble	IF	149 ± 13.6^{a}	169 ± 4.22^{a}	144 ± 6.50^{a}	138 ± 2.69^{ab}	145 ± 10.5^{a}	107 ± 2.03^{b}	142 ± 20.4
Total	SF+IF	206 ± 16.6^{a}	223 ± 6.92^{a}	198 ± 10.2^{ab}	196 ± 5.06^{ab}	205 ± 11.8^{a}	159 ± 7.60^{b}	197 ± 21.3
% Insoluble	n/a	72.3	76	73.1	70.3	70.7	67.2	71.6 ± 3.0
Antioxidant Activity (AA) (mmol TE 100 g^{-1} dw)								
Soluble	SF	0.99 ± 0.07^{a}	0.92 ± 0.07^{ab}	0.86 ± 0.13^{ab}	0.90 ± 0.18^{ab}	0.79 ± 0.03^{b}	0.54 ± 0.03^{c}	0.83 ± 0.16
Soluble-hydrolysed	SHF	1.22 ± 0.13^{a}	1.28 ± 0.12^{a}	1.39 ± 0.26^{a}	1.23 ± 0.12^{a}	0.92 ± 0.19^{a}	1.04 ± 0.00^{a}	1.18 ± 0.17
Insoluble	IF	3.12 ± 0.70^{a}	3.95 ± 0.15^{a}	2.82 ± 0.53^{ab}	3.20 ± 0.16^{a}	2.94 ± 0.13^{ab}	1.76 ± 0.26^{b}	2.97 ± 0.71
Total	SF+IF	4.11 ± 0.77^{a}	4.87 ± 0.22^{a}	3.68 ± 0.65^{a}	4.10 ± 0.35^{a}	3.73 ± 0.16^{a}	2.30 ± 0.29^{b}	3.80 ± 0.85
% Insoluble	n/a	76	81.2	76.7	78.1	78.9	76.4	77.9 ± 1.9
Ferulic acid (FA) (mg 100 g^{-1} dw) and contribution (%) for the total FA **(bold)**								
Soluble-free	SF	0.91 ± 0.14^{a} **(0.97)**	0.80 ± 0.24^{a} **(0.93)**	0.78 ± 0.00^{a} **(0.94)**	0.81 ± 0.06^{a} **(0.95)**	0.79 ± 0.18^{a} **(1.03)**	0.71 ± 0.11^{a} **(1.10)**	0.80 ± 0.07 **(0.99)**
Soluble-conjugated	SHF-SF	4.31 ± 0.45^{a} **(4.6)**	2.43 ± 0.15^{a} **(2.9)**	3.30 ± 1.09^{a} **(4.0)**	2.87 ± 1.34^{a} **(3.4)**	2.95 ± 0.18^{a} **(3.8)**	2.47 ± 0.42^{a} **(3.8)**	3.05 ± 0.69 **(3.7)**
Total soluble	SHF	5.22 ± 0.31^{a} **(5.3)**	3.23 ± 0.08^{a} **(3.8)**	4.08 ± 1.09^{a} **(4.9)**	3.68 ± 1.40^{a} **(4.4)**	3.87 ± 0.00^{a} **(4.8)**	3.18 ± 0.53^{a} **(4.9)**	3.88 ± 0.75 **(4.7)**
Insoluble	IF	89.3 ± 0.1^{a} **(94.4)**	82.1 ± 15.5^{a} **(96.2)**	79.3 ± 0.59^{a} **(95.1)**	81.8 ± 0.63^{a} **(95.7)**	75.9 ± 4.18^{a} **(95.2)**	61.4 ± 18.2^{a} **(95.1)**	78.27 ± 9.4 **(95.9)**
Total	SHF+IF	94.5 ± 2.62^{a}	85.3 ± 15.38^{a}	83.3 ± 0.51^{a}	85.5 ± 0.78^{a}	79.8 ± 1.95^{a}	64.5 ± 17.69^{a}	81.62 ± 10.06
***p*-Coumaric acid (pCA)** (mg 100 g^{-1} dw) and contribution (%) for the total pCA **(bold)**								
Soluble-free	SF	0.66 ± 0.03^{a} **(7.4)**	0.57 ± 0.09^{a} **(5.3)**	0.63 ± 0.01^{a} **(6.9)**	0.54 ± 0.03^{a} **(7.6)**	0.53 ± 0.17^{a} **(10.4)**	0.39 ± 0.03^{a} **(11.7)**	0.55 ± 0.09 **(8.2)**
Soluble-conjugated	SHF-SF	1.06 ± 0.09^{a} **(11.8)**	0.53 ± 0.00^{a} **(4.9)**	0.82 ± 0.33^{a} **(9.0)**	0.59 ± 0.34^{a} **(8.4)**	0.43 ± 0.23^{a} **(8.5)**	0.78 ± 0.10^{a} **(23.1)**	0.70 ± 0.23 **(10.9)**
Total soluble	SHF	1.72 ± 0.06^{a} **(19.2)**	1.10 ± 0.09^{a} **(10.2)**	1.45 ± 0.34^{a} **(15.9)**	1.13 ± 0.31^{a} **(16.0)**	1.08 ± 0.20^{a} **(18.9)**	1.17 ± 0.13^{a} **(34.8)**	1.28 ± 0.26 **(19.2)**

Insoluble	IF	7.30 ± 0.1^{a} **(80.8)**	9.71 ± 1.4^{a} **(89.8)**	7.66 ± 0.6^{ab} **(84.1)**	5.92 ± 0.0^{bc} **(83.9)**	4.23 ± 0.3^{cd} **(81.1)**	2.20 ± 0.48^{d} **(65.2)**	6.17 ± 2.67 **(81.3)**
Total	SHF+IF	9.03 ± 0.23^{ab}	10.80 ± 1.34^{a}	9.11 ± 0.28^{ab}	7.05 ± 0.33^{b}	5.31 ± 0.16^{bc}	3.36 ± 0.36^{c}	7.40 ± 2.79
Diferuloyl putrescine (DFP) (mg FAE 100 g^{-1} dw)								
DFP	SF	1.76 ± 0.07^{a}	1.14 ± 0.31^{a}	1.70 ± 0.15^{a}	2.52 ± 0.05^{b}	1.34 ± 0.15^{a}	0.38 ± 0.05^{c}	1.47 ± 0.71
Non-hydrolysed DFP	SHF	1.72 ± 0.05^{ab}	0.75 ± 0.08^{c}	1.56 ± 0.17^{ab}	1.87 ± 0.46^{a}	0.99 ± 0.01^{bc}	0.33 ± 0.02^{c}	1.20 ± 0.61
% Hydrolysed	n/a	3	35	8	26	26	14	19 ± 12
Coumaroyl feruloyl putrescine (CFP) (mg FAE 100 g^{-1} dw)								
CFP	SF	0.82 ± 0.06^{ab}	0.48 ± 0.15^{cd}	0.66 ± 0.02^{bc}	0.98 ± 0.05^{b}	0.52 ± 0.08^{bc}	0.20 ± 0.04^{d}	0.61 ± 0.27
Non-hydrolysed CFP	SHF	0.64 ± 0.003^{a}	0.34 ± 0.03^{bc}	0.59 ± 0.07^{ab}	0.73 ± 0.14^{a}	0.45 ± 0.08^{ab}	0.15 ± 0.01^{c}	0.48 ± 0.21
% Hydrolysed	n/a	22	29	11	25	13	27	21 ± 7
Dicoumaroyl spermidine (DCS) (mg pCAE 100 g^{-1} dw)								
DCS	SF	0.71 ± 0.04^{a}	0.51 ± 0.10^{ab}	0.64 ± 0.04^{ab}	0.45 ± 0.04^{b}	0.48 ± 0.07^{ab}	0.15 ± 0.05^{c}	0.49 ± 0.19
Non-hydrolysed DCS	SHF	0.10 ± 0.04^{a}	0.07 ± 0.002^{a}	0.09 ± 0.004^{a}	0.09 ± 0.01^{a}	0.10 ± 0.02^{a}	0.05 ± 0.0001^{a}	0.08 ± 0.02
% Hydrolysed	n/a	86	87	86	80	79	67	81 ± 7
Dehydrodiferulic acids (DFA) (mg FAE 100 g^{-1} dw)								
8-O-4′-DFA	IF	8.42 ± 0.08^{a}	6.88 ± 1.09^{a}	7.62 ± 0.73^{a}	6.72 ± 1.14^{a}	6.09 ± 0.05^{a}	3.04 ± 0.20^{b}	6.46 ± 1.86
5-5′-DFA	IF	3.27 ± 0.06^{a}	2.01 ± 0.84^{ab}	3.03 ± 0.34^{a}	2.60 ± 0.45^{ab}	2.55 ± 0.03^{ab}	1.29 ± 0.11^{b}	2.46 ± 0.72
8-5′-DFA	IF	2.90 ± 0.12^{a}	2.72 ± 0.59^{a}	2.84 ± 0.34^{a}	2.60 ± 0.25^{a}	2.43 ± 0.09^{a}	1.01 ± 0.11^{b}	2.42 ± 0.71

Table 3.4: Correlation coefficients among *broas* variables.

Very strong correlations (|*R*| > 0.8) are highlighted in bold. *p*-Value corresponds to the significance level of Pearson correlation coefficient indicated as *: significant at $p < 0.05$; **: significant at $p < 0.01$.

		SF							SHF-SF		IF						
		PCs	**AAs**	**FAf**	**pCAf**	**DFP**	**CFP**	**DCS**	**FAc**	**pCAc**	**PCi**	**AAi**	**FAi**	**pCAi**	**8-O-4′-DFA**	**5-5′-DFA**	**8-5′-DFA**
SF	**PCs**		0.397	0.492	0.249	0.579	0.566	0.314	0.327	−0.323	0.225	0.291	0.431	−0.103	0.349	0.493	0.405
	AAs			0.864*	0.908*	0.744	0.795	0.894*	0.566	0.141	0.830*	0.847*	0.993**	0.832*	0.959**	0.794	0.957**
	FAf				0.818*	0.595	0.719	0.822*	0.826*	0.426	0.576	0.571	0.915*	0.545	0.845*	0.792	0.756
	pCAf					0.620	0.678	0.995**	0.737	0.362	0.736	0.642	0.918**	0.783	0.986**	0.910*	0.940**
	DFP						0.981**	0.608	0.437	0.025	0.369	0.493	0.735	0.400	0.715	0.750	0.734
	CFP							0.663	0.564	0.173	0.377	0.494	0.802	0.426	0.763	0.792	0.746
	DCS								0.753	0.330	0.734	0.627	0.908*	0.742	0.981**	0.926**	0.939**
SHF-SF	**FAc**									0.738	0.183	0.084	0.649	0.231	0.693	0.836*	0.531
	pCAc										−0.217	−0.299	0.215	0.071	0.267	0.367	0.045
IF	**PCi**											0.955**	0.789	0.897*	0.770	0.483	0.850*
	AAi												0.797	0.867*	0.718	0.407	0.813*
	FAi													0.783	0.962**	0.826*	0.940**
	pCAi														0.793	0.481	0.820*
	8-O-4′-DFA															0.909*	0.974**
	5-5′-DFA																0.851*
	8-5′-DFA																

3.3. Comparison of traditional and commercial maize flours and *broas*

In order to evaluate the main differences among maize samples varieties and corresponding *broas*, a principal component analysis (PCA) was performed using 16 variables, described in ***Tables 3.2*** and ***3.4***. ***Figure 3.2*** shows the projection of samples and variables in the space defined by the two principal components, corresponding to 82.2% of the total variance. Three sample clusters can be identified: (i) traditional maize flours, (ii) traditional *broas* and (iii) commercial maize flour and the corresponding *broa*, which are projected along different directions.

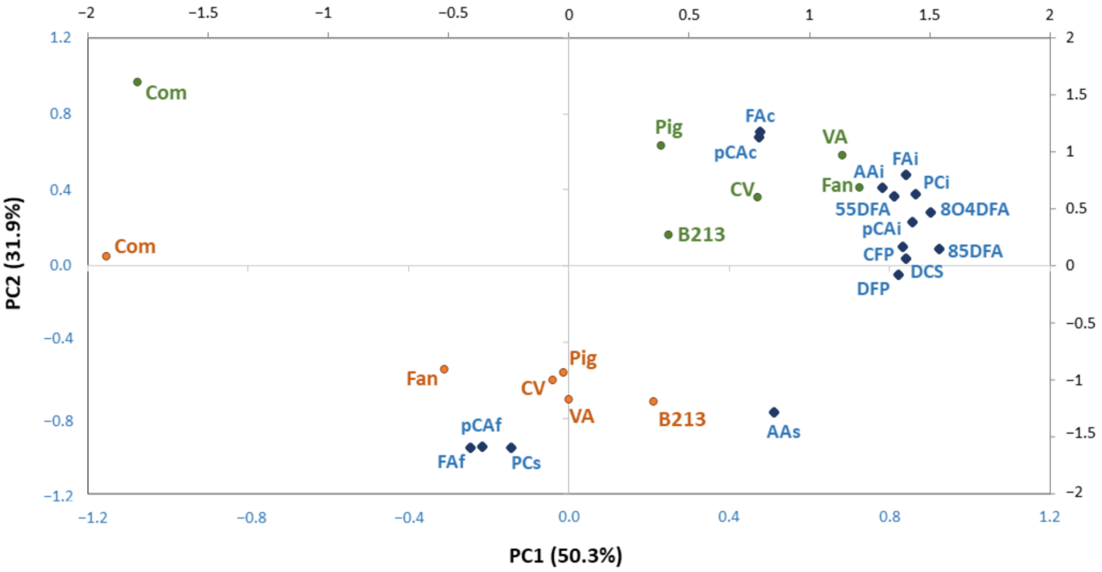

Figure 3.2: Projection of maize flours (green), *broas* (brown) and variables (blue) in the plane defined by PC1 and PC2, corresponding to 82.2% of total variance.

The commercial maize flour and corresponding *broa* were differentiated from all the other samples along PC1, mainly due to the lower contents of insoluble phenolics and hydroxycinnamic acid amides, as described in ***Tables 3.1 and 2B.3***. Indeed, post hoc analysis revealed that the contents in hydroxycinnamic acid amides, 8-O-4′- and 8-5′-dehydrodiferulic acids of the commercial *broa* were significantly lower ($p < 0.05$) than all the traditional *broas* [***Table 3.3***]. Similar results were observed for its soluble and total AA [***Table 3.3***]. These differences can be explained by the different genotype and variety type, since it was a hybrid, whereas the traditional samples were open-pollinated varieties [25]; and also by its different edaphoclimatic and agronomic growing conditions [20,23]. Moreover, as previously reported, the commercial maize flour used in the present work presented a higher mean diameter and large particle distribution than the traditional flours [2]. This could have influenced not only the extraction of maize phenolic compounds, but also the amount of phenolic compounds released during the breadmaking process [13] and, consequently, the corresponding *broa* AA. As previously reported [2], a sensory evaluation study has demonstrated a preference for traditional in detriment of hybrid maize varieties for *broa* production, suggesting that, even without previous knowledge, consumers prefer *broas* with higher contents in these health promoting compounds.

Broas were discriminated from maize flours along PC2, mainly due to their higher contents of soluble PC and AA, as well as soluble-free FA and pCA, and by lower contents of soluble-conjugated FA and pCA. These results suggest that the content of soluble-free phenolics increased as a consequence of maize processing to *broas*, as discussed below.

3.4. From raw flours to *broas*

All *broas* (n = 6) were prepared following the same recipe, which included not only maize flour, but also 20% rye and 10% wheat flours. Therefore, the values obtained for the raw flours mixture (RF) that was used for the preparation of *broas* were calculated [***Table B.5, Appendix B***] according to the expression:

$$RF = 0.70 \times M + 0.20 \times R + 0.10 \times W,$$

where M, R and W are the values presented in ***Table 3.1*** for the different varieties of maize (n = 6) and for the rye and wheat flours, respectively. Results from raw flours and *broas* were compared based on the formula:

$$100 \times B/RF,$$

which enabled the determination of the amount of AA, PC and individual phenolics remaining after raw flour (RF) processing to *broas* (B) [***Table 3.5***].

A paired-samples *t*-test was performed to compare the AA and phenolic composition of raw flours and the corresponding *broas*. The results presented in ***Table 3.5*** show that the soluble PC and AA significantly increased in *broas*, possibly due to the increase (≥ threefold) in the soluble-free FA and pCA contents. Similar results have been obtained for other maize products, particularly for tortillas, tortilla chips and cornflakes [1,18,20,31], as well as for other breads [25,39]. Some authors argued that the increase in soluble-free phenolics might be caused by the release of insoluble FA and pCA that occurs during breadmaking, especially during the fermentation process [13,18,24,25,39,40]. Therefore, a decrease in the insoluble phenolic content should be expected after processing. However, no significant differences ($p > 0.05$) were found concerning insoluble PC, AA, FA, pCA or dehydrodiferulic acids contents [***Table 3.5***]. Different hypotheses can explain these results. Firstly, the amount of FA and pCA released during the processing corresponded only to 0.6–1.1% and 4.6–9.9% of the total FA and pCA contents originally present in the raw flours, corroborating the results described for rye processing to bread [39]. Secondly, a considerable amount of insoluble phenolics can remain linked to cell walls even after hydrolysis [4]. Ultimately, the increase in soluble-free phenolics might have been caused by the hydrolysis of soluble-conjugated phenolic compounds, in particular hydroxycinnamic acid amides [25]. Indeed, after processing, the soluble-conjugated FA and pCA contents decreased by around 27 and 44%, respectively ($p < 0.05$) [***Table 3.5***].

Table 3.5: Amount (%) of phenolic content (**PC**), antioxidant activity (**AA**), ferulic acid (**FA**), *p*-coumaric acid (**pCA**), hydroxycinnamic acid amides (**HCAAs**) and dehydrodiferulic acids (**DFAs**) remaining after raw flour (70% maize + 20% rye + 10% wheat) processing to *broas*.

		Fraction	Samples						Average
			B213	**Pig**	**CV**	**VA**	**Fan**	**Com**	
PC	Soluble	SF	153	181	176	176	201	198	181 ± 17**
	Insoluble	IF	127	115	97	83	83	120	104 ± 19
	Total	SF+IF	133	126	110	99	100	137	118 ± 17*
AA	Soluble	SF	139	147	138	149	140	128	140 ± 7**
	Insoluble	IF	123	116	95	99	71	80	97 ± 20
	Total	SF+IF	127	120	103	107	79	88	104 ± 18
FA	Soluble-free	SF	298	350	333	305	353	380	337 ± 31**
	Soluble-conjugated	SHF-SF	90	57	96	59	76	62	73 ± 17*
	Total soluble	SHF	109	75	119	75	100	80	93 ± 19
	Insoluble	IF	130	94	91	81	70	123	98 ± 24
	Total	SHF+IF	128	93	92	80	69	119	97 ± 23
pCA	Soluble-free	SF	528	741	525	589	524	395	550 ± 113**
	Soluble-conjugated	SHF-SF	80	45	72	56	47	95	66 ± 20*
	Total Soluble	SHF	130	93	127	106	59	143	110 ± 31
	Insoluble	IF	115	110	97	79	60	83	91 ± 21
	Total	SHF+IF	115	108	99	82	59	95	93 ± 20
HCAAs	DFP	SF	96	131	89	107	98	117	106 ± 15
	CFP	SF	84	116	85	90	69	111	92 ± 18
	DCS	SF	100	137	98	91	87	86	100 ± 19
DFAs	8-O-4′-DFA	IF	177	98	100	74	60	110	103 ± 41
	8-5′-DFA	IF	192	71	102	81	58	97	100 ± 48
	5-5′-DFA	IF	173	103	130	102	92	104	117 ± 30

Since the soluble-conjugated FA and pCA contents decreased after processing, a decrease in the content of the main soluble-conjugated phenolics (hydroxycinnamic acid amides) would be expected. However, no differences were found between raw flours and *broas* regarding their content in diferuloyl putrescine, coumaroyl feruloyl putrescine or dicoumaroyl spermidine [***Table 3.5***]. This suggests that insoluble hydroxycinnamic acid amides, recently described in maize flours and *broas* [11], may have been released during processing, therefore increasing the content of soluble hydroxycinnamic acid amides and soluble-free FA and pCA. Additionally, other minor soluble-conjugated compounds, namely, hydroxycinnamic acid amides monoconjugates, may have been hydrolysed during processing, contributing to the higher soluble-free and lower soluble-conjugated FA and pCA contents detected in *broas*.

A comparison of traditional raw flours and *broas'* main phenolic compounds is presented in ***Figure 3.3***. No significant differences ($p > 0.05$) were found between raw flours and *broas* regarding their total FA, pCA, hydroxycinnamic acid amides and dehydrodiferulic acids contents [***Table 3.5***]. Contradictory results have been reported regarding the processing effects on the phenolic composition of other cereals, such as wheat and rye, to breads. Some authors concluded that the processing did not influence the content in the major phenolics, including FA and pCA [37]. On the other hand, some authors reported that the content of total phenolics and total FA, in particular, decreased after processing [39], while others have shown that it increased [25,41]. These discrepancies can be explained by differences in sample genotypes [13,25,39] and breadmaking processing conditions, namely, in dough pH, which influences the mechanical disaggregation of cell walls and the acidic hydrolysis that may occur during mixing [13,25,39]. No differences have been found between the dehydrodiferulic acids contents of rye flours and breads [25,39], which is in accordance to the present findings. To the best of our knowledge, hydroxycinnamic acid amides have not been considered in previous studies.

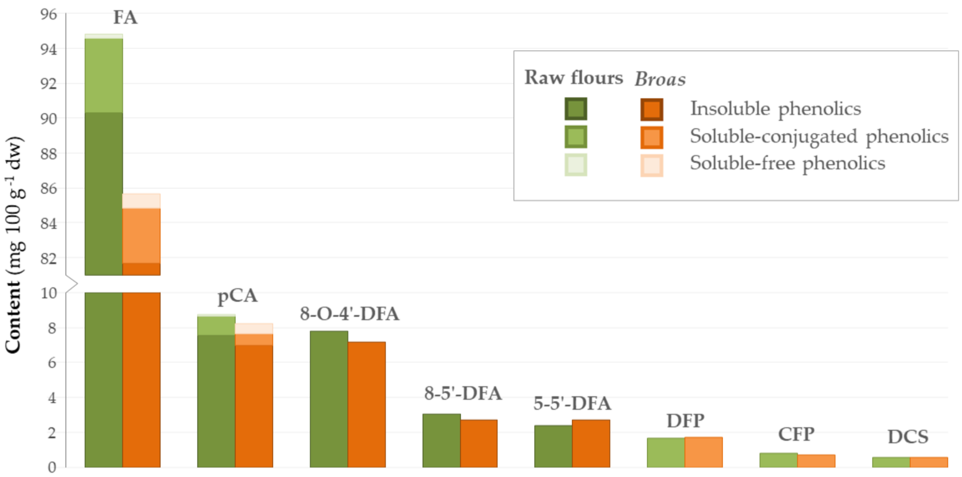

Figure 3.3: Average contents of phenolic compounds obtained for the traditional raw flours (green, n = 5) and *broas* (brown, n = 5).

On the other hand, after maize grain processing into tortillas, there is a 56–90% reduction in the total PC, mainly due to leaching of insoluble FA during the nixtamalization process [18,21,42]. The content of phenolic acids also decreased after processing of maize into toasted cornflakes due to the pressure-cooking stage and to the dry milling process, which involved the removal of maize bran and germ [31]. Therefore, the results obtained in the present work suggest that, in comparison to other maize-based foods, *broas* are an interesting source of phenolic compounds, since there were no significant losses in the total phenolics caused by the processing conditions, which were based on a traditional *broa* recipe.

4. Conclusions

Broa, a Portuguese traditional maize bread, can be considered an interesting source of antioxidant compounds, particularly hydroxycinnamic acids and hydroxycinnamic acid amides. *Broas* produced with Portuguese traditional open-pollinated maize varieties, which were, in a previous work, associated with better sensory characteristics, also showed higher phenolic content than the *broa* prepared with a commercial maize flour. The processing conditions used in *broa* preparation did not significantly change the phenolic content present in the raw flours used for its production. There was an increase in soluble-free phenolics after processing, suggesting that phenolic compounds' bioaccessibility was improved in *broas*. *In vitro* studies are currently being carried out to understand which compounds are bioavailable and may contribute to considering *broa* as a bread with health-promoting properties.

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References

1. Singh, N.; Singh, S.; Shevkani, K. Maize: Composition, bioactive constituents, and unleavened bread. In *Flour and Breads and Their Fortification in Health and Disease Prevention*; Elsevier: Amsterdam, The Netherlands, **2019**; pp. 111–121.
2. Carbas, B.; Patto, M.C.V.; Bronze, M.R.; Bento-Da-Silva, A.; Trigo, M.J.; Brites, C. Maize Flour Parameters That Are Related to the Consumer Perceived Quality of 'Broa' Specialty Bread. *Food Sci. Technol.* **2016**, *36*, 259–267.
3. Angelino, D.; Cossu, M.; Marti, A.; Zanoletti, M.; Chiavaroli, L.; Brighenti, F.; Del Rio, D.; Martini, D. Bioaccessibility and Bioavailability of Phenolic Compounds in Bread: A Review. *Food Funct.* **2017**, *8*, 2368–2393.
4. Bento-Silva, A.; Patto, M.C.V.; Bronze, M.R. Relevance, Structure and Analysis of Ferulic Acid in Maize Cell Walls. *Food Chem.* **2017**, *246*, 360–378.
5. Salinas-Moreno, Y.; García-Salinas, C.; Ramírez-Díaz, J.L.; Alemán-de la Torre, I. Phenolic Compounds in Maize Grains and Its Nixtamalized Products. In *Phenolic Compounds—Natural Sources, Importance and Applications*; Intech: London, UK, **2017**.
6. Buitimea-Cantúa, N.E.; Gutiérrez-Uribe, J.A.; Serna-Saldivar, S.O. Phenolic–Protein Interactions: Effects on Food Properties and Health Benefits. *J. Med. Food* **2018**, *21*, 188–198.
7. Choi, S.W.; Lee, S.K.; Kim, E.O.; Oh, J.H.; Yoon, K.S.; Parris, N.; Hicks, K.B.; Moreau, R.A. Antioxidant and Antimelanogenic Activities of Polyamine Conjugates from Corn Bran and Related Hydroxycinnamic Acids. *J. Agric. Food Chem.* **2007**, *55*, 3920–3925.

8. Collison, A.; Yang, L.; Dykes, L.; Murray, S.; Awika, J.M. Influence of Genetic Background on Anthocyanin and Copigment Composition and Behavior during Thermoalkaline Processing of Maize. *J. Agric. Food Chem.* **2015**, *63*, 5528–5538.
9. Xiang, J.; Zhang, M.; Apea-Bah, F.B.; Beta, T. Hydroxycinnamic Acid Amide (HCAA) Derivatives, Flavonoid C-Glycosides, Phenolic Acids and Antioxidant Properties of Foxtail Millet. *Food Chem.* **2019**, *295*, 214–223.
10. Gutiérrez-Grijalva, E.P.; Ambriz-Pére, D.L.; Leyva-López, N.; Castillo-López, R.I.; Heredia, J.B. Dietary Phenolic Compounds, Health Benefits and Bioaccessibility. *Arch. Latinoam. Nutr.* **2016**, *66*, 87–100.
11. Bento-Silva, A.; Duarte, N.; Mecha, E.; Belo, M.; Patto, M.C.V.; Bronze, M.R. Hydroxycinnamic Acids and Their Derivatives in Broa, a Traditional Ethnic Maize Bread. *Foods* **2020**, *9*, 1471.
12. Ribas-Agustí, A.; Martín-Belloso, O.; Soliva-Fortuny, R.; Elez-Martínez, P. Food Processing Strategies to Enhance Phenolic Compounds Bioaccessibility and Bioavailability in Plant-Based Foods. *Crit. Rev. Food Sci. Nutr.* **2018**, *58*, 2531–2548.
13. Wang, T.; He, F.; Chen, G. Improving Bioaccessibility and Bioavailability of Phenolic Compounds in Cereal Grains through Processing Technologies: A Concise Review. *J. Funct. Foods* **2014**, *7*, 101–111.
14. Brites, C.M.; Trigo, M.J.; Carrapiço, B.; Alviña, M.; Bessa, R.J. Maize and Resistant Starch Enriched Breads Reduce Postprandial Glycemic Responses in Rats. *Nutr. Res.* **2011**, *31*, 302–308.
15. Mussi de Mira, N.V.; Cerdeira Barros, R.M.; Schiocchet, M.A.; Noldin, J.A.; Lanfer-Marquez, U.M. Extração, Análise e Distribuição Dos Ácidos Fenólicos Em Genótipos Pigmentados e Não Pigmentados de Arroz (Oryza sativa L.). *Cienc. Technol.* **2008**, *28*, 994–1002.
16. Dobberstein, D.; Bunzel, M. Separation and Detection of Cell Wall-Bound Ferulic Acid Dehydrodimers and Dehydrotrimers in Cereals and Other Plant Materials by Reversed Phase High-Performance Liquid Chromatography with Ultraviolet Detection. *J. Agric. Food Chem.* **2010**, *58*, 8927–8935.
17. Serra, A.T.; Matias, A.A.; Frade, R.F.M.; Duarte, R.O.; Feliciano, R.P.; Bronze, M.R.; Figueira, M.E.; de Carvalho, A.; Duarte, C.M.M. Characterization of Traditional and Exotic Apple Varieties from Portugal. Part 2–Antioxidant and Antiproliferative Activities. *J. Funct. Foods* **2010**, *2*, 46–53.
18. De la Parra, C.; Serna Saldivar, S.O.; Liu, R.H. Effect of Processing on the Phytochemical Profiles and Antioxidant Activity of Corn for Production of Masa, Tortillas, and Tortilla Chips. *J. Agric. Food Chem.* **2007**, *55*, 4177–4183.
19. Lopez-Martinez, L.X.; Oliart-Ros, R.M.; Valerio-Alfaro, G.; Lee, C.-H.; Parkin, K.L.; Garcia, H.S. Antioxidant Activity, Phenolic Compounds and Anthocyanins Content of Eighteen Strains of Mexican Maize. LWT—*Food Sci. Technol.* **2009**, *42*, 1187–1192.
20. Mora-Rochin, S.; Gutiérrez-Uribe, J.A.; Serna-Saldivar, S.O.; Sánchez-Peña, P.; Reyes-Moreno, C.; Milán-Carrillo, J. Phenolic Content and Antioxidant Activity of Tortillas Produced from Pigmented Maize Processed by Conventional Nixtamalization or Extrusion Cooking. *J. Cereal Sci.* **2010**, *52*, 502–508.
21. Aguayo-Rojas, J.; Mora-Rochín, S.; Cuevas-Rodríguez, E.O.; Serna-Saldivar, S.O.; Gutierrez-Uribe, J.A.; Reyes-Moreno, C.; Milán-Carrillo, J. Phytochemicals and Antioxidant Capacity of Tortillas Obtained after Lime-Cooking Extrusion Process of Whole Pigmented Mexican Maize. *Plant Foods Hum. Nutr.* **2012**, *67*, 178–185.
22. Gutiérrez-Uribe, J.A.; Rojas-García, C.; García-Lara, S.; Serna-Saldivar, S.O. Phytochemical Analysis of Wastewater (*Nejayote*) Obtained after Lime-Cooking of Different Types of Maize Kernels Processed into Masa for Tortillas. *J. Cereal Sci.* **2010**, *52*, 410–416.
23. Urias-Lugo, D.A.; Heredia, J.B.; Serna-Saldivar, S.O.; Muy-Rangel, M.D.; Valdez-Torres, J.B. Total Phenolics, Total Anthocyanins and Antioxidant Capacity of Native and Elite Blue Maize Hybrids (*Zea mays* L.). *CyTA—J. Food* **2015**, *13*, 336–339.

24. Antognoni, F.; Mandrioli, R.; Bordoni, A.; Di Nunzio, M.; Viadel, B.; Gallego, E.; Villalba, M.P.; Tomás-Cobos, L.; Taneyo Saa, D.L.; Gianotti, A. Integrated Evaluation of the Potential Health Benefits of Einkorn-Based Breads. *Nutrients* **2017**, *9*, 1232.

25. Dynkowska,W.M.; Cyran, M.R.; Naczk, A. Soluble and CellWall-bound Phenolic Acids and Ferulic Acid Dehydrodimers in Rye Flour and Five Bread Model Systems: Insight into Mechanisms of Improved Availability. *J. Sci. Food Agric.* **2015**, *95*, 1103–1115.

26. Bastola, K.P.; Guragain, Y.N.; Bhadriraju, V.; Vadlani, P.V. Evaluation of Standards and Interfering Compounds in the Determination of Phenolics by Folin-Ciocalteu Assay Method for Effective Bioprocessing of Biomass. *Am. J. Anal. Chem.* **2017**, *8*, 416.

27. Teixeira, J.; Gaspar, A.; Garrido, E.M.; Garrido, J.; Borges, F. Hydroxycinnamic Acid Antioxidants: An Electrochemical Overview. *Biomed. Res. Int*. **2013**, *2013*.

28. Oliveira-Neto, J.R.; Rezende, S.G.; de Fátima Reis, C.; Benjamin, S.R.; Rocha, M.L.; de Souza Gil, E. Electrochemical Behavior and Determination of Major Phenolic Antioxidants in Selected Coffee Samples. *Food Chem.* **2016**, *190*, 506–512.

29. Das, A.K.; Singh, V. Antioxidative Free and Bound Phenolic Constituents in Pericarp, Germ and Endosperm of Indian Dent (*Zea mays* Var. *Indentata*) and Flint (*Zea mays* Var. *Indurata*) Maize. *J. Funct. Foods* **2015**, *13*, 363–374.

30. Ndolo, V.U.; Beta, T. Comparative Studies on Composition and Distribution of Phenolic Acids in Cereal Grain Botanical Fractions. *Cereal Chem.* **2014**, 91, 522–530.

31. Butts-Wilmsmeyer, C.J.; Mumm, R.H.; Rausch, K.D.; Kandhola, G.; Yana, N.A.; Happ, M.M.; Ostezan, A.;Wasmund, M.; Bohn, M.O. Changes in Phenolic Acid Content in Maize during Food Product Processing. *J. Agric. Food Chem.* **2018**, *66*, 3378–3385.

32. Moreau, R.A.; Nuñez, A.; Singh, V. Diferuloylputrescine and P-Coumaroyl-Feruloylputrescine, Abundant Polyamine Conjugates in Lipid Extracts of Maize Kernels. *Lipids* **2001**, *36*, 839–844.

33. Jia, Y.; He, Y.; Lu, F. The Structure-Antioxidant Activity Relationship of Dehydrodiferulates. *Food Chem*. **2018**, *269*, 480–485.

34. Gupta, K.; Dey, A.; Gupta, B. Plant Polyamines in Abiotic Stress Responses. *Acta Physiol. Plant.* **2013**, *35*, 2015–2036.

35. Butts-Wilmsmeyer, C.J.; Mumm, R.H.; Bohn, M.O. Quantitative Genetic Analysis of Hydroxycinnamic Acids in Maize (*Zea mays* L.) for Plant Improvement and Production of Health-Promoting Compounds. *J. Agric. Food Chem.* **2020**, *68*, 9585–9593.

36. Pal, M.; Janda, T. Role of Polyamine Metabolism in Plant Pathogen Interactions. *J. Plant Sci. Phytopathol.* **2017**, *1*, 95–100.

37. Mattila, P.; Pihlava, J.; Hellström, J. Contents of Phenolic Acids, Alkyl- and Alkenylresorcinols, and Avenanthramides in Commercial Grain Products. *J. Agric. Food Chem.* **2005**, *53*, 8290–8295.

38. Andreasen, M.F.; Kroon, P.A.; Williamson, G.; Garcia-Conesa, M.-T. Intestinal Release and Uptake of Phenolic Antioxidant Diferulic Acids. *Free Radic. Biol. Med.* **2001**, *31*, 304–314.

39. Hansen, H.B.; Andreasen, M.; Nielsen, M.; Larsen, L.; Knudsen, B.K.; Meyer, A.; Christensen, L. Changes in Dietary Fibre, Phenolic Acids and Activity of Endogenous Enzymes during Rye Bread-Making. *Eur. Food Res. Technol.* **2002**, *214*, 33–42.

40. Ragaee, S.; Seetharaman, K.; Abdel-Aal, E.-S.M. The Impact of Milling and Thermal Processing on Phenolic Compounds in Cereal Grains. *Crit. Rev. Food Sci. Nutr*. **2014**, *54*, 837–849.

41. Gelinas, P.; McKinnon, C.M. Effect of Wheat Variety, Farming Site, and Bread-baking on Total Phenolics. *Int. J. Food Sci. Technol*. **2006**, *41*, 329–332.

42. Del Pozo-Insfran, D.; Brenes, C.H.; Saldivar, S.O.S.; Talcott, S.T. Polyphenolic and Antioxidant Content of White and Blue Corn (*Zea mays* L.) Products. *Food Res. Int.* **2006**, *39*, 696–703.

CHAPTER 4

Shedding light on the volatile composition of **broa***, a traditional Portuguese maize bread*[1]

Abstract

In Portugal, maize has been used for centuries to produce an ethnic bread called *broa*, employing traditional maize varieties, which are preferred by the consumers in detriment of commercial hybrids. In order to evaluate the maize volatiles that can influence consumers' acceptance of *broas*, twelve *broas* were prepared from twelve maize varieties (eleven traditional and one commercial hybrid), following a traditional recipe. All maize flours and *broas* were analysed by HS-SPME-GC-MS (headspace solid-phase microextraction) and *broas* were appraised by a consumer sensory panel. In addition, the major soluble phenolics and total carotenoids contents were quantitated in order to evaluate their influence as precursors or inhibitors of volatile compounds. Results showed that the major volatiles detected in maize flours and *broas* were aldehydes and alcohols, derived from lipid oxidation, and some ketones derived from carotenoids' oxidation. Both lipid and carotenoids' oxidation reactions appeared to be inhibited by soluble phenolics. In contrast, phenolic compounds appeared to increase browning reactions during bread making and, consequently, the production of pyranones. Traditional samples, especially those with higher contents in pyranones and lower contents in aldehydes, were preferred by the consumer sensory panel. These findings suggest that, without awareness, consumers prefer *broas* prepared from traditional maize flours with higher contents in health-promoting phenolic compounds, reinforcing the importance of preserving these valuable genetic resources.

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[1] **This chapter is based on the following publication:**
Bento-Silva A, Duarte N, Belo M, Mecha E, Carbas B, Brites C, Vaz Patto MC, Bronze MR. Shedding light on the volatile composition of *broa*, a traditional Portuguese maize bread. *Biomolecules*. **2021**. *11*, 1396.
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1. Introduction

The culture of maize in Portugal started during the sixteenth century and rapidly spread across the country. For centuries, natural and human selection adapted varieties to different environments, creating a diverse maize germplasm [1]. Portuguese maize traditional open pollinated varieties (OPVs) are commonly used for the production of *broa*, a traditional Portuguese maize bread [2], which plays an important economic and social role in Central and Northern Portuguese rural communities, where it is still widely consumed [1]. This type of bread is traditionally prepared with maize flour (50–100%) and rye and/or wheat flours (0–50%) that are mixed with hot water, and leavened dough from late *broa*, acting as sourdough [2,3]. After preparation, dough is baked in a wood-fired oven [3]. Due to the progressive adoption of more productive hybrids, Portuguese traditional maize varieties are at risk of disappearing [1]. Nevertheless, traditional varieties have persisted due to their better technological capacity and aroma characteristics highly valued for the production of *broa*, in addition to their better resilience to pests, diseases and abiotic stresses, qualities exploited by the Portuguese long-term participatory maize breeding VASO program [4]. In fact, previously reported sensory evaluation results of *broas* have demonstrated a preference for traditional or participatory improved OPVs in detriment of commercial hybrid maize varieties for *broa* production [3]. Additionally, traditional maize varieties still under production have an important role in the conservation and evolution of the available genetic maize variation, meeting the future demands for increasing yields and quality in high-stress environments in the context of climate changes [4].

Quality criteria important to consumers' acceptance of breads are related to both their rheological (texture) and organoleptic (colour, volume and flavour) properties [3,5,6]. Flavour is often considered the most important attribute [7–9] and results, in part, from the perception of volatile compounds that can interact with olfactory receptors [6]. The volatile composition of breads depends on the ingredients and processing techniques employed, and mainly results from oxidation of lipids and carotenoids, fermentation by yeast and lactic acid bacteria (LAB) and browning reactions (Maillard and caramelization reactions) during baking [10,11]. Aldehydes, alcohols, ketones, esters, acids, pyrazines, pyrrolines, hydrocarbons, furans and lactones [12] have been described as the main volatiles present in cereal breads, but the exact origin of each volatile is difficult to determine [13]. For instance, aldehydes, alcohols and furans may all be derived from lipid oxidation, yeast fermentation or Maillard and caramelization reactions [14–16].

In general, lipid oxidation products are associated with off-flavours [17,18] and are produced by plants through the action of lipoxygenases in response to wounding, thus playing an important role in plants' defence strategies and signalling [7,19]. Flavour development during bread making is influenced by lipoxygenase activity [11] and by the type of sugars and amino acids present in the flour [19]. Lipoxygenases act on unsaturated fatty acids and produce unstable peroxide derivatives that can be transformed into carbonyl compounds, such as aldehydes [11,16], which can be enzymatically reduced to the corresponding alcohols [7] or be oxidized into acids and esters [16,17,20]. 4- or 5-Hydroxy carboxylic acids may further be converted to lactones [7,17], and hydrocarbons can be formed through the oxidation of the radical to the carbonium ion and decarboxylation [21]. The activity of endogenous lipoxygenases increases during grinding of the grain, after the addition of water to the flour and during kneading [15,16]. Additionally, lipid oxidation reactions may also occur by the action of enzymes associated with the metabolic activity of yeasts and LAB during fermentation [6].

Apart from lipid oxidation, aldehydes can also be formed inside the yeast cells, by the degradation of flour amino acids via the Ehrlich pathway, and originate the corresponding alcohols, acids and esters [16,17,20]. Different volatile compounds can be formed, depending on the LAB and yeasts present in the dough [11,16,22,23]. Ketones, such as geranylacetone, can be originated from carotenoids' oxidation, which have been described in maize baked products and other cereal breads [11,24].

In addition to colour development, browning reactions produce compounds that contribute to the flavour of bakery products [5]. In particular, Maillard products are formed through a reaction between amino acids and reducing sugars, leading to the formation of brown pigments (melanoidins), and a large number of volatile compounds [5,6,20]. This process consists of mainly three stages: (i) sugar degradation, where compounds such as furans, pyrones, furfurals, furanones and pyranones are formed, followed by (ii) amino acids' degradation (Strecker reaction), which generates mainly aldehydes, acids and alcohols, and (iii) further interactions, originating coloured melanoidins and other volatiles, such as pyrroles, pyridines and pyrazines [25]. These volatiles are considered important for the overall aroma profile of breads, as they generally have low odour thresholds and contribute to the desirable aroma properties [11,17,19,20,26]. Caramelization reactions are originated through sugars' degradation in the absence of amino acids and provide compounds especially related to caramel flavour [5,19]. Low levels of volatiles from nonenzymatic browning reactions and high levels of lipid oxidation products contribute to the formation of off-flavours in breads [18].

Some studies have reported that variations in wheat flour odour directly affect bread flavour [23,27]. Moreover, phenolic compounds may also contribute to the volatile composition of foods, influencing both the Maillard and lipid oxidation reactions [28–31]. In particular, the presence of ferulic acid in whole wheat breads has been reported as the main reason for the difference in the aroma of breads prepared from whole and refined wheat flours [28]. As ferulic acid is particularly abundant in maize, where it can be at least 10 times higher than in other grains [32], it is expected that the volatile composition of *broas* may be especially affected by their phenolic composition. Additionally, these compounds exhibit antioxidant properties, contributing to the prevention of non-communicable diseases [33] that are generally regarded as a major public health concern. Several studies have been published on the identification of key volatiles responsible for odour quality in maize-based foods, such as popcorn and cornflakes [24,34–37]. However, to the best of our knowledge, the volatile composition of maize-based sourdough breads has never been studied. The main objectives of this work were to: (i) characterize the volatile composition of *broas* in order to identify volatiles that may influence consumer's choice, and to (ii) shed light on the characteristics of traditional maize varieties responsible for their better suitability for *broas* production. In particular, the influence of (iia) soluble phenolic compounds, (iib) total carotenoids content and (iic) volatiles from maize flours on *broas* volatile composition was evaluated. This knowledge is important to both the baking industry and maize breeders, as cereals' composition contribution to odour can possibly become a future quality parameter in breeding [27].

2. Materials and methods

2.1. Cereal flours and *broas* samples

Twelve traditional maize flours (F1 to F12), one commercial maize flour, and the corresponding *broas* (B1 to B12) were studied. The commercial wheat and rye flours used in the preparation of *broas* were also studied. All cereal samples and *broas* are described in more detail in ***Section 7, Chapter 1***.

2.2. HS-SPME-GC-MS analysis

2.2.1. Materials and equipment

The different SPME fibres used (PDMS, PDMS/DVB, PA and DVB/CAR/PDMS) were purchased from Supelco (Sigma-Aldrich, St. Louis, MO, USA). The alkane standard mixture C_8-C_{20} solution (40 mg L^{-1} in hexane) was purchased from Fluka (Fisher Scientific, Hampton, NH, USA). Ultra-pure water (18.2 MΩ.cm^{-1}) was obtained from a Millipore-Direct equipment Q3 UV system (Millipore, Burlington, MA, USA). GC vials were sealed with a silicone/teflon-lined septum and screw cap (La-Pha-Pack, Langerwehe, Germany).

The analysis of volatile compounds was carried out using a GCMS-QP2010 Plus mass spectrometer (Shimadzu, Kyoto, Japan) and an AOC-5000 autosampler (Shimadzu, Kyoto, Japan). The compounds were separated using a Varian Factor Four DB-5MS column (30.0 m × 0.25 mm × 0.25 m film thicknesses, Agilent J&W, Santa Clara, CA, USA).

2.2.2. HS-SPME optimization of cereal flours

The commercial maize flour was used to optimize the HS-SPME extraction of volatile compounds from cereal flours. Different ratios of flour:water were studied (addition of 0, 2, 5, 8 and 10 mL of water to 2 g of flour), as well as different types of fibres (DVB/CAR/PDMS 50/30 m, PDMS 100 m, PDMS/DVB 65 m and PA 85 m), temperatures (40, 50, 60 and 70 °C) and times (10, 20, 30, 40, 50 and 60 min) of extraction, in triplicate. Additionally, sample stability in the autosampler was evaluated for 32 h. The selected conditions were also applied to the commercial wheat and rye flours.

2.2.3. HS-SPME of volatile compounds of cereal flours and *broas*

After optimization, the following conditions were used for the volatile analysis of cereal flours (maize, wheat and rye): flour (2 g) was placed in a 20 mL GC-vial, deionized water (5 mL) was added and the mixture was vortexed for 2 min before HS-SPME-GC-MS analysis. For *broas*, 4 g of sample (whole, crumb and crust) was smashed manually and placed in a 20 mL vial. A 50/30 m DVB/CARBOXEN/PDMS fibre was selected to concentrate the volatile compounds. Samples were extracted for 40 min at 60 °C and 250 rpm. Before extraction, the fibre was conditioned in the GC injector at 270 °C for 30 min, in order to remove any contaminants. Fibre blanks analyses were performed using the same SPME and chromatographic conditions. Samples were prepared in duplicate.

2.2.4. GC-MS analysis of volatile compounds of cereal flours and *broas*

The extracted volatiles were injected in the GC column in split mode (ratio 1:2). The oven temperature was kept at 35 °C for 5 min, followed by an increase of 5 °C min^{-1} to a final temperature of 230 °C, which was kept for 5 min (total run time of 48 min).

The column carrier gas was helium at a constant flow rate of 2.1 mL min^{-1}. The mass spectrometer was operated in the electron impact mode (EI) at 70 eV, scanning from *m/z* 30 to 300, at a scan rate of 555 scan s^{-1}, and the ion source temperature was set at 250 °C. Desorption time was 5 min at 260 °C (injector temperature). As previously reported [40], the identification of 11 of the most ubiquitous volatiles from maize flours was confirmed by the analysis of the respective commercial reference standards and by an additional analysis of maize flours and standards on a Sapiens-Wax.ms column (60 m × 0.2 mm × 0.25 μm) (Teknokroma, Barcelona, Spain).

2.3. Characterization of the phenolic composition and total carotenoids

Phenolic compounds of maize flours and *broas* were extracted with 50% aqueous ethanol, and the major soluble phenolic compounds (ferulic acid, *p*-coumaric acid, diferuloyl putrescine, *p*-coumaroyl feruloyl putrescine, dicoumaroyl spermidine and *bis*-diferuloyl putrescine) were quantitated by HPLC-DAD (high-performance liquid chromatography coupled to a diode array detector), following the procedures previously described [38,41].

The total carotenoids content was spectrophotometrically measured at 450 nm according to the AACC method 14-60.01 (AACC International, 2012). Results were expressed in micrograms of lutein equivalent per gram of sample, as the main carotenoid found in maize, as previously described [42].

2.4. Sensory analysis

A sensory evaluation ('appearance', 'colour', 'smell and odour', 'taste and aroma', 'texture' and 'global appreciation') of *broas* was conducted using a hedonic quantitative response scale (International Organization for Standardization, 2003) in a test room (International Organization for Standardization, 2007) with a consumer panel of 52 assessors.

The test was performed using a numeric category scale from 1 (extremely unpleasant) to 8 (extremely pleasant) for all the attributes. Further details on this hedonic test have been described elsewhere [43].

2.5. Data analysis

The software LabSolutions GCMS solution Release 2.53SU1 (Shimadzu, Kyoto, Japan) was used to analyse the data and to calculate the areas of the peak chromatograms. The identification of volatile compounds was performed by comparison of the mass spectra obtained for each compound with the mass spectra from the software library (NIST 27 and WILEY 229 and 147). The linear retention index (LRI) was calculated for each compound using the retention times of a homologous series of *n*-alkanes (C_8-C_{20}) injected under the same chromatographic conditions, and results were compared with data from the literature. A positive identification was considered when the experimental spectra shared at least an 80% similarity with spectra from the software libraries and the LRI deviation was less than 1%, when comparing to the LRI reported in the literature.

The software SPSS® version 21 was used to: (i) evaluate the differences among the results obtained during the optimization of the experimental conditions (ANOVA and Student's *t* test), and to (ii) measure the degree of association between *broas* volatile compounds and (iia) maize flours' volatiles, (iib) maize flours and *broas'* phenolic compounds, (iic) maize flours and *broas'* total carotenoids content and (iid) scores obtained in *broas* sensory analysis. A non-parametric test (Spearman correlation) was used to measure the degree of association between variables, since they did not meet the assumptions for a parametric analysis (normal distribution and homoscedasticity).

In order to identify the main differences among the twelve maize and *broas*, and to study their interdependent relations, a hierarchical cluster analysis was performed using *R* software. Since this analysis requires a number of variables lower than the number of samples, it was necessary to decrease the number of variables. Therefore, after variables' standardization, a PCA (principal component analysis) was performed, in order to group the variables according to their correlations. The number of principal components considered was based on the Kaiser criteria and a cluster analysis was then performed. The distance was measured by the Euclidean distance, and Ward's method was used to form the clusters, allowing the formation of clusters with the lowest relative standard deviations among the groups. The optimal number of clusters was estimated using two different methods, namely the elbow point and silhouette methods. A statistical significance test (*v.test*) was performed in order to identify the variables which significantly contributed to clusters' differentiation.

3. Results and discussion

3.1. HS-SPME-GC-MS optimization

The analysis of volatile components was performed by headspace solid-phase microextraction (HS-SPME) followed by gas chromatography coupled to mass spectrometry (GC-MS). The commercial maize flour was used to optimize the conditions of analysis in order to achieve a higher extraction efficiency of the volatiles. The total chromatogram areas were measured, focusing on the aldehydes' content, since they have been described as mainly responsible for the aroma of cereal flours [44]. Different types of SPME fibres were tested and a DVB/CAR/PDMS fibre coating was selected, as higher peak areas were obtained (data not shown). These results are in accordance with previous studies on cereals' volatile compounds [8,44]. Different ratios between water and flour were tested, and the highest improvement in SPME efficiency was observed when 5 mL of water was added to 2 g of flour. Different sample temperatures were tested, and the best results were obtained at 70 °C. However, the variation among the triplicates was higher [***Figure C.1, Appendix C***], probably due to changes in the sample composition, namely to the increase in lipid oxidation and Maillard reactions [44]. Thus, the temperature of 60 °C was chosen for further analyses. Different extraction times were evaluated, and results show an increase in peak areas with higher exposure times [***Figure C.2, Appendix C***], possibly due to the increase of the activity of endogenous lipoxygenase after the addition of water [16]; however, these differences were not significant ($p > 0.5$). Taking into account the time required for exposure of the fibre and chromatographic analysis, a time of exposure of 40 min was selected. The repeatability of the whole procedure (n = 6) was determined, and the relative standard deviation (RSD) was 17%, similar to values previously reported [44].

The stability of the maize flours in the autosampler was analysed for 32 h. After 20 h, compounds such as dodecanol, hexadecanoic acid and ethyl caprylate were detected in the chromatograms and tended to increase, probably due to the metabolism of bacteria and fungi [45–48]. Therefore, samples remained for a maximum of 18 h in the autosampler, in order to avoid significant changes in their volatile composition.

3.2. Identification of volatile compounds in maize flour and *broas*

All twelve maize flours and corresponding *broas* were analysed using the optimized conditions. Illustrative chromatographic profiles of a maize flour (F1), corresponding *broa* (B1) and wheat and rye flours are presented in ***Figure 4.1***. Forty-four compounds were identified in maize flours and eighty-seven in *broas* by comparison of their mass spectra with those from the software library and LRI from the literature. Their characteristic flavours, LRI, similarity indexes and retention times (RTs) are described in ***Table 4.1***.

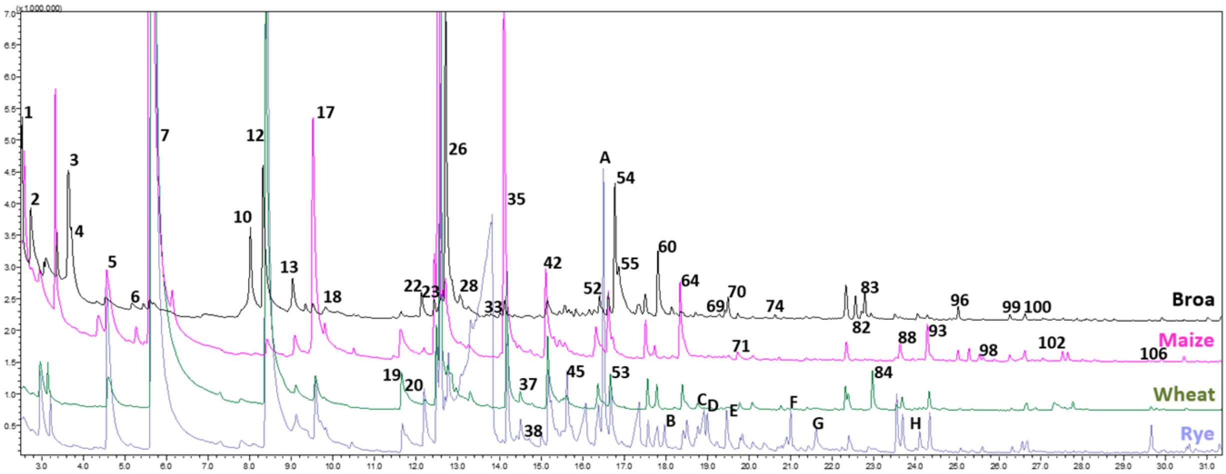

Figure 4.1: Chromatographic profiles of *broa* and maize, wheat and rye flours.

Peaks are numbered according to ***Table 4.1*** and letters represent compounds detected exclusively in rye and/or wheat flours, namely, **A**: α-terpinolene, **B**: camphor, **C**: octanoic acid, **D**: 4-terpineol, **E**: α-terpineol, **F**: linalyl acetate, **G**: nonanoic acid, **H**: 2(3H)-furanone-5-heptyldihydro.

Table 4.1: Identification of detected compounds in maize flours (**F**) and *broas* (**B**).

RT: retention time; **min**: minutes; **SI**: similarity index; **LRI**: linear retention index; **n/i**; not identified; **TI**: tentatively identified; **n/a**: not applicable; **n/f**: not found. ***Important volatile compounds described in wheat and rye breads, popcorn or extruded maize products [8,17,20,24,26,35].

Peak	B/F	RT (min)	Compound	SI (%)	LRI	LRI (literature)	Odour Description
1	B	2.56	Acetic acid*	94	-	600 (DB-5) [49]	Sour [20,49], acid, pungent [20]
2	B	3.22	Acetoin (3-hydroxy-2-butanone)	95	-	718 (DB-5) [49]	Butterscotch, buttery, yogurt, creamy [20]
3	B	3.74	Isopentanol	97	-	736 (DB-5) [49]	Balsamic, alcoholic [17,20], malty [20]
4	B	3.82	2-Methyl-1-butanol	90	-	755 (DB-5) [49]	Wine, onion [49], malty [20]
5	B	4.65	1-Pentanol	97	-	769 (DB-5MS) [50]	Fruity [20,49], balsamic, fusel-like, sweet [20]
	F			96	-		
6	B	5.56	Octane	97	809	800 (DB-5) [49]	Alkane [20,49]
	F			94	809		
7	B	5.70	Hexanal*	97	813	819 (DB-5) [51]	Green, grassy, tallowy [20,51], fruity, acorn-like, fishy, herbal, leafy [51]
	F			96	813		
8	B	6.87	Methylpyrazine*	97	840	828 (DB-5) [49]	Popcorn [49], roasted, burnt, sweet [20]
9	B	6.94	Furfural* (2-furancarboxaldehyde)	97	843	830 (DB-5) [51]	Almond, sweet [20,51], woody, fruity, flowery [51], bread-like, soil, burnt roasted, toasted [20]
10	B	8.29	Furfuryl alcohol (2-furanmethanol)	96	865	866 (HP-5) [51]	Caramel-like [25,51], weak, fermented, burnt sugar, creamy [51], sweet, fruity [25], burnt, warm oil, mild [20]
11	B	8.29	n/i	96	865	n/a	n/a
12	B	8.44	1-Hexanol	96	879	869 (DB-5 MS) [50]	Flowery [20,49], resin, green [49], green grass, woody, mild, sweet [20]
	F			96	879		
13	B	9.11	2-*n*-Butylfuran	94	895	893 (DB-5) [50]	Green [20]
14	B	9.16	2-Heptanone	91	897	895 (DB-5) [49]	Soapy [20,49], fruity, cinnamon [20]
	F			96	897		
15	B	9.45	Nonane	87	904	900 (DB-5) [49]	Alkane [49], fusel-like [51]
16	B	9.54	4-Heptenal (*Z*)*	90	906	895 (DB-5) [51]	Biscuit-like, sweet [20,51], boiled potato, creamy [51], putrid [20]
17	F	9.59	Heptanal*	97	909	900 (DB-5) [51]	Citrus, fatty, rancid [20,51], green, dry fish, pesticide, solvent, smoky, fruity [51], malty [20]
	B			97	909		

18	B	9.90	2-Acetylfuran [1-(2-Furanyl)ethanone]	95	915	910 (DB-5) [51]	Sweet [25,51], balsamic-cinnamic note, cereal [51], caramel-like, fruity [25], smoky, roasted [20]
19	B F	11.72	2-Heptenal (*Z*)	94 94	963 963	964 (DB-5) [51]	Sour, green, vegetable, fresh, fatty [10]
20	B F	11.82	Benzaldehyde*	83 95	967 967	961 (DB-5) [51]	Almond, caramel [20]
21	B	11.87	5-Methylfurfural*	82	966	978 (DB-5) [49]	Almond [20,49], caramel, burnt sugar [49], sweet, bitter [20]
22	B F	12.24	1-Heptanol	95 95	977 977	969 (DB-5) [50]	Green [20,52], woody, heavy, oily, fresh, light green, nutty [52]
23	F B	12.51	1-Octen-3-ol*	94 95	985 985	980 (DB-5) [51]	Mushroom [20], garlic, spicy, rubbery, carrots, herbaceous, dirty, dust, earthy [51]
24	F	12.67	6-Methyl-5-hepten-2-one	85	988	985 (DB-5) [51]	Mushroom, earthy, vinyl, rubbery, woody, blackcurrant, boiled fruit [51]
25	B	12.74	2-Methyl-3-octanone	90	990	985 (DB-5 MS) [50]	n/f
26	F B	12.81	2-Pentylfuran*	93 94	993 993	991 (BPX-5) or 992 (HP-5 MS) [51]	Buttery, green bean [20,51], floral, fruity [15,20], mushroom, raw nuts [20]
27	B	12.99	Hexanoic acid	88	997	1019 (DB-5)	Sweaty, cheesy, goat-like [20,51], pungent, rancid [51], fatty [20]
28	B	13.16	Decane	85	1001	1000 (DB-5) [49]	Alkane [49]
29	B	13.20	Ethyl hexanoate*	85	1003	1000 (DB-5) [50]	Apple peel, fruity [20]
30	B F	13.35	Octanal*	91 91	1007 1007	1004 (DB-5) [51]	Citrus, flowery [20,51], lemon, stew-like, boiled meat-like, rancid, soapy, green, fruity, orange [51]
31	F	13.63	2,4-Heptadienal (*E*,*E*)	87	1017	1003 (DB-5) [51]	Orange oil, oily, fatty, rancid [51]
32	B	13.69	1-Hexyl acetate	90	1017	1008 (DB-5) [51]	Fruity, spicy, herbal [20,51], sweet wine, rubbery, tobacco, acidulous, citrus, green [51]
33	B F	14.12	Limonene	87 93	1030 1030	1031 (DB-5) [51]	Citrus [20,51], licorice, green, ethereal, fruity [51]
34	B	14.12	n/i	87	1030	n/a	n/a
35	F B	14.20	3-Ethyl-2-methyl-1,3-hexadiene	89 89	1033 1033	1030 (VF-5 MS) [50]	n/f
36	B	14.52	Benzyl alcohol* (benzenemethanol)	81	1040	1039 (DB-5) [49]	Sweet, flowery [49], pleasant aromatic [20]

37	B	14.56	3-Octen-2-one	87	1043	1040 (DB-5) [49]	Nut, crushed bug [49], earthy type [20]
38	F B	14.68	2-Phenylacetaldehyde*	85 91	1045 1047	1043 (DB-5) [51]	Green [25,26], floral, hyacinths [25], metallic [26], honey-like, sweet [20]
39	B	14.72	n/i (3 compounds)	91	1047	n/a	n/a
40	F	14.77	Isooctanol (TI)	84	1049	1051 (DB-1) [51]	Fatty, orange, rose [51]
41	B	15.08	ϒ-*N*-caprolactone [5-ethyldihydro-2(3H)-furanone]	86	1058	1056 (HP-5MS) [51]	Coumarin-like, sweet [51]
42	B F	15.22	2-Octenal (*E*)*	96 96	1062 1062	1056 (DB-5) [51]	Fatty, nutty [20,51], burdock-like, sweet, sour, waxy, green, burnt, mushroom [51], roasted [20]
43	B	15.49	2-Acetylpyrrole	91	1069	1060 (DB-5), 1072 (DB-5) [51]	Herbal, nutty, anisic, sweet [51], roasted, biscuits [20]
44	F B	15.51	2-Octen-1-ol (*E*)	92 91	1069 1071	1048 (DB-1) [49], 1064 (DB-5 Interpolated)	Green, vegetable-like [20]
45	B F	15.67	1-Octanol	91 83	1075 1075	1072 (DB-5) [53]	Sharp, fatty, waxy, citrus [52], earthy, moldy vegetable [20]
46	B	15.83	2,4-Dimethyl-1-decene (TI)	84	1080	n/f	n/f
47	B	15.94	Methylpentylfuran (TI)	87	1083	n/f	n/f
48	F	16.21	2-Nonanone	89	1091	1090 (DB-5) [49]	Hot milk, soap, green [49]
49	F	16.31	6-Nonenal (*Z*)	89	1094	1101 (DB-5) [51]	Cucumber, green, melon, waxy [52]
50	F	16.35	Pantolactone [dihydro-3-hydroxy-4,4-dimethyl-2(3H)-furanone] (TI)	90	1095	990 (HP-1) [51]	Cotton candy [49], licorice, smoky, toasted bread [54]
51	B	16.39	3,5-Octadien-2-one (E,E) (TI)	79	1096	1068 (DB-5) [51]	Fresh, sweet, woody, mushroom [51]
52	B	16.51	Undecane	94	1100	1099 (DB-5) [51]	Fusel-like [51]
53	F B	16.70	Nonanal*	96 96	1107 1107	1103(DB-5), 1104(DB-5), 1108 (DB-5) [51]	Citrus, soapy [20,51], gravy, green, tallowy, fruity, gas, chlorine, floral, waxy, sweet, melon, fatty, lavender [51]
54	B	16.93	Maltol (3-hydroxy-2-methyl-4H-pyran-4-one)	95	1113	1108 (DB-5) [51]	Caramel-like [51], warm-fruity, caramelized/sweet [20]
55	B	16.98	Phenylethyl alcohol* (2-phenylethanol)	95	1115	1118 (DB-5) [49]	Rose, honey [20,49], spice, lilac [49], wilted rose [20]
56	B	16.98	n/i	95	1115	n/a	n/a
57	B	17.32	3,7-Dimethyldecane	86	1126	1127 (HP-5 MS) [50]	n/f

58	B	17.42	2,7-Dimethyl-1-octanol (TI)	84	1129	1625 (DB-Wax) [50]	n/f
59	B	17.62	2-Methoxy-2,3,3-trimethylbutane (TI)	74	1136	n/f	n/f
60	B	17.94	3-Hydroxy-2,3-dihydromaltol (2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one)	90	1144	1134 (DB-5) [51]	Caramelized [20]
61	B	17.94	n/i (2 compounds)	90	1144	n/a	n/a
62	B	18.24	3-Nonen-1-ol (*Z*)*	94	1155	1134 (DB-1) [51], 1152 (DB-5 interpolated)	Sweet, green [51], waxy [20]
63	B	18.33	1-Chloro-octadecane	80	1159	1320 (Carbowax) [51]	Sweet, green [51], waxy [20]
64	B	18.45	2-Nonenal (*E*)*	96	1163	1162 (DB-5) [51]	Green, fatty, tallowy [20,26,51], cucumber-like [20,51], soapy, floral, sweet, wet, earthy, plastic [51], paper [20]
	F			96	1163		
65	B	18.45	n/i (2 compounds)	96	1163	n/a	n/a
66	B	18.69	3-Methylundecane	93	1170	1169 (DB-5) [50]	n/f
67	B	18.82	1-Nonanol	84	1174	1174 (DB-5 MS) [51]	Citrus [20]
	F			91	1174		
68	B	18.97	1-Furfurylpyrrole	81	1179	1133 (DB-1) [51], 1166 (DB-5 interpolated)	Roasted, chocolate, green [51], vegetable [20]
69	B	19.53	Ethyl octanoate*	92	1198	1195 (DB-5) [51]	Sweet, soapy, fresh, fruity [20,51], fatty, floral, green leafy, menthol, anise, baked-fruity [51]
70	B	19.54	Dodecane	94	1200	1199 (DB-5) [51]	Fusel-like [51]
	F			94	1197		
71	F	19.80	Decanal*	96	1205	1209 (DB-5) [49]	Stewed, burnt, green, waxy, orange skin-like, floral, lemon, fatty, herbaceous, soapy [51], citrus [20]
	B			95	1208		
72	B	19.96	2,6-Dimethylundecane	91	1212	1210 (DB-5 MS) [50]	n/f
73	B	20.24	2,3-Dihydrobenzofuran	88	1222	1226 (DB-5) [50]	Musky notes [55]
74	B	20.73	2-Hexyl-1-octanol (TI)	90	1239	2162 (DB-Wax) [50]	n/f
75	B	20.80	n/i	87	1242	n/a	n/a
76	B	20.93	n/i	82	1246	n/a	n/a
77	B	21.47	n/i (ketone)	84	1265	n/a	n/a

78	B	22.02	2-Methyldodecane (TI)		1284	n/a	n/a
79	B	22.35	Indole (2,3-benzopyrrole)	93	1295	1288 (DB-5) [51]	Sweet, burnt, floral, jasmine, earthy [51], animal [20]
80	B	22.43	n/i	88	1299	n/a	n/a
81	B	22.66	n/i	92	1307	n/a	n/a
82	B	22.80	*p*-Vinylguaiacol* (2-methoxy-4-vinylphenol)	94	1312	1313 (DB-5) [51]	Clove [49,51], curry [49], phenolic, smokey [51]
83	B	22.89	n/i	89	1316	n/a	n/a
84	B	23.02	2,4-Decadienal (E,E)*	91	1321	1319 (DB-5) [51]	Fatty [49,51,52], fried, wax [49,51], citrus [51,52], meaty, pungent, green [51]
85	B	23.36	n/i	87	1333	n/a	n/a
86	B	23.49	n/i	90	1338	n/a	n/a
87	B	23.60	n/i	82	1342	n/a	n/a
88	F	23.68	n/i	88	1345	n/a	n/a
89	B	23.73	n/i	82	1347	n/a	n/a
90	B F	23.83	5-Methyltridecane	95 82	1352 1349	1355 (DB-5) [50]	n/f
91	B	24.16	γ-Nonalactone* [5-pentyl-dihydro-2(3H)-furanone]	93	1363	1360 (DB-5MS) [50]	Coconut [20,26,49], peach [49], sweet, fruity [20]
92	B F	24.25	2-Undecenal	87 83	1366 1363	1365 (DB-5) [51]	Fruity [20,51], geranium, metallic, pungent, sweet, green, fatty [51]
93	F	24.31	n/i	87	1369	n/a	n/a
94	F	24.38	2,6,11-Trimethyldodecane	91	1371	1375 (DB-5) [50]	n/f
	B	24.39	2,7,10-Trimethyldodecane		1372		
95	B F	24.93	1-Tetradecene	93 94	1392 1392	1392 (DB-5) [50,51]	n/f
96	F B	25.10	Tetradecane	96 97	1397 1400	1399 (DB-5) [51]	Mild herbaceous, sweet, fusel-like [51]
97	F	25.34	Dodecanal	86	1408	1408 (DB-5) [51]	Oily, herbal, fatty, citrus, waxy [51]
98	B F	25.74	α-Ionone		1423 1423	1426 (DB-5 [51]	Floral, violet, woody, fruity [51]

99	B	26,37	Geranylacetone	93	1448	1448 (DB-5) [51]	Fresh, floral, rosy-green, fruity odour [51]
	F			89	1448		
100	B	26,96	n/i	91	1471	n/a	n/a
101	F	26,98	Dodecanol	90	1473	1470 (DB-5) [50]	Waxy-type [20]
102	F	27,65	Pentadecane	93	1499	1500 (DB-5) [51]	Mild green, fusel-like [51]
	B			92	1499		
103	F	28,79	n/i	90	1547	n/a	n/a
104	B	28,88	2-Butyl-1-octanol (TI)	91	1550	1277 (DB-5) [50]	n/f
105	F	29,25	n/i	90	1566	n/a	n/a
106	F	29,94	Hexadecane	95	1595	1600 (DB-5) [51]	Fusel-like, fruity, sweet [51]

Aldehydes were the most abundant volatiles detected in maize flours, corresponding to 30% of the total chromatogram area, which is in accordance with results previously reported [35,40,42]. Alcohols (21%), hydrocarbons (21%) ketones (11%), terpenes (<1%) and lipid-derived furans (<1%) were also among the most important families of compounds. It was not possible to confirm the identification of seven compounds, but putative identifications were performed. Peak 49 presents a spectrum that is very similar to 6-nonenal (*Z*) and 5-undecene (*Z*), having identical similarity indexes. The presence of the ion at *m/z* 29, also present in other aldehydes, suggests that this compound is 6-nonenal. In turn, the mass spectrum of peak 50 suggests a structure corresponding to pantolactone (dihydro-3-hydroxy-4,4-dimethyl-2(3H)-furanone), with a molecular ion at *m/z* 130 and three major fragments at *m/z* 43, 57 and 71. However, it was not possible to confirm the identification of this compound, since the LRI values found in the literature were not similar (>1% deviation) to the LRI obtained in the present work. The chromatographic profiles suggested that several different compounds could be co-eluting. For peak 99, two compounds can be proposed: geranylacetone and nerylacetone, with close similarity indexes. This peak was tentatively identified as geranylacetone, since it has been widely described in several maize-based products [24,35,36].

The families of compounds identified in *broas* were mainly aldehydes (19% of total chromatogram area), alcohols (16%) and hydrocarbons (14%). Ketones (7%), furans (5%), pyrans (3%), lactones (3%), esters (3%), acids (3%) and pyrazines (1%) were also present. All these compounds have been described in other baked cereal products [11,20]. As in maize flour, it was not possible to confirm the identification of some compounds due to the absence of LRI bibliographic values for a DB-5 column, but tentative identifications were made. Peak 29 showed a mass spectrum with a base peak at *m/z* 88, and other fragments at *m/z* 43, 60, 70, 99 and 115. A structure corresponding to ethyl hexanoate, with a similarity index of 85%, was suggested. This compound has been reported in baked cereal products [11]. Peak 35 presented a spectrum similar to that of 3-ethyl-2-methyl-1,3-hexadiene, with a molecular ion at *m/z* 124, a base peak at *m/z* 67 and fragments at *m/z* 39, 41, 55, 95 and 109. This compound has been described in the crust of whole-meal wheat bread and other maize-based foods [14,56]. A tentative identification for peak 47 was methylpentylfuran, with a base peak at *m/z* 95; however, it was not possible to confirm this identification since no LRI value was described in the literature. For peak 51, two compounds were suggested: 3,5-octadien-2-one (*E,E*) and 1-octyn-3-ol, as they presented similar spectra. However, the compound 3,5-octadien-2-one was selected, due to the presence of the base peak at *m/z* 95 and the molecular ion at *m/z* 124. Compound 74 was tentatively identified as 2-hexyl-1-octanol, due to the high-spectrum similarity index (90%), and it has been described in other cereals, such as rice [57,58].

Since rye and wheat flours were also included in the recipe of *broa*, these samples were analysed in the same conditions as maize flours. The chromatographic profiles corresponding to these flours are presented in ***Figure 4.1***. Compounds such as acetoin (peak 2), hexanoic acid (27), 3-octen-2-one (37) and 2,4-decadienal (*E,E*) (84) were detected in *broas* and rye and wheat flours, but not in maize flours. Other compounds, such as 2(3H)-furanone-5-heptyldihydro (peak H) and linalyl acetate (peak F), were detected only in wheat and rye flours. 2,4-Decadienal (*E,E*) (84) was particularly abundant in the commercial wheat flour. Rye was distinguished by the presence of acids, such as octanoic (peak C) and nonanoic (G) acids, and terpenes, such as α-terpinolene (A), camphor (B), 4-terpineol (D) and α-terpineol (E).

3.3. Characterization of the volatile composition of maize flours

The majority of the compounds identified in maize flours were aldehydes (hexanal, heptanal, octanal, 2-octenal, nonanal, 2-nonenal, decanal), alcohols (1-pentanol, 1-hexanol, 1-heptanol, 1-octen-3-ol), hydrocarbons and 2-pentylfuran, which have been described in cereal flours as secondary products of lipid oxidation of unsaturated fatty acids [15,20,37,44]. Other abundantly detected volatile compounds were geranylacetone, ionone and 6-methyl-5-hepten-2-one, derived from carotenoids oxidation [40,59–61].

In order to evaluate the differences among maize flours and to study possible relations among the different volatiles, a cluster analysis was performed and the Spearman coefficients among the main volatiles were determined. Twenty-nine compounds, described in ***Table C.1, Appendix C***, were considered for these analyses, since the remaining fifteen were only present in trace amounts in all maize samples and it was not possible to accurately measure their peak areas. A PCA was performed in order to reduce the number of variables, since it was higher (29) than the number of samples. Following the Kaiser criteria, four components were retained after PCA analysis, which explained 93% of the total variance. The optimal number of clusters obtained by the elbow point and silhouette methods was two, one corresponding to all the traditional maize varieties (F1 to F11) and the other to the commercial maize sample (F12).

The commercial sample was distinguished from the traditional varieties due to the presence of benzaldehyde and higher amounts ($p < 0.05$) of other aldehydes (2-octenal (*E*), 2-nonenal (*E*), hexanal, heptanal, octanal, 2-heptenal (*Z*), 2-undecenal, decanal and 6-nonenal), alcohols (1-hexanol, 1-heptanol, 1-octen-3-ol, 1-octanol, 1-nonanol and 1-pentanol) and 3-ethyl-2-methyl-1,3-hexadiene, 2-pentylfuran, limonene, trimethyldodecane, 2-heptanone and pantolactone. The mentioned aldehydes showed very strong and positive correlations among them [***Table C.2, Appendix C***]. Since these volatiles are mainly derived from the oxidation of polyunsaturated fatty acids [***Figure 4.2***] [44,62], these results suggest that more lipid oxidation reactions were occurring in the commercial flour. Very strong and positive correlations were also found between these aldehydes and 3-ethyl-2-methyl-1,3-hexadiene [***Table C.2, Appendix C***]. Although the origin of this compound is not known, these high correlations suggest that it may also be a product of lipid oxidation. In fact, a recent study has shown that it was formed after black rice storage (>3 months) [63], and it has been recently described in purple sweet maize [64] and maize milk [65]. The highest concentration in lipid oxidation derivatives observed in F12 can be explained by its different genetic origin. In particular, the concentration of 2-octenal (*E*) and 2-nonenal (*E*), two by-products of linoleic acid oxidation, one of the most abundant lipids in maize kernel [66], was recently associated with a gene that codes for a linoleate 9S-lipoxygenase, an enzyme involved in linoleic acid metabolism [40]. Therefore, a higher enzymatic activity in F12 can explain its higher levels in 2-octenal (*E*) and 2-nonenal (*E*). Additionally, F12 was acquired already milled, and it may have been kept at room temperatures for a longer period than the traditional maize flours, contributing to its higher amounts in lipid oxidation products [67,68]. Another possibility for these differences could be the different granulometry of the commercial sample, since it presented a higher mean diameter and a large particle distribution range compared to traditional maize flours [43]. However, research has shown the opposite: finely milled cereal flours tend to be more susceptible to lipid oxidation reactions than flours with higher particle sizes [67,68], possibly due to the higher surface area that favours the contact with oxygen [67]. Thus, the different granulometry of the commercial flour was probably not the main reason for its higher content in aldehydes and alcohols. Ultimately, the lower content of lipid oxidation derivatives in the traditional maize flours may also be caused by their higher amounts in antioxidant compounds [11]. Consequently, the total carotenoids content and major soluble phenolic compounds present in maize flours (ferulic and *p*-coumaric acids, diferuloyl putrescine, coumaroyl feruloyl putrescine, dicoumaroyl spermidine and *bis*-diferuloyl putrescine) were quantitated [***Table C.3, Appendix C***] and correlated to their volatile composition [***Table C.4, Appendix C***]. Results showed strong negative correlations among several volatile compounds derived from lipid oxidation [15,17], namely 1-hexanol, 2-pentylfuran, 1-octanol, 1-nonanol, 2-undecenal and pentadecane, and some phenolic compounds, such as ferulic acid, diferuloyl putrescine, coumaroyl feruloyl putrescine and *bis*-diferuloyl putrescine, whereas no positive correlations were found. Similarly, pantolactone, also possibly derived from lipid oxidation reactions [15], showed very strong and negative correlations ($R < -0.70$, $p < 0.01$) not only with diferuloyl putrescine, but also with total carotenoids' content. Hence, phenolic compounds in traditional maize flours and, to a lesser extent, carotenoids, may have inhibited lipid oxidation reactions, and contributed to their longer preservation. In fact, ferulic acid has been approved in certain countries as a food additive to prevent lipid oxidation [69].

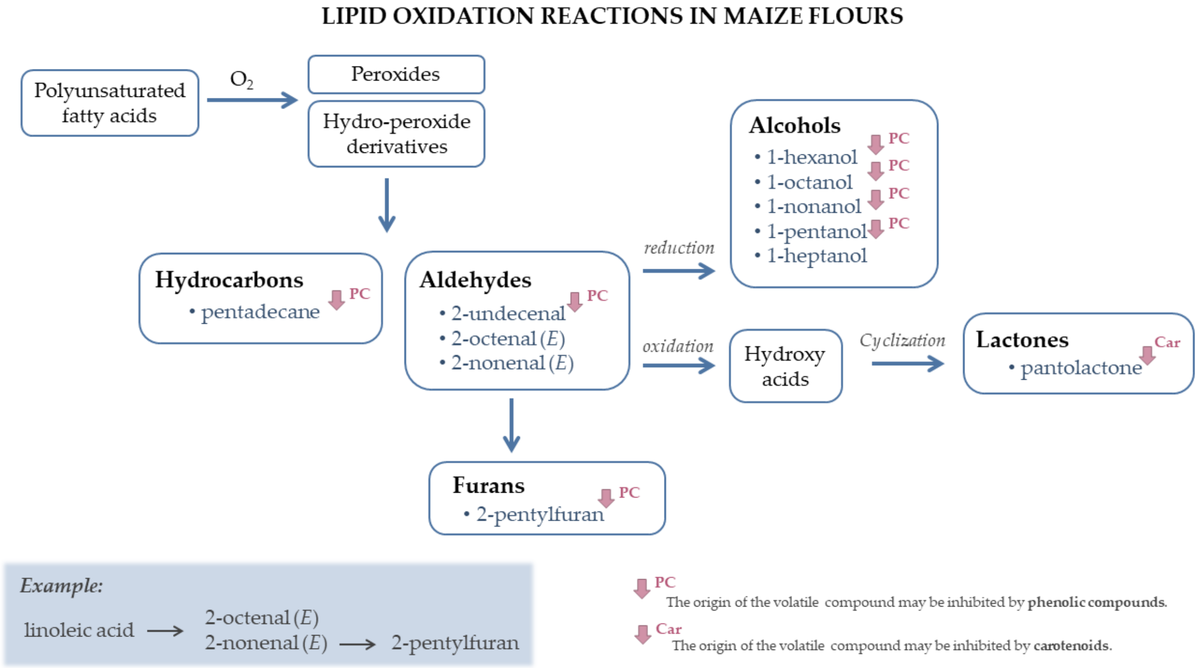

Figure 4.2: Representative scheme of lipid oxidation reactions [15,17,20,28,35] occurring in maize flour samples.

As the differences between the traditional and commercial maize flours were high, it was not possible to detect any dissimilarities among the traditional maize varieties. Thus, a second analysis was performed, excluding the commercial sample and benzaldehyde, once it was not detected in the traditional maize varieties. The analysis was performed considering the first 5 components obtained by the PCA, which explained 91.3% of the total variance. The optimal number of clusters obtained by the elbow point and silhouette methods now corresponded to 3 clusters. The resulting dendrogram is presented in ***Figure 4.3*** and the compounds that significantly contributed to discriminate the clusters are presented in ***Table 4.2***. Samples from cluster 1 (F3, F5, F6, F9 and F11) were distinguished from cluster 2 (F2, F4, F7, F8, F10) by their lower contents in ketones (α-ionone, 6-methyl-5-hepten-2-one and geranylacetone), derived from carotenoids' oxidation [40,59] and higher pantolactone contents [***Figure C.3, Appendix C***]. Geranylacetone, α-ionone and 6-methyl-5-hepten-2-one showed strong and positive correlations among them ($R > 0.7$, $p < 0.05$) [***Table C.2, Appendix C***]. Therefore, a higher content of carotenoids and/or higher carotenoids' oxidation reactions led to an increase in these compounds. Indeed, strong and positive correlations were found between total carotenoids content and both α-ionone and geranylacetone [***Table C.4, Appendix C***]. In addition, all the samples from cluster 2 were yellow kernels, showing higher total carotenoids content [***Table C.3, Appendix C***], corroborating the results from a previous study [40]. The concentrations in geranylacetone and α-ionone can also be influenced by the expression of genes encoding carotenoid cleavage dioxygenases [60,61]. Ultimately, higher amounts in compounds that inhibit carotenoids' oxidation, such as ferulic and *p*-coumaric acid or other phenolic derivatives, can also contribute to lower concentrations in these compounds. In fact, moderate negative correlations were found between geranylacetone and *p*-coumaric acid and between 6-methyl-5-hepten-2-one and ferulic acid [***Table C.4, Appendix C***].

