UNIVERSITÀ DEGLI STUDI DI SASSARI

SCUOLA DI DOTTORATO DI RICERCA
Scienze e Biotecnologie
dei Sistemi Agrari e Forestali
e delle Produzioni Alimentari

Biotecnologie microbiche agroalimentari

Ciclo XXVIII

Creating value-added cereal-based baked products: marketplace offer, laboratory-designed goods, and revisited local products

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To my beloved parents and to my sister

LIST OF PAPERS

This PhD thesis is based on the following papers:

I. Conte, P., Fadda, C., Piga, A., & Collar, C. Techno-functional and nutritional performance of commercial breads available in Europe.

II. Collar, C., Jiménez, T., Conte, P., & Fadda, C. (2014). Impact of ancient cereals, pseudocereals and legumes on starch hydrolysis and antiradical activity of technologically viable blended breads. *Carbohydrate Polymers*, *113*, 149-158.

III. Collar, C., Jiménez, T., Conte, P., & Piga, A., (2015). Significance of thermal transitions on starch digestibility and firming kinetics of restricted water mixed flour bread matrices. *Carbohydrate Polymers*, *122*, 169-179.

IV. Collar, C., Conte, P., Fadda, C., & Piga, A. (2015). Gluten-free dough-making of specialty breads: significance of blended starches, flours and additives on dough behaviour. *Food Science and Technology International*, *21*, 523-536.

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1 INTRODUCTION

Bread has been one of the staple foods most widely used and consumed around the world and one of the major constituents of the human diet since ancient times. Although we do not know precisely the time when it was invented, the history of bread has accompanied the history of the human civilization for thousands of years.

Over the centuries, many changes have characterized the process of breadmaking leading to the development of wide varieties of breads often linked to the geographical area of production in terms of both ingredients and production techniques used. This knowledge, passed down to the present day, together with our in-depth understanding of the role played by all the factors involved in baking process, have contributed to the significant increase in the variety of breads currently available in the market. In addition, in recent years, consumers' demands in the field of food production have changed considerably (Betoret, Betoret, Vidal, & Fito, 2011). The growing interest in well-being and healthy lifestyle, the increasing awareness of the relationship between non-communicable diseases and unhealthy diet, as well as the increasing prevalence of food allergies and intolerances, are other factors that, in different ways, are strictly related to the changes in consumers' food preferences and eating habits but, also linked to the growing market supply of cereal-based baked products having health-promoting and/or disease-preventing properties.

Despite the simplicity of the basic recipe (flour, water, salt and leavening agent), the long-term success of bread is easy to understand when its typical flavour, taste, and its high nutritional value are considered. Bread is a good source of energy mainly due to the high content of starch, besides protein, lipids rich in essential fatty acids, dietary fibres, antioxidants, and micronutrients (Rubel et al., 2015).

Breads encompass a wide variety of products differing in shape, size, ingredients, and method of baking.

According to the New Zealand Association of Bakers (2010), breads fall into three main categories (Rakha et al., 2013):

- Those that rise highest and have to be baked in pans;
- Those with a medium volume, like rye and French breads;
- Those that hardly rise, and consequently are called flatbreads.

The following chapters discuss several basic ingredients currently used in breadmaking, focusing our attention on the use of raw materials, which may improve the nutritional value and the disease-preventing properties in a type of bread like French bread.

1.1 Bread besides wheat

Despite the great variety of existing breads, the majority is traditionally produced from wheat flour and refined white bread can be still considered the most commonly consumed type of bread (Blandino et al., 2013). Wheat is by far the most important crop for breadmaking because of its supreme baking performance in comparison with all other cereals (Dziki et al., 2014). Only in some parts of the world, such as in Northern and Eastern Europe, bread is mainly produced and consumed from rye flours (Prättälä et al., 2001). However, during the milling process the outer layers of wheat grain, the so-called bran, rich in fibre, B vitamins, minerals, and protein are removed, resulting in a lower nutritional value of the end-product when compared to the starting material.

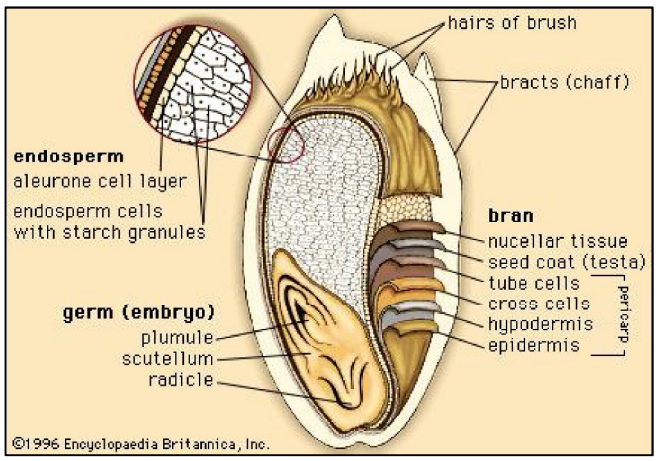

Fig. 1 Wheat grain anatomy

As a consequence, the refined white bread lacks several macro and micronutrients and provides smaller amounts of valuable nutrients needed for a healthy and balanced diet (Škrbić and Filipčev, 2008).

In order to avoid the loss of many potentially beneficial micronutrients, minerals, and dietary fibre, considerable attention has been directed to the production of whole grain breads. Furthermore, many government and health-promoting organisations provided dietary guidelines and strongly recommended consumption of whole grain foods because of their universally known health benefits (Seal, 2013). As reported by several authors, the intake of whole-grain bread is generally associated with a reduced risk of coronary heart disease and type-2 diabetes (Blandino et al., 2013). However, in spite of the positive nutritional benefits of whole grains, the consumers' acceptance of whole breads is still limited due to their lower perceived attractiveness (lower volume and denser crumb texture) with respect to that of refined breads (Noort et al., 2010).

In this context, in which novel and healthy breads have to meet the needs of consumers as well as the main quality and nutritional requirements for bakery products, the special role of cereals is being reinforced and the use of other and alternative raw materials, besides wheat, has become increasingly popular.

1.1.1 Pseudocereals and ancient cereals

The rekindled interest in some under-utilized plant species such as pseudocereals and ancient cereals derives from their excellent nutritional profile and their health-promoting attributes (Dini et al., 2012). The use of these flours as partial wheat flour substitutes in bread formulations is mainly connected to their excellent protein profile but also to their richer composition, especially regarding minor components present in grains such as dietary fibre, resistant starch, minerals, vitamins, and phenols (Angioloni and Collar, 2011). In addition, since pseudocereals seeds are naturally gluten-free, they are more and more frequently included as healthy ingredients in gluten-free diets (Alvarez-Jubete et al., 2010a).

In botanical terms, buckwheat, amaranth and quinoa are dicotyledonous species as opposed to the monocotyledonous true cereals, but since they produce small grain-like seeds they are called pseudocereals. Buckwheat belongs to genus *Fapopyrum*, which includes the two main cultivated species used around the world for human consumption: common buckwheat (*Fagopyrum esculentum*), which is the most common type in Europe, and tartary buckwheat (*Fagopyrum tataricum*). The protein content in buckwheat (12%) is very similar to that found in wheat, but buckwheat proteins are characterized by a high biological value due to the well-balanced amino acid composition (rich in lysine and arginine) (Zhang et al., 2012). Buckwheat is also rich in dietary fibre, minerals (zinc, copper and manganese), and phenolic compounds including the flavonolglycosides quercetin and, mostly, rutin, which has an important role in providing anti-inflammatory, anti-hypertensive, and antioxidant activities (Min et al., 2010). Lin et al. (2009) produced buckwheat-enhanced wheat breads and evaluated the influence of buckwheat flour supplementation on the quality and antioxidant properties of the resulting breads. These authors found that the 15% of buckwheat flours supplementation provided breads with more functional components and antioxidant properties without interfering with specific volume and bread acceptability.

Amaranth and quinoa are the other two rediscovered pseudocereals used in breadmaking as nutritious ingredients. These pseudocereals were major crops for the pre-Columbian cultures in Latin America but, after the Spanish conquest, their production fell to insignificant levels and, even today, it continues on a small scale (Bressani, 2003; Schoenlechner et al., 2008). Amaranth belongs to genus *Amaranthus* and, among the 60 species known worldwide only 3 (*Amaranthus caudatus, Amaranthus cruentus* and *Amaranthus hypochondriacus*) are cultivated today for grain production and used for human consumption. Quinoa belongs to the genus C*henopodium*, which includes sweet and bitter varieties depending on the content of saponins (Alvarez-Jubete et al., 2010b). The protein content in amaranth (14–15%) and quinoa (13–14%) is higher than that found in wheat and,

like buckwheat, they have a well-balanced amino acid composition particularly rich in lysine usually deficient in cereal grains (Dini et al., 2012). Moreover, the amino acids profile of amaranth proteins is comparable to that of whole egg (Schoenlechner et al., 2008). Regarding carbohydrates, the major fraction is represented by starch (primarily located in the perisperm), which content was found to be lower in both amaranth and quinoa with respect to major cereals (including wheat and rice). They have, also, a total mineral content (calcium, magnesium, iron, potassium, and zinc) approximately 2 times higher than common cereals and can be considered a good source of vitamins (riboflavin, folic acid and vitamin C), dietary fibres and bioactive compounds (Schoenlechner et al., 2008). Tosi et al. (2002), demonstrated that the replacement of wheat flour with whole and defatted hyperproteic amaranth flours can increase protein and lysine bread content, without detrimental effects in bread quality and acceptance, when levels of substitution used were up to 4 and 8%, respectively. Also Sanz-Penella et al. (2012) studied the effect of the addition of amaranth flours to wheat bread formulation at different levels (up to 40%). These authors found that supplemented samples were characterized by significantly increased values of protein, dietary fibre, and macro- and micro-nutrients at all substitution levels but, also, by a slightly deleterious effect in bread quality at levels of replacement between 10 and 20%.

1.1.2 Teff

Over the past few decades, there has been a rediscovered interest in the use of teff as food ingredient. This ancient cereal is especially appreciated in breadmaking for its nutritional value. In addition, being naturally gluten-free, it can be used as a healthy ingredient also in gluten-free foods.

Teff (*Eragrostis tef*) belongs to the family of *Poaceae* and genus *Eragrostis*. It is originated from Ethiopia used as basic ingredient for traditional goods such as fermented flatbread *(injera*), beer and porridge. The main constituents of teff

grain are carbohydrates and protein (8.7–11%). Among carbohydrates, starch is the major component (73%) characterized by a low tendency of starch retrogradation (Arendt and Zannini, 2013). The protein content in teff is very similar to that found in other common cereals, but with a well-balanced amino acids composition. Teff has an excellent amino acid profile that makes it similar to that of whole egg protein, except for its lower lysine content (Bultosa and Talylor, 2004). Teff is also a good source of dietary fibre, vitamins and minerals (iron, calcium and zinc). It contains a lower level of lipids (2–3%) than other cereals such as corn, oats, millet and sorghum (Arendt and Zannini, 2013) and a high percentage of unsaturated fatty acids (72.46%), among which oleic acid is predominant (32.41%), followed by linoleic acid (23.83%) (Gebremariam et al., 2014). Some reports have been published regarding the effects of the incorporation of teff grain flours into straight dough breadmaking on quality of breads. Mohammed et al. (2009) found that the supplementation of teff flour in wheat bread up to the level of 20% negatively affects the quality of breads in terms of lower specific volume, higher crumb firmness and lower sensory scores than wheat bread. Furthermore, only breads supplemented with teff flour up to a 5% level were considered acceptable from the sensory and nutritional point of view. Alaunyte et al. (2012) when assessing the effect of refined wheat flour replacement with teff flour at different levels of substitution (up to 30%) on textural and sensory properties of bread, obtained similar results. However, in the same study, the authors demonstrated that the addition of enzyme combinations (including amylase, glucose oxidase, xylanase and lipase) to both straight dough and sourdough breadmaking could improve volume, texture and sensory properties of teff-enriched breads.

Also, Ronda, Abebe, Pérez-Quirce, and Collar (2015), evaluated the impact of three Ethiopian grain teff varieties (1 brown grain teff and 2 white grain teff) at different incorporation levels (up to 40%) on the physical, sensory and nutritional performance in *ciabatta* type bread. Those authors found that the resulting supplemented breads showed enhanced nutritional value and acceptable sensory

properties with no significance effects on loaf volume, crumb hardness and cohesiveness when white grain teff varieties were added up to 30% level. Addition of brown teff variety at the same level even provided 10% higher volume than that of wheat control, while discreet negative effects were observed at levels of replacement between 30 and 40%. The authors also found that breads fortified with 40% level of teff flours showed a mineral content (mostly Fe but, also Mn, Cu, Zn, Mg, Ca, K, and P) higher than that of the 100% refined wheat breads.

1.1.3 Legumes

The use of legumes as modern and healthy ingredients in bakery food production has become highly appreciated.

Incorporation of various types of legumes in bread formulation is not a modern practice but an old traditional use that is readdressed to the current dietary needs. Now as in the past, blends of cereal and legume flours have been used to produce traditional flat breads in countries of the Indian subcontinent, the Middle East, and North Africa; also, in Europe, flours from beans, lentils and peas have been mixed with cereal flours for traditional food preparation (Qarooni, 1996). The reason of this renewed interest is due to the high nutritional value and the suitable functional properties of the legume grains.

Legumes are important source of proteins, which content ranges from 18 to 25%; only soybean is unique in containing about 35–43% proteins (Tharanathan and Mahadevamma, 2003). In addition, legumes contain high values of the essential amino acid lysine and, therefore, when consumed in combination with cereals, which are rich in sulphur-containing amino acids (methionine and cysteine), can complement proteins and provide a more nutritionally balanced final product (Qarooni, 1996; Borsuk et al., 2011). They are also good sources of minerals, vitamins, dietary fibre and, mostly, the slow release of carbohydrates, may account for the beneficial physiological effects in controlling and preventing

various metabolic diseases such as diabetes mellitus and hyperlipidaemia (Tharanathan and Mahadevamma, 2003). Angioloni and Collar (2012) explored the suitability of different legume flours (chickpea, pea and soybean) to be included, at different levels of substitution, in blended matrices with common wheat and in presence/absence of structuring agents. The authors found that high-legume breads showed enhanced nutritional quality and good sensory acceptances with no significant impairment when structuring agents are incorporated. In particular, lower starch hydrolysis and expected glycemic index of breads were promoted by the addition of pea and chickpea flours; while the higher antiradical activity by soybean flour supplementation.

1.2 Bread and specific dietary requirements

The role of diet goes beyond the hunger satisfaction and supply of nutrients towards the reduction and prevention of certain chronic diseases. For this reason, enriched/fortified bread with functional components has become quite popular.

1.2.1 High-fibre bread

The development of dietary fibre-rich products has to meet the main quality requirements for food products such as nutritional added value, safety, tasty palatability, and easy handling during processing (Collar and Angioloni, 2010). Dietary fiber (DF) undoubtedly plays a key role in bread fortification. The incorporation of various fibre-rich ingredients into bread formula has greatly increased because of their linkage to human health. DF has been associated with a decreased risk of certain chronic diseases like cardiovascular disease, colon cancer and constipation (Rakha et al., 2013). In addition, they do not constitute a defined chemical group, but represent a combination of chemically heterogeneous substances (Tharanathan and Mahadevamma, 2003). For several decades, the definition of DF has been controversial. In October 2008, the European Union

adopted the following definition of DF: “fibre means carbohydrate polymers with three or more monomeric units, which are neither digested nor absorbed in the human small intestine and belong to the following categories: edible carbohydrate polymers naturally occurring in the food as consumed; edible carbohydrate polymers which have been obtained from food raw material by physical, enzymatic or chemical means and which have a beneficial physiological effect demonstrated by generally accepted scientific evidence; edible synthetic carbohydrate polymers which have a beneficial physiological effect demonstrated by generally accepted scientific evidence” (European Union, 2008). On the basis of water solubility, DF can be divided into the two categories insoluble and soluble fibre.

The physiological properties of different DFs are closely related to their physico-chemical properties such as viscosity, capacity to form gels, and fermentation (Galisteo et al. 2008). In fact, many of the physiological effects exerted by soluble DFs like lowering blood cholesterol levels and normalization of blood glucose and insulin levels have been associated to their viscosity (Galisteo et al., 2008; Rosell et al., 2009). Instead the normal laxation and the management of intestinal disorders like constipation have been promoted by fibres that are incompletely or slowly fermented in the large intestine (Galisteo et al., 2008). Moreover, a number of studies evidenced that an increased intake of DF had positive effects in increasing postprandial satiety, body weight, energy intake and in decreasing hunger (Howarth et al., 2001).

Despite many efforts have been made to enhance the consumption of the main natural sources of DF (such as whole grain cereals, pulses, fruit, and vegetables), there is still a large gap to fill between usual and recommended intakes (38 and 25 g/day for young men and women, respectively) (Slavin, 2005). Thus, the amount of DF in diet could be improved by the addition of ingredients with high-fibre content or functional fibre in other foods such as bread.

β-glucan, one of the three major DF components has been widely studied for dietary fibre fortification in bread. β-glucan is a polymer of glucose with mixed

glucosidic bonds of both the β–1,3 and β–1,4 types. Among grains, barley and oats, in particular, contain high amounts of β-glucan (Nelson, 2001). In a study by Collar and Angioloni (2014) on composite breads, up to 40% of the wheat flour was replaced either with high β-glucan barley flour or regular commercial barley flour and the results were compared to those obtained for wheat control bread. The authors found that the incorporation of barley flour into wheat bread formulation significantly enhanced the nutritional values of blended breads in terms of dietary fibre fraction (including β-glucan and resistant starch), slowly digestible starch sub fractions, and antioxidant activity. However, notwithstanding their higher scores in overall acceptability, breads enriched with β-glucan high flour showed reduced bread volume and increased crumb hardness.

Therefore, there are many high-fibre ingredients isolated from plant sources other than cereals. Among these, inulin is often used because of its pro-health activity and functional properties. In fact, together with a small number of oligosaccharides such as transgalacto-oligosaccharides and lactulose, it has achieved the prebiotic status and, thus, all health benefits linked to this category of ingredients (including increased mineral absorption, improved immune response and an important role in cancer prevention) (Morris and Morris, 2012). Inulin, as prebiotic, can pass through the gastrointestinal tract relatively intact until reaches the large intestine where it is digested by colonic micro-flora, stimulating selectively the growth and/or activity of intestinal bacteria associated with health and well-being (Barclay et al., 2010; Morris and Morris, 2012).

It is a soluble fibre source predominately isolated from chicory root and, recently, from Jerusalem artichoke tubers (Rubel et al., 2015). The technological properties of inulin are closely related to the nature of this fructan-type polysaccharide characterized by molecules linked by β–1–2 bonds, with a terminal glucose unit and a degree of polymerization typically ranges from 2 to 60 units (Nelson, 2001). In the production of foods short-chain inulin, being soluble and sweet, can be used for partial sucrose replacement; while long-chain inulin, being less soluble and more viscous, can be used to structure low-fat foods (Tárrega et al., 2011).

However, the addition of these fibres may cause detrimental effects on the final bread quality such as reduction of loaf volume, increase of crumb hardness and dark crust appearance. Hagher et al. (2011) in producing wheat and gluten-free breads supplemented with the soluble fibres oat β-glucan and prebiotic inulin, despite the higher nutritional value of both resulting breads, observed the above mentioned effects. In particular, inulin addition caused darkening of the crust, and increased crumb hardness and rate of staling in both types of bread. Best results were obtained with the addition of oat β-glucans, which showed positive effects on softening the crumb and reducing the rate of staling, but only in gluten-free bread.

1.3 Bread and Glycemic Index

In 1981, the concept of glycemic index (GI) was proposed for the first time by Jenkins et al. (1981) as a tool for ranking carbohydrate-containing foods on the basis of their postprandial glycemic response.

Despite the qualified support given to this concept by both diabetes associations and World Health Organization (WHO), its relevance is still widely debated because many health experts considered it too variable for use in clinical practice (Atkinson et al., 2008). However, the recent changes in dietary pattern more oriented to the consumption of processed carbohydrates foods as well as the considerable increase in diseases such as obesity, diabetes and cardiovascular diseases reinforced the important role of GI. In fact, in the past decades, several epidemiological and clinical studies have demonstrated a direct link between carbohydrate-foods consumption and postprandial glucose metabolism, insulin resistance, and cardiovascular risk factors.

GI is defined as the incremental area under the blood glucose response curve of a 50g carbohydrate portion of a test food expressed as a percent of the response to the same amount of carbohydrate from a standard food (FAO/WHO, 1998; Jenkins et al., 1981). The GI is calculated within the first 2 hours after the test

food consumption, while the reference food normally used is either glucose or white wheat bread (GI = 100). Because these two reference foods have a different blood glucose release, two GI values were created (Atkinson et al., 2008). When white bread is used as a reference, the GI value are higher than when using glucose (Gropper et al., 2013) and, therefore, some foods can have GI values greater than 100.

By definition, the GI does not take into account the amount of carbohydrate present in foods; thus, to consider not only the quality but also the quantity of carbohydrates, was introduced the concept of glycemic load. It is calculated by multiplying GI of a certain food with the total amount of dietary carbohydrate available in one serving of the food (when considering glucose as a reference food) (Atkinson et al., 2008). The higher the glycemic load, the greater the rise in blood glucose level as effect of the food (Foster-Powell et al., 2002).

Changes in glycemic response after the ingestion of carbohydrates foods depend on several factors such as starch characteristics (amylose-amylopectin ratio; resistant starch), food processing (degree of gelatinization), and presence of fibre, protein, lipids and other components (WHO/FAO, 1998). Normally, foods with high GI, being rapidly digested and absorbed, cause large rises in blood glucose; while, foods with low GI release glucose gradually in the blood stream (Brand-Miller et al., 2009).

According to the "International Tables of Glycemic Index" proposed by Atkinson et al. (2008) (using white bread as reference) most refined starchy foods (breakfast cereals, white wheat bread, whole grain barley flour bread, and both gluten-free buckwheat and multigrain bread) have a high GI (>70); porridge, muesli and some types of bread (including gluten-free white bread) have intermediate GI (55–70); while, vegetable, fruits, legumes and pasta tend to have a low GI (< 55) (Atkinson et al., 2008; Wolter et al., 2013).

However, it should be noted that some foods with low GI (like chocolate) may be high in fat; therefore, in choosing carbohydrate foods both GI and food

composition (energy density and macronutrients profile) must be considered (Atkinson et al., 2008).

Thus, bread can be considered as a food with high and intermediate GI and some examples of the attempts to reduce the GI of white bread regarded the addition in its basic formula of high-fibre ingredients such as whole grains, bran fraction or legumes. In fact, whole grain intake is inversely associated with risk of type-2 diabetes, and this association is stronger for bran than for germ (De Munter et al., 2007). In the study by Marangoni and Poli (2008), fifteen healthy non-diabetic volunteers consumed, on different days, a portion equivalent to 75g of available carbohydrates of both fibre-enriched bread and biscuit and their traditional counterparts. The authors found that both the fibre-enriched products had lower GI when compared to those obtained with the equivalent controls. Also the average of blood glucose levels in the subjects tested remained at significantly lower levels than that of the control foods between 15 and 90 minutes after the carbohydrate loads. As described by Scazzina et al. (2009), another approach to reduce the GI value could be the use of sourdough leavening technique in breadmaking. In this study, eight healthy volunteers consumed four experimental breads prepared from two wheat flours (whole or white) and two different leavening techniques (sourdough or with *Saccharomyces cerevisiae*) in a portion equivalent to 50 g of available carbohydrates. The authors found that the sourdough technique ameliorates glucose response in healthy subjects and this effect was probably due to the organic acids produced by sourdough micro-flora that could delay gastric emptying without influencing starch accessibility with hydrolytic enzymes or general bioavailability.

1.4 Bread Staling

Bread is the most popular staple food worldwide, but quickly loses its desirable qualities. In fact, during the storage of bread a number of complex physical-chemical changes occur, which result in crumb hardening, loss of crust crispiness

and loss of organoleptic freshness of bread. All these alterations are commonly referred to as staling. Staling of bread, in addition to the gradually decreasing consumer acceptance is also responsible for a significant product waste all over the world (Fadda et al., 2014).

Bread staling is a complex phenomenon involving multiple mechanisms not yet fully understood. However, it seems well established that the most important causes responsible for this alteration are starch transformations, starch-gluten interactions, and moisture redistribution (Fadda et al., 2014). The moisture migration from the crumb to the crust is, generally, the cause of the staling of the crust, which results in a soft and leathery texture; whereas staling of the crumb is more complex, and less understood (Gray and Bemiller, 2003). The role of starch has been widely investigated and starch recrystallization (its reorganization during aging) is considered one of the major phenomena involved in crumb firming. The retrogradation of amylose occurs during the first hours after baking while amylopectin recrystallization requires several days. It can be considered that amylose stabilizes the initial structure and forms a more rigid insoluble network, whereas the amylopectin fraction (with reassociation of starch molecules into a partially crystalline and ordered structure) is especially responsible for the subsequent increase in crumb firmness (Giannone et al., 2016).

The relationship between crumb firming and starch retrogradation has been (and still is) a major point of discussion among researchers. Although for some of them there is no a direct cause-and-effect relationship, most agree that the amylopectin recrystallization plays a key role, but not unique, in the staling process (Gray and Bemiller, 2003). Thus, other factors seem to contribute to process of bread staling, including water and gluten network changes. In conjunction with the amylopectin retrogradation, water redistributes at a macromolecular level, shifts from gluten to starch and is partially incorporated in starch crystallites changing, therefore, the gluten network nature. However, even though the role of moisture redistribution in the staling process remains undetermined, water migration is also widely accepted to be a very important aspect of firming (Gray and Bemiller, 2003).

Although there is still much to understand on the mechanisms that regulate the process of staling, anti-staling agents such as enzymes and emulsifiers have been studied in an attempt to retard the staling process or minimize its effect. Among the enzymes, α-amylases from different origins are the most frequently used. Goesaert et al. (2009) found that unlike conventional α-amylases (which cut the long polymer chains connecting the crystalline regions and only weakening the amylopectin network) the use of maltogenic α-amylase from *Bacillus stearothermophilus* has a hindering effect on the recrystallization of amylopectin because primarily degrades its side chains. In this way it limits the formation of the permanent amylopectin network and the water immobilisation.

Another way of influencing the staling process is the addition of gluten protein into the bread formula to obtain a crumb texture with decreased firmness. Curti el al. (2014) found that higher levels (15%) of gluten protein addition positively affected the macroscopic properties of resulting bread, which retained higher softness, springiness and cohesiveness upon storage.