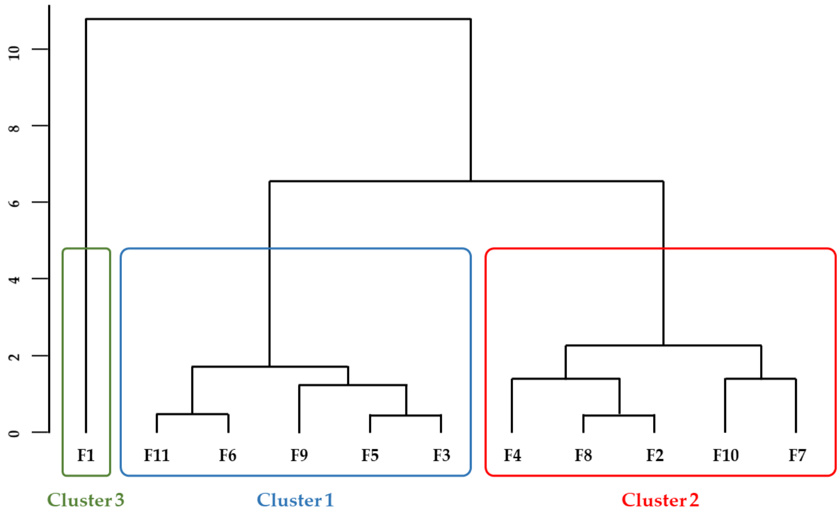

Figure 4.3: Dendrogram of cluster analysis of traditional maize flours.

Table 4.2: Maize flours volatile compounds that contributed to discriminate the different clusters.
(+) Compounds present in higher concentrations ($p < 0.05$). **(-)** Compounds present in lower concentrations ($p < 0.05$).

Chemical Class	Compound	Suggested Origin
	Cluster 1: F11, F6, F9, F5, F3	
Ketones	α-Ionone (-)	Degradation of δ-carotene [60,61]
	6-Methyl-5-hepten-2-one (-)	Degradation of phytoene, ζ-carotene, lycopene, δ-carotene [61]
	Geranylacetone (-)	Degradation of phytoene and ζ-carotene [7,61]
Alcohols	1-Nonanol (-) 1-Hexanol (-) 1-Heptanol (-) 1-Octen-3-ol (-) 1-Pentanol (-)	Lipid oxidation [17]
Aldehydes	Decanal (-) 6-Nonenal (*Z*) (-)	Lipid oxidation [15]
Hydrocarbons	Pentadecane (-)	Lipid oxidation [21]
Lactones	Pantolactone (+)	Lipid oxidation [15]
	Cluster 2: F4, F8, F2, F10, F7	
Lactones	Pantolactone (-)	Lipid oxidation [15]
Ketones	α-Ionone (+)	Degradation of δ-carotene [7,61]
	6-Methyl-5-hepten-2-one (+)	Degradation of phytoene, ζ-carotene, lycopene, δ-carotene [61]
	Geranylacetone (+)	Degradation of phytoene and ζ-carotene [7,61]
	1-Nonanol (+)	Lipid oxidation [17]

Alcohols	1-Hexanol (+)	
	Cluster 3: F1	
Aldehydes	2-Octenal (*E*) (+) Octanal (+) Heptanal (+) 2-Nonenal (+) Hexanal (+) 2-Undecenal (+) 2-Heptenal (Z) (+) Decanal (+) 6-Nonenal (+)	Lipid oxidation [15]
Terpenes	Limonene (+)	Plant metabolism and signalling [70]
Alcohols	1-Octanol	Lipid oxidation [17]
Hydrocarbons	Pentadecane 3-Ethyl-2-methyl-1,3-hexadiene	Lipid oxidation [21]
Furans	2-Pentylfuran	Lipid oxidation, from (*E*)-2-nonenal [15,17]

Finally, cluster 3, constituted by the sample F1, was distinguished by higher contents in compounds derived from lipid oxidation, similar to the commercial hybrid (F12). This sample was a synthetic open pollinated maize variety developed as an experimental higher-quality cultivar with increased precocity, obtained through the crossing of 12 maize populations (10 Portuguese traditional varieties and 2 American populations). As previously discussed for the commercial sample, the differences observed may be at least partially explained by their different genetic origin.

3.4. Characterization of the volatile composition of *broas*

The volatile compounds present in *broas* were mainly derived from lipid oxidation [***Figure 4.4***] and originated during bread making fermentation [***Figure C.4, Appendix C***] and non-enzymatic browning reactions [***Figure C.5, Appendix C***]. It is known that the increase of lipid oxidation products in breads is associated with (i) higher fermentation temperatures [15], (ii) the use of wholegrain flours, such as in *broas* preparation, probably due to the additional lipid material in cereal germ [28], and (iii) the presence of oxidative yeasts naturally present in sourdoughs, such as *Candida* spp. [16]. 4-Heptenal (*Z*) and 2,4-decadienal (*E,E*) were detected in *broas*, but not in maize flours. Both aldehydes have been reported as primary odorants to contribute to the flavour of wheat bread [56], and 2,4-decadienal has also been described as an important popcorn volatile [24]. They have also been described as lipid oxidation products and could have been formed during fermentation, due to the metabolic activity of yeasts and lactic acid bacteria [6]. Since 2,4-decadienal was particularly abundant in the commercial wheat flour used for *broas* preparation, it could also have been derived from the lipid oxidation of linoleic acid [28] present in this flour.

Benzaldehyde was detected in all *broas* despite being only detected in the commercial maize flour. It may have been produced by auto-oxidation of 2,4-decadienal [18] or formed from phenylalanine through the Ehrlich pathway and/or Strecker degradation [***Figure C.4, Appendix C***] [17,25]. Other compounds were also detected in *broas*, but not in maize flours, as acids (acetic and hexanoic acids), esters (ethyl hexanoate, ethyl octanoate, 1-hexyl acetate), ketones (octen-2-one, 3-hydroxy-2-butanone, 2-methyl-3-octanone and 3,5-octadien-2-one) and furanones (γ-*N*-caprolactone and γ-nonalactone). These compounds may have been originated by yeast and/or bacteria during fermentation [15,17,20,22]. Acetic acid is a well-known main product of fermentation by LAB and its concentration is higher when sourdough is used in breads formulation [20], such as in the preparation of *broa*. In spite of being volatilized during baking [11], acetic acid may be a product of Maillard or caramelization reactions [17,20]. Furanones can also be products of carbohydrate dehydration and fragmentation that occurs during baking [21,25]. Other compounds may have been formed during the early steps of the Maillard reaction (sugar dehydration or fragmentation), such as furans, pyranones and furfurals [5,19,25,71], and in the last stages of the reaction, such as pyrroles and pyrazines [***Figure C.4, Appendix C***] [19]. *p*-Vinylguaiacol was also detected in *broas*, but not in maize flours. It has been described as an important odorant in other breads and baked products, derived from ferulic acid decarboxylation [8,28,29]. On the contrary, some compounds were lost after processing, such as aldehydes, alcohols and ketones, which may have been volatilized during baking [16,20], or participated in further reactions during bread making [35].

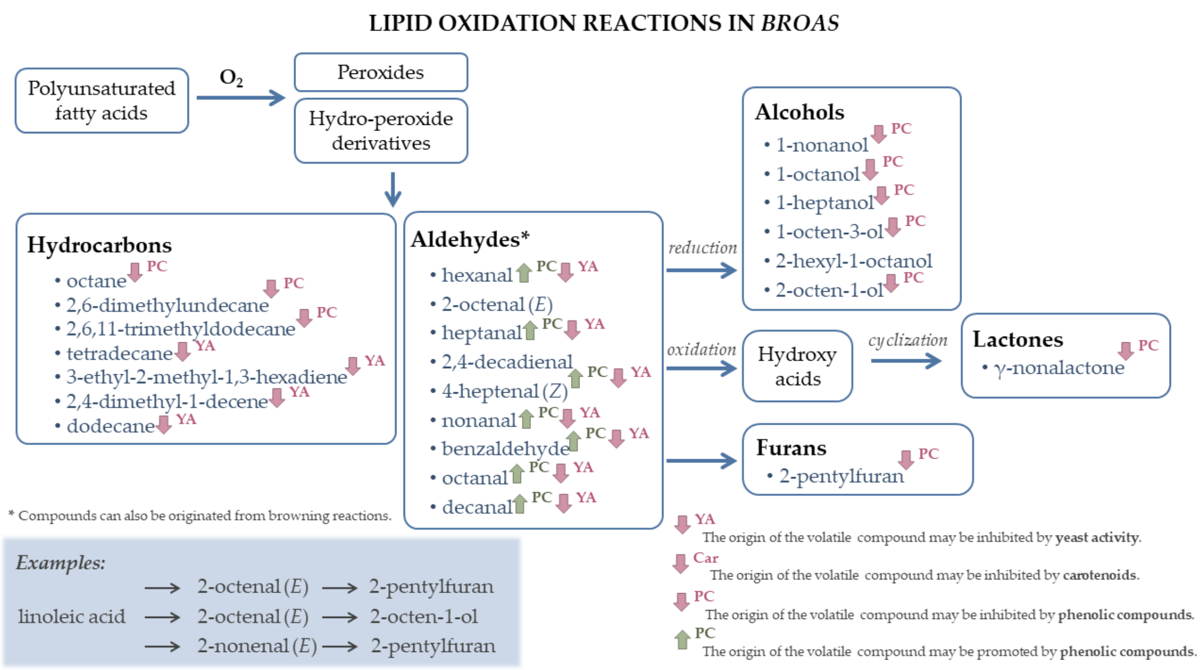

Figure 4.4: Representative scheme of lipid oxidation reactions [15,17,20,28,35] in *broas*.

The contribution of a compound to the final bread aroma depends not only on its concentration, but also on its odorant power, which is determined by its odour threshold, or odour activity value (OAV) [11]. Therefore, some compounds, although present in low concentrations, may be more flavour-active than others present in higher concentrations [11]. The most important and potent volatile compounds found in *broas* and already described in wheat bread and other maize-based foods are highlighted in ***Table 4.1***. Some of these compounds are positively associated with the pleasant aroma of breads, while others can be considered as off-flavours [15,20].

Broas' crumb and crust samples were analysed separately in the same conditions. The crust was characterized by a higher content in aldehydes, furans, pyrrolines and acids, while the crumb presented a higher content of alcohols and esters (data not shown). These results were expected, since different compounds are mainly present in the crumb, where temperatures during bread making are in general less than 100 °C, and others are formed in the crust, at temperatures of around 230–250 °C [5,11]. The main volatiles present in the crumb were compounds associated with lipid oxidation [35] and fermentation [11,12,16,72] and, in the crust, the number of volatile compounds resulting from the Maillard reaction [35] increased. Since the main objective of this work was to determine the volatile compounds of the whole bread, which may influence consumers' choice, the results discussed in the present work did not go further into the differences between the crust and crumb.

As for maize flours, the associations among different samples and the correlations among volatiles were studied. All eighty-seven compounds were considered for these analyses [***Table C.5, Appendix C***]. After sample dimension reduction by PCA and based on the Kaiser criteria, 11 components were retained, which explained 100% of the total variance. Cluster analysis was then performed. The optimal number of clusters obtained by the elbow point method was in this case of four clusters, while by the silhouette method was five clusters. However, since the average silhouette width obtained for five clusters was very similar to that obtained for four clusters, the analysis was performed considering an optimal number of four clusters, and the resulting dendrogram is presented in ***Figure 4.5***. The compounds that significantly contributed to discriminate the clusters are presented in ***Table 4.3***.

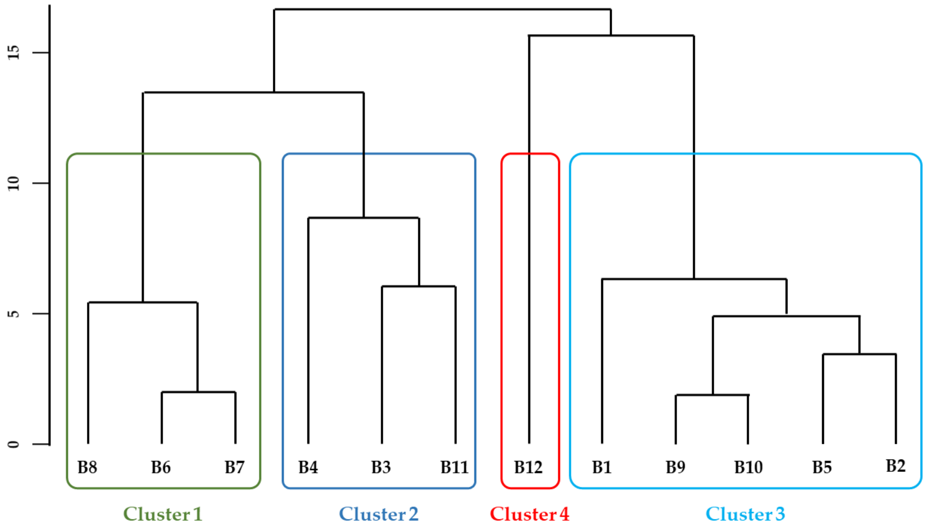

Figure 4.5: Dendrogram of cluster analysis of *broas*.

Table 4.3: *Broas* volatile compounds that contributed to discriminate the different clusters.

n/f*:* not found. **(+)** Compounds present in higher concentrations ($p < 0.05$). **(-)** Compounds present in lower concentrations ($p < 0.05$). ***** Important volatile compounds described in wheat and rye breads, popcorn, extruded maize products or other cereal-based foods [8,17,20,24,26,35].

Chemical Class	Compound	Suggested Origin
	Cluster 1: B6, B7, B8	
Furans	2-Pentylfuran* (-)	Lipid oxidation, from (*E*)-2-nonenal [17]
Hydrocarbons	1-Chlorooctadecane (+) Undecane (+) Decane (+) 3,7-Dimethyldecane (+) 3-Methylundecane (+) 2,4-Dimethyl-1-decene (+)	Lipid oxidation [21]
Pyrroles	1-Furfurylpyrrole (+) 2-Acetylpyrrole (+)	Last stages of the Maillard reaction [19] Maillard reaction, from oxidation of 2-acetyl-1-pyrroline [21]
Alcohols	2-Hexyl-1-octanol (+)	Yeast fermentation or reduction of aldehydes from lipid oxidation [17]
Pyranones	Maltol (+)	Degradation of disaccharides, such as maltose or lactose [21]
Furfurals	5-Methylfurfural* (+)	Degradation of sugars [11,20]
	Cluster 2: B3, B4, B11	
Ketones	3-Octen-2-one (-)	Lipid oxidation [20], fermentation [20] or sugar degradation [11]
Aldehydes	Octanal* (-) 2,4-Decadienal (*E,E*)* (-) Benzaldehyde* (-)	Lipid oxidation [15] Lipid oxidation of linoleic acid [28] Ehrlich pathway or Strecker degradation, from phenylalanine [17,25], or auto-oxidation of 2,4-decadienal [18]
Hydrocarbons	3-Ethyl-2-methyl-1,3-hexadiene (-) Tetradecane (+) 1-Tetradecene (+)	Lipid oxidation [21]
	Cluster 3: B1, B2, B5, B9, B10	

Alcohols	2-Octen-1-ol (-)	Yeast fermentation or reduction of aldehydes from lipid oxidation [17]
Pyrroles	1-Furfurylpyrrole (-)	Last stages of the Maillard reaction [19]
Alkanes	Nonane (-) Dodecane (-)	Lipid oxidation [21]
Aldehydes	4-Heptenal (*Z*)* (+) Heptanal* (+) Hexanal* (+) Decanal* (+)	Lipid oxidation [15,20,44]
Furans	Methylpentylfuran (+)	n/f
Cluster 4: B12		
Furans	Methylpentylfuran (-)	n/f
Aldehydes	2-Phenylacetaldehyde* (+)	Strecker reaction [16] and Ehrlich pathway [16], from phenylalanine [17,20,25]
	2-Heptenal (*Z*) (+) 2-Octenal* (*E*) (+)	Lipid oxidation [15,20,44]
Alcohols	1-Octen-3-ol* (+)	Lipid oxidation [20]
	2-Octen-1-ol (+) Benzyl alcohol* (+) 1-Pentanol (+)	Yeast fermentation or reduction of aldehydes from lipid oxidation [17]
Alkanes	2,7,10-Trimethyldodecane (+) 5-Methyltridecane (+) 2,6-Dimethylundecane (+)	Lipid oxidation [21]
Pyrroles	Indole (+)	Non-enzymatic browning reactions [12]
Terpenes	Limonene (+)	Plant metabolism and signalling [70] and yeast fermentation [16]
Pyrazines	Methylpyrazine* (+)	Last stages of the Maillard reaction [19]
Furanones	ϒ-Nonalactone* (+)	Lipid oxidation of oleic and linoleic acid [15], fermentation [22], Maillard reaction [20,21]

Broas were differentiated mainly by their content in volatiles from lipid oxidation and formed during baking. In particular, clusters 3 and 4 were characterized by higher amounts in volatiles from lipid oxidation. As observed for maize flours, the *broa* prepared from the commercial maize variety (B12) was discriminated from all the others, belonging to cluster 4 [***Figure 4.5*** and ***Table 4.3***]. This sample was characterized by higher amounts in some aldehydes, alcohols and alkanes, suggesting that more lipid oxidation reactions [20,21] had occurred, probably due to the different genetic origin of the corresponding maize flour (F12), as previously discussed. It was also characterized by lower contents in methylpentylfuran. Although it was not possible to confirm the identification of methylpentylfuran, it is possible that it had been originated by lipid oxidation, such as 2-pentylfuran [15,17], and/or during the Maillard reaction, such as other furans [19]. On the contrary, cluster 3 (B1, B2, B5, B9 and B10) was characterized by higher contents in methylpentylfuran and, similar to cluster 4, by higher amounts in several low-odour threshold aldehydes derived from lipid oxidation. Cluster 2 (B3, B4 and B11) was characterized by lower amounts in volatiles from lipid oxidation and/or fermentation reactions [15,21,28], namely 3-ethyl-2-methyl-1,3-hexadiene, 3-octen-2-one, octanal and benzaldehyde. The lower amounts in 2,4-decadienal observed in these samples can explain their lower benzaldehyde contents, since benzaldehyde can be formed by the auto-oxidation of 2,4-

decadienal (*E,E*) [18]. Finally, *broas* B6, B7 and B8 belonged to cluster 1, characterized by higher contents in compounds resulting from the baking process, particularly maltol, 1-furfurylpyrrole, 2-acetylpyrrole and 5-methylfurfural.

The correlations among the volatiles of *broas* were also evaluated [***Table C.6, Appendix C***]. Similar to maize flours, strong positive correlations were found among lipid oxidation products, such as aldehydes, alcohols and some ketones [20]. Positive correlations (R > 0.59, $p < 0.05$) were also detected among compounds formed during baking, such as methylpyrazine, 5-methylfurfural, furfurylpyrrole and 2-furanmethanol, and among volatiles which may have been originated during fermentation, namely hexanoic acid, benzyl alcohol, ethyl hexanoate, 1-hexyl acetate, ethyl octanoate, γ-nonalactone, 3-octen-2-one and 2-methyl-3-octanone. Positive correlations were also commonly found among these volatiles and several alcohols, such as isopentanol, 1-pentanol, 1-hexanol and 1-heptanol, suggesting that these compounds have also been originated by bacteria or yeast fermentation. Conversely, significant negative correlations were found among some volatiles derived from fermentation, such as 2-methyl-1-butanol, phenylethyl alcohol and 2-methyl-3-octanone, with several lipid oxidation products, such as aldehydes (hexanal, 4-heptenal (*Z*), heptanal, benzaldehyde, nonanal and decanal) and hydrocarbons (3-ethyl-2-methyl-1,3-hexadiene, 2,4-dimethyl-1-decene, dodecane and tetradecane). Other authors have reported similar negative correlations in other food products [73]. One explanation for these correlations is the lower amylase and yeast activities in some flours, which consequently provided a higher amount of active oxygen for oxidation, contributing to the increase in lipid oxidation reactions [30]. Alternatively, a higher yeast activity may have promoted the metabolization of aldehydes to the corresponding alcohols. Moskowitz *et al.* [28] have reported that *p*-vinylguaiacol can significantly reduce the generation of Maillard-type aroma compounds, but only a modest negative correlation was found between this compound and methylpyrazine in *broas* (R = –0.587, $p < 0.05$), whereas no correlations were found between this compound and other Maillard derivatives [***Table C.6, Appendix C***].

The volatile composition of maize kernels plays a major role in overall end-product quality [40]. Therefore, in order to understand the influence of maize volatiles on *broas* volatile composition, the volatile compounds detected in maize flours and *broas* were correlated [***Table C.7, Appendix C***]. In general, higher amounts of lipid oxidation products were found in *broas* prepared from maize flours that presented higher contents in these compounds. For instance, 1-octen-3-ol was detected in higher concentrations in *broas* produced from maize flours with higher contents in 1-octanol (R = 0.804, $p < 0.01$) and maize flours with higher contents in heptanal originated *broas* with higher contents in 3-ethyl-2-methyl-1,3-hexadiene (R = 0.818, $p < 0.01$).

3.4.1. Contribution of phenolic compounds to the volatile composition of *broas*

Some phenolic compounds, particularly free phenolic acids present in the outer layers of cereal grains, such as ferulic acid, may directly contribute to breads' flavour [74], increasing their bitterness and astringency [30]. However, previous studies have demonstrated that the contribution of phenolic compounds to breads' flavour is mainly due to their influence on their volatile composition [28–30]. Although most of maize phenolic compounds are insoluble and linked to arabinoxylans [32], it is believed that only the soluble phenolics are able to influence the volatile composition of breads [28,75]. Therefore, the main soluble phenolics of maize flours and *broas* were quantitated [***Table C.3, Appendix C***] and correlated with *broas* volatile composition [***Table C.8, Appendix C***]. Some examples of the influence of phenolic compounds on *broas* volatiles are represented in ***Figure 4.4***, and ***Figures C.4*** and ***C.5, Appendix C***.

Significant negative correlations were found between phenolic compounds from maize flours and *broas* and several volatiles derived from lipid oxidation reactions. In particular, strong negative correlations ($R < -0.7$, $p < 0.05$) were found between *broas'* diferuloyl putrescine and coumaroyl feruloyl putrescine contents and alkanes (octane, 2,6-dimethylundecane and 2,6,11-trimethyldodecane) and alcohols (1-octen-3-ol, 1-heptanol, 1-octanol). Negative correlations were also obtained between phenolic compounds and 1-nonanol and 1-octanol. Therefore, phenolic compounds seamed to inhibit lipid oxidation reactions not only in maize flours, as previously discussed, but also in *broas* and possibly during bread making, as described for other breads [30,33,73,76]. However, positive correlations ($R > 0.57$, $p < 0.05$) were found between both free ferulic acid and coumaroyl feruloyl putrescine in maize flours and several lipid oxidation aldehydes [15,20,44] in *broas*, such as hexanal, 4-heptenal, heptanal, nonanal, decanal and benzaldehyde. Some studies have reported that phenolics, including ferulic acid, can actually increase the generation of lipid oxidation volatiles [30], possibly because they begin to show pro-oxidant behaviour at higher concentrations [30,73]. Conversely, Moskowitz *et al.* [28] have demonstrated that the addition of ferulic acid to refined breads did not influence the generation of lipid oxidation products. The effectiveness of an antioxidant can be influenced by its location in the food matrix, survival during food processing, interactions with other food components [31] and by different hydrophilic properties of some antioxidants [30,73,77]. However, the mentioned aldehydes could have also been formed during baking [11] and not exclusively from lipid oxidation reactions.

Negative correlations were also found between phenolic compounds and some volatiles originated by yeast and/or bacteria during fermentation, such as ethyl hexanoate, ethyl octanoate, 1-hexyl acetate, benzyl alcohol, 2-methyl-1-butanol and γ-nonalactone [15,17,20,22], suggesting that hydroxycinnamic acids and hydroxycinnamic acid amides inhibited amylases and yeast activity, as it has already been described for ferulic acid [30]. These findings can also explain the positive correlations among aldehydes and phenolic compounds and the negative correlations among alcohols and phenolic compounds, since aldehydes can be reduced to alcohols by the activity of LAB and yeasts [20]. Thus, if these reduction reactions were inhibited by phenolic compounds, the levels of alcohols would be lower, whereas an increase in aldehydes' content would be expected.

On the contrary, significant positive correlations were found among phenolic compounds and furan derivatives from browning reactions (Maillard and caramelization reactions, ***Figure C.4, Appendix C***), namely, furfural, furfuryl alcohol, 2-acetylfuran and 5-methylfurfural. These findings are in accordance with previous studies, which have demonstrated an increase in furfural and 5-methylfurfural by feruloylated oligosaccharides [29], possibly due to their pro-oxidant effect at high concentrations, therefore increasing browning reactions [78]. The positive correlations of some aldehydes with phenolic compounds can also be explained by the increase in these reactions, since aldehydes can also be originated during baking [11]. Conversely, other authors have described a reduction in the content of Maillard volatiles in breads, such as furanones and other furan derivatives, by phenolic compounds, including ferulic acid [28,30,77], possibly due to the formation of adducts with dicarbonyls or scavenging reactions with radical precursors [28–30,73,77]. In fact, as previously discussed, the contents of γ-nonalactone (a furanone) were negatively correlated to some phenolics. However, taking into account the cluster analysis, γ-nonalactone was most likely originated during fermentation and not during baking.

Thus, these results suggest that phenolic compounds may: (i) act as antioxidants, inhibiting lipid oxidation reactions in both maize flours [***Figure 4.2***] and *broas* [***Figure 4.4***], (ii) inhibit amylases and yeast fermentation [***Figure C.4, Appendix C***] and (iii) act as pro-oxidants during baking, increasing the levels of Maillard and caramelization volatiles [***Figure C.5, Appendix C***]. As previously reported [41], some insoluble phenolic compounds may become soluble after maize processing to *broas*, increasing their content in soluble phenolic compounds. Therefore, the increasing levels of phenolic compounds during bread making may have contributed to their action as pro-oxidants, thus increasing the level of Maillard derivatives.

3.4.2. Contribution of carotenoids to the volatile composition of *broas*

Broas produced from maize flours with a higher content in carotenoids showed higher amounts of geranylacetone and γ-ionone ($R > 0.85$, $p < 0.01$) [***Table C.8, Appendix C***]. Thus, maize flours with higher amounts of volatiles from carotenoids' oxidation generated *broas* with higher amounts of carotenoids' oxidation volatiles [***Table C.7, Appendix C***]. For instance, although 6-methyl-5-hepten-2-one was not detected in *broas*, *broas* prepared from maize flours with higher contents in this compound showed higher geranylacetone contents (R = 0.832, $p < 0.01$). Both of these compounds can result from the degradation of phytoene and ζ-carotene [7,61].

Significant negative correlations were found between carotenoids' content and some compounds formed during bread making (fermentation and/or baking), namely γ-*N*-caprolactone, 2-phenylacetaldehyde, acetoin (3-hydroxy-2-butanone), hexanoic acid and indole [20,21,25], suggesting that carotenoids inhibited the formation of these compounds [***Figures C.4*** and ***C.5, Appendix C***].

A positive correlation was observed between γ-*N*-caprolactone present in *broas* and pantolactone in maize flours [***Table C.7, Appendix C***], which indicates that pantolactone might have participated in further reactions during bread making, originating γ-N-caprolactone. Carotenoids could have contributed to the increase in other compounds formed during bread making, such as 2,3-dihydrobenzofuran, 2-methoxy-2,3,3-trimethylbutane and acetic acid, since significant positive correlations were found between them and carotenoids' content [***Figure C.5, Appendix C***].

3.4.3. Sensory analysis and volatile composition of *broas*

The results obtained from a consumer sensory evaluation of *broas* [43] were retrieved and compared here with their volatile composition, in order to determine the volatile compounds that might contribute to *broas* sensory characteristics. The sensory analysis scores are presented in ***Table C.9, Appendix C*** and the correlations among these scores and *broas* volatile compounds are presented in ***Table C.10, Appendix C***.

As previously reported [43], the sensory analysis revealed poor discrimination among the 11 samples prepared from traditional maize varieties. The *broa* prepared from the commercial maize flour (B12) showed the lowest mean scores for all the evaluated attributes ('appearance', 'smell and odour', 'texture', 'taste and aroma', 'colour' and 'global appreciation'). In addition, the majority of the negative comments were attributed to this sample, including 'dry texture', 'weak typical flavour', 'wheat bread flavour', 'with a weak maize flavour' and 'no history'. As previously discussed, the commercial *broa* was distinguished from all the traditional *broas* [***Figure 4.5*** and ***Table 4.3***], essentially due to its higher contents in volatiles derived from lipid oxidation reactions, such as 2-octenal, which was very strongly and negatively correlated (R = –0.746, $p < 0.01$) with 'taste and aroma' scores. This aldehyde contributes to fatty, nutty, waxy and green notes [20,51]. This sample was also characterized by higher contents in benzyl alcohol, an extremely aroma-active compound associated with pleasant notes [15,17,20]. However, benzyl alcohol has been described in rye breads as a major factor responsible for their intense and bread-like flavours [16], and thus can contribute to a more bread-like flavour in *broas*. Hence, it may contribute to aroma characteristics in *broas* that are not typical of this type of bread, giving rise to some of the negative comments described above. It can also be responsible for a bitter taste [79], which was also referred to as a negative characteristic of this particular *broa*.

The *broa* B8 scored higher for the majority of the sensorial attributes (‘colour’, ‘smell and odour’, ‘texture’ and ‘global appreciation’) and was often described by the consumer panel as ‘the tastiest’ and ‘with a good maize flavour’. This sample belonged to cluster 1, together with B6 and B7, characterized by higher contents in compounds resulting from the baking process associated with positive notes [19–21,24], particularly maltol, 1-furfurylpyrrole and 2-acetylpyrrole. Interestingly, these compounds have not been reported as abundant in other breads [20], but have been described in popcorn [24,36], extruded maize products [14] and tortilla chips [80], suggesting that they can have a contribution to the characteristic maize-based foods, and particularly *broas'* sweet aroma. Significant positive correlations were found between ‘taste and aroma’ scores and pyranones content (maltol and 3-hydroxy-2,3-dihydromaltol). Despite the relatively high odour threshold of 9 mg/kg (water), maltol was very abundant in the B8 sample, which showed the highest maltol content of all *broas* [***Table C.5, Appendix C***]. It has a caramel-like odour and enhances the sweet taste of food [21]. In contrast, B12 showed the lowest contents of maltol of all *broas*, which also supports its importance on *broas* ‘taste and aroma’. B1 showed the second lowest contents in maltol and had the lowest contents in 3-hydroxy-2,3-dihydromaltol of all *broas*. Some of the comments attributed to B12, such as ‘weak typical flavour’ and ‘weak aroma and flavour’ were also attributed to B1, which was the sample with the lowest ‘taste and aroma’ scores among all *broas* prepared from traditional maize flours. As previously discussed, F1 was differentiated from all the other traditional maize flours [***Table 4.2*** and ***Figure 4.3***] due to its higher contents in volatiles from lipid oxidation, in particular 2-octenal (*E*). Similarly, B1 belonged to cluster 3, together with B2, B5, B9 and B10, characterized by higher amounts in lipid oxidation products, such as hexanal and heptanal, which were negatively correlated with ‘taste and aroma’ scores.

B11, followed by B3, showed the highest ‘taste and aroma’ scores. These samples and B4, which showed the fourth highest scores, all belonged to cluster 2 [***Figure 4.5*** and ***Table 4.3***], characterized by lower contents in volatiles from lipid oxidation reactions, in particular octanal, which has a low odour threshold and is considered an off-flavour of foods [17,24]. Indeed, a significant negative correlation was found between ‘taste and aroma’ scores and octanal. This cluster was also characterized by lower contents of 3-ethyl-2-methyl-1,3-hexadiene and benzaldehyde, which also showed negative correlations with ‘taste and aroma’ scores, along with other aldehydes derived from lipid oxidation reactions, namely 2-nonenal (*E*) and 2-undecenal, frequently described as negative contributors to the aroma of breads [20].

These results explain some differences among *broas* prepared from the traditional and commercial hybrid maize varieties and sustain the previous knowledge of producers regarding the better-quality aspects of traditional maize varieties for *broas* production [4].

In particular, according to the producers, the *broa* of traditional maize is softer, sweeter and can be preserved for longer periods than *broas* produced with hybrid varieties [81]. Indeed, higher contents in pyranones may contribute to traditional *broas* sweeter taste, and lower contents in aldehydes suggest that lower lipid oxidation reactions were occurring in traditional samples, which can not only explain their longer preservation, but also contribute to their more pleasant flavour.

Positive correlations were found between 'smell and odour' scores and 2,3-dihydrobenzofuran, which was more abundant in *broas* with higher carotenoids' content, as previously discussed. To the best of our knowledge, 2,3-dihydrobenzofuran has not been described neither in other maize-based foods nor cereal breads and was not detected in maize flours, so it was originated during the bread making process, similar to other furan derivatives [14–16]. This compound has been described in wines and soy sauce as a product of the Maillard reaction [82,83] and is associated with musky notes [55].

Very strong and positive correlations were found between the main products of carotenoids' degradation (γ-ionone and geranylacetone) and colour scores, which can be explained by the preference for *broas* yellowish colour by the panel of consumers. The 'global appreciation' of *broas* was greatly influenced not only by 'flavour and aroma', but also by 'texture', which also showed a very strong positive correlation between them [***Table C.11, Appendix C***]. *Broas* with higher contents in some volatiles associated with off-aromas, such as octanal, 2-octenal and 2-nonenal, also showed lower scores for texture, whereas samples with higher pyranones content showed higher scores. These findings may be explained by the proteolysis reactions that occur during sourdough fermentation [9,84]. Briefly, proteolysis produces amino acids and other precursors of aroma compounds during baking, enhancing the formation of volatiles related to better bread flavour [9], while simultaneously improving the dough rheology and bread texture, resulting in a large reduction of elasticity and firmness of the dough [84].

Most of the volatiles commonly described as great contributors for other cereal breads and maize-based foods, such as 2-acetylpyrroline, 2-(methylimino)-3-butanone and 2,3-butanedione [8,85], were not detected in *broas*. Therefore, their absence can account for the different sensory characteristics of *broas* [81], when comparing to other cereal breads. In particular, 2-acetyl-1-pyrroline, a compound derived from the Maillard reaction, has been referred to as the main compound responsible for the characteristic flavour of wheat bread crust [24,26,36]. However, this compound is highly volatile, may be poorly released to the headspace during extraction, degrades rapidly after baking and can be oxidized to 2-acetylpyrrole, which was detected in *broas* [21]. Furthermore, phenolic acids, such as ferulic acid, could have inhibited its production [28]. 2-Acetyl-1-pyrroline has been described as mainly responsible for the differences in the overall odours of wheat and rye breads, where it is present in much lower concentrations [26]. Therefore, its absence in *broas* can also contribute to their characteristic aroma.

4. Conclusions

The main volatile compounds present in maize flours were aldehydes and alcohols derived from lipid oxidation reactions, which were present in higher amounts in the commercial maize variety. Ketones from carotenoids' oxidation, such as γ-ionone and geranylacetone, were present in higher amounts in yellow maize varieties. The presence of higher lipid and carotenoids' oxidation volatiles in maize flours directly contributed to higher amounts of similar volatiles in *broas*. Other volatile compounds present in *broas* were esters, furans, furfurals and pyranones, derived from the bread making process (fermentation and baking). The differences in *broas* volatile composition were mainly due to lipid oxidation and browning reactions, which were influenced by soluble phenolic compounds. In particular, ferulic and *p*-coumaric acids and hydroxycinnamic acid amides inhibited lipid oxidation and fermentation reactions and promoted browning reactions. *Broas* less appreciated by consumers, especially the *broa* obtained from the commercial flour, showed higher lipid oxidation compounds, such as 2-octenal, hexanal and heptanal, and lower contents in pyranones, such as maltol and 3-hydroxy-2,3-dihydromaltol, in addition to lower phenolic compounds. Results showed that maize flours with higher levels in phenolic compounds give raise to *broas* with better sensory characteristics, especially related to 'taste and aroma'. In conclusion, the selection of maize varieties richer in health-promoting phenolic compounds originate *broas* more appreciated by the consumers. These findings are relevant not only for the industry, contributing to the selection of the best maize varieties for higher-quality bread making, but also for maize breeders, who can add these quality traits to routine breeding of maize for *broas* production. These results sustain the previous knowledge of producers regarding the better-quality aspects of traditional maize varieties for *broas* production, and can contribute to the valorisation of these varieties, which should be preserved as a safeguard against an unpredictable future.

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References

1. Patto, M.C.V.; Moreira, P.M.; Carvalho, V.; Pego, S. Collecting Maize (*Zea Mays* L. Convar. *Mays*) with Potential Technological Ability for Bread Making in Portugal. *Genet. Resour. Crop. Evol.* **2007**, *54*, 1555–1563.
2. Patto, M.C.V.; Alves, N.F.; Almeida, C.S.; Mendes, P.; Satovic, Z. Is the Bread Making Technological Ability of Portuguese Traditional Maize Landraces Associated with Their Genetic Diversity? *Maydica* **2009**, *54*, 297–311.
3. Brites, C.; Trigo, M.J.; Santos, C.; Collar, C.; Rosell, C.M. Maize-Based Gluten-Free Bread: Influence of Processing Parameters on Sensory and Instrumental Quality. *Food Bioprocess*. Technol. **2010**, *3*, 707–715.
4. Patto, M.C.V.; Mendes-Moreira, P.M.; Alves, M.L.; Mecha, E.; Brites, C.; do Rosário Bronze, M.; Pego, S. Participatory Plant Quality Breeding: An Ancient Art Revisited by Knowledge Sharing. The Portuguese Experience. In *Plant breeding from laboratories to fields*; InTechOpen: London, UK, **2013**; pp. 255–288.
5. Purlis, E. Browning Development in Bakery Products—A Review. *J. Food Eng.* **2010**, *99*, 239–249.
6. Cornell, H.; Cauvain, S.P. Bread Making: Improving Quality. In *Fish Shellfish Immunology*; Dalmo, R.A., Bogwald, J., Eds.; Woodhead Publishing: Cambridge, UK, **2003**; Volume 384.
7. Schwab, W.; Davidovich-Rikanati, R.; Lewinsohn, E. Biosynthesis of Plant-derived Flavor Compounds. *Plant J.* **2008**, *54*, 712–732.
8. Pico, J.; Gómez, M.; Bernal, J.; Bernal, J.L. Analytical Methods for Volatile Compounds in Wheat Bread. *J. Chromatogr. A* **2016**, *1428*, 55–71.
9. Galoburda, R.; Straumite, E.; Sabovics, M.; Kruma, Z. Dynamics of Volatile Compounds in Triticale Bread with Sourdough: From Flour to Bread. *Foods* **2020**, *9*, 1837.
10. Pétel, C.; Onno, B.; Prost, C. Sourdough Volatile Compounds and Their Contribution to Bread: A Review. *Trends Food Sci.* Technol. **2017**, *59*, 105–123.
11. Pozo-Bayón, M.A.; Guichard, E.; Cayot, N. *Flavor Control in Baked Cereal Products*; Taylor & Francis Group: Philadelphia, PA, USA, 2006; Volume 22, ISBN 8755-9129.
12. Paraskevopoulou, A.; Chrysanthou, A.; Koutidou, M. Characterisation of Volatile Compounds of Lupin Protein Isolate-enriched Wheat Flour Bread. *Food Res. Int.* **2012**, *48*, 568–577.
13. Martins, S.I.F.S.; Jongen, W.M.F.; Van Boekel, M.A.J.S. A Review of Maillard Reaction in Food and Implications to Kinetic Modelling. *Trends Food Sci. Technol.* **2000**, *11*, 364–373.
14. Bailey, M.E.; Gutheil, R.A.; Hsieh, F.-H.; Cheng, C.-W.; Gerhardt, K.O. Maillard reaction volatile compounds and color quality of a whey protein concentrate—Corn meal extruded product. In *Thermally Generated Flavors*; Thomas, H.P., Michael, J.M., Robert, J.M., Eds.; ACS Publications: Washington, DC, USA, **1993**; Chapter 25; pp. 315–327.
15. Birch, A.N.; Petersen, M.A.; Hansen, Å.S. The Aroma Profile of Wheat Bread Crumb Influenced by Yeast Concentration and Fermentation Temperature. *LWT-Food Sci. Technol.* **2013**, *50*, 480–488.
16. Hansen, A.; Schieberle, P. Generation of Aroma Compounds during Sourdough Fermentation: Applied and Fundamental Aspects. Trends Food Sci. *Technol.* **2005**, *16*, 85–94.

17. Birch, A.N.; Petersen, M.A.; Hansen, Å.S. Review: Aroma of Wheat Bread Crumb. *Cereal Chem.* **2014**, *91*, 105–114.

18. Jensen, S.; Oestdal, H.; Skibsted, L.H.; Larsen, E.; Thybo, A.K. Chemical Changes in Wheat Pan Bread during Storage and How It Affects the Sensory Perception of Aroma, Flavour, and Taste. J. *Cereal Sci.* **2011**, *53*, 259–268.

19. Bastos, D.M.; Monaro, É.; Siguemoto, É.; Séfora, M.; Markowicz, D.; Monaro, E.; Siguemoto, E.; Sefor, M. Maillard Reaction Products in Processed Food: Pros and Cons. In *Food Industrial Processes—Methods and Equipment*; Benjamin, V., Ed.; InTechOpen: London, UK, **2012**.

20. Pico, J.; Bernal, J.; Gómez, M. Wheat Bread Aroma Compounds in Crumb and Crust: A Review. *Food Res. Int.* **2015**, *75*, 200–215.

21. Belitz, H.-D.; Grosch,W.; Schieberle, P. Cereals and Cereal Products. *Food Chem.* **2009**, 670–745.

22. Ravyts, F.; De Vuyst, L. Prevalence and Impact of Single-Strain Starter Cultures of Lactic Acid Bacteria on Metabolite Formation in Sourdough. *Food Microbiol.* **2011**, *28*, 1129–1139.

23. Makhoul, S.; Romano, A.; Capozzi, V.; Spano, G.; Aprea, E.; Cappellin, L.; Benozzi, E.; Scampicchio, M.; Märk, T.D.; Gasperi, F. Volatile Compound Production During the Bread-Making Process: Effect of Flour, Yeast and Their Interaction. Food Bioprocess. *Technol.* **2015**, *8*, 1925–1937.

24. Park, D.; Maga, J.A. Identification of Key Volatiles Responsible for Odour Quality Differences in Popped Popcorn of Selected Hybrids. *Food Chem.* **2006**, *99*, 538–545.

25. Berger, R.G. *Flavours and Fragrances: Chemistry, Bioprocessing and Sustainability*; Springer Science & Business Media: Berlin, Germany, 2007; ISBN 3540493395.

26. Cho, I.H.; Peterson, D.G. Chemistry of Bread Aroma: A Review. *Food Sci. Biotechnol.* **2010**, *19*, 575–582.

27. Starr, G.; Hansen, T.S.; Petersen, M.A.; Bredie, W.L.P.P.; Hansen, Å.S.; Petersen, M.A.; Bredie, W.L.P.P.; Hansen, T.S.; Petersen, M.A.; Bredie,W.L.P.P. Aroma of Wheat Porridge and Bread-Crumb Is Influenced by the Wheat Variety. *LWT Food Sci. Technol.* **2015**, *63*, 590–598.

28. Moskowitz, M.R.; Bin, Q.; Elias, R.J.; Peterson, D.G. Influence of Endogenous Ferulic Acid in Whole Wheat Flour on Bread Crust Aroma. J. Agric. *Food Chem.* **2012**, *60*, 11245–11252.

29. Zhao, Q.; Yao, S.; Ou, S.Y. Maillard Volatiles in Baked Products as Affected by Feruloylated Oligosaccharides from Maize Bran. *Int. J. Food Prop.* **2017**, *20*, 3266–3273.

30. Mildner-Szkudlarz, S.; Róz˙an´ ska, M.; Piechowska, P.; Was´kiewicz, A.; Zawirska-Wojtasiak, R. Effects of Polyphenols on Volatile Profile and Acrylamide Formation in a Model Wheat Bread System. *Food Chem.* **2019**, *297*.

31. Barden, L.; Decker, E.A. Lipid Oxidation in Low-Moisture Food: A Review. *Crit. Rev. Food Sci. Nutr.* **2016**, *56*, 2467–2482.

32. Bento-Silva, A.; Patto, M.C.V.; do Rosário Bronze, M.; Vaz Patto, M.C.; do Rosário Bronze, M.; Patto, M.C.V.; do Rosário Bronze, M. Relevance, Structure and Analysis of Ferulic Acid in Maize Cell Walls. *Food Chem.* **2018**, *246*, 360–378.

33. Ibrahim, U.K.; Salleh, R.M.; Maqsood-ul-Haque, S.N.S. Bread towards Functional Food: An Overview. *ETP Int. J. Food Eng.* **2015**, *1*, 39–43.

34. Bredie, W.L.P.; Mottram, D.S.; Hassell, G.M.; Guy, R.C.E. Sensory Characterisation of the Aromas Generated in Extruded Maize and Wheat Flour. *J. Cereal Sci.* **1998**, *28*, 97–106.

35. Bredie, W.L.P.; Mottram, D.S.; Guy, R.C.E. Aroma Volatiles Generated during Extrusion Cooking of Maize Flour. *J. Agric. Food Chem.* **1998**, *46*, 1479–1487.

36. Buttery, R.G.; Ling, L.C.; Stern, D.J. Studies on Popcorn Aroma and Flavor Volatiles. J. Agric. *Food Chem.* **1997**, *45*, 837–843.

37. Paradiso, V.M.; Summo, C.; Pasqualone, A.; Caponio, F. Evaluation of Different Natural Antioxidants as Affecting Volatile Lipid Oxidation Products Related to Off-Flavours in Corn Flakes. *Food Chem.* **2009**, *113*, 543–549.

38. Bento-Silva, A.; Duarte, N.; Mecha, E.; Belo, M.; Vaz Patto, M.C.; Bronze, M.D. Hydroxycinnamic Acids and Their Derivatives in *Broa*, a Traditional Ethnic Maize Bread. *Foods* **2020**, *9*, 1471.

39. Brites, C.M.; Trigo, M.J.; Carrapiço, B.; Alviña, M.; Bessa, R.J. Maize and Resistant Starch Enriched Breads Reduce Postprandial Glycemic Responses in Rats. *Nutr. Res.* **2011**, *31*, 302–308.

40. Alves, M.L.; Bento-Silva, A.; Gaspar, D.; Paulo, M.; Brites, C.; Mendes-Moreira, P.; Bronze, M.D.R.; Malosetti, M.; Van Eeuwijk, F.; Vaz Patto, M.C. Volatilome-Genome-Wide Association Study on Wholemeal Maize Flour. *J. Agric. Food Chem.* **2020**, *68*, 7809–7818.

41. Bento-Silva, A.; Duarte, N.; Mecha, E.; Belo, M.; Serra, A.T.; Vaz Patto, M.C.; Bronze, M.R. Broa, an Ethnic Maize Bread, as a Source of Phenolic Compounds. *Antioxidants* **2021**, *10*, 672.

42. Alves, M.L.; Brites, C.; Paulo, M.; Carbas, B.; Belo, M.; Mendes-Moreira, P.M.R.; Brites, C.; Bronze, M.D.; Gunjača, J.; Šatović, Z. Setting up Decision-Making Tools toward a Quality-Oriented Participatory Maize Breeding Program. *Front. Plant. Sci.* **2017**, *8*, 2203.

43. Carbas, B.; Vaz-Patto, M.C.; Bronze, M.R.; Bento-Da-Silva, A.; Trigo, M.J.; Brites, C. Maize Flour Parameters That Are Related to the Consumer Perceived Quality of 'Broa' Specialty Bread. *Food Sci. Technol.* **2016**, *36*, 259–267.

44. Klensporf, D.; Jeleñ, H.H. Analysis of Volatile Aldehydes in Oat Flakes by SPME-GC/MS. *Polish J. food Nutr. Sci.* **2005**, *14*, 389.

45. Saerens, S.M.G.; Delvaux, F.; Verstrepen, K.J.; Van Dijck, P.; Thevelein, J.M.; Delvaux, F.R. Parameters Affecting Ethyl Ester Production by Saccharomyces Cerevisiae during Fermentation. *Appl. Environ. Microbiol.* **2008**, *74*, 454–461.

46. Lu, X.; Vora, H.; Khosla, C. Overproduction of Free Fatty Acids in E. Coli: Implications for Biodiesel Production. *Metab. Eng.* **2008**, *10*, 333–339.

47. Hamilton-Kemp, T.; Newman, M.; Collins, R.; Elgaali, H.; Yu, K.; Archbold, D. Production of the Long-Chain Alcohols Octanol, Decanol, and Dodecanol by *Escherichia Coli*. *Curr. Microbiol.* **2005**, 51, 82–86.

48. Somashekar, D.; Venkateshwaran, G.; Sambaiah, K.; Lokesh, B.R. Effect of Culture Conditions on Lipid and Gamma-Linolenic Acid Production by Mucoraceous Fungi. *Process. Biochem.* **2003**, *38*, 1719–1724.

49. Acree, T.; Arn, H. Flavornet and Human Odor Space. Available online: http://www.flavornet.org/ (accessed on 15 June 2021).

50. Linstrom, P.J.; Mallard, W.G. NIST Chemistry Webbook; NIST Standard Reference Database No. 69. **2021**. Available online: http://webbook.nist.gov/chemistry/ (accessed on 15 June 2021).

51. El-Sayed, A.M. The Pherobase: Database of Insect Pheromones and Semiochemicals. **2019**. Available online: http://www.pherobase.com (accessed on 15 June 2021).

52. Sigma-Aldrich Co. Flavors & Fragrances. 2021. Available online: http://www.sigmaaldrich.com/industries/flavors-andfragrances.html. (Accessed on 15 June 2021).

53. Goodner, K.L. Practical Retention Index Models of OV-101, DB-1, DB-5, and DB-Wax for Flavor and Fragrance Compounds. *LWT Food Sci. Technol.* **2008**, *41*, 951–958.

54. Peinado, R.A.; Moreno, J.; Medina, M.; Mauricio, J.C. Changes in Volatile Compounds and Aromatic Series in Sherry Wine with High Gluconic Acid Levels Subjected to Aging by Submerged Flor Yeast Cultures. *Biotechnol. Lett.* **2004**, *26*, 757–762.

55. Oertling, H.; Gomann, C.; Vom Ende, M. Dihydrobenzofuran Derivatives as Fragrance and/or Flavoring. Materials. Patent 9045714, 2 June 2015.

56. Izzreen, M.N.N.Q.; Hansen, Å.S.; Petersen, M.A. Volatile Compounds in Whole Meal Bread Crust: The Effects of Yeast Level and Fermentation Temperature. *Food Chem.* **2016**, *210*, 566–576.

57. Bryant, R.J.; McClung, A.M. Volatile Profiles of Aromatic and Non-Aromatic Rice Cultivars Using SPME/GC–MS. *Food Chem.* **2011**, *124*, 501–513.

58. Ghiasvand, A.R.; Setkova, L.; Pawliszyn, J. Determination of Flavour Profile in Iranian Fragrant Rice Samples Using Cold-fibre SPME–GC–TOF–MS. *Flavour Fragr. J.* **2007**, *22*, 377–391.

59. Jing, G.; Li, T.; Qu, H.; Yun, Z.; Jia, Y.; Zheng, X.; Jiang, Y. Carotenoids and Volatile Profiles of Yellow-and Red-Fleshed Papaya Fruit in Relation to the Expression of Carotenoid Cleavage Dioxygenase Genes. *Postharvest Biol. Technol.* **2015**, *109*, 114–119.

60. Simkin, A.J.; Schwartz, S.H.; Auldridge, M.; Taylor, M.G.; Klee, H.J. The Tomato Carotenoid Cleavage Dioxygenase 1 Genes Contribute to the Formation of the Flavor Volatiles Β-ionone, Pseudoionone, and Geranylacetone. *Plant J.* **2004**, *40*, 882–892.

61. Vogel, J.T.; Tan, B.-C.; McCarty, D.R.; Klee, H.J. The Carotenoid Cleavage Dioxygenase 1 Enzyme Has Broad Substrate Specificity, Cleaving Multiple Carotenoids at Two Different Bond Positions. *J. Biol. Chem.* **2008**, *283*, 11364–11373.

62. McGorrin, R.J. Key Aroma Compounds in Oats and Oat Cereals. J. Agric. *Food Chem.* **2019**, *67*, 13778–13789.

63. Choi, S.; Seo, H.-S.; Lee, K.R.; Lee, S.; Lee, J.; Lee, J. Effect of Milling and Long-Term Storage on Volatiles of Black Rice (Oryza Sativa, L.) Determined by Headspace Solid-Phase Microextraction with Gas Chromatography–Mass Spectrometry. *Food Chem.* **2019**, *276*, 572–582.

64. Feng, X.; Pan, L.;Wang, Q.; Liao, Z.;Wang, X.; Zhang, X.; Guo,W.; Hu, E.; Li, J.; Xu, J.; et al. Nutritional and Physicochemical Characteristics of Purple Sweet Corn Juice before and after Boiling. *PLoS ONE* **2020**, *15*, 1–18.

65. Supavititpatana, P.; Wirjantoro, T.I.; Raviyan, P. Characteristics and Shelf-Life of Corn Milk Yogurt. Chiang Mai Univ. *J. Nat. Sci.* **2010**, *9*, 133–150.

66. de Abreu e Lima, F.; Li, K.; Wen, W.; Yan, J.; Nikoloski, Z.; Willmitzer, L.; Brotman, Y. Unraveling Lipid Metabolism in Maize with Time-resolved Multi-omics Data. *Plant J.* **2018**, *93*, 1102–1115.

67. Martínez, M.M.; Calviño, A.; Rosell, C.M.; Gómez, M. Effect of Different Extrusion Treatments and Particle Size Distribution on the Physicochemical Properties of Rice Flour. Food Bioprocess. Technol. **2014**, *7*, 2657–2665.

68. Galliard, T.; Gallagher, D.M. The Effects of Wheat Bran Particle Size and Storage Period on Bran Flavour and Baking Quality of Bran/Flour Blends. *J. Cereal Sci.* **1988**, *8*, 147–154.

69. Singh, N.; Singh, S.; Shevkani, K. Maize: Composition, bioactive constituents, and unleavened bread. In *Flour and breads and their fortification in health and disease prevention*, 2nd ed.; Victor, P., Ronald, W., Eds.; Elsevier: Amsterdam, The Netherlands, **2019**; pp. 111–121.

70. Leppik, E.; Tammaru, T.; Frérot, B. A View of Diel Variation of Maize Odorscape. *Am. J. Plant. Sci.* **2014**, *2014*.

71. Tamanna, N.; Mahmood, N. Food Processing and Maillard Reaction Products: Effect on Human Health and Nutrition. *Int. J. Food Sci.* **2015**, *2015*.

72. Gobbetti, M.; Simonetti, M.S.; Corsetti, A.; Santinelli, F.; Rossi, J.; Damiani, P. Volatile Compound and Organic Acid Productions by Mixed Wheat Sour Dough Starters: Influence of Fermentation Parameters and Dynamics during Baking. *Food Microbiol.* **1995**, *12*, 497–507.

73. Zheng, L.W.; Chung, H.; Kim, Y.S. Effects of Dicarbonyl Trapping Agents, Antioxidants, and Reducing Agents on the Formation of Furan and Other Volatile Components in Canned-Coffee Model Systems. *Food Res. Int.* **2015**, *75*, 328–336.

74. Heiniö, R.L.; Liukkonen, K.H.; Myllymäki, O.; Pihlava, J.M.; Adlercreutz, H.; Heinonen, S.M.; Poutanen, K. Quantities of Phenolic Compounds and Their Impacts on the Perceived Flavour Attributes of Rye Grain. *J. Cereal Sci.* **2008**, *47*, 566–575.

75. Tian,W.; Chen, G.; Tilley, M.; Li, Y. Changes in Phenolic Profiles and Antioxidant Activities during the Whole Wheat Bread-Making Process. *Food Chem.* **2021**, *345*, 128851.

76. Sordini, B.; Veneziani, G.; Servili, M.; Esposto, S.; Selvaggini, R.; Lorefice, A.; Taticchi, A. A Quanti-Qualitative Study of a Phenolic Extract as a Natural Antioxidant in the Frying Processes. *Food Chem.* **2019**, *279*, 426–434.

77. Oral, R.A.; Dogan, M.; Sarioglu, K. Effects of Certain Polyphenols and Extracts on Furans and Acrylamide Formation in Model System, and Total Furans during Storage. *Food Chem.* **2014**, *142*, 423–429.

78. Favreau-farhadi, N.; Pecukonis, L.; Barrett, A. The Inhibition of Maillard Browning by Different Concentrations of Rosmarinic Acid and Epigallocatechin-3-Gallate in Model, Bakery, and Fruit Systems. *J. Food Sci.* **2015**, *80*, 2140–2146.

79. Vichi, S.; Mayer, M.N.; León-Cárdenas, M.G.; Quintanilla-Casas, B.; Tres, A.; Guardiola, F.; Batlle, I.; Romero, A. Chemical Markers to Distinguish the Homoand Heterozygous Bitter Genotype in Sweet Almond Kernels. *Foods* **2020**, *9*, 747.

80. Buttery, R.G.; Ling, L.C. Additional Studies on Flavor Components of Corn Tortilla Chips. *J. Agric. Food Chem.* **1998**, *46*, 2764–2769.

81. León, A.E.; Rosell, C.M. De Tales Harinas, Tales Panes: Granos, Harinas y Productos de Panificación En Iberoamérica; Hugo Báez: Córdoba, Argentina, **2007**; ISBN 9789871311071.

82. Burin, V.M.; Marchand, S.; De Revel, G.; Bordignon-Luiz, M.T. Development and Validation of Method for Heterocyclic Compounds in Wine: Optimization of HS-SPME Conditions Applying a Response Surface Methodology. *Talanta* **2013**, *117*, 87–93.

83. Liang, R.; Huang, J.; Wu, X.; Fan, J.; Xu, Y.; Wu, C.; Jin, Y.; Zhou, R. Investigating the Differences of Flavor Profiles between Two Types of Soy Sauce by Heat-Treatment. *Int. J. Food Prop.* **2019**, *22*, 1998–2008.

84. Arendt, E.K.; Ryan, L.A.M.; Dal Bello, F. Impact of Sourdough on the Texture of Bread. *Food Microbiol.* **2007**, *24*, 165–174.

85. Karahadian, C.; Johnson, K.A. Analysis of Headspace Volatiles and Sensory Characteristics of Fresh Corn Tortillas Made from Fresh Masa Dough and Spray-Dried Masa Flour. *J. Agric. Food Chem.* **1993**, *41*, 791–79.

CHAPTER 5

Comprehensive two-dimensional gas chromatography as a powerful strategy for the exploration of broas *volatile composition*[1]

Abstract

Broa is a Portuguese maize bread with characteristic sensory attributes that can only be achieved using traditional maize varieties. This study intends to disclose the volatile compounds that are mainly associated with the baking process of *broas* and that can be important contributors to their aroma. Twelve *broas* were prepared from twelve maize flours (eleven traditional open pollinated varieties and one commercial hybrid) and their volatile compounds were analysed by GC×GC–ToFMS (two-dimensional gas chromatography coupled with time-of-flight mass spectrometry), for an untargeted screening of the chemical compounds mainly formed during baking. It was possible to identify 128 volatiles which belong to the main chemical families formed during this stage. Among these, only 16 had been previously detected in *broas*. The most abundant were furans, furanones, and pyranones, but the most relevant for the aroma of *broas* were ascribed to sulphur-containing compounds, in particular to dimethyl trisulfide and methanethiol. Pyrazines might contribute negatively to the aroma of *broas*, since they were present in higher amounts in the commercial *broa*. In summary, this work constitutes the most detailed study so far towards the characterization of *broas* volatile compounds, particularly those formed during the Maillard reaction. These findings may contribute to the characterization of other maize-based foodstuffs, ultimately improving the production of foods with better sensory features.

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1. Introduction

Bread is considered one of the most important foodstuffs around the world [1]. In Portugal, whole maize flour is used in the production of a traditional bread known as *broa* [2], which was considered one of the 50 world's best breads by CNN Travel, in 2019 [3]. Over the last centuries, several traditional open pollinated maize varieties have been developed for the production of high-quality *broas* with improved flavours and aromas that are not possible to achieve using the presently available more productive commercial maize hybrids [2,4].

The aroma of bread plays a key role on its acceptance by consumers [1,5,6]. The nature of aroma is very complex [7,8], and the contribution of an individual compound to the overall aroma depends on several factors, as its odour description and threshold (the intensity perceived by olfaction), adsorption to the food matrices, content, and interactions with other volatiles [5,7,9–11]. In addition, the sensory description of a volatile may change depending on its concentration, therefore influencing, positively or negatively, the overall food aroma [12]. Only a small portion of the volatile compounds contribute to the overall aroma of bread [5,7,8,10–12]. These volatiles are often referred to as "key" or "character-impact" compounds [11,13,14] and are generally present at trace levels [5,15], while other compounds present in high concentrations are only scarcely perceived by the human nose [5]. Frequently, a large number of potent aroma compounds must blend to give the integrated aroma and flavour perception of a certain food [16].

The origin of bread volatiles is difficult to determine [17], once they can result from lipid oxidation, fermentation and baking reactions [1,11]. However, it is at the baking stage that some of the most valuable aroma impact compounds are generated, since they generally have low odour thresholds [18] and desirable sensory characteristics [7,9,15,19–21]. These reactions are often quoted as nonenzymatic browning reactions [21] and include mainly the Maillard reaction and, to a lower extent, caramelization reactions [7,11]. Around 2 to 3% of sugars present in the dough undergo caramelization [22] giving rise to carbonyl compounds, furans, and brown-coloured complex polymers [5,7,11,14,21,22]. The much more relevant Maillard reaction occurs between carbonyls (most often reducing sugars) and free amino groups of amino acids, peptides, or proteins [5,7,9,18,20,23]. The main steps of this reaction are summarized in ***Figure 1.4, Chapter 1***. In the initial stage, free amino groups and reducing sugars condense and originate the Amadori or Heyns products [20,24]. Then, at the intermediate stage, furans, furanones, pyrans and pyranones are formed [20,24], following by amino acids degradation (Strecker reaction), when volatile compounds are formed without the need of sugars [25]. Ultimately, condensation and polymerization occur, leading to the formation of highly coloured melanoidins and several aroma compounds, including pyrazines, pyrroles, pyridines, furans, oxazoles, thiazoles and thiophenes [7,11,15,20,21,26]. A previous study on *broas* has suggested that the volatiles produced at the baking stage, in special pyranones, may positively contribute to the taste and aroma of this ethnical bread [27].

The volatile composition of breads has been usually characterized by HS-SPMS-GC-MS techniques (1D-GC) [27]. However, taking into account the complexity of breads aroma and the presence of very low amounts of important key volatiles [5,7,8,10,11], an analysis with a highly sensitive technique, as comprehensive two-dimensional gas chromatography (GC×GC) [13,28], may allow the detection of important Maillard volatiles, otherwise difficult or even impossible to identify. GC×GC employs two independent columns to separate sample analytes, and therefore the separation potential is greatly enhanced. ToFMS (time-of-flight mass spectrometry) is used as a detector for unambiguous identification, and ensures high selectivity throughout the chromatogram [28]. GC×GC–ToFMS has been recently used for untargeted food analyses, such as fruits, rice, hazelnuts, coffee and beverages [29,30]. To the best of our knowledge, this technique was only employed for a targeted analysis of 2-acetyl-1-pyrroline in wheat and gluten-free breads [31] and has never been used for a comprehensive characterization of the volatile composition of breads.

Taking advantage of the powerful GC×GC–ToFMS technique, the present research involved an in-depth investigation of the volatile compounds of *broas*, aiming at the disclosure of those that might be important contributors to the typical aroma of this ethnic bread. The knowledge of *broas* volatiles may also be useful for the identification of relevant compounds in other foods, which currently remain unidentified. Ultimately, the data obtained in this work can be explored for the purpose of fingerprinting [29] of *broas* prepared from traditional varieties and contribute to a better knowledge of the Maillard reaction, which is essential to design foods that present sensory attributes demanded by the consumers [15,31].

2. Materials and methods

2.1. Samples

Eleven *broas* (B1 to B11) were prepared from eleven traditional open pollinated maize varieties, described in ***Table 1.4, Chapter 1***. One *broa* (B12) prepared from a commercial maize hybrid variety was also studied. *Broas* were prepared following a traditional recipe, described in ***Section 7, Chapter 1***.

2.2. HS-SPME methodology

The HS-SPME methodology used was based on the previous work developed by Bento-Silva *et al.*, 2021 [27]. Briefly, 4 g of *broas* (whole bread, including both crumb and crust) were smashed manually and placed in a 20 mL vial. A 50/30 µm DVB/CAR/PDMS (divinylbenzene/carboxen/polydimethylsiloxane) fibre purchased from Supelco (Sigma-Aldrich, USA) was used to concentrate the volatile compounds present in the headspace. Samples were extracted for 40 min in a thermostated bath adjusted to 60.0 ± 0.1 °C at 250 rpm. All samples were extracted in duplicate.

2.3. GC×GC-ToFMS analysis

The GC×GC-ToFMS experimental parameters were adapted from Costa *et al.* (2020) [32]. The equipment used was a LECO Pegasus 4D GC×GC-ToFMS system (LECO, St. Joseph, MI, USA) consisting of an Agilent GC 7890A gas chromatograph (Agilent Technologies, Inc., Wilmington, DE), with a dual stage jet cryogenic modulator (licensed from Zoex) and a secondary oven, and a mass spectrometer equipped with a Time-of-Flight (ToF) analyser.

The SPME fibre was manually introduced into the port at 250 °C for analytes desorption. The injection port was lined with a 0.75 mm I.D. glass liner. Splitless conditions (30 s) were used. An Equity-5 30 m × 0.32 mm I.D., 0.25 μm film thickness (Supelco, Bellefonte, PA, USA) was used as first-dimension column (^{1}D) and a DB-FFAP 0.79 m × 0.25 mm I.D., 0.25 μm film thickness (J&W Scientific Inc., Folsom, CA, USA) was used as a second-dimension column (^{2}D). The carrier gas was helium at a constant flow rate of 2.50 mL min^{-1}. The primary oven temperature was programmed from 35 °C (5 min) to 230 °C (2 min) at 10 °C min^{-1} and the secondary oven program was 30 °C offset above the primary one. Both the MS transfer line and MS source temperatures were set at 250 °C. The modulation period was 5 s, keeping the modulator at 20° C offset above primary oven, with hot and cold pulses by periods of 0.80 and 1.70 s, respectively. The mass spectrometer was running in the EI mode at 70 eV and detector voltage of –1530V, using an *m/z* range of 35-350.

In an initial approach, the total ion chromatograms were processed using the automated data processing software ChromaTOF® (LECO) at signal-to-noise (S/N) threshold of 100. The obtained GC×GC total ion chromatogram contour plots exhibited more than 1200 peaks. As the present study was focused on the compounds that are mainly produced during baking, in a second approach, all the compounds belonging to the characteristic chemical families associated with the Maillard and caramelization reactions were selected. In addition, the volatile compounds described in the literature as key-aromas of breads or maize-based foods and which were not detected using the automated data processing (S/N < 100) were searched on the chromatograms based on extracted ion chromatogram contour plots of the characteristic ions, and the corresponding peak areas were also included.

For identification purposes, the mass spectrum and retention times (^{1}D and ^{2}D) of the analytes were compared with the mass spectral libraries, namely an in-house library of standards and two commercial databases (Wiley 275 and US National Institute of Science and Technology

(NIST) V. 2.0 – Mainlib and Replib). Additionally, the linear retention index (LRI) was experimentally determined according to van den Dool and Kratz LRI equation [33]. A C_8-C_{20} *n*-alkanes series was used for LRI determination (the solvent *n*-hexane was used as C_6 standard), and these values were compared with those reported in the literature for chromatographic columns similar to the ^{1}D column mentioned above. A positive identification was considered when the experimental spectra shared at least an 80% similarity with spectra from the software libraries and the LRI deviation was less than 5%. This difference in LRI takes into account that (i) the literature data is obtained from a large range of GC stationary phases (several commercial GC columns are composed of 5% phenylpolysilphenylene-siloxane or equivalent stationary phases), and (ii) the modulation causes some inaccuracy in first dimension retention time, and the majority of the values reported in the literature were determined in a 1D-GC separation system [34]. In order to confirm the identification of some compounds which did not match all the criteria described above, the Log P values were calculated using ALOGPS 2.1 [35].

The DTIC (deconvoluted total ion current) GC×GC peak area data were used as an approach to estimate the relative content of each volatile component in the different samples.

2.4. Statistical Analysis

The peak areas data of all studied compounds were extracted from the chromatograms and used to build the full data matrix consisting of 12 *broas* samples and 128 variables. After data normalization, hierarchical cluster analysis (HCA) and Spearman's coefficient correlations, both combined with heatmap visualizations, were applied for this dataset, using the MetaboAnalyst 3.0 (web software, The Metabolomics Innovation Centre (TMIC), Edmonton, AB, Canada). Independent-samples *t*-tests, ANOVA followed by post hoc Tukey tests and principal component analyses (PCA) were obtained using the software SPSS version 21 (IBM, NY, USA). The limit of significance was set at $p < 0.05$.

3. Results and discussion

Eleven *broas* (B1 to B11) prepared from eleven traditional maize varieties and one *broa* (B12) prepared from a commercial hybrid maize flour, were studied [***Table 1.4, Chapter 1***] [27]. A contour plot of the total ion current chromatogram of a *broa* sample (B1) under study is presented at ***Figure 5.1***. In a first approach, the presence of volatiles belonging to the chemical families mostly associated with baking were identified in the chromatograms of all studied samples (n = 12). A total number of 128 volatile compounds belonging to these families were detected and are described in ***Table 5.1***. More than 80% of the compounds have been identified according to the criteria described in ***Section 2.3***, ***Chapter 5***.

The peak areas corresponding to the studied volatiles were measured and their average in the twelve studied *broas* is presented in ***Table 5.1***. The ratio of the peak area to odour threshold values in water (OT, in the Log_{10} form) was used as a screening method for the identification of the most relevant volatiles to the aroma of *broas*. Calculation of the odour activity value (OAV, ratio of the concentration to OT), is often carried out to evaluate the most aroma-active compounds in a food product. A compound might be sensed when OAV > 1 (or > 0, when the Log_{10} form is considered) [18,30,32,33]. Although differences in peak areas among different compounds do not give a direct information about their relative concentration, they greatly varied (sometimes more than 10,000-fold), suggesting they were present in very distinct concentrations. Thus, the higher the ratio, the higher the probability of a compound to contribute to the aroma of this breads. The table also includes some examples of foods where the compounds have been detected (visually represented in ***Figure D.1, Appendix D***).

The results will be presented as follows: firstly, the advantages of the analysis of *broas* volatile compounds by GC×GC–ToFMS will be discussed by providing some examples that allowed the identification of unreported compounds. Secondly, the compounds will be explored according to their chemical families, and those which may be more relevant to the aroma of *broas* will be suggested. Finally, the results from the analysed samples will be compared and the correlations among their volatiles will be studied, in order to elucidate which compounds may be responsible for the differences in their aroma.

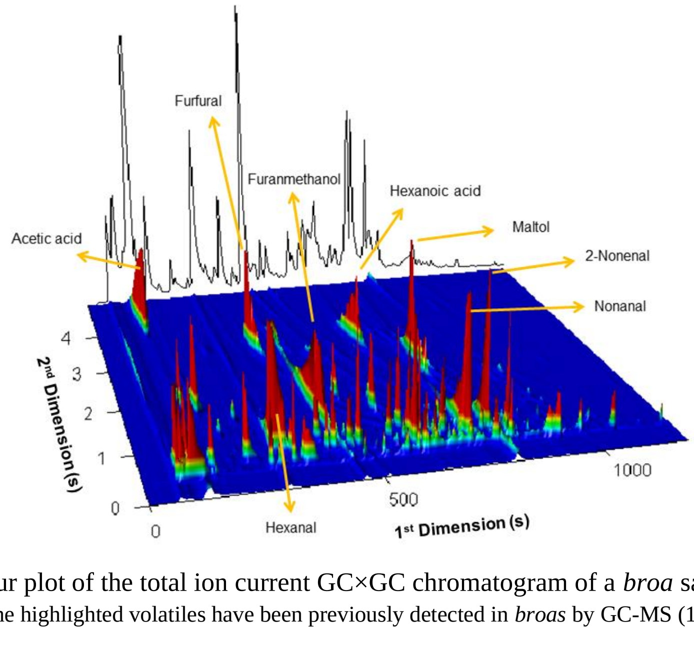

Figure 5.1: Contour plot of the total ion current GC×GC chromatogram of a *broa* sample (B1) under study. The highlighted volatiles have been previously detected in *broas* by GC-MS (1D) [27].

Table 5.1: Volatiles characteristic from browning reactions identified in *broas*, their average peak areas, odour and taste descriptors, and odour thresholds; and examples of foods where the compounds have been detected.

ID: Peak identification; **^1t_R**: retention time for the first dimension (seconds); **^2t_R**: retention time for the second dimension (seconds); **CAS**: Chemical Abstracts Service registry number; ***m/z***: mass-to-charge ratio (ions are ordered according to their decreasing intensities); **SI**: similarity index (‰); **LRI_c**: linear retention index obtained through the modulated chromatogram; **LRI_{lit}**: linear retention index reported in the literature for Equity-5 column or equivalents [27,38–46]; **OT**: Odour threshold values in water [16,36,47–54]; **PA**: average peak area (n=12); **PA/OT**: ratio between PA and OT; **TI**: tentatively identified; **n/a**: not applicable; **n/f**: not found; **n/i**: not identified. Aroma and taste descriptors were obtained from the literature [5,6,13,24,45,46,53,55–59].
Abbreviations used for other maize-based foods or breads where the compounds have been detected [5,6,7,10,16,18,24,27,31,36,37,44,47,53,60–66]:
MF: maize flour; **B**: *broa* (compounds highlighted in bold); **WB**: wheat bread; **WSB**: wheat sourdough bread; **WRB**: wheat-rye bread; **RSB**: rye sourdough bread; **GFB**: gluten-free bread; **MSB**: maize starch bread; **CWB**: Chinese white bread; **BAG**: bagels; **WFB**: whole fino bread; **TB**: triticale bread; **CB**: crisp bread; **ME**: maize extrudates; **P**: popcorn; **T**: maize tortilla; **TC**: tortilla chips; **TS**: taco shell; **WP**: maize meal extruded product with whey protein; **MJ**: maize juice; **BMJ**: boiled maize juice; **RS**: rye sourdough; **TF**: tortilla flour.