1.5 Gluten-free breads

Recently the gluten-free (GF) market is experiencing a double-digit growth, confirming it as one of the most prosperous markets in the immediate and near future (Miranda et al., 2014). This growth has occurred for two main reasons. The first one is related to the increasing number of diagnosed patients with coeliac disease and wheat allergies as well as other gluten reactions, like gluten sensitivity, in which, however, neither allergic nor autoimmune mechanisms are involved (Sapone et al., 2012). The second reason is related to the high number of people who, although not suffering from any form of gluten intolerance, have adopted the questionable choice to consume these products. This, in the mistaken belief (not supported by any scientific evidence) that these products are healthier than their conventional counterparts or represent an effective way to lose weight (Miranda et al., 2014).

1.5.1 Coeliac disease

Coeliac disease is an immune-mediated enteropathy of the small intestine that causes intestinal villi atrophy and consequent malabsorption of important nutrients such as iron, folic acid, calcium and vitamins in genetically susceptible individuals. Alongside the symptomatic cases with the classic symptoms such as chronic diarrhea, malabsorption and weight loss, increasingly commons are also the silent (or asymptomatic) forms normally discovered with serological screening (Sapone et al., 2012).

The inflammatory reaction is triggered by the ingestion of gluten and, more precisely, the gliadin fraction of wheat and prolamins from common grains such as barley (hordeins), rye (secalins) and oat (avidins). It should be pointed out that although oats are naturally gluten-free, their use in gluten-free breadmaking is controversial because of concerns of potential contamination of commercial oats that may occur during harvest, transport or during the productive process (Kupper, 2005).

In January 2009, in the regulation concerning the composition and labelling of foodstuffs suitable for people intolerant to gluten, the European Commission established as follow: “Foodstuff for people intolerant to gluten, consisting of or containing one or more ingredients made from wheat, rye, barley, oats or their crossbred varieties which have been especially processed to reduce gluten, shall not contain a level of gluten exceeding 100 mg/kg in the food as sold to the final consumer” (European Union, 2009). Such products shall bear the term ’very low gluten’ in the label. “They may bear the term ‘gluten-free’ if the gluten content does not exceed 20 mg/kg in the food as sold to the final consumer” (European Union, 2009). This regulation also established that “foodstuffs for people intolerant to gluten, consisting of or containing one or more ingredients which substitute wheat, rye, barley, oats or their crossbred varieties shall not contain a level of gluten exceeding 20 mg/kg in the food as sold to the final consumer” (European Union, 2009). Such products shall bear the term ’gluten-free’ in the label.

To date, lifelong withdrawal of gluten from the diet is the only treatment for coeliac disease.

1.5.2 The role of gluten and its replacement

The replacement of gluten in bakery products presents undoubtedly a major technological challenge. In fact, gluten is the main structure-forming complex in wheat bread and it has an exceptional ability to form cohesive viscoelastic dough capable of entrapping gas during fermentation and baking and to provide a good crumb structure in resulting breads. Thus, its removal impairs dough's capacity to properly develop during kneading, leavening and baking (Gallagher et al., 2004). The absence of gluten leads to a gluten-free (GF) doughs less cohesive and elastic and more sticky and difficult to handle than their wheat counterparts. Moreover, in order to form an acceptable consistency, GF flour require higher amount of water than wheat flour (Capriles and Arêas, 2014). The GF-doughs, having a viscosity more like to that of cake batters, are often called batters instead of doughs. The formation of dough with viscoelastic properties comparable to their gluten-containing counterparts can be obtained, therefore, enriching the gluten free bread formulations with polymeric substances. Moreover, the resulting breads showed several post-baking quality defects such as crumbling texture, lighter crumb colour and a lower volume (Gallagher et al., 2004; Houben et al., 2012; Hager et al., 2012b).

Another current concern regarding gluten-free breads is related to their nutritional improvement. Despite the considerable advances made in this field, many of the GF-products available on the market are characterized by low quality and lack of several nutritive elements; in fact, they have in general a lower protein content and inadequate amounts of vitamin B, iron, folic acid and dietary fibre than gluten-containing foods. In addition to a relatively shorter shelf-life (Torbica et al., 2010).

Thus, in gluten-free breadmaking, a consumer-satisfying structure, an adequate

nutritive value and a good taste of bread can only be achieved using a combination of different ingredients (Houben et al., 2012).

The following chapters discuss several raw materials currently used in GF breadmaking, focusing the attention on the ingredients, which may improve the nutritional and technological quality of GF breads.

1.5.3 Conventional and alternative gluten-free flours

Traditionally, GF breads were obtained using rice and corn flours, in combination with starches from different plant sources (corn, potato, or cassava) as basic ingredients, and protein and hydrocolloids as structuring agents (Capriles and Arêas, 2014).

Rice flour is probably the main ingredient used for preparing GF bakery products. In fact, natural flavour, white colour, and hypoallergenic properties together with a low-sodium levels and high amount of easily digested carbohydrates make it a suitable cereal for GF breadmaking (Rosell and Marco, 2008). However, it presents some technological limitations. The hydrophobic nature of protein and the low amount of prolamins of rice flour are responsible for its inability to form viscoelastic dough when kneaded in water. As a result, the carbon dioxide produced during fermentation cannot be retained, leading to a product with a lower specific volume and a very compact crumb (Rosell and Marco, 2008).

With regard to the corn (*Zea mays*) flour, despite it is often used as a basic ingredient in gluten-free breadmaking, presents some limitations mainly due to its distinctive flavour and characteristic yellow colour. More suitable could be white maize varieties, in which pigments responsible for yellow colouring are absent (Hager et al., 2012a).

In recent years a great deal of attention has been focused on the improvement of physical and sensory properties as well as the nutritional value of GF breads through the use of nutrient-dense alternative raw materials such as pseudocereals, ancient cereals and legumes (Capriles et al., 2015).

The properties of these raw materials have been discussed in previous chapters of this thesis; thus, in this section, are only reported some studies related to their use in the field of GF breadmaking.

Among pesudocereals, buckwheat flour with a more pleasant aroma and taste than amaranth and quinoa is undoubtedly the most studied ingredient for GF bread production; while only little research is available on the production of breads from quinoa (Capriles and Arêas, 2014).

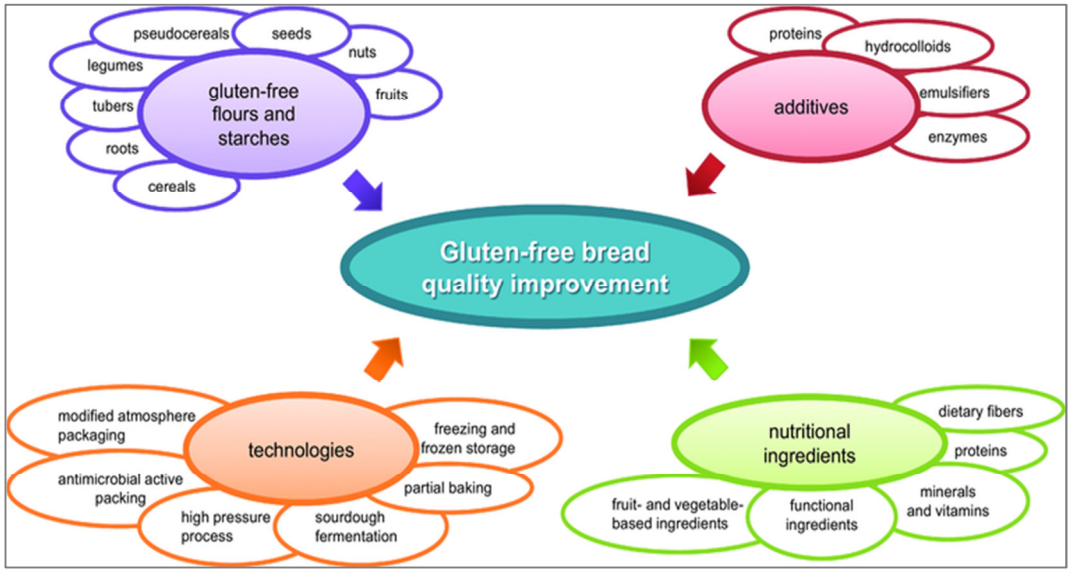

Fig. 2 Approaches to improve gluten-free bread quality (Capriles and Arêas, 2014)

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Torbica et al. (2010) prepared rice-based gluten-free breads with the addition of both husked and unhusked buckwheat flours at different levels (up to 30%) and evaluated the effects on the rheological, textural and sensory properties of resulting breads. These authors found that the addition of hydrocolloids was unnecessary for the dough structuration effect when buckwheat flour is included into the bread formula. They also observed a reduction in the starch retrogradation degree suggesting an improvement in the anti-staling properties of GF breads

supplemented with buckwheat flours. Regarding the sensory properties all breads were found to be acceptable but lower scores were given for the unhusked buckwheat supplemented breads at higher levels of buckwheat addition.

Alvarez-Jubete et al. (2010c) produced three types of breads containing 50% of rice flour and 50% of buckwheat, amaranth or quinoa flours (as substitutes of potato starch), respectively, and evaluated the influence of pseudocereals flour supplementation on the technological properties of resulting breads. The GF breads containing pseudocereals showed a softer and more cohesive crumb and a desirable darkening of the crust colour when compared to the gluten-free control bread (50% rice flour and 50% potato starch) without negative effects on the sensory properties. In another study by the same authors (Alvarez-Jubete et al., 2010a), these same pseudocereals-containing GF breads have also showed significantly higher antioxidant capacity and total phenol content compared to the GF control.

Manzatti Machado Alencar et al. (2015) evaluated the influence of a partial substitution of a mixture of starches (sour tapioca, cassava, potato, and rice flour) by pseudocereals (amaranth and quinoa) and sweeteners (sucralose, stevia and sucralose/acesulfame-K blend) in GF bread formulations. The resulting breads containing amaranth and quinoa flours (20%) showed higher nutritional profile in terms of greater amount of proteins, lipids and ash when compared to the control bread. While, values for specific volume, firmness and water activity were found to be similar to those of the control formulation, except for a lower firmness obtained in bread with quinoa and stevia.

This study is of particular interest because, always more frequently, there is a simultaneous presence of other autoimmune diseases (such as diabetes) in patients suffering from coeliac disease and, in these cases, they must consume not only gluten-free but also sugar-free foods.

In contemporary times, the legume flours are receiving increasing attention in breadmaking process and several authors obtained promising results. Miñarro et al. (2012) explored the feasibility of using legume proteins of different sources in

GF breadmaking; they produced gluten-free breads with chickpea flour, pea isolate, carob germ flour or soy flour. The authors, for the breads with legume flours, generally observed good physico-chemical characteristics and an adequate sensory acceptance. The poorest results (viscoelastic behaviour but batter structure thicker than others, lowest specific volume, highest hardness and more compact microstructure) with an overall poor quality were observed for carob germ flour bread; while chickpea flour and pea isolate showed very good results in all aspects analyzed.

1.5.4 Native and modified starches

Starch-containing flours and/or starches from different plant sources are traditionally used in the production of GF breads. Among those, potato, corn, tapioca, rice, and wheat are the most widely used either as single starch or as composite starch mixtures, in presence or absence of flours and other ingredients (Capriles and Arêas, 2014). It has to be pointed out that although the use of GF wheat starch is not considered harmful for coeliac patients, its application in GF-products remains questionable (Houben et al., 2012; Capriles and Arêas, 2014).

The absence of gluten increases the role of starch in providing structure and texture to GF breads (Witczak et al., 2015). In fact, during baking, starch binds water and creates a gas-permeable structure influencing, therefore, dough rheology, water retention and final structure and quality of the gluten-free breads (Houben et al., 2012). The properties of final and intermediate products are closely linked to the type of starch used in terms of origin, species, particle size, amylose/amylopectin content, starch treatment and combination of different starch sources and other ingredients (Witczak et al., 2015).

Another way to use starch in breadmaking is related to the use of starch modified by chemical, physical or enzymatic treatment, through which adverse physical and chemical characteristics of native starch (low thermal stability, susceptibility to extreme pH conditions, tendency to retrogradation) can be eliminated (Witczak et

al., 2012).

Inclusion of chemically modified starch such as amylose corn starch (HACS), acetylated distarch adipate (ADA), and hydroxypropyl distarch phosphate (HDP) in gluten-free bread formulation can improve structure stability and delay bread staling. Chemically modified starches are classified as food additives and labelled with "E numbers" (Witczak et al., 2015); this led the consumers to consider these additives as unnatural constituents explaining, at least in part, their limited use in GF-products (Ziobro et al., 2012). Conversely, physically modified starches are classified, like native starch, as food components give a "clean label", which is preferred by the consumer. Among those pre-gelatinized tapioca starch is the most used, affecting positively bread volume and crumb softness (Witczak et al., 2015).

1.5.5 Hydrocolloids

In a starch-based GF bread formulation, other polymeric substances such as hydrocolloids and non-gluten proteins from both animal and plant origin, can be used as gluten replacer in order to mimic the viscoelastic properties of gluten and to improve the overall quality of the end products.

Hydrocolloids, also known as food gums, include different polysaccharides and proteins, which are able to perform a range of functions including gelling, thickening and emulsifying, when they are incorporated in a food system (Pegg, 2012). Moreover, hydrocolloids are included in the concept of soluble fibre adding a nutritional function to this group of substances (Matos and Rosell, 2014). Although a regulatory category for food hydrocolloids does not exist, they are currently classified as food additives, with some exceptions like gelatine and starches classified, instead, as food ingredients (Pegg, 2012).

According to the either plant origin or chemical synthetic forms, hydrocolloids can be classified as follows: a) from marine algae (agar-agar and carrageen); b) plant extracts (pectin and β-glucan), c) plant exudates like gum arabic, and d) seed mucilages (locust bean gum, guar gum and psyllium), as it regards the natural

forms, and e) chemical synthesized cellulose derivatives like hydroxypropyl methylcellulose (HPMC), carboxymethylcellulose (CMC) and methylcellulose (MC), f) microbial biosynthetic like xanthan gum, as it regards the synthetic forms (Houben et al., 2012; Collar and Angioloni, 2010).

The effects of hydrocolloids addition in GF bread formula include several aspects; they modify dough behaviour improving its development and increasing gas binding capacity (by raising in system viscosity), improve moisture retention and stability of the system, affect swelling, gelatinization and pasting properties of the dough and delay starch retrogradation. They also increase loaf volume and improve structure, texture and acceptability of final breads (Lazaridou et al., 2007; Houben et al., 2012). However, it is worth highlighting that their effects on dough and bread properties are dependent on the type of additive and related percentages used (normally up to 2%), on the interactions with other food polymers added in the formula (mostly starch and protein), and on the parameters of the process (Lazaridou et al., 2007; Houben et al., 2012; Capriles and Arêas, 2014).

1.5.6 Proteins

The addition of proteins in gluten-free bread formula is important for two main reasons. The first one is related to their important functional role: proteins are primarily used as a structure and texture forming agents to build up a network similar to that formed by gluten in bread production. The second reason is related to their positive nutritional impact (increasing protein content and supplying essential amino acids) on the end products (Houben et al., 2012; Ziobro et al., 2013).

The proteins most commonly used are taken from both plant (like cereals, legumes and pseudocereals) and animal origin (like egg albumins and milk proteins). Among those, milk proteins are widely used because of their chemical structure very similar to that of gluten proteins and their high nutritional levels (Houben et al., 2012). However, the effects on the quality of final product are

closely linked to the kind of dairy proteins used. In fact, they are characterized by different functional properties, which include emulsifying and stabilizing ability of caseinates, gelling properties of isolated and concentrated whey proteins, and a high water-binding capacity of high-temperature skim milk powders (Houben et al., 2012). Krupa-Kozak et al. (2013) produced GF bread supplemented with low-lactose dairy proteins at two different levels (12% and 24%) and evaluated the effects on the quality of resulting breads. The authors found that supplemented breads were characterized by significantly increased values of protein at all supplementation levels. In particular, breads supplemented with 12% of milk powders showed a significantly decreased hardness, increased specific volume, crust darkening and crumb lightness and, mostly, a protein content 5 times richer than that of the control.

Since coeliac disease are often linked to a secondary lactose intolerance caused by absence or reduction of lactase secretion (resulted from the villous atrophy), the use of such ingredients in developing bakery products for a low-protein diet must be carefully considered (Krupa-Kozak et al., 2013; Houben et al., 2012).

Other two ingredients often used in the production of gluten-free bread are egg and soy proteins. Addition of eggs as a gluten replacer is related to their foaming ability, stabilizing effect and emulsifying properties (Capriles and Arêas, 2014). Whereas, soy proteins can be used for its effects in increasing crumb texture and bread volume. They can be added into the bread formula in two different forms: as high-protein soy flour or as soy protein isolate (Capriles and Arêas, 2014).

1.5.7 Enzymes

Another possibility for improving dough properties and enhancing the final quality and shelf life of GF breads is related to the use of enzymes as exogenous ingredients.

The most commonly enzymes used in GF breadmaking are those that act either on starch fraction (amylase and cyclodextrin glycosyltransferase) or proteins network

(transglutaminase and glucose oxidase), and proteases (Gómez and Sciarini, 2015; Houben et al., 2012).

The protein-connecting transglutaminase has the ability to induce cross-links between proteins leading, indirectly, to a conversion of soluble proteins to insoluble protein polymers with high molecular weight (Gallagher, 2009). Renzetti et al. (2008) evaluated the effects of the addition of transglutaminase (TGase) at different levels (0, 1, and 10 U of TGase/g of protein) on the quality of GF breads whose included in their formulation buckwheat, brown rice, oat, sorghum, teff and corn flours without addition of any hydrocolloids. Those authors found that the efficiency of the enzyme is dependent on the protein source. Buckwheat and brown rice flours were the optimal substrates for TGase application resulting in breads with improving structure and texture; no effects (or very slight) were found on breads from oat, sorghum and teff; while in bread from corn flours the enzyme addition caused detrimental effects on batters behaviour but, the resulting bread were of high quality.

Also Moore et al. (2006) evaluated the impact of TGase (0, 0.1, 1, and 10 U of TGase/g of protein) on GF bread quality with the simultaneous presence of various protein sources (skim milk powder, soya flour, and egg powder) in bread formula. Those authors obtained breads with improved quality but observed that the efficiency of the enzyme is dependent on both protein sources and levels of enzyme addition.

Cyclodextrins with their hydrophilic surface and hydrophobic interior can form complexes with lipids or hydrophobic proteins improving crumb structure and gas-binding capacity in GF breads, particularly those containing rice flour. Furthermore, such both cyclodextrins and amylases are useful in reducing the staling rate of GF bread. They reduce the amount of amylopectin available to retrograde and also affect both protein-starch and protein-protein interactions (Gallagher, 2009; Houben et al., 2012).

1.6 Gluten-free bread and Glycemic Index

Since there seems to be an increasing prevalence of type I diabetes among coeliac patients, a good glycemic control is of particular importance in people suffering from coeliac disease. The likely explanation for the simultaneous occurrence of these two immune-mediated disorders is a common genetic background (Scaramuzza et al., 2013).

GF breads, being starchy-based foods are normally characterized by high glycemic index (GI) with only some exception such as white GF bread (GI=57) (Atkinson et al., 2008). Matos and Rosell (2011) in evaluating the chemical composition and starch digestibility of eleven GF commercial breads found that estimated GI ranged from 83.3 to 96.1. Thus, in the attempt to reduce the GI of GF-products, the choice of suitable raw materials and/or functional ingredients to be included in GF bread formula becomes extremely important.

Capriles and Arêas (2013) found that the addition of inulin-type fructans (ITFs) in GF bread (rice flour 50%; potato starch 50%; egg 10.5% as main ingredients) up to the level of 12% resulted in a product with low GI, high quality and enhanced sensory acceptability. In particular, the addition of 12% of these inulin-type fructans enriched the resulting breads with the 8% of dietary fibres (despite, during baking, one-third of the ITFs was lost) and reduced the GI from a value of 71 to 48, a drop of 23 points.

1.7 Gluten-free bread staling

A rapid onset staling (more than in wheat bread) and a short shelf-life are included among the major disadvantages normally associated with GF breads. This is mainly due to the high amount of isolated starches (almost 100% of the flour base) included in their formulation. Furthermore, as a consequence of the high hydration required for their good development, a greater amount of water is available causing an increase in crumb firmness and softer crust (Arendt et al., 2008).

Several authors adopted different promising approaches for delaying the staling of GF bread including the use of enzymes (α-amylases and cyclodextrin glycoxyltransferase), some hydrocolloids (such as HPMC and CMC), emulsifiers and sourdough technology (Gujral et al., 2003; Moore et al., 2007). Mariotti et al., (2013) evaluated the effects of the addition of buckwheat flour (40%) and HPMC (0.5%) on the breadmaking performance of two commercial GF bread mixtures with particular attention to their influence on the properties of the resulting optimized doughs and breads. The authors found that the addition of buckwheat flour improved the baking performance of the two optimized commercial GF-mixtures; while the addition of HPMC resulted in a slower staling kinetics during storage mainly due to its ability in reducing diffusion and loss of water from bread crumb as well as the interactions among starch and protein.

2 AIM OF RESEARCH

The ancient origin of bread and its countless varieties currently spread throughout the world could give the mistaken impression that bread is a sufficiently well known food with lost significance in terms of new advances on both product development and process innovation.

On the contrary, its centuries-old history (dating back to ancient Egypt sometime around 7000 BC) through different cultures to the present day, makes it this food characterized by a continuous technological and nutritional development, which constantly renews and evolves along with the changes in human civilisation. To consider how different were the earliest forms of breads from those available in industrialized countries today helps realize how this food involves itself both change and improvement. Although in some parts of the world bread still retains its traditional form as for flat breads of the Middle East (Cauvain, 2015).

Since the industrial revolution, because of the continuous growing economic development and the rise of globalization, the evolution of breadmaking process has progressed further than in other periods. These changes occur in the social economy have inevitably induced deep changes in people lifestyle and, especially, in food consumption dynamics, making the consumers more aware of the significant impact of food upon health. In this context, a relatively inexpensive and high nutritious staple food like bread, which represents an important part of the human diet, is a main player.

The overall objective of this thesis was to create cereal-based baked products with added value with respect to those currently available on the European market, satisfying the consumers' needs in terms of both sensory acceptability and superior nutritional profile. The research project was divided into three different parts, all of which aimed at achieving the same overall objective following, however, their own lines of investigation and specific goals.

In the first part of the work a market study was performed to get a comprehensive, realistic and detailed overview of the current bread-market supply in both gluten-containing and gluten-free products market, in order to obtain an overall quality

picture of the available offer in this prioritised food industry area. For this purpose, a representative selection of 20 commercial sliced breads (10 gluten-containing and 10 gluten-free samples), selected from the major brands currently available on the European market, has been assessed by physical-chemical, technological, nutritional, and sensory determinations (chapter I).

Hereupon, two other different lines of research for the improvement of both gluten-containing and gluten-free breads were undertaken.

The first one, as described in chapter II and III, evaluated the impact of wheat flour replacement up to 45% (weight basis) by incorporation of nutrience-dense raw materials (such as ancient cereals, pseudocereals and legumes) in improving wheat bread quality.

In particular, in chapter II, the specific purpose was to explore the competences and exploit the suitability of non-breadmaking whole grains (such as teff, buckwheat and green pea flours) with unique nutritional components to be simultaneously included in mixed wheat matrices, to obtain novel and healthy fermented baked goods meeting the functional and sensory restrictions of viscoelastic breadmaking systems. Bread functional and nutritional profiles, with special emphasis on starch hydrolysis kinetics and relevant starch nutritional fractions, were assessed in quaternary wheat blended matrices using wheat flour bread for comparative purposes (control).

In chapter III, our attention has been focused on a) the investigation of the thermal transitions that occur during starch gelatinization and retrogradation in these multicomponent bread matrices baked at restricted water availability, b) on the impact of non-breadmaking whole grains in the transition phases, and c) on the exploration of the relationships between thermal properties and starch digestibility and firming kinetics of these multigrain bread matrices.

Currently, little is known about the thermal transitions of multicomponent bread matrices baked at restricted water conditions, as well as information about the possible relationships between thermal properties, textural behaviour and the

susceptibility of starch to enzymatic digestion in those heterogeneous matrices lack.

The second line of research evaluated the impact of different flours, starches, polymeric substances, and surfactants on gluten-free dough performance to achieve GF-flat breads. The specific objective was to explore the capability of different GF basic formulations made of different flour (rice, amaranth and chickpea) and starch (corn and cassava) blends, to make processable and viscoelastic GF-doughs in absence/presence of single hydrocolloids (guar gam, locust bean and psyllium fibre), proteins (milk and egg white) and surfactant (vegetable oil) in order to select the most promising formulations for producing the so called *Spianata,* a typical and widely consumed Sardinian flat bread.

Without doubt, development and innovation of GF-products due to the growing number of diagnosed patients with coeliac disease constitute areas of increasing interest. However, to better understand our choice of investigate gluten-free flat bread, it is worth highlighting that the prevalence of coeliac disease in Sardinia (0.31%) (a major Mediterranean island) not only is the highest among the Italian regions but, also, shows a strong and steady increase in the number of diagnosed cases (Ministero della Salute, 2012). Hence, either the importance or the desire to make available this typical and widely consumed Sardinian flat bread (traditionally made from durum wheat) for people suffering from coeliac disease is being emphasized.

3 WORK PLAN

The research project was divided into three different parts regarding a) characterization and classification of commercial breads, b) production and characterization of multigrain breads, and c) evaluation of gluten-free dough performance to achieve GF-flat bread, respectively. In this section a brief description of the materials used and experimental analysis performed are reported.

3.1 Study Materials

I. *Techno-functional and nutritional performance of commercial breads available in Europe*: The first study was conducted on twenty sliced and packaged commercial breads from the major brands currently available in the European market. Samples, consisting of gluten (n=10) and gluten-free (n=10) breads, were selected and purchased in supermarkets of different European countries (Italy, Spain, and Germany).

II. *Impact of ancient cereals, pseudocereals and legumes on starch hydrolysis and antiradical activity of technologically viable blended breads*.

III. *Significance of thermal transitions on starch digestibility and firming kinetics of restricted water mixed flour bread matrices*: The second and third studies were performed using commercial flours from refined common wheat (*Triticum aestivum*), whole teff, (*Eragrostis tef*), green pea (*Pisum sativum*) and buckwheat (*Fagopyrum esculentum*) purchased from the Spanish market. Other ingredients used were: water, yeast, salt, vegetable fat-margarine, sugar, sourdough (Ireks), milk powder, α-amylase (Novozymes), and Calcium propionate (Sigma–Aldrich).

Bread making and Experimental Design. For the preparation of multigrain breads, all ingredients were blended with composite flours following a straight-dough bread-making process. Wheat flour was replaced from 22.5% to 45%, by simultaneous incorporation of teff, green pea, and

buckwheat flours added at two different levels of substitution: low (corresponding to 7.5%) and high (corresponding to 15%). It was decided to use these levels of wheat flours substitution on the basis of the results obtained in previous studies (Angioloni and Collar, 2012). Preparation of blended breads was in according to a Multilevel Factorial Design with three experimental factors (teff, green pea and buckwheat flours) at two levels (low and high wheat flour replacement), and five error degrees of freedom, set as experimental design for the study. Thus a total of eight flour combinations (plus one wheat bread control) were obtained.