ID	^1t_R	^2t_R	Compound	Odour and taste descriptors	CAS	Formula	*m/z*	SI	LRI_c	LRI_{lit}	PA	OT (ppb)	Log_{10} PA/OT	Foods
Furans														
F1	95	0.38	2,3-Dihydrofuran	Pungent	1191-99-7	C_4H_6O	70, 41, 39, 29	891	600	n/f	3100662 ± 2605740	n/f	n/a	WRB
F2	100	0.40	2-Methylfuran or 3-Methylfuran (TI)	Ethereal, acetone, chocolate n/f	534-22-5 930-27-8	C_5H_6O	82, 53, 39, 27	912 900	606	605 611	3101036 ± 1363010	90450 n/f	1.5 n/a	P, RSB, TB, WB, WRB RSB, WB
F3	130	0.43	2,3-Dihydro-5-methylfuran	n/f	1487-15-6	C_5H_8O	43, 84, 39, 27, 69	872	641	n/f	1630190 ± 846164	n/f	n/a	n/i
F4	140	0.40	Tetrahydrofuran	Ethereal	109-99-9	C_4H_8O	42, 72, 27	942	647	633	15225039 ± 4244701	7375-177000	1.9-3.3	WB, WP
F5	155	0.47	2-Ethylfuran	Rubbery, pungent, acid, sweet	3208-16-0	C_6H_8O	81, 53, 96, 39	975	662	689	2595043 ± 621119	n/f	n/a	P, RS, WB
F6	160	0.47	2,5-Dimethylfuran	Chemical, ethereal, meaty, gravy, juice, bacon	625-86-5	C_6H_8O	43, 96, 53, 81, 27, 39	916	667	667	843893 ± 397252	n/f	n/a	WRB
F7	270	0.58	2-Propylfuran	n/f	4229-91-8	$C_7H_{10}O$	81, 53, 110, 39, 27	973	780	792	613397 ± 229105	n/f	n/a	WB
F8	295	0.59	2-Ethyl-5-methylfuran	Fresh, gassy, burnt	1703-52-2	$C_7H_{10}O$	95, 110, 43	877	803	803	357713 ± 172466	n/f	n/a	n/i
F9	325	0.60	2,3,5-Trimethylfuran	n/f	10504-04-8	$C_7H_{10}O$	110, 109, 43, 95, 67	896	819	817	64938 ± 50557	n/f	n/a	WB
F10	325	3.65	3-Furfural [3-furancarboxaldehyde]	Almond-like	498-60-2	$C_5H_4O_2$	95, 39, 67, 29	942	820	832	4539412 ± 1121048	n/f	n/a	GFB, NSB, P, RSB, WB
F11	355	0.80	2-Vinyl-5-methylfuran	n/f	10504-13-9	C_7H_8O	108, 65, 43, 79, 56, 93	889	835	826	295635 ± 120061	n/f	n/a	n/i
F12	360	3.43	**Furfural [2-furancarboxaldehyde]**	Woody, almond, sweet, fruity, flowery	98-01-1	$C_5H_4O_2$	96, 39, 29	966	839	843	200344626 ± 78855174	3000-23000	3.9-4.8	B, BAG, CWB, ME, GFB, NSB, P, T, TB, TC, S, WB, WSB
F13	425	1.70	**Furfuryl alcohol [2-furanmethanol]**	Weak, fermented, creamy, caramel	98-00-0	$C_5H_6O_2$	39, 41, 53, 81, 70, 98	949	872	865	404124163 ± 136284119	2000	5.3	B, CWB, ME, GFB, NSB, P, RSB, T, TB, TC, TF, TS, WB, WP, WSB

F14	465	0.56	**2-*n*-Butyl furan**	Green	4466-24-4	$C_8H_{12}O$	81, 53, 39, 27	951	892	898	5012989 ± 1768099	50800	2.0	B, RS, WB
F15	490	1.77	Furfuryl formate	Ethereal	13493-97-5	$C_6H_6O_3$	81, 53, 39, 44	918	909	902	643862 ± 233472	n/f	n/a	WB
F16	495	1.77	**2-Acetylfuran [1-(2-furanyl)-ethanone]**	Smoky, roasty	1192-62-7	$C_6H_6O_2$	95, 39, 43, 67	921	912	915	49074458 ± 10718656	10000	3.7	B, CWB, ME, GFB, P, RSB, T, TC, TF, WB, WP, WSB
F17	555	1.37	1-(2-Furyl)-2-propanone [2-furyl acetone]	Herbal, caramel, fruity, spicy, radish, green, burnt	6975-60-6	$C_7H_8O_2$	81, 43, 53, 124	915	957	954	2719968 ± 2692555	n/f	n/a	WB
F18	565	1.61	**5-Methyl furfural [5-methyl-2-furancarboxaldehyde]**	Almond, sweet, bitter	620-02-0	$C_6H_6O_2$	110, 53, 39, 43, 81	979	964	966	33471223 ± 8857491	500	4.8	B, ME, GFB, NSB, P, RSB, TB, WB
F19	565	2.98	5-methyl-2-furanmethanol [5-methylfurfuryl alcohol]	Bread-like, honey, sweet	3857-25-8	$C_6H_8O_2$	95, 112, 43, 69, 53	913	965	966	8379336 ± 4226245	11.9	5.8	BAG, CWB, WB
F20	600	0.53	**2-Pentylfuran**	Butter, green bean, floral, fruity, mushroom, raw nuts	3777-69-3	$C_9H_{14}O$	81, 53, 39	934	989	993	55139336 ± 14203476	6	7.0	B, BAG, ME, GFB, MF, MJ, NSB, P, RS, TC, TF, TS, WB, WP, WRB
F21	605	1.05	Benzofuran	Styrene, aromatic	271-89-6	C_8H_6O	118, 89, 63, 39, 51, 45	892	993	996	470358 ± 286276	n/f	n/a	n/i
F22	605	1.53	Isomaltol [2-acetyl-3-hydroxyfuran]	Caramel-like	3420-59-5	$C_6H_6O_3$	111, 126, 43, 55, 84	918	994	989	392890 ± 417850	n/f	n/a	n/i
F23	610	1.12	Furfuryl acetate [2-furanmethanol acetate]	Sweet, fruity, banana-like, horseradish, ethereal, green	623-17-6	$C_7H_8O_3$	81, 98, 43, 52, 140	920	997	998	5202352 ± 2702948	n/f	n/a	WB
F24	625	1.21	1-(2-Furanyl)-1-propanone	Fruity	3194-15-8	$C_7H_8O_2$	95, 39, 45, 74, 67, 57	872	1011	1016	2589462 ± 1364329	n/f	n/a	WP
F25	645	1.22	2-Acetyl-5-methylfuran	Sweet, nutty with a caramel nuance, cocoa-like with a toasted bready nuance	1193-79-9	$C_7H_8O_2$	109, 124, 43, 53	928	1030	1042	7472295 ± 7428702	n/f	n/a	n/i
F26	655	1.13	2,2'-Bifuran [2-(2-furanyl)furan]	Vegetable, garlic	5905-00-0	$C_8H_6O_2$	134, 78, 105, 51, 39	893	1039	1047	998680 ± 662678	n/f	n/a	WB
F27	705	0.50	**Methylpentylfuran**	n/f	-	$C_{10}H_{20}O$	95, 152, 43, 67	892	1086	1083	3099292 ± 2322514	n/f	n/a	B
F28	705	0.95	2-Furfurylfuran [2,2'-Methylenebisfuran]	Rich, roasted, aromatic	1197-40-6	$C_9H_8O_2$	91, 148, 120, 39, 65	863	1087	1086	451285 ± 232810	n/f	n/a	n/i
F29	710	3.24	5-Formylfurfural [2,5-furandicarboxaldehyde]	n/f	823-82-5	$C_6H_4O_3$	124, 77	879	1094	1084	5731606 ± 2639042	n/f	n/a	WB
F30	715	0.51	2-Hexylfuran	n/f	3777-70-6	$C_{10}H_{16}O$	81, 53, 39, 41, 95, 123	905	1096	1096	463776 ± 178720	n/f	n/a	n/i
F31	715	0.77	2-Butyl-tetrahydrofuran	n/f	1004-29-1	$C_8H_{16}O$	71, 41, 55	864	1096	1096	3919696 ± 3110078	n/f	n/a	n/i
F32	715	3.29	Furyl hydroxymethyl ketone [1-(2-furanyl)-2-hydroxyethanone]	n/f	17678-19-2	$C_6H_6O_3$	95, 39, 126, 29, 67	948	1098	1088	21691749 ± 9667403	n/f	n/a	n/i
F33	805	1.32	1-(5-Methyl-2-furanyl)-2-hydroxyethanone	n/f	-	$C_7H_8O_3$	109, 56, 69, 43, 140	833	1191	n/f	1550219 ± 734005	n/f	n/a	n/i
F34	865	4.97	**2,3-Dihydrobenzofuran**	Musky notes	496-16-2	C_8H_8O	120, 91, 65, 51	911	1265	1222	2807760 ± 735738	n/f	n/a	B, BMJ
F35	905	1.17	Difurfuryl ether [2,2'-[oxybis(methylene)]*bis*-furan]	Coffee, mushroom-like, nutty, earthy	4437-22-3	$C_{10}H_{10}O_3$	81, 56, 27, 39, 97, 110	944	1308	1305	240009 ± 95353	n/f	n/a	n/i

F36	935	4.45	Hydroxymethylfurfural [5-(Hydroxymethyl)furfural]	Fatty, buttery, musty, waxy, caramel, herbal, tobacco	67-47-0	$C_6H_6O_3$	97, 126, 41, 69, 53	907	1325	1266	22784687 ± 12185059	1000000	1.4	P, RSB, WSB
						Furanones								
Fo1	245	3.32	2-Furanone (TI) [2(3H)-furanone]	n/f	20825-71-2	$C_4H_4O_2$	55, 84, 27, 53, 39, 44	873	757	914 (DB-1)	2079421 ± 625998	n/f	n/a	n/i
Fo2	305	1.58	Dihydro-2-methyl-3-(2H)-furanone [2-methyltetrahydro-3-furanone]	Spicy, rancid, butter	3188-00-9	$C_5H_8O_2$	43, 72, 100	974	809	812	19616932 ± 16891970	n/f	n/a	WB, WP
Fo3	420	3.11	5-Methyl-2(3H)-furanone	Sweet, oily, coconut, tobacco, creamy, vanilla, hay	591-12-8	$C_5H_6O_2$	55, 98, 43, 27, 70	851	870	869	235195 ± 69497	n/f	n/a	n/i
Fo4	480	0.83	5-Methyl-5-furfuryl-2(5H)-furanone [5-(2-furanylmethyl)-5-methyl-2(5H)-furanone] (TI)	n/f	31969-27-4	$C_{10}H_{10}O_3$	81, 53, 39, 69	751	901	n/f	1263633 ± 1567577	n/f	n/a	WB
Fo5	505	4.88	2(5H)-Furanone [2,5-dihydrofuranone]	n/f	497-23-4	$C_4H_4O_2$	55, 84, 27, 29, 39	940	922	918	43176086 ± 16137613	n/f	n/a	WB, CWB
Fo6	540	2.77	5-Methyl-2(5H)-furanone	n/f	591-11-7	$C_5H_6O_2$	69, 41, 39, 98	942	943	938	6155177 ± 2897005	n/f	n/a	WB
Fo7	550	1.31	3-Methyl-2,5-furandione or 2,5-Dimethyl-3(2H)-furanone (TI)	n/f n/f	616-02-4 14400-67-0	$C_5H_4O_3$	39, 68, 40, 28, 53, 112	706 926	953	949 924 (DB-1)	1818359 ± 629312	n/f	n/a	WB n/i
Fo8	555	1.93	γ-Valerolactone [dihydro-5-methyl-2(3H)-furanone]	Herbal, sweet, warm, tobacco, cocoa, woody, coconut	108-29-2	$C_5H_8O_2$	56, 85, 41, 43	958	956	956	3864613 ± 1759554	n/f	n/a	WB, CWB
Fo9	555	1.91	5,5-Dimethyl-2(5H)-furanone	n/f	20019-64-1	$C_6H_8O_2$	97, 69, 43, 54, 26, 112	891	957	958	250199 ± 71863	n/f	n/a	n/i
Fo10	560	1.90	Dihydro-3-methyl-2(3H)-furanone or Dihydro-4-methyl-2(3H)-furanone [3-methylbutyrolactone] (TI)	n/f n/f	1679-47-6 1679-49-8	$C_5H_8O_2$	41, 56, 27, 100	910 917	961	958 919	2130675 ± 842009	n/f n/f	n/a n/a	n/i n/i
Fo11	565	1.84	Dihydro-3-methyl-2(3H)-furanone or Dihydro-4-methyl-2(3H)-furanone [3-methylbutyrolactone] (TI)	n/f n/f	1679-47-6 1679-49-8	$C_5H_8O_2$	56, 85, 41, 43, 100	895 891	964	958 919	502907 ± 283902	n/f n/f	n/a n/a	n/ n/i
Fo12	570	1.87	n/i	n/a	-	$C_6H_8O_2$	97, 69, 43, 26, 54	n/a	968	n/a	183534 ± 115610	n/a	n/a	n/a
Fo13	575	1.24	5-Ethyl-(3H)-furan-2-one [2-ethylbutenolide]	Spicy	2313-01-1	$C_6H_8O_2$	55, 112, 83, 97	910	971	954	612612 ± 196297	n/f	n/a	WB
Fo14	615	1.64	2,5-Dihydro-3,5-dimethyl 2-furanone	n/f	5584-69-0	$C_6H_8O_2$	69, 41, 115, 97	884	1002	993	973653 ± 442005	n/f	n/a	n/i
Fo15	655	1.95	5-Ethyl-2(5H)-Furanone (TI)	Spicy	2407-43-4	$C_6H_8O_2$	28, 83, 18, 55, 44	897	1040	984 (DB-1)	5650222 ± 2621703	n/f	n/a	WB
Fo16	655	1.79	3,4-Dimethyl-2,5-furandione [2,3-dimethyl maleic anhydride]	n/f	766-39-2	$C_6H_6O_3$	39, 54, 82, 126	882	1040	1038	3343389 ± 1471465	n/f	n/a	n/i
Fo17	665	4.90	***R*-Pantolactone**	Cotton candy, licorice, smoky, toasted bread	599-04-2	$C_6H_{10}O_3$	71, 43, 29, 57	951	1047	1043	2268814 ± 1125826	50	4.7	MF
Fo18	670	2.82	4-Methyl-2(5H)-furanone	n/f	6124-79-4	$C_5H_6O_2$	69, 41, 39, 98	924	1055	n/f	2855095 ± 885123	n/f	n/a	n/i

Fo19	675	1.45	**γ-*N*-Caprolactone [γ-hexalactone, 5-ethyldihydro-2(3H)-furanone]**	Coumarin-like, sweet	695-06-7	$C_6H_{10}O_2$	85, 42, 56, 70, 114	942	1059	1058	15427139 ± 6696866	50	5.5	B, CWB
Fo20	705	4.02	Furaneol [2,5-dimethyl-4-hydroxy-3(2H)-furanone]	Caramel, strawberry	3658-77-3	$C_6H_8O_3$	43, 57, 128, 85	918	1090	1090	3801345 ± 2827164	60	4.8	WB, BAG, CWB, GFB, NSB, P, RS, TC
Fo21	765	3.00	Solerone (TI) [5-acetyldihydro-2(3H)-furanone]	n/f	29393-32-6	$C_6H_8O_3$	85, 29, 43, 57, 128	937	1151	1299 (SE-54)	2040510 ± 929704	n/f	n/a	n/i
Fo22	775	1.26	γ-Heptalactone [dihydro-5-propyl-2(3H)-furanone]	Sweet, coconut, nutty, caramel, creamy, milky, tobacco	105-21-5	$C_7H_{12}O_2$	85, 29, 56, 41	948	1159	1163	833364 ± 298059	499	3.2	n/i
Fo23	870	1.17	γ-Octalactone [5-butyldihydro-2(3H)-furanone]	Sweet, coconut, waxy, creamy, milky, soapy, fruity	104-50-7	$C_8H_{14}O_2$	85, 41, 56, 100	955	1266	1264	1318130 ± 558277	8	5.2	CWB, TC
Fo24	875	0.89	5-Pentyl-2(3H)-furanone [3-nonen-4-olide]	Tropical, fruity, milky, dairy	51352-68-2	$C_9H_{14}O_2$	98, 111, 55, 83, 70, 154	840	1272	1273	3499672 ± 1292082	n/f	n/a	n/i
Fo25	940	1.29	5-Pentyl-2(5H)-furanone [4-hydroxy-2-nonenoic acid lactone]	Minty, fruity	21963-26-8	$C_9H_{14}O_2$	29, 28, 45, 57, 100, 113, 126, 85, 72	823	1352	1358	10012186 ± 4179765	n/f	n/a	n/i
Fo26	955	1.10	γ-Nonalactone [dihydro-5-pentyl-2(3H)-furanone]	Coconut-like, sweet, fruity	104-61-0	$C_9H_{16}O_2$	85, 114, 41, 55, 99, 137	946	1370	1363	9304844 ± 4182379	9.7-27	5.5-6.0	B, BMJ, CWB, MJ, WSB
							Pyrans							
Pn1	240	0.53	3,4-Dihydro-6-methyl-2H-pyran	n/f	16015-11-5	$C_6H_{10}O$	43, 55, 98, 83	913	749	n/f	134400 ± 30229	n/f	n/a	n/i
Pn2	675	1.50	5,6-Dihydro-2H-pyran-2-carboxaldehyde or 3,4-Dihydro-2H-pyran-2-carboxaldehyde (TI)	n/f n/f	53897-26-0 100-73-2	$C_6H_8O_2$	83, 55, 29, 112, 39	867 860	1059	n/f 853 (OV-101)	1444173 ± 653900	n/f n/f	n/a n/a	n/i
Pn3	915	1.02	2-(1-Butenyl)-tetrahydropyran	n/f	95652-24-7	$C_9H_{16}O$	111, 140, 83, 98, 125	801	1320	n/f	129456 ± 54493	n/f	n/a	n/i
							Pyranones							
Po1	450	1.47	Dihydro-2H-pyran-3(4H)-one (TI)	n/f	23462-75-1	$C_5H_8O_2$	42, 27, 71, 55	947	885	1439 (HP-Wax)	54028 ± 25761	n/f	n/a	n/i
Po2	585	3.05	2H-Pyran-2-one [α-pyrone]	Herbal	504-31-4	$C_5H_4O_2$	39, 68, 96	877	980	978	34118 ± 13808	n/f	n/a	WP
Po3	650	1.58	n/i	n/a	n/a	n/a	68, 39, 98, 53	801 756	1035	n/a	5592787 ± 4970053	n/a n/a	n/a n/a	n/i
Po4	655	2.74	5,6-Dihydro-2H-pyran-2-one (TI)	n/f	3393-45-1	$C_5H_6O_2$	68, 39, 98, 53	929	1041	1838 (DB-Wax)	1621497 ± 558094	n/f	n/a	n/i
Po5	675	2.03	δ-Valerolactone (TI) [tetrahydro-2H-pyran-2-one]	n/f	542-28-9	$C_5H_8O_2$	42, 41, 27, 56, 100, 70	947	1059	965	916387 ± 142550	n/f	n/a	n/i
Po6	715	1.60	δ-Hexalactone [tetrahydro-6-methyl-2H-pyran-2-one, δ-caprolactone]	Creamy, fruity, coconut, spicy	823-22-3	$C_6H_{10}O_2$	42, 70, 55, 99	944	1097	1084	840593 ± 226566	n/f	n/a	WB

Po7	740	2.64	**Maltol [3-hydroxy-2-methyl-4H-pyran-4-one]**	Warmy-fruity, caramel-sweet	118-71-8	$C_6H_6O_3$	126, 71, 43, 55, 97	974	1124	1113	117672149 ± 40223031	2500	4.7	B, P, TB, TC, WB
Po8	795	0.20	**3-Hydroxy-2,3-dihydromaltol [2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one]**	Caramelized	28564-83-2	$C_6H_8O_4$	43, 144, 101, 73, 55	972	1179	1144	29961549 ± 16035481	n/f	n/a	B, P, WB
Po9	975	2.40	2-Hydroxy-3-methyl-4H-pyran-4-one	n/f	61892-88-4	$C_6H_6O_3$	126, 71, 43, 55, 97	818	1397	n/f	37421 ± 25172	n/f	n/a	n/i
							Pyrazines							
Pz1	190	1.25	Pyrazine	Roasted	290-37-9	$C_4H_4N_2$	80, 26, 53	960	704	739	1631354 ± 797679	180000	1.0	WB, ME, GFB, NSB, P, TC, TS, WP
Pz2	345	1.34	**2-Methylpyrazine**	Roasted, burnt, sweet	109-08-0	$C_5H_6N_2$	94, 67, 39, 26, 53	974	830	840	15773787 ± 11169354	60-105000	2.2-5.4	B, WB, BAG, ME, GFB, NSB, P, T, TC, TS, WFB, WP, WRB
Pz3	495	0.90	2,6-Dimethylpyrazine	Roasted	108-50-9	$C_6H_8N_2$	42, 108, 39, 40, 28, 18	972	912	915	23375159 ± 12540414	200-9000	3.4-5.1	WB, CWB, GFB, NSB, P, T, TC, WFB, WP, WRB
Pz4	500	0.91	2-Ethylpyrazine	Popcorn, nutty	13925-00-3	$C_6H_8N_2$	107, 108, 80, 53, 39, 28	926	915	915	2149106 ± 1864070	6000-22000	2.0-2.6	WB, ME, GFB, NSB, P, RSB, T, TC, TS, WRB
Pz5	505	0.93	2,3-Dimethylpyrazine	Popcorn, roasted	5910-89-4	$C_6H_8N_2$	108, 67, 42	929	919	919	2413964 ± 1493485	2500-35000	1.8-3.0	WB, ME, GFB, NSB, P, T, TC, TS
Pz6	615	0.75	2-Ethyl-3-methylpyrazine or 2,3,5-trimethylpyrazine (TI)	Potato-like, earthy Nutty, roasted, sweet	15707-23-0 14667-55-1	$C_7H_{10}N_2$	42, 122, 81, 39	954 936	1001	1001 1005	5256433 ± 2326845	400-1800 130	4.6 3.5-4.1	WB, ME, GFB, NSB, P, RSB, TC, WFB, WP, WRB WB, BAG, ME, GFB, TC
Pz7	630	0.90	2-Ethenyl-6-methylpyrazine [2-methyl-6-vinylpyrazine]	Coffee	13925-09-2	$C_7H_8N_2$	120, 52, 39, 94	834	1015	1023	918205 ± 550329	n/f	n/a	WB, ME, P, T, WP
Pz8	640	1.35	Acetylpyrazine	Biscuit, cracker-like, crust-like, sweet, roasted	22047-25-2	$C_6H_6N_2O$	43, 52, 80, 122, 94, 28, 15	965	1025	1031	418788 ± 223595	62	3.8	WB, GFB, NSB, RSB, WRB
Pz9	700	0.65	2-Ethyl-3,6-dimethylpyrazine [3-ethyl-2,5-dimethylpyrazine] or 2-Ethyl-3,5-dimethylup pyrazine [3-ethyl-2,6-dimethylpyrazine]	Potato, cocoa, roasted, nutty Burnt, roasted, nutty, coffee, caramel, cocoa, maize	13360-65-1 13925-07-0	$C_8H_{12}N_2$	135, 136, 42, 39, 56, 108	918 885	1082	1082 1082	749792 ± 503023	8.6 0.04-1	4.9 5.9-7.3	WB, ME, P, RSB WB, ME, P, T, TC, WRB, WSB
Pz10	740	1.07	2-Acetyl-3-methylpyrazine	Nutty, roasted, hazelnut, corn chip, caramel, potato chip	23787-80-6	$C_7H_8N_2O$	43, 93, 136, 94, 42, 67, 52	864	1122	1128	207115 ± 107124	n/f	n/a	n/i
Pz11	760	0.83	5H-5-Methyl-6,7-dihydrocyclopentapyrazine	Earthy, baked potato, sweet, roasted, corn with savory	23747-48-0	$C_8H_{10}N_2$	119, 134	907	1143	1149	511503 ± 176711	n/f	n/a	n/i
Pz12	875	1.40	2-(2'-Furyl)-pyrazine (TI) [2-(furan-2-yl)pyrazine]	n/f	32736-95-1	$C_8H_6N_2O$	146, 93, 63, 38, 39	816	1272	1255 (DB-1)	293650 ± 139715	n/f	n/a	n/i
							Pyridines and Pyrimidines							
Pd1	350	1.05	2-Methylpyridine	Sweat, astringent, hazelnut, nutty	109-06-8	C_6H_7N	93, 66, 39, 78, 51	958	832	824	349470 ± 195878	n/f	n/a	n/i

Pd2	420	0.98	4-Methylpyrimidine	n/f	3438-46-8	$C_5H_6N_2$	94, 40, 53, 67, 79	802	869	853	9105 ± 6947	n/f	n/a	n/i
Pd3	460	0.79	2,6-Dimethylpyridine [2,6-lutidine]	Nutty, ammoniacal, woody, bready, cocoa, coffee, musty	108-48-5	C_7H_9N	107, 106, 39, 66, 79, 93	924	890	890	306301 ± 109246	n/f	n/a	n/i
Pd4	490	0.79	2-Ethylpyridine (TI)	Green, grassy	100-71-0	C_7H_9N	106, 107, 79, 52, 66, 39	702	908	906	135706 ± 64908	n/f	n/a	T
Pd5	550	0.83	2,3-Dimethylpyridine [2,3-lutidine]	Coffee, caramel	583-61-9	C_7H_9N	107, 106, 39, 66, 79, 92	937	952	952	29778 ± 9134	n/f	n/a	n/i
Pd6	605	0.71	2,4,6-Trimethylpyridine	Aromatic odor	108-75-8	$C_8H_{11}N$	121, 79, 39, 106	842	993	993	167322 ± 96358	n/f	n/a	n/i
Pd7	650	1.18	2-Acetylpyridine [1-(2-pyridinyl)-ethanone]	Biscuit-like, toasted, cracker-like, crust-like, roasted	1122-62-9	C_7H_7NO	79, 121, 93, 43, 51	939	1035	1050	1141684 ± 1282175	19	4.8	ME, P, T, TC
Pd8	810	1.13	1-Acetyl-1,2,3,4-tetrahydropyridine	Nutty	19615-27-1	$C_7H_{11}NO$	85, 125, 83, 68, 43, 54	882	1196	1189	1504556 ± 534232	n/f	n/a	n/i
Pd9	815	0.65	2-Pentylpyridine [2-propylpyridine]	Green, fatty, roasted, tobacco, nutty	622-39-9	$C_8H_{11}N$	93, 106, 120, 79, 65, 39	850	1201	1202	89596 ± 34399	0.6	5.2	n/i
							Pyrroles							
Py1	315	1.04	1-Ethyl-1H-pyrrole	Burnt	617-92-5	C_6H_9N	80, 95, 67, 39, 27, 53	879	814	815	68416 ± 27880	n/f	n/a	ME
Py2	615	1.48	*N*-Methyl-2-formylpyrrole [1-methyl-1H-pyrrole-2-carboxaldehyde]	Roasted, nutty	1192-58-1	C_6H_7NO	109, 108, 53, 80, 39	920	1004	1010	848932 ± 340434	37	4.4	P
Py3	665	1.14	1-Ethyl-2-formyl-1H-pyrrole [1-ethyl-1H-pyrrole-2-carboxaldehyde]	Burnt, roasted, smoky	2167-14-8	C_7H_9NO	123, 94, 39, 108, 66, 53, 80	816	1047	1046	664513 ± 343483	n/f	n/a	ME
Py4	665	0.14	2-Formyl-1H-pyrrole [1H-pyrrole-2-carboxaldehyde]	Musty	1003-29-8	C_5H_5NO	95, 94, 66, 39	949	1048	1047	2142059 ± 1055032	n/f	n/a	ME, P, WB
Py5	670	1.38	1-Methyl-2-pyrrolidinone	Fishlike	872-50-4	C_5H_9NO	44, 42, 99, 98, 28	849	1057	1046	331816 ± 211791	n/f	n/a	n/i
Py6	690	3.46	**2-Acetylpyrrole [1-(1H-pyrrol-2-yl)-ethanone]**	Musty	1072-83-9	C_6H_7NO	94, 109, 66, 39, 43, 53	954	1076	1069	21389485 ± 7698915	170000	2.1	B, BAG, CWB, GFB, NSB, P, TC, WB
Py7	735	1.14	1-Ethyl-2-pyrrolidinone (TI)	Slight amine	2687-91-4	$C_6H_{11}NO$	98, 113, 70, 41, 28	907	1117	1856 (FFAP)	209715 ± 147230	n/f	n/a	n/i
Py8	740	1.42	Ethyl pyrrole 1-acetate (TI)	n/f	5145-67-5	$C_8H_{11}NO_2$	80, 153, 53, 57, 71	718	1123	n/f	995860 ± 1013831	n/f	n/a	n/i
Py9	800	1.20	***N*-Furfurylpyrrole [1-(2-furanylmethyl)-1H-pyrrole]**	Vegetable, plastic, waxy, fruity, cereal, bready, potato	1438-94-4	C_9H_9NO	81, 147, 53, 27, 39	955	1186	1179	775590 ± 358385	100	3.9	B, ME, P, WB, WRB
Py10	910	4.10	**Indole [benzopyrrole]**	Animal, naphthyl, fecal, pungent, musty; in low concentrations: powerful floral notes and pleasant radiation	120-72-9	C_8H_7N	117, 90, 63, 39, 50	954	1318	1295	6532212 ± 1981606	140	4.7	B, WB
Py11	980	1.18	5-Acetyl-2,3-dihydro-1H-pyrrolizine (TI)	Amine, grass, hay, smoky	55041-85-5	$C_9H_{11}NO$	134, 149, 106, 79, 51	910	1402	1382 (DB-1)	807263 ± 424045	n/f	n/a	P

Py12	985	3.01	Skatole [3-methyl-1H-indole]	Animal, fecal, warm, sweet, over-ripe fruit; in low concentrations: may give a note of 'overmature flower'	83-34-1	C_9H_9N	130, 131, 77, 51, 65, 103, 39	940	1411	1410	50749 ± 33736	0.2	5.4	P
Py13	1000	1.49	1-Furfuryl-2-formyl pyrrole [1-methyl-1H-pyrrole-2-carboxaldehyde]	Slightly burnt taste, acid n/f	19377-82-3	$C_{10}H_9NO_2$	81, 175, 53, 39, 147	851	1429	1384	284459 ± 165711	97	3.5	P, WRB
Oxazoles														
Ox1	235	0.93	4,5-Dimethyloxazole	n/f	7064-40-6	C_5H_7NO	97, 43, 55	821	745	750	29777 ± 23570	n/f	n/a	n/i
Ox2	410	0.77	Trimethyloxazole	Nutty, roasted, shellfish, mustard burnt, oily, mushroom	20662-84-4	C_6H_9NO	111, 43, 68, 55	868	864	863	109136 ± 176271	n/f	n/a	WP
Ox3	735	1.34	Benzoxazole (TI)	n/f	273-53-0	C_7H_5NO	119, 64, 91	853	1117	1067 (DB-1)	182454 ± 98420	n/f	n/a	n/i
Thiazoles														
Tz1	195	1.49	Thiazole	Fishy, nutty, meaty	288-47-1	C_3H_3NS	85, 58, 45	967	704	694	294112 ± 230369	n/f	n/a	WB
Tz2	635	1.46	2-Acetylthiazole	Roasty	24295-03-2	C_5H_5NOS	43, 127, 99, 58, 85	944	1020	1018	1155525 ± 300637	10	5.1	WB, ME
Tz3	840	1.45	Benzothiazole	Sulfurous	95-16-9	C_7H_5NS	135, 108, 69, 45	956	1231	1231	1124234 ± 601368	80	4.1	WB
Thiophenes														
Tp1	235	0.72	3-Methylthiophene	Fatty, winey	616-44-4	C_5H_6S	97, 98, 45	968	744	770	22399 ± 9459	n/f	n/a	n/i
Tp2	600	1.97	3-Thiophenecarboxaldehyde	n/f	498-62-4	C_5H_4OS	111, 112, 83, 39	847	990	1003	252769 ± 141357	n/f	n/a	n/i
Tp3	610	1.98	2-Thiophenecarboxaldehyde [2-formylthiophene, thenaldehyde]	Sulfurous, almond, bitter, cherry	98-03-3	C_5H_4OS	111, 112, 83, 58	952	998	1001	762146 ± 260839	n/f	n/a	ME
Tp4	705	1.31	2-Formyl-3-methylthiophene [3-methyl-2-thiophenecarboxaldehyde]	Saffron, camphoreous	5834-16-2	C_6H_6OS	125, 126, 97, 45	845	1087	1109	123821 ± 40906	n/f	n/a	n/i
Tp5	705	1.56	2-Acetylthiophene [1-(2-thienyl)-ethanone]	Sulfurous, nutty, hazelnut, onion	88-15-3	C_6H_6OS	111, 126	829	1087	1085	70861 ± 22816	n/f	n/a	WB
Tp6	745	1.48	2-Formyl-5-methylthiophene (TI) [5-methyl-2-thiophenecarboxaldehyde]	Rancid, fatty, grass	13679-70-4	C_6H_6OS	125, 126, 97, 45	760	1128	1124	90000 ± 35587	n/f	n/a	WP, ME
Other sulphur-containing compounds														
S1	70	0.32	Methanethiol	Rotting cabbage; in low concentrations: tropical fruit, may contribute for the aroma of sweet maize	74-93-1	CH_4S	47, 45, 48	991	575	< 500	6115559 ± 1459463	0.04-82	4.9-8.2	WB, CB
S2	80	0.35	Dimethyl sulphide	Cabbage-like, in low concentrations: canned maize	75-18-3	C_2H_6S	47, 62, 35	978	585	565	1689726 ± 724639	0.3-1	6.2-6.8	WB, CB, P, RS
S3	195	0.69	Dimethyl disulphide	Garlic; in low concentrations: contributes to maize flavor	624-92-0	$C_2H_6S_2$	94, 45, 79, 61	973	703	718	9175781 ± 4840433	12	5.9	WB, CB, ME, GFB, P, RS, TC, WP
S4	220	1.56	1-Methylthiopropane (TI)	Alliceous, creamy, green, leek	3877-15-4	$C_4H_{10}S$	61, 90, 48	714	730	715	100912	n/f	n/a	n/i

			[methyl propyl sulphide]								± 23581			
S5	375	1.58	Methylthio-2-propanone [acetonyl methyl sulphide]	Melon, cabbage, garlic	14109-72-9	C_4H_8OS	43, 61, 104	923	846	863	17904 ± 16085	n/f	n/a	n/i
S6	480	1.10	1,4-Oxathiane, or 1,3-Oxathiane, or 1,2-Oxathiane [thioxane] (TI)	Characteristic n/f Green, grassy, leafy, cortex, foliage, aromatic, vegetable, floral, juicy mango, tropical	15980-15-1 646-12-8 57917-36-9	C_4H_8OS	46, 104, 74, 61	811 863 n/a	901	885 n/f n/f	57599 ± 47389	n/f n/f n/f	n/a n/a n/a	n/i n/i n/i
S7	485	1.52	Methional [3-methylthiopropanal]	Boiled-potato, cooked-potato, malty, waxy	3268-49-3	C_4H_8OS	48, 104, 61, 76	930	905	903	806522 ± 660313	0.2	6.6	WB, CB, CWB, NSB, P, T, TC, TS, WP
S8	565	0.85	Dimethyl trisulphide	Cabbage-like, in low concentrations: tropical fruit/grapefruit	3658-80-8	$C_2H_6S_3$	126, 79, 45, 111	947	964	964	2195136 ± 1508373	0.01	8.3	WB, ME, GFB, P, TC, WFB

3.1. The potentialities of GC×GC–ToFMS in the identification of *broas* volatile compounds

In a previous study on *broas* volatile composition by GC-MS (1D) [27], only 16 out of the 128 volatiles described in the present work were detected [***Table 5.1***], which confirms that GC×GC-ToFMS provides a much higher potential for the detection and identification of foods volatiles [34,67]. It should also be noted that, in this work, a relatively high signal-to-noise (S/N) threshold (100) was used, which limited the number of detected compounds. ***Figures 5.2*** and ***5.3*** illustrate the advantages of GC×GC-ToFMS, which allowed the separation of analytes with similar volatility and common product ions through the secondary ^{2}D column. Considering the set of columns used (non-polar/polar), the decreasing in volatility (high ^{1}tR) is mainly related to the increasing in the number of carbons [32,34], whereas the increase in the ^2t_R corresponds to polarity increasing [32,34]. For instance, 2-butyl-tetrahydrofuran (F31) was not detected in the previous study [27]. This compound was co-eluting with 2-nonanone (^1t_R: 715 s), and both presented similar mass spectra, with identical product ions at *m/z* 71 and 43 [***Figure 5.2***]. However, since they present different polarities, their separation was achieved through the ^{2}D column. 2-Butyl-tetrahydrofuran (F31) exhibits a slightly lower Log P than 2-nonanone (Log P: 2.93 and 3.08, respectively), which supports its relatively high polarity, and therefore the higher retention time in the ^{2}D (^2t_R: 0.77 vs. 0.56 s). Thus, despite the high similarity index of 2-nonanone with the library spectra (89%) obtained in the previous analysis by GC-MS [27], the compound 2-butyl-tetrahydrofuran was also contributing to the peak area considered for 2-nonanone. Without the high resolving power of GC×GC, the separation, identification and relative quantitation of these two compounds would have been a challenge.

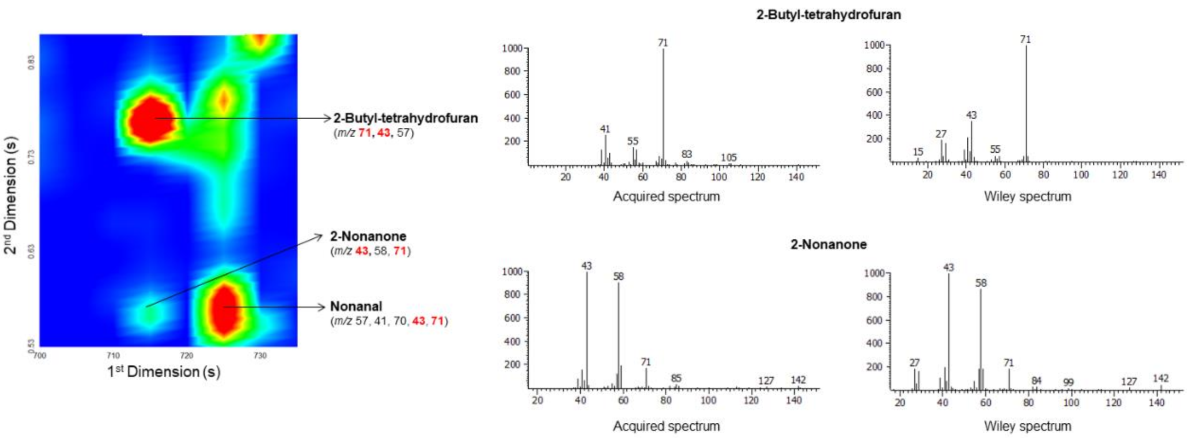

Figure 5.2: Blow-up of a part of a contour plot extracted ion chromatogram at *m/z* 71 from a *broa* sample, showing the separation, through the ^{2}D column, of 2-nonanone and 2-butyl-tetrahydrofuran, which showed the same retention time in the ^{1}D column and shared similar mass spectra.

Another example is the identification of methylpentylfuran (F27). This compound has not been commonly detected in foods, but was previously tentatively identified in *broas* by GC-MS [27]. The results obtained in the present work corroborate this earlier identification. The mass spectrum of F27 showed a high similarity index with the spectra from methylpentylfuran from the Wiley database [***Table 5.1***]. Furthermore, this compound showed a very low retention time of 0.50 s in the ^{2}D column [***Figure 5.3***], suggesting it has a low polarity, which was confirmed by its calculated Log P of 4.43. Although methylpentylfuran usually refers to 3-methyl-2-pentylfuran, F27 can also correspond to other isomers, as 2-methyl-5-pentylfuran or 4-methyl-2-pentylfuran. In addition, several other volatiles, belonging to chemical families which usually arise during baking, were eluting at this ^1t_R (705 s), namely furfurylfuran (F28), 2-formyl-3-methylthiophene (Tp4), 2-acetylthiophene (Tp5) and furaneol (Fo20). Since they show different polarities (Log P of 2.52, 1.38, 1.28 and –0.33, respectively), they were separated through the ^{2}D column (^2t_R: 0.95, 1.31, 1.56 and 4.02, respectively). As can be seen in ***Figure 5.3A***, the peaks intensities of methylpentylfuran (F27) and 3-nonanone were much higher than F28, Tp4, Tp5 and Fo20. Thus, it would have not been possible to identify these compounds without the orthogonal separation through the ^{2}D column [32,34]. The identification of volatile sulphur compounds (***Figure 5.3B***) is particularly relevant, since they are typically present in foods at extremely low levels, often at sub parts-per-billion concentrations, but provide background sensory nuances to the flavour and are often considered "character-impact compounds" [13].

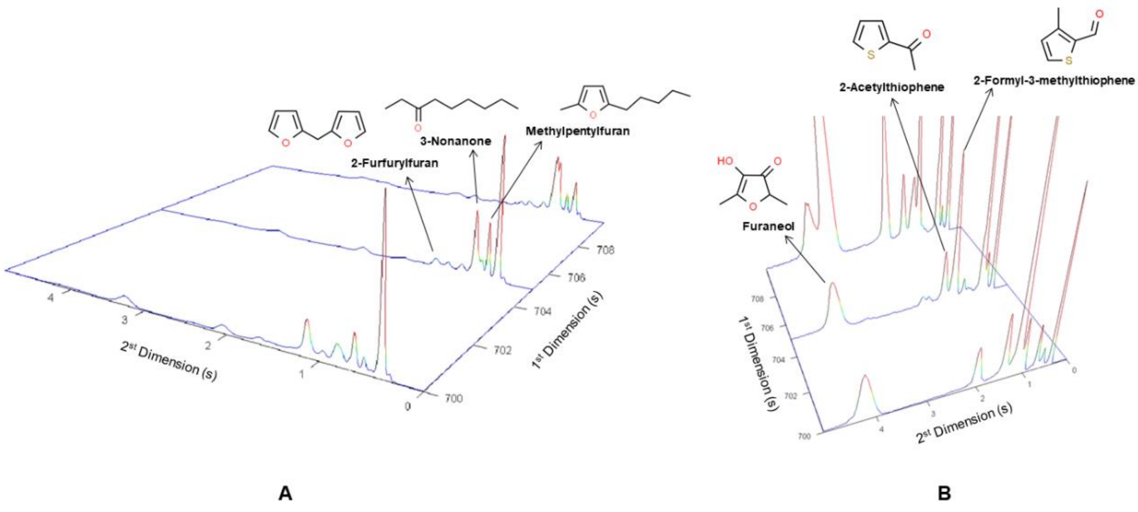

Figure 5.3: Blow-up of (A) total ion GC×GC chromatogram and (B) extracted ion chromatogram at *m/z* 126 and 128, through the ^{2}D column from a *broa* sample, showing the separation of methylpentylfuran (F27), furfurylfuran (F28), 2-formyl-3-methylthiophene (Tp4), 2-acetylthiophene (Tp5), and furaneol (Fo20).

These results show the potentialities of GC×GC-ToFMS for the characterization of food volatiles and demonstrated the complexity of the volatile composition of *broas*. Moreover, this technique also allowed the identification of several compounds belonging to the characteristic chemical classes of the baking process, which have not been commonly described neither in breads nor maize-based foods.

3.2. Exploring the volatile compounds associated with baking

The most relevant compounds of *broas*, selected based on their abundance and, more importantly, their OTs, will be discussed below.

3.2.1. Furans and furanones

Furan and furanone derivatives are among the most common volatiles of heated foods [20,68], and give burnt, pungent, sweet and caramel aroma to foods [9,15,20]. The major furans included 2-pentylfuran (F20) and 2-butylfuran (F14), which have been previously detected in *broas* [27]. 2-Pentylfuran (F20) was particularly abundant and it is a potent odorant [52], giving floral-fruity notes [64], which may significantly contribute to their aroma. It has been reported as the most common aroma-active furan in wheat bread crumb [64] and a likely contributor to the total aroma and flavour of maize tortilla chips [47] and popcorn [37].

Several furans with oxygenated substituents, as furfurals and furanones, were also identified in *broas*. Among all the compounds described in the present work, the highest peak areas were obtained for 2-furanmethanol (F13). Although it is not a very strong odorant [36,52], this compound may also be relevant for the aroma of *broas* due to its abundance. It is usually associated with pleasant, creamy and caramel notes [45], and may be relevant for the aroma of popcorn [37]. Other oxygenated furans include 5-methyl-2-furanmethanol (F19), 3-furfural (F10), and isomaltol (F22), which had not been previously reported in *broas* [27]. Among these, 5-methyl-2-furanmethanol shows a relatively low OT and was confirmed as a key aroma compound of Chinese white breads [53]. It was not possible to confirm the OTs of 3-furfural and isomaltol, but they have been considered important compounds to the aroma of popcorn [37] and wheat breads [5,21], respectively.

Only two out of the twenty-six furanones detected in the present work have been previously detected in *broas*, namely *N*-caprolactone (Fo19), and γ-nonalactone (Fo26). Both of them are possible contributors for *broas* sweet aroma [5,45], since they were present in high amounts in *broas* and are potent odorants [48,51]. γ-Octalactone (Fo23), detected in *broas* for the first time, may also contribute to their aroma, taking into account its abundance and low OT.

3.2.2. Pyrans and pyranones

Pyrans have not been described in similar food products, but some of them may also be important odorants, specially tetrahydropyrans [52]. In contrast, pyranones occur in the volatiles of all heated foods [20], conferring sweet, burnt, pungent and caramel-like flavours and aromas [15]. Maltol (Po7) and 3-hydroxy-2,3-dihydromaltol (Po8) were the most relevant pyranones. These compounds have been previously described as possible positive contributors for the ‘taste and aroma’ of *broas* [27]. Maltol imparts desirable, caramel-like, sweet and fruity characteristics to foods [14,20,21], it is able to mask bitter flavours [14], and it is considered a key odorant in cereal products [69]. Although it has a relatively high OT of 2500-9000 µg L^{-1} [14,47], it may be important for the aroma of *broas* due to its abundance.

3.2.3. Pyrazines

In the present study, twelve pyrazines were identified in *broas*, although only 2-methyl pyrazine (Pz2) had been previously described [27]. Pyrazines are considered impact odorants, with characteristic pleasant nutty and roasted odour notes [7,11,20,63,70] and significantly contribute to the flavour of baked products [11], as breads [5,7,31], popcorn, rye crisp bread [70] and maize products [24]. *Broas* appear to have a lack of pyrazines, when comparing to similar foods. Recent studies have shown that the amount of pyrazines varies greatly between different cereal breads [31,60,71].

Although the OTs values of alkylpyrazines are relatively high (above 1000 µg L^{-1}), [20,70], replacing one or more of the methyl groups with ethyl can give a marked decrease in the OT, and some ethyl-substituted pyrazines have sufficiently low threshold values for them to be important in the roast aroma of cooked foods [20]. Taking into account the OTs described for pyrazines and their amount in *broas*, 2-methylpyrazine (Pz2), 2,6-dimethylpyrazine (Pz3) and, especially, 2-ethyl-dimethylpyrazine (Pz9), may be relevant for their overall aroma. The former shows nutty, cocoa, and roasted meat aromas [70], however, it was described as a possible off-volatile of popcorn [37]. 2,6-Dimethylpyrazine was also considered an important volatile in maize meal extruded product with whey protein [24]. Lastly, Pz9 was tentatively identified as 2-ethyl-3,6-dimethylpyrazine or 2-ethyl-3,5-dimethylpyrazine, since both of them show similar SI and LRI [***Table 5.1***]. Both compounds are potent odorants and contribute to cocoa, nutty, potato and roasted notes [55]. 2-Ethyl-3,5-dimethylpyrazine is a key odorant of maize tortilla chips, popcorn and rye bread crust [7,47,72], whilst 2-ethyl-3,6-dimethylpyrazine is as a key odorant of maize tortilla chips, taco shell [36,47] and popcorn [37].

Some pyrazines, such as 2-acetyl-3-methylpyrazine (Pz10), 5H-5-methyl-6,7-dihydrocyclopentapyrazine (Pz11) and 2-(2'-furyl)-pyrazine (Pz12), have not been detected in related food products, but may still be relevant to the aroma of *broas,* since Pz10 and Pz11 may give maize-like aromas to foods [55].

3.2.4. Pyridines and pyrimidines

Pyridines and pyrimidines were detected in *broas* for the first time. Although they were not present in high amounts, they might still have some relevance for their overall aroma, due to their low OTs. Pyridines give roasted and popcorn odours to foods [14,47].

2-Acetyltetrahydropyridines (or 6-acetyltetrahydropyridines) are possibly the most referred pyridines in similar foodstuffs. They are potent Maillard flavour compounds which contribute substantially to caramel, roasty, and bready aromas [55,73] in several bakery products [14,16,36,73], and are formed by the degradation of proline and hydroxyproline [20]. However, these compounds were not detected in *broas*. Instead, 1-acetyl-1,2,3,4-tetrahydropyridine (Pd8) was identified as the main pyridine. Although it exhibits a similar mass spectrum to that of 2-acetyltetrahydropyridine, Pd8 showed a very intense peak at *m/z* 68 [***Figure D.2, Appendix D***], which is not expected in 2-acetyltetrahydropyridine [74]. This compound has also been identified in the crusts of wheat bread [46] and lupin protein isolate-enriched wheat bread as an important contributor to its aroma profile [46]. Thus, it might also be an important *broa* volatile, contributing to a nutty odour [46].

2-Pentylpyridine (Pd9) may also be relevant for *broas* aroma, contributing to roasted and nutty odours [55]. Although it was present in lower amounts than other pyridines, it shows a very low OT [52]. This compound has not been described in similar foods, probably due to its low concentration, but it has been described in several fried foods [75].

4-Methylpyrimidine (Pd2) was the only pyrimidine detected in *broas*. This compound has not been described neither in breads nor in other maize-based foods, and it was not possible to obtain any information regarding its odour characteristics.

3.2.5. Pyrroles, pyrrolines and oxazoles

Pyrrole derivatives are responsible for roasted odours [14]. Skatole (Py12) has a very low OT of 0.2 µg L^{-1} [16] and may be relevant to the aroma of *broas*. It is usually described as an off-flavour, however, in low concentrations, it may introduce a natural note of ‘overmature flower’ [55].

Possibly one of the most striking results to emerge from this study was the absence of pyrrolines in *broas*. These compounds are abundant breads volatiles, significantly contributing to their flavour [5,7]. Pyrrolines have been described as character impact odorants of the ‘roasted’ and ‘popcorn-like’ notes [7]. Important pyrrolidines described in cereal breads and maize-based foods are 2-acetyl-1-pyrroline and its precursor 1-pyrroline [7,14,20,63]. However, recent studies have shown that the amount of 2-acetyl-1-pyrroline varies greatly among different cereal breads [71]. For instance, 2-acetyl-1-pyrroline seems not to be so relevant to the aroma properties of rye breads [7] and it was not detected in gluten-free breads, which were analysed by GC×GC [31]. By contrast, other authors have found that 2-acetyl-1-pyrroline was higher in gluten-free breads [60]. These differences can be explained by the presence or absence of precursors or interferents in the cereal flours. For instance, differences in the ornithine content of yeasts may play a role in the formation of pyrrolines, since this amino acid has been ascribed as the most important precursor for the formation of 2-acetyl-1-pyrroline during baking [7,11,20]. 2-Acetylpyrroline may also be formed by the degradation of proline and hydroxyproline, similar to 2-acetyl-tetrahydropyridines [20], which were also not found in *broas*, as previously discussed. Thus, differences in the amounts of proline and hydroxyproline may also influence the production of 2-acetylpyrroline and 2-acetyl-tetrahydropyridines. In addition, phenolic acids may inhibit the production of 2-acetyl-1-pyrroline [76], and whole maize flours were used in the preparation of *broas*, which have higher amounts of phenolic compounds, when comparing to other cereals or with refined maize flours [77]. Lastly, 2-acetyl-1-pyrroline is highly volatile and can be oxidized to 2-acetylpyrrole [14,20], which was one of the major volatile compounds detected in the present work.

Three oxazoles were detected in *broas*, but they probably have a low impact on their overall aroma, as it has been reported for other foods [20]. They were present in *broas* in very low amounts, when compared to other compounds.

3.2.6. Sulphur-containing compounds

Sulphur compounds had not been previously reported in *broas* [27], possible due to their low concentrations. Usually, foods from cereals show very low contents in sulphur-containing compounds as a result of the low amounts in sulphur amino acids [20]. However, sulphur-containing Maillard odorants constitute the most powerful aroma compounds of foods, even though they are present at trace levels [14,15]. These compounds have traditionally been associated as unpleasant and noxious off-flavours [13,14], but they contribute to positive flavour characteristics at low concentrations (< 1 µg kg^{-1}), giving tropical, fruity, and savoury aromas to foods [13]. They are considered “character-impact compounds” of bread crust, popcorn and toasted cereal grains [13,15].

Dimethyl sulphide (S2), dimethyl disulphide (S3), and, in particular, dimethyl trisulphide (S8) may be extremely relevant for the aroma of *broas*. At low levels, they all show positive aroma characteristics. Dimethyl trisulphide (S8) has an extremely low OT of 0.01 µg L^{-1} [47] and, when present at low concentrations, is associated to tropical fruit and grapefruit aromas [13]. The most abundant sulphur compound detected in *broas* was dimethyl disulphide (S3), an important contributor to maize flavour [24]. Dimethyl sulphide (S2) is considered a flavour impact compound of sweet maize and conveys the typical flavour impression of canned maize, when present at reduced levels [13]. Methanethiol (S1) was the second most abundant sulphur-containing compound and may also have a significant impact on the aroma of *broas*. It has been described as a possible contributor to the aroma of sweet maize [13].

Another sulphur-containing compound detected in *broas* was the very well-known methional (S7) [70], possibly also one of the most relevant volatiles for the aroma of *broas*. It is a potent [5] and desired odorant [21], with a characteristic potato-like aroma [7,63]. It has been considered a key odorant of fried foods [47], of wheat, rye [7] and Chinese white [53] breads, and may be responsible in part for typical popcorn [37], maize tortilla chips [47] and taco shell [36] aroma characteristics. Furthermore, it has flavour-modifying characteristics, since it has been shown to suppress flavour and aroma [63]. Other aliphatic sulphur compounds detected in *broas* were 1-methylthiopropane (S4) and methylthio-2-propanone (S5). These compounds have not been described in similar foods, and their impact on the overall aroma of *broas* is unknown.

Regarding aromatic sulphur heterocycles derivatives, it was possible to detect three thiazoles and six thiophenes. Thiazoles usually confer green, vegetable-like, cocoa and nutty aromas to foods [20], while thiophenes contribute to sulphurous, nutty and fatty aromas [55]. Among them, 2-acetylthiazole (Tz2) may have a high importance in the aroma of *broas*. It has been described as key volatile of popcorn and maize flour extrudates [44]. It was possible to detect six thiophenes in *broas*, but they have not been so commonly described in similar foods and it was not possible to find information regarding their OTs in the literature.

Another sulphur heterocycle compound detected in *broas* was an oxathiane derivative (S6), which may refer to 1,2-, 1,3- or 1,4-oxathiane. However, taking into account their polarities (Log P of 1.14, 0.43 and 0.36, respectively) and the $^{2}t_{R}$ of S6 (1.10 s), it was tentatively identified as 1,2-oxathiane. This compound has not been described in foods, but oxathiane derivatives have been ascribed as important aroma compounds. The most relevant example is 2-methyl-4-propyl-1,3-oxathiane, a key aroma compound of passion fruit, with a low OT of 3 μg L^{-1} [13]. Recently, this compound and 2,4,4,6-tetramethyl-1,3-oxathiane have been detected in wines [78]. Although the impact of oxathiane on the aroma of foods is currently unknown, it may impact the aroma of *broas*, and this compound is worth further study.

3.3. An overall view of the volatiles associated with baking in *broas*

As a previous study pointed out [79], a sensorial analysis revealed that the *broa* prepared from the available commercial maize hybrid variety (B12) showed the lowest scores for ‘smell and odour’ and ‘taste and aroma’, whereas the traditional *broas* (obtained from the traditional open pollinated varieties) were poorly discriminated among them [79]. A following study revealed that these differences can at least be partially explained by their volatile composition [27]. Thus, in order to further explore these differences, a PCA (principal component analysis) was performed for an easy, rapid and global assessment of the main differences on the volatile composition among the studied *broas*. ***Figure 5.4*** shows the projection of samples and variables in the space defined by the two principal components, corresponding to 59.2% of the total variance. A hierarchical cluster analysis combined with a heatmap representation was also constructed and shown in ***Figure D.3, Appendix D***.

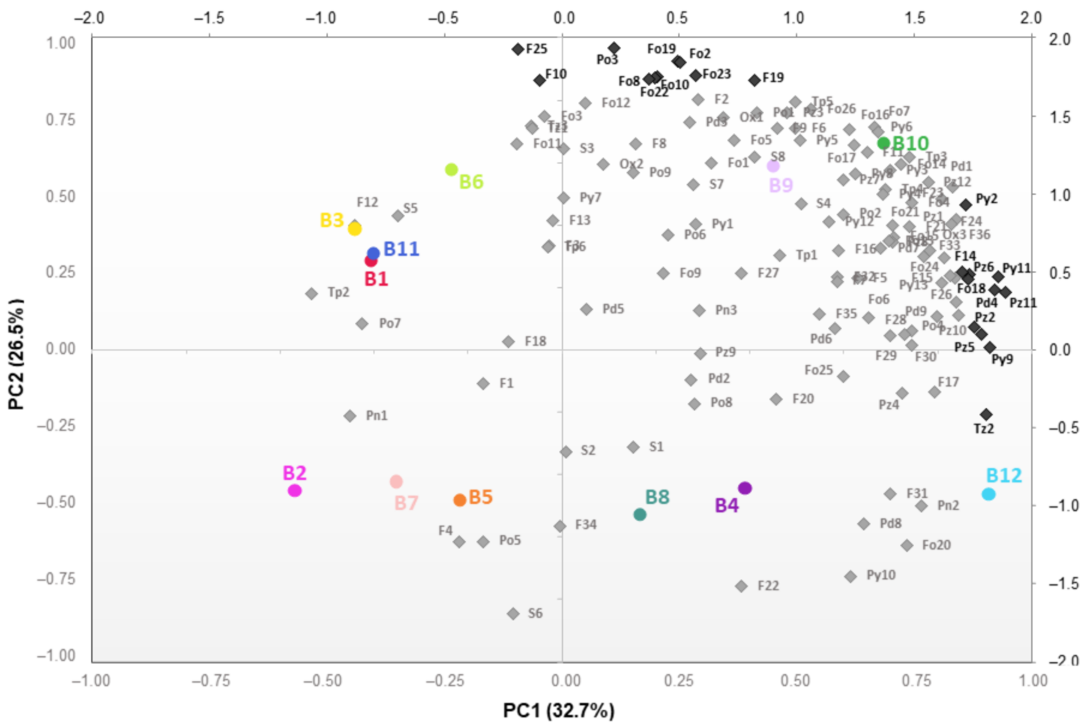

Figure 5.4: Projection of *broas* (coloured dots), and variables (grey) [***Table 5.1***] in the plane defined by PC1 and PC2, corresponding to 59.2% of total variance.

The volatiles that strongly (PC > 0.85) contributed to differentiate the samples along the two PCs are represented in black.

Since all *broas* were prepared following the same procedure, and submitted to the same temperature of kneading and baking, it can be stated that the differences observed among the different *broas* were caused by differences in the corresponding maize flours which affect the Maillard reaction. In order to shed some light on the volatile compounds which may had been formed from the same precursors, a matrix correlation analysis was conducted and represented by a heatmap [***Figure 5.5*** and ***Table D.3***].

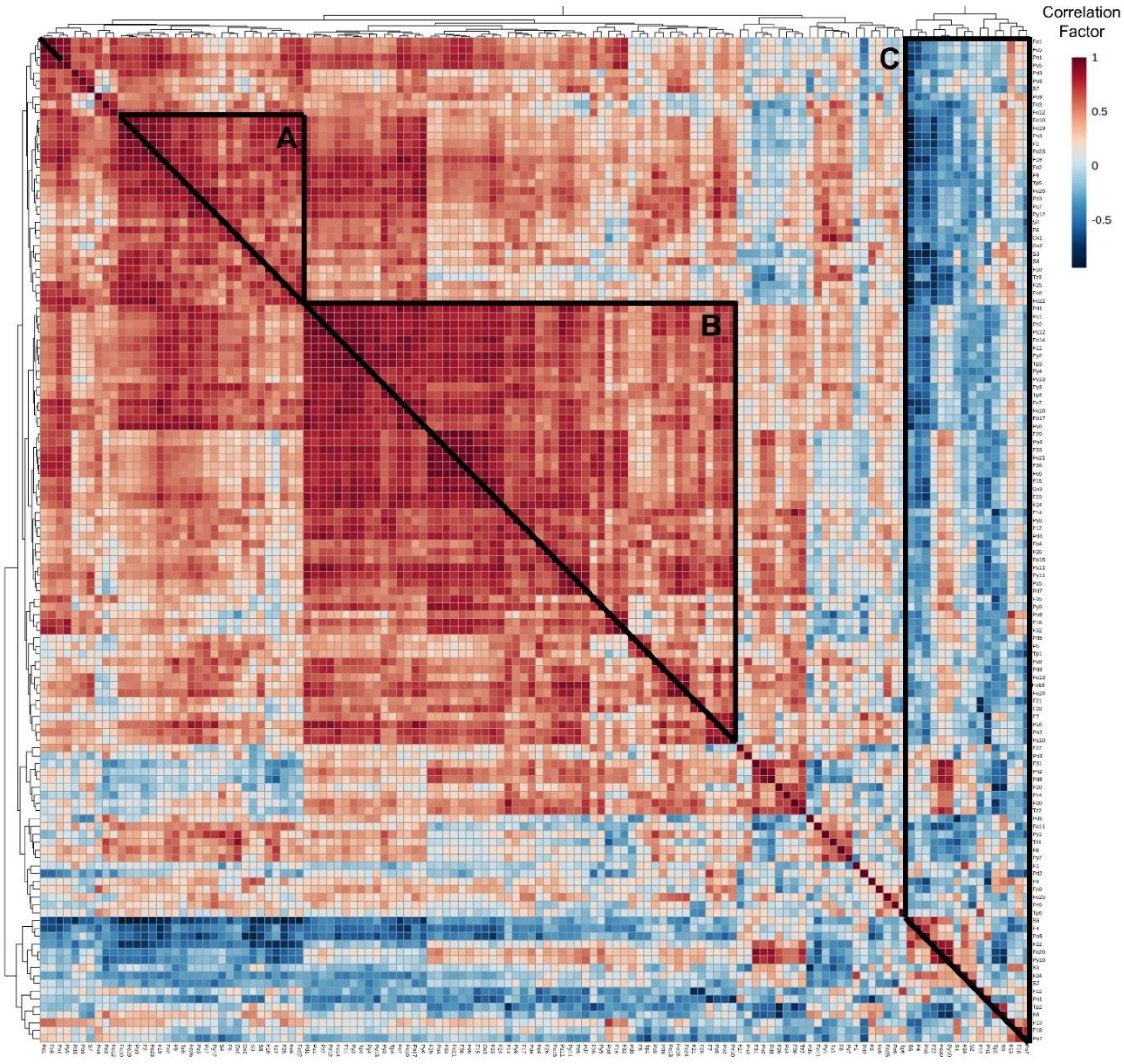

Figure 5.5: Correlation heatmap of the 128 volatile compounds in *broas*.

The correlation coefficients of volatiles are represented through a chromatic scale from deep blue (−1), corresponding to negative correlations, to red (1), corresponding to positive correlations.

The volatiles that strongly contributed to differentiate the samples along PC2 [***Figure 5.4***] are represented in group A of the correlation matrix [***Figure 5.5***] and highlighted in ***Table D.3, Appendix D***. This group is characterized by a simple pattern of strong and positive correlations amongst several furans and furanones. In low-moisture starchy foods, as *broas*, the Maillard reaction seems to be the main route of their formation [80,81]. Thus, higher amounts in precursors of the Maillard reaction, as amino acids and sugars, may have generated *broas* with higher amounts in furans. Alternatively, the presence of interferents, for instance, phenolic compounds [27], may explain these correlations, since they may promote [27,82] or inhibit [76,82] the reaction. In particular, very strong correlations (R > 0.85, $p < 0.05$) were observed among 2-methylfuran (F2), 5-methyl-2-furanmethanol (F19) and 2-acetyl-5-methylfuran (F25), which are usually produced by caramelization reactions and by the breakdown of the Amadori or Heyns intermediates, in the early stages of the Maillard reaction [***Figure 1.4***] [14,19,20]. Strong and positive (R > 0.7, $p < 0.05$) correlations were also found between dihydro-2-methyl-3-(2H)-furanone (Fo2) and sulphur-containing heterocycles. Higher amounts in dihydro-2-methyl-3-(2H)-furanone, which reacts with ammonia and hydrogen sulphide (produced from cysteine by hydrolysis or by Strecker degradation) [19,20,24,83], may directly cause higher amounts of thiazoles and thiophenes.

The volatile compounds which strongly contributed to differentiate the samples along PC1 [***Figure 5.4***], are placed in group B of the correlation heatmap [***Figure 5.5*** and ***Table D.3, Appendix D***], and consisted, among other, in several pyrazines. The major route for the formation of pyrazines is thought to be from α-aminoketones, which are products of the condensation of a dicarbonyl with an amino compound via Strecker degradation, during the Maillard reaction [20,24]. Very strong and positive correlations were found between Pz7 (2-methyl-5-vinylpyrazine) and Pz3 (2,6-dimethylpyrazine), both products from the reaction between leucine and fructose [63]. The *broa* prepared from the available commercial hybrid variety maize flour (B12) showed a higher content in pyrazines ($p < 0.05$) than all the traditional varieties [***Table D.1, Appendix D*** and ***Figure 5.6***], especially in 2-methylpyrazine (Pz2) and 3-dimethylpyrazine (Pz5) [***Table D.2, Appendix D***]. It is possible that the higher amounts in total ferulic acid in the traditional maize varieties [84] might have inhibited the formation of pyrazines [82] in the corresponding *broas*.

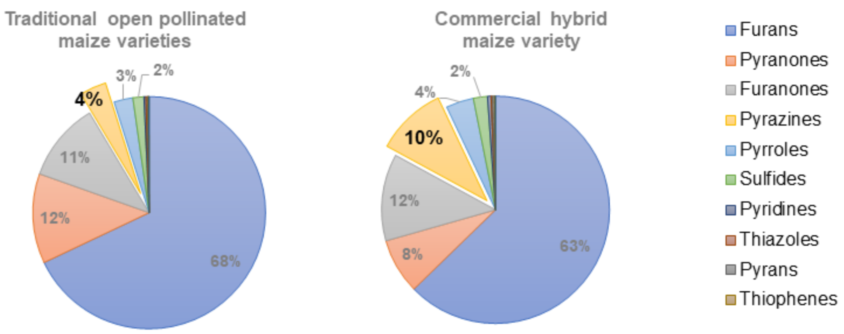

Figure 5.6: Representation of the percentage of chromatogram area for the families of chemical compounds studied in *broas* prepared from traditional maize varieties (n = 11) and from a commercial maize flour.

Lastly, the volatiles which negatively contributed to PC1 and PC2 are located in group C [***Figure 5.5***]. These volatiles show strong and negative correlations with several of the volatiles which belong to groups A and B. Furfural (F12) was strongly and negatively correlated to both benzofuran (F21) and furfurylfuran (F28). Thus, *broas* with higher amounts of furfural also showed lower amounts of benzofuran and furfurylfuran, and vice-versa. Furfural is mainly generated in the initial stages of the Maillard reaction and participates in further reactions [14,24,85]; thus, differences in the precursors which reacted with furfural and generated benzofuran and furfurylfuran, or the presence of interferents of this reaction [27,82], may have influenced its extent. Similarly, negative correlations were found among several sulphur compounds and both furans and furanones, which have been described as important precursors of thiazoles and thiophenes [24]; and between oxathiane (S6) and sulphur-containing compounds, as dimethyl disulphide (S3), dimethyl trisulphide (S8), 2-acetylthiophene (Tp5) and benzothiazole (Tz3), which might have been precursors of the formation of oxathiane. A recent study in wines has proposed that thiols act as precursors of the formation of oxathiane [78].

Taken together, these results suggest that the main differences among the volatile compounds of the studied *broas* are likely due to the presence of specific precursors or interferents which affect the extent of some reaction pathways of the Maillard reaction, as the formation of pyrazines and oxathiane, rather than the overall Maillard reaction. These results can at least partially explain the contradictory reports on the effect of phenolic compounds on the Maillard reaction [27,76,82]. Differences in precursors or interferent of certain pathways of this reaction in maize varieties can therefore cause differences on *broas* aroma.

As previously stated, the analysed commercial hybrid variety sample (B12) showed the lowest scores in a sensorial analysis [79]. The results from the present study have shown that this sample showed a higher content in pyrazines [***Figure 5.6***], particularly in 2-methylpyrazine ($p < 0.05$). These results were somewhat surprising, since pyrazines are strong odorants usually associated with positive sensorial characteristics, giving roasted and nutty aromas to breads [5,55]. However, pyrazines may contribute to aroma characteristics in *broas* that are not typical of this type of bread, giving rise to some of the negative comments associated with B12, as ‘weak typical flavour’, ‘wheat bread flavour’, ‘with a weak maize flavour’ and ‘no history’ [27]. Similarly, it has been reported that most pyrazines contributed negatively to the aroma of popcorn [37]. Besides the higher amount in pyrazines, a closer examination of ***Table D.2, Appendix D*** also showed that 2-butyltetrahydrofuran (F31) was present in B12 in higher ($p < 0.05$) amounts. However, this compound has not been described in similar foods and it was not possible to infer about its impact on *broas* aroma. Therefore, these data should be further explored not only for the characterization of *broas* aroma, but also for the purpose of fingerprinting [29], since both 2-butyltetrahydrofuran and 2-methylpyrazine may be potential biomarkers of the authenticity of *broas* prepared from traditional maize varieties.

Other compounds can contribute to the lower sensorial attributes of B12, namely the higher contents in some pyridines and pyrroles, including the powerful skatole (Py12), often associated with off-aromas [55], and lower contents in maltol (Po7), associated to higher ‘taste and aroma’ [27]. Although the ANOVA showed that these results were not statistically different from some traditional varieties ($p > 0.05$) [***Table D.2, Appendix D***], combinations of volatiles can yield different characteristics than those expected from individual compounds [12,30], and therefore contribute to the lower sensorial attributes of *broas* prepared from the commercial maize hybrid variety under study.

4. Conclusions

The purpose of this study was to extend previous research on the volatile compounds of *broas*, focusing on those mainly associated to the baking process. Almost 90% of the compounds identified in this work have not been previously detected in *broas*. One of the most relevant study findings was the complete absence of pyrrolidines and lack of pyrazines in *broas*, especially when prepared from traditional open pollinated maize varieties. Thus, the absence of pyrrolidines may contribute to the distinctive aroma of *broas* and high amounts of pyrazines may confer negative characteristics associated to the commercial hybrid maize *broa* analysed. Sulphur compounds, as dimethyl trisulphide and methanethiol, were identified as the most likely contributors to the aroma of this ethnic bread. Some volatiles, as oxathiane, have not been previously reported in similar foods and their relevance to the overall aroma of foods is currently unknown. These data obtained in this study can be further explored for the purpose of fingerprinting of traditional *broas*, since some compounds (2-methylpyrazine and 2-butyltetrahydrofuran) were present in significantly higher amounts in the *broa* prepared from the analysed commercial maize hybrid variety. In conclusion, this work represents the most detailed study on the volatile composition of *broas* and may contribute to the disclosure of possible volatiles of other breads and maize-based foods. These findings may have a number of important implications for future practice, since a better knowledge of the volatile compounds that are produced along the Maillard reaction may help to achieve the production of foods with sensory characteristics more appreciated by the consumers.

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References

1. Pico, J.; Gómez, M.; Bernal, J.; Bernal, J.L. Analytical Methods for Volatile Compounds in Wheat Bread. *J. Chromatogr. A* **2016**, *1428*, 55–71.

2. Vaz Patto, M.C.; Alves, M.L.; Almeida, N.F.; Santos, C.; Moreira, P.M.; Satovic, Z.; Brites, C.; Patto, V.; Alves, N.F.; Almeida, C.S.; et al. Is the Bread Making Technological Ability of Portuguese Traditional Maize Landraces Associated with Their Genetic Diversity? *Maydica* **2009**, *54*, 297–311.

3. Smith, J.R. 50 of the World's Best Breads. Available online: https://edition.cnn.com/travel/article/world-50-best-breads/index.html (accessed on 26 March 2022).

4. Vaz Patto, M.C.; Mendes-Moreira, P.M.; Alves, M.L.; Mecha, E.; Brites, C.; Bronze, R.; Pego, S. Participatory Plant Quality Breeding: An Ancient Art Revisited by Knowledge Sharing. The Portuguese Experience. In *Plant breeding from laboratories to fields*; InTechOpen, 2013; pp. 255–288.

5. Pico, J.; Bernal, J.; Gómez, M. Wheat Bread Aroma Compounds in Crumb and Crust: A Review. *Food Res. Int.* **2015**, *75*, 200–215.

6. Lasekan, O.; Dabaj, F.; Muniandy, M.; Juhari, N.H.; Lasekan, A. Characterization of the Key Aroma Compounds in Three Types of Bagels by Means of the Sensomics Approach. *BMC Chem.* **2021**, *15*, 1–13.

7. Cho, I.H.; Peterson, D.G. Chemistry of Bread Aroma: A Review. *Food Sci. Biotechnol.* **2010**, *19*, 575–582.

8. Raffo, A.; Carcea, M.; Castagna, C.; Magrì, A. Improvement of a Headspace Solid Phase Microextraction-Gas Chromatography/Mass Spectrometry Method for the Analysis of Wheat Bread Volatile Compounds. *J. Chromatogr. A* **2015**, *1406*, 266–278.

9. Starowicz, M.; Zieliński, H. How Maillard Reaction Influences Sensorial Properties (Color, Flavor and Texture) of Food Products? *Food Rev. Int.* **2019**, *35*, 707–725.

10. Pétel, C.; Onno, B.; Prost, C. Sourdough Volatile Compounds and Their Contribution to Bread: A Review. *Trends Food Sci. Technol.* **2017**, *59*, 105–123.

11. Pozo-Bayón, M.A.; Guichard, E.; Cayot, N. *Flavor Control in Baked Cereal Products*; 2006; Vol. 22; ISBN 8755912060.

12. Chambers IV, E.; Koppel, K. Associations of Volatile Compounds with Sensory Aroma and Flavor: The Complex Nature of Flavor. *Molecules* **2013**, *18*, 4887–4905.

13. McGorrin, R.J. The Significance of Volatile Sulfur Compounds in Food Flavors. *ACS Symp. Ser.* **2011**, *1068*, 3–31.

14. Belitz, H.-D.; Grosch, W.; Schieberle, P. Cereals and Cereal Products. *Food Chem.* **2009**, 670–745.

15. Bastos, D.M.; Monaro, É.; Siguemoto, É.; Séfora, M.; Markowicz, D.; Monaro, E.; Siguemoto, E.; Sefor, M. Maillard Reaction Products in Processed Food: Pros and Cons. *Food Ind. Process. - Methods Equip.* **2012**.

16. Buttery, R.G.; Ling, L.C.; Stern, D.J. Studies on Popcorn Aroma and Flavor Volatiles. *J. Agric. Food Chem.* **1997**, *45*, 837–843.

17. Martins, S.I.F.S.; Jongen, W.M.F.; Van Boekel, M.A.J.S. A Review of Maillard Reaction in Food and Implications to Kinetic Modelling. *Trends Food Sci. Technol.* **2000**, *11*, 364–373.

18. Birch, A.N.; Petersen, M.A.; Hansen, Å.S. REVIEW: Aroma of Wheat Bread Crumb. *Cereal Chem.* **2014**, *91*, 105–114.

19. Hellwig, M.; Henle, T. Baking, Ageing, Diabetes: A Short History of the Maillard Reaction. *Angew. Chemie - Int. Ed.* **2014**, *53*, 10316–10329.

20. Berger, R.G. *Flavours and Fragrances: Chemistry, Bioprocessing and Sustainability*; Springer Science & Business Media, 2007; ISBN 3540493395.

21. Purlis, E. Browning Development in Bakery Products–a Review. *J. Food Eng.* **2010**, *99*, 239–249.

22. Cornell, H.; Cauvain, S.P. Bread Making: Improving Quality. Woodhead Publ. Cambridge Dalmo, RA, Bogwald, *J Fish Shellfish Immunol.* **2003**, *384*.

23. Tamanna, N.; Mahmood, N. Food Processing and Maillard Reaction Products: Effect on Human Health and Nutrition. *Int. J. Food Sci.* **2015**, *2015*.

24. Bailey, M.E.; Gutheil, R.A.; Hsieh, F.-H.; Cheng, C.-W.; Gerhardt, K.O. Maillard reaction volatile compounds and color quality of a whey protein concentrate—Corn meal extruded product. In *Thermally Generated Flavors*; Thomas, H.P., Michael, J.M., Robert, J.M., Eds.; ACS Publications: Washington, DC, USA, 1993; Chapter 25; pp. 315–327.

25. Seok, Y.-J.; Kim, Y.-G.; Jeong, S.Y.; Lee, J.; Yoon, H.-J. Furan in Thermally Processed Foods-a Review. *Toxicol. Res.* **2015**, *31*, 241.

26. Rizzi, G.P. The Strecker Degradation of Amino Acids: Newer Avenues for Flavor Formation. *Food Rev. Int.* **2008**, *24*, 416–435.

27. Bento-Silva, A.; Duarte, N.; Belo, M.; Mecha, E.; Carbas, B.; Brites, C.; Vaz Patto, M.C.; Bronze, M.R. Shedding Light on the Volatile Composition of *Broa*, a Traditional Portuguese Maize Bread. *Biomolecules* **2021**, *11*, 1–31.

28. Adahchour, M.; Beens, J.; Brinkman, U.A.T. Recent Developments in the Application of Comprehensive Two-Dimensional Gas Chromatography. *J. Chromatogr. A* **2008**, *1186*, 67–108.

29. Tranchida, P.Q.; Purcaro, G.; Maimone, M.; Mondello, L. Impact of Comprehensive Two-Dimensional Gas Chromatography with Mass Spectrometry on Food Analysis. *J. Sep. Sci.* **2016**, *39*, 149–161.

30. Rocha, S.M.; Costa, C.P.; Martins, C. Aroma Clouds of Foods: A Step Forward to Unveil Food Aroma Complexity Using GC × GC. *Front. Chem.* **2022**, *10*, 1–26.

31. Pacyński, M.; Wojtasiak, R.Z.; Mildner-Szkudlarz, S. Improving the Aroma of Gluten-Free Bread. *Lwt* **2015**, *63*, 706–713.

32. Costa, C.P.; Bezerra, A.R.; Almeida, A.; Rocha, S.M. Candida Species (Volatile) Metabotyping through Advanced Comprehensive Two-Dimensional Gas Chromatography. *Microorganisms* **2020**, *8*, 1–23.

33. Van den Dool, H.; Kratz, P.D. A Generalization of the Retention Index System Including Linear Temperature Programmed Gas—Liquid Partition Chromatography. *J. Chromatogr. A* **1963**, *11*, 463–471.

34. Rocha, S.M.; Freitas, R.; Cardoso, P.; Santos, M.; Martins, R.; Figueira, E. Exploring the Potentialities of Comprehensive Two-Dimensional Gas Chromatography Coupled to Time-of-Flight Mass Spectrometry to Distinguish Bivalve Species: Comparison of Two Clam Species (*Venerupis Decussata* and *Venerupis Philippinarum*). *J. Chromatogr. A* **2013**, *1315*, 152–161.

35. Tetko, I. V.; Tanchuk, V.Y. Application of Associative Neural Networks for Prediction of Lipophilicity in ALOGPS 2.1 Program. *J. Chem. Inf. Comput. Sci.* **2002**, *42*, 1136–1145.

36. Buttery, R.G.; Ling, L.C. Volatile Flavor Components of Corn Tortillas and Related Products. *J. Agric. Food Chem.* **1995**, *43*, 1878–1882.

37. Park, D.; Maga, J.A. Identification of Key Volatiles Responsible for Odour Quality Differences in Popped Popcorn of Selected Hybrids. *Food Chem.* **2006**, *99*, 538–545.

38. Linstrom, P.J.; Mallard, W.G. NIST Chemistry Webbook; NIST Standard Reference Database No. 69 Available online: http://webbook.nist.gov/chemistry/ (accessed on 26 March 2022).

39. Lee, J.; Hong, S.J.; Cho, J.-J.; Boo, C.G.; And, D.-S.K.; Shin, E.-C. Peanut Coffee: Enhancement of Nutritional, Physicochemical, and Sensory Characteristics in Coffee Brewed with Conventional and High-Oleic Peanut Extracts. *Foods* **2020**, *9*, 1664.

40. Wang, Z.; Xiao, Q.; Zhuang, J.; Feng, T.; Ho, C.T.; Song, S. Characterization of Aroma-Active Compounds in Four Yeast Extracts Using Instrumental and Sensory Techniques. *J. Agric. Food Chem.* **2020**, *68*, 267–278.

41. Elmore, J.S.; Mottram, D.S.; Hierro, E. Two-Fibre Solid-Phase Microextraction Combined with Gas Chromatography-Mass Spectrometry for the Analysis of Volatile Aroma Compounds in Cooked Pork. *J. Chromatogr. A* **2001**, *905*, 233–240.

42. Mildner-Szkudlarz, S.; Różańska, M.; Gaca, A.; Jeleń, H.H. Changes in Volatile Compound Profiles of Cold-Pressed Berry Seed Oils Induced by Roasting. *Lwt* **2021**, *148*.

43. Farag, M.A.; Otify, A.M.; El-Sayed, A.M.; Michel, C.G.; ElShebiney, S.A.; Ehrlich, A.; Wessjohann, L.A. Sensory Metabolite Profiling in a Date Pit Based Coffee Substitute and in Response to Roasting as Analyzed via Mass Spectrometry Based Metabolomics. *Molecules* **2019**, *24*, 3377.

44. Bredie, W.L.P.; Mottram, D.S.; Guy, R.C.E. Aroma Volatiles Generated during Extrusion Cooking of Maize Flour. *J. Agric. Food Chem.* **1998**, *46*, 1479–1487.

45. El-Sayed, A.M. The Pherobase: Database of Insect Pheromones and Semiochemicals Available online: http://www.pherobase.com (accessed on 22 March 2022).

46. Paraskevopoulou, A.; Chrysanthou, A.; Koutidou, M. Characterisation of Volatile Compounds of Lupin Protein Isolate-enriched Wheat Flour Bread. Food Res. Int. **2012**, 48, 568–577.

47. Buttery, R.G.; Ling, L.C. Additional Studies on Flavor Components of Corn Tortilla Chips. *J. Agric. Food Chem.* **1998**, *46*, 2764–2769.

48. Miller, G.H. *Whisky Science - A Condensed Distillation*; Springer Nature, 2019; ISBN 978-3-030-13732-8.

49. Vázquez-Araújo, L.; Enguix, L.; Verdú, A.; E., G.-G.; Carbonell-Barrachina, A.A. Investigation of Aromatic Compounds in Toasted Almonds Used for the Manufacture of Turrón. *Eur Food Res Technol* **2008**, *227*, 243–254.

50. Ruth, J.O.N.H. Odor Thresholds and Irritation Levels of Several Chemical Substances: A Review. *Am. Ind. Hyg. Assoc. J.* **1986**, 47, A-142–A-151.

51. Buttery, R.G.; Orts, W.J.; Takeoka, G.R.; Nam, Y. Volatile Flavor Components of Rice Cakes. **1999**.

52. Leffingwell, J.C.; Leffingwell, D. GRAS Flavor Chemicals Detection Thresholds. *Perfumer&Flavorist* **1991**, *16*, 1–13.

53. Pu, D.; Zhang, H.; Zhang, Y.; Sun, B.; Ren, F.; Chen, H. Characterization of the Key Aroma Compounds in White Bread by Aroma Extract Dilution Analysis, Quantitation, and Sensory Evaluation Experiments. *J. Food Process. Preserv.* **2019**, *43*.

54. de Lerma, N.L.; Martínez, T.G.; Moreno, J.; Mauricio, J.C.; Peinado, R.A. Sweet Wines with Great Aromatic Complexity Obtained by Partial Fermentation of Must from Tempranillo Dried Grapes. *Eur. Food Res. Technol.* **2012**, *234*, 695–701.

55. The Good Scents Company TGSC Information System Available online: http://www.thegoodscentscompany.com/search2.html (accessed on 17 January 2022).

56. Laukaleja, I.; Koppel, K. Aroma Active Compound Perception in Differently Roasted and Brewed Coffees by Gas Chromatography–Olfactometry. *J. Sens. Stud.* **2021**, *36*.

57. Oertling, H.; Gomann, C.; Vom Ende, M. Dihydrobenzofuran Derivatives as Fragrance and/or Flavoring. Materials. Patent 9045714, 2 June 2015.

58. Acree, T.; Arn, H. Flavornet and Human Odor Space Available online: http://www.flavornet.org/ (accessed on 5 March 2022).

59. Peinado, R.A.; Moreno, J.; Medina, M.; Mauricio, J.C. Changes in Volatile Compounds and Aromatic Series in Sherry Wine with High Gluconic Acid Levels Subjected to Aging by Submerged Flor Yeast Cultures. *Biotechnol. Lett.* **2004**, *26*, 757–762.

60. Drakula, S.; Mustač, N.Č.; Novotni, D.; Voučko, B.; Krpan, M.; Hruškar, M.; Ćurić, D. Optimization and Validation of a HS-SPME/GC–MS Method for the Analysis of Gluten-Free Bread Volatile Flavor Compounds. *Food Anal. Methods* **2021**, 1–16.

61. Feng, X.; Pan, L.; Wang, Q.; Liao, Z.; Wang, X.; Zhang, X.; Guo, W.; Hu, E.; Li, J.; Xu, J.; et al. Nutritional and Physicochemical Characteristics of Purple Sweet Corn Juice before and after Boiling. *PLoS One* **2020**, 15, 1–18.

62. Galoburda, R.; Straumite, E.; Sabovics, M.; Kruma, Z. Dynamics of Volatile Compounds in Triticale Bread with Sourdough: From Flour to Bread. *Foods* **2020**, *9*.

63. Karahadian, C.; Johnson, K.A. Analysis of Headspace Volatiles and Sensory Characteristics of Fresh Corn Tortillas Made from Fresh Masa Dough and Spray-Dried Masa Flour. *J. Agric. Food Chem.* **1993**, *41*, 791–799.

64. Pico, J.; Martinez, M.M.; Bernal, J.; Gomez, M. Evolution of Volatile Compounds in Gluten-Free Bread: From Dough to Crumb. *Food Chem.* **2017**, *227*, 179–186.

65. Pico, J.; Antolín, B.; Román, L.; Bernal, J.; Gómez, M. Selection of the Most Suitable Mixture of Flours and Starches for the Improvement of Gluten-Free Breads through Their Volatile Profiles. *Eur. Food Res. Technol.* ***2019***, *245*, 1755–1766.

66. Hussein, A.M.S.; Ibrahim, G.E. Effects of Various Brans on Quality and Volatile Compounds of Bread. *Foods Raw Mater.* **2019**, *7*, 42–50.

67. Rocha, S.M.; Coelho, E.; Zrostlíková, J.; Delgadillo, I.; Coimbra, M.A. Comprehensive Two-Dimensional Gas Chromatography with Time-of-Flight Mass Spectrometry of Monoterpenoids as a Powerful Tool for Grape Origin Traceability. *J. Chromatogr. A* **2007**, *1161*, 292–299.

68. Vranová, J.; Ciesarová, Z. Furan in Food–a Review. *Czech J Food Sci* **2009**, *27*, 1–10.

69. Mohsen, S.M.; Ammar, A.S.M. Total Phenolic Contents and Antioxidant Activity of Corn Tassel Extracts. *Food Chem.* **2009**, *112*, 595–598.

70. Starowicz, M. Analysis of Volatiles in Food Products. *Separations* **2021**, *8*.

71. Pico, J.; Antolín, B.; Román, L.; Gómez, M.; Bernal, J. Analysis of Volatile Compounds in Gluten-Free Bread Crusts with an Optimised and Validated SPME-GC/QTOF Methodology. *Food Res. Int.* **2018**, *106*, 686–695.

72. Obuchowski, W.; Makowska, A.; Mildner-Szkudlarz, S.; Szwengiel, A.; Majcher, M.; Remiszewski, M. Effect of Triticale Grain Characteristics, Scouring, and Extrusion Conditions on Physico-Chemical Properties, Antioxidant Activity, and Volatile Compounds of Flat Bread. *Acta Aliment.* **2015**, *44*, 511–519.

73. Deblander, J.; Van Aeken, S.; Adams, A.; De Kimpe, N.; Abbaspour Tehrani, K. New Short and General Synthesis of Three Key Maillard Flavour Compounds: 2-Acetyl-1-Pyrroline, 6-Acetyl-1,2,3,4-Tetrahydropyridine and 5-Acetyl-2,3-Dihydro-4H-1,4-Thiazine. *Food Chem.* **2015**, *168*, 327–331.

74. Sanders, R.A.; Zyzak, D. V.; Morsch, T.R.; Rizzi, G.P. Elucidation of Tautomer Structures of 2-Acetyltetrahydropyridine Using Gas Chromatography/Mass Spectrometry and Gas Chromatography/Infrared Spectroscopy. *Eur. J. Mass Spectrom.* **2005**, *11*, 217–220.

75. Zhang, Q.; Wan, C.; Wang, C.; Chen, H.; Liu, Y.; Li, S.; Lin, D.; Wu, D.; Qin, W. Evaluation of the Non-Aldehyde Volatile Compounds Formed during Deep-Fat Frying Process. *Food Chem.* **2018**, *243*, 151–161.

76. Moskowitz, M.R.; Bin, Q.; Elias, R.J.; Peterson, D.G. Influence of Endogenous Ferulic Acid in Whole Wheat Flour on Bread Crust Aroma. *J. Agric. Food Chem.* **2012**, *60*, 11245–11252.

77. Bento-Silva, A.; Patto, M.C.V.; do Rosário Bronze, M.; Vaz Patto, M.C.; do Rosário Bronze, M.; Patto, M.C.V.; do Rosário Bronze, M. Relevance, Structure and Analysis of Ferulic Acid in Maize Cell Walls. *Food Chem.* **2018**, *246*, 360–378.

78. Wang, X.; Capone, D.L.; Roland, A.; Jeffery, D.W. Chiral Analysis of Cis-2-Methyl-4-Propyl-1,3-Oxathiane and Identification of Cis-2,4,4,6-Tetramethyl-1,3-Oxathiane in Wine. *Food Chem.* **2021**, *357*.

79. Carbas, B.; Vaz-Patto, M.C.; Bronze, M.R.; Bento-Da-Silva, A.; Trigo, M.J.; Brites, C. Maize Flour Parameters That Are Related to the Consumer Perceived Quality of 'Broa'Specialty Bread. *Food Sci. Technol.* **2016**, *36*, 259–267.

80. Mariotti, M.; Granby, K.; Fromberg, A.; Risum, J.; Agosin, E.; Pedreschi, F. Furan Occurrence in Starchy Food Model Systems Processed at High Temperatures: Effect of Ascorbic Acid and Heating Conditions. *J. Agric. Food Chem.* **2012**, *60*, 10162–10169.

81. Van Lancker, F.; Adams, A.; Owczarek-Fendor, A.; De Meulenaer, B.; De Kimpe, N. Mechanistic Insights into Furan Formation in Maillard Model Systems. *J. Agric. Food Chem.* **2011**, *59*, 229–235.