Table 1 Multilevel factorial design for multigrain breads.

	Levels		**Amount g/100g solids**	
Factors	**Low**	**High**	**Low**	**High**
Teff	0	1	7.5	15
Green pea	0	1	7.5	15
Buckwheat	0	1	7.5	15

IV. *Gluten-free dough-making of specialty breads: significance of blended starches, flours and additives on dough behaviour*: The fourth study was conducted on six basic formulations -(flour(s)/starch(es)-, which include additionally different flours, hydrocolloids, proteins and surfactants. Rice flour (RF), corn starch (CS), cassava starch (CaS), milk proteins (MP), guar gam GG), diacetyl tartaric of mono- and diglycerides (DATA), psyllium fibre (PF) and locust bean gum (LB) were from Chimab Campodarsego (PD, Italy). Amaranth flour (AF), egg white proteins (EP), and chickpea flour (CF) were from Molini Bongiovanni S.p.A. (TO, Italy). Sodium stearoyl-2-lactylate (SSL) was from Dupont™ Danisco®, and sunflower oil (SF) was from Carapelli Firenze (Italy).

Dough making and Experimental Design. For the preparation of gluten-free doughs, all ingredients were blended at different levels of addition

using a Kitchen-Aid Artisan mixer. Qualitative and quantitative composition on a 100 g solid basis of the six different basic formulations (coded A-F) was:

- Formulation A: RF (50%) + CS (50%)
- Formulation B: RF (50%) + CaS (50%)
- Formulation C: RF (45%) + CS (45%) + CF (10%)
- Formulation D: RF (45%) + CaS (45%) + CF (10%)
- Formulation E: RF (30%) + CS (30%) + AF (40%)
- Formulation F: RF (30%) + CaS (30%) + AF (40%)

All other ingredients were added singly to each basic formulation at two levels of addition (low and high) as it follows: GG (1/2), LB (1/2), PF (1/2), MP (5/10), EP (5/10), DATA (0.5/1.0), SSL (0.5/1.0), and SF (4/8). Thus, a total of 102 GF dough formulations were obtained. It was decided to use these ingredients at these levels of addition after a thorough study of the recent literature of this research area.

3.2 Experimental Analysis

All analysis were carried out in the four studies either using ICC and AACC official methods or according to procedures previously reported in the literature for bread analysis. A detailed description of methods and statistical analysis of the results is presented in the respective sections of material and methods for each study reported in this thesis.

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5 CHAPTER I

Techno-functional and nutritional performance of commercial breads available in Europe

Techno-functional and nutritional performance of commercial breads available in Europe

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Abstract

In recent years, the growing interest for well-being and healthy lifestyle together with an increasing awareness of the close relationship between food and health have boosted the production of an increasing number of novel goods to be placed in both gluten-containing and gluten-free products market. The objective of this study was to provide a realistic and detailed overview of the current bread-market supply, in order to evaluate the overall quality of the available offer in this prioritised food industry area. Twenty commercial breads consisting of gluten (n=10) and gluten-free (n=10) samples currently available in the European market have been assessed by physical-chemical, technological, nutritional, and sensory determinations. The quality parameters obtained were related to each other by using Pearson correlations, while sample classification was achieved by applying factor analysis. Although the main distinction was between gluten and gluten-free samples as it was expected, classification of breads allowed differentiating samples with different formulations in terms of presence/absence of alternative, innovative and nutrient-dense raw materials.

***Keywords*:** bread, gluten-free, physico-chemical characteristics, nutritional features, sensory analysis.

Introduction

Bread has been one of the staple foods most widely used and consumed around the world and one of the major constituents of the human diet since ancient times. Although the simplicity of the basic recipe (flour, water, salt and leavening agent) the long-term success of bread is easy to understand when considering its typical flavour, taste, and high nutritional value. Bread is a good source of energy mainly due to the high content of starch, besides protein, lipids rich in essential fatty acids, dietary fibres, antioxidants, and micronutrients (Rubel et al., 2015). Nowadays, despite some differences between countries depending on bread type or region are observed, the general trend is that level of bread consumption in the world has been declining (Cauvain, 2015). This is in contrast to the gluten-free market that is experiencing a double-digit growth, confirming it as one of the most prosperous markets in the immediate and near future (Miranda et al., 2014). Even in Europe, bread market has been showing contrasting patterns within countries (Collar, 2015). A study for European Commission in 2010 (made through 27 European Union states) reported that bread consumption patterns differ widely within the European Union but most countries have an average consumption of 50 kg of bread per capita per year (Bakers-Federation, 2013). This slightly decline can be caused by several factors including the changes in consumers' food preferences (increasing consumption of alternative and energy-dense foods often rich in fat) and in eating habits (growth of out-of-home meals) often associated with a lack of physical activity. Over the past decades these changes in people's lifestyles have also resulted in a dramatic increase of several non-communicable diseases including obesity, type 2 diabetes, cardiovascular diseases, and certain forms of cancer (Thondre, 2009). However, consumers' demands in the field of food production have changed considerably (Betoret, Betoret, Vidal, & Fito, 2011). In recent years, the increasing awareness of the

relationship between food and health, the growing demand for healthy, natural and innovative foods as well as the increasing prevalence of food intolerances (in particular the coeliac disease) led both scientific research and bakery industry to make considerable efforts in order to meet the needs of consumers, and to improve the variety, quality and taste of bakery products available in the market. Thus, breads made from grains, grain flours, and bran alternative to wheat or containing other functional ingredients are acquiring a privileged position in the bakery market. The use of whole grains as partially substituted of wheat flour in bread formulations is of nutritional interest because of their lower glycemic index and health-related composition including dietary fibre, minerals, vitamins and antioxidants. The intake of whole-grain bread, which provides more health benefits than refined-grain bread, is generally associated with a reduced risk of coronary heart disease and type-2 diabetes (Blandino et al., 2013). Bread products enriched/fortified with functional components such as ω-3 fatty acids (Gökmen, 2011), prebiotic oligosaccharides (Angioloni and Collar 2011a), inulin (Rubel et al., 2015), and calcium (Salinas and Puppo, 2015) as well as multigrain breads obtained by the addition of minor cereals, pseudocereals, and grain legumes flours (Collar et al., 2014a), are also in good agreement with the current nutritional and nutraceutical dietary trends. Conversely, in case of allergies and food intolerances such as the coeliac disease, the production of bread products made with gluten-free alternative raw materials becomes a necessity. Furthermore, in gluten-free breadmaking, a consumer-satisfying structure, an adequate nutritive value and a good taste of bread can only be achieved using a combination of different ingredients (Houben et al., 2012). Apart from most basic gluten-free ingredients such as rice and corn flour blended with structuring agents (hydrocolloids) and dairy proteins (Lazaridou et al., 2007), also different gluten-free flours (corn, teff, buckwheat, quinoa, sorghum) (Hager et al., 2012) and starches (corn, cassava, potato) (Collar et al., 2014b) as well as enzymes (transglutaminase, proteases) (Hamada et al., 2013), and other non-gluten proteins (from both animal and plant origin, e.g. milk protein, egg albumins and soy protein) (Ziobro et al., 2013) are

being used in order to mimic the viscoelastic properties of gluten and to improve the overall quality of gluten-free bakery products.

In this context, the objective of the present study was to provide a comprehensive, realistic and detailed overview of the current bread-market supply through the physical-chemical, technological, nutritional, and sensory characterization of twenty European commercial breads, in order to obtain an overall quality picture of the available offer in this prioritised food industry area.

Materials and Methods

Materials

20 commercial breads (10 gluten-containing and 10 gluten-free samples) from major brands were selected and purchased from the European market (Fig.1). The chemical composition and nutrition facts of breads were retrieved from the labels provided by the manufacturers, with the only exception of both moisture and ash contents. Moisture determination was performed according to the AACC method 44-15.02 (AACC, 2005), while the ash content was estimated by difference. The ingredient composition of breads is compiled in Table 1. A four digit bread sample code was defined for commercial breads according to their crumb colour (1st digit), absence/presence of seeds (2nd digit), and sample number (3rd and 4th digits). The first digit of the code was set referring to white bread (1), mixed bread (2), and dark bread (3), the second digit to absence (1) or presence (2) of seeds, and the third digit to sample number (from 1 to 10 for gluten breads and from 11 to 20 for gluten-free breads), as it follows: 1101, 2202, 1103, 3104, 1105, 1206, 1107, 3208, 3209, 3210, 1211, 1112, 2213, 1114, 1115, 2116, 1117, 1118, 1119, 3220 (Fig. 1).

Methods

Bread measurements

Physical-chemical properties. The volume of bread samples was measured according to the AACC 10-05.01 method of rapeseed displacement (AACC, 2005). The specific volume was calculated as bread volume (mL) / bread weight (g). Aspect ratio was calculated as width/height ratio of central slices.

Colour measurements were determined on both crumb and crust using a Photoshop system in accordance with the method previously described by Angioloni and Collar (2009) and the results were expressed in accordance to the Hunter Lab colour space. The Photoshop (PS Adobe Photoshop CS5 extended) system (L, a, b colour coordinates) was calibrated using colour sheets from Pantone®Formula Guide (Pantone, Inc., USA). Pantone colour sheets and bread slices (three slices per sample) were used for calibration and for colour measurement, respectively. Images were acquired at 300 pixel resolution with a ScanJet II cx flatbed scanner (Hewlett-Packard, USA). Parameters determined were L (L = 0 [black] and L = 100[white]), a ($-a$ = greenness and $+a$ = redness), b ($-b$ = blueness and $+b$ = yellowness), WI - whiteness index (crumb), and BI - Browning Index (crust), as described earlier (Collar and Angioloni, 2014). Hunter Lab colour space parameters from Minolta colorimeter were calculated from the calibration linear equation Colorimeter *vs* Photoshop (Angioloni and Collar, 2009).

Crumb grain characteristics were assessed in bread slices using a digital image analysis system. Images were previously acquired with a ScanJet II cx flatbed scanner (Hewlett-Packard, USA). The analysis was performed on 40x40 mm or 60x60 mm squares (depending on the size of breads) taken from the centre of the images and data were processed using SigmaScan Pro 5 (Jandel Corporation, USA). The crumb grain parameters determined were: cell area, cell density (cell/cm^2), cell/total area ratio, and wall to total area ratio (Collar, Bollaìn, & Angioloni, 2005). According to the pre-selected cell size range (<0.4 mm^2, 0.4-1.0 mm^2, 1-10 mm^2, 10-80 mm^2, and >80 mm^2), cell area distribution and cell number distribution were also determined.

Bread primary and secondary mechanical characteristics (Texture Profile Analysis, TPA, using a double compression cycle) of breads were recorded in a TA-XT2 texture analyser (Stable Micro System, Surrey, UK) using a 25 mm diameter probe, a 30 Kg load cell, 50% penetration depth and a 30 s gap between compressions on slices of 25 mm width (Collar et al., 2005).

For stress relaxation (SR) measurements, samples from the centre of the crumb slices were cut into cubes (2x2x2 cm) and compressed using a TA-XTplus texture analyser (Stable Micro System, Surrey, UK). Samples were compressed using a cylindrical upper die of 50 mm diameter at a cross speed 0.5 mm/sec. The strain used was 20% and the whole relaxation experiment lasted 10 min. The obtained stress relaxation curves were normalized and converted to linear form according to the Peleg and Pollak (1982) model, previously applied by Angioloni and Collar (2009) for bread:

$$\frac{F_0\, t}{F_0 - F(t)} = k_1 + k_2 t \qquad (1)$$

where F_0 is the initial force, $F(t)$ the momentary force at time (t) and k_1 (s), k_2 are constants related to the initial rate of relaxation (intercept) and to the extent of relaxation (slope), respectively. Relaxation time (RT) was calculated as the time required for the maximum force to drop to 60% of its value. All measurements were made in triplicate.

Enzymatic/Biochemical determinations. Bioaccessible polyphenols were determined in commercial breads using an *in vitro* digestive enzymatic mild extraction that mimics the conditions in the gastrointestinal tract according to the procedure of Glahn, Lee, Yeung, Goldman, and Miller (1998) and adapted by Angioloni and Collar (2011b) for breads. The enzymes used to simulate the gastric and intestinal digestion were pepsin and bile/pancreatin solution, respectively. The obtained digestive extracts were used for the determination of bioavailable polyphenols after removing the proteins by addition of trichloroacetic

acid (20% w/w), precipitation and centrifugation. The same extracts were used to determine the radical scavenging capacity of breads using the DPPH• (2,2-diphenyl-1-picrylhydrazyl) method (Brand-Williams, Cuvelier, & Berset, 1995), modified by Sánchez-Moreno, Larrauri, and Saura-Calixto (1998) and adapted by Collar, Jiménez, Conte, and Fadda (2014a). In brief, aliquots of 0.1 mL were taken, and 3.9 ml of a solution of DPPH 0.025 g/L (equivalent to 0.0634 μmol/mL) was added. Tubes were gently shaken, and 4 mL of each tube were added to 4 mL cuvettes, and A515 nm was read at 1 min and every 5–10 min until the plateau was reached. A cuvette containing 4 mL of DPPH 0.247 μmol in methanol was read at the same periods. A blank of methanol was used. Lectures were taken in duplicated samples. Plots of μmol DPPH *vs* time (min) were drawn, and calculations were made to know the antiradical activity (AR). AR = [([DPPH] $_{INITIAL}$ − [DPPH] $_{PLATEAU}$) × 100]/[DPPH] $_{INITIAL}$.

In vitro starch hydrolysis kinetics and relevant starch nutritional fractions were determined in accordance with the AACC (2005) method 32-40 with the modification reported by Angioloni and Collar (2011a). As stated by Englyst et al. (2003) different fractions of starch were determined: rapidly digestible starch (RDS) and slowly digestible starch (SDS) were measured after incubation for 20 and 120 min; total digestible starch (DS) was determined after 16 h of incubation while resistant starch (RS) was determined in the pellet as the starch remaining after 16 h incubation.

The starch hydrolysis kinetics and expected glycemic index (eGI) of breads were calculated in accordance with the procedure followed by Chung, Liu, Pauls, Fan, and Yada (2008) based on the method established by Goñi, Garcia-Alonso, and Saura-Calixto (1997), and applied previously (Angioloni and Collar, 2011a). A first order kinetic equation [$C = C_\infty (1 - e^{-kt})$] was applied to describe the kinetics of starch hydrolysis, where C was the hydrolysis degree at each time, C_∞ the equilibrium concentration or maximum hydrolysis extent, and k the kinetic constant. The hydrolysis index (HI) was calculated as the relation between the area under the hydrolysis curve (0–16 h) of breads and the area of a standard

material (white bread) (Chung et al., 2008). The eGI was calculated using the equation proposed by Granfeldt, Björck, Drews, and Tovar (1992): eGI = 8.198 + 0.862HI.

Sensory evaluation

Sensory analysis of fresh breads was performed with a panel of eight trained judges (four males and four females aged 24–55) using a semi-structured scale, scored 1–10 in which extremes (lowest: 1; highest: 10) were described for each sensory attribute according to Setser (1996). Evaluated attributes were grouped into visual, textural and organoleptic characteristics (Collar et al., 2005).

Statistical analysis

Statistical analysis of the results was performed using Statgraphics V.7.7 program (Bitstream, Cambridge, MN). Pearson correlation analysis for relationship between bread properties and factor analysis for breads classification were used.

Results and Discussion

Relationships between biochemical, physical and sensory parameters of breads

Associations between all the evaluated bread quality parameters were analyzed by using Pearson correlations. Biochemical *vs* physical properties (Table 2) and biochemical *vs* sensory ratings (Table 3) explicited major significant relationships.

Values of correlation coefficients (*r*) revealed significant relationships ($0.01<p<0.05$) between biochemical and physical properties of breads (r= 0.46 - 0.77), especially for starch hydrolysis parameters, protein and bio-accessible polyphenol contents with mechanical characteristics of breads, respectively (Table 2). C_∞ and eGI as well as H_{90} and HI positively affected all bread primary and secondary mechanical characteristics ($0.50 < r < 0.76$), while only a few correlations were found for the starch nutritional fractions. DS negatively correlated with springiness (r= -0.51) and RS positively correlated with kinetic

parameters for stress relaxation k_1 (r= 0.73), k_2 (r= 0.65), and RT (r= 0.77) (Table 2). Protein and bio-accessible polyphenol contents negatively correlated with hardness (r = -0.53, -0.54, $p<0.05$) and positively correlated with springiness (r = 0.55, $p<0.05$) and cohesiveness (r = 0.61, 0.68, $p<0.01$), respectively.

Crumb texture is an important attribute of bread quality, and the protein fraction plays a key role in the formation of the structure, gas retention and volume of breads (Scanlon and Zghan, 2001). In this study, commercial breads analyzed showed wide variation, with gluten-free breads exhibiting inferior crumb texture profile compared to wheat-based breads (softer and springier crumb with high cohesiveness). Hardness, springiness, and cohesiveness values for gluten breads ranged from 4.5 to 9.7 (N), from 0.8 to 1 and from 0.59 to 0.68, respectively (except for sample 3210, which showed the highest hardness (96 N) and the lowest cohesiveness (0.18) values, respectively); while in gluten-free breads the following intervals were found: 8.5-47.1 for hardness, 0.7-0-9 for springiness, and 0.38-0.6 for cohesiveness. This is probably due to the lack of a coherent and continuous protein matrix that, in gluten-free breadmaking, led to a low dough development important in determining the crumb structure and, consequently, the mechanical properties of bread. Also, changes in the structure can be linked to changes in starch digestibility. Bread can be considered as a composite material in which the protein network does not represent an isolate system but interacts with other constituents like starch granules (Guerrieri et al., 1997). Depending on the kind of protein, starch and lipid interactions may block enzyme active sites with a consequent reduction of starch hydrolysis rate and expected glycemic index.

Moreover, significant correlations were found between protein and polyphenols content and cell to total area ratio (r = 0.76, $p<0.01$), as well as between eGI and cell to total area ratio (r= -0.47, $p<005$) (Table 2).

Sensory attributes grouped into visual, textural (tactil and biting) and organoleptic characteristics were correlated with biochemical properties of breads, and, although *r* values were discreet, significant ($0.01<p<0.05$) correlations (from 0.46

to 0.77) were found (Table 3). Relationship between these properties evidenced that the digestible carbohydrates and dietary fibre content were the bread nutritional fractions that most influenced visual and taste and aroma properties.

It is a common agreement that sensory visual and tactile perception of breads play a key role in the consumers' acceptability (Angioloni and Collar, 2009); besides, several authors (Scanlon and Zghan, 2001; Angioloni and Collar, 2009; Hager and Arendt, 2013) pointed out how the crumb feels to the touch or in the mouth is greatly influenced by the grain or cell structure of the crumb (cell size, cell uniformity and thin-walled cells).

In this work, higher content of dietary fibre corresponding to low cell uniformity (r = -0.54) high cell size (r = 0.50), thickness (r = 0.70), aroma and taste intensity (r =0.46, 0.48), and saltiness (r=0.69); instead, the contrary occurred for the digestible carbohydrate content ($0.50 < r < 0.63$) (Table 3). The effect of dietary fibre addition (using ingredients with high-fibre content or adding functional fibre) on crumb grain characteristics have been studied in several works and, in the most of these studies, the authors did not find a clear trend. Angioloni and Collar (2011a) reported heterogeneity in the values related to crumb grain structure for unsupplemented and fibre-supplemented breads; but, the authors, also pointed out how the overall acceptability ratings seem to depend more on organoleptic and textural than on tactile and visual characteristics.

Protein and bio-accessible polyphenols content influenced the organoleptic properties but, unlike fibre and carbohydrates, these fractions were in good accordance with aroma (for both r= 0.49, $p<0.05$) and taste (r= 0.69, 0.79, $p<0.01$) quality and aftertaste (r= -0.51, -0.72) (Table 3).

In bread, sensory texture parameters are often connected and well predicted by instrumental measurement such as TPA (Bollaín et al., 2005). Consistent with this and in accordance with the correlations previously reported (biochemical *vs* texture properties) good correlations between starch hydrolysis parameters, protein and polyphenols content and sensory texture characteristics were also found. The higher C_∞, eGI, H_{90} and HI, the lower sensory cohesiveness (-0.46< r

<-0.57) and elasticity (-0.48< r <-0.55); while, the higher protein and polyphenols content, the higher sensory cohesiveness (r=0.58, 0.66 p<0.01) and gumminess (r=0.64, 0.74 p<0.01) (Table 3).

Classification of breads

Classification of 20 European commercial breads (10 with gluten and 10 gluten-free) on the basis of their distinctive and significant responses in terms of crumb and crust colour features, rheological behaviour, relevant nutritional fractions, bioactive components, and sensory ratings was achieved by means of multivariate data handling.

From more than 70 functional variables analyzed in the different commercial breads, 17 independent variables were selected to perform sample classification using factor analysis (FA). FA grouped techno-functional and nutritional bread parameters into five different factors that explained 78.22% of the cumulative variance (VE), with the first three factors explaining 59.83% of the variability of the results (Table 4).

Factor 1, which makes the highest contribution accounted for 31.87% of the total variation, grouped bioactive components and taste and aroma sensory features, factor 2 (16,53%) grouped mechanical properties and starch hydrolysis parameters, while factor 3 (11,43%) included biting and tactil sensory properties (Fig. 2). Factor 1 correlated positively with protein content, bio-accessible polyphenols, aroma and taste quality. Factor 2 correlated positively with hardness, C_∞ and eGI. Factor 3 showed negative relationship with softness and smoothness sensory characteristics. Plots of scores of Factor 1 *vs* Factor 2 and Factor 1 *vs* Factor 3 illustrating variable and sample location in the scatterplots are depicted in Fig. 3. In both plots, the separation between gluten and gluten-free breads was observed clearly according to the factor 1, located along the x axis. Gluten breads were located in the positive zone (side) of the x axis in both of plots; while, gluten-free breads were located in the negative zone (side). The plot 1 (Fig. 3) allowed to identify three different groups of samples as described below.

In the positive side of the x axis is located the group I that included all gluten breads with the exception of sample 3210, which general behaviour appeared closer to that of gluten-free breads. The samples of this group exhibited higher values for protein and bio-accessible polyphenols content, and aroma and taste quality. The group II (2213, 1114, 2116, 1117, 1118, 3220 samples) that showed intermediate and low (2116, 1117, 1118) values of the above-mentioned characteristics (especially in terms of protein and polyphenols content), and group III (1211, 1112, 1115, 1119 and 3210) in which the values for variables in factor 1 are always very low, were instead located in the left side of the x axis.

Most of the gluten bread formulations were based primarily on common wheat flour except for samples 3104, 1105 and 1107 based on whole wheat flour, durum wheat remilled semolina and Khorosan kamut wheat flour, respectively (Table 1); but, it is noticeable that the protein content of breads of group I, which ranged from 8.5 to 12.5 (g/100 g bread, as is), was found to be highest in 1206 (12.5 g/100g) closely followed by 3209 (12g/100g). This highest level of protein content is probably due to the presence of soybean grain/seeds, which are an excellent source of high-quality protein and isoflavones in bread formulation. These values are consistent with the significant increase in protein content for soy-supplemented wheat breads previously observed by Dhingra and Jood (2001). Moreover, the presence of flaxseeds and flaxseed oil, as ingredients, in 3208 closely followed by these same samples 1206 and 3209 could be responsible for the high content of both protein (Marpalle et al., 2014) and polyphenols (Meral et al., 2013). Among the samples grouped in the other two groups, it should be noticed the prominent level of both protein and polyphenols provided by the gluten-free sample 1112 (8.5 g/100g; 1474 mg of gallic acid/100g of fresh bread) (group III), but also the relevant polyphenol content observed in gluten-free bread 2213 (1444 mg of gallic acid/100 g) (group II). Sample 1112 includes in its formulation eggs and soy protein isolate (ingredients normally used as source of protein in gluten-free bread), while sample 2213 includes flaxseeds. With regard to the sensory parameters, breads of group I were scored for taste and aroma

quality higher than those of group II and III. In the group I values ranged between 5.6-7.1 for aroma quality and between 5.1-6.6 for taste quality highlighting the clear preference given to these breads; in group II and III, instead, the only samples to be awarded a score higher than 5 for both of parameters were 2213 (5.7-5.5), 3220 (5.3-5.4) (group II), and 3210 (6.4-6.3) (group III). This result showed that, among the gluten-free breads, the judges gave a high acceptability to those samples that, in their formulations, included either flours of minor cereals and pseudocereals or seeds in significant percentages (Table 1).

Furthermore, considering Factor 2, it is possible to clearly identify these three groups of breads also in terms of mechanical properties and starch hydrolysis parameters (Fig. 3). From the top along the y axis, the groups were characterized by gradually decreasing values of eGI, C_∞, and hardness: once again, group I and group III exhibited the best and the poorest behaviour, respectively. All samples of group I showed low and moderate eGI with values ranging from 52.2 (1105) to 72.52 (3209), with the only exception of sample 1101 (91.20) (Table 5). The reason of this results is probably due to the poorer formulation of this bread that includes only refined wheat flour in its recipe and that, therefore, showed a glycemic response very close to that of white bread (GI=100) generally used as a reference food. All breads belonging to the other two groups can be classified as high glycemic index (GI) showing values of eGI higher than 70 (group II) and 86 (group III), with the only exception of sample 1114 (65.22) in group II (Table 5). This extreme variability shown by commercial breads in eGI values, is directly related to the degree and rate of carbohydrate digestion. In fact, the starch digestion of cereal products is a complex process and the rate of digestion of the starch seems to be influenced by several factors such as characteristics of the starch, food processing, and the presence of fibre, protein, lipids and their interactions (Singh et al., 2010; Annor et al., 2013). As above, group I showed a low extent of starch hydrolysis with the lowest values for C_∞ and eGI (Table 5). It seems that the high protein content of these breads may account for the reduced digestibility of the starch and for the low eGI. In fact, in several cereal products,

starch-protein interactions lead to the formation of protein network that surrounds the starch granules reducing the availability to enzyme attack. Therefore, it should be noticed the rather low eGI (55.2) reported of sample 1105 made from durum wheat semolina, which is characterized by stronger starch-protein interactions. This fact, probably, may contribute to a further reduction in the degree of starch digestion.

Despite Factor 3 explained less than 12 % of the cumulative variance, it is useful to further classify the samples in terms of sensory textural parameters such as smoothness (tactil parameter) and softness (biting parameter). Thus, considering the plot of Factor 1 *vs* Factor 3 (Fig. 3) three groups can be defined: a first group (A) in which, along with most of the gluten-free bread (1211,1112,2213,1115,1117,1119,3220) were grouped some of the gluten breads (1103, 3104 and 3210); a second group (B) formed by the most of gluten breads (1101, 2202, 1105, 1206, 1107, 3208, 3209); and a third group (C) that showed the highest values (data not shown) for sensory smoothness and softness, composed of 1114, 2116 and 1118 gluten-free breads. It should be noticed the higher values showed by the three gluten-free breads when compared to all other samples, including gluten-breads.