82. Zhao, Q.; Yao, S.; Ou, S.Y. Maillard Volatiles in Baked Products as Affected by Feruloylated Oligosaccharides from Maize Bran. *Int. J. Food Prop.* **2017**, *20*, 3266–3273.

83. Frankel, E. *Lipid Oxidation*; Elsevier: Amsterdam, 2014; ISBN 9780953194988.

84. Bento-Silva, A.; Duarte, N.; Mecha, E.; Belo, M.; Serra, A.T.; Vaz Patto, M.C.; Bronze, M.R. *Broa*, an Ethnic Maize Bread, as a Source of Phenolic Compounds. *Antioxidants* **2021**, *10*, 1–21.

85. Ozolina, V.; Kunkulberga, D.; Cieslak, B.; Obiedzinski, M. Furan Derivatives Dynamic in Rye Bread Processing. *Procedia Food Sci.* **2011**, *1*, 1158–1164.

CHAPTER 6

Final discussion and future perspectives

1. Concluding remarks

The main purpose of this thesis was to contribute to the valorisation of traditional Portuguese maize varieties and *broas*, by exploring their phenolic and volatile composition. Portuguese traditional open pollinated varieties (OPVs) are currently in risk of disappearing due to the progressive introduction of maize hybrids [1]. The loss that would come with the extinction of these valuable genetic resources would be unquestionable. Although they show lower yields than commercial hybrids, they are more resilient to biotic and abiotic stresses and are able to resist in marginal and stress environments [2]. Moreover, traditional varieties are able to produce high-quality *broas*, which show outstanding flavours and aromas that are not possible to achieve using the presently available commercial hybrids [2]. Portugal strongly relies on food supply from other countries; therefore, the cultivation of local foods, in particular Portuguese OPVs, is of foremost importance in facing food security challenges posed by future uncertainties, as those associated with climate changes, population growth, environmental degradation, major conflicts or sudden onset of pandemic crises [3]. Thus, the erosion of these resources would result not only in a severe risk to food security, but also in a loss of a traditional and very much appreciated Portuguese bread.

In this study, the phenolic and volatile composition of traditional Portuguese maize OPVs were studied, after milling the grain to whole maize flour. These flours were used for the preparation of *broas*, following a traditional recipe, and the impact of maize processing on the phenolic and volatile composition was appraised. These two compositional properties were chosen considering their significance on the overall quality of food products that show a major influence on consumers' preferences. Phenolic compounds contribute to the prevention of non-transmissible diseases [4,5], whilst volatile compounds contribute to the sensory properties of the final products, as odour and aroma [6].

There is a wide range of scientific evidence on the health benefits of polyphenols, attributed to their antioxidant properties [4,5]. In comparison to other cereals, maize has a particular abundant content in phenolic compounds, mostly hydroxycinnamic acids derivatives [34], which are partially dependent on the maize genotype [7–9]. The phenolic composition of raw maize flour is distinct from that of the final processed food and, depending on the techniques employed, phenolics can be degraded during processing, or suffer changes that affect their bioaccessibility [10,11]. Soluble phenolic compounds are mainly absorbed by the human GI, while insoluble phenolics are usually bound to dietary fibres and show a very low bioaccessibility [12]. Thus, depending on their form, they may either exhibit systemic health benefits, or act locally in the colon [13]. The potential of Portuguese maize OPVs and *broas* to be a valuable source of phenolic compounds was, until now, undisclosed. Thus, throughout the studies developed in this thesis, employing liquid chromatography and mass spectrometry tools, their phenolic composition was evaluated and the effects of maize processing on the phenolic composition of *broas* was revealed.

Results showed that Portuguese maize OPVs and *broas* were a valuable source of phenolic compounds, in particular ferulic acid, followed by *p*-coumaric acid. These compounds were mostly conjugated to amines, as putrescine and spermidine, forming the underexplored hydroxycinnamic acid amides. A new compound was putatively identified as *bis-N,N′*-diferuloyl putrescine and it was also possible to detect, for the first time, several insoluble hydroxycinnamic acid amides, consisting on structural isomers and stereoisomers of ferulic acid dehydrodimers and trimers. The presence of these compounds in *broas* shows that they may also contribute to the total phenolic content and antioxidant properties of this maize-based bread and potential associated health benefits.

Rather unexpectedly, the total phenolic content of maize flours and their ascribed antioxidant activity resisted to the processing of maize flours to *broas*. More importantly, the bioaccessibility of phenolic compounds was improved, since the content in free phenolic acids (ferulic and *p*-coumaric acids) increased in the final product. *Broas* prepared from maize traditional varieties showed a higher phenolic content than the *broa* prepared from a commercial maize flour, suggesting that they may exhibit more positive health benefits than those prepared from available commercial hybrids. These findings are relevant not only for the industry, contributing to the selection of the best maize varieties for higher-quality bread making, but also for maize breeders, who can add these quality traits to routine breeding of maize for *broas* production.

Despite the increasing public awareness about the impact of food choices on health [144], the aroma is often considered the most important quality attribute of breads [6]. The volatile compounds present in breads are responsible for their aroma [14], and result mainly from the oxidation of lipids and carotenoids, fermentation by yeast and lactic acid bacteria, and browning reactions (Maillard and caramelization reactions) during baking [15,16]. Thus, variations in the precursors [17] of these reactions in maize flours directly affects the type of volatiles generated. Furthermore, variations in flour odour can directly impact bread aroma [18,19]. Although several hundreds of volatiles are usually present in breads, only a small portion of them, usually present at trace levels, significantly contribute to their aroma [20,21]. In this work, the volatile composition of maize OPVs and *broas* was uncovered through gas chromatography coupled to mass spectrometry (GC-MS), in order to understand their influence on consumers' acceptance of this final product.

Overall, results provided important insights into the volatile composition of traditional maize OPVs and *broas*. The main volatile compounds present in maize flours were aldehydes, alcohols, hydrocarbons and ketones, mainly derived from lipids and carotenoids oxidation reactions. Higher amounts of aldehydes and alcohols from lipid oxidation reactions were detected in the commercial variety, whilst the yellow maize varieties showed higher contents in ketones from carotenoids oxidation. The presence of higher lipid and carotenoids oxidation volatiles in maize flours directly contributed to higher amounts of similar volatiles in *broas*. Other volatile compounds were present in these breads, mainly esters, derived from fermentation reactions, and furans, furfurals and pyranones, from browning reactions. The evidence from this study suggests that a higher content in lipid oxidation compounds, as 2-octenal, hexanal and heptanal, and lower contents in pyranones, namely maltol and 3-hydroxy-2,3-dihydromaltol, were associated to lower 'taste and aroma' scores obtained by a sensorial analysis. Furthermore, the used commercial *broa* was less appreciated by the panel of consumers, sustaining the previous general knowledge regarding the higher-quality characteristics of *broas* prepared from traditional maize varieties. In addition, phenolic compounds appeared to inhibit lipid oxidation reactions and increase browning reactions during bread making. Taken together, these findings suggest that maize varieties richer in health-promoting phenolic compounds originate *broas* which are more appreciated by the consumers. Thus, the better sensory attributes of *broas* prepared from traditional maize varieties may be, at least partially, caused by the higher amounts of phenolic compounds present in these varieties.

Although several volatile compounds were identified in *broas* by the most conventional technique (GC-MS), the volatiles mainly related to the baking process of *broas* (browning reactions) were further explored by means of GC×GC–ToFMS, taking advantage of the high resolving power and sensitivity of this more recent technique [22,23]. It is at the baking stage that some of the most valuable aroma impact compounds are generated, since they generally are strong odorants [24] with desirable sensory characteristics [6,25,26]. This technique allowed the detection of more than one hundred volatiles associated to the baking stage, of which almost 90% had not been previously detected by GC-MS. Although the most abundant volatiles were furans, furanones, and pyranones, the most relevant for the aroma of *broas* were ascribed to sulphur-containing compounds, in particular to dimethyl trisulphide and methanethiol, based on their strong odorant powers. One of the most relevant findings was the complete absence of pyrrolidines and lack of pyrazines in *broas*, especially when prepared from traditional maize varieties. Thus, the absence of pyrrolidines may contribute to the typical aroma of *broas*, and high amounts of pyrazines may confer negative characteristics associated to *broas* prepared from the used commercial maize varieties. Another important study finding was the identification of an oxathiane, since oxathiane derivatives have been identified as key volatile compounds, which significantly affect the aroma of foods [23]. Moreover, this study has shown that an exhaustive GC×GC–ToFMS analysis of breads volatiles may shed some light on the Maillard reaction and the origin of each compound, which is of uttermost importance in the development of foods with improved sensory characteristics.

In conclusion, the studies conducted throughout this thesis disclosed the phenolic and volatile composition of maize OPVs and *broas*, employing multiple chemical characterization approaches. The presence of phenolic compounds in *broas* suggests that their consumption on a regular basis may be associated to health benefits, which can stimulate the consumption of *broas* as part of a healthy diet. When comparing to the *broa* produced from a commercial hybrid maize variety, *broas* produced from traditional Portuguese OPVs show higher amounts of phenolic compounds and are also more appreciated by the consumers, mainly due to their better taste/aroma and smell/odour. Thus, these results reinforce the importance in preserving these traditional landraces, which are considered precious resources as a safeguard against an unpredictable future.

2. Ongoing work and future perspectives

The work presented in this thesis makes several noteworthy contributions to the characterization of Portuguese maize OPVs and *broas*. Notwithstanding the value of these contributions, this research has thrown up many questions in need of further research and serves as a base for future studies in the phenolic and volatile composition of *broas*.

Using a low-resolution mass spectrometer, several new hydroxycinnamic acid derivatives were identified for the first time. A possible area of future research would be to investigate more hydroxycinnamic acids derivatives, particularly other hydroxycinnamic acid amides, present in maize and *broas*, taking advantage of advanced and high-resolution mass spectrometry tools, which would almost certainly add considerable information to the characterization of the phenolic composition of maize and *broas*.

Although several phenolic compounds resisted to the processing of maize flours to *broas*, the associated health benefits depend on their bioaccessibility and bioavailability, but the stability of these compounds during the digestion process is currently unknown. Thus, future assays are being planned in order to enlighten the effects of digestion on *broas'* phenolic compounds. A standardised and practical static digestion *in vitro* method based on physiologically relevant conditions is going to be applied to simulate the digestion process of *broas*. The compounds present along the digestion process (after oral, gastric and intestinal digestion) will be identified and quantitated, focusing on hydroxycinnamic acid amides and the release of ferulic acid from the food matrix. In addition, permeability assays are going to be performed, by the evaluation of the intestinal release and uptake of the phenolic compounds which resisted to the simulated *in vitro* digestion.

The results obtained in this thesis have revealed that most of the phenolic compounds of *broas* were bound to the food matrix. There is some literature evidence suggesting that bound phenolics, in general, have a very low bioavailability [27,28]. Nevertheless, digestion assays must be performed, in order to confirm this theory. On the other hand, bound phenolics may also reach the colon in their intact form, be metabolized by the microbiota, and finally be absorbed [13]. As a result, the effects of the microbiota on these compounds should be evaluated. Furthermore, these compounds may also impact the gut microbiota, potentially having a role on the prevention of chronic diseases [13,29]. Thus, the effects of these compounds on the microbiota should also be addressed. Future *in vivo* bioavailability studies could further explore the bioavailability of *broas'* phenolics. After recruiting healthy volunteers, the bioavailability of the phenolic compounds of *broas* could be confirmed by the collection of blood and urine at different times, before and after the consumption of *broa* (e.g., after 1, 2, 4, 6 and 8 h) [30].

In order to understand the possible health-promoting properties of *broas*, *in vitro* studies should be performed, after simulated digestion process. Preliminary studies are currently being carried out, in order to evaluate the cytotoxicity and antiproliferative effects of the phenolic compounds present in *broas*. Scientific evidence on the health benefits of *broas* will certainly stimulate its consumption as part of a healthy diet, and eventually promote the cultivation of traditional maize varieties.

As far as the volatile composition is concerned, the present work suggests that some *broas* volatile compounds may be responsible for the better sensorial attributes of *broas* prepared from traditional maize varieties. However, only a small number of samples (n = 12) was used, and the analysis through advanced comprehensive two-dimensional gas chromatography has shown that possibly around 90% of the compounds present in *broas* were not previously identified and therefore, the most powerful odorants may have not been detected by 1D GC-MS. Thus, it showed the importance of evaluating the volatile composition of breads by high-resolving and sensitive techniques. Further research needs to examine more closely the links between the volatile composition of *broas* and their sensorial attributes. Although sulphur-containing compounds were pointed out as the strongest contributors to the aroma of *broas*, these data should be explored. The odour activity values of the compounds which most likely contribute to the aroma of *broas* should be experimentally determined. Ultimately, more advanced and cutting-edge techniques, as smell digitalization and sensomics [31], can be applied in order to deeper study the sensory attributes of *broas*. In this way, it would be possible to ascertain the character-impact volatiles of *broas*, which give them their remarkable and characteristic aromas. Furthermore, the data obtained by this technique can be fruitfully explored for the purpose of fingerprinting of traditional *broas*, since some compounds were present in significantly higher amounts in the *broa* prepared from the commercial maize variety analysed.

The findings presented in this thesis also have important implications for future studies in the phenolic composition of other cereals, since they draw attention to the presence of unreported phenolic acid derivatives. Aside from simple phenolic acids, attention must be given to hydroxycinnamic acid amides. In addition, this work also highlights the importance of an in-depth study of the volatile composition of foods, by exposing several compounds which had not been detected so far, and that may have an important sensorial impact on food aroma.

In closing, the studies conducted throughout this thesis have contributed not only for the worldwide dissemination of a traditional Portuguese bread, but also for the valorisation of maize Portuguese varieties. It is expected that the dissemination of their good characteristics among farmers and consumers can stimulate the production of traditional varieties. The results obtained can be useful not only for researchers, for the study of other cereal foods, processing effects, and bioaccessibility and bioavailability assays, but also for the food industry, farmers and breeders. The information is equally relevant for consumers, who are nowadays more aware of the need of preserving traditional farming due to environmental factors [2], as well as more demanding of high-quality food products, associated with positive health effects [32].

References

1. Patto, M.C.V.; Moreira, P.M.; Carvalho, V.; Pego, S. Collecting Maize (*Zea Mays* L. *Convar. Mays*) with Potential Technological Ability for Bread Making in Portugal. *Genet. Resour. Crop. Evol.* **2007**, *54*, 1555–1563.
2. Vaz Patto, M.C.; Mendes-Moreira, P.M.; Alves, M.L.; Mecha, E.; Brites, C.; Bronze, R.; Pego, S. Participatory Plant Quality Breeding : An Ancient Art Revisited by Knowledge Sharing. The Portuguese Experience. In *Plant breeding from laboratories to fields*; InTechOpen, 2013; pp. 255–288.
3. Henry, R. Innovations in Agriculture and Food Supply in Response to the COVID-19 Pandemic. *Mol. Plant* **2020**.
4. Buanafina, M.M. de O. Feruloylation in Grasses: Current and Future Perspectives. *Mol. Plant* **2009**, *2*, 861–872.
5. Mancuso, C.; Santangelo, R. Ferulic Acid: Pharmacological and Toxicological Aspects. *Food Chem. Toxicol.* **2014**, *65*, 185–195.
6. Purlis, E. Browning Development in Bakery Products–a Review. *J. Food Eng.* **2010**, *99*, 239–249.

7. Majumdar, R.; Minocha, R.; Lebar, M.D.; Rajasekaran, K.; Long, S.; Carter-Wientjes, C.; Minocha, S.C.; Cary, J.W. Contribution of Maize Polyamine and Amino Acid Metabolism towards Resistance against Aspergillus Flavus Infection and Aflatoxin Production. *Front. Plant Sci.* **2019**, *10*, 692.

8. Burt, A.J.; Arnason, J.T.; García-Lara, S. Natural Variation of Hydroxycinnamic Acid Amides in Maize Landraces. *J. Cereal Sci.* **2019**.

9. Tiburcio, A.F.; Alcázar, R. *Polyamines - Methods and Protocols*; Springer: New York, U.S.A., 2018; ISBN 9781493973972.

10. Food and Agriculture Organization of the United Nations (FAO) *Maize in Human Nutrition*; FAO: Rome, Italy, **1992**; ISBN 9789251030134.

11. La Frano, M.R.; de Moura, F.F.; Boy, E.; Lönnerdal, B.; Burri, B.J. Bioavailability of Iron, Zinc, and Provitamin A Carotenoids in Biofortified Staple Crops. *Nutr. Rev.* **2014**, *72*, 289–307.

12. Yang, L.; Wang, D.; Zhou, D.; Zhang, Y. Effect of Different Isolation Methods on Structure and Properties of Lignin from Valonea of Quercus Variabilis. *Int. J. Biol. Macromol.* **2016**, *85*, 417–424.

13. Aura, A.M. Microbial Metabolism of Dietary Phenolic Compounds in the Colon. *Phytochem. Rev.* **2008**, *7*, 407–429.

14. Cornell, H.; Cauvain, S.P. Bread Making: Improving Quality. Woodhead Publ. Cambridge Dalmo, RA, Bogwald, *J Fish Shellfish Immunol.* **2003**, *384*.

15. Pétel, C.; Onno, B.; Prost, C. Sourdough Volatile Compounds and Their Contribution to Bread: A Review. *Trends Food Sci. Technol.* **2017**, *59*, 105–123.

16. Pozo-Bayón, M.A.; Guichard, E.; Cayot, N. Flavor Control in Baked Cereal Products. *Food Reviews International.* **2006**, *22*, 335–379.

17. Galoburda, R.; Straumite, E.; Sabovics, M.; Kruma, Z. Dynamics of Volatile Compounds in Triticale Bread with Sourdough: From Flour to Bread. *Foods* **2020**, *9*.

18. Starr, G.; Hansen, T.S.; Petersen, M.A.; Bredie, W.L.P.P.; Hansen, Å.S.; Petersen, M.A.; Bredie, W.L.P.P.; Hansen, T.S.; Petersen, M.A.; Bredie, W.L.P.P. Aroma of Wheat Porridge and Bread-Crumb Is Influenced by the Wheat Variety. *LWT - Food Sci. Technol.* **2015**, *63*, 590–598.

19. Makhoul, S.; Romano, A.; Capozzi, V.; Spano, G.; Aprea, E.; Cappellin, L.; Benozzi, E.; Scampicchio, M.; Märk, T.D.; Gasperi, F. Volatile Compound Production During the Bread-Making Process: Effect of Flour, Yeast and Their Interaction. *Food Bioprocess Technol.* **2015**, *8*, 1925–1937.

20. Chambers IV, E.; Koppel, K. Associations of Volatile Compounds with Sensory Aroma and Flavor: The Complex Nature of Flavor. *Molecules* **2013**, *18*, 4887–4905.

21. Pico, J.; Bernal, J.; Gómez, M. Wheat Bread Aroma Compounds in Crumb and Crust: A Review. *Food Res. Int.* **2015**, *75*, 200–215.

22. Adahchour, M.; Beens, J.; Brinkman, U.A.T. Recent Developments in the Application of Comprehensive Two-Dimensional Gas Chromatography. *J. Chromatogr. A* **2008**, *1186*, 67–108.

23. McGorrin, R.J. The Significance of Volatile Sulfur Compounds in Food Flavors. *ACS Symp. Ser.* **2011**, *1068*, 3–31.

24. Birch, A.N.; Petersen, M.A.; Hansen, Å.S. Review: Aroma of Wheat Bread Crumb. *Cereal Chem.* **2014**, *91*, 105–114.

25. Starowicz, M.; Zieliński, H. How Maillard Reaction Influences Sensorial Properties (Color, Flavor and Texture) of Food Products? *Food Rev. Int.* **2019**, *35*, 707–725.

26. Cho, I.H.; Peterson, D.G. Chemistry of Bread Aroma: A Review. *Food Sci. Biotechnol.* **2010**, *19*, 575–582.

27. Zhao, Z.; Moghadasian, M.H. Chemistry, Natural Sources, Dietary Intake and Pharmacokinetic Properties of Ferulic Acid: A Review. *Food Chem.* **2008**, *109*, 691–702.

28. Gutiérrez-Grijalva, E.P.; Ambriz-Pére, D.L.; Leyva-López, N.; Castillo-López, R.I.; Heredia, J.B. Review: Dietary Phenolic Compounds, Health Benefits and Bioaccessibility. *Arch. Latinoam. Nutr.* **2016**, *66*, 87–100.

29. Ou, J. ying; Huang, J. qing; Song, Y.; Yao, S. wen; Peng, X. chun; Wang, M. fu; Ou, S. yi Feruloylated Oligosaccharides from Maize Bran Modulated the Gut Microbiota in Rats. *Plant Foods Hum. Nutr.* **2016**, *71*, 123–128.

30. Mecha, E.; Feliciano, R.P.; Rodriguez-Mateos, A.; Silva, S.D.; Figueira, M.E.; Vaz Patto, M.C.; Bronze, M.R. Human Bioavailability of Phenolic Compounds Found in Common Beans: The Use of High-Resolution MS to Evaluate Inter-Individual Variability. *Br. J. Nutr.* **2020**, *123*, 273–292.

31. Rocha, S.M.; Costa, C.P.; Martins, C. Aroma Clouds of Foods: A Step Forward to Unveil Food Aroma Complexity Using GC×GC. *Front. Chem.* **2022**, *10*, 1–26.

32. Domínguez Díaz, L.; Fernández-Ruiz, V.; Cámara, M. An International Regulatory Review of Food Health-Related Claims in Functional Food Products Labeling. *J. Funct. Foods* **2020**, *68*, 10389.

APPENDIX A

Hydroxycinnamic acids and their derivatives in broa*, a traditional ethnic maize bread*[1]

[1]**Supplementary information included in the following publication:**
Bento-Silva A, Duarte N, Mecha E, Belo M, Vaz Patto MC, Bronze MR. Hydroxycinnamic Acids and Their Derivatives in *Broa*, a Traditional Ethnic Maize Bread. *Foods*. **2020**, *9*, 1471.
DOI: 10.3390/foods9101471.

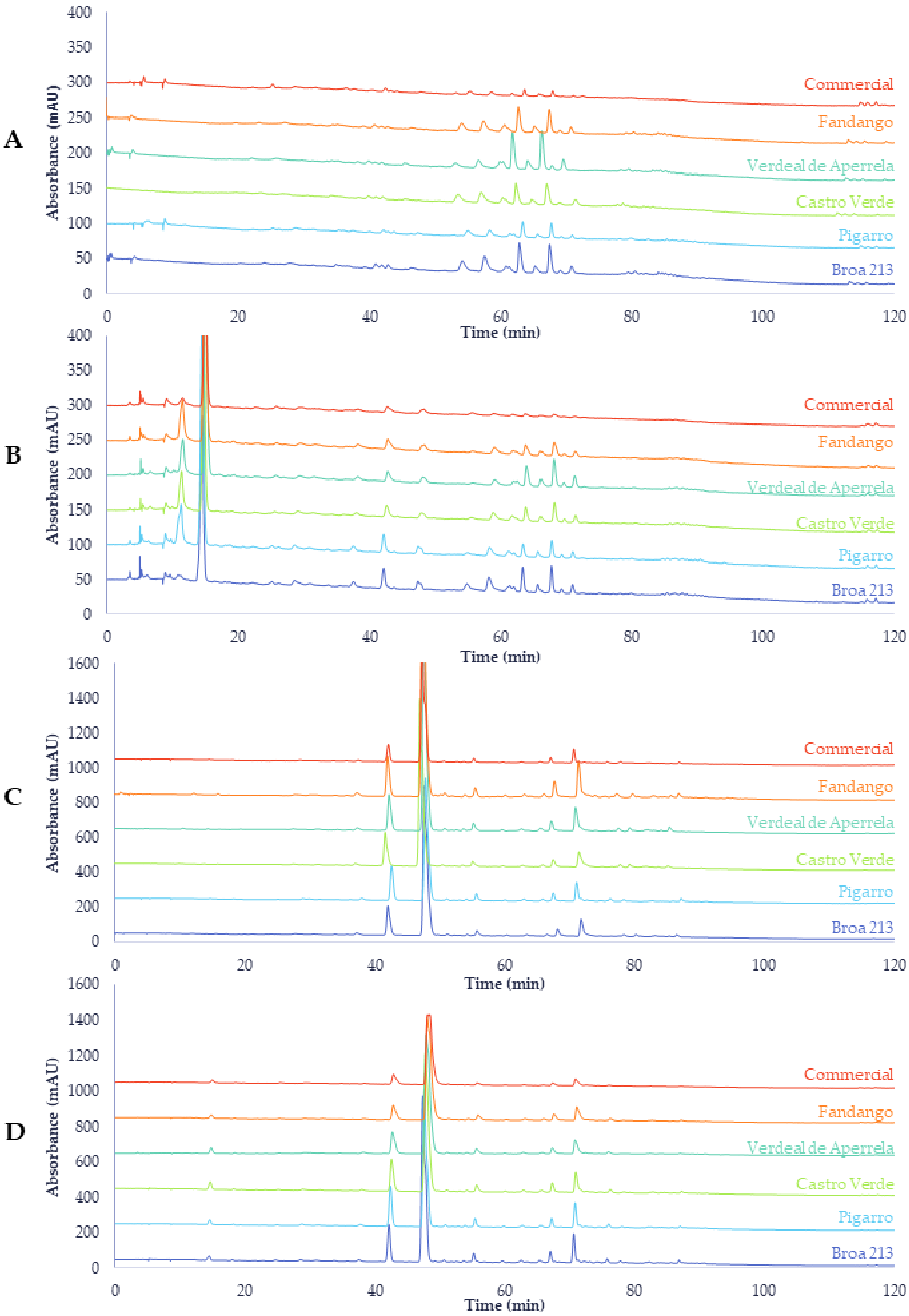
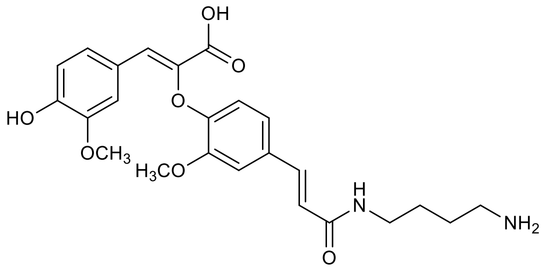
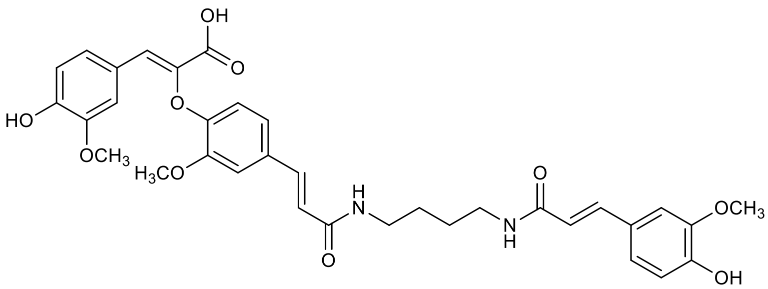


Figure A.1: Comparison of chromatographic profiles of (**A**) maize flours soluble fraction, (**B**) *broas* soluble fraction, (**C**) maize flour insoluble fraction and (**D**) *broas* insoluble fraction at 280 nm.



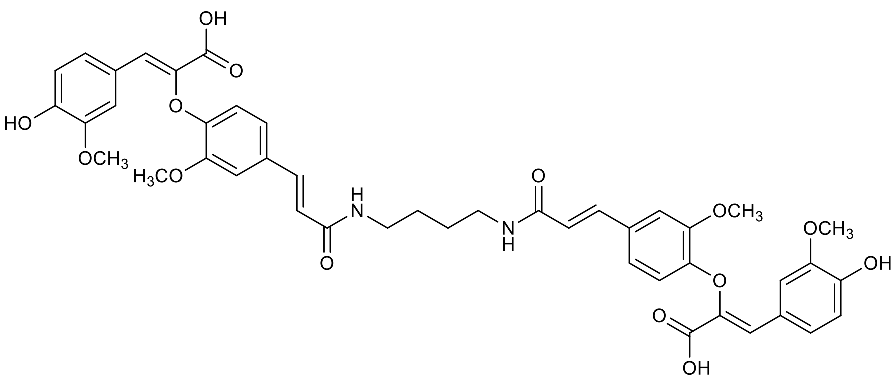
***N*-8-O-4'-Dehydrodiferuloyl putrescine**

m/z 457 $[M+H]^+$



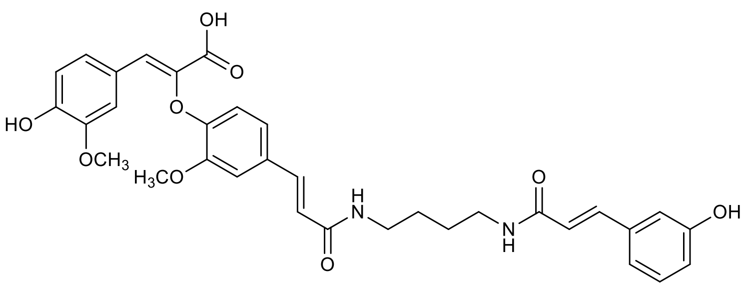
***N,N'*-Feruloyl 8-O-4'-didehydrodiferuloyl putrescine**

m/z 633 $[M+H]^+$



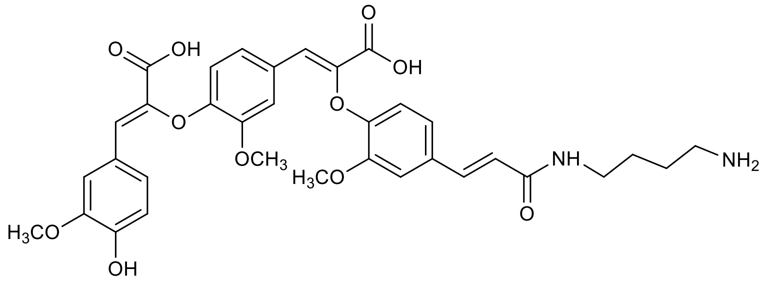
***N,N'*-8-O-4'-Didehydrodiferuloyl putrescine**

m/z 825 $[M+H]^+$



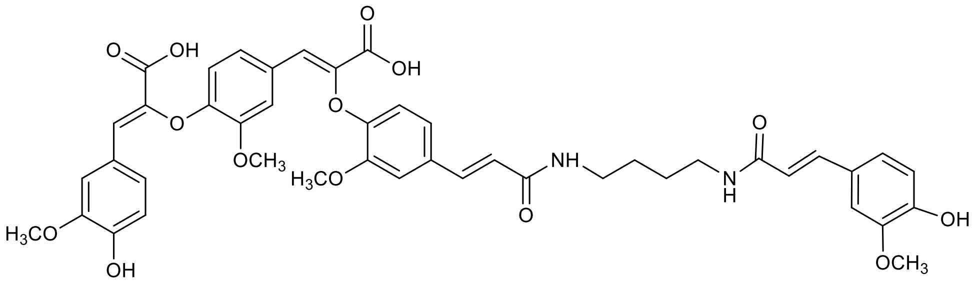
***N,N'*-Coumaroyl 8-O-4'-dehydrodiferuloyl putrescine**

m/z 603 $[M+H]^+$



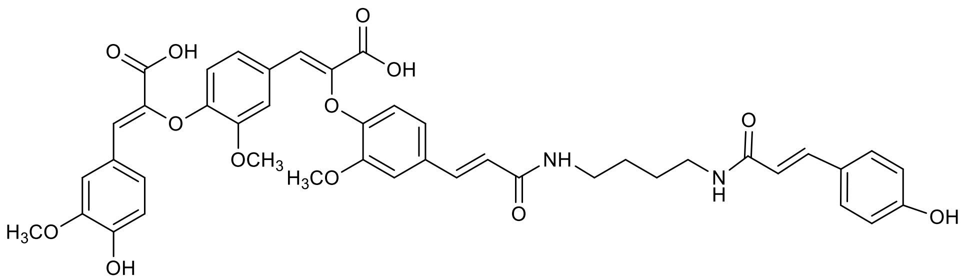
***N*-8-O-4'/4-O-8''-Dehydrotriferuloyl putrescine**

m/z 649 $[M+H]^+$



***N,N′*-Feruloyl 8-O-4'/4-O-8''-dehydrotriferuloyl putrescine**

m/z 825 $[M+H]^+$



***N,N′*-Coumaroyl 8-O-4'/4-O-8''-dehydrotriferuloyl putrescine**

m/z 795 $[M+H]^+$

Figure A.2: Chemical structures suggested for 8-O-4′-dehydrodiferulic and 8-O-4′/4-O-8″-dehydrotriferulic acid putrescines.

Table A.1: Details of the MRM conditions applied in the HPLC-DAD-MS/MS analysis.

(+) Compounds analysed in positive ion mode; (-) Compounds analysed in negative ion mode.

Compound	*m/z*	MS/MS ions	Cone Voltage (V)	Collision Energy (eV)
Ferulic acid (-)	193	134	30	10
p-Coumaric acid (-)	163	93	20	20
o-Coumaric acid (-)	163	93	20	20
m-Coumaric acid (-)	163	93	20	20
p-Hydroxybenzoic acid (-)	137	93	30	10
Caffeic acid (-)	179	135	20	10
Syringic acid (+)	199	140	20	10
Vanillic acid (+)	169	93	20	10
Protocatechuic acid (-)	153	109	20	10
Citric acid (-)	191	111	10	10
Gallic acid (-)	169	125	20	10
Syringaldehyde (+)	183	123	20	10
Vanillin (+)	153	93	20	10
Quercetin (-)	301	151	30	20
Kaempferol (-)	285	178	40	30
Citric acid (-)	191	111	10	10

APPENDIX B

Broa, *an ethnic maize bread, as a source of phenolic compounds*[1]

[1] **Supplementary information included in the following publication:**
Bento-Silva, A.; Duarte, N.; Mecha, E.; Belo, M.; Serra, A.T.; Vaz Patto, M.C.; Bronze, M.R. *Broa*, an Ethnic Maize Bread, as a Source of Phenolic Compounds. *Antioxidants*. **2021**, *10*, 672.
DOI: 10.3390/antiox10050672

Table B.1: Increase (%) in the antioxidant activity (**AA**) and phenolic content (**PC**) after hydrolysis of the soluble compounds (from the soluble [SF] to the soluble-hydrolysed [SHF] fractions) of cereal flours.

*****: Significant difference between SF and SHF (p < 0.001).

Samples	AA increase (%)	PC increase (%)
Broa-213	89	9
Pigarro	112	15
Castro Verde	72	13
Verdial de Aperrela	93	27
Fandango	128	11
Commercial	199	21
Average	*115 ± 45 **	*16 ± 7 **
Wheat	*- 25*	*20*
Rye	*12*	*7*

Table B.2: Contribution (%) of soluble-free, total soluble and insoluble ferulic (**FA**) and *p*-coumaric (**pCA**) acids for the antioxidant activity of the soluble (**SF**), soluble-hydrolysed (**SHF**) and insoluble fractions (**IF**) of cereal flours extracts, respectively.

Others: other compounds or synergistic effects; **FA**: 15.6 µmol TE mg^{-1}; **pCA**: 23 µmol TE mg^{-1}.

Sample	Soluble fraction (SF)			Soluble-hydrolysed fraction (SHF)			Insoluble fraction (IF)		
	FA	pCA	Others	FA	pCA	Others	FA	pCA	Others
Broa-213	0.6	0.4	99.0	6.5	2.8	90.7	44.0	6.1	49.9
Pigarro	0.5	0.3	99.2	5.9	2.5	91.6	40.8	6.1	53.0
Castro Verde	0.5	0.5	99.0	5.9	3.1	91.1	47.4	6.4	46.2
Verdial de Aperrela	0.6	0.4	99.0	7.8	2.6	89.6	50.7	5.5	43.7
Fandango	0.5	0.4	99.0	5.6	2.1	92.3	41.4	4.0	54.6
Commercial	0.6	0.6	98.8	6.2	2.1	91.7	37.1	2.9	60.0
Average	*0.6 ± 0.1*	*0.4 ± 0.1*	*99.0 ± 0.1*	*6.3 ± 0.8*	*2.5 ± 0.4*	*91.2 ± 0.9*	*43.6 ± 4.9*	*5.2 ± 1.4*	*51.3 ± 5.9*
Wheat	0.9	0.1	99.0	4.3	0.2	95.5	15.9	0.6	83.5
Rye	1.0	0.4	98.7	4.5	1.3	94.2	15.0	1.7	83.3

Table B.3: Increase (%) in the antioxidant activity (**AA**) and phenolic content (**PC**) after hydrolysis of the soluble compounds (from the soluble [**SF]** to the soluble-hydrolysed [**SHF**] fractions) of *broas*.

*****: Significant difference between SF and SHF (p < 0.001).

Samples	AA increase (%)	PC increase (%)
Broa-213	24	- 2
Pigarro	39	17
Castro Verde	62	27
Verdial de Aperrela	37	20
Fandango	16	- 7
Commercial	91	0
Average	*45 ± 28 **	*9 ± 14*

Table B.4: Contribution (%) of soluble-free, total soluble and insoluble ferulic (**FA**) and *p*-coumaric (**pCA**) acids for the antioxidant activity of the soluble (**SF**), soluble-hydrolysed (**SHF**) and insoluble fractions (**IF**) of *broas* extracts, respectively.

Others: other compounds or synergistic effects. **FA**: 15.6 µmol TE mg^{-1}; **pCA**: 23 µmol TE mg^{-1}.

Sample	Soluble fraction (SF)			Soluble-hydrolysed fraction (SHF)			Insoluble fraction (IF)		
	FA	pCA	Others	FA	pCA	Others	FA	pCA	Others
Broa-213	1.4	1.5	97.0	6.7	3.3	90.1	44.6	5.4	50.0
Pigarro	1.4	1.4	97.2	3.9	2.0	94.1	32.4	5.6	62.0
Castro Verde	1.4	1.7	96.9	4.6	2.4	93.0	43.8	6.2	50.0
Verdial de Aperrela	1.4	1.4	97.2	4.7	2.1	93.2	39.8	4.3	55.9
Fandango	1.6	1.5	96.9	6.6	2.7	90.7	40.3	3.3	56.4
Commercial	2.0	1.7	96.3	4.8	2.6	92.7	54.4	2.9	42.8
Average	*1.5 ± 0.3*	*1.5 ± 0.1*	*96.9±0.3*	*5.2 ± 1.1*	*2.5 ± 0.5*	*92.3±1.6*	*42.5±7.2*	*4.6 ± 1.4*	*52.8±6.7*

Table B.5: Calculated values obtained for raw flours (**RF** = 70% maize + 20% rye + 10% wheat) and used for direct comparison with the corresponding *broas*.

Values reported as: **PC**: mg GAE (gallic acid equivalents) 100 g^{-1} dw; **AA**: mmol of TE (Trolox equivalents) 100 g^{-1} dw; **pCA** and **FA**: mg 100 g^{-1} dw; **DCS**: mg pCAE (pCA equivalents) 100 g^{-1} dw; **CFP**, **DFP**, and **DFAs**: mg FAE (FA equivalents) 100 g^{-1} dw.

Variable		Fraction	Samples (raw flours)					
			Broa-213	Pigarro	Castro Verde	Verdial de Aperrela	Fandango	Commercial
PC	PCs	SF	37.3	29.6	30.3	32.9	29.7	26.3
	PCi	IF	117	147	149	165	174	89.1
AA	AAs	SF	0.71	0.62	0.62	0.61	0.56	0.42
	AAi	IF	2.53	3.42	2.97	3.22	4.16	2.20
FA	FAf	SF	0.31	0.23	0.24	0.27	0.22	0.19
	FAc	SHF-SF	4.78	4.30	3.44	4.90	3.89	3.96
	FAi	IF	68.6	86.9	86.9	101.3	107.9	50.2
pCA	pCAf	SF	0.13	0.08	0.12	0.09	0.10	0.10
	pCAc	SHF-SF	1.33	1.18	1.14	1.07	0.91	0.82
	pCAi	IF	6.37	8.79	7.93	7.45	7.11	2.64
HCAAs	DCS	SF	0.71	0.37	0.65	0.50	0.55	0.18
	CFP	SF	0.98	0.41	0.78	1.09	0.75	0.18
	DFP	SF	1.83	0.87	1.91	2.36	1.36	0.33
DFAs	8-O-4′-	IF	4.85	7.09	7.69	9.10	10.21	2.91
	8-5′-	IF	1.72	2.69	2.24	2.61	2.69	1.06
	5-5′-	IF	1.73	2.85	3.00	3.24	4.42	1.37

APPENDIX C

Shedding light on the volatile composition of broa*, a traditional Portuguese maize bread*[1]

[1] **Supplementary information included in the following publication:**
Bento-Silva A, Duarte N, Belo M, Mecha E, Carbas B, Brites C, Vaz Patto MC, Bronze MR. Shedding light on the volatile composition of *broa*, a traditional Portuguese maize bread. *Biomolecules*. **2021**. *11*, 1396.
DOI: 10.3390/biom11101396

1. HS-SPME-GC-MS Optimization

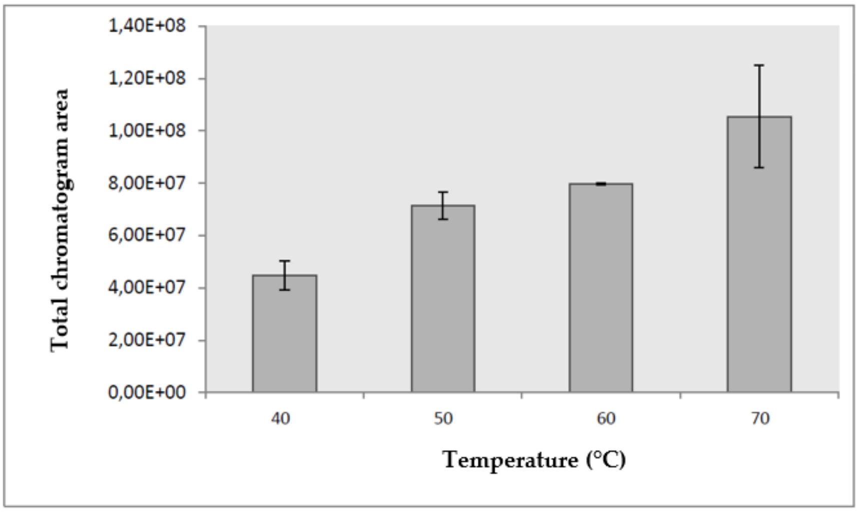


Figure C.1: Average of the total chromatogram areas (n = 3) at different temperatures.

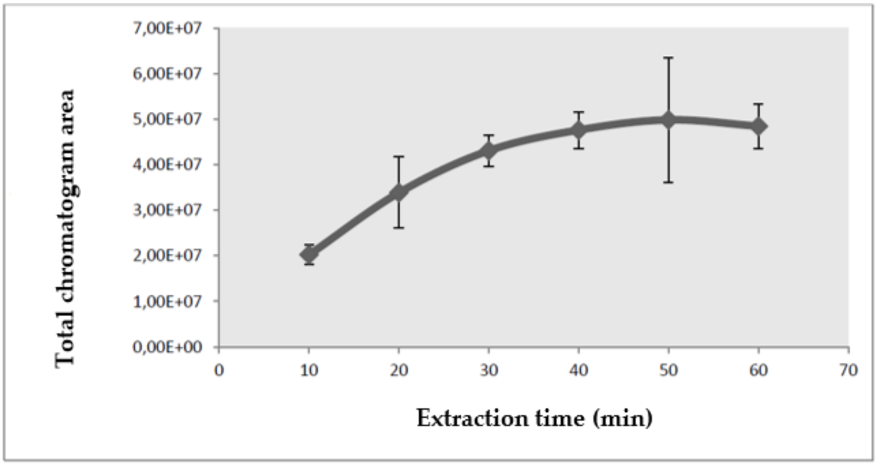


Figure C.2: Average of the total chromatogram areas (n = 3) at different extraction times.

2. Volatile composition of maize flours

Table C.1: Average peak areas obtained for each maize flour and considered for the cluster analysis.

Peaks are numbered according to ***Table 4.1***. *****: Not detected. A peak area of 3400 was considered, which corresponded to the half of the area obtained for the lowest detected peak.

Peak	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
5	1658377	1009855	1256498	1217227	1060382	1327411	1799584	1476005	919320	1838126	725925	2210163
6	94738	110760	117426	164492	99848	355394	296692	68028	55465	174295	308121	139354
7	16595178	8977672	5575694	4341115	6491413	6763045	9356316	7880485	4373802	8572044	2648452	81446621
12	781602	1130851	1117981	877255	446660	790109	1226260	1158610	534814	1022466	673474	12351643
14	244418	320968	294144	125473	264506	270256	306814	243268	91076	337048	235060	472126
17	4731308	1662092	919518	533459	1220885	1038858	1605374	1136067	725771	2045836	393957	11657972
19	1690755	1096634	593988	376191	694269	567770	872046	583080	276485	566205	380358	7174363
20	3400*	3400*	3400*	3400*	3400*	3400*	3400*	3400*	3400*	3400*	3400*	635057
22	153824	126149	116886	83802	52492	71816	177935	131352	47218	93224	52360	571579
23	1863520	1693657	832024	676149	782413	1146345	1428193	1079132	498389	1451812	735445	5543102
24	392873	631673	192640	348560	239456	281681	303880	974572	102258	662634	211448	724874
26	362023	130821	135200	117157	166565	266458	240408	99853	62655	237295	155139	1399370
30	136901	35237	25917	16085	19342	24093	41639	31926	18972	41795	3400*	1091192
33	189790	50216	43185	59953	41795	30543	52713	63620	34159	54659	58150	473815
35	6014598	2146759	1100882	685711	939887	1515439	2303204	1383751	707293	2532928	459037	19002344
42	4284828	1251491	571410	725409	862704	958432	1386846	716032	361203	1334293	390485	36536915
45	260713	87435	49191	89162	55616	82610	102763	109371	21953	69462	149627	590549
49	72082	35303	27496	24509	31146	20784	34279	32704	7350	46674	17329	126399
50	190169	3400*	232639	3400*	348155	355868	3400*	6907	135364	3400*	362948	3986645
64	4231824	2130422	1237578	1737192	1475089	1908541	2614240	1333316	731441	1909602	1054486	19169040
67	20702	119436	66271	183689	49393	76753	137860	159094	3400*	140627	88015	362653
71	472376	287434	146411	329358	157311	169237	209568	282677	205773	197245	214246	567680
92	101510	36743	25168	43967	26273	34320	41865	33899	35971	74969	39171	184084
94	209606	278910	210798	204543	151520	207405	261172	346156	248025	219035	208088	745170
95	40161	47346	67717	31560	76296	161301	90450	79728	100375	227738	136649	39949
96	1048673	848752	833964	583538	896301	1760036	1186079	792172	1153657	1963731	1474996	1103037
98	83655	66810	3400*	71098	19757	3400*	24464	74100	21156	94637	3400*	3400*
99	271686	510639	55346	466517	107719	86793	137806	437938	79753	486880	66329	277122
102	253946	140318	87333	134232	95153	96632	222702	121057	94501	125287	87087	197216

Table C.2: Spearman correlation coefficients among maize flours volatile compounds.

P: Peak (numbered according to ***Table 4.1***); *p*-Value corresponds to the significance level of Spearman correlation coefficient indicated as *: significant at $p < 0.05$; **: significant at $p < 0.01$.

P	6	7	12	14	17	19	22	23	24	26	30	33	35	42	45	49	50	64	67	71	92	94	95	96	98	99	102
5	0.070	0.661*	0.551	0.449	0.571	0.415	0.695*	0.620*	0.419	0.621*	0.563	0.368	0.614*	0.527	0.269	0.667*	-0.478	0.609*	0.316	0.234	0.565	0.192	0.222	0.252	0.513	0.326	0.642*
6		-0.214	0.076	0.268	-0.236	-0.184	-0.047	0.040	-0.274	0.380	-0.252	-0.241	-0.145	-0.143	0.094	-0.219	0.324	0.042	0.224	-0.311	-0.095	-0.223	0.473	0.587	-0.443	-0.332	-0.011
7			0.255	0.383	0.964**	0.933**	0.711*	0.875**	0.338	0.751**	0.951**	0.807**	0.969**	0.940**	0.681*	0.921**	-0.237	0.923**	-0.154	0.667*	0.760**	0.128	-0.200	0.003	0.557	0.291	0.844**
12				0.534	0.083	0.199	0.782**	0.491	0.576	0.042	0.107	-0.016	0.175	0.047	0.035	0.264	-0.667*	0.209	0.649*	0.082	0.019	0.634*	-0.053	-0.108	0.333	0.466	0.324
14					0.293	0.408	0.466	0.652*	0.363	0.457	0.182	0.007	0.315	0.212	0.110	0.463	-0.022	0.316	0.211	-0.213	0.107	0.047	0.324	0.344	0.086	0.137	0.178
17						0.911**	0.566	0.802**	0.208	0.779**	0.983**	0.888**	0.990**	0.981**	0.740**	0.932**	-0.117	0.910**	-0.287	0.698*	0.866**	-0.039	-0.150	0.069	0.543	0.224	0.799**
19							0.676*	0.851**	0.199	0.693*	0.894**	0.802**	0.911**	0.914**	0.729*	0.866**	-0.094	0.911**	-0.223	0.673*	0.641*	0.027	-0.385	-0.144	0.407	0.202	0.804**
22								0.731*	0.416	0.419	0.605*	0.476	0.632*	0.559	0.455	0.638*	-0.547	0.678*	0.298	0.450	0.362	0.486	-0.334	-0.233	0.391	0.307	0.797**
23									0.475	0.706*	0.746**	0.580	0.844**	0.769**	0.580	0.826**	-0.337	0.836**	0.107	0.512	0.648*	0.238	0.000	0.188	0.553	0.444	0.738**
24										-0.024	0.176	0.143	0.224	0.130	0.164	0.397	-0.601	0.147	0.601	0.312	0.197	0.696*	0.070	-0.057	0.731*	0.802**	0.160
26											0.744**	0.641*	0.807**	0.810**	0.672*	0.743**	0.189	0.848**	-0.177	0.364	0.725*	-0.318	0.203	0.453	0.192	-0.058	0.663*
30												0.926**	0.982**	0.982**	0.773**	0.897**	-0.132	0.909**	-0.291	0.758**	0.851**	0.013	-0.245	-0.019	0.516	0.189	0.825**
33													0.879**	0.927**	0.908**	0.833**	-0.045	0.828**	-0.235	0.870**	0.843**	-0.041	-0.342	-0.131	0.508	0.191	0.758**
35														0.981**	0.762**	0.922**	-0.163	0.939**	-0.222	0.711*	0.871**	0.020	-0.132	0.102	0.539	0.240	0.839**
42															0.816**	0.907**	-0.071	0.953**	-0.263	0.766**	0.864**	-0.095	-0.230	0.021	0.498	0.190	0.840**
45																0.715*	0.078	0.778**	-0.089	0.799**	0.726*	0.009	-0.241	-0.008	0.355	0.125	0.703*
49																	-0.227	0.878**	-0.002	0.659*	0.825**	-0.022	-0.131	0.012	0.662*	0.406	0.761**
50																		-0.163	-0.631*	-0.335	-0.206	-0.639*	0.153	0.251	-0.711*	-0.768**	-0.398
64																			-0.066	0.719*	0.784**	-0.066	-0.244	0.015	0.472	0.237	0.910**
67																				0.043	-0.111	0.399	0.053	-0.110	0.426	0.665*	0.049
71																					0.712*	0.197	-0.513	-0.342	0.676*	0.494	0.713*
92																						-0.102	0.105	0.285	0.661*	0.343	0.701*
94																							-0.111	-0.193	0.334	0.433	0.152
95																								0.937**	-0.074	-0.086	-0.331
96																									-0.103	-0.175	-0.113
98																										0.905**	0.475
99																											0.241

Table C.3: Major soluble phenolic compounds and total carotenoids content of maize flours (**F**) and *broas* (**B**).

cc: *cis,cis* isomer; ***ct***: *cis.trans* isomer; ***tt***: *trans,trans* isomer; **$_T$**: Total of isomeric forms; **Carot**: Total carotenoids content (mg lutein equivalents 100 g^{-1}).

Sample	pCA	FA	DCS*ct*	DCS*tt*	DCS$_T$	DFP*cc*	DFP*ct*	DFP*tt*	DFP$_T$	CFP*ct*	CFP*tt*	CFP$_T$	*bis*DFP	Carot
F1	0.41 ± 0.01	0.42 ± 0.03	0.82 ± 0.10	2.73 ± 0.50	3.55 ± 0.40	0.06 ± 0.01	1.52 ± 0.09	3.30 ± 0.79	4.89 ± 0.69	0.30 ± 0.03	0.89 ± 0.14	1.18 ± 0.17	0.07 ± 0.00	27.01 ± 0.84
B1	1.53 ± 0.19	1.80 ± 0.22	0.44 ± 0.01	1.65 ± 0.20	2.08 ± 0.20	0.06 ± 0.01	0.75 ± 0.01	2.26 ± 0.41	3.08 ± 0.41	0.19 ± 0.01	0.60 ± 0.09	0.79 ± 0.09	< 0.01	8.83 ± 0.03
F2	0.26 ± 0.02	0.33 ± 0.02	0.86 ± 0.03	1.80 ± 0.30	2.66 ± 0.27	0.17 ± 0.06	2.26 ± 0.01	3.27 ± 0.98	5.70 ± 0.93	0.37 ± 0.01	0.95 ± 0.19	1.33 ± 0.19	0.08 ± 0.02	46.01 ± 0.05
B2	1.02 ± 0.04	1.30 ± 0.09	0.35 ± 0.01	1.26 ± 0.11	1.61 ± 0.12	0.06 ± 0.01	0.97 ± 0.04	3.12 ± 0.53	4.15 ± 0.56	0.19 ± 0.01	0.81 ± 0.13	1.00 ± 0.13	< 0.01	15.21 ± 0.33
F3	0.36 ± 0.02	0.37 ± 0.03	0.86 ± 0.17	2.61 ± 0.50	3.46 ± 0.67	0.07 ± 0.01	2.09 ± 0.37	5.10 ± 0.51	7.26 ± 0.89	0.32 ± 0.05	1.09 ± 0.13	1.40 ± 0.18	0.11 ± 0.01	10.83 ± 0.35
B3	1.51 ± 0.11	1.44 ± 0.10	0.37 ± 0.03	1.50 ± 0.13	1.87 ± 0.15	0.05 ± 0.01	0.81 ± 0.11	3.74 ± 0.39	4.60 ± 0.49	0.15 ± 0.02	0.81 ± 0.09	0.96 ± 0.10	< 0.01	5.20 ± 0.08
F4	0.16 ± 0.00	0.28 ± 0.02	0.39 ± 0.02	0.61 ± 0.11	0.99 ± 0.09	0.15 ± 0.05	1.39 ± 0.12	1.98 ± 0.42	3.52 ± 0.25	0.25 ± 0.01	0.75 ± 0.10	0.99 ± 0.11	0.06 ± 0.00	33.49 ± 0.22
B4	0.75 ± 0.04	1.21 ± 0.08	0.22 ± 0.06	0.94 ± 0.10	1.16 ± 0.11	0.05 ± 0.01	0.81 ± 0.06	3.47 ± 0.57	4.33 ± 0.54	0.17 ± 0.01	1.08 ± 0.16	1.25 ± 0.15	< 0.01	11.63 ± 0.32
F5	0.38 ± 0.01	0.43 ± 0.02	0.76 ± 0.24	2.38 ± 0.48	3.13 ± 0.72	0.07 ± 0.01	1.86 ± 0.38	4.65 ± 0.13	6.58 ± 0.27	0.32 ± 0.07	0.98 ± 0.00	1.30 ± 0.07	0.10 ± 0.00	28.52 ± 0.15
B5	1.31 ± 0.08	1.46 ± 0.08	0.42 ± 0.03	1.86 ± 0.24	2.28 ± 0.27	0.05 ± 0.01	0.98 ± 0.02	4.21 ± 0.61	5.24 ± 0.63	0.22 ± 0.00	0.95 ± 0.11	1.16 ± 0.11	< 0.01	10.17 ± 0.46
F6	0.27 ± 0.00	0.36 ± 0.01	0.83 ± 0.07	1.78 ± 0.10	2.61 ± 0.17	0.11 ± 0.01	1.69 ± 0.31	2.45 ± 0.30	4.26 ± 0.62	0.33 ± 0.05	0.80 ± 0.12	1.14 ± 0.17	0.07 ± 0.00	10.10 ± 0.02
B6	1.05 ± 0.05	1.25 ± 0.06	0.40 ± 0.02	1.02 ± 0.06	1.43 ± 0.07	0.08 ± 0.01	0.87 ± 0.03	1.88 ± 0.15	2.83 ± 0.18	0.16 ± 0.00	0.57 ± 0.04	0.73 ± 0.04	< 0.01	4.44 ± 0.02
F7	0.33 ± 0.05	0.39 ± 0.03	0.69 ± 0.01	2.11 ± 0.03	2.79 ± 0.01	0.08 ± 0.00	2.13 ± 0.03	4.97 ± 0.07	7.18 ± 0.10	0.36 ± 0.00	1.32 ± 0.06	1.68 ± 0.06	0.06 ± 0.01	17.67 ± 0.02
B7	0.98 ± 0.09	1.36 ± 0.11	0.37 ± 0.05	1.13 ± 0.59	1.49 ± 0.61	0.05 ± 0.00	0.91 ± 0.17	3.55 ± 0.27	4.51 ± 0.40	0.18 ± 0.01	0.90 ± 0.07	1.08 ± 0.08	< 0.01	7.24 ± 0.06
F8	0.24 ± 0.01	0.28 ± 0.04	0.70 ± 0.12	1.72 ± 0.37	2.41 ± 0.48	0.12 ± 0.01	1.91 ± 0.16	3.42 ± 0.36	5.45 ± 0.50	0.29 ± 0.02	0.81 ± 0.07	1.09 ± 0.09	0.08 ± 0.00	61.39 ± 0.53
B8	0.97 ± 0.14	1.14 ± 0.15	0.47 ± 0.03	1.15 ± 0.23	1.62 ± 0.26	0.13 ± 0.02	1.38 ± 0.11	2.40 ± 0.61	3.91 ± 0.69	0.21 ± 0.01	0.60 ± 0.12	0.82 ± 0.13	< 0.01	19.47 ± 0.66
F9	0.27 ± 0.01	0.42 ± 0.02	0.63 ± 0.06	0.79 ± 0.14	1.41 ± 0.20	0.38 ± 0.01	3.22 ± 0.37	4.45 ± 0.71	8.05 ± 1.09	0.37 ± 0.10	1.08 ± 0.13	1.45 ± 0.23	0.07 ± 0.00	9.15 ± 0.55
B9	0.93 ± 0.02	1.19 ± 0.02	0.34 ± 0.02	0.83 ± 0.05	1.17 ± 0.06	0.10 ± 0.02	1.62 ± 0.22	4.43 ± 0.57	6.15 ± 0.63	0.21 ± 0.02	0.95 ± 0.10	1.15 ± 0.10	< 0.01	3.31 ± 0.05
F10	0.26 ± 0.00	0.37 ± 0.02	0.77 ± 0.07	1.28 ± 0.09	2.05 ± 0.15	0.21 ± 0.02	2.05 ± 0.12	2.27 ± 0.15	4.53 ± 0.29	0.35 ± 0.03	0.69 ± 0.07	1.04 ± 0.10	0.09 ± 0.01	50.74 ± 0.37
B10	0.87 ± 0.06	1.24 ± 0.09	0.35 ± 0.03	0.99 ± 0.08	1.35 ± 0.11	0.08 ± 0.00	0.97 ± 0.06	2.36 ± 0.20	3.41 ± 0.26	0.16 ± 0.01	0.63 ± 0.05	0.79 ± 0.05	< 0.01	15.54 ± 0.36
F11	0.30 ± 0.04	0.39 ± 0.06	0.92 ± 0.04	2.40 ± 0.31	3.33 ± 0.35	0.06 ± 0.01	1.63 ± 0.09	2.88 ± 0.44	4.58 ± 0.51	0.27 ± 0.03	0.65 ± 0.07	0.92 ± 0.10	0.12 ± 0.01	8.16 ± 0.15
B11	1.30 ± 0.10	1.67 ± 0.13	0.51 ± 0.08	1.48 ± 0.14	1.99 ± 0.21	0.07 ± 0.02	1.04 ± 0.17	2.67 ± 0.24	3.78 ± 0.38	0.17 ± 0.05	0.58 ± 0.05	0.75 ± 0.07	< 0.01	3.53 ± 0.31
F12	0.28 ± 0.02	0.34 ± 0.02	0.53 ± 0.06	1.17 ± 0.13	1.70 ± 0.19	0.06 ± 0.02	0.76 ± 0.05	1.05 ± 0.04	1.87 ± 0.11	0.22 ± 0.01	0.44 ± 0.05	0.66 ± 0.05	0.06 ± 0.00	2.11 ± 0.51
B12	0.62 ± 0.06	1.00 ± 0.12	0.25 ± 0.10	0.57 ± 0.11	0.82 ± 0.17	0.07 ± 0.01	0.46 ± 0.02	0.69 ± 0.08	1.22 ± 0.07	0.13 ± 0.01	0.29 ± 0.05	0.42 ± 0.05	< 0.01	1.21 ± 0.17

Table C.4: Spearman correlation coefficients between maize flours volatile compounds and the content in major phenolics and total carotenoids.

Var: Variables; **V**: Maize flours volatiles (peaks numbered according to ***Table 4.1***); ***cc***: *cis,cis* isomer; ***ct***: *cis.trans* isomer; ***tt***: *trans,trans* isomer; $_T$: Total of isomeric forms; **Carot**: Carotenoids. *p*-Value corresponds to the significance level of Spearman correlation coefficient indicated as *: significant at $p < 0.05$; **: significant at $p < 0.01$.

Var / V	pCA	FA	DCS*ct*	DCS*tt*	DCS$_T$	DFP*cc*	DFP*ct*	DFP*tt*	DFP$_T$	CFP*ct*	CFP*tt*	CFP$_T$	bisDFP	Carot
5	0.05	-0.20	-0.35	-0.10	-0.10	-0.18	-0.28	-0.24	-0.38	-0.19	-0.20	-0.20	-0.45	0.13
6	-0.05	-0.16	0.24	0.03	0.03	-0.19	-0.28	-0.39	-0.50	-0.12	-0.38	-0.33	-0.04	-0.27
7	0.19	-0.07	-0.14	0.10	0.10	-0.20	-0.07	-0.11	-0.17	0.08	-0.01	0.05	-0.43	0.12
12	-0.28	-0.64*	-0.21	-0.19	-0.19	-0.02	0.02	-0.14	-0.20	-0.11	-0.07	-0.08	-0.39	0.14
14	0.11	-0.17	0.13	0.09	0.09	-0.13	0.05	-0.17	-0.20	0.15	-0.10	-0.03	-0.06	0.04
17	0.21	0.02	-0.12	0.09	0.09	-0.17	-0.10	-0.17	-0.20	0.07	-0.08	-0.03	-0.28	0.19
19	0.48	0.02	0.08	0.43	0.43	-0.54	-0.17	0.09	-0.04	-0.09	0.11	0.09	-0.19	-0.01
20	0.04	-0.22	-0.39	-0.31	-0.31	-0.40	-0.48	-0.48	-0.48	-0.48	-0.48	-0.48	-0.40	-0.48
22	0.10	-0.33	-0.20	0.12	0.12	-0.32	-0.17	-0.05	-0.18	-0.20	0.01	-0.03	-0.45	0.15
23	0.16	-0.17	0.11	0.20	0.20	-0.29	-0.18	-0.27	-0.34	-0.01	-0.20	-0.13	-0.26	0.09
24	-0.43	-0.60*	-0.26	-0.28	-0.28	0.04	-0.31	-0.50	-0.55	-0.29	-0.47	-0.47	-0.31	0.52
26	0.52	0.23	0.08	0.32	0.32	-0.62*	-0.50	-0.28	-0.45	-0.21	-0.28	-0.24	-0.25	-0.34
30	0.18	-0.09	-0.11	0.10	0.10	-0.18	-0.06	-0.12	-0.17	0.05	-0.05	0.00	-0.32	0.15
33+34	-0.09	-0.33	-0.28	-0.06	-0.06	-0.41	-0.62*	-0.44	-0.52	-0.71**	-0.54	-0.62*	-0.27	0.11
35	0.17	-0.07	-0.09	0.07	0.07	-0.17	-0.10	-0.20	-0.25	0.08	-0.10	-0.02	-0.38	0.08
42	0.19	-0.06	-0.18	0.06	0.06	-0.28	-0.32	-0.34	-0.43	-0.06	-0.19	-0.14	-0.50	0.08
45	0.05	-0.27	-0.09	0.11	0.11	-0.60*	-0.66*	-0.44	-0.57	-0.64*	-0.53	-0.57	-0.34	-0.13
49	0.14	-0.14	-0.12	0.11	0.11	-0.24	-0.21	-0.22	-0.28	-0.12	-0.17	-0.16	-0.25	0.29
50	0.49	0.27	0.23	0.28	0.28	-0.71*	-0.49	-0.11	-0.23	-0.47	-0.34	-0.36	0.22	-0.74**
64+65	0.12	-0.16	-0.19	0.03	0.03	-0.25	-0.33	-0.37	-0.45	-0.08	-0.19	-0.14	-0.56	0.08
67	-0.60*	-0.78**	-0.40	-0.52	-0.52	0.05	-0.37	-0.59*	-0.64*	-0.47	-0.56	-0.60*	-0.36	0.24
71	-0.24	-0.39	-0.35	-0.24	-0.24	-0.20	-0.50	-0.55	-0.50	-0.47	-0.46	-0.46	-0.55	0.00
92	-0.07	-0.08	-0.34	-0.25	-0.25	-0.16	-0.48	-0.66*	-0.60*	-0.29	-0.54	-0.47	-0.54	-0.12
94	-0.25	-0.36	-0.20	-0.24	-0.24	0.14	0.31	-0.01	0.10	0.14	0.01	0.06	-0.26	0.02
95	-0.06	0.29	0.35	0.03	0.03	0.30	0.41	0.12	0.17	0.47	0.00	0.12	0.39	-0.05
96	0.18	0.41	0.23	0.03	0.03	-0.01	0.08	-0.20	-0.15	0.32	-0.23	-0.06	0.06	-0.39
98	-0.35	-0.13	-0.23	-0.17	-0.17	0.45	0.12	-0.09	-0.03	0.17	0.02	0.03	-0.16	0.80**
99	-0.596*	-0.56	-0.30	-0.43	-0.43	0.40	-0.08	-0.50	-0.42	0.00	-0.31	-0.28	-0.34	0.69*
102	-0.01	-0.20	-0.38	-0.08	-0.08	-0.07	-0.22	-0.26	-0.30	-0.03	-0.03	-0.01	-0.71*	0.26

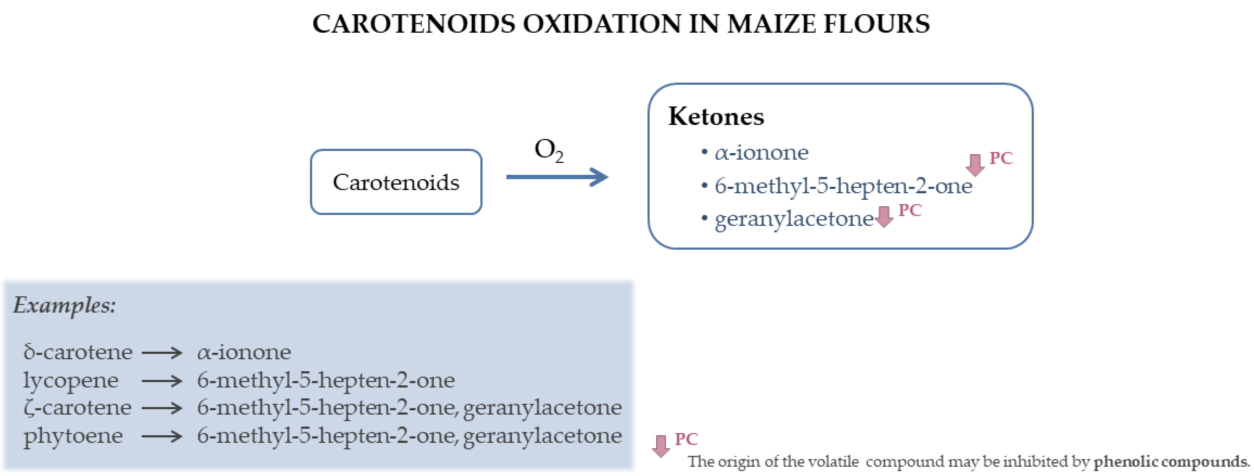


Figure C.3: Representative scheme of carotenoids oxidation reactions [60,61] occurring in maize flour samples.

3. Volatile composition of *broas*

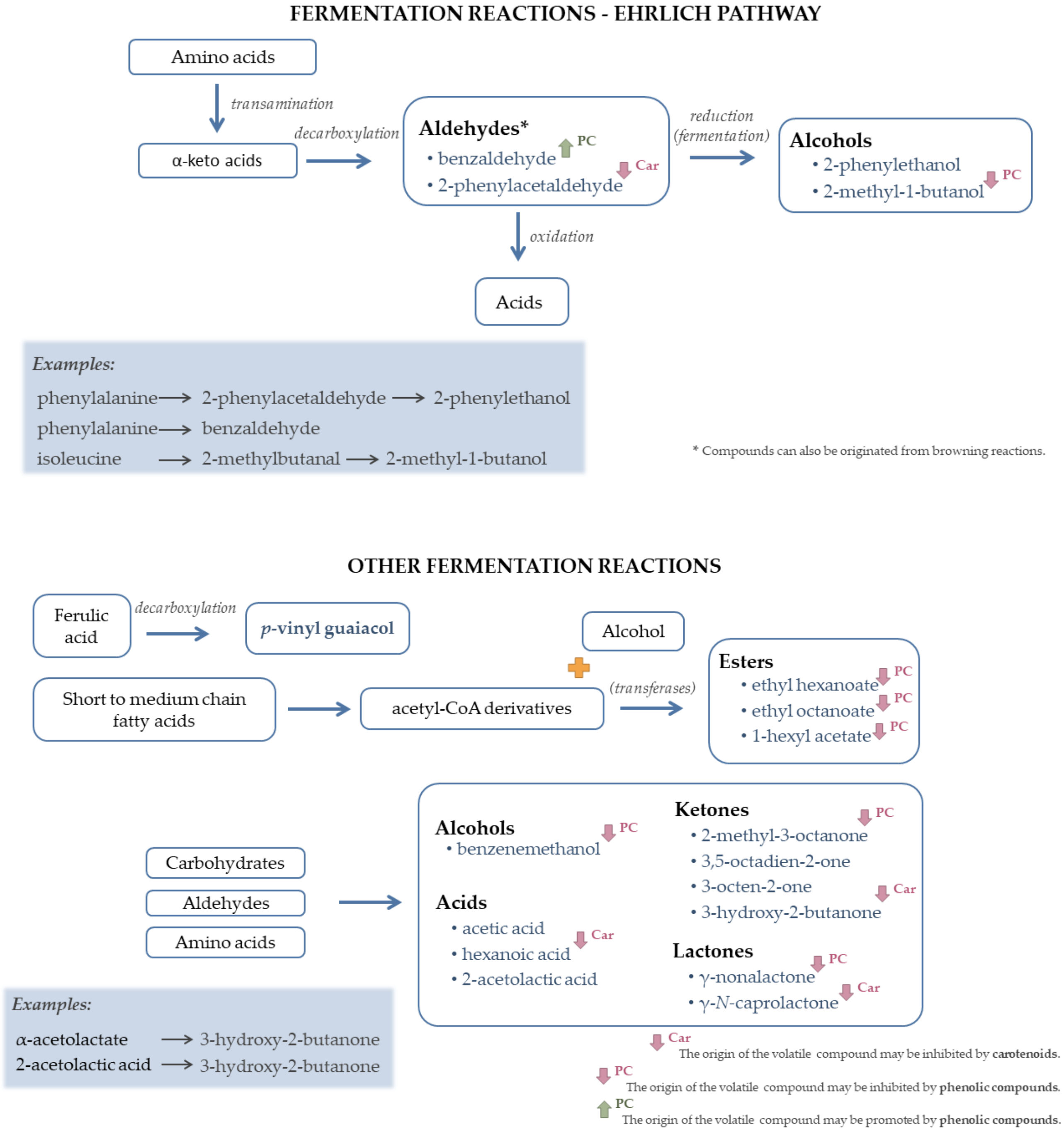
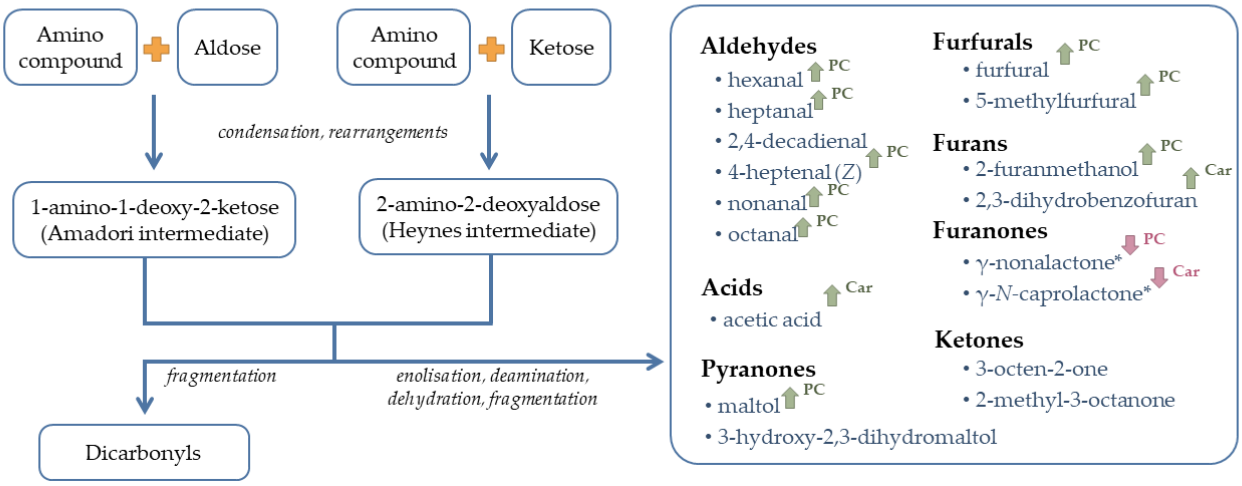


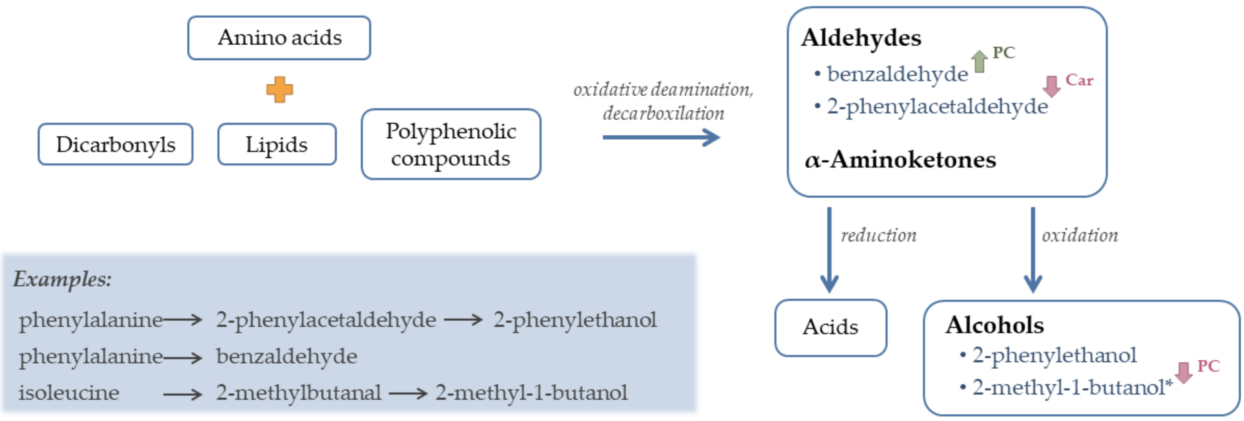
Figure C.4: Representative scheme of fermentation [1–6] reactions in *broas*.

MAILLARD REACTION – EARLY STAGES



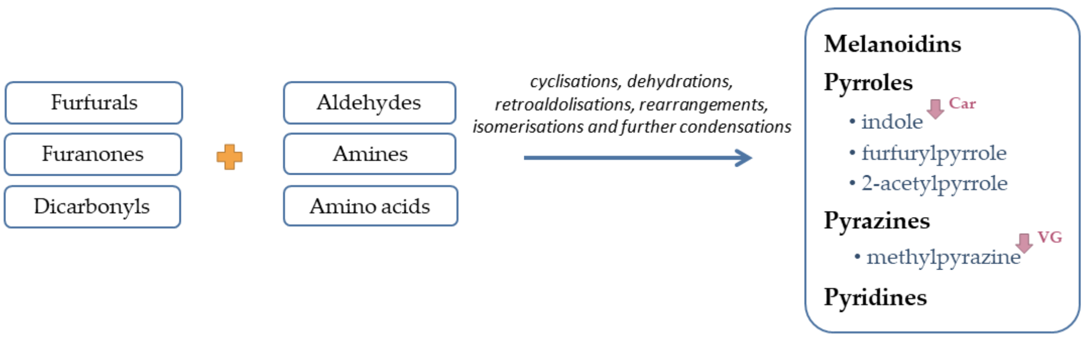
* The compound can also be originated from lipid oxidation reactions and/or during fermentation.

MAILLARD REACTION – STRECKER DEGRADATION



* The compound can also be originated by the Ehrlich pathway during fermentation.

MAILLARD REACTION – LAST STAGES



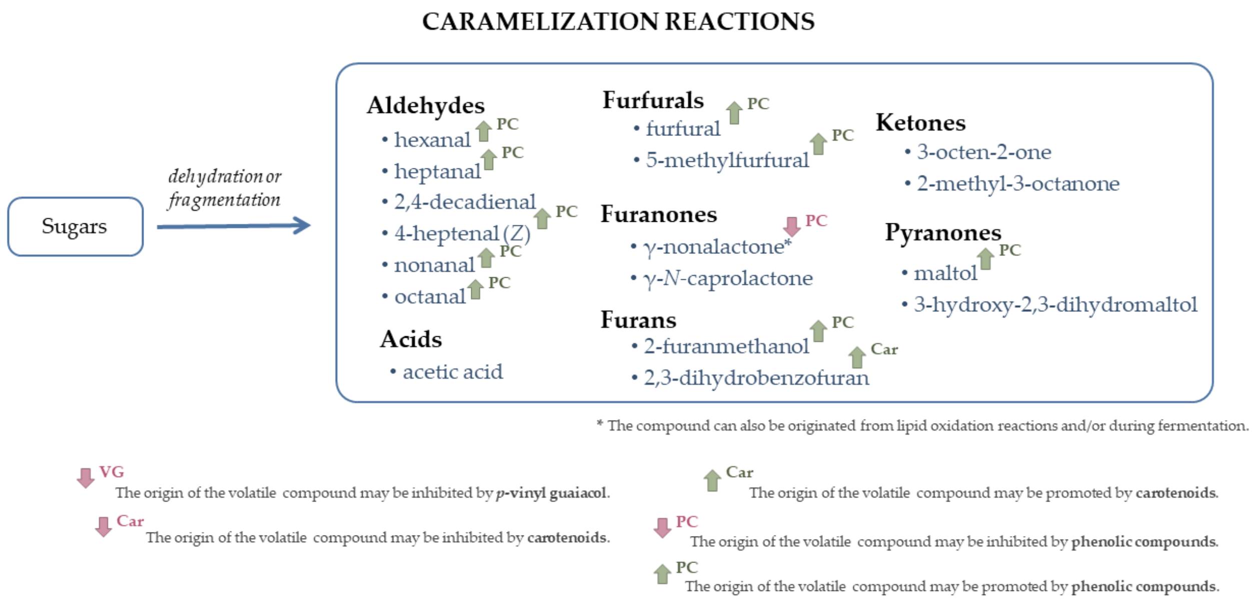


Figure C.5: Representative scheme of non-enzymatic browning reactions [3,5–13].

Table C.5: Average peak areas obtained for each *broa* and considered for the cluster analysis.

Peaks are numbered according to ***Table 4.1***. *****: Not detected. A peak area of 3400 was considered, which corresponded to the half of the area obtained for the lowest detected peak.