Conclusions

In conclusion, characterization of different commercial breads evidenced that the main distinction, as it was expected, was between gluten and gluten-free breads, although the latters have shown a great variability in terms of overall quality. Classification of breads also allowed differentiating samples with different formulations. In fact, highest values for the most significant variables were observed for breads characterized by rich formulations in terms of presence of other and alternative flours (rye, buckwheat, quinoa, millet, durum wheat semolina, and whole wheat), grains (soybean) and seeds (flaxseed, sunflower, and sesame). Among these samples, the best overall behaviour was observed for gluten breads (1206, 3209, 2202 and 3208 particularly) but, intermediate values

were also found for gluten-free samples 1112, 3220 and 2213. Conversely, lowest values were found in gluten-free breads characterized by poorer formulations in terms of absence or presence of low percentages of the above-mentioned ingredients (1211, 1115, and 1119). Although the efforts of research and bakery industry are moving in the right direction to develop and produce gluten and gluten-free high-quality products, data obtained in this study confirm that, from a nutritional point of view, there is still a substantial room for improvement on both of areas.

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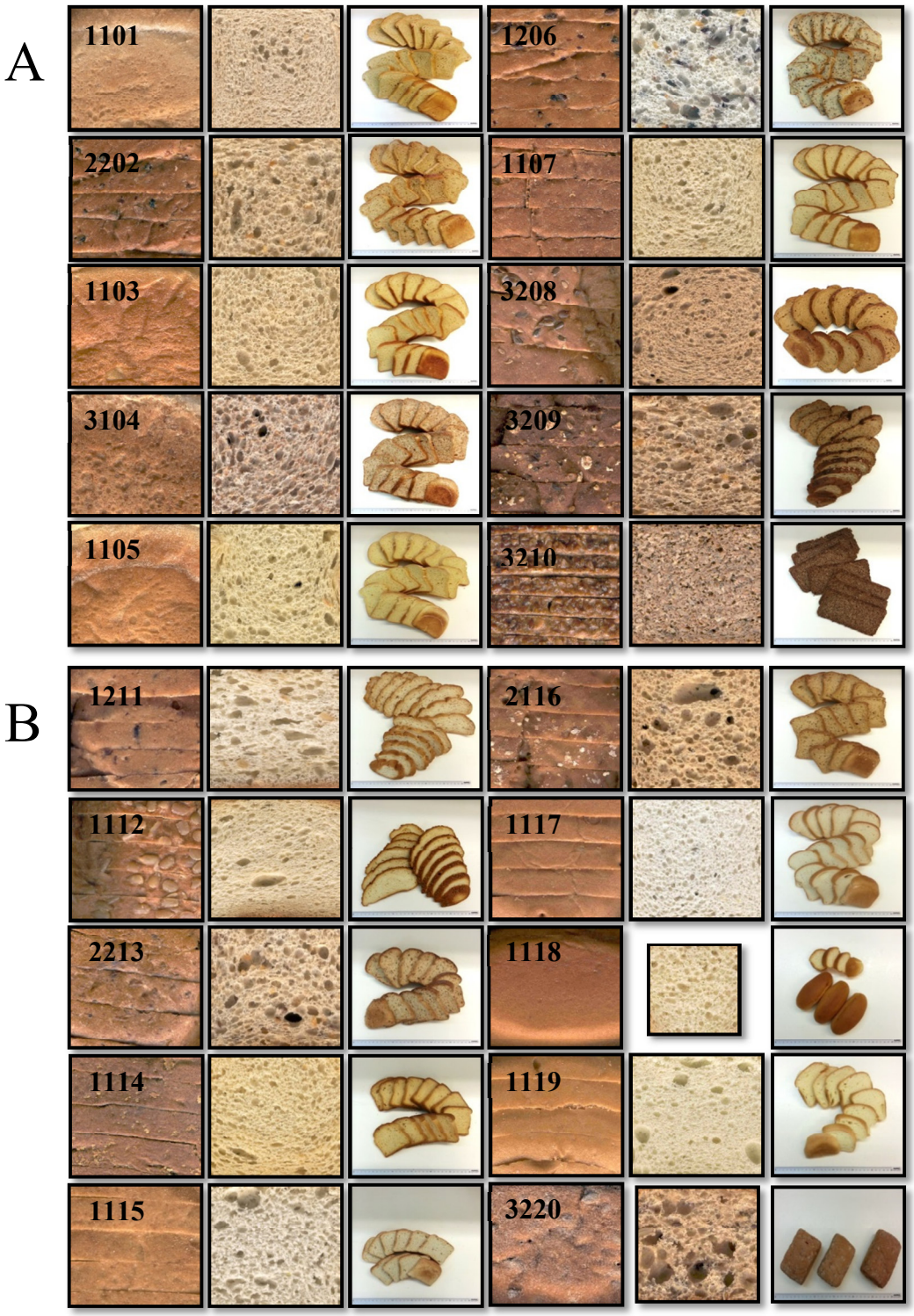

Fig. 1 Crust and crumb slice digitalized images of gluten (A) and gluten-free (B) commercial breads. A four digit bread sample code refers to white (1), mixed (2), and dark (3) crumb colour (1st digit); absence (1) or presence (2) of seeds (2nd digit), and sample number (3rd and 4th digits) from 1 to 10 for gluten breads and from 11 to 20 for gluten-free breads.

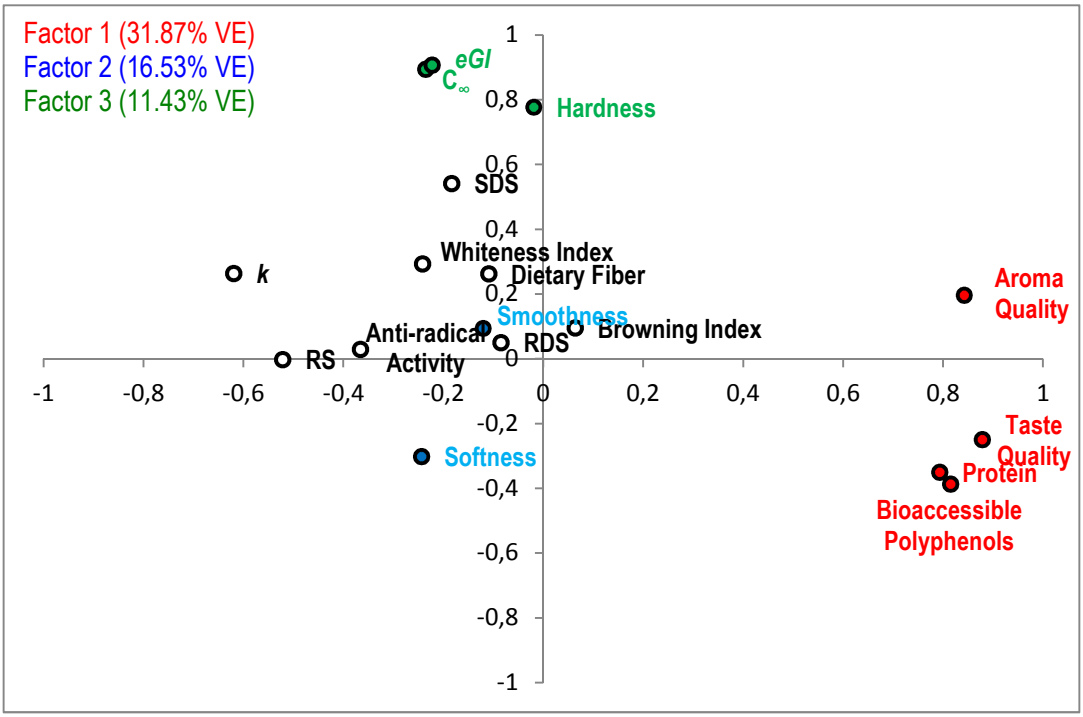

Fig. 2 Scatterplots of technofunctional, nutritional and sensory parameters of commercial breads from Factor Analysis scores.

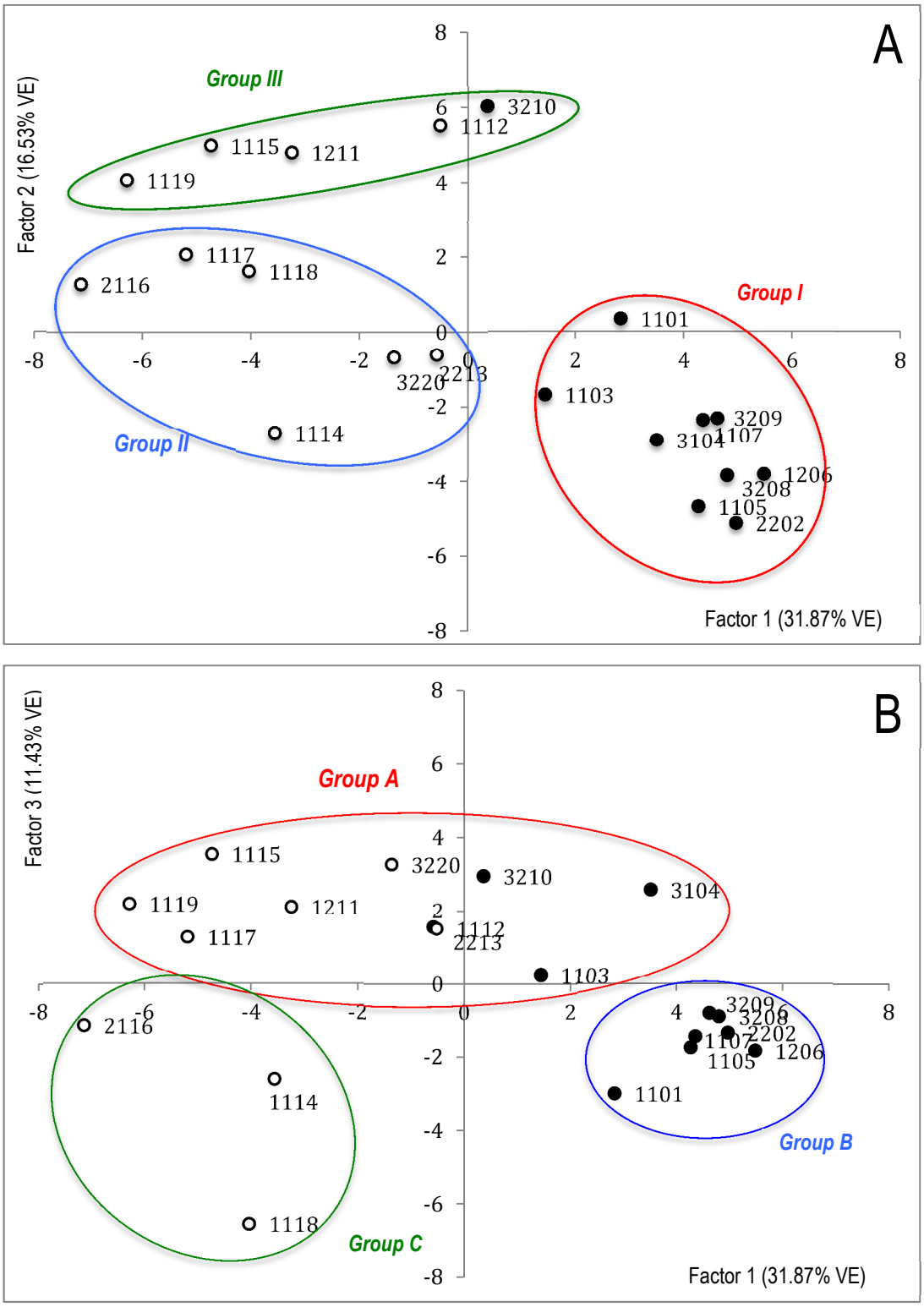

Fig. 3 Scatterplots of scores of factor 1 *vs* Factor 2 (A) and Factor 1 *vs* factor 3 (B) of commercial breads.

Table 1
Ingredients of gluten and gluten-free breads. A four digit bread sample code refers to white (1), mixed (2), and dark (3) crumb color (1st digit); absence (1) or presence (2) of seeds (2nd digit), and sample number (3rd and 4th digits) from 1 to 10 for gluten breads and from 11 to 20 for gluten-free breads.