Peak	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
1	4052460	6913655	3400	5878768	3059599	3436765	2782261	3277620	3400	4707432	1877325	3400*
2	1613111	1620132	3900906	2643630	1468109	2776033	3882538	1373954	6166869	2463561	30935558	3394857
3	42809453	39384900	33613872	34737284	26545801	28408077	25012446	32308492	30243685	36939700	31307981	40425174
4	1391288	1847294	2180311	11175028	1462992	2194962	2376009	3637860	1917772	1856100	2435142	2685667
5	3593791	3686261	3972399	4820196	2761784	2702190	3046064	2477990	2769137	3338531	3405233	5004677
6	1291798	1471542	1850174	2083018	1177605	1413696	2148679	1968306	2227936	2065355	2722728	1925435
7	41683618	27962542	10011568	6495596	28208587	23023617	14924908	6775093	33668744	15145745	7077303	11052841
8	198480	193930	311064	181077	445379	303176	485323	253979	213735	148576	140761	709668
9	34897282	26314065	26882267	2273011	37324793	52388869	35154083	15673955	22721271	12099642	9750833	7089576
10+11	8031932	15653699	32243251	15761638	17793289	24282608	25086285	22056034	16016763	12749030	19141693	8549643
12	37377696	38822350	51998922	60982592	33481342	32710156	29479398	24333969	30882438	35750860	49383172	63521909
13	1164009	1349253	1255192	1672298	1106670	864794	977344	1018060	1306491	1202917	954805	1054406
14	2149843	1955902	2463292	6311790	1685502	1452278	1903953	1764546	1969608	2341862	2434417	3421223
15	113944	134862	261796	415480	178634	191245	214951	452640	186123	211217	223172	248711
16	446277	283641	74232	3400*	317254	152965	148377	32667	390962	238175	98585	105878
17	32489764	21278051	10737694	3468487	23923175	14278293	13260280	6812942	23952379	17195874	7717869	11536969
18	1735450	2057507	4662797	2570542	3061797	3857207	3131023	3733842	2249823	1377301	1731491	1151421
19	1623548	1465513	1290231	1566770	1111399	1562207	1337130	1225524	1277881	1355121	956488	2258959
20	3144333	2446348	1497460	458360	2525714	3378573	2520405	1111221	2636028	1459251	1263346	1507561
21	2595959	1737963	3879275	267453	2325498	5775428	3852010	2784448	1749929	1171298	1173912	1415271
22	4311779	2332057	4041739	9207260	2756138	2029140	2017175	2113637	2069868	3220536	5199669	7341500

23	3768645	3048444	3083768	3935878	2703577	3354148	3269097	3549377	2973530	3283096	3414662	5159205
25	2051450	2148189	1800744	1915352	1709455	1535459	1701481	1643097	2286783	2099377	1189440	2142055
26	31676334	28989143	27251115	33325932	25999215	23244700	24141763	21986265	30210938	28265876	23382118	28739581
27	4953777	5037512	5052697	4710193	4735489	5259447	5154612	3933640	5574949	5467128	4887885	5750972
28	100780	81779	158659	114860	206489	283017	252524	182043	177354	194603	193142	182601
29	462177	353456	390378	522654	325111	379800	271951	247557	336935	394589	345915	448016
30	4188404	3020244	2089020	739941	2897347	3273912	2690390	1588579	3239119	3107094	2130140	3773725
32	298606	218204	429272	2468688	135767	195278	145557	91797	93674	884731	1833196	2194559
33+34	621269	483667	696457	744337	534387	468188	510564	633148	667322	486793	656622	1222280
35	8302680	6047662	2618490	1973207	4356173	4162317	4602592	3059521	5098051	4959568	2473066	7522055
36	98102	84972	311543	191446	72314	392731	326022	381897	459506	364234	288649	997204
37	587522	755951	297704	272196	486277	471636	529033	268413	484596	613623	297630	715258
38+39	1598227	1802087	1647842	1514611	1446428	1739374	1662019	1320210	1900977	1780575	2046604	2916241
41	59738	46123	350006	227259	290495	432200	351480	168764	276482	268761	412327	373893
42	8695448	5976580	5714487	6971412	6441891	7782569	7318207	7045146	7957922	7468878	5688109	9934246
43	174532	400291	317189	330852	324537	424027	501831	392050	347390	325182	395272	313054
44	238209	193748	259734	567444	285827	385678	374888	303588	209579	249207	317469	408199
45	1535769	893236	1295200	1928160	1294623	1588531	1619058	1512016	1233514	1319706	1268380	1928022
46	376254	178579	438269	319181	332838	878159	666754	640722	107136	3400*	3400*	3400*
47	1268841	1442951	1048583	1195958	1184681	994363	1221829	1195409	1635686	1676642	1310337	472280
51	976209	1257881	1127429	1378532	1040453	1502233	1252689	1081818	1171470	1222260	1297878	961650
52	1503007	283172	2068925	1164557	819799	4132068	2798608	2812151	446526	265389	508945	367096
53	15152537	11609414	8828916	5020070	10862974	12566136	13518938	6185419	13483629	10616912	12359645	8839444
54	540800	5129324	6599299	2262882	6032707	13884318	6531884	15783039	4701947	6569573	9450744	400543
55+56	8450949	14285894	18616582	14589386	12678874	14041615	10873452	13614795	13201407	12280016	15421156	16179171
57	20041	3400*	93277	31646	45008	225202	169023	143060	19116	10995	22289	17029
58	1011329	2516272	973607	1094614	1101677	1548085	850607	1543379	598885	813143	460842	1151660
59	1412029	2624523	1393247	1607556	1273287	1900710	1314660	1708459	1054199	1137389	1091069	1052786
60+61	718680	4156615	3168889	1902561	2752217	5673703	3021665	9812370	834929	3902311	8548717	2165964
62	1863121	3400*	1733556	2152229	2313533	1720956	1831554	1804888	2133698	2657191	2395628	3474196
63	51189	3400*	120961	44869	20411	347098	209081	191003	10491	3400*	3400*	14602
64+65	6339166	5642795	3417544	2707100	4269184	5385376	5911011	3113892	5152787	4961316	4767411	5000905
66	74630	57873	158578	129116	292359	597594	642937	586295	255356	402545	458564	464240
67	438183	640477	515117	857963	450077	555275	586652	948866	604684	451767	646392	706207
68	95629	64393	109861	101058	72714	140880	124854	108057	78194	62189	65775	109235
69	2330773	1478157	1553139	2171127	1476484	1637940	1276584	1296740	1401814	1158143	1222309	1758262
70	2526163	3656598	4313685	3129476	2656395	3715235	3358593	3912802	2052527	2587557	2717313	3115555
71	3212529	2364740	1870765	1478061	3005185	2484220	2415535	1769081	2288348	2605161	2273039	1980932
72	2566407	138938	337016	1119054	220113	368650	309619	242157	84277	235418	466327	2533421
73	454356	621107	362993	797011	430308	737478	533188	779705	276825	536760	513292	221482
74	416078	317656	470235	383909	380686	729395	634770	624754	259102	172866	164255	239652
75	37184	58201	39284	20787	55507	42719	51099	28537	87530	73321	90025	28895
76	187247	76902	163165	159803	125155	322680	279348	270960	3400*	3400*	3400*	3400*
77	1613542	1520422	1193258	1115091	1266597	1592534	1611110	1365599	1679614	1771210	1273830	1953320
78	206958	136615	255803	222412	183613	178479	160586	103178	106388	128061	140773	176374
79	1829113	1773109	1673559	1972275	1575570	1675712	1596893	1385864	1898293	1581061	1798724	2975183

80	6635587	4319722	5277130	5450636	6300769	9207526	8274601	7910861	2274495	2209702	2223696	3286119
81	3906205	2154931	3052472	2992268	3601337	6323672	5602433	5357264	352941	317403	232080	574600
82	2831539	3293831	3296997	3952307	3270635	722472	3479460	2979964	3750725	3659228	4729299	1768510
83	3654169	1940086	3016515	3235971	3351406	5973824	5256780	5163522	503478	357077	419552	594503
84	3206104	3052760	2175799	2146376	2181693	2296852	3079223	2531329	3633080	3540861	2206438	3447821
85	20733	37824	25370	37319	124243	135042	178924	195143	162155	254445	240083	327804
86	39752	30773	3400*	28294	100447	92256	135648	148839	98077	171831	152263	183248
87	1524488	1369420	1115381	1385528	1237920	1296943	1385626	1281608	1520064	1604085	1009771	1981223
89	566510	772346	759028	638117	499344	589963	436567	508654	545421	419994	419998	770465
90	229693	305503	324023	304323	295081	273278	331966	318051	282425	380979	457308	476522
91	1320265	1308923	1191385	1699429	1491772	1579126	1491461	1586679	1566635	1528966	1417740	1918638
92	359465	272754	213875	178524	181084	229743	262689	221401	257947	252175	208731	253418
94	1488997	1156215	1069183	1328720	1399041	1496208	1585530	1631865	1555529	1818639	1884353	2487290
95	292897	451798	516811	472471	319294	246098	324599	278938	226035	253007	350927	247127
96	2098644	2928697	3068897	2813205	2532200	2209901	2515595	2274340	2319640	2493556	3335083	2744327
98	209390	167197	3400*	114012	113658	75642	84664	266659	102979	234135	68232	3400*
99	981776	1003767	522505	839491	774144	600560	722266	1225579	555970	1197652	479132	736538
100	194157	108831	90697	159954	74380	101802	84468	85110	3400*	3400*	3400*	3400*
102	352367	265805	222085	318640	326795	350290	323164	255323	267612	277907	349331	302100
104	290772	253259	243965	378274	395475	305004	390790	379881	195570	229084	261919	203555

Table C.6: Spearman correlation coefficients among *broas* volatile compounds.

P: Peak (numbered according to ***Table 4.1***); *p*-Value corresponds to the significance level of Spearman correlation coefficient indicated as *: significant at $p < 0.05$; **: significant at $p < 0.01$.

P	2	3	4	5	6	7	8	9	10+11	12	13	14	15	16	17	18	19	20	21	22	23
1	-0.634*	0.324	-0.190	0.000	-0.289	0.070	-0.528	0.000	-0.373	-0.014	0.303	-0.148	-0.268	0.099	0.085	-0.092	0.345	-0.106	-0.275	0.035	0.127
2		-0.252	0.273	0.273	0.643*	-0.175	-0.014	-0.189	0.329	0.252	-0.112	0.406	0.196	-0.175	-0.189	-0.063	-0.147	0.000	-0.042	0.049	-0.070
3			-0.119	0.678*	-0.217	0.014	-0.308	-0.490	-0.762**	0.615*	0.455	0.587*	-0.056	0.042	0.056	-0.580*	.636*	-0.217	-0.427	0.643*	0.524
4				0.126	0.559	-0.881**	0.056	-0.580*	0.301	0.147	-0.224	0.336	0.888**	-0.881**	-0.902**	0.126	0.021	-0.643*	-0.168	0.210	.587*
5					0.056	-0.238	-0.091	-0.531	-0.427	0.916**	0.497	0.867**	0.154	-0.231	-0.210	-0.441	0.552	-0.315	-0.476	0.762**	0.392
6						-0.469	-0.350	-0.587*	0.168	-0.049	-0.007	0.385	0.462	-0.385	-0.434	-0.217	-0.308	-0.490	-0.413	0.028	0.119
7							0.105	0.601*	-0.357	-0.294	0.105	-0.448	-0.944**	0.986**	0.993**	-0.161	0.119	0.867**	0.224	-0.371	-0.497
8								0.441	0.315	-0.161	-0.308	-0.280	0.084	0.007	0.063	0.378	0.119	0.385	0.594*	-0.315	-0.112
9									0.413	-0.531	-0.329	-0.776**	-0.559	0.510	0.545	0.573	-0.126	.783**	0.811**	-0.678*	-0.552
10+11										-0.385	-0.399	-0.357	0.399	-0.434	-0.406	.832**	-0.559	-0.042	0.643*	-0.503	-0.357
12											0.364	0.825**	0.182	-0.287	-0.280	-0.427	0.434	-0.343	-0.510	.874**	0.406
13												0.455	-0.105	0.105	0.154	-0.133	0.224	-0.210	-0.441	0.315	-0.196
14													0.420	-0.406	-0.399	-0.469	0.329	-0.559	-0.615*	.839**	0.490
15														-0.951**	-0.937**	0.245	-0.119	-0.783**	-0.091	0.280	0.469
16															0.993**	-0.287	0.077	.797**	0.098	-0.322	-0.476
17																-0.224	0.105	.811**	0.154	-0.336	-0.503
18																	-0.287	0.126	0.769**	-0.517	-0.329
19																		0.196	-0.112	0.301	0.566
20																			0.545	-0.510	-0.378
21																				-0.643*	-0.273
22																					0.580*

P	25	26	27	28	29	30	32	33_34	35	36	37	38+39	41	42	43	44	45	46	47	51	52
1	0.077	0.246	-0.387	-0.261	0.289	-0.049	0.120	-0.542	0.035	-0.493	0.162	-0.331	-0.613*	-0.085	0.183	-0.127	0.085	0.085	0.331	0.394	-0.092
2	-0.035	0.000	0.497	0.154	0.035	-0.021	0.301	0.357	-0.210	0.385	-0.126	0.664*	0.643*	-0.098	0.203	0.168	-0.091	-0.254	0.070	0.322	-0.091
3	0.552	0.594*	0.077	-0.699*	0.727**	0.308	0.559	0.259	0.441	-0.077	0.406	0.182	-0.517	0.252	-0.594*	-0.224	0.105	-0.444	0.119	-0.336	-0.455
4	-0.364	-0.259	-0.161	0.133	-0.021	-0.538	0.280	0.503	-0.580*	0.455	-0.580*	0.021	0.322	-0.077	0.273	0.783**	0.538	0.028	-0.364	0.280	0.287
5	0.448	0.650*	0.161	-0.559	0.741**	0.014	.832**	0.545	0.119	-0.161	0.308	0.343	-0.098	-0.049	-0.462	0.126	0.224	-0.465	-0.070	-0.098	-0.434
6	-0.042	-0.021	0.126	0.077	-0.182	-0.378	0.168	0.350	-0.329	0.378	-0.322	0.399	0.203	-0.119	0.378	0.189	-0.014	-0.373	0.448	0.364	-0.182
7	0.441	0.294	0.364	-0.028	-0.035	0.762**	-0.413	-0.462	0.741**	-0.161	0.608*	0.126	-0.196	0.420	-0.154	-0.650*	-0.392	0.014	0.294	-0.273	-0.217
8	-0.042	-0.252	0.294	0.357	-0.266	0.182	-0.329	0.119	0.224	0.301	0.168	-0.119	0.378	0.315	-0.112	0.287	0.336	0.444	-0.720**	-0.483	0.371
9	-0.266	-0.336	0.042	0.357	-0.371	0.287	-0.615*	-0.643*	0.182	-0.217	0.147	-0.315	0.147	0.035	0.217	-0.252	-0.154	0.711**	-0.203	-0.014	0.503
10+11	-0.664*	-0.692*	-0.168	0.434	-0.580*	-0.552	-0.385	-0.105	-0.650*	0.105	-0.587*	-0.259	0.483	-0.490	0.524	0.238	-0.056	0.627*	-0.329	0.350	0.671*
12	0.259	0.497	0.063	-0.427	0.762**	-0.007	0.895**	0.538	-0.063	-0.196	0.161	0.336	0.070	-0.168	-0.503	0.231	0.161	-0.521	-0.210	-0.035	-0.420
13	0.755**	0.818**	-0.042	-0.755**	0.413	-0.147	0.210	0.280	0.119	-0.315	0.168	-0.084	-0.678*	-0.105	-0.350	-0.434	-0.217	-0.324	0.378	-0.042	-0.483
14	0.336	0.566	0.112	-0.469	0.692*	-0.161	0.846**	0.748**	-0.119	0.042	-0.014	0.315	-0.028	-0.049	-0.503	0.203	0.238	-0.592*	0.056	-0.077	-0.420
15	-0.357	-0.301	-0.224	0.070	-0.014	-0.685*	0.273	0.573	-0.664*	0.378	-0.636*	-0.161	0.231	-0.231	0.035	0.629*	0.441	0.049	-0.385	0.084	0.287
16	0.455	0.308	0.350	-0.021	-0.042	0.755**	-0.371	-0.462	0.755**	-0.182	0.636*	0.175	-0.224	0.406	-0.154	-0.671*	-0.427	-0.106	0.406	-0.273	-0.322
17	0.490	0.329	0.371	-0.049	-0.021	0.748**	-0.385	-0.448	0.762**	-0.175	0.643*	0.133	-0.245	0.413	-0.196	-0.692*	-0.413	-0.056	0.364	-0.308	-0.294
18	-0.476	-0.441	-0.308	0.175	-0.399	-0.455	-0.510	-0.161	-0.510	-0.035	-0.559	-0.608*	0.126	-0.308	0.329	0.126	0.063	0.866**	-0.434	0.224	0.811**
19	0.434	0.552	0.357	-0.280	0.769**	0.503	0.483	0.056	0.469	0.154	0.469	0.147	-0.154	0.615*	-0.294	0.224	0.622*	0.000	-0.224	-0.063	-0.077
20	0.147	0.049	0.434	0.217	-0.084	0.755**	-0.441	-0.455	0.580*	0.042	0.434	0.119	0.168	0.483	0.028	-0.308	-0.126	0.338	-0.077	-0.133	0.168

21	-0.392	-0.510	0.042	0.273	-0.406	0.098	-0.608*	-0.322	0.007	0.140	-0.154	-0.336	0.245	0.070	0.203	-0.056	0.035	0.859**	-0.462	-0.105	0.776**	
22	0.147	0.476	-0.203	-0.434	0.720**	-0.105	0.839**	0.650*	-0.140	-0.238	-0.049	0.098	-0.084	-0.112	-0.657*	0.259	0.231	-0.570	-0.112	-0.196	-0.371	
23	-0.147	0.112	-0.112	-0.140	0.545	0.042	0.573	0.413	-0.056	0.266	-0.168	0.021	0.035	0.357	-0.252	0.601*	0.727**	-0.099	-0.301	-0.070	0.147	
25		0.797**	0.483	-0.559	0.371	0.427	0.112	0.189	0.671*	0.084	0.629*	0.343	-0.503	0.406	-0.399	-0.510	-0.196	-0.500	0.371	-0.329	-0.692*	
26			0.154	-0.692*	0.671*	0.252	0.413	0.350	0.399	-0.231	0.371	0.126	-0.517	0.280	-0.490	-0.266	0.056	-0.416	0.329	-0.140	-0.503	
27				0.252	0.189	0.664*	0.105	-0.035	0.545	0.650*	0.580*	0.727**	0.399	0.580*	-0.049	-0.098	0.028	-0.275	0.042	-0.091	-0.357	
28					-0.406	0.084	-0.217	-0.364	-0.189	0.343	-0.070	0.063	0.741**	0.112	0.371	0.448	0.217	0.218	-0.266	0.175	0.266	
29						0.287	0.811**	0.301	0.140	-0.063	0.224	0.154	-0.119	0.280	-0.566	0.161	0.420	-0.310	-0.077	0.007	-0.266	
30							-0.028	-0.259	0.839**	0.273	0.699*	0.462	0.091	0.755**	-0.273	-0.273	0.000	-0.169	0.035	-0.329	-0.266	
32								0.413	-0.147	-0.084	0.112	0.357	0.154	-0.077	-0.350	0.392	0.364	-0.521	-0.063	0.168	-0.378	
33_34									-0.210	0.224	-0.336	0.084	0.084	0.035	-0.531	0.322	0.245	-0.352	-0.273	-0.364	-0.098	
35										0.119	0.860**	0.350	-0.294	0.671*	-0.294	-0.490	-0.098	-0.218	0.217	-0.531	-0.420	
36											-0.063	0.427	0.441	0.587*	0.098	0.280	0.287	-0.049	-0.203	-0.070	0.077	
37												0.462	-0.168	0.392	-0.182	-0.392	-0.119	-0.338	0.224	-0.301	-0.601*	
38_39													0.406	0.189	0.126	-0.070	-0.238	-0.641*	0.238	0.140	-0.594*	
41														0.014	0.217	0.573	0.210	0.056	-0.476	0.210	0.238	
42															-0.280	0.070	0.462	-0.007	-0.091	-0.385	-0.021	
43																0.133	-0.126	0.324	0.196	0.748**	0.266	
44																	0.790**	0.169	-0.636*	0.238	0.392	
45																		0.303	-0.524	-0.014	0.413	
46																			-0.444	0.127	0.916**	
47																				0.231	-0.538	
51																					0.133	
P	**53**	**54**	**55+56**	**57**	**58**	**59**	**60+61**	**62**	**63**	**64+65**	**66**	**67**	**68**	**69**	**70**	**71**	**72**	**73**	**74**	**75**	**76**	**77**
1	-0.077	-0.035	-0.317	-0.197	0.423	0.690*	0.120	-0.155	-0.092	0.092	-0.366	-0.049	-0.359	0.148	0.035	0.239	-0.028	0.775**	0.106	-0.148	0.208	-0.141
2	0.224	0.021	0.427	0.021	-0.622*	-0.517	-0.056	0.091	-0.141	0.077	0.182	0.133	0.196	-0.189	-0.056	-0.280	0.105	-0.420	-0.252	0.413	-0.306	0.077
3	-0.231	-0.510	0.182	-0.685*	0.196	0.063	-0.245	0.224	-0.380	0.084	-0.573	0.056	-0.301	0.462	-0.133	-0.084	0.406	-0.056	-0.357	-0.322	-0.324	0.273
4	-0.538	0.203	0.517	0.343	0.035	-0.035	0.280	0.098	0.246	-0.524	0.476	0.846**	0.441	-0.028	0.441	-0.825**	0.350	0.336	0.105	-0.455	0.093	-0.224
5	-0.280	-0.629*	0.517	-0.503	-0.049	-0.168	-0.399	0.294	-0.345	-0.042	-0.510	0.147	-0.070	0.476	-0.007	-0.329	0.490	-0.252	-0.371	-0.252	-0.381	-0.028
6	-0.028	0.112	0.140	-0.077	-0.671*	-0.441	0.098	0.322	-0.275	-0.182	0.266	0.531	-0.105	-0.545	-0.154	-0.483	-0.077	0.049	-0.357	0.308	-0.367	0.077
7	0.727**	-0.350	-0.594*	-0.294	0.021	-0.056	-0.434	-0.084	-0.134	0.713**	-0.294	-0.706*	-0.252	0.133	-0.559	0.825**	-0.322	-0.399	-0.035	0.413	-0.057	0.469
8	0.028	-0.112	0.021	0.427	0.322	-0.140	-0.189	-0.168	0.563	0.133	0.434	-0.098	0.685*	0.231	0.294	0.028	0.028	-0.441	0.469	-0.350	0.359	0.154
9	0.566	0.259	-0.455	0.503	0.231	0.350	0.028	-0.608*	0.535	0.483	0.147	-0.671*	0.329	0.063	0.147	0.650*	-0.238	-0.070	0.629*	0.147	0.623*	-0.035
10+11	-0.056	.734**	0.210	0.811**	-0.077	0.203	0.490	-0.573	0.570	-0.259	0.483	0.070	0.545	-0.322	0.643*	-0.273	-0.203	0.140	0.566	0.049	0.523	-0.503
12	-0.392	-0.476	0.671*	-0.441	0.007	-0.182	-0.245	0.357	-0.394	-0.231	-0.469	0.133	-0.126	0.483	0.007	-0.322	0.538	-0.231	-0.455	-0.203	-0.441	-0.175
13	-0.315	-0.531	0.098	-0.559	-0.014	0.098	-0.490	0.042	-0.408	-0.189	-0.839**	0.007	-0.413	0.280	-0.182	-0.210	-0.273	-0.014	-0.259	-0.077	-0.352	-0.161
14	-0.413	-0.462	0.517	-0.448	-0.378	-0.392	-0.350	0.483	-0.394	-0.322	-0.378	0.266	-0.133	0.245	-0.140	-0.503	0.490	-0.231	-0.503	-0.203	-0.513	-0.042
15	-0.741**	0.329	0.552	0.357	-0.056	-0.063	0.322	0.070	0.254	-0.734**	0.378	0.671*	0.392	-0.112	0.531	-0.853**	0.273	0.238	0.112	-0.490	0.082	-0.343
16	0.734**	-0.364	-0.636*	-0.385	-0.063	-0.133	-0.420	0.042	-0.254	0.706*	-0.294	-0.685*	-0.378	0.035	-0.657*	0.846**	-0.336	-0.392	-0.161	0.490	-0.164	0.510
17	0.699*	-0.371	-0.622*	-0.364	-0.035	-0.105	-0.448	-0.014	-0.204	0.692*	-0.322	-0.720**	-0.329	0.077	-0.608*	0.832**	-0.350	-0.413	-0.105	0.441	-0.125	0.497
18	-0.119	0.545	0.056	.832**	0.259	0.538	0.252	-0.797**	0.775**	-0.238	0.203	-0.056	0.622*	0.077	0.671*	-0.189	-0.189	0.280	.839**	-0.287	0.769**	-0.566
19	0.014	-0.608*	0.014	-0.259	0.399	0.196	-0.448	0.007	0.141	0.378	-0.203	-0.021	0.273	0.720**	-0.014	0.070	0.545	0.070	0.133	-0.531	0.128	0.399
20	0.783**	-0.196	-0.455	0.105	0.140	0.035	-0.343	-0.315	0.261	0.755**	0.021	-0.629*	0.217	0.273	-0.280	0.727**	-0.112	-0.364	0.301	0.238	0.267	0.406
21	0.322	0.434	-0.154	0.713**	0.266	0.371	0.182	-0.776**	0.796**	0.280	0.343	-0.329	0.692*	0.119	0.497	0.196	-0.049	-0.070	.818**	-0.168	0.772**	-0.070
22	-0.462	-0.448	0.469	-0.413	-0.133	-0.266	-0.287	0.566	-0.401	-0.413	-0.441	0.168	-0.259	0.399	-0.196	-0.322	0.622*	-0.147	-0.517	-0.301	-0.459	-0.203
23	-0.273	-0.203	0.217	0.000	0.133	0.014	-0.056	0.273	0.162	-0.126	0.154	0.441	0.273	0.413	0.070	-0.357	0.846**	0.238	0.000	-0.643*	0.085	0.133

25	0.014	-0.734**	-0.063	-0.790**	0.014	-0.224	-0.594*	0.238	-0.507	0.259	-0.587*	-0.070	-0.378	0.238	-0.434	0.063	-0.280	-0.371	-0.420	0.077	-0.534	0.483
26	0.021	-0.867**	-0.049	-0.636*	-0.105	-0.105	-0.804**	0.266	-0.387	0.154	-0.776**	-0.098	-0.315	0.524	-0.497	0.014	0.126	-0.196	-0.329	-0.126	-0.377	0.147
27	0.329	-0.259	0.014	-0.315	-0.189	-0.490	-0.238	0.154	-0.155	0.497	0.217	-0.210	0.140	-0.049	-0.245	0.224	-0.091	-0.531	-0.224	0.315	-0.324	0.776**
28	0.189	0.469	-0.217	0.510	-0.126	-0.273	0.329	0.133	0.296	0.042	0.853**	-0.182	0.294	-0.462	0.021	0.315	-0.077	0.000	0.161	0.252	0.189	0.175
29	-0.189	-0.545	0.238	-0.392	0.028	0.000	-0.462	0.238	-0.162	0.007	-0.483	-0.133	-0.035	.650*	-0.182	-0.028	0.629*	-0.021	-0.210	-0.357	-0.185	0.098
30	.608*	-0.434	-0.336	-0.378	0.098	-0.238	-0.378	0.140	-0.120	0.748**	-0.021	-0.469	-0.035	0.287	-0.503	0.657*	0.126	-0.455	-0.154	0.189	-0.157	0.797**
32	-0.329	-0.357	0.497	-0.371	-0.168	-0.210	-0.161	0.462	-0.352	-0.189	-0.252	0.140	-0.119	0.301	-0.063	-0.259	.643*	-0.014	-0.448	-0.168	-0.384	-0.056
33_34	-0.462	-0.399	0.538	-0.112	-0.280	-0.490	-0.413	0.399	-0.099	-0.510	-0.161	0.441	0.154	0.301	-0.077	-0.685*	0.392	-0.420	-0.280	-0.385	-0.356	-0.154
35	0.510	-0.587*	-0.441	-0.566	0.147	-0.189	-0.441	0.154	-0.232	0.755**	-0.189	-0.350	-0.189	0.189	-0.469	0.559	-0.084	-0.448	-0.189	0.126	-0.214	0.804**
36	-0.042	0.126	0.161	0.091	-0.084	-0.371	0.084	0.070	0.183	0.035	0.601*	0.336	0.434	-0.133	0.056	-0.287	0.035	-0.196	0.021	-0.077	-0.078	0.587*
37	0.378	-0.538	-0.280	-0.636*	0.154	-0.175	-0.294	0.231	-0.394	0.685*	-0.203	-0.392	-0.301	0.070	-0.343	0.573	-0.133	-0.364	-0.315	0.266	-0.320	0.664*
38_39	0.287	-0.217	0.329	-0.545	-0.273	-0.524	0.021	0.343	-0.570	0.385	0.084	0.154	-0.203	-0.182	-0.273	0.000	0.000	-0.434	-0.622*	0.545	-0.644*	0.559
41	0.140	0.301	0.336	0.441	-0.231	-0.427	0.210	0.105	0.225	-0.007	.678*	-0.007	0.476	-0.154	0.133	-0.028	0.252	-0.308	0.028	0.203	0.021	0.056
42	0.329	-0.469	-0.406	-0.147	0.084	-0.252	-0.510	0.196	0.190	0.483	0.210	-0.133	0.266	0.329	-0.420	0.301	0.238	-0.252	0.077	-0.252	0.032	0.811**
43	0.252	0.524	-0.063	0.357	0.091	0.364	0.573	-0.420	0.169	0.196	0.448	0.336	0.140	-0.462	0.364	-0.070	-0.364	0.538	0.287	0.329	0.310	-0.112
44	-0.350	0.063	0.343	0.503	0.133	-0.063	0.084	0.189	0.423	-0.350	0.566	0.448	0.580*	0.203	0.315	-0.413	0.587*	0.245	0.231	-0.510	0.260	-0.203
45	-0.210	-0.217	-0.021	0.378	0.189	0.063	-0.252	0.105	0.570	-0.063	0.378	0.189	0.636*	0.441	0.182	-0.189	0.692*	0.245	0.413	-0.755**	0.434	0.091
46	0.155	0.408	-0.246	0.845**	0.380	0.641*	0.155	-0.803**	.929**	0.120	0.296	-0.176	0.718**	0.218	0.577*	0.085	0.049	0.352	0.986**	-0.423	0.975**	-0.310
47	0.343	-0.070	-0.441	-0.559	-0.517	-0.112	-0.049	0.203	-0.620*	0.259	-0.357	-0.119	-0.748**	-0.510	-0.545	0.259	-0.448	0.119	-0.483	0.636*	-0.438	0.238
51	0.049	0.385	0.168	0.217	-0.035	0.399	0.399	-0.308	0.014	-0.028	0.091	0.231	0.014	-0.182	0.266	-0.147	-0.084	0.643*	0.119	0.259	0.164	-0.336
52	0.070	0.476	-0.084	0.937**	0.224	0.483	0.196	-0.657*	0.944**	-0.063	0.420	0.000	.790**	0.217	0.552	-0.112	0.287	0.322	0.902**	-0.490	0.915**	-0.378
53		-0.126	-0.587*	0.007	-0.273	-0.098	-0.238	-0.147	0.056	0.874**	0.077	-0.503	0.000	-0.070	-0.448	.706*	-0.042	-0.266	0.091	0.497	0.139	0.448
54			0.084	0.573	-0.007	0.301	0.867**	-0.434	0.303	-0.294	0.510	0.063	0.154	-0.566	0.538	-0.105	-0.245	0.413	0.322	0.196	0.367	-0.329
55_56				-0.042	0.161	-0.042	0.273	-0.028	-0.148	-0.503	-0.084	0.531	0.175	0.217	0.503	-0.734**	0.231	-0.105	-0.203	-0.168	-0.260	-0.385
57					0.112	0.322	0.273	-0.524	.880**	-0.189	0.573	-0.007	0.755**	0.014	0.538	-0.112	0.161	0.280	0.811**	-0.336	0.815**	-0.441
58						0.671*	0.224	-0.427	0.310	0.028	-0.035	0.203	0.259	0.490	0.531	-0.049	0.014	0.371	0.427	-0.517	0.409	-0.112
59							0.343	-0.776**	0.437	0.042	-0.210	0.056	0.196	0.315	0.608*	-0.028	-0.021	0.748**	0.650*	-0.378	0.691*	-0.434
60_61							1.000	-0.301	0.035	-0.238	0.441	0.308	-0.021	-0.538	0.601*	-0.196	-0.210	0.476	0.091	0.196	0.160	-0.238
62									-0.620*	-0.189	0.035	0.084	-0.490	-0.210	-0.671*	0.042	0.161	-0.343	-0.790**	0.182	-0.747**	0.322
63										0.035	0.444	-0.070	0.887**	0.317	0.535	-0.049	0.289	0.239	0.951**	-0.599*	0.928**	-0.197
64_65											0.014	-0.420	0.049	0.098	-0.287	.664*	0.014	-0.210	0.112	0.301	0.142	0.636*
66												0.203	0.510	-0.434	0.245	-0.028	0.063	0.077	0.280	-0.007	0.295	0.252
67													0.119	-0.049	0.357	-0.839**	0.077	0.336	-0.105	-0.301	-0.132	-0.161
68														0.385	0.566	-0.259	0.406	0.007	0.776**	-0.580*	0.712**	-0.063
69															0.091	-0.070	0.573	-0.063	0.308	-0.664*	0.278	-0.112
70																-0.503	0.056	0.413	0.601*	-0.434	0.573	-0.476
71																	-0.140	-0.175	0.007	0.420	0.082	0.406
72																		0.000	0.112	-0.559	0.210	-0.021
73																			0.371	-0.273	0.459	-0.371
74																				-0.531	0.975**	-0.266
75																					-0.513	0.210
76																						-0.288
P	**78**	**79**	**80**	**81**	**82**	**83**	**84**	**85**	**86**	**87**	**89**	**90**	**91**	**92**	**94**	**95**	**96**	**98**	**99**	**100**	**102**	**104**
1	-0.007	-0.127	0.169	0.148	-0.035	0.169	-0.120	-0.310	-0.282	0.127	0.063	-0.331	-0.028	0.092	-0.324	0.204	-0.183	0.720**	0.711**	0.602*	0.155	0.310
2	0.049	0.448	-0.385	-0.406	0.490	-0.385	0.056	0.217	0.042	-0.084	-0.035	0.357	-0.119	-0.042	0.210	0.035	0.434	-0.771**	-0.874**	-0.434	-0.007	-0.483
3	0.112	0.455	-0.371	-0.350	-0.182	-0.371	0.245	-0.126	-0.070	0.476	0.476	0.105	-0.035	0.329	-0.021	0.091	0.049	0.245	0.448	0.274	-0.154	-0.497

4	-0.077	0.196	0.084	0.007	0.189	0.084	-0.294	0.406	0.224	-0.112	0.063	0.455	0.622*	-0.455	0.371	0.084	0.245	-0.245	-0.126	-0.121	-0.175	0.112
5	0.483	0.671*	-0.420	-0.434	0.175	-0.420	-0.098	-0.182	-0.224	0.280	0.559	0.357	-0.119	0.049	-0.168	0.483	0.587*	-0.336	-0.098	0.160	-0.126	-0.434
6	-0.448	0.224	-0.441	-0.497	0.776**	-0.441	0.182	0.497	0.329	0.049	-0.399	0.490	0.196	-0.112	0.483	0.007	0.287	-0.137	-0.315	-0.502	-0.189	-0.259
7	-0.049	0.028	0.035	0.084	-0.315	0.035	0.517	-0.280	-0.119	0.329	0.000	-0.587*	-0.329	0.615*	-0.196	-0.427	-0.483	0.165	0.042	0.050	0.308	-0.161
8	0.238	-0.133	0.490	0.538	-0.587*	0.490	0.035	0.056	0.091	0.091	0.259	0.042	0.175	0.112	0.000	-0.196	-0.147	-0.445	-0.182	-0.089	-0.063	0.196
9	0.217	-0.469	0.643*	0.699*	-0.476	.643*	-0.063	-0.413	-0.308	-0.238	-0.056	-0.552	-0.427	0.259	-0.399	-0.105	-0.413	-0.049	-0.140	0.295	0.308	0.420
10+11	0.077	-0.497	0.427	0.441	0.105	0.427	-0.497	-0.056	-0.196	-0.699*	-0.168	0.063	-0.189	-0.378	-0.182	0.252	0.168	-0.385	-0.497	-0.018	-0.182	0.385
12	0.552	0.629*	-0.462	-0.490	0.126	-0.462	-0.294	-0.140	-0.175	0.063	0.517	0.336	-0.049	-0.231	-0.140	0.448	0.650*	-0.420	-0.189	0.093	-0.007	-0.392
13	0.140	0.308	-0.392	-0.371	0.364	-0.392	0.000	-0.476	-0.545	0.238	0.420	-0.203	-0.133	0.042	-0.580*	0.336	0.245	0.294	0.273	0.274	-0.490	-0.350
14	0.322	.629*	-0.594*	-0.615*	0.441	-0.594*	-0.070	0.021	-0.042	0.245	0.252	0.462	0.063	-0.182	0.070	0.364	0.538	-0.277	-0.161	-0.085	-0.189	-0.510
15	0.014	-0.049	0.000	-0.021	0.217	0.000	-0.385	0.322	0.161	-0.224	0.007	0.545	0.441	-0.573	0.245	0.238	0.322	-0.200	-0.070	-0.146	-0.420	0.070
16	-0.133	0.028	-0.070	-0.028	-0.224	-0.070	0.573	-0.189	-0.014	0.364	-0.105	-0.510	-0.322	0.615*	-0.098	-0.434	-0.455	0.224	0.084	-0.036	0.329	-0.182
17	-0.084	0.007	-0.042	0.014	-0.259	-0.042	0.559	-0.245	-0.077	0.371	-0.049	-0.531	-0.343	0.622*	-0.168	-0.413	-0.462	0.214	0.091	0.004	0.266	-0.196
18	0.266	-0.483	0.699*	0.727**	-0.175	0.699*	-0.545	-0.448	-0.538	-0.573	0.112	-0.378	-0.126	-0.294	-0.538	0.224	-0.126	-0.102	-0.189	0.406	-0.196	0.503
19	0.364	0.566	0.147	0.154	-0.441	0.147	0.238	-0.182	-0.175	0.699*	0.587*	-0.140	0.273	0.420	-0.077	-0.133	-0.245	0.004	0.280	0.448	0.196	-0.231
20	0.126	0.063	0.364	0.399	-0.510	0.364	0.357	-0.280	-0.168	0.210	0.105	-0.615*	-0.210	0.545	-0.175	-0.476	-0.538	-0.151	-0.224	0.132	0.455	-0.014
21	0.175	-0.427	0.734**	0.797**	-0.559	.734**	-0.112	-0.308	-0.287	-0.308	0.105	-0.364	-0.273	0.203	-0.273	-0.105	-0.399	-0.207	-0.231	0.295	0.021	0.315
22	0.455	0.531	-0.462	-0.510	0.203	-0.462	-0.280	-0.070	-0.007	0.077	0.231	0.294	0.098	-0.364	0.035	0.371	0.469	-0.137	0.007	0.046	0.091	-0.238
23	0.168	0.448	0.084	0.021	-0.196	0.084	-0.021	0.196	0.231	0.322	0.168	0.224	0.503	-0.056	0.413	-0.056	-0.112	0.014	0.203	0.192	0.259	-0.014
25	-0.140	0.455	-0.490	-0.441	0.042	-0.490	0.580*	-0.091	-0.119	0.678*	0.420	-0.084	0.021	0.503	-0.154	-0.189	-0.021	0.175	0.266	-0.057	-0.385	-0.657*
26	0.287	.678*	-0.350	-0.364	0.224	-0.350	0.217	-0.427	-0.399	0.559	0.420	-0.266	-0.021	0.273	-0.350	0.126	0.063	0.154	0.161	0.278	-0.028	-0.406
27	-0.161	0.322	-0.329	-0.252	-0.147	-0.329	0.685*	0.385	0.287	0.601*	0.119	0.210	0.112	0.497	0.329	-0.559	-0.133	-0.431	-0.308	-0.466	-0.070	-0.706*
28	-0.126	-0.434	0.238	0.252	-0.133	0.238	0.028	0.545	0.552	-0.098	-0.580*	0.210	0.280	-0.231	0.483	-0.406	-0.203	-0.277	-0.266	-0.495	0.378	0.322
29	0.566	0.629*	-0.245	-0.245	-0.049	-0.245	-0.035	-0.287	-0.273	0.441	0.434	-0.056	0.098	0.014	-0.168	0.126	0.077	-0.077	0.077	0.342	0.196	-0.385
30	-0.049	0.336	-0.063	-0.021	-0.538	-0.063	0.692*	0.091	0.217	0.622*	0.140	-0.266	0.028	0.671*	0.238	-0.671*	-0.510	-0.070	0.007	-0.100	0.406	-0.427
32	0.455	0.573	-0.455	-0.490	0.259	-0.455	-0.203	0.049	0.007	0.196	0.231	0.441	0.070	-0.231	0.091	0.364	0.524	-0.312	-0.126	0.028	0.154	-0.329
33_34	0.259	0.545	-0.322	-0.364	0.259	-0.322	-0.147	0.042	0.007	0.056	0.266	0.294	0.308	-0.343	0.112	0.161	0.371	-0.378	-0.294	-0.192	-0.259	-0.308
35	-0.217	0.231	-0.112	-0.056	-0.448	-0.112	0.804**	0.063	0.203	0.727**	0.196	-0.140	-0.091	0.860**	0.154	-0.476	-0.413	0.175	0.301	-0.057	0.084	-0.392
36	-0.406	0.175	-0.084	-0.049	-0.189	-0.084	0.510	0.622*	0.469	0.392	0.000	0.266	0.594*	0.154	0.608*	-0.706*	-0.322	-0.287	-0.196	-0.491	-0.224	-0.476
37	-0.091	0.161	-0.252	-0.189	-0.294	-0.252	.615*	0.112	0.196	0.629*	0.196	0.112	-0.217	0.706*	0.070	-0.203	-0.056	0.039	0.252	-0.107	0.063	-0.364
38_39	-0.308	0.538	-0.636*	-0.643*	0.168	-0.636*	0.503	0.510	0.385	0.308	0.105	0.448	0.000	0.343	0.469	-0.294	0.308	-0.501	-0.434	-0.552	0.007	-0.748**
41	0.175	0.070	0.042	0.028	-0.049	0.042	-0.140	0.420	0.343	-0.224	-0.182	0.336	0.175	-0.301	0.399	-0.210	0.182	-0.820**	-0.762**	-0.477	0.336	-0.028
42	-0.119	0.350	0.133	0.161	-0.490	0.133	0.720**	0.217	0.301	.839**	0.084	-0.231	0.510	0.545	0.392	-0.790**	-0.734**	0.091	0.196	-0.082	0.252	-0.287
43	-0.441	-0.252	0.280	0.231	0.203	0.280	-0.070	0.210	0.028	-0.301	-0.203	0.028	-0.007	0.056	0.077	0.007	0.028	0.004	-0.133	-0.004	0.028	0.308
44	0.329	0.189	0.322	0.245	-0.077	0.322	-0.413	0.287	0.224	-0.049	0.021	0.294	0.629*	-0.510	0.308	0.035	0.126	-0.399	-0.203	-0.025	0.315	0.364
45	0.406	0.224	0.462	0.434	-0.287	0.462	-0.105	0.070	0.091	0.399	0.119	0.077	0.580*	-0.098	0.196	-0.070	-0.259	-0.091	0.140	0.256	0.308	0.294
46	0.282	-0.444	0.937**	0.958**	-0.444	0.937**	-0.345	-0.451	-0.437	-0.310	0.085	-0.479	-0.106	0.028	-0.408	0.085	-0.430	0.046	0.007	0.581*	0.113	0.641*
47	-0.531	-0.007	-0.524	-0.524	0.629*	-0.524	0.455	0.063	0.063	0.217	-0.434	-0.021	-0.329	0.350	0.077	-0.007	-0.021	0.511	0.210	-0.167	-0.084	-0.287
51	-0.035	0.049	0.056	-0.007	0.427	0.056	-0.343	-0.056	-0.273	-0.308	-0.070	-0.070	-0.014	-0.238	-0.161	0.224	0.217	-0.063	-0.231	0.192	0.154	0.140
52	0.308	-0.315	0.874**	0.860**	-0.350	0.874**	-0.441	-0.322	-0.308	-0.406	-0.007	-0.364	0.042	-0.175	-0.203	0.063	-0.371	-0.091	-0.161	0.466	0.210	0.622*
53	-0.105	0.105	0.175	0.168	-0.091	0.175	0.469	-0.105	0.021	0.196	-0.252	-0.371	-0.392	.657*	0.091	-0.322	-0.406	-0.067	-0.287	0.004	0.573	-0.007
54	-0.287	-0.713**	0.224	0.252	0.028	0.224	-0.322	0.210	0.112	-0.664*	-0.420	0.133	-0.133	-0.357	0.105	0.035	-0.021	0.028	-0.112	-0.110	-0.147	0.294
55_56	0.238	0.364	-0.301	-0.329	0.091	-0.301	-0.469	0.063	-0.147	-0.385	0.552	0.420	0.091	-0.462	-0.084	0.350	.713**	-0.613*	-0.420	-0.075	-0.378	-0.343
57	0.287	-0.427	0.790**	0.776**	-0.210	0.790**	-0.517	-0.161	-0.175	-0.503	-0.175	-0.210	0.091	-0.371	-0.126	0.077	-0.224	-0.217	-0.287	0.238	0.210	.678*
58	0.091	-0.098	0.510	0.510	-0.734**	0.510	-0.210	-0.203	-0.224	-0.035	0.629*	-0.273	0.231	0.070	-0.308	-0.028	-0.168	0.182	0.469	0.516	-0.091	0.287
59	0.147	-0.273	0.615*	0.608*	-0.315	0.615*	-0.420	-0.566	-0.615*	-0.301	0.371	-0.483	-0.182	0.049	-0.601*	0.329	-0.182	0.413	0.413	0.847**	-0.049	0.462

60_61	-0.420	-0.566	0.056	0.063	-0.035	0.056	-0.259	0.378	0.252	-0.573	-0.217	0.364	-0.084	-0.252	0.217	0.098	0.189	0.046	0.056	-0.132	-0.217	0.182
62	-0.133	0.287	-0.636*	-0.671*	0.322	-0.636*	0.287	0.559	0.664*	0.399	-0.371	0.483	0.364	-0.182	0.601*	-0.189	0.203	-0.014	0.063	-0.634*	0.189	-0.245
63	0.359	-0.275	0.908**	0.923**	-0.486	0.908**	-0.296	-0.296	-0.282	-0.155	0.099	-0.345	0.141	-0.042	-0.204	-0.035	-0.458	-0.106	-0.070	0.462	0.162	0.556
64_65	-0.112	0.189	0.175	0.196	-0.322	0.175	0.608*	-0.070	0.021	0.462	0.056	-0.252	-0.329	0.888**	0.070	-0.322	-0.399	-0.014	-0.035	0.128	0.406	-0.147
66	-0.308	-0.350	0.329	0.329	-0.210	0.329	0.119	0.685*	0.643*	-0.035	-0.448	0.371	0.434	-0.105	0.664*	-0.434	-0.245	-0.263	-0.189	-0.459	0.175	0.273
67	-0.364	0.280	-0.091	-0.182	0.203	-0.091	-0.133	0.385	0.189	-0.084	0.238	0.385	0.538	-0.245	0.301	0.070	0.308	-0.042	0.063	-0.103	-0.364	-0.035
68	0.385	0.035	0.706*	0.713**	-0.455	0.706*	-0.217	-0.112	-0.182	-0.049	0.301	-0.091	0.252	-0.021	-0.056	-0.077	-0.231	-0.473	-0.329	0.263	0.077	0.259
69	0.692*	0.573	0.350	0.322	-0.490	0.350	-0.231	-0.615*	-0.559	0.203	0.769**	-0.490	0.133	0.077	-0.469	0.091	-0.133	-0.147	0.028	0.669*	0.231	0.000
70	0.161	-0.329	0.469	0.490	-0.273	0.469	-0.545	-0.133	-0.308	-0.497	0.406	0.175	-0.063	-0.231	-0.301	0.413	0.252	-0.203	-0.014	0.374	-0.406	0.287
71	0.028	-0.252	0.133	0.182	-0.301	0.133	0.371	-0.119	0.105	0.245	-0.350	-0.385	-0.350	0.448	-0.014	-0.322	-0.476	0.228	0.133	0.018	0.573	0.133
72	0.580*	0.483	0.196	0.140	-0.231	0.196	-0.224	-0.098	0.000	0.140	0.203	0.098	0.168	-0.084	0.175	0.140	0.000	-0.294	-0.140	0.310	0.510	0.070
73	-0.133	-0.287	0.378	0.329	0.077	0.378	-0.357	-0.105	-0.196	-0.189	-0.077	-0.168	0.196	-0.217	-0.154	0.217	-0.140	0.553	0.497	0.502	0.035	0.503
74	0.308	-0.350	0.937**	0.958**	-0.469	0.937**	-0.315	-0.448	-0.455	-0.203	0.182	-0.462	-0.014	0.056	-0.406	0.070	-0.441	0.042	0.049	0.619*	0.070	0.587*
75	-0.441	-0.126	-0.538	-0.524	0.455	-0.538	0.315	0.273	0.245	-0.147	-0.476	0.126	-0.434	0.154	0.182	-0.140	0.203	-0.105	-0.357	-0.555	0.070	-0.315
76	0.317	-0.381	0.954**	0.961**	-0.459	0.954**	-0.352	-0.434	-0.402	-0.238	0.085	-0.441	-0.061	0.046	-0.349	0.117	-0.445	0.105	0.096	0.659*	0.210	0.676*
77	-0.455	0.217	-0.238	-0.182	-0.294	-0.238	0.951**	0.517	0.566	0.818**	-0.105	0.140	0.231	0.727**	0.601*	-0.755**	-0.490	0.056	0.154	-0.416	0.091	-0.531
78		0.252	0.301	0.294	-0.154	0.301	-0.601*	-0.650*	-0.573	-0.161	0.385	-0.238	-0.203	-0.301	-0.559	0.490	0.217	-0.403	-0.294	0.516	0.336	0.231
79			-0.315	-0.392	0.119	-0.315	0.161	-0.091	-0.147	0.413	0.510	-0.042	0.231	0.175	0.049	-0.063	0.182	-0.375	-0.315	0.068	0.203	-0.510
80				0.986**	-0.559	1.000**	-0.315	-0.385	-0.315	-0.182	0.119	-0.497	0.077	0.049	-0.294	0.007	-0.490	0.091	0.119	0.605*	0.308	0.748**
81					-0.608*	0.986**	-0.259	-0.385	-0.315	-0.147	0.119	-0.483	0.028	0.098	-0.315	-0.021	-0.524	0.102	0.147	0.584*	0.231	0.692*
82						-0.559	-0.126	0.077	-0.035	-0.175	-0.406	0.287	-0.168	-0.315	0.014	0.399	0.517	0.004	-0.266	-0.295	-0.168	-0.140
83							-0.315	-0.385	-0.315	-0.182	0.119	-0.497	0.077	0.049	-0.294	0.007	-0.490	0.091	0.119	0.605*	0.308	0.748**
84								0.455	0.497	0.776**	-0.168	0.077	0.133	0.769**	0.524	-0.706*	-0.483	0.210	0.210	-0.424	-0.056	-0.552
85									0.937**	0.245	-0.441	0.706*	0.490	-0.021	0.916**	-0.497	0.028	-0.112	0.007	-0.826**	-0.084	-0.245
86										0.294	-0.552	0.615*	0.420	0.042	0.944**	-0.524	-0.105	-0.004	0.105	-0.787**	0.112	-0.119
87											0.091	0.028	0.378	0.608*	0.336	-0.510	-0.434	0.217	0.371	-0.110	0.063	-0.420
89												-0.189	0.021	0.168	-0.503	0.196	0.203	-0.263	0.000	0.505	-0.287	-0.273
90													0.070	-0.147	0.573	0.189	0.587*	-0.329	-0.105	-0.605*	-0.322	-0.259
91														-0.273	0.476	-0.545	-0.301	0.060	0.210	-0.238	0.035	0.021
92															0.091	-0.329	-0.420	0.182	0.210	0.117	0.028	-0.329
94																-0.566	-0.161	-0.074	-0.014	-0.719**	0.175	-0.210
95																	0.713**	-0.088	-0.070	0.431	-0.175	0.301
96																		-0.480	-0.378	-0.182	-0.301	-0.154
98																			0.876**	0.298	-0.095	0.259
99																				0.303	-0.189	0.217
100																					0.146	0.399
102																						0.378

Table C.7: Spearman correlation coefficients among the volatile compounds from traditional maize flours and *broas*.

M: Maize flour (peaks numbered according to ***Table 4.1***); **B**: *Broas* (peaks numbered according to ***Table 4.1***); *p*-Value corresponds to the significance level of Spearman correlation coefficient indicated as *: significant at $p < 0.05$; **: significant at $p < 0.01$.

B \ F	5	6	7	12	14	17	19	20	22	23	24	26	30	33+34	35	42	45	49	50	64+65	67	71	92	94	95	96	98	99	102
1	0.04	0.10	0.15	0.00	0.06	0.20	0.01	-0.40	0.07	0.24	0.48	-0.04	0.10	0.11	0.15	0.30	0.08	0.26	-0.60*	0.35	0.23	0.25	0.23	-0.21	-0.11	-0.15	.674*	0.75**	0.44
2	-0.27	0.41	-0.39	-0.06	-0.11	-0.48	-0.36	0.13	-0.29	-0.34	-0.64*	-0.03	-0.34	-0.24	-0.31	-0.36	-0.13	-0.50	0.29	-0.34	-0.14	-0.18	0.04	0.01	0.26	0.40	-0.62*	-0.64*	-0.41
3	0.31	-0.22	0.42	0.29	0.29	0.50	0.37	0.39	0.45	0.57	0.56	0.19	0.53	0.65*	0.48	0.42	0.42	0.62*	-0.06	0.47	0.27	0.64*	0.58*	0.31	-0.54	-0.24	0.36	0.52	0.45
4	0.10	0.33	-0.25	0.45	-0.19	-0.41	-0.30	0.31	0.12	-0.26	0.20	-0.20	-0.25	0.36	-0.27	-0.21	0.37	-0.24	0.05	-0.15	0.71**	0.29	0.09	0.20	-0.12	-0.21	-0.21	0.03	-0.08
5	0.11	0.12	0.13	0.31	0.29	0.17	0.29	0.48	0.34	0.27	0.11	0.18	0.22	0.46	0.17	0.28	0.29	0.34	0.02	0.36	0.32	0.48	0.52	0.11	-0.68*	-0.28	-0.10	0.19	0.29
6	-0.19	0.22	-0.41	0.09	-0.33	-0.49	-0.60*	-0.04	-0.22	-0.45	-0.18	-0.40	-0.35	0.13	-0.39	-0.41	0.06	-0.40	-0.22	-0.38	0.27	0.11	0.22	0.22	0.31	0.23	0.00	-0.12	-0.23
7	-0.06	-0.36	0.34	-0.45	0.08	0.42	0.27	-0.13	-0.12	0.25	-0.21	0.26	0.26	-0.39	0.33	0.27	-0.29	0.16	0.04	0.22	-0.72**	-0.14	0.03	-0.08	0.13	0.33	0.13	-0.06	0.19
8	0.42	-0.10	0.43	0.37	0.38	0.34	0.55	0.48	0.44	0.25	-0.01	0.37	0.36	-0.12	0.36	0.32	0.00	0.24	0.33	0.29	0.01	-0.15	-0.21	0.27	-0.22	-0.10	-0.43	-0.25	0.19
9	-0.01	-0.01	0.20	-0.24	0.12	0.15	0.30	-0.39	-0.03	0.12	-0.34	0.28	0.08	-0.62*	0.16	0.13	-0.34	-0.06	0.09	0.08	-0.63*	-0.55	-0.48	-0.27	0.27	0.19	-0.15	-0.35	0.01
10+11	-0.22	0.27	-0.42	0.06	-0.15	-0.57	-0.26	-0.39	-0.20	-0.45	-0.49	-0.26	-0.43	-0.52	-0.44	-0.50	-0.36	-0.57	0.07	-0.51	-0.14	-0.66*	-0.74**	-0.14	0.38	-0.03	-0.46	-0.59*	-0.52
12	-0.03	0.22	-0.07	0.10	0.21	0.02	0.17	0.48	0.10	0.14	0.03	0.20	0.02	0.38	0.00	0.14	0.25	0.18	0.29	0.20	0.28	0.35	0.38	-0.13	-0.58*	-0.23	-0.29	0.06	0.03
13	-0.24	-0.48	-0.15	-0.07	-0.11	-0.03	-0.15	-0.13	-0.09	-0.17	-0.03	-0.50	-0.06	-0.01	-0.13	-0.14	-0.40	0.05	-0.53	-0.08	-0.05	0.13	0.06	0.00	-0.49	-0.52	0.40	0.37	0.10
14	0.08	0.10	-0.15	0.18	0.01	-0.09	-0.10	0.39	0.13	-0.03	0.03	-0.01	0.02	0.53	-0.06	-0.01	0.24	0.12	0.03	0.04	0.36	0.40	0.50	0.06	-0.46	-0.20	-0.03	0.06	0.04
15	0.18	0.20	-0.29	0.48	-0.10	-0.38	-0.28	0.22	0.15	-0.27	0.18	-0.27	-0.18	0.36	-0.27	-0.29	0.19	-0.16	0.01	-0.27	0.66*	0.06	-0.09	0.19	-0.11	-0.34	-0.15	-0.03	-0.22
16	-0.07	-0.35	0.33	-0.47	0.08	0.43	0.24	-0.13	-0.14	0.25	-0.17	0.25	0.26	-0.32	0.32	0.27	-0.24	0.19	0.01	0.22	-0.68*	-0.09	0.11	-0.07	0.17	0.38	0.20	-0.01	0.20
17	-0.03	-0.38	0.35	-0.43	0.11	0.45	0.27	-0.13	-0.10	0.27	-0.17	0.24	0.29	-0.35	0.35	0.28	-0.29	0.21	-0.01	0.22	-0.69*	-0.13	0.07	-0.06	0.14	0.34	0.20	-0.01	0.20
18	-0.15	-0.03	-0.29	0.01	-0.22	-0.42	-0.14	-0.48	-0.10	-0.37	-0.35	-0.30	-0.32	-0.53	-0.32	-0.38	-0.44	-0.44	-0.09	-0.37	-0.24	-0.57	-0.79**	-0.23	0.09	-0.34	-0.21	-0.38	-0.30
19	0.57	0.14	.587*	0.39	0.40	0.55	0.43	0.48	0.55	0.63*	0.47	0.52	0.58*	0.38	0.65*	0.72**	0.38	0.55	-0.07	0.78**	0.33	0.57	0.66*	0.16	-0.51	-0.03	0.18	0.47	0.74**
20	0.02	-0.11	0.33	-0.34	0.09	0.30	0.31	-0.04	-0.06	0.24	-0.32	0.43	0.20	-0.50	0.32	0.28	-0.21	0.01	0.26	0.24	-0.69*	-0.22	-0.08	-0.13	0.17	0.39	-0.19	-0.31	0.16
21	0.14	-0.06	0.19	0.10	0.07	0.03	0.29	-0.22	0.19	0.10	-0.24	0.17	0.13	-0.42	0.16	-0.01	-0.18	-0.10	0.19	-0.03	-0.42	-0.45	-0.57	0.03	0.18	0.00	-0.28	-0.48	-0.06
22	0.01	0.06	-0.14	-0.04	-0.05	-0.01	0.04	0.39	0.06	0.03	0.15	0.13	-0.03	.629*	-0.08	0.07	0.40	0.20	0.26	0.10	0.31	0.46	0.45	-0.21	-0.57	-0.30	-0.02	0.13	0.03
23	0.48	0.24	0.24	0.37	-0.01	0.15	0.13	0.48	0.45	0.32	0.56	0.35	0.27	0.82**	0.27	0.36	0.80**	0.32	0.18	0.41	0.61*	0.72**	0.64*	0.13	-0.38	-0.10	0.12	0.29	0.42
25	0.08	-0.50	0.31	0.09	0.22	0.42	0.15	0.31	0.14	0.24	0.15	-0.12	0.37	0.06	0.34	0.23	-0.21	0.36	-0.30	0.26	-0.05	0.30	0.36	0.39	-0.36	-0.12	0.30	0.41	0.34
26	-0.05	-0.34	0.08	-0.13	-0.10	0.17	0.03	0.13	0.05	0.06	0.01	-0.08	0.13	0.22	0.10	0.19	-0.04	0.21	-0.31	0.25	-0.08	0.45	0.50	-0.05	-0.59*	-0.28	0.35	0.33	0.39
27	0.36	0.19	0.36	0.20	0.50	0.36	0.10	0.48	0.10	0.34	-0.09	0.40	0.41	-0.22	0.48	0.31	-0.18	0.17	0.18	0.28	-0.06	-0.10	0.31	0.38	0.28	0.63*	-0.28	-0.13	0.10
28	0.25	0.57	-0.01	-0.06	0.21	-0.02	-0.12	0.04	-0.17	-0.05	-0.13	0.41	-0.06	-0.34	0.03	0.10	-0.09	-0.16	0.29	-0.01	0.06	-0.50	-0.13	-0.19	0.66*	0.66*	-0.34	-0.30	-0.20
29	0.27	0.20	0.12	0.02	0.16	0.20	0.08	0.31	0.16	0.29	0.17	0.36	0.23	0.37	0.27	0.36	0.20	0.29	0.05	0.41	0.18	0.38	0.59*	-0.22	-0.46	-0.06	0.11	0.24	0.32
30	0.33	-0.07	0.58*	-0.12	0.34	0.62*	0.41	0.39	0.16	0.58*	0.10	0.64*	0.54	-0.02	0.64*	0.56	0.13	0.39	0.34	0.52	-0.32	0.18	0.43	0.15	0.08	0.54	-0.04	0.02	0.36
32	0.15	0.50	-0.06	0.15	0.22	0.01	0.01	0.39	0.12	0.17	0.13	0.31	0.06	0.47	0.06	0.24	0.36	0.20	0.14	0.28	0.45	0.37	0.59*	-0.17	-0.35	0.03	-0.11	0.16	0.13
33+34	-0.04	-0.26	-0.27	0.08	-0.31	-0.27	-0.12	0.48	0.05	-0.31	-0.11	-0.21	-0.17	0.45	-0.27	-0.27	0.20	-0.11	0.29	-0.24	0.21	0.33	0.15	0.10	-0.54	-0.45	-0.25	-0.23	-0.15
35	0.38	-0.37	0.78**	0.15	0.45	0.82**	0.61*	0.39	0.44	0.70*	0.33	0.44	0.74**	0.16	0.76**	0.65*	0.18	.657*	-0.01	.063*	-0.18	0.36	0.48	0.48	-0.15	0.25	0.25	0.31	.601*
36	0.40	0.10	0.14	0.35	0.12	0.03	-0.19	0.48	0.10	0.07	0.12	0.09	0.20	0.02	0.23	-0.01	0.03	-0.07	0.24	-0.03	0.26	0.01	0.13	0.51	0.34	0.38	-0.24	-0.15	-0.07
37	0.36	-0.03	0.73**	0.24	0.73**	0.82**	0.63*	0.39	0.41	0.74**	0.31	0.52	0.706*	0.06	0.74**	0.73**	0.09	0.70*	-0.09	0.71**	-0.01	0.22	0.49	0.37	-0.11	0.34	0.15	0.41	0.57
38+39	-0.08	0.31	0.08	0.10	0.30	0.08	-0.01	0.48	-0.08	0.20	-0.12	0.22	0.10	-0.03	0.15	0.08	0.11	-0.01	0.31	0.10	0.06	0.17	0.40	0.36	0.23	.587*	-0.42	-0.12	-0.08
41	0.04	.657*	-0.20	-0.04	0.13	-0.27	-0.12	0.31	-0.21	-0.15	-0.43	0.41	-0.22	-0.28	-0.13	-0.08	0.01	-0.36	0.67*	-0.13	0.00	-0.37	-0.10	-0.19	0.41	0.55	-0.82**	-0.66*	-0.40
42	.615*	-0.17	.587*	0.13	0.16	0.55	0.23	0.48	0.34	0.45	0.33	0.48	0.57	0.23	.636*	0.55	0.24	0.39	0.10	0.52	0.03	0.36	0.55	0.31	-0.04	0.32	0.20	0.18	0.54

43	-0.29	0.43	-0.18	0.13	-0.08	-0.36	-0.29	-0.39	-0.19	-0.20	-0.11	-0.25	-0.33	-0.42	-0.25	-0.21	-0.08	-0.41	-0.30	-0.16	0.11	-0.17	-0.24	0.08	0.50	0.27	-0.10	0.01	-0.10
44	0.29	.587*	-0.10	0.27	-0.01	-0.20	-0.07	0.39	0.14	-0.09	0.13	0.29	-0.15	0.27	-0.10	0.13	0.41	-0.10	0.28	0.15	.601*	0.18	0.20	-0.18	-0.15	-0.01	-0.36	-0.06	0.08
45	.685*	0.36	0.32	0.43	0.15	0.21	0.18	0.39	0.51	0.27	0.40	0.48	0.31	0.47	0.35	0.51	0.48	0.30	0.00	0.53	0.57	0.37	0.48	-0.02	-0.34	-0.07	0.08	0.22	0.55
46	0.15	0.03	0.11	0.13	-0.08	-0.08	0.16	-0.40	0.21	0.00	-0.08	0.06	0.01	-0.30	0.05	0.03	-0.10	-0.12	-0.11	0.04	-0.19	-0.30	-0.48	-0.15	0.03	-0.21	-0.02	-0.20	0.12
47	-0.29	-0.15	-0.13	-0.27	-0.20	-0.05	-0.37	-0.48	-0.29	-0.13	-0.06	-0.36	-0.08	-0.05	-0.10	-0.16	-0.19	-0.06	-0.58*	-0.16	-0.21	0.06	0.24	0.08	0.35	0.28	0.58	0.27	0.01
51	-0.41	0.64*	-0.50	-0.13	-0.21	-0.58*	-0.57	-0.48	-0.45	-0.37	-0.27	-0.25	-0.55	-0.41	-0.45	-0.31	-0.20	-0.57	-0.29	-0.24	0.10	-0.19	-0.10	-0.36	0.37	0.23	-0.09	0.00	-0.23
52	0.11	0.11	-0.08	0.06	-0.29	-0.28	0.01	-0.31	0.13	-0.15	-0.14	0.05	-0.15	-0.13	-0.12	-0.13	0.08	-0.27	0.11	-0.11	-0.12	-0.22	-0.43	-0.22	0.04	-0.20	-0.15	-0.38	-0.03
53	-0.08	0.05	0.25	-0.33	-0.06	0.17	0.17	-0.22	-0.06	0.18	-0.34	0.36	0.13	-0.27	0.22	0.22	0.05	-0.03	0.08	0.20	-0.62*	-0.03	0.17	-0.06	0.34	0.58*	-0.02	-0.30	0.20
54	-0.14	0.31	-0.35	0.00	-0.08	-0.41	-0.35	-0.48	-0.27	-0.26	-0.12	-0.22	-0.31	-0.35	-0.31	-0.45	-0.22	-0.41	0.05	-0.49	-0.01	-0.58*	-0.62*	-0.09	0.69*	0.20	-0.15	-0.29	-0.56
55+56	-0.27	0.22	-0.37	0.23	0.05	-0.39	-0.10	0.39	-0.09	-0.19	-0.13	-0.20	-0.31	0.05	-0.34	-0.35	0.03	-0.27	0.42	-0.28	0.31	0.04	-0.20	0.07	-0.29	-0.31	-0.65*	-0.22	-0.43
57	0.06	0.27	-0.22	0.01	-0.24	-0.38	-0.11	-0.31	-0.03	-0.30	-0.27	0.03	-0.29	-0.28	-0.25	-0.21	-0.06	-0.39	0.15	-0.22	-0.08	-0.42	-0.52	-0.31	0.20	-0.08	-0.29	-0.47	-0.20
58	0.16	-0.11	0.39	0.35	0.31	0.34	0.48	0.22	0.36	0.41	0.51	0.10	0.26	0.01	0.31	0.34	0.16	0.31	0.02	0.40	0.28	0.22	-0.17	0.16	-0.39	-0.41	-0.01	0.45	0.34
59	-0.08	0.01	0.06	0.15	-0.03	-0.04	0.11	-0.48	0.15	0.13	0.24	-0.18	-0.02	-0.14	0.01	0.04	-0.01	0.01	-0.40	0.13	0.04	0.03	-0.31	-0.12	-0.17	-0.42	0.28	0.34	0.22
60+61	-0.12	0.38	-0.16	0.24	0.16	-0.22	-0.14	-0.22	-0.08	0.00	0.18	-0.16	-0.16	-0.13	-0.15	-0.25	0.04	-0.17	0.05	-0.26	0.28	-0.27	-0.41	0.15	0.52	0.15	-0.16	0.01	-0.38
62	0.15	0.06	0.01	-0.13	0.06	0.19	-0.07	0.48	-0.07	0.01	0.18	0.21	0.07	0.47	0.04	0.18	0.28	0.24	0.18	0.12	0.31	0.31	0.59*	-0.01	-0.05	0.25	0.08	0.18	0.06
63	0.35	0.10	0.15	0.23	-0.07	-0.05	0.16	-0.13	0.31	0.03	-0.02	0.20	0.08	-0.11	0.12	0.11	0.06	-0.07	0.04	0.11	-0.03	-0.18	-0.30	-0.10	-0.05	-0.18	-0.11	-0.25	0.17
64+65	0.21	0.06	0.62*	0.06	0.32	0.52	0.47	0.04	0.31	0.57	0.02	0.52	0.50	-0.10	0.59*	0.56	0.19	0.34	-0.04	0.57	-0.34	0.20	0.38	0.24	0.13	0.50	0.08	0.04	0.53
66	0.40	0.47	0.13	0.31	0.18	0.00	-0.03	0.22	0.13	0.06	0.11	0.31	0.08	-0.03	0.13	0.10	0.21	-0.07	0.26	0.04	0.32	-0.20	-0.05	0.20	0.53	0.49	-0.30	-0.22	-0.06
67	-0.14	0.02	-0.20	0.43	-0.22	-0.33	-0.23	0.31	0.09	-0.21	0.31	-0.45	-0.24	0.37	-0.29	-0.27	0.37	-0.18	-0.06	-0.17	0.66*	0.50	0.08	0.42	-0.23	-0.36	-0.09	0.25	-0.03
68	0.38	0.23	0.16	0.39	0.06	-0.06	0.20	0.22	0.36	0.07	-0.08	0.28	0.11	-0.06	0.15	0.13	0.13	-0.08	0.25	0.15	0.11	-0.08	-0.17	0.08	-0.13	-0.10	-0.41	-0.36	0.13
69	0.12	-0.17	0.20	0.01	-0.05	0.17	0.36	0.31	0.27	0.22	0.08	0.27	0.15	0.20	0.20	0.30	0.22	0.18	0.23	0.38	-0.06	0.41	0.19	-0.20	-0.76**	-0.46	-0.10	0.06	0.38
70	0.08	0.24	-0.01	0.57	0.24	-0.17	0.16	-0.04	0.31	0.07	0.15	-0.13	-0.01	-0.10	-0.03	-0.10	0.01	-0.06	-0.04	-0.03	0.36	-0.17	-0.48	0.19	-0.11	-0.39	-0.28	-0.01	-0.09
71	0.16	0.03	0.39	-0.40	0.26	0.50	0.31	-0.22	-0.04	0.38	-0.06	0.55	0.33	-0.27	0.42	0.46	-0.11	0.29	0.03	0.37	-0.52	-0.23	0.14	-0.27	0.34	0.55	0.21	0.01	0.25
72	0.38	0.36	0.17	0.16	0.01	0.10	0.27	0.39	0.38	0.29	0.20	0.57	0.20	.629*	0.22	0.37	0.71**	0.24	0.41	0.41	0.27	0.50	0.52	-0.17	-0.42	-0.04	-0.15	-0.08	0.33
73	-0.02	0.29	-0.14	0.15	-0.16	-0.22	-0.29	-0.48	-0.01	-0.08	0.38	-0.27	-0.20	0.05	-0.15	-0.03	0.10	-0.10	-0.55	0.03	0.42	0.11	-0.04	-0.17	0.10	-0.18	0.46	0.52	0.16
74	0.24	0.03	0.16	0.22	-0.05	-0.03	0.18	-0.31	0.29	0.05	-0.01	0.07	0.08	-0.23	0.11	0.09	-0.06	-0.06	-0.15	0.12	-0.09	-0.21	-0.39	-0.08	-0.06	-0.26	0.01	-0.13	0.21
75	-0.48	0.14	-0.27	-0.45	-0.01	-0.17	-0.31	-0.31	-0.57	-0.22	-0.48	-0.12	-0.27	-0.51	-0.24	-0.29	-0.40	-0.32	0.03	-0.35	-0.46	-0.42	-0.13	-0.08	.671*	.636*	-0.16	-0.29	-0.45
76	0.25	0.12	0.18	0.19	-0.05	-0.01	0.21	-0.36	0.31	0.10	0.05	0.15	0.09	-0.14	0.13	0.15	0.07	-0.02	-0.14	0.17	-0.06	-0.15	-0.31	-0.15	-0.03	-0.20	0.07	-0.09	0.25
77	0.55	-0.05	0.66*	0.24	0.43	.657*	0.27	0.48	0.34	.587*	0.34	0.48	.671*	0.18	0.72**	0.55	0.21	0.49	0.05	0.50	0.05	0.27	0.587*	0.56	0.24	.615*	0.17	0.22	0.44
78	0.06	0.21	-0.08	-0.14	0.02	-0.06	0.24	0.04	0.08	-0.01	-0.29	0.36	-0.06	0.00	-0.04	0.16	0.00	0.01	0.26	0.18	-0.15	-0.08	-0.01	-0.58*	-0.52	-0.31	-0.29	-0.28	0.08
79	-0.12	0.03	-0.03	-0.05	-0.17	-0.06	-0.02	0.48	0.00	0.01	-0.10	0.11	-0.05	0.29	-0.01	0.09	0.31	-0.05	0.24	0.18	0.06	.601*	0.57	0.03	-0.48	-0.03	-0.22	-0.02	0.20
80	0.23	0.03	0.24	0.14	-0.08	0.06	0.29	-0.22	0.31	0.11	0.08	0.20	0.08	-0.12	0.14	0.22	0.14	0.01	-0.04	0.24	-0.06	-0.06	-0.28	-0.15	-0.13	-0.23	0.01	-0.06	0.31
81	0.31	0.01	0.30	0.18	0.02	0.13	0.34	-0.22	0.35	0.17	0.10	0.23	0.17	-0.16	0.22	0.25	0.06	0.08	-0.06	0.26	-0.09	-0.14	-0.31	-0.10	-0.08	-0.20	0.04	-0.06	0.31
82	-0.50	0.13	-0.65*	-0.27	-0.42	-0.60*	-0.67*	-0.39	-0.49	-0.64*	-0.41	-0.55	-0.57	-0.07	-0.63*	-0.55	-0.26	-0.48	-0.38	-0.52	0.01	-0.11	0.04	-0.20	0.19	0.03	0.12	-0.10	-0.37
83	0.23	0.03	0.24	0.14	-0.08	0.06	0.29	-0.22	0.31	0.11	0.08	0.20	0.08	-0.12	0.14	0.22	0.14	0.01	-0.04	0.24	-0.06	-0.06	-0.28	-0.15	-0.13	-0.23	0.01	-0.06	0.31
84	0.39	-0.25	0.56	0.16	0.28	0.57	0.16	0.31	0.24	0.45	0.28	0.22	0.58*	0.13	0.59*	0.38	0.09	0.41	-0.12	0.34	-0.06	0.25	0.50	0.61*	0.27	0.52	0.32	0.24	0.38
85	0.31	0.29	0.15	0.31	0.29	0.15	-0.10	0.48	0.06	0.14	0.33	0.16	0.17	0.23	0.17	0.10	0.27	0.13	0.19	0.04	0.51	0.09	0.29	0.47	0.47	0.54	-0.12	0.11	-0.08
86	0.39	0.19	0.28	0.20	0.29	0.32	0.06	0.48	0.14	0.25	0.38	0.33	0.29	0.36	0.29	0.25	0.39	0.29	0.26	0.16	0.40	0.15	0.37	0.38	0.41	0.55	-0.01	0.12	0.04
87	0.64*	-0.11	0.64*	0.30	0.36	0.66*	0.25	0.48	0.45	0.54	0.47	0.39	0.67*	0.37	0.70*	0.66*	0.23	0.59*	-0.25	0.66*	0.25	0.49	0.78**	0.42	-0.17	0.27	0.43	0.50	0.71*
89	-0.03	-0.20	0.20	0.33	0.20	0.14	0.38	0.39	0.31	0.26	0.12	-0.01	0.17	0.04	0.17	0.15	0.06	0.16	0.11	0.27	0.10	0.34	-0.01	0.22	-0.70*	-0.53	-0.26	0.15	0.23
90	0.28	0.39	0.10	0.57	0.48	0.11	0.10	0.48	0.30	0.20	0.27	0.13	0.21	0.40	0.13	0.11	0.31	0.27	0.09	0.09	0.64*	0.11	0.27	0.46	0.09	0.19	-0.21	0.08	-0.06

91	0.31	0.05	0.01	0.17	-0.12	-0.01	-0.24	0.48	0.01	-0.09	0.38	0.01	-0.03	0.26	0.02	0.08	0.21	-0.03	0.10	0.08	0.56	0.31	0.27	0.12	-0.06	-0.01	0.02	0.28	0.13
92	0.33	-0.23	0.73**	0.29	0.36	0.64*	0.51	0.13	0.50	0.66*	0.26	0.31	0.69*	0.10	0.71*	0.55	0.21	0.52	-0.23	0.57	-0.19	0.35	0.41	0.58*	-0.01	0.28	0.30	0.25	.622*
94	0.38	0.25	0.22	0.21	0.14	0.20	-0.05	0.48	0.13	0.20	0.34	0.31	0.23	0.43	0.24	0.19	0.50	0.17	0.30	0.13	0.40	0.27	0.47	0.40	0.41	0.58*	-0.04	0.03	0.05
95	-0.30	0.14	-0.27	0.12	-0.02	-0.28	0.09	-0.31	0.09	-0.16	-0.15	-0.24	-0.24	0.10	-0.33	-0.16	0.02	-0.03	-0.27	-0.09	0.11	-0.01	-0.17	-0.24	-0.43	-0.55	-0.01	0.01	-0.05
96	-0.42	0.27	-0.39	0.12	0.12	-0.34	-0.05	0.13	-0.13	-0.24	-0.25	-0.23	-0.34	0.02	-0.41	-0.29	-0.03	-0.17	0.09	-0.24	0.24	-0.03	-0.10	-0.05	-0.24	-0.24	-0.44	-0.13	-0.35
98	0.11	-0.42	0.18	-0.06	-0.15	0.25	-0.07	-0.44	0.08	0.12	0.54	-0.29	0.18	0.22	0.14	0.12	0.02	0.29	-0.66*	0.11	0.08	0.21	0.11	0.06	0.02	-0.21	0.93**	0.69*	0.34
99	0.41	-0.34	0.48	0.30	0.22	0.55	0.26	-0.04	0.42	0.44	0.83**	-0.05	0.48	0.42	0.44	0.43	0.21	0.62*	-0.56	0.43	0.41	0.38	0.25	0.27	-0.22	-0.30	0.83**	0.89**	0.57
100	-0.01	-0.09	0.12	0.05	-0.13	0.03	0.24	-0.36	0.26	0.16	0.14	-0.01	0.06	0.06	0.08	0.18	0.12	0.11	-0.31	0.27	-0.09	0.23	-0.06	-0.26	-0.51	-0.54	0.30	0.25	0.40
102	0.03	0.41	0.06	-0.45	-0.16	0.06	0.09	-0.04	-0.13	0.10	-0.14	.615*	-0.07	0.06	0.06	0.33	0.38	-0.03	0.35	0.30	-0.24	0.14	0.36	-0.57	0.10	0.44	-0.09	-0.18	0.21
104	-0.01	0.12	-0.04	-0.07	-0.20	-0.11	0.11	-0.39	0.07	-0.14	0.07	0.03	-0.20	-0.02	-0.18	0.06	0.15	-0.06	-0.16	0.06	0.06	-0.10	-0.28	-0.41	-0.07	-0.27	0.14	0.06	0.15

3.1. Contribution of phenolic compounds and total carotenoids content to the volatile composition of *broas*

Table C.8: Spearman correlation coefficients between *broas* volatile compounds and (i) the major phenolic compounds and (ii) total carotenoids content of both broas and maize flours.

Var: Variables; **V**: *Broas* volatiles (peaks numbered according to ***Table 4.1***); ***cc***: *cis,cis* isomer; ***ct***: *cis.trans* isomer; ***tt***: *trans,trans* isomer; $_{\mathbf{T}}$: total; **bisDFP**: *bis*-diferuloyl putrescine. *p*-Value corresponds to the significance level of Spearman correlation coefficient indicated as *****: significant at $p < 0.05$; ******: significant at $p < 0.01$.

	Maize flours													
V \ Var	pCA	FA	DCS*ct*	DCS*tt*	DCS$_T$	DFP*cc*	DFP*ct*	DFP*tt*	DFP$_T$	CFP*ct*	CFP*tt*	CFP$_T$	bisDFP	Carot
1	-0.459	-0.383	0.099	-0.106	-0.106	0.384	-0.077	-0.373	-0.352	0.134	-0.183	-0.141	-0.097	0.739**
2	0.158	0.208	0.098	-0.028	-0.028	-0.106	0.140	0.049	0.147	0.067	0.028	0.084	0.004	-0.783**
3	-0.140	-0.352	0.039	-0.049	-0.049	-0.116	-0.364	-0.510	-0.441	-0.326	-0.455	-0.434	-0.099	0.154
4	-0.523	-0.648*	-0.417	-0.497	-0.497	-0.028	-0.357	-0.350	-0.420	-0.568	-0.399	-0.483	-0.334	-0.147
5	0.039	-0.254	-0.067	-0.035	-0.035	-0.279	-0.357	-0.371	-0.322	-0.379	-0.266	-0.294	-0.199	-0.238
6	-0.365	-0.085	-0.242	-0.399	-0.399	0.268	0.182	-0.098	0.049	-0.011	-0.140	-0.112	-0.096	-0.224
7	0.460	0.662*	0.144	0.252	0.252	0.085	0.315	0.245	0.357	0.596*	0.371	0.497	-0.018	-0.077
8	0.414	0.120	-0.340	0.070	0.070	-0.328	0.014	0.364	0.210	-0.018	0.385	0.322	-0.345	-0.308
9	0.551	0.486	0.340	0.552	0.552	-0.102	0.350	0.608*	0.476	0.533	0.650*	0.678*	0.099	0.007
10+11	0.102	0.004	0.238	0.252	0.252	-0.011	0.371	0.601*	0.455	0.179	0.490	0.420	0.259	-0.112
12	0.046	-0.243	0.077	0.000	0.000	-0.384	-0.538	-0.490	-0.476	-0.540	-0.469	-0.510	0.018	-0.308
13	-0.295	-0.151	-0.224	-0.308	-0.308	0.504	0.252	-0.014	0.196	0.196	0.203	0.196	-0.068	0.350
14	-0.098	-0.229	-0.179	-0.196	-0.196	-0.183	-0.392	-0.406	-0.343	-0.516	-0.406	-0.455	-0.092	-0.245
15	-0.418	-0.602*	-0.301	-0.336	-0.336	-0.046	-0.252	-0.140	-0.252	-0.572	-0.273	-0.406	-0.057	0.014
16	0.432	0.687*	0.140	0.231	0.231	0.095	0.301	0.189	0.322	0.579*	0.294	0.427	0.018	-0.049
17	0.446	0.673*	0.130	0.238	0.238	0.113	0.336	0.238	0.364	0.604*	0.357	0.483	0.011	-0.028
18	0.042	-0.095	0.095	0.196	0.196	0.120	0.322	0.615*	0.448	0.207	0.622*	0.531	0.075	0.161
19	-0.119	-0.363	-0.305	-0.259	-0.259	-0.067	-0.441	-0.573	-0.580*	-0.186	-0.301	-0.245	-0.700*	-0.056
20	0.537	0.585*	0.126	0.287	0.287	-0.092	0.196	0.273	0.280	0.502	0.406	0.517	-0.195	-0.336
21	0.425	0.176	0.259	0.476	0.476	-0.190	0.294	0.643*	0.455	0.291	0.608*	0.587*	0.021	-0.105
22	0.018	-0.176	-0.060	-0.021	-0.021	-0.430	-0.713**	-0.517	-0.545	-0.765**	-0.601*	-0.692*	0.068	-0.133
23	-0.232	-0.504	-0.284	-0.217	-0.217	-0.384	-0.811**	-0.671*	-0.797**	-0.793**	-0.706*	-0.755**	-0.441	-0.098
25	-0.154	0.007	-0.333	-0.399	-0.399	0.413	0.259	-0.161	0.112	0.316	0.077	0.168	-0.291	0.049
26	-0.056	0.042	-0.347	-0.266	-0.266	0.194	-0.098	-0.252	-0.070	0.014	0.000	0.028	-0.384	0.021
27	0.098	0.176	-0.123	-0.266	-0.266	0.134	0.217	-0.175	-0.021	0.368	-0.035	0.126	-0.288	-0.531
28	0.158	0.261	-0.039	-0.014	-0.014	-0.113	-0.035	0.021	-0.112	0.077	-0.077	-0.049	0.025	-0.231
29	-0.049	-0.218	-0.077	-0.133	-0.133	-0.152	-0.573	-0.615*	-0.608*	-0.389	-0.469	-0.455	-0.266	-0.105
30	0.344	0.373	0.028	0.049	0.049	-0.152	-0.112	-0.252	-0.175	0.214	-0.147	0.007	-0.266	-0.371
32	-0.084	-0.285	0.021	-0.098	-0.098	-0.307	-0.608*	-0.657*	-0.664*	-0.551	-0.629*	-0.643*	-0.082	-0.238
33+34	0.011	-0.120	-0.448	-0.252	-0.252	-0.286	-0.385	-0.105	-0.098	-0.646*	-0.196	-0.322	-0.160	-0.392
35	0.267	0.282	-0.109	0.028	0.028	-0.018	0.126	-0.070	0.056	0.330	0.070	0.196	-0.320	-0.077
36	-0.284	-0.222	-0.371	-0.538	-0.538	0.173	0.021	-0.245	-0.182	-0.028	-0.245	-0.168	-0.359	-0.413
37	0.228	0.201	0.035	0.056	0.056	-0.004	0.147	-0.168	-0.042	0.382	0.007	0.140	-0.195	-0.035
38+39	-0.021	0.063	0.147	-0.189	-0.189	-0.025	0.063	-0.392	-0.175	0.140	-0.357	-0.196	-0.043	-0.650*
41	0.302	0.204	0.095	0.070	0.070	-0.413	-0.217	-0.056	-0.168	-0.175	-0.175	-0.161	0.028	-0.755**
42	0.028	0.092	-0.536	-0.371	-0.371	0.028	-0.224	-0.322	-0.287	-0.004	-0.175	-0.084	-0.679*	-0.217
43	-0.375	-0.215	0.158	-0.126	-0.126	0.384	0.434	0.091	0.140	0.456	0.175	0.259	-0.078	0.063
44	-0.165	-0.380	-0.413	-0.301	-0.301	-0.335	-0.657*	-0.420	-0.615*	-0.653*	-0.434	-0.524	-0.433	-0.280
45	-0.105	-0.363	-0.574	-0.287	-0.287	-0.250	-0.629*	-0.399	-0.601*	-0.533	-0.294	-0.364	-0.718**	-0.049
46	0.166	-0.057	0.056	0.310	0.310	-0.050	0.148	0.507	0.275	0.173	0.556	0.493	-0.182	0.197
47	-0.214	0.243	0.151	-0.070	-0.070	0.526	0.490	0.021	0.273	0.519	0.077	0.196	0.195	0.308
51	-0.470	-0.299	0.238	-0.196	-0.196	0.402	0.140	-0.231	-0.168	0.249	-0.091	-0.014	-0.011	0.035
52	0.165	-0.081	0.025	0.287	0.287	-0.236	-0.098	0.392	0.119	-0.137	0.336	0.238	-0.153	0.007
53	0.530	0.676*	0.245	0.406	0.406	-0.138	0.252	0.273	0.315	0.505	0.336	0.469	-0.131	-0.350
54	-0.154	-0.144	0.504	0.231	0.231	0.102	0.273	0.273	0.154	0.133	0.056	0.042	0.558	0.196
55+56	-0.214	-0.500	0.161	-0.133	-0.133	-0.198	-0.238	-0.259	-0.245	-0.439	-0.329	-0.385	0.185	-0.392
57	0.172	0.000	-0.007	0.231	0.231	-0.208	-0.049	0.420	0.147	-0.105	0.336	0.231	-0.060	-0.049
58	-0.274	-0.563	-0.025	-0.112	-0.112	0.032	-0.168	-0.210	-0.287	-0.074	-0.077	-0.091	-0.234	0.315
59	-0.312	-0.496	0.284	0.147	0.147	0.233	0.098	0.063	-0.028	0.168	0.203	0.189	-0.043	0.573
60+61	-0.333	-0.408	0.546	0.133	0.133	0.071	0.168	-0.021	-0.084	0.039	-0.210	-0.196	0.515	0.224
62	0.074	0.246	-0.385	-0.280	-0.280	-0.240	-0.483	-0.448	-0.413	-0.477	-0.573	-0.594*	-0.018	-0.252
63	0.170	-0.110	-0.159	0.169	0.169	-0.188	-0.092	0.345	0.077	-0.081	0.373	0.289	-0.358	0.014
64+65	0.375	0.373	0.179	0.280	0.280	-0.081	0.224	0.070	0.133	0.516	0.238	0.399	-0.334	-0.238
66	-0.021	-0.046	-0.172	-0.126	-0.126	-0.148	-0.070	-0.014	-0.161	-0.091	-0.140	-0.133	-0.167	-0.245
67	-0.684*	-0.722**	-0.357	-0.573	-0.573	0.162	-0.161	-0.371	-0.301	-0.396	-0.378	-0.420	-0.277	-0.021
68	0.154	-0.201	-0.238	0.021	0.021	-0.282	-0.168	0.182	-0.035	-0.186	0.231	0.168	-0.483	-0.343
69	0.140	-0.190	-0.193	0.007	0.007	-0.303	-0.524	-0.238	-0.322	-0.372	-0.063	-0.112	-0.465	-0.168
70	-0.235	-0.644*	0.235	0.112	0.112	-0.035	0.091	0.168	0.007	-0.084	0.154	0.077	0.064	0.168
71	0.526	0.673*	0.280	0.427	0.427	-0.099	0.112	0.140	0.119	0.453	0.182	0.287	0.103	0.063
72	0.235	-0.162	-0.046	0.189	0.189	-0.705*	-0.853**	-0.434	-0.643*	-0.768**	-0.490	-0.559	-0.316	-0.350

73	-0.670*	-0.620*	0.028	-0.231	-0.231	0.413	-0.056	-0.280	-0.336	-0.007	-0.182	-0.189	-0.107	0.657*
74	0.088	-0.162	-0.049	0.203	0.203	-0.018	0.098	0.420	0.196	0.126	0.510	0.448	-0.309	0.196
75	0.168	0.556	0.462	0.175	0.175	0.215	0.531	0.182	0.385	0.593*	0.119	0.259	0.508	-0.217
76	0.118	-0.149	0.046	0.303	0.303	-0.111	-0.004	0.356	0.100	0.055	0.413	0.349	-0.257	0.238
77	0.042	0.176	-0.231	-0.252	-0.252	0.088	0.070	-0.287	-0.154	0.249	-0.203	-0.042	-0.380	-0.238
78	0.474	0.113	0.056	0.371	0.371	-0.511	-0.469	0.056	-0.147	-0.368	0.112	0.000	-0.089	-0.210
79	-0.049	-0.088	-0.280	-0.315	-0.315	-0.152	-0.413	-0.510	-0.371	-0.309	-0.350	-0.301	-0.497	-0.580*
80	0.158	-0.088	-0.102	0.231	0.231	-0.169	-0.091	0.315	0.063	0.000	0.385	0.315	-0.366	0.175
81	0.189	-0.067	-0.070	0.259	0.259	-0.138	-0.014	0.371	0.119	0.074	0.441	0.378	-0.323	0.210
82	-0.225	0.120	-0.025	-0.168	-0.168	0.325	0.266	0.063	0.224	0.105	0.042	0.035	0.227	0.021
83	0.158	-0.088	-0.102	0.231	0.231	-0.169	-0.091	0.315	0.063	0.000	0.385	0.315	-0.366	0.175
84	-0.018	0.229	-0.235	-0.266	-0.266	0.258	0.280	-0.133	0.063	0.393	-0.049	0.112	-0.302	-0.112
85	-0.274	-0.123	-0.210	-0.406	-0.406	0.032	-0.063	-0.378	-0.315	-0.133	-0.517	-0.455	-0.018	-0.203
86	-0.046	0.081	-0.196	-0.217	-0.217	-0.162	-0.189	-0.322	-0.315	-0.214	-0.510	-0.469	0.014	-0.161
87	-0.130	-0.021	-0.567	-0.469	-0.469	0.201	-0.112	-0.434	-0.308	0.084	-0.210	-0.105	-0.675*	-0.014
89	-0.123	-0.475	-0.049	-0.126	-0.126	-0.042	-0.126	-0.175	-0.133	-0.112	0.028	0.021	-0.323	-0.147
90	-0.109	-0.254	0.007	-0.091	-0.091	-0.212	-0.077	-0.217	-0.217	-0.291	-0.378	-0.399	0.160	-0.168
91	-0.547	-0.426	-0.760**	-0.818**	-0.818**	0.194	-0.448	-0.587*	-0.580*	-0.425	-0.524	-0.538	-0.561	-0.049
92	0.179	0.165	0.046	0.126	0.126	0.085	0.343	0.084	0.203	0.505	0.252	0.399	-0.362	-0.042
94	-0.102	0.014	-0.214	-0.266	-0.266	-0.180	-0.266	-0.406	-0.392	-0.288	-0.580*	-0.524	-0.110	-0.301
95	0.095	-0.176	0.315	0.413	0.413	-0.201	0.007	0.252	0.161	-0.161	0.231	0.105	0.288	0.238
96	-0.011	-0.165	0.256	0.105	0.105	-0.183	0.000	-0.021	0.028	-0.204	-0.091	-0.154	0.366	-0.203
98	-0.411	-0.160	-0.118	-0.154	-0.154	0.489	0.147	-0.060	-0.007	0.185	-0.007	0.004	0.007	0.876**
99	-0.442	-0.405	-0.231	-0.238	-0.238	0.342	-0.042	-0.252	-0.245	0.000	-0.168	-0.175	-0.149	0.853**
100	-0.032	-0.303	0.135	0.253	0.253	-0.022	-0.146	0.057	-0.064	-0.030	0.235	0.185	-0.235	0.388
102	0.432	0.440	0.084	0.301	0.301	-0.487	-0.545	-0.238	-0.378	-0.214	-0.245	-0.217	-0.188	-0.308
104	0.116	0.018	-0.067	0.273	0.273	-0.190	-0.161	0.287	0.035	-0.140	0.245	0.119	-0.053	0.399

	Broas												
V \ Var	**pCA**	**FA**	**DCS*ct***	**DCS*tt***	**DCS$_T$**	**DFP*cc***	**DFP*ct***	**DFP*tt***	**DFP$_T$**	**CFP*ct***	**CFP*tt***	**CFP$_T$**	**Carot**
1	-0.042	0.077	-0.060	0.099	0.014	-0.068	-0.081	-0.254	-0.232	0.174	0.149	0.166	0.754**
2	-0.077	0.035	-0.204	-0.322	-0.294	-0.029	0.004	0.224	0.147	-0.461	-0.046	-0.140	-0.776**
3	-0.147	-0.119	-0.277	-0.098	-0.168	0.064	-0.519	-0.524	-0.517	-0.320	-0.313	-0.340	0.161
4	-0.573	-0.559	-0.172	-0.524	-0.497	0.136	-0.053	-0.168	-0.154	-0.345	-0.148	-0.095	-0.119
5	-0.182	0.007	-0.540	-0.168	-0.294	-0.476	-0.681*	-0.133	-0.175	-0.563	-0.004	-0.063	-0.217
6	-0.497	-0.287	-0.193	-0.503	-0.420	0.236	0.386	0.231	0.196	-0.106	0.148	0.098	-0.217
7	0.371	0.322	0.042	0.224	0.238	0.061	0.067	0.091	0.084	0.405	0.084	0.046	-0.133
8	0.014	-0.175	-0.084	-0.035	-0.049	-0.233	-0.302	0.056	0.098	-0.070	-0.067	-0.018	-0.343
9	0.671*	0.497	0.411	0.524	0.510	-0.204	0.021	0.175	0.189	0.299	0.056	0.081	-0.014
10+11	0.301	0.140	0.344	0.224	0.238	-0.140	0.274	0.413	0.420	-0.021	0.123	0.161	-0.098
12	-0.091	0.063	-0.393	-0.077	-0.189	-0.408	-0.621*	-0.210	-0.266	-0.609*	-0.134	-0.186	-0.266
13	-0.210	-0.154	-0.642*	-0.126	-0.238	-0.265	-0.077	0.427	0.441	0.123	0.650*	0.599*	0.294
14	-0.329	-0.126	-0.481	-0.308	-0.371	-0.240	-0.460	-0.077	-0.119	-0.567	0.018	-0.067	-0.224
15	-0.434	-0.476	-0.102	-0.329	-0.322	0.050	-0.060	-0.049	-0.021	-0.398	-0.077	-0.046	0.042
16	0.322	0.329	0.056	0.210	0.238	0.097	0.130	0.077	0.063	0.430	0.081	0.035	-0.098
17	0.336	0.315	0.018	0.217	0.231	0.061	0.084	0.105	0.098	0.405	0.112	0.063	-0.084
18	0.364	0.084	0.239	0.287	0.252	-0.200	0.098	0.392	0.441	0.155	0.278	0.343	0.140
19	-0.301	-0.252	-0.540	-0.413	-0.517	-0.111	-0.828**	-0.594*	-0.580*	-0.475	-0.243	-0.280	-0.077
20	0.420	0.294	0.105	0.154	0.168	0.021	-0.102	-0.014	-0.021	0.211	-0.084	-0.102	-0.385
21	0.552	0.224	0.435	0.364	0.371	-0.025	-0.060	0.056	0.098	0.085	-0.162	-0.105	-0.126
22	-0.112	0.063	-0.193	-0.007	-0.063	-0.276	-0.477	-0.245	-0.287	-0.394	-0.116	-0.147	-0.084
23	-0.343	-0.280	-0.049	-0.336	-0.329	0.147	-0.547	-0.734**	-0.727**	-0.451	-0.524	-0.497	-0.056
25	-0.385	-0.350	-0.723**	-0.392	-0.448	0.054	-0.137	0.112	0.126	0.032	0.278	0.207	-0.028
26	-0.217	-0.056	-0.660*	-0.238	-0.329	-0.283	-0.379	0.133	0.119	0.004	0.422	0.350	-0.028
27	-0.329	-0.315	-0.495	-0.573	-0.566	0.265	-0.214	-0.196	-0.217	-0.493	-0.264	-0.389	-0.580*
28	-0.056	-0.007	0.249	-0.105	-0.028	0.132	0.179	-0.112	-0.126	-0.141	-0.225	-0.249	-0.210
29	-0.140	-0.007	-0.467	-0.231	-0.343	-0.218	-0.768**	-0.434	-0.462	-0.595*	-0.137	-0.231	-0.098
30	0.056	0.021	-0.095	-0.161	-0.133	0.297	-0.291	-0.476	-0.490	-0.109	-0.446	-0.504	-0.406
32	-0.238	0.035	-0.375	-0.231	-0.322	-0.311	-0.621*	-0.371	-0.434	-0.732**	-0.200	-0.287	-0.182
33+34	-0.301	-0.301	-0.319	-0.280	-0.273	-0.122	-0.232	0.147	0.140	-0.229	0.088	0.088	-0.399
35	-0.049	-0.056	-0.204	-0.112	-0.105	0.175	-0.211	-0.287	-0.273	0.116	-0.211	-0.238	-0.126
36	-0.566	-0.720**	-0.270	-0.755**	-0.664*	0.666*	0.021	-0.329	-0.301	-0.401	-0.429	-0.466	-0.455
37	-0.084	0.049	-0.337	-0.084	-0.140	-0.097	-0.302	-0.245	-0.259	-0.113	-0.137	-0.200	-0.056
38+39	-0.301	-0.168	-0.316	-0.469	-0.434	0.297	-0.011	-0.231	-0.301	-0.447	-0.397	-0.483	-0.643*
41	0.021	0.042	0.116	-0.175	-0.126	0.004	-0.098	-0.119	-0.175	-0.507	-0.383	-0.424	-0.720**
42	-0.371	-0.427	-0.309	-0.538	-0.490	0.401	-0.330	-0.476	-0.441	-0.102	-0.295	-0.333	-0.280
43	-0.098	-0.063	0.133	-0.140	-0.105	0.168	0.463	0.126	0.119	0.158	0.053	0.098	0.077
44	-0.371	-0.280	-0.116	-0.364	-0.371	-0.168	-0.379	-0.308	-0.315	-0.447	-0.204	-0.172	-0.238
45	-0.378	-0.301	-0.260	-0.399	-0.441	-0.190	-0.674*	-0.462	-0.434	-0.437	-0.165	-0.161	-0.042
46	0.416	0.176	0.350	0.317	0.289	-0.187	-0.120	0.070	0.127	0.174	0.067	0.148	0.183
47	-0.077	0.147	-0.049	-0.028	0.014	0.222	0.502	0.252	0.224	0.349	0.320	0.245	0.294
51	-0.091	0.049	-0.088	-0.189	-0.224	-0.014	0.175	0.070	0.028	-0.165	0.144	0.116	0.063
52	0.378	0.147	0.463	0.259	0.273	-0.089	-0.116	-0.021	0.021	0.070	-0.084	0.004	0.014
53	0.420	0.490	0.288	0.189	0.252	0.050	0.084	0.007	-0.035	0.268	-0.098	-0.130	-0.364
54	0.266	0.098	0.607*	0.266	0.329	0.322	0.481	0.000	0.014	-0.011	-0.239	-0.203	0.238
55+56	-0.147	-0.238	-0.228	-0.175	-0.231	-0.075	-0.204	-0.049	-0.077	-0.553	-0.225	-0.210	-0.357
57	0.308	0.140	0.432	0.224	0.245	-0.157	0.004	0.119	0.147	0.039	0.011	0.081	-0.035
58	-0.035	-0.273	-0.056	0.035	-0.042	0.014	-0.256	-0.364	-0.301	0.056	-0.214	-0.095	0.308
59	0.280	0.112	0.179	0.301	0.210	-0.107	-0.098	-0.119	-0.070	0.173	0.056	0.147	0.580*
60+61	0.084	-0.028	0.491	0.168	0.210	0.358	0.411	-0.224	-0.217	-0.106	-0.422	-0.361	0.287

62	-0.420	-0.147	-0.189	-0.301	-0.238	0.054	-0.039	-0.140	-0.182	-0.187	-0.077	-0.151	-0.231
63	0.232	0.007	0.240	0.106	0.092	-0.144	-0.297	-0.077	-0.021	-0.032	-0.060	0.007	0.000
64+65	0.224	0.280	0.035	0.021	0.028	0.043	-0.189	-0.245	-0.266	0.060	-0.236	-0.266	-0.259
66	-0.238	-0.273	0.260	-0.280	-0.175	0.326	0.151	-0.273	-0.259	-0.194	-0.415	-0.389	-0.224
67	-0.622*	-0.643*	-0.228	-0.510	-0.476	0.283	0.175	-0.098	-0.077	-0.063	-0.091	0.000	-0.007
68	0.028	-0.175	-0.007	-0.168	-0.189	-0.132	-0.449	-0.147	-0.112	-0.338	-0.200	-0.158	-0.357
69	0.119	0.000	-0.277	-0.007	-0.112	-0.319	-0.740**	-0.259	-0.245	-0.180	-0.056	-0.025	-0.189
70	0.091	-0.133	0.112	0.112	0.035	-0.143	-0.165	-0.077	-0.035	-0.296	-0.165	-0.081	0.196
71	0.448	0.531	0.288	0.378	0.392	-0.050	-0.011	-0.119	-0.140	0.254	-0.067	-0.119	0.056
72	0.077	0.161	0.095	-0.014	-0.042	-0.215	-0.754**	-0.601*	-0.629*	-0.532	-0.485	-0.483	-0.294
73	-0.161	-0.126	0.095	-0.042	-0.077	0.089	0.119	-0.196	-0.161	0.102	0.084	0.144	0.685*
74	0.301	0.063	0.221	0.196	0.154	-0.186	-0.221	0.014	0.077	0.095	0.063	0.140	0.175
75	0.182	0.329	0.158	0.119	0.189	0.190	0.600*	0.322	0.252	0.180	0.074	-0.007	-0.210
76	0.374	0.185	0.355	0.292	0.256	-0.199	-0.234	-0.089	-0.036	0.099	-0.020	0.062	0.238
77	-0.371	-0.336	-0.242	-0.497	-0.427	0.512	-0.098	-0.462	-0.455	-0.165	-0.418	-0.490	-0.280
78	0.413	0.448	-0.081	0.329	0.196	-0.762**	-0.737**	0.042	0.014	-0.345	0.141	0.116	-0.189
79	-0.301	-0.182	-0.530	-0.476	-0.503	-0.047	-0.442	-0.182	-0.231	-0.320	-0.084	-0.126	-0.594*
80	0.315	0.126	0.326	0.259	0.238	-0.204	-0.214	-0.084	-0.028	0.229	-0.004	0.105	0.168
81	0.336	0.119	0.312	0.273	0.245	-0.190	-0.232	-0.084	-0.021	0.190	-0.014	0.081	0.196
82	-0.189	0.105	-0.182	-0.112	-0.098	-0.157	0.411	0.566	0.510	0.070	0.545	0.469	0.035
83	0.315	0.126	0.326	0.259	0.238	-0.204	-0.214	-0.084	-0.028	0.229	-0.004	0.105	0.168
84	-0.357	-0.329	-0.232	-0.448	-0.364	0.551	0.116	-0.252	-0.238	0.053	-0.228	-0.294	-0.168
85	-0.594*	-0.497	-0.032	-0.517	-0.399	0.551	0.302	-0.336	-0.343	-0.236	-0.443	-0.462	-0.182
86	-0.427	-0.308	0.144	-0.315	-0.182	0.483	0.249	-0.392	-0.399	-0.099	-0.478	-0.487	-0.133
87	-0.566	-0.434	-0.593*	-0.615*	-0.622*	0.197	-0.379	-0.378	-0.357	-0.187	-0.077	-0.158	-0.070
89	-0.056	-0.245	-0.463	-0.147	-0.273	-0.204	-0.540	-0.133	-0.112	-0.268	-0.063	-0.021	-0.175
90	-0.399	-0.217	-0.109	-0.245	-0.231	0.000	-0.032	-0.168	-0.196	-0.518	-0.299	-0.333	-0.112
91	-0.776**	-0.804**	-0.382	-0.741**	-0.685*	0.415	-0.032	-0.329	-0.287	-0.113	-0.127	-0.098	-0.077
92	0.035	0.021	-0.098	-0.098	-0.091	0.193	-0.151	-0.245	-0.231	0.092	-0.207	-0.228	-0.084
94	-0.462	-0.350	0.130	-0.441	-0.294	0.580*	0.182	-0.483	-0.497	-0.201	-0.569	-0.585*	-0.273
95	0.336	0.476	0.056	0.497	0.371	-0.759**	-0.200	0.364	0.336	-0.070	0.376	0.399	0.294
96	-0.014	0.161	-0.182	0.112	0.035	-0.483	-0.032	0.322	0.259	-0.310	0.169	0.154	-0.147
98	-0.098	-0.091	0.121	0.116	0.133	0.269	0.315	-0.067	-0.004	0.564	0.231	0.270	0.855**
99	-0.273	-0.280	-0.063	-0.007	-0.035	0.175	0.004	-0.308	-0.231	0.303	0.046	0.098	0.839**
100	0.374	0.278	0.055	0.342	0.221	-0.384	-0.445	-0.096	-0.064	0.106	0.152	0.216	0.388
102	0.322	0.503	0.347	0.203	0.238	-0.157	-0.246	-0.329	-0.385	0.035	-0.236	-0.242	-0.266
104	0.301	0.322	0.467	0.469	0.462	-0.397	0.074	0.140	0.168	0.440	0.243	0.357	0.434

3.2. Sensory analysis and volatile composition of *broas*

Table C.9: Average sensorial analysis scores of *broas*.

Broas	Appearance	Colour	Smell and odour	Flavour and aroma	Texture	Global appreciation
1	5.90 ± 0.90	6.00 ± 0.83	5.89 ± 1.01	5.73 ± 1.20	5.90 ± 1.10	5.67 ± 1.14
2	6.48 ± 0.95	6.71 ± 0.87	5.87 ± 0.82	6.04 ± 0.87	6.17 ± 0.91	6.06 ± 0.73
3	6.13 ± 0.70	5.88 ± 0.87	5.91 ± 0.93	6.25 ± 0.93	6.08 ± 0.82	6.15 ± 0.85
4	6.42 ± 0.79	6.29 ± 0.90	5.98 ± 0.77	6.17 ± 1.00	6.31 ± 0.80	6.13 ± 0.87
5	6.40 ± 0.99	6.36 ± 0.87	5.85 ± 0.87	5.98 ± 1.09	6.15 ± 1.00	6.02 ± 0.94
6	6.15 ± 0.93	5.89 ± 0.84	5.96 ± 0.76	6.13 ± 0.95	6.13 ± 0.88	6.00 ± 0.93
7	6.19 ± 0.68	5.98 ± 0.77	5.93 ± 0.83	5.85 ± 1.04	6.04 ± 0.93	5.87 ± 0.97
8	6.72 ± 1.08	6.89 ± 1.11	6.20 ± 0.88	6.17 ± 1.09	6.36 ± 0.87	6.28 ± 0.99
9	5.98 ± 0.69	5.76 ± 0.71	5.93 ± 0.70	5.84 ± 0.90	5.90 ± 1.10	5.89 ± 0.96
10	6.76 ± 1.05	6.84 ± 1.09	6.16 ± 0.91	6.20 ± 0.97	6.17 ± 0.91	6.27 ± 1.12
11	6.00 ± 0.85	5.73 ± 0.78	5.86 ± 0.93	6.36 ± 0.88	6.08 ± 0.82	6.20 ± 0.89
12	5.36 ± 1.05	5.00 ± 0.93	5.39 ± 0.95	4.67 ± 0.88	6.31 ± 0.80	4.58 ± 0.97

Table C.10: Spearman correlation coefficients between *broas* volatile compounds (**V**) and sensorial analysis scores (**SA**).

p-Value corresponds to the significance level of Spearman correlation coefficient indicated as *: significant at $p < 0.05$; **: significant at $p < 0.01$.

SA / V	Appearance	Colour	Smell and odour	Flavour and aroma	Texture	Global appreciation
1	0.648*	0.746**	0.370	0.141	0.585*	0.254
2	-0.531	-0.832**	-0.196	0.158	-0.406	-0.154
3	-0.126	0.084	-0.137	-0.133	-0.042	-0.056
4	0.049	-0.210	0.305	0.270	0.301	0.231
5	-0.322	-0.350	-0.410	-0.056	-0.224	-0.217
6	-0.007	-0.294	0.277	0.287	0.140	0.245
7	-0.266	0.014	-0.298	-0.599*	-0.545	-0.545
8	-0.315	-0.266	-0.291	-0.539	-0.580*	-0.552
9	-0.035	0.105	-0.077	-0.231	-0.301	-0.308
10+11	0.140	-0.112	0.242	0.455	0.140	0.301
12	-0.322	-0.392	-0.487	0.119	-0.091	-0.077
13	0.196	0.252	0.081	-0.028	0.070	0.084
14	-0.287	-0.378	-0.154	0.172	-0.056	0.042
15	0.168	-0.077	0.368	0.473	0.385	0.462
16	-0.231	0.042	-0.305	-0.571	-0.490	-0.497
17	-0.224	0.056	-0.284	-0.578*	-0.517	-0.503
18	0.224	0.161	0.382	0.249	0.140	0.189
19	-0.231	-0.084	-0.014	-0.480	-0.336	-0.545
20	-0.441	-0.231	-0.280	-0.627*	-0.685*	-0.706*
21	-0.154	-0.056	0.098	-0.126	-0.315	-0.203
22	-0.252	-0.217	-0.336	0.161	0.105	0.077
23	-0.224	-0.147	0.116	-0.049	0.091	-0.084
25	-0.119	0.007	-0.147	-0.497	-0.392	-0.343
26	-0.224	-0.056	-0.147	-0.389	-0.280	-0.371
27	-0.406	-0.510	-0.144	-0.389	-0.685*	-0.497
28	0.077	-0.126	0.077	0.067	-0.021	-0.035
29	-0.252	-0.175	-0.070	-0.046	-0.126	-0.203
30	-0.517	-0.287	-0.343	-0.676*	-0.685*	-0.692*
32	-0.189	-0.322	-0.224	0.242	0.056	0.014
33+34	-0.476	-0.497	-0.207	-0.049	-0.203	-0.077
35	-0.322	-0.028	-0.343	-0.788**	-0.629*	-0.650*
36	-0.266	-0.406	0.294	-0.172	-0.322	-0.154
37	-0.119	0.007	-0.448	-0.564	-0.483	-0.524
38+39	-0.448	-0.643*	-0.413	-0.133	-0.434	-0.280
41	-0.427	-0.706*	-0.256	0.109	-0.315	-0.196
42	-0.399	-0.175	0.095	-0.746**	-0.573	-0.657*
43	0.371	0.105	0.319	0.231	0.294	0.182
44	-0.091	-0.287	0.067	0.074	0.112	-0.091
45	-0.098	-0.077	0.252	-0.228	-0.070	-0.315
46	0.134	0.225	0.363	-0.035	0.007	-0.085
47	0.280	0.287	0.224	0.137	0.210	0.252
51	0.350	0.028	0.378	0.515	0.441	0.315
52	-0.035	0.021	0.357	0.084	0.049	-0.014
53	-0.434	-0.273	-0.235	-0.459	-0.552	-0.573
54	0.441	0.231	0.445	0.711**	0.531	0.685*
55+56	-0.196	-0.462	-0.270	0.389	0.070	0.224
57	0.049	-0.021	0.319	0.193	0.105	0.063
58	0.252	0.364	-0.028	-0.235	0.133	-0.105
59	0.490	0.573	0.392	0.200	0.448	0.238
60+61	0.497	0.259	0.245	0.662*	0.601*	0.678*
62	-0.210	-0.252	-0.266	-0.084	-0.063	-0.063
63	-0.049	0.028	0.356	-0.116	-0.113	-0.197
64+65	-0.357	-0.175	-0.249	-0.606*	-0.608*	-0.678*
66	0.014	-0.175	0.207	0.018	-0.021	-0.021
67	0.119	-0.077	0.172	0.144	0.336	0.245
68	-0.315	-0.350	0.154	-0.193	-0.350	-0.364
69	-0.434	-0.196	-0.231	-0.431	-0.350	-0.524
70	0.308	0.133	0.228	0.399	0.301	0.329
71	-0.049	0.175	-0.210	-0.354	-0.287	-0.364
72	-0.497	-0.392	-0.172	-0.067	-0.161	-0.287
73	0.734**	0.650*	0.676*	0.438	0.797**	0.510
74	0.119	0.210	0.403	-0.088	-0.021	-0.133
75	0.000	-0.161	-0.231	0.179	-0.070	0.119
76	0.149	0.260	0.390	-0.025	0.071	-0.085
77	-0.301	-0.189	-0.049	-0.588*	-0.545	-0.497

78	-0.322	-0.252	-0.294	-0.007	-0.217	-0.287
79	-0.657*	-0.636*	-0.350	-0.378	-0.462	-0.531
80	0.070	0.224	0.238	-0.235	-0.021	-0.252
81	0.098	0.259	0.266	-0.231	-0.056	-0.238
82	0.189	-0.063	0.161	0.487	0.329	0.420
83	0.070	0.224	0.238	-0.235	-0.021	-0.252
84	-0.210	-0.077	0.028	-0.557	-0.476	-0.392
85	0.056	-0.161	0.025	0.035	0.056	0.126
86	-0.021	-0.098	-0.098	-0.102	-0.007	0.021
87	-0.175	-0.021	0.081	-0.648*	-0.434	-0.538
89	-0.259	-0.196	-0.270	-0.287	-0.294	-0.336
90	0.063	-0.217	-0.175	0.308	0.112	0.266
91	0.042	-0.028	0.305	-0.228	0.098	-0.091
92	-0.252	-0.021	-0.102	-0.623*	-0.552	-0.552
94	-0.175	-0.259	-0.004	-0.091	-0.063	-0.028
95	0.231	0.140	-0.151	0.476	0.357	0.336
96	0.014	-0.280	-0.448	0.455	0.175	0.287
98	0.651*	0.886**	0.533	0.014	0.546	0.357
99	0.636*	0.860**	0.378	-0.119	0.462	0.231
100	0.135	0.352	0.194	-0.036	0.157	-0.078
102	-0.357	-0.224	-0.259	-0.249	-0.182	-0.441
104	0.378	0.448	0.147	0.067	0.399	0.098

Table C.11: Spearman correlation coefficients among *broas* sensorial analysis scores.

p-Value corresponds to the significance level of Spearman correlation coefficient indicated as *: significant at $p < 0.05$; **: significant at $p < 0.01$.

	Colour	**Smell and odour**	**Flavour and aroma**	**Texture**	**Global appreciation**
Appearance	0.874**	0.595*	0.490	0.825**	0.699*
Colour		0.522	0.203	0.685*	0.503
Smell and odour			0.368	0.525	0.494
Flavour and aroma				0.739**	0.897**
Texture					0.874**

References

1. Pétel, C.; Onno, B.; Prost, C. Sourdough Volatile Compounds and Their Contribution to Bread: A Review. *Trends Food Sci.* Technol. **2017**, *59*, 105–123.
2. Birch, A.N.; Petersen, M.A.; Hansen, Å.S. The Aroma Profile of Wheat Bread Crumb Influenced by Yeast Concentration and Fermentation Temperature. *LWT-Food Sci. Technol.* **2013**, *50*, 480–488.
3. Hansen, A.; Schieberle, P. Generation of Aroma Compounds during Sourdough Fermentation: Applied and Fundamental Aspects. Trends Food Sci. *Technol.* **2005**, *16*, 85–94.
4. Birch, A.N.; Petersen, M.A.; Hansen, Å.S. Review: Aroma of Wheat Bread Crumb. *Cereal Chem.* **2014**, *91*, 105–114.
5. Bastos, D.M.; Monaro, É.; Siguemoto, É.; Séfora, M.; Markowicz, D.; Monaro, E.; Siguemoto, E.; Sefor, M. Maillard Reaction Products in Processed Food: Pros and Cons. In *Food Industrial Processes—Methods and Equipment*; Benjamin, V., Ed.; InTechOpen: London, UK, **2012**.
6. Berger, R.G. *Flavours and Fragrances: Chemistry, Bioprocessing and Sustainability*; Springer Science & Business Media: Berlin, Germany, 2007; ISBN 3540493395.
7. Purlis, E. Browning Development in Bakery Products—A Review. *J. Food Eng.* **2010**, *99*, 239–249.

8. Pozo-Bayón, M.A.; Guichard, E.; Cayot, N. *Flavor Control in Baked Cereal Products*; Taylor & Francis Group: Philadelphia, PA, USA, 2006; Volume 22, ISBN 8755-9129.
9. Martins, S.I.F.S.; Jongen, W.M.F.; Van Boekel, M.A.J.S. A Review of Maillard Reaction in Food and Implications to Kinetic Modelling. *Trends Food Sci. Technol.* **2000**, *11*, 364–373.
10. Bailey, M.E.; Gutheil, R.A.; Hsieh, F.-H.; Cheng, C.-W.; Gerhardt, K.O. Maillard reaction volatile compounds and color quality of a whey protein concentrate—Corn meal extruded product. In *Thermally Generated Flavors*; Thomas, H.P., Michael, J.M., Robert, J.M., Eds.; ACS Publications: Washington, DC, USA, **1993**; Chapter 25; pp. 315–327.
11. Jensen, S.; Oestdal, H.; Skibsted, L.H.; Larsen, E.; Thybo, A.K. Chemical Changes in Wheat Pan Bread during Storage and How It Affects the Sensory Perception of Aroma, Flavour, and Taste. J. *Cereal Sci.* **2011**, *53*, 259–268.
12. Belitz, H.-D.; Grosch,W.; Schieberle, P. Cereals and Cereal Products. *Food Chem.* **2009**, 670–745.
13. Cho, I.H.; Peterson, D.G. Chemistry of Bread Aroma: A Review. *Food Sci. Biotechnol.* **2010**, *19*, 575–582.

APPENDIX D

Comprehensive two-dimensional gas chromatography as a powerful strategy for the exploration of broas *volatile composition*[1]

[1] **Supplementary information included in a submitted publication to *Molecules*, currently under revision:**
Bento-Silva A, Duarte N, Santos M, Costa CP, Vaz Patto MC, Rocha SM, Bronze MR. Comprehensive two-dimensional gas chromatography as a powerful strategy for the exploration of *broas* volatile composition.

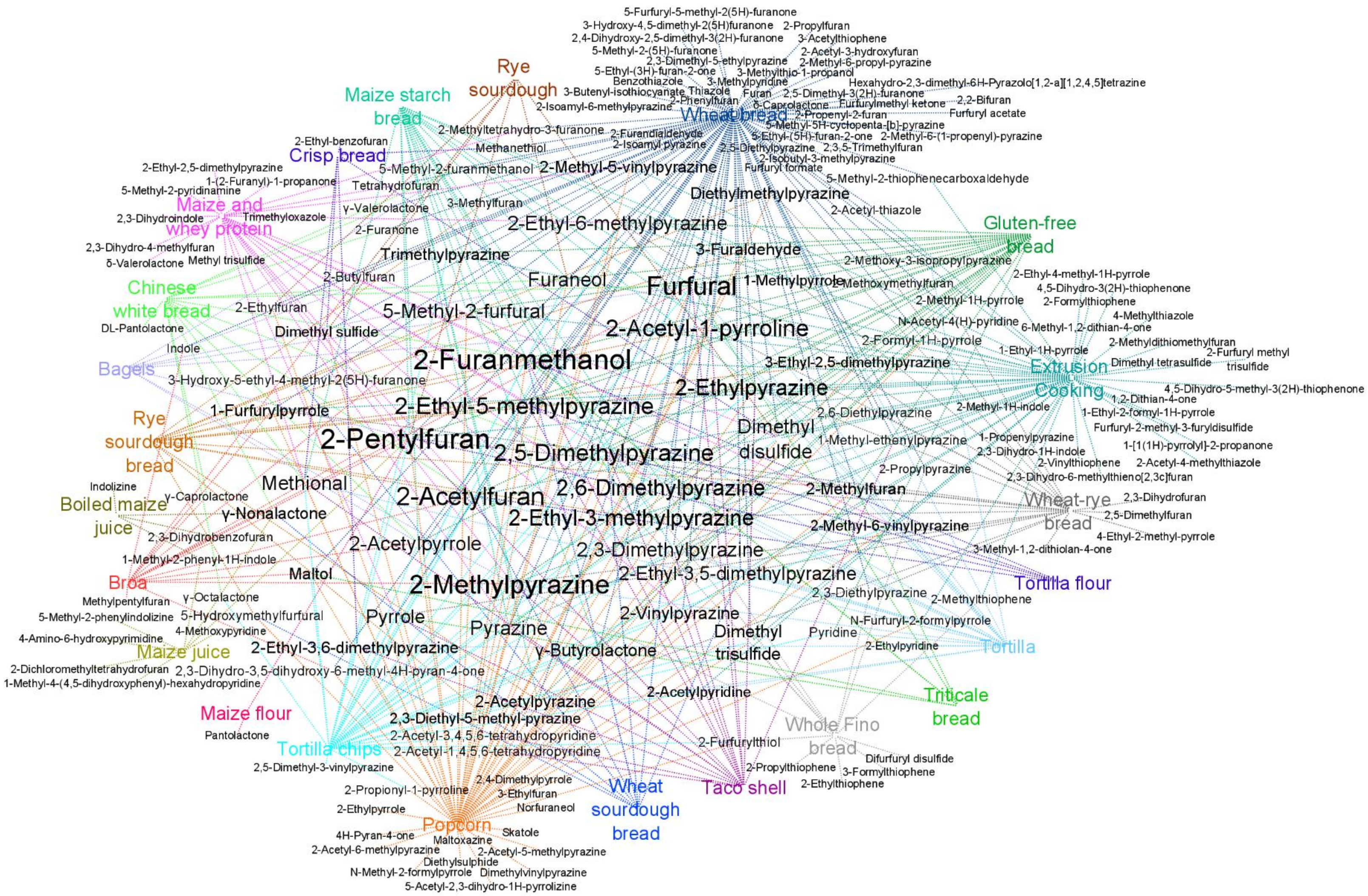


Figure D.1: Visual representation of the baking volatile compounds reported in the literature [1–21] for several breads and maize-based foods (nodes).

The volatiles more frequently reported (in different foods) are represented with larger font sizes.

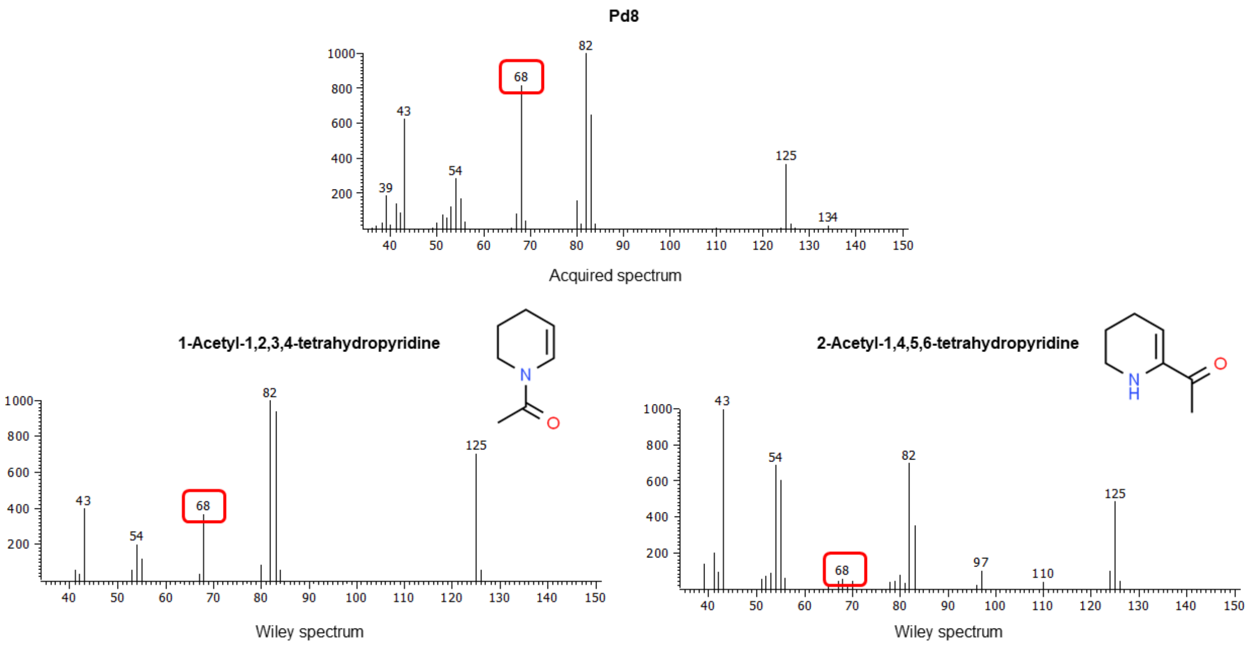


Figure D.2: Electron ionization mass of Pd8 (1-acetyl-1,2,3,4-tetrahydropyridine) and mass spectra of 1-acetyl-1,2,3,4-tetrahydropyridine and 2-acetyl-1,4,5,6-tetrahydropyridine from the Wiley library.

Table D.1: Average total peak areas and % of chromatogram area for the families of chemical compounds studied in *broas*.

Mean values within rows with no letters (a–f or A–G) in common are significantly different ($p < 0.05$).

Broa	Furans	Furanones	Pyrans	Pyranones	Pyrazines	Pyridines	Pyrroles	Oxazoles	Thiazoles	Thiophenes	Sulfides
B1	763602279 ± 58932402^{abcd}	123703256 ± 10093713^{b}	1222564 ± 221783^{ab}	127142718 ± 31923812^{ab}	54598819 ± 1318758^{cd}	1899809 ± 436122^{a}	30763217 ± 6724318^{abc}	245164 ± 3953^{ab}	4025450 ± 16575^{c}	1310212 ± 237537^{abcde}	15195450 ± 4374617^{abc}
%	**68 ± 0.1^{AB}**	**11.0 ± 0.03^{BCD}**	**0.11 ± 0.028^{ABCD}**	**11.2 ± 2.0^{AB}**	**4.9 ± 0.50^{BC}**	**0.17 ± 0.026^{A}**	**2.8 ± 0.82^{AB}**	**0.022 ± 0.001^{BC}**	**0.36 ± 0.027^{G}**	**0.12 ± 0.030^{AB}**	**1.4 ± 0.50^{AB}**
B2	572947817 ± 769839^{a}	55576697 ± 3171058^{a}	1078252 ± 60845^{a}	128987045 ± 14171961^{ab}	21759529 ± 1018204^{a}	1685579 ± 150533^{a}	18855416 ± 228942^{a}	22441 ± 8866^{a}	1979246 ± 214345^{ab}	916267 ± 184068^{ab}	9535746 ± 788673^{a}
%	**70 ± 1.6^{AB}**	**6.8 ± 0.24^{A}**	**0.13 ± 0.005^{BCDE}**	**15.8 ± 1.4^{B}**	**2.7 ± 0.07^{A}**	**0.21 ± 0.014^{AB}**	**2.3 ± 0.02^{A}**	**0.003 ± 0.001^{A}**	**0.24 ± 0.021^{EF}**	**0.11 ± 0.025^{AB}**	**1.2 ± 0.12^{A}**
B3	902541570 ± 34657093^{bcde}	142700122 ± 37193201^{b}	1142308 ± 171976^{a}	205620499 ± 65909180^{abc}	45442047 ± 7198963^{abc}	2270359 ± 566836^{ab}	28317141 ± 1652035^{abc}	773377 ± 557754^{b}	2382071 ± 133763^{ab}	1541447 ± 71150^{cde}	23907365 ± 2549614^{bcd}
%	**67 ± 6.5^{AB}**	**10.5 ± 2.12^{ABC}**	**0.08 ± 0.008^{ABC}**	**15.0 ± 4.0^{AB}**	**3.3 ± 0.33^{AB}**	**0.17 ± 0.032^{A}**	**2.1 ± 0.003^{A}**	**0.056 ± 0.038^{C}**	**0.18 ± 0.020^{ABCD}**	**0.11 ± 0.012^{AB}**	**1.8 ± 0.08^{AB}**
B4	733337645 ± 74541267^{abc}	133042906 ± 3270531^{b}	2558263 ± 200734de	171823016 ± 2086398^{abc}	38240052 ± 1938303^{abc}	4156503 ± 217381^{bcd}	37025735 ± 2403180^{bcd}	357337 ± 130283^{ab}	1980961 ± 173030^{ab}	1229722 ± 50077^{abcd}	12066114 ± 716982^{ab}
%	**64 ± 2.9^{AB}**	**11.7 ± 0.95^{CD}**	**0.23 ± 0.005^{FG}**	**15.2 ± 1.0^{AB}**	**3.4 ± 0.36^{AB}**	**0.37 ± 0.040^{CD}**	**3.3 ± 0.40^{AB}**	**0.031 ± 0.010^{BC}**	**0.18 ± 0.025^{ABCD}**	**0.11 ± 0.011^{AB}**	**1.1 ± 0.12^{A}**
B5	804136125 ± 40227364^{abcde}	100982157 ± 8422559^{ab}	1978429 ± 179562^{bcd}	148529804 ± 14247976^{ab}	29554242 ± 2034746^{abc}	3573341 ± 330181^{abc}	27248066 ± 3480151^{ab}	162081 ± 17281^{ab}	1741250 ± 213838^{a}	1203493 ± 15558^{abcd}	18415325 ± 183388^{abc}
%	**71 ± 0.8^{AB}**	**8.9 ± 0.20^{ABC}**	**0.17 ± 0.005^{EF}**	**13.0 ± 0.5^{AB}**	**2.6 ± 0.02^{A}**	**0.31 ± 0.010^{BC}**	**2.4 ± 0.16^{AB}**	**0.014 ± 0.001^{AB}**	**0.15 ± 0.010^{ABC}**	**0.11 ± 0.005^{AB}**	**1.6 ± 0.11^{AB}**
B6	1297623398 ± 153937928^{f}	202268961 ± 4702710^{bd}	1537947 ± 91283^{abc}	212328658 ± 12374856^{bc}	52262611 ± 426677^{bcd}	3052932 ± 173244^{abc}	39025017 ± 1655587^{bcde}	218189 ± 101997^{ab}	2601717 ± 34763^{b}	1294486 ± 81170^{abcde}	21481118 ± 276979^{abc}
%	**71 ± 3.1^{AB}**	**11.1 ± 1.09^{BCD}**	**0.08 ± 0.001^{AB}**	**11.6 ± 1.6^{AB}**	**2.9 ± 0.24^{AB}**	**0.17 ± 0.003^{A}**	**2.1 ± 0.07^{A}**	**0.012 ± 0.005^{BC}**	**0.14 ± 0.009^{AB}**	**0.07 ± 0.010^{A}**	**1.2 ± 0.07^{A}**
B7	737501117 ± 104722371^{abc}	91056203 ± 14966619^{ab}	1482743 ± 108720^{abc}	104928027 ± 31520596^{ab}	29054400 ± 1637310^{ab}	2929451 ± 665040^{abc}	24838544 ± 4584499^{ab}	83542 ± 6726^{ab}	1983132 ± 274075^{ab}	743113 ± 198427^{a}	16790956 ± 1254191^{abc}
%	**73 ± 1.0^{B}**	**9.0 ± 0.08^{ABC}**	**0.15 ± 0.012^{DE}**	**10.3 ± 1.5^{AB}**	**2.9 ± 0.29^{AB}**	**0.29 ± 0.021^{ABC}**	**2.5 ± 0.07^{AB}**	**0.008 ± 0.001^{AB}**	**0.20 ± 0003^{BCDE}**	**0.07 ± 0.008^{A}**	**1.7 ± 0.39^{AB}**
B8	1068994852 ± 23244752^{ef}	122063687 ± 4286803^{b}	2127752 ± 122437^{cd}	265824225 ± 39293874^{c}	33485937 ± 3666921^{abc}	3995059 ± 132931^{bcd}	31619349 ± 737329^{abc}	177188 ± 57610^{ab}	2383771 ± 124811^{ab}	1054969 ± 40806^{abc}	18834727 ± 115241^{abc}
%	**69 ± 1.7^{AB}**	**7.9 ± 0.09^{AB}**	**0.14 ± 0.002^{CDE}**	**17.1 ± 1.7^{B}**	**2.2 ± 0.14^{A}**	**0.26 ± 0.003^{ABC}**	**2.0 ± 0.05^{A}**	**0.011 ± 0.003^{AB}**	**0.15 ± 0.001^{AB}**	**0.07 ± 0.001^{A}**	**1.2 ± 0.06^{A}**
B9	786239757 ± 118338835^{abcde}	227934913 ± 5599334^{d}	1701839 ± 230339^{abc}	139028903 ± 24613043^{ab}	77368869 ± 4220626de	4423825 ± 259076^{cd}	50731991 ± 7429054de	598940 ± 77307^{ab}	3892719 ± 45114^{c}	1894113 ± 300276^{e}	23380191 ± 6648156^{bcd}
%	**60 ± 5.3^{A}**	**17.4 ± 1.51^{E}**	**0.13 ± 0.026^{BCDE}**	**10.6 ± 2.5^{AB}**	**5.9 ± 0.69^{C}**	**0.34 ± 0.041^{BC}**	**3.9 ± 0.81^{B}**	**0.046 ± 0.009^{BC}**	**0.30 ± 0.022^{FG}**	**0.14 ± 0.032^{B}**	**1.8 ± 0.39^{AB}**
B10	1047140092 ± 17830892^{def}	232492424 ± 7300115^{d}	1565362 ± 262668^{abc}	129678111 ± 8817368^{ab}	95899669 ± 1443621^{ef}	8142898 ± 1287932^{e}	56436164 ± 3171211^{e}	666369 ± 43723^{ab}	3471267 ± 13590^{c}	1721907 ± 95202de	24904193 ± 4639753^{cd}
%	**65 ± 1.1^{AB}**	**14.5 ± 0.46^{DE}**	**0.10 ± 0.016^{ABCD}**	**8.1 ± 0.6^{A}**	**6.0 ± 0.09^{C}**	**0.51 ± 0.080^{D}**	**3.5 ± 0.20^{AB}**	**0.042 ± 0.003^{BC}**	**0.22 ± 0.001^{DEF}**	**0.11 ± 0.006^{AB}**	**1.6 ± 0.29^{AB}**
B11	1019649140 ± 39507727^{cdef}	144921482 ± 20397144^{bc}	964489 ± 204627^{a}	191245724 ± 13182486^{abc}	51949320 ± 19237817^{bc}	3075796 ± 352928^{abc}	31335663 ± 7621939^{abc}	194332 ± 8127^{ab}	2053386 ± 253974^{ab}	1492655 ± 182577^{bcde}	34456252 ± 202457^{cd}
%	**69 ± 0.8^{AB}**	**9.8 ± 0.89^{ABC}**	**0.06 ± 0.011^{A}**	**12.9 ± 1.5^{AB}**	**3.5 ± 1.12^{AB}**	**0.21 ± 0.013^{AB}**	**2.1 ± 0.41^{A}**	**0.013 ± 0.001^{AB}**	**0.14 ± 0.010^{A}**	**0.10 ± 0.017^{AB}**	**2.3 ± 0.13^{B}**
B12	720002606 ± 60458791^{ab}	141869673 ± 13541962^{b}	3136408 ± 396934^{e}	96622336 ± 343407^{a}	114770778 ± 3969717^{f}	5596651 ± 7186^{d}	45016538 ± 4665850^{cde}	357452 ± 134274^{ab}	2391480 ± 272829^{ab}	1461570 ± 82806^{bcde}	22942230 ± 4061405^{bcd}
%	**62 ± 1.4^{AB}**	**12.3 ± 0.41^{CD}**	**0.27 ± 0.018^{G}**	**8.4 ± 0.5^{A}**	**10.0 ± 0.97^{D}**	**0.49 ± 0.030^{D}**	**3.9 ± 0.16^{B}**	**0.031 ± 0.010^{BC}**	**0.21 ± 0.011^{CDE}**	**0.13 ± 0.001^{AB}**	**2.0 ± 0.48^{AB}**
Range (%)	60 – 73	6.8 – 17.4	0.06 – 0.27	8.1 – 17.1	2.2 – 10	0.17 – 0.51	2.0 – 3.9	0.003 – 0.056	0.14 – 0.36	0.07 – 0.14	1.1 – 2.3
Average (%)	**67 ± 3.9**	**11 ± 2.9**	**0.14 ± 0.06**	**12 ± 2.9**	**4.2 ± 2.2**	**0.29 ± 0.12**	**2.7 ± 0.7**	**0.02 ± 0.02**	**0.20 ± 0.07**	**0.10 ± 0.02**	**1.56 ± 0.38**

Table D.2: Peak areas obtained for each sample (average of duplicates) and considered for the statistical analysis. Peaks are numbered according to ***Table 5.1***.

*: Peak areas of B12 are significantly different ($p < 0.05$).

	***Broa* 1**	***Broa* 2**	***Broa* 3**	***Broa* 4**	***Broa* 5**	***Broa* 6**	***Broa* 7**	***Broa* 8**	***Broa* 9**	***Broa* 10**	***Broa* 11**	***Broa* 12**
F1	6691949	615338	1524867	2907057	9430278	697865	3007692	2254879	1869877	3858576	3342831	1006736
F2	3855653	760479	3129887	3127175	1382390	4510440	1352344	2784558	4916862	3907464	4613681	2871501
F3	1564893	477422	3770771	1089328	1697488	1321111	952693	1381521	1052209	1847419	2267586	2139840
F4	21688854	21250167	9601637	17453662	17648469	9818783	17879138	17560166	11664935	13661400	11023845	13449413
F5	2321265	1981779	2407285	2047850	2162221	1731681	3078880	2568511	3238308	3818400	2573213	3211120
F6	1035198	378707	715749	607644	464959	852327	825584	628717	1613649	1603575	631408	769200
F7	613214	405566	546782	584720	896700	501804	327758	260853	816380	842887	582574	981525
F8	541598	415078	291348	196372	339360	446174	140171	266720	546593	696045	202210	210884
F9	126038	20869	39151	46182	19503	84103	17774	37977	104130	182536	39122	61878
F10	5038337	3390059	5938643	3103550	2984852	5378781	4788557	3819086	6366782	5244858	4698748	3720697
F11	286728	187491	325055	299002	231218	375150	52497	252741	477892	465918	218651	375275
F12	243673923	173235728	224239502	97906758	247142336	401411249	205310585	193980303	188920874	175250853	140367303	112696099
F13	307535937	221549424	431424479	335784907	335995242	597568015	323162386	544448792	275385258	523653196	629111351	323870967
F14	4012814	3390190	3812794	5664162	2971315	4583546	4639505	6035995	6520826	9029608	3197879	6297233
F15	449543	305656	689062	954860	522983	559080	285260	636521	779624	952141	656882	934735
F16	40838453	35885319	42990320	62085584	45772044	53724887	28614781	59575993	57927654	60992699	54187945	46297816
F17	867065	412820	2097674	5152339	487365	745117	310839	6142932	6890261	2295240	529062	6708900
F18	30080154	26218804	47092492	32673318	33740731	41690627	21432196	48563747	33432526	20351278	33398063	32980737
F19	6387321	3504340	8036589	6809433	4394092	12544810	4362250	5490856	14379319	16203874	11434070	7005084
F20	40072221	50709157	40723631	44116974	42190514	61205631	73769044	77574347	55148421	70595715	39777299	65789076
F21	305653	393783	240636	453018	253483	241365	320697	357474	936899	1130620	394057	616611
F22	102038	246465	83552	1201833	652265	38535	360365	1125856	46181	208905	32905	615788
F23	3042589	2616761	4401703	5337709	3913548	4055291	2620028	3950549	9721303	10880388	4643749	7244604
F24	1479001	1330751	2246068	3213051	1727106	1733592	1068891	2317975	5272061	4816347	2265095	3603605
F25	11744406	364180	13408359	834582	348034	15716957	453993	559970	11943832	16644897	17052679	595660
F26	571613	461504	698154	1694769	609352	180361	461779	1314331	1947285	2192950	580382	1271681
F27	1103478	4637031	1704494	3468107	1072908	2936404	2247103	5270820	5241802	8116638	792745	599978
F28	279200	426296	281036	915023	329032	355566	156699	369893	469223	874177	370897	588379
F29	3062804	1308846	5825253	11445330	5174130	6948361	3295742	6650606	6878153	7173904	3894500	7121649
F30	417748	473592	261994	520235	211931	382397	449441	630675	530164	829115	246224	611802
F31*	1582645	2730735	1646021	3365522	4945510	2295342	3356299	5253821	3730074	3660913	1482510	12986965
F32	9124542	5842499	18043675	34269351	19224900	25244578	13277867	33263785	30865001	32155446	22712000	16277351
F33	951176	624451	1382940	2906089	1020722	1807511	557674	1467504	2077353	2576416	1327184	1903610
F34	3282095	2289354	2054910	2823143	3805170	1721847	3549608	4082695	2201446	2851387	2559006	2472457
F35	197494	137709	246269	455023	147310	271196	221291	264933	242785	365691	120823	209588
F36	8674641	3969466	20618789	37823994	10226671	33942922	10793716	27848757	32083820	37208625	18320671	31904173
Fo1	1416333	917722	2188718	2625361	1836114	2916887	1769231	2083380	2617219	2819450	2349961	1412684
Fo2	21589399	2311547	34509222	6097889	3737198	42364060	4595524	4912079	42733413	44547955	13573723	14431175
Fo3	313733	207928	359807	219479	171318	267612	136672	208136	288518	301337	182264	165539
Fo4	223625	251073	776411	668022	300607	154510	362123	480842	4168335	4547388	815185	2415477
Fo5	27283680	17061444	40048767	51991492	34225043	65947012	33205283	42302007	61425654	65750920	51122357	27749381
Fo6	3565710	2717831	5443714	13243918	4980244	6726264	4017751	5980801	7070348	9705259	4341841	6068441
Fo7	1471081	746602	1774065	1784995	1235977	2402724	1166578	1550675	2590886	2864570	1977732	2254429
Fo8	3480035	1601196	3851382	2448360	2863359	6396902	3750601	2339302	5316795	7382578	4528913	2415935
Fo9	281958	156610	293402	288714	339805	271929	150199	181153	365960	183629	208990	280036
Fo10	2439856	1008121	2062364	1435480	1255945	3635684	1640213	1736757	2534154	3592115	2452807	1774605
Fo11	910587	316923	505991	254669	240466	671116	584748	119616	286959	1024379	715242	404184
Fo12	188622	47503	297550	171356	88889	414100	114481	119387	168235	362105	152851	77328
Fo13	586504	442896	357194	405198	446784	657112	603530	712418	767863	1035825	531556	804472
Fo14	717734	482684	1011281	1288692	593262	1264964	372989	524594	1513285	1683617	876186	1354553
Fo15	5393372	1463893	5250051	5421374	6488077	3183829	1359268	6888726	8928801	9793314	6347362	7284597
Fo16	2495148	1232673	3438660	3159910	3008151	4813505	1042741	2866037	5278547	5969719	3129652	3685929

Fo17	2437671	636654	1245638	2166751	1550380	3319877	1100931	2022183	4519971	3664409	2024502	2536806
Fo18	2258487	1350660	1878907	2585103	2598141	3113998	2271811	3227531	3643853	3997732	2921421	4413501
Fo19	15553948	7443467	15486010	9468744	10658071	21800415	10022706	11109564	26558986	26136459	20147214	10740090
Fo20	1022822	1830177	1299325	6088783	4785472	1704967	4596384	7249791	3251885	3968800	210305	9607426
Fo21	1276671	274500	2319515	3265982	1592619	2928300	1009895	2002399	2912255	2955028	1441297	2507664
Fo22	879132	391425	729777	681532	588925	1204385	630808	731231	1321939	1157641	1118531	565049
Fo23	1527187	683573	1201631	1001792	846184	1752950	776295	978398	2531772	1927246	1613543	976987
Fo24	3244663	2081387	2130452	2433202	2343718	3457588	2887274	3795027	4700976	5556173	3457370	5908237
Fo25	11368478	5964188	6398430	7988065	8443830	9285562	8278210	11775168	16072152	6399859	8397390	19774899
Fo26	11776818	3954020	7841859	5858050	5763584	11612716	4609962	6166489	16366158	15164925	10283294	12260257
Pn1	128721	164838	132077	118514	115605	142426	211042	141521	138760	115073	104861	99362
Pn2	854156	770248	891949	2299826	1751247	1306766	1223631	1830585	1421491	1263005	815491	2901688
Pn3	239687	143165	118282	139923	111577	88755	48071	155647	141588	187285	44138	135359
Po1	39416	21631	65223	65247	27185	92053	30893	45243	76576	100794	45481	38596
Po2	28105	21174	26837	44089	39469	32495	23171	8777	58917	50568	33806	42012
Po3	6576263	482803	7836481	709701	1018669	10989002	943673	1167002	13469699	11253499	10162696	2503960
Po4	863864	713807	1377747	2505068	1519070	1843557	1369072	1783058	2355182	2048686	1149389	1929465
Po5	987323	1089567	653389	886066	1009525	890442	1068723	1123177	847614	853004	747275	840535
Po6	739294	496635	461982	796668	891235	1207374	853089	1004493	1033968	1016578	956892	628915
Po7	107105240	109561012	171474298	115185328	115644962	163141630	79813166	192598823	91506723	83230534	125669543	57134529
Po8	8331390	14438103	21355513	46454551	24388393	31001539	17848051	63810414	26214143	27939120	50536109	27221265
Po9	26697	5809	84414	59774	34441	54675	12704	27734	62404	54605	15556	10246
Pz1	1593679	837242	1418950	1625846	860505	1668279	957239	1055195	2705312	2838027	996329	3019652
Pz2*	11366618	6623650	10100276	13871874	9133466	12323112	8176939	12291280	22040199	28520900	9606416	45230718
Pz3	30531761	7618190	24334797	12123377	10583455	26950791	10864691	10338605	35309745	44011053	31747791	36087651
Pz4	1084374	1416652	1339397	1130510	1215550	1123698	2386891	1370981	2850949	3171803	1104668	7593796
Pz5	1110181	1481207	1431379	2380468	1799076	1985463	1511212	1668634	3512562	4218684	1700557	6168152
Pz6	4867019	2559376	4047867	4621055	4444498	4712331	3156703	4061757	6938815	8746235	4399120	10522425
Pz7	1325194	348275	913191	436317	235384	861382	516394	455157	1446934	1646952	939177	1894102
Pz8	620205	236437	256309	413386	111582	424409	194709	598426	553190	827785	186801	602217
Pz9	1194268	231396	852879	431641	346272	1155999	778895	683574	726667	246223	382162	1967528
Pz10	199414	75250	118028	203713	237314	169824	107191	141060	251290	353318	177017	451967
Pz11	391966	228872	369168	626039	464914	528529	304595	548245	633890	766596	468103	807122
Pz12	314141	102980	259807	375830	122228	358797	98943	273025	399318	552095	241181	425452
Pd1	257752	110243	251056	310865	235471	374522	150685	268402	668170	710614	284372	571485
Pd2	22664	8855	4387	5361	9068	3789	5140	3410	7617	8456	6430	24087
Pd3	336126	108250	226514	246390	255700	355284	258639	276468	414519	474652	477590	245480
Pd4	94098	73629	107609	126738	94380	128577	82481	136483	223971	211331	77727	271447
Pd5	38838	34892	36190	49097	23323	28660	15629	22958	24706	34289	23656	25099
Pd6	41370	18579	218063	118391	95460	107907	232797	205511	311505	163167	173208	321905
Pd7	120531	474230	424975	872845	655666	691329	693501	900879	1250385	4973064	794265	1848534
Pd8	860844	819664	967771	2323907	2107323	1281307	1422393	2081962	1418476	1434034	1180093	2156897
Pd9	127585	37237	33794	102910	96952	81559	68186	98988	104479	133293	58458	131719
Py1	105715	79069	66900	39302	19828	78637	62546	43250	81720	93742	42327	107957
Py2	715664	506628	785394	898007	589260	760489	413685	750007	1302663	1533553	685704	1246135
Py3	573938	363224	687486	481540	309593	639073	462350	491480	1396012	1187878	410619	970965
Py4	2202592	951949	2167007	3642265	698738	2753303	958906	1225934	3359658	3332886	1510001	2901475
Py5	241791	43798	275423	490598	236390	579406	33728	315608	596795	681427	299826	187009
Py6	21342173	9004823	17160728	20235766	16263386	26353008	13456932	16950393	32633721	35698940	22526622	25047325
Py7	289382	402083	127891	68465	67201	411181	116514	130409	186503	481574	93345	142034
Py8	348245	257967	1540564	537328	329821	440653	477825	573086	2480093	3421058	296613	1247075
Py9	397774	548147	561906	1285431	496122	804005	487235	915915	1040690	1267756	294266	1207833
Py10	3736442	6238638	4111672	8043309	7456520	5135785	7685556	9398938	6091910	6559038	4297102	9631639
Py11	477967	321395	463463	891510	582781	702182	590217	641636	1151719	1635629	679147	1549511
Py12	90463	13094	43289	28958	18707	64934	15544	29492	76551	68983	38108	120864
Py13	241070	124600	325418	383259	179722	302364	77510	153205	333960	473703	161985	656718
Ox1	52214	13395	38277	8894	4430	35407	24683	9388	67321	70531	4430	28355
Ox2	72555	1085	551104	14116	7058	7058	7058	7058	299659	289879	19229	33775
Ox3	120395	7962	183996	334327	150593	175724	51801	160743	231961	305959	170673	295323

Tz1	652472	198748	554124	61430	44492	446190	322918	43604	500951	502178	14762	187477
Tz2	898987	954719	867782	1356396	1117765	807859	1080597	1473119	1404647	1602954	780704	1520775
Tz3	2473991	825779	960165	563136	578994	1347668	579617	867049	1987122	1366136	1257920	683228
Tp1	23851	12412	24036	19935	12314	9617	34909	13451	33196	36899	19646	28521
Tp2	274934	291071	360986	151372	413347	197151	131751	95375	201387	153420	583758	178681
Tp3	701088	430162	815657	828866	518663	803099	384527	680956	1235079	1148656	656055	942946
Tp4	102411	87452	160409	105888	77205	108464	83873	108783	191958	177546	104739	177125
Tp5	80581	48045	72588	76253	36419	73881	45258	48791	102897	110820	81453	73353
Tp6	127348	47125	107771	47409	145545	102275	62797	107615	129597	94568	47004	60945
S1	5183873	3682260	5638402	5201407	7695280	6003347	6389799	9573231	5550541	5467429	6541864	6459281
S2	706027	1563140	1854872	1463810	2508621	748310	2458145	2707726	1195649	2555254	1543694	971464
S3	7807314	3373056	14252565	4245342	6942347	10015802	5212575	4735382	11739271	10865322	20073686	10846713
S4	98446	97302	100179	71783	58688	112405	100532	87601	133405	123900	88229	138477
S5	30161	13520	63274	13355	13628	13142	2591	2591	13499	14101	22650	12333
S6	40737	146590	20376	110645	115345	11715	78222	52739	13040	17339	8077	76373
S7	124612	140916	35734	342249	233037	1677600	1379107	494292	1555969	1604271	1401192	689287
S8	1204280	518964	1941963	617525	848380	2898799	1169987	1181168	3178819	4256579	4776862	3748305

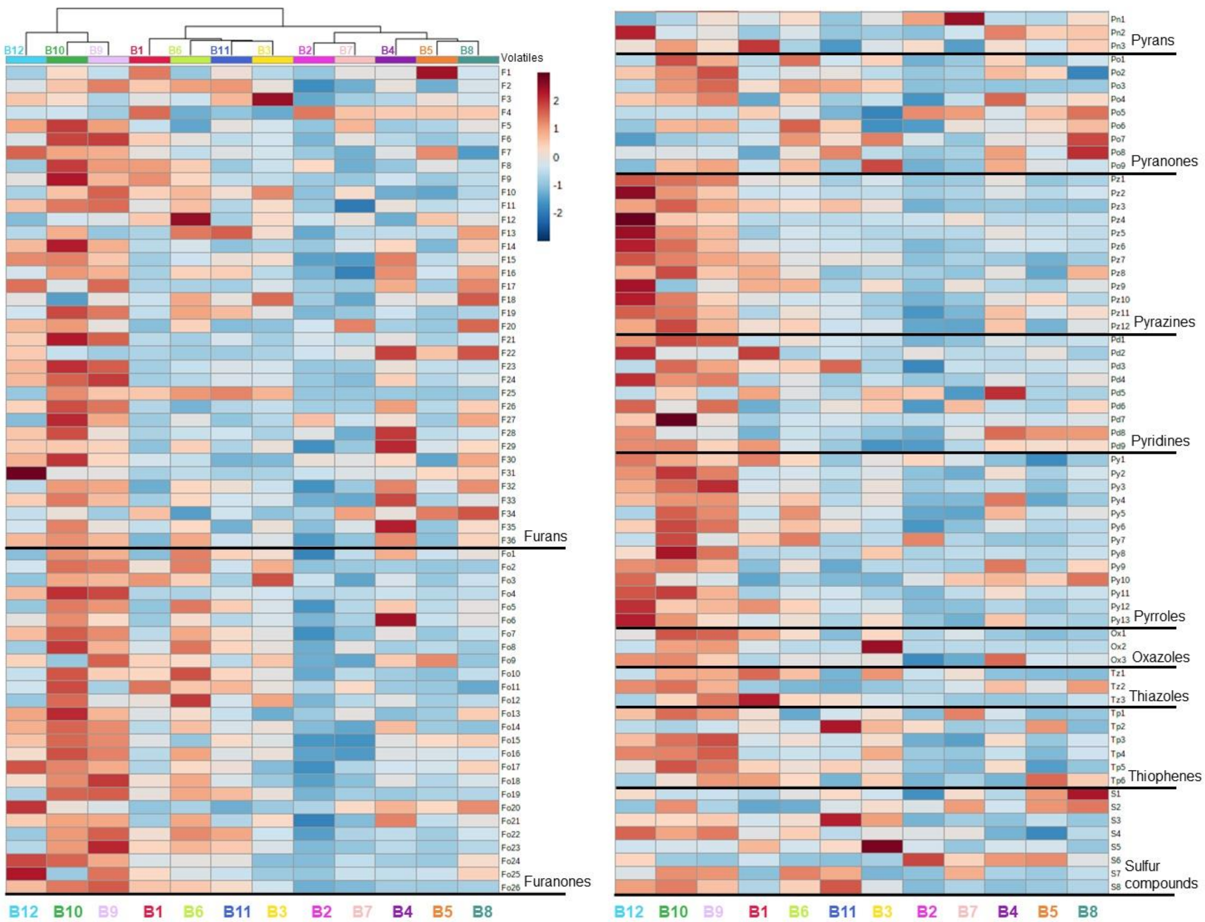


Figure D.3: Heatmap and hierarchical cluster analysis representation of the 128 volatiles identified in *broas*.
The content of each metabolite is illustrated through a chromatic scale (from deep blue, minimum, to deep red, maximum).

Table D.3: Spearman correlation coefficients among the 128 studied *broas* volatile compounds, ordered according to the heatmap representation [***Figure 5.5***].

The green cells highlight the volatiles which strongly contributed to differentiate the samples along PC1 and the purple cells highlight those which strongly contributed to differentiate the samples along PC2.

	Fo1	**Fo5**	**Po1**	**Py5**	**Pd3**	**Po6**	**S7**	**Po9**	**Fo3**	**Fo12**	**Fo10**	**Fo19**	**Po3**	**F2**	**Fo23**	**F19**	**Fo2**	**F9**	**Tp5**	**Fo26**	**Pz3**	**Pz7**	**Py12**	**S4**	**F6**	**Ox1**	**Ox2**	**S3**	**S8**	**F10**	**Tz3**	**F25**
Fo1	1.000	**0.979**	**0.916**	**0.888**	0.587	0.699	0.615	0.748	0.441	0.783	0.643	0.601	0.601	0.692	0.762	0.762	0.629	0.455	0.573	0.399	0.371	0.147	0.182	0.091	0.371	0.207	0.288	0.371	0.392	0.441	0.280	0.692
Fo5	**0.979**	1.000	**0.902**	**0.902**	0.629	0.790	0.720	0.692	0.350	0.692	0.664	0.643	0.643	0.692	0.762	0.783	0.629	0.441	0.566	0.455	0.399	0.196	0.217	0.168	0.399	0.207	0.256	0.385	0.455	0.441	0.287	0.657
Po1	**0.916**	**0.902**	1.000	**0.930**	0.580	0.587	0.573	0.734	0.636	**0.860**	0.797	0.741	0.748	0.804	**0.888**	**0.895**	**0.853**	0.720	0.769	0.657	0.594	0.462	0.483	0.357	0.594	0.508	0.548	0.497	0.559	0.636	0.510	0.818
Py5	**0.888**	**0.902**	**0.930**	1.000	0.629	0.699	0.545	0.713	0.608	0.734	0.692	0.727	0.699	0.762	**0.867**	0.818	0.734	0.678	0.748	0.622	0.476	0.322	0.406	0.168	0.462	0.382	0.431	0.371	0.462	0.476	0.504	0.685
Pd3	0.587	0.629	0.580	0.629	1.000	0.811	0.713	0.182	0.189	0.483	0.755	0.797	0.727	0.748	0.762	0.629	0.538	0.455	0.685	0.580	0.552	0.441	0.371	0.189	0.608	0.228	0.260	0.504	0.664	0.455	0.636	0.657
Po6	0.699	0.790	0.587	0.699	0.811	1.000	0.839	0.315	0.056	0.392	0.601	0.650	0.587	0.545	0.615	0.531	0.413	0.294	0.406	0.413	0.280	0.140	0.168	0.161	0.455	0.137	-0.050	0.210	0.399	0.336	0.378	0.378
S7	0.615	0.720	0.573	0.545	0.713	0.839	1.000	0.084	-0.147	0.287	0.643	0.573	0.573	0.517	0.538	0.615	0.469	0.322	0.469	0.476	0.510	0.392	0.252	0.497	0.531	0.214	0.000	0.322	0.587	0.350	0.280	0.476
Po9	0.748	0.692	0.734	0.713	0.182	0.315	0.084	1.000	0.685	0.720	0.406	0.469	0.490	0.538	0.622	0.580	0.587	0.399	0.350	0.336	0.196	0.056	0.224	0.021	0.301	0.333	0.537	0.350	0.126	0.504	0.231	0.434
Fo3	0.441	0.350	0.636	0.608	0.189	0.056	-0.147	0.685	1.000	0.790	0.524	0.552	0.531	0.552	0.664	0.524	0.692	0.692	0.552	0.476	0.294	0.301	0.448	0.154	0.441	0.662	0.634	0.329	0.196	0.643	0.678	0.559
Fo12	0.783	0.692	**0.860**	0.734	0.483	0.392	0.287	0.720	0.790	1.000	0.755	0.664	0.650	0.699	0.790	0.706	0.790	0.678	0.608	0.504	0.441	0.336	0.434	0.217	0.580	0.550	0.513	0.427	0.392	0.678	0.580	0.769
Fo10	0.643	0.664	0.797	0.692	0.755	0.601	0.643	0.406	0.524	0.755	1.000	**0.944**	**0.944**	**0.888**	**0.909**	**0.888**	**0.909**	0.755	0.762	0.832	0.769	0.727	0.720	0.601	0.818	0.634	0.566	0.734	0.832	0.804	0.811	**0.874**
Fo19	0.601	0.643	0.741	0.727	0.797	0.650	0.573	0.469	0.552	0.664	**0.944**	1.000	**0.986**	**0.895**	**0.930**	**0.867**	**0.874**	0.713	0.748	**0.853**	0.727	0.685	0.699	0.510	0.790	0.613	0.623	0.769	0.811	0.790	**0.853**	0.790
Po3	0.601	0.643	0.748	0.699	0.727	0.587	0.573	0.490	0.531	0.650	**0.944**	**0.986**	1.000	**0.895**	**0.916**	**0.909**	**0.902**	0.706	0.734	**0.867**	0.776	0.727	0.720	0.580	0.783	0.627	0.676	0.839	**0.853**	0.804	0.804	0.811
F2	0.692	0.692	0.804	0.762	0.748	0.545	0.517	0.538	0.552	0.699	**0.888**	**0.895**	**0.895**	1.000	**0.965**	**0.902**	0.839	0.734	**0.867**	0.804	0.748	0.629	0.664	0.378	0.664	0.441	0.651	0.762	0.762	0.678	0.734	**0.888**
Fo23	0.762	0.762	**0.888**	**0.867**	0.762	0.615	0.538	0.622	0.664	0.790	**0.909**	**0.930**	**0.916**	**0.965**	1.000	**0.923**	**0.902**	0.804	**0.881**	0.825	0.727	0.615	0.657	0.399	0.727	0.567	0.662	0.685	0.713	0.727	0.776	**0.867**
F19	0.762	0.783	**0.895**	0.818	0.629	0.531	0.615	0.580	0.524	0.706	**0.888**	**0.867**	**0.909**	**0.902**	**0.923**	1.000	**0.923**	0.769	0.832	0.846	0.832	0.706	0.685	0.545	0.671	0.546	0.694	0.776	0.825	0.664	0.608	**0.881**
Fo2	0.629	0.629	**0.853**	0.734	0.538	0.413	0.469	0.587	0.692	0.790	**0.909**	**0.874**	**0.902**	0.839	**0.902**	**0.923**	1.000	**0.881**	0.797	**0.895**	0.825	0.783	0.804	0.671	0.839	0.799	0.779	0.706	0.741	0.811	0.741	0.811
F9	0.455	0.441	0.720	0.678	0.455	0.294	0.322	0.399	0.692	0.678	0.755	0.713	0.706	0.734	0.804	0.769	**0.881**	1.000	**0.853**	**0.881**	0.790	0.741	0.846	0.552	0.734	0.753	0.669	0.441	0.573	0.552	0.720	0.664
Tp5	0.573	0.566	0.769	0.748	0.685	0.406	0.469	0.350	0.552	0.608	0.762	0.748	0.734	**0.867**	**0.881**	0.832	0.797	**0.853**	1.000	0.811	0.811	0.713	0.664	0.406	0.650	0.522	0.662	0.566	0.692	0.517	0.692	0.825
Fo26	0.399	0.455	0.657	0.622	0.580	0.413	0.476	0.336	0.476	0.504	0.832	**0.853**	**0.867**	0.804	0.825	0.846	**0.895**	**0.881**	0.811	1.000	**0.909**	**0.895**	**0.937**	0.685	0.811	0.694	0.747	0.692	0.818	0.622	0.727	0.671
Pz3	0.371	0.399	0.594	0.476	0.552	0.280	0.510	0.196	0.294	0.441	0.769	0.727	0.776	0.748	0.727	0.832	0.825	0.790	0.811	**0.909**	1.000	**0.930**	0.846	0.699	0.755	0.588	0.747	0.755	**0.874**	0.510	0.559	0.720
Pz7	0.147	0.196	0.462	0.322	0.441	0.140	0.392	0.056	0.301	0.336	0.727	0.685	0.727	0.629	0.615	0.706	0.783	0.741	0.713	**0.895**	**0.930**	1.000	**0.888**	0.804	0.790	0.690	0.755	0.713	**0.853**	0.594	0.629	0.629
Py12	0.182	0.217	0.483	0.406	0.371	0.168	0.252	0.224	0.448	0.434	0.720	0.699	0.720	0.664	0.657	0.685	0.804	0.846	0.664	**0.937**	0.846	**0.888**	1.000	0.664	0.734	0.662	0.726	0.615	0.727	0.531	0.650	0.552
S4	0.091	0.168	0.357	0.168	0.189	0.161	0.497	0.021	0.154	0.217	0.601	0.510	0.580	0.378	0.399	0.545	0.671	0.552	0.406	0.685	0.699	0.804	0.664	1.000	0.776	0.781	0.473	0.476	0.601	0.650	0.469	0.371
F6	0.371	0.399	0.594	0.462	0.608	0.455	0.531	0.301	0.441	0.580	0.818	0.790	0.783	0.664	0.727	0.671	0.839	0.734	0.650	0.811	0.755	0.790	0.734	0.776	1.000	0.844	0.623	0.545	0.636	0.818	0.741	0.566
Ox1	0.207	0.207	0.508	0.382	0.228	0.137	0.214	0.333	0.662	0.550	0.634	0.613	0.627	0.441	0.567	0.546	0.799	0.753	0.522	0.694	0.588	0.690	0.662	0.781	0.844	1.000	0.640	0.382	0.410	0.795	0.715	0.424
Ox2	0.288	0.256	0.548	0.431	0.260	-0.050	0.000	0.537	0.634	0.513	0.566	0.623	0.676	0.651	0.662	0.694	0.779	0.669	0.662	0.747	0.747	0.755	0.726	0.473	0.623	0.640	1.000	0.758	0.619	0.630	0.534	0.619
S3	0.371	0.385	0.497	0.371	0.504	0.210	0.322	0.350	0.329	0.427	0.734	0.769	0.839	0.762	0.685	0.776	0.706	0.441	0.566	0.692	0.755	0.713	0.615	0.476	0.545	0.382	0.758	1.000	**0.881**	0.643	0.559	0.769
S8	0.392	0.455	0.559	0.462	0.664	0.399	0.587	0.126	0.196	0.392	0.832	0.811	**0.853**	0.762	0.713	0.825	0.741	0.573	0.692	0.818	**0.874**	**0.853**	0.727	0.601	0.636	0.410	0.619	**0.881**	1.000	0.559	0.601	0.797
F10	0.441	0.441	0.636	0.476	0.455	0.336	0.350	0.504	0.643	0.678	0.804	0.790	0.804	0.678	0.727	0.664	0.811	0.552	0.517	0.622	0.510	0.594	0.531	0.650	0.818	0.795	0.630	0.643	0.559	1.000	0.783	0.664
Tz3	0.280	0.287	0.510	0.504	0.636	0.378	0.280	0.231	0.678	0.580	0.811	**0.853**	0.804	0.734	0.776	0.608	0.741	0.720	0.692	0.727	0.559	0.629	0.650	0.469	0.741	0.715	0.534	0.559	0.601	0.783	1.000	0.664
F25	0.692	0.657	0.818	0.685	0.657	0.378	0.476	0.434	0.559	0.769	**0.874**	0.790	0.811	**0.888**	**0.867**	**0.881**	0.811	0.664	0.825	0.671	0.720	0.629	0.552	0.371	0.566	0.424	0.619	0.769	0.797	0.664	0.664	1.000
Fo8	0.734	0.713	0.748	0.615	0.720	0.594	0.629	0.524	0.434	0.748	0.846	0.818	0.839	0.776	0.811	0.804	0.783	0.524	0.615	0.587	0.643	0.497	0.399	0.462	0.734	0.532	0.516	0.713	0.664	0.755	0.601	0.783
Fo22	0.762	0.776	0.832	0.832	0.846	0.755	0.601	0.573	0.587	0.769	**0.888**	**0.916**	**0.867**	**0.895**	**0.944**	0.797	0.790	0.650	0.755	0.692	0.545	0.469	0.504	0.315	0.720	0.497	0.473	0.566	0.615	0.755	0.783	0.783
Pd1	0.636	0.706	0.790	0.783	0.559	0.566	0.678	0.392	0.301	0.483	0.720	0.692	0.713	0.727	0.769	**0.867**	0.790	0.790	0.797	**0.860**	0.818	0.706	0.727	0.552	0.615	0.462	0.534	0.490	0.713	0.385	0.420	0.636
Pz1	0.448	0.490	0.692	0.594	0.301	0.294	0.469	0.371	0.378	0.497	0.657	0.573	0.615	0.594	0.643	0.762	0.818	0.846	0.685	**0.860**	0.818	0.783	0.846	0.699	0.685	0.637	0.623	0.427	0.615	0.441	0.392	0.531
Pz2	0.455	0.517	0.664	0.643	0.287	0.343	0.455	0.371	0.308	0.399	0.545	0.504	0.531	0.524	0.587	0.706	0.713	0.776	0.643	0.804	0.727	0.678	0.769	0.552	0.545	0.494	0.541	0.322	0.545	0.280	0.280	0.434
Pz12	0.476	0.517	0.699	0.692	0.315	0.329	0.420	0.392	0.427	0.469	0.580	0.545	0.566	0.573	0.650	0.741	0.762	**0.867**	0.727	0.832	0.755	0.685	0.783	0.538	0.566	0.564	0.577	0.329	0.538	0.308	0.378	0.490
Fo14	0.601	0.622	0.769	0.720	0.336	0.315	0.455	0.531	0.441	0.517	0.629	0.608	0.671	0.699	0.734	**0.874**	0.818	0.811	0.769	0.818	0.825	0.678	0.713	0.545	0.545	0.525	0.691	0.552	0.629	0.392	0.357	0.629
F11	0.517	0.559	0.734	0.699	0.252	0.329	0.378	0.594	0.552	0.538	0.650	0.657	0.706	0.636	0.713	0.811	**0.860**	0.832	0.643	**0.853**	0.727	0.671	0.790	0.636	0.636	0.690	0.687	0.490	0.552	0.531	0.462	0.510
Py2	0.545	0.580	0.762	0.734	0.231	0.266	0.350	0.573	0.517	0.504	0.559	0.559	0.608	0.587	0.671	0.797	0.797	0.790	0.692	0.790	0.713	0.650	0.706	0.531	0.524	0.585	0.701	0.441	0.538	0.413	0.343	0.531
Tp3	0.510	0.538	0.741	0.706	0.231	0.238	0.315	0.587	0.545	0.504	0.573	0.580	0.629	0.636	0.699	0.797	0.818	0.825	0.720	0.825	0.741	0.678	0.755	0.559	0.573	0.620	0.747	0.469	0.531	0.455	0.392	0.531
Py4	0.573	0.566	0.769	0.685	0.287	0.252	0.385	0.510	0.469	0.559	0.545	0.462	0.490	0.643	0.678	0.727	0.741	0.811	0.783	0.713	0.699	0.601	0.650	0.483	0.566	0.518	0.612	0.308	0.427	0.385	0.315	0.566

Py13	0.406	0.406	0.594	0.524	0.063	0.056	0.210	0.469	0.392	0.413	0.427	0.385	0.462	0.483	0.517	0.692	0.692	0.748	0.587	0.713	0.734	0.615	0.713	0.483	0.413	0.473	0.669	0.413	0.469	0.217	0.168	0.434
Py3	0.385	0.434	0.685	0.573	0.273	0.252	0.371	0.469	0.559	0.531	0.706	0.685	0.727	0.594	0.678	0.755	**0.888**	0.797	0.629	**0.860**	0.741	0.811	0.818	0.804	0.797	0.848	0.751	0.531	0.615	0.727	0.580	0.538
Tp4	0.455	0.531	0.713	0.671	0.259	0.287	0.399	0.476	0.483	0.420	0.622	0.636	0.685	0.587	0.657	0.783	0.790	0.685	0.636	0.790	0.664	0.713	0.692	0.650	0.566	0.630	0.683	0.531	0.636	0.580	0.462	0.559
Fo7	0.727	0.783	**0.860**	0.811	0.580	0.573	0.699	0.490	0.371	0.580	0.811	0.776	0.818	0.811	0.839	**0.958**	**0.860**	0.762	0.797	**0.853**	0.832	0.706	0.699	0.580	0.629	0.487	0.591	0.643	0.790	0.517	0.476	0.762
Fo16	0.699	0.741	0.811	0.769	0.371	0.455	0.552	0.608	0.434	0.559	0.699	0.692	0.762	0.706	0.755	**0.916**	0.832	0.713	0.657	0.776	0.741	0.594	0.636	0.552	0.524	0.508	0.605	0.615	0.664	0.483	0.378	0.650
Fo17	0.573	0.629	0.734	0.748	0.622	0.608	0.629	0.392	0.385	0.531	0.769	0.762	0.755	0.776	0.818	0.818	0.818	**0.874**	0.804	**0.909**	0.811	0.699	0.804	0.559	0.734	0.560	0.523	0.455	0.650	0.455	0.573	0.587
Py6	0.629	0.671	0.804	0.755	0.657	0.552	0.657	0.399	0.427	0.608	**0.874**	0.839	**0.860**	**0.860**	**0.881**	**0.937**	**0.902**	**0.874**	**0.867**	**0.937**	**0.909**	0.797	0.818	0.622	0.748	0.585	0.630	0.657	0.818	0.559	0.629	0.776
F29	0.657	0.692	0.699	0.671	0.154	0.371	0.469	0.524	0.182	0.413	0.350	0.280	0.329	0.364	0.441	0.622	0.524	0.504	0.434	0.483	0.476	0.322	0.399	0.287	0.252	0.207	0.327	0.168	0.329	0.084	-0.084	0.350
Po4	0.629	0.685	0.650	0.671	0.182	0.455	0.483	0.580	0.147	0.322	0.287	0.287	0.322	0.357	0.441	0.566	0.483	0.455	0.406	0.476	0.420	0.266	0.350	0.287	0.308	0.228	0.324	0.112	0.231	0.112	-0.084	0.217
F33	0.678	0.713	0.769	0.790	0.238	0.385	0.434	0.580	0.343	0.448	0.420	0.399	0.434	0.517	0.587	0.713	0.608	0.629	0.615	0.594	0.538	0.392	0.476	0.280	0.294	0.277	0.448	0.245	0.385	0.168	0.098	0.455
Fo21	0.776	0.783	0.818	0.790	0.224	0.420	0.448	0.692	0.385	0.587	0.462	0.406	0.448	0.510	0.594	0.720	0.643	0.608	0.531	0.538	0.497	0.315	0.434	0.273	0.329	0.305	0.416	0.245	0.336	0.231	0.070	0.469
F36	0.790	0.818	0.832	0.776	0.301	0.490	0.587	0.587	0.266	0.545	0.483	0.392	0.427	0.497	0.573	0.706	0.608	0.538	0.545	0.504	0.490	0.343	0.371	0.329	0.364	0.273	0.342	0.217	0.378	0.259	0.049	0.497
Fo6	0.755	0.790	0.783	0.783	0.252	0.483	0.510	0.650	0.280	0.483	0.406	0.378	0.413	0.462	0.552	0.678	0.580	0.531	0.504	0.510	0.462	0.294	0.371	0.273	0.322	0.263	0.370	0.196	0.315	0.196	0.014	0.392
F15	0.545	0.559	0.664	0.650	0.147	0.140	0.259	0.510	0.308	0.350	0.329	0.322	0.385	0.483	0.510	0.685	0.552	0.552	0.615	0.559	0.594	0.462	0.469	0.231	0.196	0.200	0.598	0.385	0.455	0.105	0.028	0.490
Ox3	0.636	0.643	0.741	0.678	0.175	0.217	0.357	0.580	0.322	0.462	0.420	0.371	0.441	0.524	0.559	0.741	0.636	0.601	0.608	0.594	0.636	0.483	0.510	0.329	0.308	0.280	0.591	0.399	0.469	0.196	0.042	0.524
F23	0.608	0.657	0.741	0.713	0.399	0.357	0.504	0.490	0.287	0.399	0.566	0.580	0.643	0.664	0.685	**0.853**	0.720	0.643	0.734	0.755	0.790	0.657	0.608	0.455	0.455	0.357	0.680	0.601	0.692	0.322	0.245	0.622
F24	0.510	0.594	0.657	0.720	0.343	0.378	0.434	0.448	0.266	0.259	0.448	0.517	0.552	0.566	0.608	0.734	0.608	0.587	0.664	0.713	0.643	0.566	0.566	0.364	0.357	0.298	0.584	0.441	0.573	0.231	0.224	0.462
F14	0.315	0.406	0.531	0.504	0.252	0.364	0.504	0.210	0.182	0.238	0.385	0.357	0.357	0.259	0.392	0.469	0.545	0.559	0.483	0.594	0.510	0.573	0.497	0.622	0.601	0.585	0.374	0.070	0.343	0.343	0.224	0.210
Py8	0.406	0.455	0.615	0.531	0.133	0.203	0.280	0.552	0.413	0.406	0.427	0.455	0.504	0.336	0.469	0.594	0.678	0.517	0.413	0.608	0.531	0.580	0.524	0.580	0.580	0.648	0.683	0.378	0.420	0.524	0.231	0.329
F17	0.287	0.364	0.517	0.573	0.126	0.189	0.168	0.434	0.364	0.224	0.343	0.406	0.420	0.434	0.483	0.538	0.552	0.601	0.517	0.685	0.497	0.538	0.664	0.364	0.364	0.382	0.580	0.266	0.385	0.252	0.245	0.273
Pd4	0.378	0.483	0.545	0.559	0.168	0.385	0.427	0.413	0.210	0.259	0.434	0.455	0.490	0.357	0.448	0.587	0.608	0.573	0.378	0.699	0.545	0.545	0.657	0.552	0.483	0.466	0.445	0.266	0.441	0.287	0.175	0.217
Fo4	0.273	0.343	0.420	0.399	0.259	0.119	0.322	0.231	0.077	0.049	0.294	0.378	0.448	0.385	0.399	0.587	0.455	0.322	0.531	0.538	0.636	0.608	0.378	0.406	0.315	0.263	0.655	0.566	0.608	0.231	0.112	0.406
F26	0.357	0.406	0.476	0.573	0.189	0.196	0.147	0.462	0.273	0.161	0.154	0.294	0.308	0.287	0.392	0.469	0.406	0.406	0.476	0.483	0.427	0.371	0.343	0.133	0.231	0.249	0.580	0.238	0.294	0.084	0.049	0.203
Fo15	0.273	0.378	0.385	0.552	0.413	0.399	0.336	0.231	0.126	0.056	0.329	0.497	0.497	0.406	0.469	0.545	0.441	0.483	0.517	0.671	0.580	0.510	0.538	0.224	0.301	0.214	0.481	0.385	0.538	0.049	0.238	0.245
Pz11	0.455	0.552	0.573	0.629	0.371	0.462	0.552	0.266	0.070	0.210	0.441	0.448	0.469	0.469	0.510	0.650	0.545	0.601	0.580	0.713	0.664	0.566	0.622	0.399	0.385	0.242	0.392	0.294	0.552	0.077	0.133	0.350
Py11	0.552	0.643	0.629	0.615	0.497	0.566	0.762	0.210	-0.035	0.259	0.517	0.469	0.497	0.504	0.538	0.692	0.566	0.559	0.622	0.671	0.720	0.587	0.524	0.504	0.497	0.270	0.331	0.322	0.601	0.161	0.126	0.434
Pz5	0.441	0.545	0.448	0.483	0.273	0.476	0.671	0.168	-0.189	0.042	0.301	0.287	0.343	0.322	0.343	0.559	0.385	0.392	0.406	0.517	0.573	0.399	0.378	0.420	0.266	0.116	0.178	0.217	0.441	-0.049	-0.091	0.210
Pd7	0.357	0.490	0.427	0.476	0.371	0.483	0.671	0.042	-0.203	-0.028	0.280	0.294	0.315	0.252	0.308	0.483	0.329	0.301	0.434	0.483	0.510	0.462	0.308	0.406	0.301	0.137	0.199	0.182	0.483	0.021	-0.021	0.217
F35	0.685	0.664	0.727	0.657	0.091	0.343	0.308	0.650	0.441	0.636	0.308	0.210	0.210	0.217	0.385	0.427	0.483	0.420	0.287	0.245	0.154	0.091	0.168	0.182	0.308	0.371	0.221	-0.084	0.021	0.294	0.014	0.280
Py9	0.420	0.469	0.524	0.545	-0.112	0.182	0.280	0.413	0.210	0.210	0.112	0.077	0.119	0.112	0.238	0.406	0.378	0.441	0.308	0.350	0.280	0.217	0.273	0.308	0.147	0.298	0.235	-0.098	0.070	0.014	-0.133	0.091
Po8	0.608	0.678	0.510	0.594	0.420	0.552	0.524	0.231	-0.098	0.203	0.280	0.252	0.238	0.350	0.343	0.427	0.161	0.091	0.308	0.196	0.161	0.042	0.063	-0.161	-0.098	-0.336	-0.053	0.147	0.364	-0.105	-0.091	0.385
F16	0.720	0.762	0.713	0.839	0.448	0.552	0.462	0.490	0.245	0.385	0.350	0.385	0.364	0.497	0.566	0.601	0.413	0.448	0.594	0.434	0.357	0.189	0.252	-0.056	0.119	-0.004	0.253	0.154	0.336	0.014	0.084	0.441
F32	0.797	0.832	0.713	0.825	0.427	0.629	0.462	0.615	0.224	0.434	0.301	0.343	0.315	0.420	0.510	0.517	0.343	0.280	0.420	0.280	0.175	0.007	0.084	-0.147	0.091	-0.060	0.157	0.084	0.203	0.063	-0.007	0.357
Pd6	0.063	0.168	0.196	0.056	0.077	0.077	0.315	0.126	-0.189	-0.084	0.231	0.238	0.315	0.189	0.147	0.329	0.301	0.028	0.119	0.378	0.413	0.531	0.350	0.552	0.378	0.210	0.452	0.462	0.483	0.350	-0.035	0.161
F5	0.049	0.147	0.231	0.189	0.413	0.210	0.392	-0.021	-0.049	-0.028	0.357	0.448	0.476	0.287	0.322	0.427	0.420	0.273	0.420	0.566	0.636	0.713	0.441	0.559	0.566	0.413	0.573	0.524	0.636	0.357	0.273	0.273
Tp1	0.021	0.035	0.252	0.091	0.168	-0.070	0.182	0.077	0.140	0.133	0.259	0.259	0.301	0.182	0.245	0.329	0.448	0.350	0.406	0.448	0.580	0.664	0.385	0.608	0.622	0.599	0.648	0.357	0.392	0.427	0.210	0.238
Pz8	0.140	0.175	0.462	0.455	0.238	0.168	0.196	0.119	0.504	0.406	0.497	0.455	0.420	0.329	0.462	0.441	0.636	0.811	0.566	0.713	0.552	0.664	0.748	0.538	0.615	0.718	0.456	0.105	0.378	0.371	0.545	0.315
Pd9	0.133	0.189	0.315	0.392	0.336	0.301	0.308	0.049	0.140	0.161	0.308	0.336	0.301	0.266	0.364	0.364	0.448	0.678	0.517	0.664	0.601	0.559	0.650	0.371	0.517	0.431	0.356	0.042	0.322	0.021	0.252	0.105
Fo13	0.161	0.308	0.322	0.350	0.531	0.587	0.706	-0.133	-0.077	0.077	0.538	0.545	0.524	0.301	0.385	0.448	0.483	0.476	0.399	0.671	0.587	0.650	0.573	0.671	0.657	0.487	0.178	0.238	0.587	0.315	0.406	0.210
Fo18	0.378	0.524	0.455	0.517	0.504	0.622	0.720	0.091	-0.126	0.091	0.504	0.531	0.545	0.420	0.455	0.601	0.483	0.441	0.441	0.685	0.629	0.573	0.566	0.483	0.441	0.214	0.246	0.364	0.657	0.140	0.189	0.301
Fo24	0.294	0.427	0.476	0.483	0.580	0.566	0.699	0.000	0.000	0.189	0.636	0.622	0.615	0.497	0.531	0.615	0.594	0.587	0.566	0.797	0.727	0.755	0.720	0.629	0.643	0.413	0.345	0.399	0.734	0.322	0.413	0.406
F21	0.133	0.224	0.266	0.350	0.301	0.231	0.448	-0.133	-0.112	-0.154	0.140	0.182	0.189	0.238	0.266	0.364	0.238	0.378	0.580	0.441	0.517	0.469	0.266	0.343	0.245	0.179	0.249	0.098	0.357	-0.063	0.084	0.182
F28	0.322	0.378	0.392	0.490	0.063	0.161	0.336	0.098	0.021	-0.021	0.070	0.063	0.098	0.224	0.252	0.413	0.224	0.392	0.504	0.329	0.371	0.238	0.189	0.154	-0.042	0.025	0.149	0.000	0.210	-0.210	-0.091	0.210
F7	0.035	0.049	0.119	0.161	0.112	0.000	0.070	0.126	0.049	0.007	0.175	0.259	0.315	0.294	0.273	0.392	0.357	0.483	0.371	0.559	0.643	0.476	0.566	0.266	0.259	0.214	0.498	0.385	0.378	-0.091	0.077	0.119
Pz6	0.315	0.364	0.497	0.504	0.413	0.357	0.434	0.217	0.238	0.336	0.573	0.566	0.580	0.566	0.594	0.657	0.685	0.818	0.657	**0.860**	0.825	0.720	0.846	0.538	0.622	0.494	0.527	0.392	0.587	0.224	0.392	0.392
Po2	0.497	0.517	0.531	0.552	0.378	0.357	0.448	0.413	0.147	0.266	0.392	0.441	0.490	0.573	0.573	0.671	0.552	0.587	0.643	0.636	0.720	0.476	0.490	0.336	0.420	0.270	0.527	0.427	0.455	0.126	0.140	0.385
Pz10	0.252	0.308	0.350	0.420	0.329	0.287	0.336	0.203	0.056	0.119	0.336	0.406	0.434	0.427	0.441	0.545	0.483	0.601	0.531	0.706	0.727	0.566	0.657	0.329	0.392	0.249	0.491	0.364	0.497	-0.021	0.140	0.231
F27	0.406	0.441	0.441	0.559	0.182	0.427	0.280	0.322	0.385	0.280	0.126	0.182	0.119	0.049	0.259	0.168	0.210	0.217	0.231	0.056	-0.133	-0.105	-0.161	0.070	0.182	0.361	-0.053	-0.294	-0.161	0.259	0.224	0.056
Pn3	-0.140	-0.140	0.105	0.259	-0.028	-0.077	-0.252	0.035	0.524	0.119	0.014	0.105	0.028	-0.021	0.147	0.007	0.210	0.510	0.308	0.294	0.077	0.196	0.308	0.070	0.196	0.487	0.224	-0.273	-0.126	0.056	0.385	-0.077
F31	-0.112	0.028	-0.112	0.042	-0.189	0.175	0.161	-0.035	-0.364	-0.420	-0.266	-0.154	-0.140	-0.322	-0.231	-0.084	-0.119	-0.070	-0.224	0.098	-0.014	0.007	0.070	0.133	-0.070	-0.046	-0.110	-0.266	-0.091	-0.336	-0.385	-0.490

Pn2	0.196	0.280	0.168	0.238	-0.112	0.231	0.203	0.252	-0.224	-0.084	-0.091	-0.077	-0.056	-0.063	-0.021	0.112	0.056	0.098	-0.063	0.196	0.105	0.035	0.224	0.056	-0.014	-0.116	0.011	-0.168	-0.021	-0.280	-0.413	-0.238
Pd8	0.203	0.266	0.098	0.161	-0.049	0.210	0.238	0.126	-0.399	-0.161	-0.210	-0.203	-0.182	-0.168	-0.126	0.035	-0.091	-0.056	-0.084	0.035	0.084	-0.049	0.014	-0.091	-0.126	-0.287	-0.068	-0.203	-0.042	-0.455	-0.580	-0.252
F20	0.049	0.175	0.084	0.084	0.014	0.357	0.469	-0.147	-0.280	-0.126	0.014	-0.035	-0.049	-0.294	-0.147	-0.042	0.014	-0.056	-0.175	0.028	-0.056	0.070	-0.042	0.385	0.210	0.214	-0.274	-0.322	-0.021	0.049	-0.147	-0.252
Pz4	-0.168	-0.042	-0.028	-0.049	-0.189	-0.007	0.266	-0.147	-0.238	-0.343	-0.063	-0.021	0.049	-0.231	-0.140	0.091	0.112	0.028	-0.042	0.203	0.231	0.343	0.119	0.601	0.217	0.361	0.142	0.021	0.154	0.077	-0.133	-0.217
F30	0.056	0.154	0.273	0.329	0.056	0.175	0.301	-0.063	0.077	-0.028	0.098	0.091	0.070	-0.028	0.119	0.189	0.252	0.385	0.322	0.357	0.259	0.378	0.280	0.420	0.294	0.410	0.135	-0.203	0.126	0.063	0.098	-0.014
Tz2	-0.021	0.084	0.091	0.210	-0.042	0.147	0.189	0.000	-0.112	-0.210	-0.112	-0.021	-0.021	-0.182	-0.035	0.084	0.105	0.217	0.105	0.280	0.217	0.245	0.210	0.259	0.154	0.231	0.139	-0.196	0.035	-0.182	-0.168	-0.266
Pd5	0.119	-0.021	0.287	0.210	-0.280	-0.427	-0.392	0.301	0.657	0.441	0.070	-0.035	-0.014	0.189	0.217	0.189	0.308	0.538	0.378	0.154	0.168	0.112	0.252	0.007	0.021	0.333	0.352	-0.042	-0.126	0.084	0.189	0.280
Fo11	0.182	0.112	0.329	0.119	0.476	0.070	0.294	-0.112	0.308	0.517	0.636	0.483	0.490	0.455	0.448	0.441	0.531	0.497	0.524	0.420	0.601	0.587	0.413	0.455	0.587	0.504	0.363	0.497	0.573	0.504	0.594	0.664
Py1	-0.224	-0.182	0.133	0.035	0.049	-0.105	0.126	-0.196	0.315	0.126	0.434	0.371	0.392	0.231	0.273	0.315	0.538	0.664	0.413	0.643	0.587	0.755	0.720	0.811	0.636	0.799	0.424	0.245	0.413	0.455	0.587	0.224
Tz1	0.084	0.007	0.329	0.161	0.021	-0.119	-0.098	0.350	0.713	0.566	0.441	0.399	0.413	0.287	0.392	0.315	0.622	0.594	0.322	0.427	0.364	0.448	0.469	0.559	0.678	**0.897**	0.562	0.259	0.140	0.713	0.601	0.301
F8	0.231	0.245	0.378	0.490	0.336	0.350	0.168	0.301	0.643	0.420	0.510	0.622	0.587	0.427	0.559	0.448	0.580	0.657	0.448	0.559	0.350	0.329	0.455	0.357	0.510	0.694	0.327	0.238	0.252	0.490	0.741	0.287
Py7	0.112	0.147	0.350	0.322	0.224	0.238	0.336	-0.056	0.455	0.357	0.538	0.462	0.441	0.273	0.392	0.371	0.538	0.629	0.413	0.490	0.357	0.462	0.448	0.629	0.545	0.750	0.128	0.084	0.301	0.510	0.678	0.329
F1	0.070	0.014	-0.035	0.056	0.441	0.168	-0.063	0.042	-0.007	0.112	0.000	0.126	0.056	0.084	0.119	-0.028	-0.035	0.021	0.140	0.042	0.133	-0.007	-0.007	-0.385	0.105	-0.137	0.164	0.098	0.063	-0.168	0.042	0.021
Pd2	-0.524	-0.517	-0.406	-0.336	-0.133	-0.343	-0.224	-0.434	-0.161	-0.399	-0.175	-0.105	-0.084	-0.105	-0.154	-0.112	-0.063	0.224	0.105	0.224	0.329	0.294	0.315	0.161	0.035	0.105	0.153	0.028	0.063	-0.322	0.035	-0.231
F3	0.119	0.091	0.203	0.126	0.140	-0.154	-0.112	0.175	0.210	0.259	0.336	0.350	0.413	0.336	0.280	0.427	0.357	0.231	0.231	0.385	0.476	0.441	0.462	0.042	0.063	0.025	0.559	0.699	0.615	0.119	0.168	0.483
Fo9	0.238	0.203	0.252	0.301	-0.028	-0.007	-0.210	0.664	0.392	0.245	0.140	0.280	0.322	0.420	0.378	0.350	0.350	0.336	0.224	0.378	0.273	0.126	0.399	-0.021	0.140	0.133	0.552	0.371	0.091	0.154	0.105	0.126
Fo25	-0.070	0.056	0.028	0.112	0.308	0.371	0.287	-0.056	-0.168	-0.112	0.287	0.350	0.315	0.280	0.224	0.175	0.210	0.266	0.147	0.524	0.329	0.399	0.594	0.294	0.371	0.088	0.121	0.168	0.343	0.091	0.224	-0.035
Pz9	-0.112	-0.112	0.084	-0.126	-0.063	-0.133	-0.028	0.077	0.126	0.231	0.336	0.203	0.231	0.210	0.140	0.147	0.357	0.308	0.042	0.392	0.322	0.469	0.622	0.483	0.469	0.361	0.331	0.252	0.259	0.392	0.217	0.140
Tp6	0.077	0.084	0.091	0.189	0.098	0.224	-0.168	0.517	0.406	0.252	0.168	0.364	0.329	0.147	0.245	0.091	0.273	0.189	-0.084	0.266	0.000	0.014	0.280	0.028	0.336	0.361	0.306	0.147	-0.049	0.343	0.308	-0.133
S6	-0.629	-0.650	**-0.734**	-0.643	**-0.776**	-0.566	-0.580	-0.392	-0.448	-0.671	**-0.937**	**-0.909**	**-0.909**	**-0.909**	**-0.874**	**-0.839**	**-0.783**	-0.566	**-0.713**	**-0.713**	-0.664	-0.629	-0.587	-0.427	-0.657	-0.413	-0.537	**-0.811**	**-0.846**	**-0.762**	**-0.755**	**-0.909**
F4	-0.629	-0.650	-0.657	-0.517	-0.238	-0.273	-0.406	-0.566	-0.280	-0.483	-0.608	-0.552	-0.650	-0.643	-0.580	**-0.762**	-0.608	-0.294	-0.378	-0.462	-0.476	-0.392	-0.378	-0.364	-0.259	-0.175	-0.481	**-0.741**	-0.643	-0.517	-0.224	-0.699
Po5	-0.357	-0.329	-0.497	-0.322	-0.182	0.084	-0.147	-0.406	-0.315	-0.378	-0.517	-0.483	-0.594	-0.657	-0.552	**-0.727**	-0.629	-0.455	-0.566	-0.587	**-0.734**	-0.643	-0.566	-0.413	-0.343	-0.277	**-0.790**	**-0.853**	**-0.706**	-0.427	-0.266	**-0.706**
F22	-0.322	-0.308	-0.406	-0.287	-0.531	-0.280	-0.350	-0.182	-0.392	-0.469	**-0.741**	**-0.713**	**-0.734**	**-0.727**	-0.643	-0.587	-0.573	-0.378	-0.504	-0.469	-0.476	-0.462	-0.378	-0.420	-0.517	-0.403	-0.388	**-0.734**	-0.629	**-0.734**	**-0.748**	**-0.734**
Fo20	-0.147	-0.049	-0.189	-0.098	-0.343	0.014	0.063	-0.119	-0.483	-0.441	-0.448	-0.427	-0.413	-0.504	-0.427	-0.266	-0.301	-0.217	-0.350	-0.140	-0.168	-0.154	-0.112	-0.021	-0.238	-0.224	-0.263	-0.476	-0.280	-0.517	-0.636	-0.573
Py10	-0.168	-0.063	-0.238	-0.161	-0.301	0.021	0.147	-0.280	-0.629	-0.524	-0.476	-0.490	-0.476	-0.538	-0.490	-0.308	-0.399	-0.322	-0.357	-0.231	-0.196	-0.182	-0.224	-0.049	-0.315	-0.347	-0.384	-0.497	-0.252	-0.587	-0.692	-0.545
S1	0.014	0.119	-0.154	-0.098	0.175	0.301	0.224	-0.091	-0.524	-0.266	-0.021	0.049	0.056	-0.084	-0.147	-0.049	-0.217	-0.448	-0.357	-0.077	-0.098	-0.091	-0.077	-0.175	-0.189	-0.487	-0.199	0.196	0.238	-0.189	-0.287	-0.119
F34	-0.280	-0.266	-0.392	-0.231	0.098	0.049	-0.175	-0.315	-0.357	-0.322	-0.420	-0.308	-0.406	-0.455	-0.378	-0.497	-0.476	-0.350	-0.301	-0.315	-0.315	-0.294	-0.301	-0.504	-0.224	-0.368	-0.299	-0.427	-0.301	-0.504	-0.308	-0.476
S2	0.014	0.049	-0.133	-0.021	-0.056	0.077	-0.049	0.000	-0.189	-0.210	-0.336	-0.203	-0.217	-0.434	-0.301	-0.238	-0.329	-0.497	-0.371	-0.406	-0.392	-0.371	-0.545	-0.350	-0.343	-0.252	-0.210	-0.175	-0.189	-0.224	-0.336	-0.273
F12	0.070	0.035	-0.021	-0.035	0.112	0.231	-0.077	0.266	0.259	0.336	0.210	0.252	0.210	0.021	0.084	-0.077	0.105	-0.049	-0.315	-0.049	-0.224	-0.203	0.007	-0.042	0.224	0.210	-0.093	0.049	-0.119	0.357	0.287	-0.070
Pn1	-0.098	-0.098	-0.161	-0.189	-0.203	0.063	-0.042	-0.014	0.021	-0.028	-0.182	-0.217	-0.266	-0.329	-0.259	-0.413	-0.259	-0.357	-0.427	-0.462	-0.615	-0.462	-0.483	-0.014	-0.021	0.098	-0.466	-0.483	-0.538	0.231	0.000	-0.336
Tp2	-0.140	-0.196	-0.203	-0.168	-0.007	-0.238	-0.308	0.014	0.119	-0.063	0.028	0.133	0.175	0.224	0.084	0.063	-0.035	-0.077	0.014	-0.014	0.049	-0.049	0.000	-0.203	-0.210	-0.172	0.164	0.448	0.147	0.014	0.196	0.175
S5	0.063	-0.060	0.137	0.123	0.109	-0.284	-0.392	0.270	0.592	0.361	0.221	0.305	0.322	0.371	0.347	0.273	0.298	0.305	0.350	0.200	0.259	0.179	0.200	-0.137	0.063	0.211	0.538	0.501	0.228	0.245	0.438	0.438
F13	0.594	0.594	0.462	0.441	0.448	0.441	0.378	0.259	0.056	0.441	0.427	0.364	0.364	0.357	0.350	0.427	0.231	0.028	0.161	0.119	0.147	0.028	0.049	-0.196	-0.049	-0.256	0.007	0.385	0.462	0.084	0.063	0.538
F18	0.217	0.259	0.140	0.217	-0.042	0.182	-0.098	0.455	0.154	0.126	0.112	0.203	0.210	0.182	0.140	0.126	0.056	-0.140	-0.210	0.035	-0.217	-0.189	0.077	-0.224	-0.189	-0.224	0.032	0.217	0.063	0.133	0.014	0.056
Po7	0.308	0.266	0.154	0.245	-0.007	0.112	-0.224	0.392	0.287	0.273	0.007	0.049	0.014	0.091	0.084	-0.014	-0.112	-0.238	-0.189	-0.280	-0.462	-0.483	-0.280	-0.587	-0.427	-0.382	-0.178	0.007	-0.147	0.007	0.007	0.147
	Fo8	**Fo22**	**Pd1**	**Pz1**	**Pz2**	**Pz12**	**Fo14**	**F11**	**Py2**	**Tp3**	**Py4**	**Py13**	**Py3**	**Tp4**	**Fo7**	**Fo16**	**Fo17**	**Py6**	**F29**	**Po4**	**F33**	**Fo21**	**F36**	**Fo6**	**F15**	**Ox3**	**F23**	**F24**	**F14**	**Py8**	**F17**	**Pd4**
Fo1	0.734	0.762	0.636	0.448	0.455	0.476	0.601	0.517	0.545	0.510	0.573	0.406	0.385	0.455	0.727	0.699	0.573	0.629	0.657	0.629	0.678	0.776	0.790	0.755	0.545	0.636	0.608	0.510	0.315	0.406	0.287	0.378
Fo5	0.713	0.776	0.706	0.490	0.517	0.517	0.622	0.559	0.580	0.538	0.566	0.406	0.434	0.531	0.783	0.741	0.629	0.671	0.692	0.685	0.713	0.783	0.818	0.790	0.559	0.643	0.657	0.594	0.406	0.455	0.364	0.483
Po1	0.748	0.832	0.790	0.692	0.664	0.699	0.769	0.734	0.762	0.741	0.769	0.594	0.685	0.713	**0.860**	0.811	0.734	0.804	0.699	0.650	0.769	0.818	0.832	0.783	0.664	0.741	0.741	0.657	0.531	0.615	0.517	0.545
Py5	0.615	0.832	0.783	0.594	0.643	0.692	0.720	0.699	0.734	0.706	0.685	0.524	0.573	0.671	0.811	0.769	0.748	0.755	0.671	0.671	0.790	0.790	0.776	0.783	0.650	0.678	0.713	0.720	0.504	0.531	0.573	0.559
Pd3	0.720	0.846	0.559	0.301	0.287	0.315	0.336	0.252	0.231	0.231	0.287	0.063	0.273	0.259	0.580	0.371	0.622	0.657	0.154	0.182	0.238	0.224	0.301	0.252	0.147	0.175	0.399	0.343	0.252	0.133	0.126	0.168
Po6	0.594	0.755	0.566	0.294	0.343	0.329	0.315	0.329	0.266	0.238	0.252	0.056	0.252	0.287	0.573	0.455	0.608	0.552	0.371	0.455	0.385	0.420	0.490	0.483	0.140	0.217	0.357	0.378	0.364	0.203	0.189	0.385
S7	0.629	0.601	0.678	0.469	0.455	0.420	0.455	0.378	0.350	0.315	0.385	0.210	0.371	0.399	0.699	0.552	0.629	0.657	0.469	0.483	0.434	0.448	0.587	0.510	0.259	0.357	0.504	0.434	0.504	0.280	0.168	0.427
Po9	0.524	0.573	0.392	0.371	0.371	0.392	0.531	0.594	0.573	0.587	0.510	0.469	0.469	0.476	0.490	0.608	0.392	0.399	0.524	0.580	0.580	0.692	0.587	0.650	0.510	0.580	0.490	0.448	0.210	0.552	0.434	0.413
Fo3	0.434	0.587	0.301	0.378	0.308	0.427	0.441	0.552	0.517	0.545	0.469	0.392	0.559	0.483	0.371	0.434	0.385	0.427	0.182	0.147	0.343	0.385	0.266	0.280	0.308	0.322	0.287	0.266	0.182	0.413	0.364	0.210
Fo12	0.748	0.769	0.483	0.497	0.399	0.469	0.517	0.538	0.504	0.504	0.559	0.413	0.531	0.420	0.580	0.559	0.531	0.608	0.413	0.322	0.448	0.587	0.545	0.483	0.350	0.462	0.399	0.259	0.238	0.406	0.224	0.259
Fo10	0.846	**0.888**	0.720	0.657	0.545	0.580	0.629	0.650	0.559	0.573	0.545	0.427	0.706	0.622	0.811	0.699	0.769	**0.874**	0.350	0.287	0.420	0.462	0.483	0.406	0.329	0.420	0.566	0.448	0.385	0.427	0.343	0.434
Fo19	0.818	**0.916**	0.692	0.573	0.504	0.545	0.608	0.657	0.559	0.580	0.462	0.385	0.685	0.636	0.776	0.692	0.762	0.839	0.280	0.287	0.399	0.406	0.392	0.378	0.322	0.371	0.580	0.517	0.357	0.455	0.406	0.455
Po3	0.839	**0.867**	0.713	0.615	0.531	0.566	0.671	0.706	0.608	0.629	0.490	0.462	0.727	0.685	0.818	0.762	0.755	**0.860**	0.329	0.322	0.434	0.448	0.427	0.413	0.385	0.441	0.643	0.552	0.357	0.504	0.420	0.490

F2	0.776	0.895	0.727	0.594	0.524	0.573	0.699	0.636	0.587	0.636	0.643	0.483	0.594	0.587	0.811	0.706	0.776	0.860	0.364	0.357	0.517	0.510	0.497	0.462	0.483	0.524	0.664	0.566	0.259	0.336	0.434	0.357
Fo23	0.811	0.944	0.769	0.643	0.587	0.650	0.734	0.713	0.671	0.699	0.678	0.517	0.678	0.657	0.839	0.755	0.818	0.881	0.441	0.441	0.587	0.594	0.573	0.552	0.510	0.559	0.685	0.608	0.392	0.469	0.483	0.448
F19	0.804	0.797	0.867	0.762	0.706	0.741	0.874	0.811	0.797	0.797	0.727	0.692	0.755	0.783	0.958	0.916	0.818	0.937	0.622	0.566	0.713	0.720	0.706	0.678	0.685	0.741	0.853	0.734	0.469	0.594	0.538	0.587
Fo2	0.783	0.790	0.790	0.818	0.713	0.762	0.818	0.860	0.797	0.818	0.741	0.692	0.888	0.790	0.860	0.832	0.818	0.902	0.524	0.483	0.608	0.643	0.608	0.580	0.552	0.636	0.720	0.608	0.545	0.678	0.552	0.608
F9	0.524	0.650	0.790	0.846	0.776	0.867	0.811	0.832	0.790	0.825	0.811	0.748	0.797	0.685	0.762	0.713	0.874	0.874	0.504	0.455	0.629	0.608	0.538	0.531	0.552	0.601	0.643	0.587	0.559	0.517	0.601	0.573
Tp5	0.615	0.755	0.797	0.685	0.643	0.727	0.769	0.643	0.692	0.720	0.783	0.587	0.629	0.636	0.797	0.657	0.804	0.867	0.434	0.406	0.615	0.531	0.545	0.504	0.615	0.608	0.734	0.664	0.483	0.413	0.517	0.378
Fo26	0.587	0.692	0.860	0.860	0.804	0.832	0.818	0.853	0.790	0.825	0.713	0.713	0.860	0.790	0.853	0.776	0.909	0.937	0.483	0.476	0.594	0.538	0.504	0.510	0.559	0.594	0.755	0.713	0.594	0.608	0.685	0.699
Pz3	0.643	0.545	0.818	0.818	0.727	0.755	0.825	0.727	0.713	0.741	0.699	0.734	0.741	0.664	0.832	0.741	0.811	0.909	0.476	0.420	0.538	0.497	0.490	0.462	0.594	0.636	0.790	0.643	0.510	0.531	0.497	0.545
Pz7	0.497	0.469	0.706	0.783	0.678	0.685	0.678	0.671	0.650	0.678	0.601	0.615	0.811	0.713	0.706	0.594	0.699	0.797	0.322	0.266	0.392	0.315	0.343	0.294	0.462	0.483	0.657	0.566	0.573	0.580	0.538	0.545
Py12	0.399	0.504	0.727	0.846	0.769	0.783	0.713	0.790	0.706	0.755	0.650	0.713	0.818	0.692	0.699	0.636	0.804	0.818	0.399	0.350	0.476	0.434	0.371	0.371	0.469	0.510	0.608	0.566	0.497	0.524	0.664	0.657
S4	0.462	0.315	0.552	0.699	0.552	0.538	0.545	0.636	0.531	0.559	0.483	0.483	0.804	0.650	0.580	0.552	0.559	0.622	0.287	0.287	0.280	0.273	0.329	0.273	0.231	0.329	0.455	0.364	0.622	0.580	0.364	0.552
F6	0.734	0.720	0.615	0.685	0.545	0.566	0.545	0.636	0.524	0.573	0.566	0.413	0.797	0.566	0.629	0.524	0.734	0.748	0.252	0.308	0.294	0.329	0.364	0.322	0.196	0.308	0.455	0.357	0.601	0.580	0.364	0.483
Ox1	0.532	0.497	0.462	0.637	0.494	0.564	0.525	0.690	0.585	0.620	0.518	0.473	0.848	0.630	0.487	0.508	0.560	0.585	0.207	0.228	0.277	0.305	0.273	0.263	0.200	0.280	0.357	0.298	0.585	0.648	0.382	0.466
Ox2	0.516	0.473	0.534	0.623	0.541	0.577	0.691	0.687	0.701	0.747	0.612	0.669	0.751	0.683	0.591	0.605	0.523	0.630	0.327	0.324	0.448	0.416	0.342	0.370	0.598	0.591	0.680	0.584	0.374	0.683	0.580	0.445
S3	0.713	0.566	0.490	0.427	0.322	0.329	0.552	0.490	0.441	0.469	0.308	0.413	0.531	0.531	0.643	0.615	0.455	0.657	0.168	0.112	0.245	0.245	0.217	0.196	0.385	0.399	0.601	0.441	0.070	0.378	0.266	0.266
S8	0.664	0.615	0.713	0.615	0.545	0.538	0.629	0.552	0.538	0.531	0.427	0.469	0.615	0.636	0.790	0.664	0.650	0.818	0.329	0.231	0.385	0.336	0.378	0.315	0.455	0.469	0.692	0.573	0.343	0.420	0.385	0.441
F10	0.755	0.755	0.385	0.441	0.280	0.308	0.392	0.531	0.413	0.455	0.385	0.217	0.727	0.580	0.517	0.483	0.455	0.559	0.084	0.112	0.168	0.231	0.259	0.196	0.105	0.196	0.322	0.231	0.343	0.524	0.252	0.287
Tz3	0.601	0.783	0.420	0.392	0.280	0.378	0.357	0.462	0.343	0.392	0.315	0.168	0.580	0.462	0.476	0.378	0.573	0.629	-0.084	-0.084	0.098	0.070	0.049	0.014	0.028	0.042	0.245	0.224	0.224	0.231	0.245	0.175
F25	0.783	0.783	0.636	0.531	0.434	0.490	0.629	0.510	0.531	0.531	0.566	0.434	0.538	0.559	0.762	0.650	0.587	0.776	0.350	0.217	0.455	0.469	0.497	0.392	0.490	0.524	0.622	0.462	0.210	0.329	0.273	0.217
Fo8	1.000	0.804	0.524	0.420	0.287	0.336	0.531	0.476	0.392	0.406	0.399	0.308	0.497	0.385	0.671	0.629	0.566	0.699	0.273	0.266	0.294	0.413	0.420	0.371	0.238	0.357	0.490	0.280	0.210	0.371	0.035	0.210
Fo22	0.804	1.000	0.636	0.476	0.427	0.469	0.510	0.538	0.476	0.497	0.510	0.252	0.559	0.524	0.706	0.580	0.713	0.748	0.301	0.336	0.427	0.455	0.483	0.441	0.287	0.350	0.490	0.448	0.350	0.371	0.350	0.343
Pd1	0.524	0.636	1.000	0.909	0.930	0.930	0.916	0.860	0.888	0.874	0.846	0.797	0.769	0.818	0.965	0.881	0.937	0.944	0.811	0.783	0.874	0.818	0.832	0.825	0.790	0.832	0.909	0.881	0.734	0.650	0.748	0.811
Pz1	0.420	0.476	0.909	1.000	0.965	0.958	0.895	0.916	0.909	0.916	0.888	0.888	0.888	0.825	0.860	0.818	0.881	0.874	0.783	0.734	0.804	0.783	0.769	0.755	0.734	0.811	0.811	0.762	0.776	0.734	0.776	0.853
Pz2	0.287	0.427	0.930	0.965	1.000	0.979	0.881	0.895	0.930	0.916	0.867	0.881	0.818	0.832	0.846	0.804	0.867	0.825	0.860	0.825	0.888	0.832	0.818	0.832	0.811	0.853	0.846	0.860	0.811	0.741	0.867	0.916
Pz12	0.336	0.469	0.930	0.958	0.979	1.000	0.916	0.916	0.944	0.937	0.888	0.902	0.818	0.818	0.853	0.825	0.895	0.860	0.818	0.776	0.881	0.825	0.783	0.804	0.804	0.839	0.839	0.839	0.769	0.699	0.825	0.853
Fo14	0.531	0.510	0.916	0.895	0.881	0.916	1.000	0.923	0.944	0.951	0.881	0.937	0.783	0.811	0.923	0.944	0.853	0.895	0.804	0.769	0.881	0.853	0.790	0.818	0.867	0.909	0.937	0.853	0.608	0.678	0.713	0.748
F11	0.476	0.538	0.860	0.916	0.895	0.916	0.923	1.000	0.951	0.965	0.818	0.888	0.909	0.881	0.860	0.916	0.853	0.846	0.748	0.748	0.818	0.811	0.727	0.776	0.734	0.797	0.818	0.797	0.678	0.776	0.797	0.867
Py2	0.392	0.476	0.888	0.909	0.930	0.944	0.944	0.951	1.000	0.986	0.874	0.916	0.867	0.916	0.867	0.895	0.797	0.811	0.839	0.811	0.916	0.867	0.818	0.853	0.888	0.909	0.909	0.902	0.748	0.832	0.860	0.860
Tp3	0.406	0.497	0.874	0.916	0.916	0.937	0.951	0.965	0.986	1.000	0.902	0.923	0.881	0.895	0.853	0.881	0.825	0.825	0.783	0.783	0.881	0.832	0.769	0.811	0.853	0.881	0.888	0.874	0.713	0.797	0.860	0.832
Py4	0.399	0.510	0.846	0.888	0.867	0.888	0.881	0.818	0.874	0.902	1.000	0.839	0.755	0.734	0.797	0.741	0.811	0.790	0.776	0.769	0.860	0.825	0.825	0.804	0.804	0.860	0.804	0.755	0.706	0.643	0.741	0.671
Py13	0.308	0.252	0.797	0.888	0.881	0.902	0.937	0.888	0.916	0.923	0.839	1.000	0.734	0.727	0.769	0.839	0.734	0.748	0.811	0.748	0.839	0.825	0.720	0.769	0.860	0.902	0.846	0.769	0.566	0.671	0.727	0.762
Py3	0.497	0.559	0.769	0.888	0.818	0.818	0.783	0.909	0.867	0.881	0.755	0.734	1.000	0.916	0.776	0.769	0.755	0.783	0.580	0.580	0.643	0.622	0.615	0.608	0.587	0.650	0.713	0.692	0.776	0.860	0.755	0.804
Tp4	0.385	0.524	0.818	0.825	0.832	0.818	0.811	0.881	0.916	0.895	0.734	0.727	0.916	1.000	0.832	0.825	0.699	0.755	0.678	0.657	0.776	0.692	0.706	0.706	0.755	0.755	0.832	0.867	0.762	0.853	0.853	0.832
Fo7	0.671	0.706	0.965	0.860	0.846	0.853	0.923	0.860	0.867	0.853	0.797	0.769	0.776	0.832	1.000	0.944	0.888	0.958	0.769	0.720	0.832	0.811	0.818	0.797	0.769	0.825	0.916	0.839	0.622	0.643	0.657	0.741
Fo16	0.629	0.580	0.881	0.818	0.804	0.825	0.944	0.916	0.895	0.881	0.741	0.839	0.769	0.825	0.944	1.000	0.804	0.874	0.790	0.748	0.839	0.853	0.790	0.818	0.783	0.846	0.895	0.811	0.538	0.678	0.636	0.762
Fo17	0.566	0.713	0.937	0.881	0.867	0.895	0.853	0.853	0.797	0.825	0.811	0.734	0.755	0.699	0.888	0.804	1.000	0.951	0.657	0.678	0.741	0.713	0.685	0.699	0.601	0.671	0.762	0.734	0.650	0.531	0.671	0.741
Py6	0.699	0.748	0.944	0.874	0.825	0.860	0.895	0.846	0.811	0.825	0.790	0.748	0.783	0.755	0.958	0.874	0.951	1.000	0.636	0.594	0.720	0.699	0.685	0.664	0.650	0.713	0.832	0.741	0.580	0.552	0.601	0.671
F29	0.273	0.301	0.811	0.783	0.860	0.818	0.804	0.748	0.839	0.783	0.776	0.811	0.580	0.678	0.769	0.790	0.657	0.636	1.000	0.944	0.951	0.965	0.958	0.972	0.867	0.930	0.818	0.797	0.678	0.685	0.699	0.818
Po4	0.266	0.336	0.783	0.734	0.825	0.776	0.769	0.748	0.811	0.783	0.769	0.748	0.580	0.657	0.720	0.748	0.678	0.594	0.944	1.000	0.923	0.923	0.916	0.972	0.804	0.867	0.790	0.811	0.727	0.713	0.734	0.839
F33	0.294	0.427	0.874	0.804	0.888	0.881	0.881	0.818	0.916	0.881	0.860	0.839	0.643	0.776	0.832	0.839	0.741	0.720	0.951	0.923	1.000	0.958	0.937	0.965	0.930	0.951	0.888	0.902	0.685	0.685	0.804	0.804
Fo21	0.413	0.455	0.818	0.783	0.832	0.825	0.853	0.811	0.867	0.832	0.825	0.825	0.622	0.692	0.811	0.853	0.713	0.699	0.965	0.923	0.958	1.000	0.958	0.979	0.853	0.930	0.818	0.776	0.608	0.671	0.678	0.769
F36	0.420	0.483	0.832	0.769	0.818	0.783	0.790	0.727	0.818	0.769	0.825	0.720	0.615	0.706	0.818	0.790	0.685	0.685	0.958	0.916	0.937	0.958	1.000	0.972	0.825	0.902	0.811	0.776	0.706	0.685	0.657	0.748
Fo6	0.371	0.441	0.825	0.755	0.832	0.804	0.818	0.776	0.853	0.811	0.804	0.769	0.608	0.706	0.797	0.818	0.699	0.664	0.972	0.972	0.965	0.979	0.972	1.000	0.853	0.916	0.832	0.825	0.692	0.713	0.713	0.804
F15	0.238	0.287	0.790	0.734	0.811	0.804	0.867	0.734	0.888	0.853	0.804	0.860	0.587	0.755	0.769	0.783	0.601	0.650	0.867	0.804	0.930	0.853	0.825	0.853	1.000	0.972	0.930	0.909	0.573	0.678	0.776	0.692
Ox3	0.357	0.350	0.832	0.811	0.853	0.839	0.909	0.797	0.909	0.881	0.860	0.902	0.650	0.755	0.825	0.846	0.671	0.713	0.930	0.867	0.951	0.930	0.902	0.916	0.972	1.000	0.930	0.867	0.608	0.713	0.741	0.741
F23	0.490	0.490	0.909	0.811	0.846	0.839	0.937	0.818	0.909	0.888	0.804	0.846	0.713	0.832	0.916	0.895	0.762	0.832	0.818	0.790	0.888	0.818	0.811	0.832	0.930	0.930	1.000	0.944	0.636	0.727	0.748	0.748
F24	0.280	0.448	0.881	0.762	0.860	0.839	0.853	0.797	0.902	0.874	0.755	0.769	0.692	0.867	0.839	0.811	0.734	0.741	0.797	0.811	0.902	0.776	0.776	0.825	0.909	0.867	0.944	1.000	0.706	0.734	0.881	0.818
F14	0.210	0.350	0.734	0.776	0.811	0.769	0.608	0.678	0.748	0.713	0.706	0.566	0.776	0.762	0.622	0.538	0.650	0.580	0.678	0.727	0.685	0.608	0.706	0.692	0.573	0.608	0.636	0.706	1.000	0.825	0.734	0.811
Py8	0.371	0.371	0.650	0.734	0.741	0.699	0.678	0.776	0.832	0.797	0.643	0.671	0.860	0.853	0.643	0.678	0.531	0.552	0.685	0.713	0.685	0.671	0.685	0.713	0.678	0.713	0.727	0.734	0.825	1.000	0.748	0.818
F17	0.035	0.350	0.748	0.776	0.867	0.825	0.713	0.797	0.860	0.860	0.741	0.727	0.755	0.853	0.657	0.636	0.671	0.601	0.699	0.734	0.804	0.678	0.657	0.713	0.776	0.741	0.748	0.881	0.734	0.748	1.000	0.867

Pd4	0.210	0.343	0.811	**0.853**	**0.916**	**0.853**	0.748	**0.867**	**0.860**	0.832	0.671	0.762	0.804	0.832	0.741	0.762	0.741	0.671	0.818	0.839	0.804	0.769	0.748	0.804	0.692	0.741	0.748	0.818	0.811	0.818	**0.867**	1.000
Fo4	0.301	0.231	0.622	0.517	0.566	0.538	0.657	0.524	0.678	0.643	0.504	0.573	0.552	0.713	0.629	0.601	0.413	0.524	0.517	0.531	0.594	0.455	0.504	0.531	0.762	0.685	0.839	0.818	0.573	0.706	0.601	0.538
F26	0.126	0.252	0.629	0.545	0.678	0.664	0.650	0.608	0.769	0.727	0.587	0.636	0.538	0.685	0.552	0.566	0.483	0.455	0.671	0.734	0.755	0.636	0.615	0.713	0.818	0.741	0.783	**0.867**	0.671	0.776	0.783	0.685
Fo15	0.182	0.336	0.734	0.580	0.713	0.706	0.664	0.636	0.706	0.671	0.455	0.608	0.510	0.650	0.650	0.629	0.657	0.622	0.573	0.615	0.664	0.524	0.476	0.587	0.685	0.608	0.769	**0.860**	0.566	0.580	0.734	0.727
Pz11	0.224	0.371	**0.923**	0.839	**0.937**	**0.895**	0.818	0.769	0.839	0.804	0.741	0.783	0.636	0.741	0.825	0.762	0.818	0.769	**0.860**	0.846	**0.881**	0.790	0.797	0.832	0.818	0.825	**0.867**	**0.909**	0.748	0.636	0.818	**0.881**
Py11	0.413	0.434	**0.923**	0.811	**0.860**	0.818	0.797	0.685	0.755	0.720	0.748	0.699	0.601	0.664	**0.853**	0.748	0.811	0.797	0.832	0.825	0.818	0.762	0.832	0.818	0.741	0.790	**0.853**	0.818	0.769	0.601	0.629	0.769
Pz5	0.259	0.203	0.811	0.692	0.769	0.727	0.755	0.643	0.685	0.650	0.608	0.699	0.455	0.559	0.748	0.741	0.692	0.657	0.818	0.832	0.783	0.734	0.748	0.790	0.706	0.748	0.790	0.769	0.622	0.490	0.552	0.748
Pd7	0.175	0.245	0.762	0.608	0.720	0.650	0.594	0.504	0.636	0.566	0.531	0.497	0.483	0.643	0.671	0.566	0.573	0.559	0.713	0.734	0.706	0.580	0.699	0.699	0.671	0.650	0.755	0.811	0.797	0.615	0.622	0.720
F35	0.294	0.378	0.517	0.566	0.601	0.580	0.497	0.559	0.636	0.573	0.636	0.497	0.531	0.531	0.497	0.517	0.420	0.378	0.783	0.748	0.720	0.811	0.839	0.811	0.559	0.657	0.469	0.469	0.657	0.685	0.497	0.601
Py9	0.007	0.098	0.664	0.692	0.790	0.769	0.692	0.706	0.811	0.755	0.713	0.734	0.580	0.692	0.580	0.650	0.510	0.441	**0.888**	**0.881**	**0.881**	0.839	0.832	**0.874**	0.783	0.804	0.678	0.748	0.769	0.713	0.734	0.797
Po8	0.126	0.364	0.552	0.294	0.448	0.371	0.343	0.224	0.371	0.280	0.322	0.224	0.098	0.378	0.538	0.413	0.350	0.378	0.629	0.538	0.622	0.566	0.664	0.615	0.566	0.538	0.531	0.594	0.294	0.210	0.427	0.413
F16	0.238	0.497	0.762	0.538	0.692	0.685	0.657	0.545	0.699	0.636	0.650	0.545	0.364	0.587	0.706	0.636	0.615	0.587	0.804	0.783	**0.881**	0.804	0.825	0.846	0.811	0.776	0.762	0.832	0.538	0.476	0.671	0.601
F32	0.280	0.517	0.629	0.406	0.559	0.524	0.510	0.448	0.580	0.510	0.531	0.392	0.287	0.490	0.601	0.552	0.490	0.448	0.769	0.783	0.797	0.783	0.818	0.839	0.685	0.685	0.636	0.706	0.483	0.483	0.566	0.552
Pd6	0.168	0.119	0.385	0.434	0.427	0.280	0.322	0.357	0.406	0.399	0.308	0.301	0.538	0.580	0.399	0.336	0.224	0.287	0.357	0.413	0.308	0.245	0.371	0.343	0.399	0.413	0.510	0.510	0.545	0.664	0.517	0.552
F5	0.343	0.266	0.490	0.441	0.441	0.392	0.406	0.371	0.448	0.427	0.294	0.315	0.552	0.559	0.462	0.357	0.385	0.455	0.238	0.301	0.266	0.147	0.245	0.252	0.385	0.343	0.580	0.566	0.622	0.657	0.434	0.469
Tp1	0.336	0.154	0.357	0.469	0.392	0.378	0.399	0.364	0.448	0.455	0.441	0.385	0.594	0.476	0.329	0.266	0.287	0.350	0.224	0.280	0.231	0.175	0.259	0.238	0.357	0.364	0.469	0.385	0.643	0.699	0.336	0.343
Pz8	0.140	0.378	0.643	0.776	0.769	0.804	0.552	0.685	0.692	0.678	0.629	0.580	0.769	0.671	0.524	0.448	0.678	0.615	0.455	0.399	0.517	0.448	0.441	0.427	0.420	0.434	0.448	0.517	0.762	0.608	0.678	0.671
Pd9	0.112	0.252	0.699	0.734	0.790	0.804	0.601	0.615	0.636	0.636	0.615	0.643	0.552	0.462	0.517	0.441	0.755	0.615	0.552	0.587	0.573	0.497	0.462	0.524	0.490	0.504	0.545	0.594	0.720	0.504	0.629	0.692
Fo13	0.315	0.406	0.678	0.629	0.643	0.601	0.434	0.517	0.476	0.441	0.350	0.329	0.608	0.559	0.587	0.455	0.678	0.629	0.392	0.420	0.371	0.301	0.392	0.378	0.231	0.273	0.462	0.510	0.755	0.510	0.462	0.685
Fo18	0.301	0.399	0.839	0.706	0.790	0.720	0.643	0.636	0.650	0.601	0.483	0.552	0.566	0.657	0.769	0.678	0.748	0.720	0.685	0.685	0.664	0.587	0.636	0.657	0.573	0.594	0.734	0.776	0.685	0.552	0.643	0.825
Fo24	0.343	0.497	0.839	0.776	0.804	0.748	0.608	0.636	0.636	0.608	0.545	0.510	0.685	0.692	0.755	0.594	0.797	0.776	0.559	0.545	0.566	0.476	0.559	0.531	0.476	0.504	0.664	0.699	0.762	0.566	0.650	0.776
F21	0.056	0.140	0.629	0.483	0.566	0.580	0.552	0.385	0.538	0.517	0.545	0.448	0.364	0.504	0.510	0.399	0.504	0.483	0.448	0.504	0.559	0.357	0.448	0.462	0.594	0.510	0.650	0.706	0.664	0.399	0.510	0.434
F28	-0.042	0.063	0.657	0.517	0.636	0.664	0.671	0.497	0.657	0.615	0.629	0.622	0.308	0.538	0.573	0.566	0.483	0.469	0.685	0.657	0.783	0.629	0.643	0.671	0.783	0.713	0.706	0.762	0.524	0.343	0.573	0.497
F7	0.189	0.021	0.504	0.524	0.531	0.573	0.650	0.552	0.517	0.559	0.413	0.727	0.336	0.280	0.448	0.531	0.552	0.524	0.392	0.413	0.420	0.385	0.217	0.350	0.504	0.504	0.573	0.490	0.189	0.273	0.378	0.448
Pz6	0.371	0.420	**0.853**	**0.881**	**0.874**	**0.895**	0.825	0.811	0.762	0.790	0.734	0.825	0.685	0.594	0.762	0.720	**0.916**	**0.853**	0.636	0.629	0.671	0.636	0.559	0.608	0.601	0.657	0.720	0.678	0.594	0.497	0.650	0.748
Po2	0.476	0.371	0.762	0.664	0.678	0.713	**0.853**	0.692	0.706	0.741	0.720	0.783	0.476	0.483	0.734	0.755	0.755	0.734	0.657	0.727	0.720	0.692	0.615	0.699	0.720	0.755	0.818	0.720	0.434	0.448	0.497	0.552
Pz10	0.252	0.238	0.762	0.720	0.776	0.783	0.776	0.692	0.699	0.713	0.608	0.797	0.497	0.490	0.664	0.664	0.769	0.706	0.636	0.664	0.664	0.601	0.504	0.608	0.671	0.678	0.755	0.727	0.490	0.462	0.615	0.692
F27	0.133	0.357	0.238	0.126	0.203	0.252	0.147	0.245	0.315	0.252	0.245	0.000	0.287	0.371	0.203	0.203	0.189	0.119	0.273	0.357	0.357	0.322	0.392	0.399	0.168	0.154	0.154	0.294	0.545	0.413	0.259	0.266
Pn3	-0.224	0.091	0.196	0.280	0.343	0.441	0.189	0.329	0.371	0.364	0.280	0.245	0.357	0.322	0.049	0.056	0.273	0.147	0.084	0.112	0.231	0.112	0.042	0.112	0.168	0.084	0.084	0.259	0.448	0.308	0.448	0.287
F31	-0.343	-0.259	0.294	0.287	0.455	0.357	0.203	0.308	0.343	0.287	0.105	0.301	0.210	0.308	0.147	0.217	0.203	0.042	0.483	0.587	0.399	0.322	0.315	0.441	0.315	0.294	0.294	0.469	0.545	0.448	0.510	0.678
Pn2	-0.203	-0.077	0.469	0.497	0.643	0.524	0.392	0.448	0.490	0.455	0.406	0.517	0.280	0.350	0.336	0.371	0.385	0.224	0.748	0.797	0.636	0.629	0.608	0.692	0.531	0.573	0.448	0.552	0.524	0.469	0.636	0.762
Pd8	-0.175	-0.182	0.385	0.343	0.504	0.392	0.308	0.238	0.357	0.294	0.301	0.420	0.070	0.168	0.252	0.259	0.245	0.126	0.706	0.734	0.559	0.552	0.566	0.636	0.524	0.545	0.434	0.483	0.448	0.378	0.427	0.573
F20	-0.070	-0.021	0.231	0.252	0.315	0.217	0.007	0.154	0.182	0.091	0.063	-0.007	0.294	0.301	0.140	0.091	0.126	0.042	0.350	0.385	0.210	0.203	0.357	0.322	0.028	0.084	0.084	0.196	0.692	0.462	0.231	0.504
Pz4	-0.056	-0.210	0.280	0.336	0.364	0.308	0.280	0.343	0.385	0.336	0.154	0.294	0.448	0.490	0.231	0.294	0.133	0.133	0.308	0.378	0.259	0.168	0.238	0.280	0.273	0.259	0.357	0.413	0.650	0.608	0.350	0.531
F30	-0.133	0.084	0.524	0.545	0.636	0.615	0.385	0.448	0.573	0.510	0.490	0.378	0.545	0.615	0.371	0.294	0.406	0.322	0.504	0.524	0.538	0.392	0.497	0.490	0.455	0.413	0.434	0.587	**0.895**	0.629	0.636	0.636
Tz2	-0.189	-0.112	0.455	0.462	0.601	0.559	0.385	0.434	0.538	0.476	0.343	0.455	0.406	0.469	0.287	0.308	0.350	0.224	0.552	0.629	0.524	0.413	0.427	0.524	0.483	0.441	0.462	0.601	0.769	0.636	0.601	0.699
Pd5	-0.007	0.014	0.126	0.301	0.231	0.364	0.378	0.329	0.364	0.406	0.497	0.483	0.231	0.168	0.126	0.217	0.168	0.189	0.189	0.077	0.301	0.315	0.182	0.175	0.343	0.343	0.154	0.084	0.000	0.063	0.182	-0.021
Fo11	0.636	0.399	0.224	0.273	0.077	0.168	0.231	0.126	0.084	0.098	0.196	0.133	0.280	0.084	0.308	0.189	0.287	0.462	-0.112	-0.266	-0.105	-0.042	-0.007	-0.140	-0.049	0.021	0.126	-0.112	0.028	0.014	-0.252	-0.168
Py1	0.147	0.154	0.392	0.601	0.476	0.531	0.406	0.531	0.434	0.476	0.392	0.427	0.685	0.504	0.343	0.315	0.483	0.490	0.056	0.021	0.126	0.056	0.042	0.014	0.098	0.126	0.231	0.217	0.497	0.364	0.350	0.392
Tz1	0.455	0.329	0.126	0.378	0.189	0.287	0.294	0.455	0.322	0.385	0.336	0.308	0.601	0.308	0.182	0.259	0.287	0.315	-0.035	-0.021	0.014	0.112	0.028	0.021	-0.028	0.070	0.063	-0.049	0.252	0.406	0.091	0.140
F8	0.427	0.510	0.357	0.336	0.287	0.427	0.406	0.552	0.399	0.427	0.231	0.294	0.483	0.378	0.378	0.462	0.531	0.497	0.049	0.098	0.196	0.203	0.063	0.147	0.049	0.070	0.203	0.224	0.217	0.238	0.210	0.287
Py7	0.301	0.378	0.371	0.441	0.343	0.441	0.315	0.455	0.350	0.343	0.287	0.217	0.552	0.441	0.371	0.350	0.455	0.469	0.077	0.014	0.154	0.140	0.147	0.091	-0.014	0.035	0.119	0.126	0.427	0.238	0.161	0.280
F1	0.210	0.140	-0.035	-0.147	-0.105	-0.070	-0.070	-0.189	-0.140	-0.140	-0.119	-0.070	-0.238	-0.329	-0.091	-0.161	0.056	0.021	-0.112	-0.063	-0.126	-0.098	-0.154	-0.084	-0.042	-0.070	0.007	-0.056	-0.140	-0.077	-0.175	-0.182
Pd2	-0.210	-0.350	0.042	0.126	0.105	0.182	0.168	0.091	0.042	0.105	0.021	0.287	-0.014	-0.119	-0.063	-0.014	0.161	0.105	-0.161	-0.133	-0.105	-0.203	-0.336	-0.231	0.007	-0.049	0.049	0.021	-0.063	-0.168	0.007	-0.007
F3	0.252	0.112	0.252	0.259	0.245	0.238	0.343	0.266	0.308	0.273	0.070	0.406	0.224	0.287	0.343	0.371	0.147	0.336	0.196	0.000	0.182	0.189	0.091	0.098	0.406	0.364	0.420	0.301	-0.140	0.224	0.210	0.189
Fo9	0.189	0.217	0.273	0.315	0.322	0.343	0.490	0.524	0.434	0.524	0.385	0.545	0.294	0.259	0.301	0.455	0.378	0.315	0.294	0.406	0.385	0.420	0.210	0.364	0.413	0.434	0.399	0.378	-0.056	0.252	0.441	0.343
Fo25	-0.042	0.259	0.427	0.441	0.490	0.392	0.210	0.357	0.245	0.294	0.231	0.217	0.350	0.294	0.308	0.189	0.538	0.385	0.203	0.301	0.210	0.140	0.147	0.189	0.091	0.119	0.224	0.350	0.350	0.182	0.538	0.566
Pz9	0.077	0.126	0.175	0.469	0.343	0.259	0.147	0.322	0.189	0.259	0.294	0.273	0.469	0.238	0.161	0.105	0.280	0.252	0.105	0.070	0.028	0.098	0.098	0.035	-0.007	0.112	0.042	-0.007	0.203	0.245	0.308	0.343
Tp6	0.210	0.294	0.014	0.091	0.091	0.098	0.063	0.329	0.147	0.196	-0.042	0.098	0.280	0.112	0.021	0.154	0.217	0.091	-0.021	0.140	-0.007	0.091	-0.077	0.077	-0.119	-0.063	-0.028	0.035	0.035	0.294	0.217	0.308
S6	**-0.797**	**-0.881**	-0.601	-0.469	-0.378	-0.392	-0.497	-0.476	-0.420	-0.434	-0.413	-0.259	-0.545	-0.545	**-0.727**	-0.587	-0.608	**-0.755**	-0.224	-0.161	-0.322	-0.336	-0.385	-0.294	-0.294	-0.343	-0.517	-0.413	-0.203	-0.301	-0.280	-0.273

F4	-0.566	-0.483	-0.531	-0.462	-0.413	-0.371	-0.594	-0.552	-0.538	-0.524	-0.420	-0.476	-0.497	-0.622	**-0.706**	**-0.741**	-0.385	-0.566	-0.510	-0.413	-0.524	-0.573	-0.573	-0.524	-0.559	-0.615	-0.643	-0.524	-0.133	-0.427	-0.371	-0.420
Po5	-0.510	-0.322	-0.517	-0.531	-0.441	-0.448	-0.699	-0.552	-0.580	-0.615	-0.538	-0.657	-0.517	-0.559	-0.643	-0.664	-0.413	-0.608	-0.385	-0.294	-0.455	-0.441	-0.392	-0.371	-0.650	-0.657	**-0.720**	-0.538	-0.105	-0.413	-0.385	-0.301
F22	**-0.713**	-0.643	-0.217	-0.140	0.035	-0.035	-0.238	-0.217	-0.077	-0.140	-0.119	-0.007	-0.301	-0.231	-0.399	-0.336	-0.308	-0.483	0.217	0.252	0.091	0.056	0.049	0.133	0.084	0.035	-0.161	-0.007	0.175	0.056	0.126	0.140
Fo20	-0.490	-0.441	0.154	0.196	0.364	0.245	0.063	0.112	0.210	0.140	0.091	0.224	0.035	0.126	-0.014	0.028	0.021	-0.140	0.497	0.552	0.350	0.301	0.336	0.413	0.287	0.280	0.168	0.315	0.490	0.343	0.385	0.531
Py10	-0.510	-0.483	0.112	0.105	0.273	0.147	-0.021	-0.035	0.098	0.014	0.021	0.105	-0.084	0.049	-0.049	-0.056	-0.063	-0.189	0.434	0.462	0.280	0.210	0.301	0.336	0.238	0.217	0.126	0.259	0.441	0.224	0.266	0.406
S1	-0.049	-0.035	-0.007	-0.147	-0.042	-0.196	-0.203	-0.175	-0.175	-0.238	-0.378	-0.210	-0.189	-0.056	0.014	-0.035	-0.112	-0.091	0.070	0.056	-0.070	-0.056	0.000	0.014	-0.049	-0.056	0.028	0.077	-0.112	-0.007	0.021	0.175
F34	-0.350	-0.252	-0.273	-0.343	-0.203	-0.245	-0.448	-0.455	-0.350	-0.406	-0.385	-0.364	-0.420	-0.427	-0.427	-0.517	-0.259	-0.385	-0.175	-0.119	-0.259	-0.294	-0.259	-0.203	-0.238	-0.308	-0.294	-0.182	0.000	-0.126	-0.147	-0.140
S2	-0.112	-0.203	-0.259	-0.413	-0.280	-0.315	-0.308	-0.308	-0.168	-0.294	-0.462	-0.329	-0.266	-0.091	-0.238	-0.175	-0.441	-0.392	-0.028	-0.021	-0.105	-0.112	-0.063	-0.014	-0.042	-0.126	-0.084	-0.007	0.014	0.168	-0.168	-0.070
F12	0.301	0.245	-0.301	-0.217	-0.308	-0.294	-0.308	-0.063	-0.301	-0.273	-0.385	-0.308	-0.042	-0.273	-0.210	-0.119	-0.077	-0.119	-0.322	-0.280	-0.406	-0.196	-0.301	-0.266	-0.566	-0.441	-0.448	-0.490	-0.329	-0.133	-0.350	-0.126
Pn1	-0.105	-0.014	-0.490	-0.420	-0.476	-0.490	-0.559	-0.350	-0.448	-0.434	-0.336	-0.622	-0.168	-0.266	-0.455	-0.441	-0.399	-0.476	-0.406	-0.287	-0.434	-0.350	-0.252	-0.308	-0.629	-0.559	-0.622	-0.538	-0.063	-0.168	-0.364	-0.308
Tp2	0.140	-0.021	-0.266	-0.343	-0.413	-0.322	-0.056	-0.147	-0.252	-0.182	-0.322	-0.098	-0.287	-0.259	-0.119	-0.007	-0.175	-0.049	-0.462	-0.483	-0.343	-0.336	-0.497	-0.434	-0.182	-0.238	-0.119	-0.224	**-0.748**	-0.476	-0.350	-0.462
S5	0.326	0.193	-0.077	-0.084	-0.172	-0.025	0.175	0.084	0.067	0.112	-0.004	0.154	0.021	-0.014	0.046	0.137	-0.004	0.147	-0.266	-0.340	-0.112	-0.098	-0.270	-0.224	0.081	0.018	0.088	-0.046	-0.441	-0.105	-0.144	-0.329
F13	0.385	0.392	0.287	0.091	0.140	0.098	0.154	0.063	0.126	0.028	0.000	0.056	-0.014	0.147	0.392	0.322	0.140	0.287	0.343	0.147	0.266	0.343	0.378	0.301	0.266	0.287	0.287	0.210	-0.098	0.049	0.014	0.119
F18	-0.042	0.203	0.014	-0.035	0.042	-0.042	-0.021	0.154	0.077	0.070	-0.119	-0.021	0.056	0.196	0.098	0.175	-0.007	-0.014	0.126	0.126	0.133	0.175	0.105	0.154	0.077	0.077	0.042	0.154	-0.203	0.070	0.294	0.252
Po7	-0.049	0.189	-0.231	-0.357	-0.280	-0.287	-0.252	-0.182	-0.175	-0.217	-0.280	-0.294	-0.280	-0.098	-0.119	-0.070	-0.273	-0.224	-0.070	-0.154	-0.035	0.028	-0.021	-0.028	-0.070	-0.105	-0.196	-0.119	-0.462	-0.231	-0.070	-0.196
	Fo4	**F26**	**Fo15**	**Pz11**	**Py11**	**Pz5**	**Pd7**	**F35**	**Py9**	**Po8**	**F16**	**F32**	**Pd6**	**F5**	**Tp1**	**Pz8**	**Pd9**	**Fo13**	**Fo18**	**Fo24**	**F21**	**F28**	**F7**	**Pz6**	**Po2**	**Pz10**	**F27**	**Pn3**	**F31**	**Pn2**	**Pd8**	**F20**
Fo1	0.273	0.357	0.273	0.455	0.552	0.441	0.357	0.685	0.420	0.608	0.720	0.797	0.063	0.049	0.021	0.140	0.133	0.161	0.378	0.294	0.133	0.322	0.035	0.315	0.497	0.252	0.406	-0.140	-0.112	0.196	0.203	0.049
Fo5	0.343	0.406	0.378	0.552	0.643	0.545	0.490	0.664	0.469	0.678	0.762	0.832	0.168	0.147	0.035	0.175	0.189	0.308	0.524	0.427	0.224	0.378	0.049	0.364	0.517	0.308	0.441	-0.140	0.028	0.280	0.266	0.175
Po1	0.420	0.476	0.385	0.573	0.629	0.448	0.427	0.727	0.524	0.510	0.713	0.713	0.196	0.231	0.252	0.462	0.315	0.322	0.455	0.476	0.266	0.392	0.119	0.497	0.531	0.350	0.441	0.105	-0.112	0.168	0.098	0.084
Py5	0.399	0.573	0.552	0.629	0.615	0.483	0.476	0.657	0.545	0.594	0.839	0.825	0.056	0.189	0.091	0.455	0.392	0.350	0.517	0.483	0.350	0.490	0.161	0.504	0.552	0.420	0.559	0.259	0.042	0.238	0.161	0.084
Pd3	0.259	0.189	0.413	0.371	0.497	0.273	0.371	0.091	-0.112	0.420	0.448	0.427	0.077	0.413	0.168	0.238	0.336	0.531	0.504	0.580	0.301	0.063	0.112	0.413	0.378	0.329	0.182	-0.028	-0.189	-0.112	-0.049	0.014
Po6	0.119	0.196	0.399	0.462	0.566	0.476	0.483	0.343	0.182	0.552	0.552	0.629	0.077	0.210	-0.070	0.168	0.301	0.587	0.622	0.566	0.231	0.161	0.000	0.357	0.357	0.287	0.427	-0.077	0.175	0.231	0.210	0.357
S7	0.322	0.147	0.336	0.552	0.762	0.671	0.671	0.308	0.280	0.524	0.462	0.462	0.315	0.392	0.182	0.196	0.308	0.706	0.720	0.699	0.448	0.336	0.070	0.434	0.448	0.336	0.280	-0.252	0.161	0.203	0.238	0.469
Po9	0.231	0.462	0.231	0.266	0.210	0.168	0.042	0.650	0.413	0.231	0.490	0.615	0.126	-0.021	0.077	0.119	0.049	-0.133	0.091	0.000	-0.133	0.098	0.126	0.217	0.413	0.203	0.322	0.035	-0.035	0.252	0.126	-0.147
Fo3	0.077	0.273	0.126	0.070	-0.035	-0.189	-0.203	0.441	0.210	-0.098	0.245	0.224	-0.189	-0.049	0.140	0.504	0.140	-0.077	-0.126	0.000	-0.112	0.021	0.049	0.238	0.147	0.056	0.385	0.524	-0.364	-0.224	-0.399	-0.280
Fo12	0.049	0.161	0.056	0.210	0.259	0.042	-0.028	0.636	0.210	0.203	0.385	0.434	-0.084	-0.028	0.133	0.406	0.161	0.077	0.091	0.189	-0.154	-0.021	0.007	0.336	0.266	0.119	0.280	0.119	-0.420	-0.084	-0.161	-0.126
Fo10	0.294	0.154	0.329	0.441	0.517	0.301	0.280	0.308	0.112	0.280	0.350	0.301	0.231	0.357	0.259	0.497	0.308	0.538	0.504	0.636	0.140	0.070	0.175	0.573	0.392	0.336	0.126	0.014	-0.266	-0.091	-0.210	0.014
Fo19	0.378	0.294	0.497	0.448	0.469	0.287	0.294	0.210	0.077	0.252	0.385	0.343	0.238	0.448	0.259	0.455	0.336	0.545	0.531	0.622	0.182	0.063	0.259	0.566	0.441	0.406	0.182	0.105	-0.154	-0.077	-0.203	-0.035
Po3	0.448	0.308	0.497	0.469	0.497	0.343	0.315	0.210	0.119	0.238	0.364	0.315	0.315	0.476	0.301	0.420	0.301	0.524	0.545	0.615	0.189	0.098	0.315	0.580	0.490	0.434	0.119	0.028	-0.140	-0.056	-0.182	-0.049
F2	0.385	0.287	0.406	0.469	0.504	0.322	0.252	0.217	0.112	0.350	0.497	0.420	0.189	0.287	0.182	0.329	0.266	0.301	0.420	0.497	0.238	0.224	0.294	0.566	0.573	0.427	0.049	-0.021	-0.322	-0.063	-0.168	-0.294
Fo23	0.399	0.392	0.469	0.510	0.538	0.343	0.308	0.385	0.238	0.343	0.566	0.510	0.147	0.322	0.245	0.462	0.364	0.385	0.455	0.531	0.266	0.252	0.273	0.594	0.573	0.441	0.259	0.147	-0.231	-0.021	-0.126	-0.147
F19	0.587	0.469	0.545	0.650	0.692	0.559	0.483	0.427	0.406	0.427	0.601	0.517	0.329	0.427	0.329	0.441	0.364	0.448	0.601	0.615	0.364	0.413	0.392	0.657	0.671	0.545	0.168	0.007	-0.084	0.112	0.035	-0.042
Fo2	0.455	0.406	0.441	0.545	0.566	0.385	0.329	0.483	0.378	0.161	0.413	0.343	0.301	0.420	0.448	0.636	0.448	0.483	0.483	0.594	0.238	0.224	0.357	0.685	0.552	0.483	0.210	0.210	-0.119	0.056	-0.091	0.014
F9	0.322	0.406	0.483	0.601	0.559	0.392	0.301	0.420	0.441	0.091	0.448	0.280	0.028	0.273	0.350	0.811	0.678	0.476	0.441	0.587	0.378	0.392	0.483	0.818	0.587	0.601	0.217	0.510	-0.070	0.098	-0.056	-0.056
Tp5	0.531	0.476	0.517	0.580	0.622	0.406	0.434	0.287	0.308	0.308	0.594	0.420	0.119	0.420	0.406	0.566	0.517	0.399	0.441	0.566	0.580	0.504	0.371	0.657	0.643	0.531	0.231	0.308	-0.224	-0.063	-0.084	-0.175
Fo26	0.538	0.483	0.671	0.713	0.671	0.517	0.483	0.245	0.350	0.196	0.434	0.280	0.378	0.566	0.448	0.713	0.664	0.671	0.685	0.797	0.441	0.329	0.559	**0.860**	0.636	0.706	0.056	0.294	0.098	0.196	0.035	0.028
Pz3	0.636	0.427	0.580	0.664	0.720	0.573	0.510	0.154	0.280	0.161	0.357	0.175	0.413	0.636	0.580	0.552	0.601	0.587	0.629	0.727	0.517	0.371	0.643	0.825	0.720	0.727	-0.133	0.077	-0.014	0.105	0.084	-0.056
Pz7	0.608	0.371	0.510	0.566	0.587	0.399	0.462	0.091	0.217	0.042	0.189	0.007	0.531	0.713	0.664	0.664	0.559	0.650	0.573	0.755	0.469	0.238	0.476	0.720	0.476	0.566	-0.105	0.196	0.007	0.035	-0.049	0.070
Py12	0.378	0.343	0.538	0.622	0.524	0.378	0.308	0.168	0.273	0.063	0.252	0.084	0.350	0.441	0.385	0.748	0.650	0.573	0.566	0.720	0.266	0.189	0.566	0.846	0.490	0.657	-0.161	0.308	0.070	0.224	0.014	-0.042
S4	0.406	0.133	0.224	0.399	0.504	0.420	0.406	0.182	0.308	-0.161	-0.056	-0.147	0.552	0.559	0.608	0.538	0.371	0.671	0.483	0.629	0.343	0.154	0.266	0.538	0.336	0.329	0.070	0.070	0.133	0.056	-0.091	0.385
F6	0.315	0.231	0.301	0.385	0.497	0.266	0.301	0.308	0.147	-0.098	0.119	0.091	0.378	0.566	0.622	0.615	0.517	0.657	0.441	0.643	0.245	-0.042	0.259	0.622	0.420	0.392	0.182	0.196	-0.070	-0.014	-0.126	0.210
Ox1	0.263	0.249	0.214	0.242	0.270	0.116	0.137	0.371	0.298	-0.336	-0.004	-0.060	0.210	0.413	0.599	0.718	0.431	0.487	0.214	0.413	0.179	0.025	0.214	0.494	0.270	0.249	0.361	0.487	-0.046	-0.116	-0.287	0.214
Ox2	0.655	0.580	0.481	0.392	0.331	0.178	0.199	0.221	0.235	-0.053	0.253	0.157	0.452	0.573	0.648	0.456	0.356	0.178	0.246	0.345	0.249	0.149	0.498	0.527	0.527	0.491	-0.053	0.224	-0.110	0.011	-0.068	-0.274
S3	0.566	0.238	0.385	0.294	0.322	0.217	0.182	-0.084	-0.098	0.147	0.154	0.084	0.462	0.524	0.357	0.105	0.042	0.238	0.364	0.399	0.098	0.000	0.385	0.392	0.427	0.364	-0.294	-0.273	-0.266	-0.168	-0.203	-0.322
S8	0.608	0.294	0.538	0.552	0.601	0.441	0.483	0.021	0.070	0.364	0.336	0.203	0.483	0.636	0.392	0.378	0.322	0.587	0.657	0.734	0.357	0.210	0.378	0.587	0.455	0.497	-0.161	-0.126	-0.091	-0.021	-0.042	-0.021
F10	0.231	0.084	0.049	0.077	0.161	-0.049	0.021	0.294	0.014	-0.105	0.014	0.063	0.350	0.357	0.427	0.371	0.021	0.315	0.140	0.322	-0.063	-0.210	-0.091	0.224	0.126	-0.021	0.259	0.056	-0.336	-0.280	-0.455	0.049
Tz3	0.112	0.049	0.238	0.133	0.126	-0.091	-0.021	0.014	-0.133	-0.091	0.084	-0.007	-0.035	0.273	0.210	0.545	0.252	0.406	0.189	0.413	0.084	-0.091	0.077	0.392	0.140	0.140	0.224	0.385	-0.385	-0.413	-0.580	-0.147
F25	0.406	0.203	0.245	0.350	0.434	0.210	0.217	0.280	0.091	0.385	0.441	0.357	0.161	0.273	0.238	0.315	0.105	0.210	0.301	0.406	0.182	0.210	0.119	0.392	0.385	0.231	0.056	-0.077	-0.490	-0.238	-0.252	-0.252
Fo8	0.301	0.126	0.182	0.224	0.413	0.259	0.175	0.294	0.007	0.126	0.238	0.280	0.168	0.343	0.336	0.140	0.112	0.315	0.301	0.343	0.056	-0.042	0.189	0.371	0.476	0.252	0.133	-0.224	-0.343	-0.203	-0.175	-0.070

Fo22	0.231	0.252	0.336	0.371	0.434	0.203	0.245	0.378	0.098	0.364	0.497	0.517	0.119	0.266	0.154	0.378	0.252	0.406	0.399	0.497	0.140	0.063	0.021	0.420	0.371	0.238	0.357	0.091	-0.259	-0.077	-0.182	-0.021
Pd1	0.622	0.629	0.734	**0.923**	**0.923**	0.811	0.762	0.517	0.664	0.552	0.762	0.629	0.385	0.490	0.357	0.643	0.699	0.678	0.839	0.839	0.629	0.657	0.504	**0.853**	0.762	0.762	0.238	0.196	0.294	0.469	0.385	0.231
Pz1	0.517	0.545	0.580	0.839	0.811	0.692	0.608	0.566	0.692	0.294	0.538	0.406	0.434	0.441	0.469	0.776	0.734	0.629	0.706	0.776	0.483	0.517	0.524	**0.881**	0.664	0.720	0.126	0.280	0.287	0.497	0.343	0.252
Pz2	0.566	0.678	0.713	**0.937**	**0.860**	0.769	0.720	0.601	0.790	0.448	0.692	0.559	0.427	0.441	0.392	0.769	0.790	0.643	0.790	0.804	0.566	0.636	0.531	**0.874**	0.678	0.776	0.203	0.343	0.455	0.643	0.504	0.315
Pz12	0.538	0.664	0.706	**0.895**	0.818	0.727	0.650	0.580	0.769	0.371	0.685	0.524	0.280	0.392	0.378	0.804	0.804	0.601	0.720	0.748	0.580	0.664	0.573	**0.895**	0.713	0.783	0.252	0.441	0.357	0.524	0.392	0.217
Fo14	0.657	0.650	0.664	0.818	0.797	0.755	0.594	0.497	0.692	0.343	0.657	0.510	0.322	0.406	0.399	0.552	0.601	0.434	0.643	0.608	0.552	0.671	0.650	0.825	**0.853**	0.776	0.147	0.189	0.203	0.392	0.308	0.007
F11	0.524	0.608	0.636	0.769	0.685	0.643	0.504	0.559	0.706	0.224	0.545	0.448	0.357	0.371	0.364	0.685	0.615	0.517	0.636	0.636	0.385	0.497	0.552	0.811	0.692	0.692	0.245	0.329	0.308	0.448	0.238	0.154
Py2	0.678	0.769	0.706	0.839	0.755	0.685	0.636	0.636	0.811	0.371	0.699	0.580	0.406	0.448	0.448	0.692	0.636	0.476	0.650	0.636	0.538	0.657	0.517	0.762	0.706	0.699	0.315	0.371	0.343	0.490	0.357	0.182
Tp3	0.643	0.727	0.671	0.804	0.720	0.650	0.566	0.573	0.755	0.280	0.636	0.510	0.399	0.427	0.455	0.678	0.636	0.441	0.601	0.608	0.517	0.615	0.559	0.790	0.741	0.713	0.252	0.364	0.287	0.455	0.294	0.091
Py4	0.504	0.587	0.455	0.741	0.748	0.608	0.531	0.636	0.713	0.322	0.650	0.531	0.308	0.294	0.441	0.629	0.615	0.350	0.483	0.545	0.545	0.629	0.413	0.734	0.720	0.608	0.245	0.280	0.105	0.406	0.301	0.063
Py13	0.573	0.636	0.608	0.783	0.699	0.699	0.497	0.497	0.734	0.224	0.545	0.392	0.301	0.315	0.385	0.580	0.643	0.329	0.552	0.510	0.448	0.622	0.727	0.825	0.783	0.797	0.000	0.245	0.301	0.517	0.420	-0.007
Py3	0.552	0.538	0.510	0.636	0.601	0.455	0.483	0.531	0.580	0.098	0.364	0.287	0.538	0.552	0.594	0.769	0.552	0.608	0.566	0.685	0.364	0.308	0.336	0.685	0.476	0.497	0.287	0.357	0.210	0.280	0.070	0.294
Tp4	0.713	0.685	0.650	0.741	0.664	0.559	0.643	0.531	0.692	0.378	0.587	0.490	0.580	0.559	0.476	0.671	0.462	0.559	0.657	0.692	0.504	0.538	0.280	0.594	0.483	0.490	0.371	0.322	0.308	0.350	0.168	0.301
Fo7	0.629	0.552	0.650	0.825	**0.853**	0.748	0.671	0.497	0.580	0.538	0.706	0.601	0.399	0.462	0.329	0.524	0.517	0.587	0.769	0.755	0.510	0.573	0.448	0.762	0.734	0.664	0.203	0.049	0.147	0.336	0.252	0.140
Fo16	0.601	0.566	0.629	0.762	0.748	0.741	0.566	0.517	0.650	0.413	0.636	0.552	0.336	0.357	0.266	0.448	0.441	0.455	0.678	0.594	0.399	0.566	0.531	0.720	0.755	0.664	0.203	0.056	0.217	0.371	0.259	0.091
Fo17	0.413	0.483	0.657	0.818	0.811	0.692	0.573	0.420	0.510	0.350	0.615	0.490	0.224	0.385	0.287	0.678	0.755	0.678	0.748	0.797	0.504	0.483	0.552	**0.916**	0.755	0.769	0.189	0.273	0.203	0.385	0.245	0.126
Py6	0.524	0.455	0.622	0.769	0.797	0.657	0.559	0.378	0.441	0.378	0.587	0.448	0.287	0.455	0.350	0.615	0.615	0.629	0.720	0.776	0.483	0.469	0.524	**0.853**	0.734	0.706	0.119	0.147	0.042	0.224	0.126	0.042
F29	0.517	0.671	0.573	**0.860**	0.832	0.818	0.713	0.783	**0.888**	0.629	0.804	0.769	0.357	0.238	0.224	0.455	0.552	0.392	0.685	0.559	0.448	0.685	0.392	0.636	0.657	0.636	0.273	0.084	0.483	0.748	0.706	0.350
Po4	0.531	0.734	0.615	0.846	0.825	0.832	0.734	0.748	**0.881**	0.538	0.783	0.783	0.413	0.301	0.280	0.399	0.587	0.420	0.685	0.545	0.504	0.657	0.413	0.629	0.727	0.664	0.357	0.112	0.587	0.797	0.734	0.385
F33	0.594	0.755	0.664	**0.881**	0.818	0.783	0.706	0.720	**0.881**	0.622	**0.881**	0.797	0.308	0.266	0.231	0.517	0.573	0.371	0.664	0.566	0.559	0.783	0.420	0.671	0.720	0.664	0.357	0.231	0.399	0.636	0.559	0.210
Fo21	0.455	0.636	0.524	0.790	0.762	0.734	0.580	0.811	0.839	0.566	0.804	0.783	0.245	0.147	0.175	0.448	0.497	0.301	0.587	0.476	0.357	0.629	0.385	0.636	0.692	0.601	0.322	0.112	0.322	0.629	0.552	0.203
F36	0.504	0.615	0.476	0.797	0.832	0.748	0.699	0.839	0.832	0.664	0.825	0.818	0.371	0.245	0.259	0.441	0.462	0.392	0.636	0.559	0.448	0.643	0.217	0.559	0.615	0.504	0.392	0.042	0.315	0.608	0.566	0.357
Fo6	0.531	0.713	0.587	0.832	0.818	0.790	0.699	0.811	**0.874**	0.615	0.846	0.839	0.343	0.252	0.238	0.427	0.524	0.378	0.657	0.531	0.462	0.671	0.350	0.608	0.699	0.608	0.399	0.112	0.441	0.692	0.636	0.322
F15	0.762	0.818	0.685	0.818	0.741	0.706	0.671	0.559	0.783	0.566	0.811	0.685	0.399	0.385	0.357	0.420	0.490	0.231	0.573	0.476	0.594	0.783	0.504	0.601	0.720	0.671	0.168	0.168	0.315	0.531	0.524	0.028
Ox3	0.685	0.741	0.608	0.825	0.790	0.748	0.650	0.657	0.804	0.538	0.776	0.685	0.413	0.343	0.364	0.434	0.504	0.273	0.594	0.504	0.510	0.713	0.504	0.657	0.755	0.678	0.154	0.084	0.294	0.573	0.545	0.084
F23	0.839	0.783	0.769	**0.867**	**0.853**	0.790	0.755	0.469	0.678	0.531	0.762	0.636	0.510	0.580	0.469	0.448	0.545	0.462	0.734	0.664	0.650	0.706	0.573	0.720	0.818	0.755	0.154	0.084	0.294	0.448	0.434	0.084
F24	0.818	**0.867**	**0.860**	**0.909**	0.818	0.769	0.811	0.469	0.748	0.594	0.832	0.706	0.510	0.566	0.385	0.517	0.594	0.510	0.776	0.699	0.706	0.762	0.490	0.678	0.720	0.727	0.294	0.259	0.469	0.552	0.483	0.196
F14	0.573	0.671	0.566	0.748	0.769	0.622	0.797	0.657	0.769	0.294	0.538	0.483	0.545	0.622	0.643	0.762	0.720	0.755	0.685	0.762	0.664	0.524	0.189	0.594	0.434	0.490	0.545	0.448	0.545	0.524	0.448	0.692
Py8	0.706	0.776	0.580	0.636	0.601	0.490	0.615	0.685	0.713	0.210	0.476	0.483	0.664	0.657	0.699	0.608	0.504	0.510	0.552	0.566	0.399	0.343	0.273	0.497	0.448	0.462	0.413	0.308	0.448	0.469	0.378	0.462
F17	0.601	0.783	0.734	0.818	0.629	0.552	0.622	0.497	0.734	0.427	0.671	0.566	0.517	0.434	0.336	0.678	0.629	0.462	0.643	0.650	0.510	0.573	0.378	0.650	0.497	0.615	0.259	0.448	0.510	0.636	0.427	0.231
Pd4	0.538	0.685	0.727	**0.881**	0.769	0.748	0.720	0.601	0.797	0.413	0.601	0.552	0.552	0.469	0.343	0.671	0.692	0.685	0.825	0.776	0.434	0.497	0.448	0.748	0.552	0.692	0.266	0.287	0.678	0.762	0.573	0.504
Fo4	1.000	0.818	0.734	0.643	0.650	0.594	0.755	0.203	0.490	0.350	0.531	0.413	0.678	0.832	0.692	0.273	0.378	0.406	0.587	0.531	0.734	0.594	0.448	0.420	0.608	0.566	0.147	0.077	0.343	0.259	0.350	0.147
F26	0.818	1.000	0.839	0.734	0.622	0.573	0.713	0.490	0.706	0.406	0.741	0.671	0.448	0.622	0.531	0.455	0.601	0.357	0.566	0.476	0.657	0.629	0.462	0.497	0.615	0.650	0.392	0.420	0.552	0.538	0.566	0.217
Fo15	0.734	0.839	1.000	0.839	0.699	0.692	0.748	0.196	0.524	0.455	0.692	0.552	0.357	0.643	0.329	0.497	0.720	0.615	0.811	0.713	0.664	0.594	0.650	0.706	0.664	0.832	0.189	0.371	0.601	0.517	0.504	0.182
Pz11	0.643	0.734	0.839	1.000	**0.930**	**0.888**	**0.860**	0.483	0.755	0.622	0.797	0.664	0.441	0.497	0.301	0.601	0.776	0.678	**0.909**	0.839	0.678	0.727	0.566	0.832	0.727	0.839	0.175	0.224	0.587	0.720	0.657	0.343
Py11	0.650	0.622	0.699	**0.930**	1.000	**0.923**	**0.902**	0.497	0.685	0.594	0.734	0.629	0.476	0.559	0.420	0.497	0.706	0.720	**0.888**	0.839	0.727	0.692	0.483	0.776	0.762	0.762	0.203	0.042	0.462	0.594	0.615	0.413
Pz5	0.594	0.573	0.692	**0.888**	**0.923**	1.000	**0.853**	0.378	0.720	0.524	0.657	0.552	0.392	0.420	0.245	0.308	0.615	0.608	**0.853**	0.692	0.685	0.755	0.594	0.720	0.797	0.790	0.126	-0.042	0.615	0.678	0.692	0.378
Pd7	0.755	0.713	0.748	**0.860**	**0.902**	**0.853**	1.000	0.406	0.685	0.622	0.706	0.615	0.580	0.685	0.462	0.420	0.608	0.734	**0.874**	0.797	0.825	0.720	0.315	0.545	0.566	0.615	0.343	0.112	0.643	0.587	0.650	0.594
F35	0.203	0.490	0.196	0.483	0.497	0.378	0.406	1.000	0.769	0.420	0.615	0.713	0.182	0.049	0.231	0.483	0.343	0.224	0.308	0.287	0.133	0.336	-0.084	0.287	0.259	0.175	0.601	0.245	0.252	0.504	0.441	0.476
Py9	0.490	0.706	0.524	0.755	0.685	0.720	0.685	0.769	1.000	0.420	0.692	0.636	0.294	0.210	0.273	0.538	0.545	0.364	0.545	0.441	0.545	0.769	0.287	0.504	0.517	0.504	0.510	0.371	0.615	0.699	0.615	0.490
Po8	0.350	0.406	0.455	0.622	0.594	0.524	0.622	0.420	0.420	1.000	0.832	0.832	0.245	0.112	-0.196	0.091	0.154	0.266	0.608	0.476	0.315	0.504	-0.070	0.203	0.231	0.252	0.196	-0.175	0.252	0.483	0.517	0.238
F16	0.531	0.741	0.692	0.797	0.734	0.657	0.706	0.615	0.692	0.832	1.000	**0.944**	0.161	0.231	0.056	0.371	0.483	0.322	0.636	0.517	0.559	0.734	0.231	0.483	0.573	0.538	0.441	0.217	0.336	0.538	0.552	0.175
F32	0.413	0.671	0.552	0.664	0.629	0.552	0.615	0.713	0.636	0.832	**0.944**	1.000	0.189	0.154	0.000	0.224	0.329	0.238	0.545	0.399	0.357	0.538	0.049	0.308	0.448	0.371	0.504	0.084	0.336	0.566	0.580	0.259
Pd6	0.678	0.448	0.357	0.441	0.476	0.392	0.580	0.182	0.294	0.245	0.161	0.189	1.000	0.734	0.622	0.147	0.154	0.434	0.517	0.531	0.322	0.112	0.098	0.217	0.224	0.252	-0.084	-0.259	0.392	0.406	0.357	0.399
F5	0.832	0.622	0.643	0.497	0.559	0.420	0.685	0.049	0.210	0.112	0.231	0.154	0.734	1.000	0.839	0.371	0.476	0.664	0.601	0.664	0.629	0.224	0.350	0.413	0.406	0.483	0.105	0.119	0.350	0.147	0.238	0.350
Tp1	0.692	0.531	0.329	0.301	0.420	0.245	0.462	0.231	0.273	-0.196	0.056	0.000	0.622	0.839	1.000	0.420	0.434	0.427	0.266	0.406	0.524	0.154	0.280	0.336	0.371	0.336	0.154	0.210	0.147	0.035	0.133	0.280
Pz8	0.273	0.455	0.497	0.601	0.497	0.308	0.420	0.483	0.538	0.091	0.371	0.224	0.147	0.371	0.420	1.000	0.783	0.657	0.497	0.685	0.406	0.336	0.266	0.692	0.252	0.469	0.371	0.748	0.259	0.273	0.105	0.371
Pd9	0.378	0.601	0.720	0.776	0.706	0.615	0.608	0.343	0.545	0.154	0.483	0.329	0.154	0.476	0.434	0.783	1.000	0.706	0.678	0.734	0.608	0.469	0.643	**0.874**	0.615	0.832	0.175	0.573	0.524	0.545	0.510	0.315
Fo13	0.406	0.357	0.615	0.678	0.720	0.608	0.734	0.224	0.364	0.266	0.322	0.238	0.434	0.664	0.427	0.657	0.706	1.000	**0.853**	**0.930**	0.545	0.280	0.280	0.657	0.343	0.538	0.273	0.266	0.510	0.357	0.294	0.664
Fo18	0.587	0.566	0.811	**0.909**	**0.888**	**0.853**	**0.874**	0.308	0.545	0.608	0.636	0.545	0.517	0.601	0.266	0.497	0.678	**0.853**	1.000	**0.930**	0.594	0.524	0.462	0.741	0.580	0.741	0.133	0.063	0.622	0.629	0.580	0.497

Fo24	0.531	0.476	0.713	0.839	0.839	0.692	0.797	0.287	0.441	0.476	0.517	0.399	0.531	0.664	0.406	0.685	0.734	**0.930**	**0.930**	1.000	0.587	0.406	0.364	0.769	0.469	0.657	0.140	0.196	0.448	0.462	0.378	0.497
F21	0.734	0.657	0.664	0.678	0.727	0.685	0.825	0.133	0.545	0.315	0.559	0.357	0.322	0.629	0.524	0.406	0.608	0.545	0.594	0.587	1.000	0.825	0.406	0.504	0.615	0.587	0.336	0.336	0.413	0.259	0.364	0.287
F28	0.594	0.629	0.594	0.727	0.692	0.755	0.720	0.336	0.769	0.504	0.734	0.538	0.112	0.224	0.154	0.336	0.469	0.280	0.524	0.406	0.825	1.000	0.399	0.476	0.629	0.559	0.343	0.287	0.406	0.413	0.448	0.161
F7	0.448	0.462	0.650	0.566	0.483	0.594	0.315	-0.084	0.287	-0.070	0.231	0.049	0.098	0.350	0.280	0.266	0.643	0.280	0.462	0.364	0.406	0.399	1.000	0.783	0.811	**0.909**	-0.343	0.140	0.336	0.364	0.378	-0.252
Pz6	0.420	0.497	0.706	0.832	0.776	0.720	0.545	0.287	0.504	0.203	0.483	0.308	0.217	0.413	0.336	0.692	**0.874**	0.657	0.741	0.769	0.504	0.476	0.783	1.000	0.783	**0.916**	-0.049	0.308	0.343	0.490	0.385	0.084
Po2	0.608	0.615	0.664	0.727	0.762	0.797	0.566	0.259	0.517	0.231	0.573	0.448	0.224	0.406	0.371	0.252	0.615	0.343	0.580	0.469	0.615	0.629	0.811	0.783	1.000	**0.874**	0.007	0.035	0.273	0.413	0.448	-0.112
Pz10	0.566	0.650	0.832	0.839	0.762	0.790	0.615	0.175	0.504	0.252	0.538	0.371	0.252	0.483	0.336	0.469	0.832	0.538	0.741	0.657	0.587	0.559	**0.909**	**0.916**	**0.874**	1.000	-0.119	0.217	0.497	0.580	0.573	0.014
F27	0.147	0.392	0.189	0.175	0.203	0.126	0.343	0.601	0.510	0.196	0.441	0.504	-0.084	0.105	0.154	0.371	0.175	0.273	0.133	0.140	0.336	0.343	-0.343	-0.049	0.007	-0.119	1.000	0.545	0.224	0.042	0.014	0.538
Pn3	0.077	0.420	0.371	0.224	0.042	-0.042	0.112	0.245	0.371	-0.175	0.217	0.084	-0.259	0.119	0.210	0.748	0.573	0.266	0.063	0.196	0.336	0.287	0.140	0.308	0.035	0.217	0.545	1.000	0.231	0.042	-0.070	0.168
F31	0.343	0.552	0.601	0.587	0.462	0.615	0.643	0.252	0.615	0.252	0.336	0.336	0.392	0.350	0.147	0.259	0.524	0.510	0.622	0.448	0.413	0.406	0.336	0.343	0.273	0.497	0.224	0.231	1.000	0.818	0.769	0.650
Pn2	0.259	0.538	0.517	0.720	0.594	0.678	0.587	0.504	0.699	0.483	0.538	0.566	0.406	0.147	0.035	0.273	0.545	0.357	0.629	0.462	0.259	0.413	0.364	0.490	0.413	0.580	0.042	0.042	0.818	1.000	**0.909**	0.455
Pd8	0.350	0.566	0.504	0.657	0.615	0.692	0.650	0.441	0.615	0.517	0.552	0.580	0.357	0.238	0.133	0.105	0.510	0.294	0.580	0.378	0.364	0.448	0.378	0.385	0.448	0.573	0.014	-0.070	0.769	**0.909**	1.000	0.434
F20	0.147	0.217	0.182	0.343	0.413	0.378	0.594	0.476	0.490	0.238	0.175	0.259	0.399	0.350	0.280	0.371	0.315	0.664	0.497	0.497	0.287	0.161	-0.252	0.084	-0.112	0.014	0.538	0.168	0.650	0.455	0.434	1.000
Pz4	0.594	0.462	0.413	0.392	0.427	0.510	0.643	0.168	0.531	-0.063	0.056	0.007	0.587	0.650	0.601	0.294	0.329	0.566	0.476	0.427	0.566	0.392	0.217	0.217	0.231	0.287	0.322	0.189	0.685	0.329	0.336	0.685
F30	0.483	0.615	0.517	0.615	0.580	0.483	0.741	0.504	0.734	0.259	0.476	0.371	0.343	0.504	0.497	0.734	0.643	0.657	0.545	0.615	0.720	0.608	0.056	0.392	0.203	0.329	0.636	0.636	0.594	0.420	0.378	0.720
Tz2	0.545	0.755	0.706	0.671	0.587	0.622	0.755	0.399	0.727	0.196	0.455	0.385	0.364	0.559	0.476	0.538	0.734	0.608	0.622	0.545	0.664	0.559	0.399	0.476	0.399	0.587	0.427	0.517	**0.874**	0.664	0.685	0.650
Pd5	-0.077	0.070	-0.133	0.000	-0.084	-0.112	-0.294	0.273	0.287	-0.231	0.098	-0.042	-0.420	-0.371	0.007	0.343	0.119	-0.385	-0.357	-0.266	0.007	0.294	0.196	0.203	0.189	0.077	0.070	0.441	-0.385	-0.168	-0.252	-0.455
Fo11	0.056	-0.238	-0.126	-0.063	0.126	-0.091	-0.091	-0.056	-0.287	-0.203	-0.175	-0.280	-0.098	0.231	0.364	0.287	0.112	0.224	-0.007	0.217	0.028	-0.161	0.105	0.259	0.105	0.049	-0.147	-0.021	-0.622	-0.566	-0.469	-0.189
Py1	0.189	0.063	0.210	0.273	0.252	0.161	0.168	0.000	0.203	-0.378	-0.168	-0.364	0.168	0.371	0.469	0.748	0.504	0.566	0.273	0.504	0.322	0.147	0.315	0.552	0.168	0.308	0.063	0.510	0.049	-0.105	-0.287	0.196
Tz1	-0.028	-0.007	-0.126	-0.119	-0.084	-0.210	-0.273	0.259	0.056	-0.608	-0.280	-0.287	-0.035	0.119	0.462	0.490	0.189	0.105	-0.196	0.014	-0.147	-0.245	0.126	0.259	0.098	0.021	0.196	0.399	-0.308	-0.315	-0.462	-0.056
F8	0.021	0.154	0.350	0.175	0.105	0.105	-0.014	0.105	0.147	-0.245	0.105	0.021	-0.322	0.077	0.042	0.510	0.371	0.364	0.189	0.245	0.112	0.105	0.322	0.448	0.294	0.301	0.420	0.580	-0.014	-0.196	-0.364	-0.028
Py7	-0.035	-0.070	0.084	0.168	0.196	0.105	0.119	0.210	0.231	-0.168	0.000	-0.119	-0.168	0.091	0.147	0.678	0.315	0.531	0.217	0.399	0.203	0.168	-0.021	0.350	0.014	0.049	0.469	0.531	-0.077	-0.252	-0.420	0.322
F1	0.077	0.231	0.280	0.007	0.028	-0.070	-0.021	-0.126	-0.308	-0.028	0.105	0.126	-0.189	0.252	0.189	-0.056	0.322	0.014	0.021	0.007	0.028	-0.217	0.350	0.161	0.238	0.329	-0.161	0.070	-0.049	-0.028	0.217	-0.259
Pd2	0.112	0.056	0.294	0.126	0.056	0.189	0.000	-0.524	-0.077	-0.476	-0.245	-0.483	-0.189	0.175	0.175	0.168	0.462	0.140	0.077	0.091	0.343	0.203	0.755	0.455	0.385	0.545	-0.406	0.294	0.168	-0.028	0.014	-0.294
F3	0.399	0.252	0.385	0.252	0.126	0.084	0.049	-0.084	-0.070	0.238	0.161	0.070	0.224	0.280	0.105	0.105	0.070	0.007	0.245	0.210	-0.105	-0.021	0.427	0.287	0.182	0.357	-0.497	-0.147	-0.112	0.028	0.063	-0.371
Fo9	0.182	0.364	0.350	0.273	0.105	0.210	-0.098	0.077	0.189	-0.063	0.217	0.210	0.084	-0.042	-0.028	0.000	0.196	-0.203	0.084	-0.035	-0.084	0.091	0.615	0.427	0.580	0.517	-0.252	0.007	0.098	0.336	0.168	-0.510
Fo25	0.070	0.161	0.462	0.531	0.406	0.357	0.343	-0.056	0.091	0.238	0.182	0.140	0.406	0.280	0.000	0.378	0.531	0.594	0.629	0.678	0.147	0.000	0.308	0.573	0.224	0.497	-0.231	0.063	0.455	0.552	0.329	0.217
Pz9	-0.140	-0.175	-0.133	0.133	0.084	-0.049	-0.119	0.119	-0.021	-0.147	-0.238	-0.224	0.420	0.056	0.175	0.364	0.189	0.203	0.126	0.315	-0.308	-0.357	0.070	0.343	-0.056	0.105	-0.434	-0.098	-0.049	0.245	-0.007	0.042
Tp6	-0.126	0.182	0.245	0.014	-0.140	-0.112	-0.217	0.119	-0.035	-0.280	-0.070	0.063	0.007	0.049	0.000	0.168	0.217	0.112	0.063	0.035	-0.371	-0.420	0.252	0.224	0.105	0.217	0.077	0.245	0.231	0.210	0.014	-0.028
S6	-0.322	-0.112	-0.273	-0.329	-0.399	-0.175	-0.217	-0.161	0.056	-0.392	-0.350	-0.322	-0.287	-0.336	-0.161	-0.273	-0.077	-0.371	-0.413	-0.524	-0.077	-0.007	-0.035	-0.371	-0.280	-0.189	-0.028	0.161	0.385	0.189	0.280	0.133
F4	-0.448	-0.217	-0.224	-0.385	-0.399	-0.378	-0.266	-0.308	-0.273	-0.490	-0.392	-0.406	-0.476	-0.168	-0.042	0.007	0.168	-0.077	-0.378	-0.294	0.007	-0.217	-0.105	-0.231	-0.343	-0.182	0.091	0.441	0.119	-0.140	-0.028	0.105
Po5	-0.636	-0.343	-0.322	-0.378	-0.385	-0.336	-0.210	-0.056	-0.168	-0.168	-0.252	-0.133	-0.462	-0.378	-0.378	-0.084	-0.056	0.000	-0.259	-0.252	-0.210	-0.280	-0.490	-0.420	-0.566	-0.434	0.364	0.273	0.231	0.000	0.021	0.427
F22	-0.056	0.294	0.105	0.126	0.007	0.126	0.196	0.231	0.392	0.084	0.133	0.175	-0.028	-0.098	-0.035	0.021	0.259	-0.077	0.007	-0.126	0.119	0.203	0.028	-0.098	-0.105	0.091	0.126	0.259	0.664	0.608	0.699	0.392
Fo20	0.217	0.448	0.371	0.483	0.399	0.524	0.573	0.343	0.615	0.287	0.301	0.329	0.343	0.196	0.119	0.168	0.434	0.329	0.455	0.294	0.336	0.378	0.175	0.189	0.140	0.343	0.161	0.133	**0.916**	0.846	**0.867**	0.664
Py10	0.224	0.357	0.294	0.434	0.406	0.517	0.615	0.259	0.538	0.364	0.287	0.301	0.343	0.203	0.098	0.070	0.336	0.322	0.441	0.287	0.399	0.413	0.063	0.084	0.070	0.245	0.140	0.021	0.839	0.748	0.832	0.685
S1	0.105	0.035	0.245	0.175	0.126	0.168	0.252	-0.140	-0.168	0.504	0.112	0.231	0.427	0.210	-0.210	-0.301	-0.112	0.217	0.427	0.273	-0.189	-0.231	-0.049	-0.077	-0.140	0.070	-0.329	-0.517	0.378	0.399	0.434	0.238
F34	-0.084	0.175	0.182	-0.042	-0.084	-0.140	0.098	-0.084	-0.175	0.063	0.035	0.098	-0.112	0.175	0.056	-0.014	0.280	0.112	0.035	0.014	0.042	-0.203	-0.028	-0.119	-0.203	0.056	0.042	0.217	0.343	0.203	0.413	0.238
S2	0.238	0.308	0.175	-0.112	-0.112	-0.063	0.210	0.119	0.056	0.196	0.126	0.259	0.077	0.252	0.091	-0.231	-0.182	0.000	0.021	-0.140	0.028	-0.035	-0.259	-0.441	-0.259	-0.210	0.399	0.028	0.343	0.035	0.252	0.392
F12	-0.594	-0.448	-0.315	-0.413	-0.427	-0.427	-0.566	-0.007	-0.427	-0.343	-0.413	-0.210	-0.273	-0.315	-0.308	-0.098	-0.210	-0.056	-0.231	-0.203	**-0.783**	**-0.783**	-0.175	-0.133	-0.322	-0.266	-0.056	-0.105	-0.203	-0.175	-0.315	-0.042
Pn1	-0.552	-0.483	-0.657	-0.580	-0.476	-0.497	-0.378	0.112	-0.189	-0.322	-0.420	-0.210	-0.140	-0.357	-0.175	-0.189	-0.476	-0.161	-0.469	-0.378	-0.406	-0.455	**-0.790**	-0.608	-0.615	**-0.769**	0.455	-0.007	-0.161	-0.287	-0.399	0.350
Tp2	-0.098	-0.315	-0.105	-0.378	-0.434	-0.287	-0.531	-0.678	-0.566	-0.301	-0.329	-0.406	-0.329	-0.238	-0.343	-0.483	-0.434	-0.476	-0.350	-0.420	-0.301	-0.210	0.280	-0.133	0.063	-0.035	-0.524	-0.287	-0.497	-0.531	-0.531	**-0.839**
S5	0.095	0.021	0.035	-0.259	-0.326	-0.347	-0.459	-0.284	-0.340	-0.326	-0.116	-0.217	-0.364	-0.049	0.032	-0.056	-0.151	-0.413	-0.375	-0.336	-0.172	-0.123	0.319	0.028	0.154	0.063	-0.221	0.140	-0.599	-0.585	-0.539	**-0.802**
F13	0.105	0.056	0.175	0.245	0.252	0.161	0.189	0.266	0.000	0.748	0.469	0.531	0.049	-0.021	-0.287	-0.070	-0.133	0.077	0.336	0.238	-0.189	0.007	-0.133	0.028	-0.014	0.028	-0.063	-0.378	-0.140	0.105	0.182	0.000
F18	-0.112	0.021	0.098	0.070	-0.140	-0.077	-0.133	0.098	0.000	0.399	0.189	0.322	0.182	-0.252	-0.510	-0.161	-0.301	-0.154	0.119	-0.007	-0.483	-0.210	-0.175	-0.112	-0.189	-0.105	-0.112	-0.238	0.119	0.308	0.063	-0.098
Po7	-0.329	-0.161	-0.189	-0.252	-0.399	-0.392	-0.378	0.112	-0.175	0.406	0.189	0.329	-0.280	-0.538	-0.685	-0.301	-0.545	-0.476	-0.259	-0.357	-0.580	-0.238	-0.483	-0.448	-0.427	-0.448	0.077	-0.161	-0.280	-0.091	-0.189	-0.273
	Pz4	**F30**	**Tz2**	**Pd5**	**Fo11**	**Py1**	**Tz1**	**F8**	**Py7**	**F1**	**Pd2**	**F3**	**Fo9**	**Fo25**	**Pz9**	**Tp6**	**S6**	**F4**	**Po5**	**F22**	**Fo20**	**Py10**	**S1**	**F34**	**S2**	**F12**	**Pn1**	**Tp2**	**S5**	**F13**	**F18**	**Po7**
Fo1	-0.168	0.056	-0.021	0.119	0.182	-0.224	0.084	0.231	0.112	0.070	-0.524	0.119	0.238	-0.070	-0.112	0.077	-0.629	-0.629	-0.357	-0.322	-0.147	-0.168	0.014	-0.280	0.014	0.070	-0.098	-0.140	0.063	0.594	0.217	0.308
Fo5	-0.042	0.154	0.084	-0.021	0.112	-0.182	0.007	0.245	0.147	0.014	-0.517	0.091	0.203	0.056	-0.112	0.084	-0.650	-0.650	-0.329	-0.308	-0.049	-0.063	0.119	-0.266	0.049	0.035	-0.098	-0.196	-0.060	0.594	0.259	0.266
Po1	-0.028	0.273	0.091	0.287	0.329	0.133	0.329	0.378	0.350	-0.035	-0.406	0.203	0.252	0.028	0.084	0.091	**-0.734**	-0.657	-0.497	-0.406	-0.189	-0.238	-0.154	-0.392	-0.133	-0.021	-0.161	-0.203	0.137	0.462	0.140	0.154

Py5	-0.049	0.329	0.210	0.210	0.119	0.035	0.161	0.490	0.322	0.056	-0.336	0.126	0.301	0.112	-0.126	0.189	-0.643	-0.517	-0.322	-0.287	-0.098	-0.161	-0.098	-0.231	-0.021	-0.035	-0.189	-0.168	0.123	0.441	0.217	0.245
Pd3	-0.189	0.056	-0.042	-0.280	0.476	0.049	0.021	0.336	0.224	0.441	-0.133	0.140	-0.028	0.308	-0.063	0.098	**-0.776**	-0.238	-0.182	-0.531	-0.343	-0.301	0.175	0.098	-0.056	0.112	-0.203	-0.007	0.109	0.448	-0.042	-0.007
Po6	-0.007	0.175	0.147	-0.427	0.070	-0.105	-0.119	0.350	0.238	0.168	-0.343	-0.154	-0.007	0.371	-0.133	0.224	-0.566	-0.273	0.084	-0.280	0.014	0.021	0.301	0.049	0.077	0.231	0.063	-0.238	-0.284	0.441	0.182	0.112
S7	0.266	0.301	0.189	-0.392	0.294	0.126	-0.098	0.168	0.336	-0.063	-0.224	-0.112	-0.210	0.287	-0.028	-0.168	-0.580	-0.406	-0.147	-0.350	0.063	0.147	0.224	-0.175	-0.049	-0.077	-0.042	-0.308	-0.392	0.378	-0.098	-0.224
Po9	-0.147	-0.063	0.000	0.301	-0.112	-0.196	0.350	0.301	-0.056	0.042	-0.434	0.175	0.664	-0.056	0.077	0.517	-0.392	-0.566	-0.406	-0.182	-0.119	-0.280	-0.091	-0.315	0.000	0.266	-0.014	0.014	0.270	0.259	0.455	0.392
Fo3	-0.238	0.077	-0.112	0.657	0.308	0.315	0.713	0.643	0.455	-0.007	-0.161	0.210	0.392	-0.168	0.126	0.406	-0.448	-0.280	-0.315	-0.392	-0.483	-0.629	-0.524	-0.357	-0.189	0.259	0.021	0.119	0.592	0.056	0.154	0.287
Fo12	-0.343	-0.028	-0.210	0.441	0.517	0.126	0.566	0.420	0.357	0.112	-0.399	0.259	0.245	-0.112	0.231	0.252	-0.671	-0.483	-0.378	-0.469	-0.441	-0.524	-0.266	-0.322	-0.210	0.336	-0.028	-0.063	0.361	0.441	0.126	0.273
Fo10	-0.063	0.098	-0.112	0.070	0.636	0.434	0.441	0.510	0.538	0.000	-0.175	0.336	0.140	0.287	0.336	0.168	**-0.937**	-0.608	-0.517	**-0.741**	-0.448	-0.476	-0.021	-0.420	-0.336	0.210	-0.182	0.028	0.221	0.427	0.112	0.007
Fo19	-0.021	0.091	-0.021	-0.035	0.483	0.371	0.399	0.622	0.462	0.126	-0.105	0.350	0.280	0.350	0.203	0.364	**-0.909**	-0.552	-0.483	**-0.713**	-0.427	-0.490	0.049	-0.308	-0.203	0.252	-0.217	0.133	0.305	0.364	0.203	0.049
Po3	0.049	0.070	-0.021	-0.014	0.490	0.392	0.413	0.587	0.441	0.056	-0.084	0.413	0.322	0.315	0.231	0.329	**-0.909**	-0.650	-0.594	**-0.734**	-0.413	-0.476	0.056	-0.406	-0.217	0.210	-0.266	0.175	0.322	0.364	0.210	0.014
F2	-0.231	-0.028	-0.182	0.189	0.455	0.231	0.287	0.427	0.273	0.084	-0.105	0.336	0.420	0.280	0.210	0.147	**-0.909**	-0.643	-0.657	**-0.727**	-0.504	-0.538	-0.084	-0.455	-0.434	0.021	-0.329	0.224	0.371	0.357	0.182	0.091
Fo23	-0.140	0.119	-0.035	0.217	0.448	0.273	0.392	0.559	0.392	0.119	-0.154	0.280	0.378	0.224	0.140	0.245	**-0.874**	-0.580	-0.552	-0.643	-0.427	-0.490	-0.147	-0.378	-0.301	0.084	-0.259	0.084	0.347	0.350	0.140	0.084
F19	0.091	0.189	0.084	0.189	0.441	0.315	0.315	0.448	0.371	-0.028	-0.112	0.427	0.350	0.175	0.147	0.091	**-0.839**	**-0.762**	**-0.727**	-0.587	-0.266	-0.308	-0.049	-0.497	-0.238	-0.077	-0.413	0.063	0.273	0.427	0.126	-0.014
Fo2	0.112	0.252	0.105	0.308	0.531	0.538	0.622	0.580	0.538	-0.035	-0.063	0.357	0.350	0.210	0.357	0.273	**-0.783**	-0.608	-0.629	-0.573	-0.301	-0.399	-0.217	-0.476	-0.329	0.105	-0.259	-0.035	0.298	0.231	0.056	-0.112
F9	0.028	0.385	0.217	0.538	0.497	0.664	0.594	0.657	0.629	0.021	0.224	0.231	0.336	0.266	0.308	0.189	-0.566	-0.294	-0.455	-0.378	-0.217	-0.322	-0.448	-0.350	-0.497	-0.049	-0.357	-0.077	0.305	0.028	-0.140	-0.238
Tp5	-0.042	0.322	0.105	0.378	0.524	0.413	0.322	0.448	0.413	0.140	0.105	0.231	0.224	0.147	0.042	-0.084	**-0.713**	-0.378	-0.566	-0.504	-0.350	-0.357	-0.357	-0.301	-0.371	-0.315	-0.427	0.014	0.350	0.161	-0.210	-0.189
Fo26	0.203	0.357	0.280	0.154	0.420	0.643	0.427	0.559	0.490	0.042	0.224	0.385	0.378	0.524	0.392	0.266	**-0.713**	-0.462	-0.587	-0.469	-0.140	-0.231	-0.077	-0.315	-0.406	-0.049	-0.462	-0.014	0.200	0.119	0.035	-0.280
Pz3	0.231	0.259	0.217	0.168	0.601	0.587	0.364	0.350	0.357	0.133	0.329	0.476	0.273	0.329	0.322	0.000	-0.664	-0.476	**-0.734**	-0.476	-0.168	-0.196	-0.098	-0.315	-0.392	-0.224	-0.615	0.049	0.259	0.147	-0.217	-0.462
Pz7	0.343	0.378	0.245	0.112	0.587	0.755	0.448	0.329	0.462	-0.007	0.294	0.441	0.126	0.399	0.469	0.014	-0.629	-0.392	-0.643	-0.462	-0.154	-0.182	-0.091	-0.294	-0.371	-0.203	-0.462	-0.049	0.179	0.028	-0.189	-0.483
Py12	0.119	0.280	0.210	0.252	0.413	0.720	0.469	0.455	0.448	-0.007	0.315	0.462	0.399	0.594	0.622	0.280	-0.587	-0.378	-0.566	-0.378	-0.112	-0.224	-0.077	-0.301	-0.545	0.007	-0.483	0.000	0.200	0.049	0.077	-0.280
S4	0.601	0.420	0.259	0.007	0.455	0.811	0.559	0.357	0.629	-0.385	0.161	0.042	-0.021	0.294	0.483	0.028	-0.427	-0.364	-0.413	-0.420	-0.021	-0.049	-0.175	-0.504	-0.350	-0.042	-0.014	-0.203	-0.137	-0.196	-0.224	-0.587
F6	0.217	0.294	0.154	0.021	0.587	0.636	0.678	0.510	0.545	0.105	0.035	0.063	0.140	0.371	0.469	0.336	-0.657	-0.259	-0.343	-0.517	-0.238	-0.315	-0.189	-0.224	-0.343	0.224	-0.021	-0.210	0.063	-0.049	-0.189	-0.427
Ox1	0.361	0.410	0.231	0.333	0.504	0.799	**0.897**	0.694	0.750	-0.137	0.105	0.025	0.133	0.088	0.361	0.361	-0.413	-0.175	-0.277	-0.403	-0.224	-0.347	-0.487	-0.368	-0.252	0.210	0.098	-0.172	0.211	-0.256	-0.224	-0.382
Ox2	0.142	0.135	0.139	0.352	0.363	0.424	0.562	0.327	0.128	0.164	0.153	0.559	0.552	0.121	0.331	0.306	-0.537	-0.481	**-0.790**	-0.388	-0.263	-0.384	-0.199	-0.299	-0.210	-0.093	-0.466	0.164	0.538	0.007	0.032	-0.178
S3	0.021	-0.203	-0.196	-0.042	0.497	0.245	0.259	0.238	0.084	0.098	0.028	0.699	0.371	0.168	0.252	0.147	**-0.811**	**-0.741**	**-0.853**	**-0.734**	-0.476	-0.497	0.196	-0.427	-0.175	0.049	-0.483	0.448	0.501	0.385	0.217	0.007
S8	0.154	0.126	0.035	-0.126	0.573	0.413	0.140	0.252	0.301	0.063	0.063	0.615	0.091	0.343	0.259	-0.049	**-0.846**	-0.643	**-0.706**	-0.629	-0.280	-0.252	0.238	-0.301	-0.189	-0.119	-0.538	0.147	0.228	0.462	0.063	-0.147
F10	0.077	0.063	-0.182	0.084	0.504	0.455	0.713	0.490	0.510	-0.168	-0.322	0.119	0.154	0.091	0.392	0.343	**-0.762**	-0.517	-0.427	**-0.734**	-0.517	-0.587	-0.189	-0.504	-0.224	0.357	0.231	0.014	0.245	0.084	0.133	0.007
Tz3	-0.133	0.098	-0.168	0.189	0.594	0.587	0.601	0.741	0.678	0.042	0.035	0.168	0.105	0.224	0.217	0.308	**-0.755**	-0.224	-0.266	**-0.748**	-0.636	-0.692	-0.287	-0.308	-0.336	0.287	0.000	0.196	0.438	0.063	0.014	0.007
F25	-0.217	-0.014	-0.266	0.280	0.664	0.224	0.301	0.287	0.329	0.021	-0.231	0.483	0.126	-0.035	0.140	-0.133	**-0.909**	-0.699	**-0.706**	**-0.734**	-0.573	-0.545	-0.119	-0.476	-0.273	-0.070	-0.336	0.175	0.438	0.538	0.056	0.147
Fo8	-0.056	-0.133	-0.189	-0.007	0.636	0.147	0.455	0.427	0.301	0.210	-0.210	0.252	0.189	-0.042	0.077	0.210	**-0.797**	-0.566	-0.510	**-0.713**	-0.490	-0.510	-0.049	-0.350	-0.112	0.301	-0.105	0.140	0.326	0.385	-0.042	-0.049
Fo22	-0.210	0.084	-0.112	0.014	0.399	0.154	0.329	0.510	0.378	0.140	-0.350	0.112	0.217	0.259	0.126	0.294	**-0.881**	-0.483	-0.322	-0.643	-0.441	-0.483	-0.035	-0.252	-0.203	0.245	-0.014	-0.021	0.193	0.392	0.203	0.189
Pd1	0.280	0.524	0.455	0.126	0.224	0.392	0.126	0.357	0.371	-0.035	0.042	0.252	0.273	0.427	0.175	0.014	-0.601	-0.531	-0.517	-0.217	0.154	0.112	-0.007	-0.273	-0.259	-0.301	-0.490	-0.266	-0.077	0.287	0.014	-0.231
Pz1	0.336	0.545	0.462	0.301	0.273	0.601	0.378	0.336	0.441	-0.147	0.126	0.259	0.315	0.441	0.469	0.091	-0.469	-0.462	-0.531	-0.140	0.196	0.105	-0.147	-0.343	-0.413	-0.217	-0.420	-0.343	-0.084	0.091	-0.035	-0.357
Pz2	0.364	0.636	0.601	0.231	0.077	0.476	0.189	0.287	0.343	-0.105	0.105	0.245	0.322	0.490	0.343	0.091	-0.378	-0.413	-0.441	0.035	0.364	0.273	-0.042	-0.203	-0.280	-0.308	-0.476	-0.413	-0.172	0.140	0.042	-0.280
Pz12	0.308	0.615	0.559	0.364	0.168	0.531	0.287	0.427	0.441	-0.070	0.182	0.238	0.343	0.392	0.259	0.098	-0.392	-0.371	-0.448	-0.035	0.245	0.147	-0.196	-0.245	-0.315	-0.294	-0.490	-0.322	-0.025	0.098	-0.042	-0.287
Fo14	0.280	0.385	0.385	0.378	0.231	0.406	0.294	0.406	0.315	-0.070	0.168	0.343	0.490	0.210	0.147	0.063	-0.497	-0.594	-0.699	-0.238	0.063	-0.021	-0.203	-0.448	-0.308	-0.308	-0.559	-0.056	0.175	0.154	-0.021	-0.252
F11	0.343	0.448	0.434	0.329	0.126	0.531	0.455	0.552	0.455	-0.189	0.091	0.266	0.524	0.357	0.322	0.329	-0.476	-0.552	-0.552	-0.217	0.112	-0.035	-0.175	-0.455	-0.308	-0.063	-0.350	-0.147	0.084	0.063	0.154	-0.182
Py2	0.385	0.573	0.538	0.364	0.084	0.434	0.322	0.399	0.350	-0.140	0.042	0.308	0.434	0.245	0.189	0.147	-0.420	-0.538	-0.580	-0.077	0.210	0.098	-0.175	-0.350	-0.168	-0.301	-0.448	-0.252	0.067	0.126	0.077	-0.175
Tp3	0.336	0.510	0.476	0.406	0.098	0.476	0.385	0.427	0.343	-0.140	0.105	0.273	0.524	0.294	0.259	0.196	-0.434	-0.524	-0.615	-0.140	0.140	0.014	-0.238	-0.406	-0.294	-0.273	-0.434	-0.182	0.112	0.028	0.070	-0.217
Py4	0.154	0.490	0.343	0.497	0.196	0.392	0.336	0.231	0.287	-0.119	0.021	0.070	0.385	0.231	0.294	-0.042	-0.413	-0.420	-0.538	-0.119	0.091	0.021	-0.378	-0.385	-0.462	-0.385	-0.336	-0.322	-0.004	0.000	-0.119	-0.280
Py13	0.294	0.378	0.455	0.483	0.133	0.427	0.308	0.294	0.217	-0.070	0.287	0.406	0.545	0.217	0.273	0.098	-0.259	-0.476	-0.657	-0.007	0.224	0.105	-0.210	-0.364	-0.329	-0.308	-0.622	-0.098	0.154	0.056	-0.021	-0.294
Py3	0.448	0.545	0.406	0.231	0.280	0.685	0.601	0.483	0.552	-0.238	-0.014	0.224	0.294	0.350	0.469	0.280	-0.545	-0.497	-0.517	-0.301	0.035	-0.084	-0.189	-0.420	-0.266	-0.042	-0.168	-0.287	0.021	-0.014	0.056	-0.280
Tp4	0.490	0.615	0.469	0.168	0.084	0.504	0.308	0.378	0.441	-0.329	-0.119	0.287	0.259	0.294	0.238	0.112	-0.545	-0.622	-0.559	-0.231	0.126	0.049	-0.056	-0.427	-0.091	-0.273	-0.266	-0.259	-0.014	0.147	0.196	-0.098
Fo7	0.231	0.371	0.287	0.126	0.308	0.343	0.182	0.378	0.371	-0.091	-0.063	0.343	0.301	0.308	0.161	0.021	**-0.727**	**-0.706**	-0.643	-0.399	-0.014	-0.049	0.014	-0.427	-0.238	-0.210	-0.455	-0.119	0.046	0.392	0.098	-0.119
Fo16	0.294	0.294	0.308	0.217	0.189	0.315	0.259	0.462	0.350	-0.161	-0.014	0.371	0.455	0.189	0.105	0.154	-0.587	**-0.741**	-0.664	-0.336	0.028	-0.056	-0.035	-0.517	-0.175	-0.119	-0.441	-0.007	0.137	0.322	0.175	-0.070
Fo17	0.133	0.406	0.350	0.168	0.287	0.483	0.287	0.531	0.455	0.056	0.161	0.147	0.378	0.538	0.280	0.217	-0.608	-0.385	-0.413	-0.308	0.021	-0.063	-0.112	-0.259	-0.441	-0.077	-0.399	-0.175	-0.004	0.140	-0.007	-0.273
Py6	0.133	0.322	0.224	0.189	0.462	0.490	0.315	0.497	0.469	0.021	0.105	0.336	0.315	0.385	0.252	0.091	**-0.755**	-0.566	-0.608	-0.483	-0.140	-0.189	-0.091	-0.385	-0.392	-0.119	-0.476	-0.049	0.147	0.287	-0.014	-0.224
F29	0.308	0.504	0.552	0.189	-0.112	0.056	-0.035	0.049	0.077	-0.112	-0.161	0.196	0.294	0.203	0.105	-0.021	-0.224	-0.510	-0.385	0.217	0.497	0.434	0.070	-0.175	-0.028	-0.322	-0.406	-0.462	-0.266	0.343	0.126	-0.070
Po4	0.378	0.524	0.629	0.077	-0.266	0.021	-0.021	0.098	0.014	-0.063	-0.133	0.000	0.406	0.301	0.070	0.140	-0.161	-0.413	-0.294	0.252	0.552	0.462	0.056	-0.119	-0.021	-0.280	-0.287	-0.483	-0.340	0.147	0.126	-0.154
F33	0.259	0.538	0.524	0.301	-0.105	0.126	0.014	0.196	0.154	-0.126	-0.105	0.182	0.385	0.210	0.028	-0.007	-0.322	-0.524	-0.455	0.091	0.350	0.280	-0.070	-0.259	-0.105	-0.406	-0.434	-0.343	-0.112	0.266	0.133	-0.035

Fo21	0.168	0.392	0.413	0.315	-0.042	0.056	0.112	0.203	0.140	-0.098	-0.203	0.189	0.420	0.140	0.098	0.091	-0.336	-0.573	-0.441	0.056	0.301	0.210	-0.056	-0.294	-0.112	-0.196	-0.350	-0.336	-0.098	0.343	0.175	0.028
F36	0.238	0.497	0.427	0.182	-0.007	0.042	0.028	0.063	0.147	-0.154	-0.336	0.091	0.210	0.147	0.098	-0.077	-0.385	-0.573	-0.392	0.049	0.336	0.301	0.000	-0.259	-0.063	-0.301	-0.252	-0.497	-0.270	0.378	0.105	-0.021
Fo6	0.280	0.490	0.524	0.175	-0.140	0.014	0.021	0.147	0.091	-0.084	-0.231	0.098	0.364	0.189	0.035	0.077	-0.294	-0.524	-0.371	0.133	0.413	0.336	0.014	-0.203	-0.014	-0.266	-0.308	-0.434	-0.224	0.301	0.154	-0.028
F15	0.273	0.455	0.483	0.343	-0.049	0.098	-0.028	0.049	-0.014	-0.042	0.007	0.406	0.413	0.091	-0.007	-0.119	-0.294	-0.559	-0.650	0.084	0.287	0.238	-0.049	-0.238	-0.042	-0.566	-0.629	-0.182	0.081	0.266	0.077	-0.070
Ox3	0.259	0.413	0.441	0.343	0.021	0.126	0.070	0.070	0.035	-0.070	-0.049	0.364	0.434	0.119	0.112	-0.063	-0.343	-0.615	-0.657	0.035	0.280	0.217	-0.056	-0.308	-0.126	-0.441	-0.559	-0.238	0.018	0.287	0.077	-0.105
F23	0.357	0.434	0.462	0.154	0.126	0.231	0.063	0.203	0.119	0.007	0.049	0.420	0.399	0.224	0.042	-0.028	-0.517	-0.643	**-0.720**	-0.161	0.168	0.126	0.028	-0.294	-0.084	-0.448	-0.622	-0.119	0.088	0.287	0.042	-0.196
F24	0.413	0.587	0.601	0.084	-0.112	0.217	-0.049	0.224	0.126	-0.056	0.021	0.301	0.378	0.350	-0.007	0.035	-0.413	-0.524	-0.538	-0.007	0.315	0.259	0.077	-0.182	-0.007	-0.490	-0.538	-0.224	-0.046	0.210	0.154	-0.119
F14	0.650	**0.895**	0.769	0.000	0.028	0.497	0.252	0.217	0.427	-0.140	-0.063	-0.140	-0.056	0.350	0.203	0.035	-0.203	-0.133	-0.105	0.175	0.490	0.441	-0.112	0.000	0.014	-0.329	-0.063	**-0.748**	-0.441	-0.098	-0.203	-0.462
Py8	0.608	0.629	0.636	0.063	0.014	0.364	0.406	0.238	0.238	-0.077	-0.168	0.224	0.252	0.182	0.245	0.294	-0.301	-0.427	-0.413	0.056	0.343	0.224	-0.007	-0.126	0.168	-0.133	-0.168	-0.476	-0.105	0.049	0.070	-0.231
F17	0.350	0.636	0.601	0.182	-0.252	0.350	0.091	0.210	0.161	-0.175	0.007	0.210	0.441	0.538	0.308	0.217	-0.280	-0.371	-0.385	0.126	0.385	0.266	0.021	-0.147	-0.168	-0.350	-0.364	-0.350	-0.144	0.014	0.294	-0.070
Pd4	0.531	0.636	0.699	-0.021	-0.168	0.392	0.140	0.287	0.280	-0.182	-0.007	0.189	0.343	0.566	0.343	0.308	-0.273	-0.420	-0.301	0.140	0.531	0.406	0.175	-0.140	-0.070	-0.126	-0.308	-0.462	-0.329	0.119	0.252	-0.196
Fo4	0.594	0.483	0.545	-0.077	0.056	0.189	-0.028	0.021	-0.035	0.077	0.112	0.399	0.182	0.070	-0.140	-0.126	-0.322	-0.448	-0.636	-0.056	0.217	0.224	0.105	-0.084	0.238	-0.594	-0.552	-0.098	0.095	0.105	-0.112	-0.329
F26	0.462	0.615	0.755	0.070	-0.238	0.063	-0.007	0.154	-0.070	0.231	0.056	0.252	0.364	0.161	-0.175	0.182	-0.112	-0.217	-0.343	0.294	0.448	0.357	0.035	0.175	0.308	-0.448	-0.483	-0.315	0.021	0.056	0.021	-0.161
Fo15	0.413	0.517	0.706	-0.133	-0.126	0.210	-0.126	0.350	0.084	0.280	0.294	0.385	0.350	0.462	-0.133	0.245	-0.273	-0.224	-0.322	0.105	0.371	0.294	0.245	0.182	0.175	-0.315	-0.657	-0.105	0.035	0.175	0.098	-0.189
Pz11	0.392	0.615	0.671	0.000	-0.063	0.273	-0.119	0.175	0.168	0.007	0.126	0.252	0.273	0.531	0.133	0.014	-0.329	-0.385	-0.378	0.126	0.483	0.434	0.175	-0.042	-0.112	-0.413	-0.580	-0.378	-0.259	0.245	0.070	-0.252
Py11	0.427	0.580	0.587	-0.084	0.126	0.252	-0.084	0.105	0.196	0.028	0.056	0.126	0.105	0.406	0.084	-0.140	-0.399	-0.399	-0.385	0.007	0.399	0.406	0.126	-0.084	-0.112	-0.427	-0.476	-0.434	-0.326	0.252	-0.140	-0.399
Pz5	0.510	0.483	0.622	-0.112	-0.091	0.161	-0.210	0.105	0.105	-0.070	0.189	0.084	0.210	0.357	-0.049	-0.112	-0.175	-0.378	-0.336	0.126	0.524	0.517	0.168	-0.140	-0.063	-0.427	-0.497	-0.287	-0.347	0.161	-0.077	-0.392
Pd7	0.643	0.741	0.755	-0.294	-0.091	0.168	-0.273	-0.014	0.119	-0.021	0.000	0.049	-0.098	0.343	-0.119	-0.217	-0.217	-0.266	-0.210	0.196	0.573	0.615	0.252	0.098	0.210	-0.566	-0.378	-0.531	-0.459	0.189	-0.133	-0.378
F35	0.168	0.504	0.399	0.273	-0.056	0.000	0.259	0.105	0.210	-0.126	-0.524	-0.084	0.077	-0.056	0.119	0.119	-0.161	-0.308	-0.056	0.231	0.343	0.259	-0.140	-0.084	0.119	-0.007	0.112	-0.678	-0.284	0.266	0.098	0.112
Py9	0.531	0.734	0.727	0.287	-0.287	0.203	0.056	0.147	0.231	-0.308	-0.077	-0.070	0.189	0.091	-0.021	-0.035	0.056	-0.273	-0.168	0.392	0.615	0.538	-0.168	-0.175	0.056	-0.427	-0.189	-0.566	-0.340	0.000	0.000	-0.175
Po8	-0.063	0.259	0.196	-0.231	-0.203	-0.378	-0.608	-0.245	-0.168	-0.028	-0.476	0.238	-0.063	0.238	-0.147	-0.280	-0.392	-0.490	-0.168	0.084	0.287	0.364	0.504	0.063	0.196	-0.343	-0.322	-0.301	-0.326	0.748	0.399	0.406
F16	0.056	0.476	0.455	0.098	-0.175	-0.168	-0.280	0.105	0.000	0.105	-0.245	0.161	0.217	0.182	-0.238	-0.070	-0.350	-0.392	-0.252	0.133	0.301	0.287	0.112	0.035	0.126	-0.413	-0.420	-0.329	-0.116	0.469	0.189	0.189
F32	0.007	0.371	0.385	-0.042	-0.280	-0.364	-0.287	0.021	-0.119	0.126	-0.483	0.070	0.210	0.140	-0.224	0.063	-0.322	-0.406	-0.133	0.175	0.329	0.301	0.231	0.098	0.259	-0.210	-0.210	-0.406	-0.217	0.531	0.322	0.329
Pd6	0.587	0.343	0.364	-0.420	-0.098	0.168	-0.035	-0.322	-0.168	-0.189	-0.189	0.224	0.084	0.406	0.420	0.007	-0.287	-0.476	-0.462	-0.028	0.343	0.343	0.427	-0.112	0.077	-0.273	-0.140	-0.329	-0.364	0.049	0.182	-0.280
F5	0.650	0.504	0.559	-0.371	0.231	0.371	0.119	0.077	0.091	0.252	0.175	0.280	-0.042	0.280	0.056	0.049	-0.336	-0.168	-0.378	-0.098	0.196	0.203	0.210	0.175	0.252	-0.315	-0.357	-0.238	-0.049	-0.021	-0.252	-0.538
Tp1	0.601	0.497	0.476	0.007	0.364	0.469	0.462	0.042	0.147	0.189	0.175	0.105	-0.028	0.000	0.175	0.000	-0.161	-0.042	-0.378	-0.035	0.119	0.098	-0.210	0.056	0.091	-0.308	-0.175	-0.343	0.032	-0.287	-0.510	-0.685
Pz8	0.294	0.734	0.538	0.343	0.287	0.748	0.490	0.510	0.678	-0.056	0.168	0.105	0.000	0.378	0.364	0.168	-0.273	0.007	-0.084	0.021	0.168	0.070	-0.301	-0.014	-0.231	-0.098	-0.189	-0.483	-0.056	-0.070	-0.161	-0.301
Pd9	0.329	0.643	0.734	0.119	0.112	0.504	0.189	0.371	0.315	0.322	0.462	0.070	0.196	0.531	0.189	0.217	-0.077	0.168	-0.056	0.259	0.434	0.336	-0.112	0.280	-0.182	-0.210	-0.476	-0.434	-0.151	-0.133	-0.301	-0.545
Fo13	0.566	0.657	0.608	-0.385	0.224	0.566	0.105	0.364	0.531	0.014	0.140	0.007	-0.203	0.594	0.203	0.112	-0.371	-0.077	0.000	-0.077	0.329	0.322	0.217	0.112	0.000	-0.056	-0.161	-0.476	-0.413	0.077	-0.154	-0.476
Fo18	0.476	0.545	0.622	-0.357	-0.007	0.273	-0.196	0.189	0.217	0.021	0.077	0.245	0.084	0.629	0.126	0.063	-0.413	-0.378	-0.259	0.007	0.455	0.441	0.427	0.035	0.021	-0.231	-0.469	-0.350	-0.375	0.336	0.119	-0.259
Fo24	0.427	0.615	0.545	-0.266	0.217	0.504	0.014	0.245	0.399	0.007	0.091	0.210	-0.035	0.678	0.315	0.035	-0.524	-0.294	-0.252	-0.126	0.294	0.287	0.273	0.014	-0.140	-0.203	-0.378	-0.420	-0.336	0.238	-0.007	-0.357
F21	0.566	0.720	0.664	0.007	0.028	0.322	-0.147	0.112	0.203	0.028	0.343	-0.105	-0.084	0.147	-0.308	-0.371	-0.077	0.007	-0.210	0.119	0.336	0.399	-0.189	0.042	0.028	**-0.783**	-0.406	-0.301	-0.172	-0.189	-0.483	-0.580
F28	0.392	0.608	0.559	0.294	-0.161	0.147	-0.245	0.105	0.168	-0.217	0.203	-0.021	0.091	0.000	-0.357	-0.420	-0.007	-0.217	-0.280	0.203	0.378	0.413	-0.231	-0.203	-0.035	**-0.783**	-0.455	-0.210	-0.123	0.007	-0.210	-0.238
F7	0.217	0.056	0.399	0.196	0.105	0.315	0.126	0.322	-0.021	0.350	0.755	0.427	0.615	0.308	0.070	0.252	-0.035	-0.105	-0.490	0.028	0.175	0.063	-0.049	-0.028	-0.259	-0.175	**-0.790**	0.280	0.319	-0.133	-0.175	-0.483
Pz6	0.217	0.392	0.476	0.203	0.259	0.552	0.259	0.448	0.350	0.161	0.455	0.287	0.427	0.573	0.343	0.224	-0.371	-0.231	-0.420	-0.098	0.189	0.084	-0.077	-0.119	-0.441	-0.133	-0.608	-0.133	0.028	0.028	-0.112	-0.448
Po2	0.231	0.203	0.399	0.189	0.105	0.168	0.098	0.294	0.014	0.238	0.385	0.182	0.580	0.224	-0.056	0.105	-0.280	-0.343	-0.566	-0.105	0.140	0.070	-0.140	-0.203	-0.259	-0.322	-0.615	0.063	0.154	-0.014	-0.189	-0.427
Pz10	0.287	0.329	0.587	0.077	0.049	0.308	0.021	0.301	0.049	0.329	0.545	0.357	0.517	0.497	0.105	0.217	-0.189	-0.182	-0.434	0.091	0.343	0.245	0.070	0.056	-0.210	-0.266	**-0.769**	-0.035	0.063	0.028	-0.105	-0.448
F27	0.322	0.636	0.427	0.070	-0.147	0.063	0.196	0.420	0.469	-0.161	-0.406	-0.497	-0.252	-0.231	-0.434	0.077	-0.028	0.091	0.364	0.126	0.161	0.140	-0.329	0.042	0.399	-0.056	0.455	-0.524	-0.221	-0.063	-0.112	0.077
Pn3	0.189	0.636	0.517	0.441	-0.021	0.510	0.399	0.580	0.531	0.070	0.294	-0.147	0.007	0.063	-0.098	0.245	0.161	0.441	0.273	0.259	0.133	0.021	-0.517	0.217	0.028	-0.105	-0.007	-0.287	0.140	-0.378	-0.238	-0.161
F31	0.685	0.594	**0.874**	-0.385	-0.622	0.049	-0.308	-0.014	-0.077	-0.049	0.168	-0.112	0.098	0.455	-0.049	0.231	0.385	0.119	0.231	0.664	**0.916**	0.839	0.378	0.343	0.343	-0.203	-0.161	-0.497	-0.599	-0.140	0.119	-0.280
Pn2	0.329	0.420	0.664	-0.168	-0.566	-0.105	-0.315	-0.196	-0.252	-0.028	-0.028	0.028	0.336	0.552	0.245	0.210	0.189	-0.140	0.000	0.608	0.846	0.748	0.399	0.203	0.035	-0.175	-0.287	-0.531	-0.585	0.105	0.308	-0.091
Pd8	0.336	0.378	0.685	-0.252	-0.469	-0.287	-0.462	-0.364	-0.420	0.217	0.014	0.063	0.168	0.329	-0.007	0.014	0.280	-0.028	0.021	0.699	**0.867**	0.832	0.434	0.413	0.252	-0.315	-0.399	-0.531	-0.539	0.182	0.063	-0.189
F20	0.685	0.720	0.650	-0.455	-0.189	0.196	-0.056	-0.028	0.322	-0.259	-0.294	-0.371	-0.510	0.217	0.042	-0.028	0.133	0.105	0.427	0.392	0.664	0.685	0.238	0.238	0.392	-0.042	0.350	**-0.839**	**-0.802**	0.000	-0.098	-0.273
Pz4	1.000	0.685	0.755	-0.273	-0.154	0.427	0.091	0.091	0.266	-0.294	0.189	-0.126	-0.182	0.077	-0.077	-0.049	0.196	-0.028	-0.042	0.259	0.573	0.566	0.035	-0.028	0.336	-0.350	-0.007	-0.413	-0.399	-0.329	-0.273	-0.594
F30	0.685	1.000	0.832	0.035	-0.105	0.476	0.084	0.182	0.462	-0.210	0.028	-0.238	-0.287	0.203	-0.042	-0.147	0.063	0.126	0.140	0.371	0.566	0.559	-0.182	0.140	0.175	-0.490	-0.014	**-0.748**	-0.459	-0.196	-0.294	-0.413
Tz2	0.755	0.832	1.000	-0.140	-0.329	0.266	-0.056	0.140	0.133	0.091	0.252	-0.084	0.000	0.280	-0.133	0.126	0.287	0.196	0.133	0.608	0.804	0.734	0.063	0.378	0.357	-0.378	-0.252	-0.608	-0.413	-0.210	-0.217	-0.476
Pd5	-0.273	0.035	-0.140	1.000	0.252	0.308	0.504	0.287	0.294	-0.168	0.210	0.077	0.301	-0.378	0.077	-0.112	0.042	-0.035	-0.294	-0.070	-0.301	-0.378	**-0.804**	-0.427	-0.462	-0.182	-0.154	0.154	0.515	-0.217	-0.217	0.014
Fo11	-0.154	-0.105	-0.329	0.252	1.000	0.483	0.531	0.287	0.490	0.196	0.154	0.308	-0.259	-0.203	0.189	-0.210	-0.552	-0.140	-0.364	-0.643	-0.629	-0.566	-0.322	-0.224	-0.294	0.112	-0.175	0.168	0.445	0.182	-0.490	-0.294
Py1	0.427	0.476	0.266	0.308	0.483	1.000	0.650	0.545	0.790	-0.294	0.469	0.035	-0.056	0.273	0.434	0.049	-0.217	0.021	-0.203	-0.294	-0.105	-0.161	-0.448	-0.343	-0.455	-0.063	-0.077	-0.105	0.081	-0.406	-0.357	-0.580
Tz1	0.091	0.084	-0.056	0.504	0.531	0.650	1.000	0.608	0.594	-0.056	0.119	-0.007	0.203	-0.140	0.378	0.413	-0.238	-0.035	-0.210	-0.392	-0.406	-0.545	-0.629	-0.364	-0.315	0.392	0.217	0.014	0.403	-0.357	-0.252	-0.280

F8	0.091	0.182	0.140	0.287	0.287	0.545	0.608	1.000	0.741	0.021	0.273	-0.014	0.259	0.070	-0.105	0.510	-0.322	-0.021	-0.021	-0.406	-0.322	-0.455	-0.420	-0.252	-0.140	0.364	0.014	0.210	0.406	-0.147	-0.049	-0.070
Py7	0.266	0.462	0.133	0.294	0.490	0.790	0.594	0.741	1.000	-0.378	0.105	-0.168	-0.280	0.021	0.098	0.014	-0.322	-0.021	0.063	-0.392	-0.238	-0.259	-0.490	-0.385	-0.245	0.126	0.217	-0.161	0.042	-0.133	-0.266	-0.231
F1	-0.294	-0.210	0.091	-0.168	0.196	-0.294	-0.056	0.021	-0.378	1.000	0.259	0.273	0.154	0.028	-0.217	0.308	-0.007	0.357	0.063	0.140	-0.049	-0.084	0.175	0.720	0.273	0.175	-0.406	0.105	0.336	0.154	-0.238	-0.105
Pd2	0.189	0.028	0.252	0.210	0.154	0.469	0.119	0.273	0.105	0.259	1.000	0.126	0.231	0.168	-0.028	0.028	0.308	0.406	-0.105	0.091	0.056	0.014	-0.280	0.098	-0.308	-0.224	-0.524	0.357	0.305	-0.524	-0.490	-0.643
F3	-0.126	-0.238	-0.084	0.077	0.308	0.035	-0.007	-0.014	-0.168	0.273	0.126	1.000	0.294	0.063	0.196	0.084	-0.413	-0.510	-0.671	-0.231	-0.182	-0.217	0.378	-0.042	0.070	0.007	**-0.748**	0.399	0.532	0.580	0.308	0.224
Fo9	-0.182	-0.287	0.000	0.301	-0.259	-0.056	0.203	0.259	-0.280	0.154	0.231	0.294	1.000	0.287	0.231	0.601	-0.133	-0.343	-0.497	-0.098	-0.056	-0.252	-0.042	-0.266	-0.350	0.161	-0.378	0.413	0.399	-0.105	0.420	0.112
Fo25	0.077	0.203	0.280	-0.378	-0.203	0.273	-0.140	0.070	0.021	0.028	0.168	0.063	0.287	1.000	0.580	0.371	-0.252	-0.063	-0.014	0.014	0.308	0.231	0.448	0.126	-0.385	0.105	-0.203	-0.196	-0.403	-0.007	0.364	-0.147
Pz9	-0.077	-0.042	-0.133	0.077	0.189	0.434	0.378	-0.105	0.098	-0.217	-0.028	0.196	0.231	0.580	1.000	0.252	-0.266	-0.231	-0.266	-0.168	-0.014	-0.105	0.105	-0.245	-0.615	0.280	0.014	-0.175	-0.175	-0.056	0.259	-0.126
Tp6	-0.049	-0.147	0.126	-0.112	-0.210	0.049	0.413	0.510	0.014	0.308	0.028	0.084	0.601	0.371	0.252	1.000	-0.077	0.028	0.091	-0.021	0.007	-0.224	0.140	0.154	0.049	0.727	0.105	0.105	0.179	-0.112	0.434	0.154
S6	0.196	0.063	0.287	0.042	-0.552	-0.217	-0.238	-0.322	-0.322	-0.007	0.308	-0.413	-0.133	-0.252	-0.266	-0.077	1.000	0.699	0.580	0.811	0.552	0.538	-0.133	0.413	0.266	-0.147	0.189	-0.168	-0.273	-0.545	-0.266	-0.203
F4	-0.028	0.126	0.196	-0.035	-0.140	0.021	-0.035	-0.021	-0.021	0.357	0.406	-0.510	-0.343	-0.063	-0.231	0.028	0.699	1.000	0.776	0.531	0.217	0.224	-0.259	0.671	0.140	0.035	0.259	-0.203	-0.147	-0.573	-0.517	-0.329
Po5	-0.042	0.140	0.133	-0.294	-0.364	-0.203	-0.210	-0.021	0.063	0.063	-0.105	-0.671	-0.497	-0.014	-0.266	0.091	0.580	0.776	1.000	0.510	0.315	0.336	0.021	0.573	0.322	0.280	0.608	-0.392	-0.483	-0.252	-0.119	0.070
F22	0.259	0.371	0.608	-0.070	-0.643	-0.294	-0.392	-0.406	-0.392	0.140	0.091	-0.231	-0.098	0.014	-0.168	-0.021	0.811	0.531	0.510	1.000	**0.853**	0.818	0.140	0.629	0.406	-0.259	-0.028	-0.538	-0.487	-0.210	-0.091	-0.112
Fo20	0.573	0.566	0.804	-0.301	-0.629	-0.105	-0.406	-0.322	-0.238	-0.049	0.056	-0.182	-0.056	0.308	-0.014	0.007	0.552	0.217	0.315	**0.853**	1.000	**0.965**	0.343	0.434	0.343	-0.308	-0.105	-0.657	**-0.718**	-0.112	0.014	-0.266
Py10	0.566	0.559	0.734	-0.378	-0.566	-0.161	-0.545	-0.455	-0.259	-0.084	0.014	-0.217	-0.252	0.231	-0.105	-0.224	0.538	0.224	0.336	0.818	**0.965**	1.000	0.378	0.441	0.378	-0.434	-0.091	-0.664	**-0.778**	-0.049	-0.077	-0.280
S1	0.035	-0.182	0.063	**-0.804**	-0.322	-0.448	-0.629	-0.420	-0.490	0.175	-0.280	0.378	-0.042	0.448	0.105	0.140	-0.133	-0.259	0.021	0.140	0.343	0.378	1.000	0.364	0.392	0.175	-0.196	-0.042	-0.364	0.580	0.573	0.315
F34	-0.028	0.140	0.378	-0.427	-0.224	-0.343	-0.364	-0.252	-0.385	0.720	0.098	-0.042	-0.266	0.126	-0.245	0.154	0.413	0.671	0.573	0.629	0.434	0.441	0.364	1.000	0.545	0.049	-0.091	-0.343	-0.217	0.035	-0.175	-0.070
S2	0.336	0.175	0.357	-0.462	-0.294	-0.455	-0.315	-0.140	-0.245	0.273	-0.308	0.070	-0.350	-0.385	-0.615	0.049	0.266	0.140	0.322	0.406	0.343	0.378	0.392	0.545	1.000	0.007	0.091	-0.210	-0.102	0.273	0.042	0.217
F12	-0.350	-0.490	-0.378	-0.182	0.112	-0.063	0.392	0.364	0.126	0.175	-0.224	0.007	0.161	0.105	0.280	0.727	-0.147	0.035	0.280	-0.259	-0.308	-0.434	0.175	0.049	0.007	1.000	0.413	0.182	0.137	0.133	0.378	0.343
Pn1	-0.007	-0.014	-0.252	-0.154	-0.175	-0.077	0.217	0.014	0.217	-0.406	-0.524	**-0.748**	-0.378	-0.203	0.014	0.105	0.189	0.259	0.608	-0.028	-0.105	-0.091	-0.196	-0.091	0.091	0.413	1.000	-0.287	-0.375	-0.308	0.049	0.175
Tp2	-0.413	**-0.748**	-0.608	0.154	0.168	-0.105	0.014	0.210	-0.161	0.105	0.357	0.399	0.413	-0.196	-0.175	0.105	-0.168	-0.203	-0.392	-0.538	-0.657	-0.664	-0.042	-0.343	-0.210	0.182	-0.287	1.000	0.760	0.028	0.182	0.245
S5	-0.399	-0.459	-0.413	0.515	0.445	0.081	0.403	0.406	0.042	0.336	0.305	0.532	0.399	-0.403	-0.175	0.179	-0.273	-0.147	-0.483	-0.487	**-0.718**	**-0.778**	-0.364	-0.217	-0.102	0.137	-0.375	0.760	1.000	0.056	-0.049	0.182
F13	-0.329	-0.196	-0.210	-0.217	0.182	-0.406	-0.357	-0.147	-0.133	0.154	-0.524	0.580	-0.105	-0.007	-0.056	-0.112	-0.545	-0.573	-0.252	-0.210	-0.112	-0.049	0.580	0.035	0.273	0.133	-0.308	0.028	0.056	1.000	0.462	0.601
F18	-0.273	-0.294	-0.217	-0.217	-0.490	-0.357	-0.252	-0.049	-0.266	-0.238	-0.490	0.308	0.420	0.364	0.259	0.434	-0.266	-0.517	-0.119	-0.091	0.014	-0.077	0.573	-0.175	0.042	0.378	0.049	0.182	-0.049	0.462	1.000	0.776
Po7	-0.594	-0.413	-0.476	0.014	-0.294	-0.580	-0.280	-0.070	-0.231	-0.105	-0.643	0.224	0.112	-0.147	-0.126	0.154	-0.203	-0.329	0.070	-0.112	-0.266	-0.280	0.315	-0.070	0.217	0.343	0.175	0.245	0.182	0.601	0.776	1.000

References

1. Pico, J.; Bernal, J.; Gómez, M. Wheat Bread Aroma Compounds in Crumb and Crust: A Review. *Food Res. Int.* **2015**, *75*, 200–215.
2. Lasekan, O.; Dabaj, F.; Muniandy, M.; Juhari, N.H.; Lasekan, A. Characterization of the Key Aroma Compounds in Three Types of Bagels by Means of the Sensomics Approach. *BMC Chem.* **2021**, *15*, 1–13.
3. Cho, I.H.; Peterson, D.G. Chemistry of Bread Aroma: A Review. *Food Sci. Biotechnol.* **2010**, *19*, 575–582.
4. Pétel, C.; Onno, B.; Prost, C. Sourdough Volatile Compounds and Their Contribution to Bread: A Review. *Trends Food Sci. Technol.* **2017**, *59*, 105–123.
5. Buttery, R.G.; Ling, L.C.; Stern, D.J. Studies on Popcorn Aroma and Flavor Volatiles. *J. Agric. Food Chem.* **1997**, *45*, 837–843.
6. Birch, A.N.; Petersen, M.A.; Hansen, Å.S. REVIEW: Aroma of Wheat Bread Crumb. *Cereal Chem.* **2014**, *91*, 105–114.
7. Bailey, M.E.; Gutheil, R.A.; Hsieh, F.-H.; Cheng, C.-W.; Gerhardt, K.O. Maillard reaction volatile compounds and color quality of a whey protein concentrate—Corn meal extruded product. In *Thermally Generated Flavors*; Thomas, H.P., Michael, J.M., Robert, J.M., Eds.; ACS Publications: Washington, DC, USA, **1993**; Chapter 25; pp. 315–327.
8. Bento-Silva, A.; Duarte, N.; Belo, M.; Mecha, E.; Carbas, B.; Brites, C.; Vaz Patto, M.C.; Bronze, M.R. Shedding Light on the Volatile Composition of *Broa*, a Traditional Portuguese Maize Bread. *Biomolecules* **2021**, *11*, 1–31.
9. Pacyński, M.; Wojtasiak, R.Z.; Mildner-Szkudlarz, S. Improving the Aroma of Gluten-Free Bread. *Lwt* **2015**, *63*, 706–713.
10. Buttery, R.G.; Ling, L.C. Volatile Flavor Components of Corn Tortillas and Related Products. *J. Agric. Food Chem.* **1995**, *43*, 1878–1882.
11. Park, D.; Maga, J.A. Identification of Key Volatiles Responsible for Odour Quality Differences in Popped Popcorn of Selected Hybrids. *Food Chem.* **2006**, *99*, 538–545.
12. Bredie, W.L.P.; Mottram, D.S.; Guy, R.C.E. Aroma Volatiles Generated during Extrusion Cooking of Maize Flour. *J. Agric. Food Chem.* **1998**, *46*, 1479–1487.
13. Buttery, R.G.; Ling, L.C. Additional Studies on Flavor Components of Corn Tortilla Chips. J. Agric. *Food Chem.* **1998**, *46*, 2764–2769.
14. Pu, D.; Zhang, H.; Zhang, Y.; Sun, B.; Ren, F.; Chen, H. Characterization of the Key Aroma Compounds in White Bread by Aroma Extract Dilution Analysis, Quantitation, and Sensory Evaluation Experiments. *J. Food Process.* Preserv. **2019**, *43*.
15. Drakula, S.; Mustač, N.Č.; Novotni, D.; Voučko, B.; Krpan, M.; Hruškar, M.; Ćurić, D. Optimization and Validation of a HS-SPME/GC–MS Method for the Analysis of Gluten-Free Bread Volatile Flavor Compounds. *Food Anal. Methods* **2021**.
16. Feng, X.; Pan, L.; Wang, Q.; Liao, Z.; Wang, X.; Zhang, X.; Guo, W.; Hu, E.; Li, J.; Xu, J.; et al. Nutritional and Physicochemical Characteristics of Purple Sweet Corn Juice before and after Boiling. *PLoS One* **2020**, *15*, 1–18.
17. Galoburda, R.; Straumite, E.; Sabovics, M.; Kruma, Z. Dynamics of Volatile Compounds in Triticale Bread with Sourdough: From Flour to Bread. *Foods* **2020**, *9*.
18. Karahadian, C.; Johnson, K.A. Analysis of Headspace Volatiles and Sensory Characteristics of Fresh Corn Tortillas Made from Fresh Masa Dough and Spray-Dried Masa Flour. *J. Agric. Food Chem.* **1993**, *41*, 791–799.
19. Pico, J.; Martinez, M.M.; Bernal, J.; Gomez, M. Evolution of Volatile Compounds in Gluten-Free Bread: From Dough to Crumb. *Food Chem.* **2017**, *227*, 179–186.

20. Pico, J.; Antolín, B.; Román, L.; Bernal, J.; Gómez, M. Selection of the Most Suitable Mixture of Flours and Starches for the Improvement of Gluten-Free Breads through Their Volatile Profiles. *Eur. Food Res. Technol.* **2019**, *245*, 1755–1766.
21. Hussein, A.M.S.; Ibrahim, G.E. Effects of Various Brans on Quality and Volatile Compounds of Bread. *Foods Raw Mater.* **2019**, *7*, 42–50.

FROM MAIZE FLOUR TO BREAD: ASSESSING THE IMPACT OF PROCESSING ON PHENOLIC AND VOLATILE COMPOSITION

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