Breads	Ingredients
1101	Wheat Flour Tipe "I", Water, Extra-Virgin Olive Oil (3%), Rye Sourdough, Brewer's Yeast, Iodized Salt, Ethyl Alcohol
2202	Wheat Flour Tipe "0" (56.8%), Water, Cereal Flakes/Flours Mixture 5.7% (Oat, Barley, Durum Wheat, Toasted Corn, Rice, Rye, Millet), Soybean Grain (5.5%), Sunflower Oil (3.7%), Sourdough, Wheat Gluten, Salt, Malted Barley Flour, Malt Barley Extract, Ethyl Alcohol
1103	Wheat Flour Tipe "0", Water, Selected Wheat Bran (9.3%), Malted Barley Flour, Extra-Virgin Olive Oil (3.9%), Sourdough, Wheat Gluten, Salt, Glucose, Malted Barley Flour, Ethyl Alcohol
3104	Whole Wheat Flour (64.2%), Water, Sunflower Oil (4.3 %), Wheat Gluten, Sourdough, Dextrose (1.5%), Salt, Malted Barley Flour, Ethyl Alcohol
1105	Durum Wheat Remilled Semolina (68.3%), Water, Extra-Virgin Olive Oil (2.9%), Sourdough, Salt, Glucose, Wheat Gluten, Malted Barley Flour, Wheat Flour Tipe "0", Ethyl Alcohol
1206	Wheat Flour Tipe "0" (55%), Water, Flax-seeds (4.9%), Soybean Grain (4.3%), Malted Barley Flour, Sunflower seeds (3.3%), Wheat Gluten, Olive Oil (1.2%), Sourdough, Dextrose, Flax Seed Oil (0.9%), Salt, Wheat Fiber (0.6%), Malted Barley Flour, Vitamin E (0.03%), Ethyl Alcohol.
1107	Khorasan Kamut Wheat Flour, Water,Rice Oil (2.8%), Salt, Yeast, Extra-Virgin Olive Oil (1.2%), Rice Flour, Malted Wheat Flour, Acacia Fiber, Ethyl Alcohol
3208	Wheat Flour Tipe "0", Water, Rye Flour (18.7%), Sunflower seeds (4%), Vegetable Oil (3.4%), Wheat Gluten, Sesame Seeds (2%), Sourdough, Flax-seeds (1.8%), Dextrose, Salt, Malt Barley Extract, Malted Barley Flour, Ethyl Alcohol
3209	Water, Wheat Flour Tipe "0" (34.5%), Whole Wheat Flour (16.5%), Seeds 9.5 (Sunflower seeds 3.7%, Soybeans 3.5, Flax-seeds 2.1%, Sesame Seeds 0.2%),Cereal Flours 6.3% (Oat Flour 1.6%, Barley Flour 1.6%, Rice Flour 1.6%, Rye Flour 0.9%, Millet Flour 0.4%, Corn Flour 0.2%), Vegetable Oil (2.8%), Sourdough, Dextrose (1.3%), Wheat Gluten, Salt, Oat Flakes 0.2%, Barley Flakes 0.2%), Rice Flakes (0.2%), Malted Barley Flour, Malt Barley Extract, Ethyl Alcohol
3210	Whole Rye Groats, Water, Sourdough, Flaxseeds (3%), Barley Flakes (3%), Oat Flakes (3%), Salt, Yeast
1211	Water, Corn Starch, Rice Flour, Sunflower seeds (5%), No-Hydrogenated Vegetable Margarine (refined palm oil, water), sugar, Brewer's Yeast, Thickening Agents: Guar Seed Flour-HPMC, Iodized Salt, Lupine Protein, Psyllium Fiber, Tartaric Acid, Flavouring
1112	Sourdough (Rice Flour, Corn Starch, Buckwheat Flour, Salt, Lactobacillus sanfranciscensis, Lactobacillus plantarum), Water, Sunflower seeds (5.4%), Potato Starch, Corn Starch, Glycerol, Inulin, Sunflower Oil, Olive Oil (3.2%), Egg White, Soy Protein Isolate, Sugar, Brewer's Yeast, Xanthan Gum, Glucose/Fructose Syrup, Salt, Guar Gum, Soy Lecithin, Mono and Diacetyltartaric Acid Esters of Mono and Diglycerides of Fatty Acids
2213	Corn Starch, Rice Starch, Sourdough 22.5% (Rice Flour), Water, Rice Flour, Millet Flour (2.3%), Quinoa Flour (1.6%), Apple Fiber, Rice Syrup, Beet Sugar Syrup, Sunflower seeds (1.8%), Soy Flakes (1.8%), Soy Bran (1.7%), Millet Flakes (1.2%),
1114	Water, Corn Starch, Rice Flour, Sugar, Eggs, No-Hydrogenated Vegetable Margarine (Palm Oil, Coconut Oil, Colza Oil), Glucose Syrup, Milk Powder, Apple Fiber, HPMC, Guar Gum, Mono and Diglycerides of Fatty Acids, Yeast, Tartaric Acid, Salt
1115	Potato Starch, Water, Rice Flour, Corn Flour, Vegetable Fibers, Non-Hydrogenated Vegetable Oils, HPMC, Xanthan Gum, Eggs, Yeast, Teff Flour (2.5%), Sugar, Modified Rice Starch, Maltodextrins, Salt, Invert Sugar, Potassium Sorbate, Quinoa Flour
2116	Water, Potato Starch, Corn Starch, Refined Sunflower Oil, Tapioca Starch, Egg White (Powder), Rice Bran, Yeast, Cellulose, Salt, Beet Pulp, Millet Flakes , Wine Vinegar, Xanthan Gum, HPMC, CMC, Calcium Propionate, Sorbic Acid, Potassium sorbate
1117	Water, Wheat Starch (No Gluten), Rice Starch, Cellulose Fiber, Guar Gum, HPMC, Soy Protein, Apple Fiber, Rice Flour, Millet Flour, Yeast, Sunflower Oil, Quinoa Flour, Sugar, Rice Syrup, Salt, Palm Oil, Honey, Folic Acid, Calcium Citrate
1118	Rice Flour, Corn Starch, Sugar, Eggs, Water, Vegetable Margarine (Palm fat, Coco fat, Canola oil, Salt, Mono and Diglycerides of Fatty Acids, Natural Flavour), Rice Starch, Glucose Syrup, Mono and Diglycerides of Fatty Acids, Guar Gum Seeds Flour, HPMC, Yeast, Salt, Flavouring, Citric Acid
1119	Corn Starch, Water, Rice Flour, Sunflower Oil, Sugar, Guar Gum Seeds Flour, HPMC, Lupine Protein, Yeast, Salt, Apple Fiber, Flavour, Mono and Diacetyltartaric Acid Esters of Mono and Diglycerides of Fatty Acids
3220	Corn Starch, Water, Rice Flour, Sunflower seeds (7,5%), Buckwheat Flour (7%), Flaxseeds (5,5%), Sugar beet Syrup, Rice Starch, Yeast, Apple Extract, HPMC, Soy Protein, Salt, Sunflower Oil, Tartaric Acid

Table 2
Significant Pearson correlations (p<0.05 *, p <0.01 **) between biochemical and physical properties of commercial breads.

	Moisture	Protein	Dietary Fibre	C_∞	k	H_{90}	HI	eGI	DS	RS	TS	Bioaccessible Polyphenols	Soluble Polyphenols	Insoluble Polyphenols	Antiradical activity
Whiteness Index									0.6046**		0.5603*	-0.4649*		-0.4773*	
Cell to total area ratio	-0.5241*	0.7566**					-0.4699*	-0.4699*				0.7647**		0.5898**	
Hardness	0.5027*	-0.5296*		0.6345**	0.5206*	0.5552*	0.6446**	0.6446**				-0.5372*			
Springiness	-0.6687**	0.5511*		-0.6908**		-0.6824**	-0.7607**	-0.7606**	-0.5149*		-0.5536*	0.553*		0.6395**	
Cohesiveness	-0.7385**	0.6105**		-0.6706**		-0.5409*	-0.7127**	-0.7127**				0.6771**	0.5062*	0.4721*	
Chewiness			0.4718*	0.5479*	0.4907*	0.4983*	0.5201*	0.5201*							
Resilience	-0.6625**			-0.6219**		-0.5486*	-0.6782**	-0.6782**				0.5076*	0.4668*		
F_0	0.6285**			0.5183*		0.5135*	0.5572*	0.5571*							
k_1										0.7303*					0.5512*
k_2		-0.6013**								0.6516**		-0.6605**			
RT		-0.5911**								0.7726**		-0.6425**		-0.4567*	

C_∞: equilibrium concentration; k: kinetic constant; H_{90}: total starch hydrolysis at 90 min; HI: hydrolysis index; eGI: expected glycemic index; DS: digestible starch; RS: resistant starch; TS: total starch; F_0: initial force; k_1: stress decay rate; k_2: residual stress; RT: relaxation time.

Table 3
Significant Pearson correlations (p<0.05 *, p <0.01 **) between biochemical properties and sensory parameters of breads.

	Moisture	Protein	Digestible carbohydrates	Dietary Fibre	Ash	C_∞	k	H_{90}	HI	eGI	RS	TS	Bioaccessible Polyphenols
Cell Uniformity			0.5771**	-0.5433*									
Cell Size			-0.5048*	0.5022*									
Thickness	0.4678*		-0.5576*	0.6986**		0.4934*	0.6657**				0.4838*		-0.494*
Moistness	0.47*				-0.4628*								
Elasticity	-0.4752*					-0.487*		-0.5169*	-0.5461*	-0.546*			
Softness					-0.6159**								
Coarseness				0.5251*			0.533*						
Cohesiveness	-0.5713*	0.5825**				-0.5664*	-0.4763*	-0.4628*	-0.5507*	-0.5506*			0.6636**
Gumminess		0.6428**										-0.4703*	0.7445**
Mouth Dryness				0.4769*									
Aroma Intensity	0.506*		-0.5087*	0.4659*							0.4741*		-0.4934*
Aroma Quality		0.4929*											0.4936*
Taste Intensity			-0.5074*	0.482*									
Taste Quality		0.6924**					-0.4994*					-0.4807*	0.7931**
Saltiness			-0.6274**	0.6943**									
Aftertaste	0.68**	-0.5102*					0.6155**						-0.7242*

C_∞: equilibrium concentration; k: kinetic constant; H_{90}: total starch hydrolysis at 90 min; HI: hydrolysis index; eGI: expected glycemic index; RS: resistant starch; TS: total starch.

Table 4
Loading Matrix After Varimax Rotation in Factor Analysis.

	Factor 1 (31,87%VE)	Factor 2 (16,53%VE)	Factor 3 (11,43%VE)	Factor 4 (10,42%VE)	Factor 5 (7,96%VE)
Protein	0,7936	-0,3507	-0,0866	0,2366	-0,0771
Dietary Fibre	-0,1094	0,2613	0,6121	0,4758	0,1232
Whiteness Index	-0,2417	0,2927	-0,2245	-0,7495	-0,2201
Browning Index	0,0636	0,0950	-0,2123	0,7734	-0,0792
Hardness	-0,0188	0,7767	0,1689	0,3922	-0,0873
C_∞	-0,2348	0,8939	0,0937	-0,0934	0,0967
k	-0,6198	0,2631	0,6117	0,0558	0,0031
eGI	-0,2222	0,9066	0,0765	-0,1256	0,0782
RDS	-0,0849	0,0492	0,0531	-0,2954	-0,8372
SDS	-0,1837	0,5407	-0,0024	-0,2606	0,6930
RS	-0,5215	-0,0032	0,5213	-0,2754	0,3216
Bioaccessible Polyphenols	0,8154	-0,3875	0,0010	0,2058	-0,1695
Antiradical activity	-0,3663	0,0279	-0,1678	-0,0997	0,4971
Aroma Quality	0,8424	0,1960	0,1465	-0,2081	-0,0291
Smoothness	-0,1202	0,0937	-0,8387	0,1749	0,1280
Softness	-0,2433	-0,3026	-0,7280	-0,0723	0,2525
Taste Quality	0,8786	-0,2498	0,1073	0,1387	-0,0208

VE: variance explained

Table 5
Starch hydrolysis kinetics parameters and expected glycemic index values of gluten and gluten-free commercial breads.

Bread samples [a,b]	Characteristics				
	C_∞	*k*	H_{90}	*HI*	*eGI*
Gluten Breads					
1101	80±1p	0.010±0.003a	71±1n	96±1l	91±2i
2202	49±1b	0.010±0.010a	47±0b	58±2b	58±1b
1103	60±1f	0.100±0.010b	53±1e	67±2d	66±1d
3104	55±0d	0.100±0.006b	50±1cd	65±2cd	65±1cd
1105	42±1a	0.010±0.009a	36±1a	51±0a	52±0a
1206	53±1c	0.010±0.004a	51±1d	63±3c	63±3c
1107	64±1h	0.010±0.003a	59±1i	73±4fg	71±1ef
3208	59±0e	0.010±0.010a	55±1g	68±1de	66±2d
3209	66±1i	0.010±0.006a	71±1n	75±1g	73±2g
3210	80±1p	0.100±0.006b	73±1o	95±4l	90±1i
Gluten-free Breads					
1211	82±0q	0.100±0.003b	69±0m	97±1lm	92±3il
1112	90±0s	0.100±0.005b	79±0p	100m	94±0l
2213	62±1g	0.100±0.008b	57±1h	74±0fg	72±0f
1114	56±0d	0.100±0.005b	53±1ef	66±4cd	65±1cd
1115	86±1r	0.100±0.007b	80±1p	100±0m	94±0l
2116	73±0n	0.100±0.005b	58±0h	80±1h	78±1g
1117	68±0l	0.100±0.009b	62±1l	80±0h	77±0g
1118	71±0m	0.010±0.001a	49±1c	82±3h	79±3h
1119	78±1o	0.100±0.002b	71±0n	90±1i	86±2i
3220	64±0h	0.100±0.002b	50±1d	71±3ef	70±3e

[a] Mean values ± standard deviation. Within rows, values (mean of three replicates) with the same following letter do not differ significantly from each other ($p > 0.05$).

[b] A four digit bread sample code refers to white (1), mixed (2), and dark (3) crumb color (1st digit); absence (1) or presence (2) of seeds (2nd digit), and sample number (3rd and 4th digits) from 1 to 10 for gluten breads and from 11 to 20 for gluten-free breads.

C_∞: equilibrium concentration; *k*: kinetic constant; H_{90}: total starch hydrolysis at 90 min; *HI*: hydrolysis index; *eGI*: expected glycemic index.

6 CHAPTER II

Impact of ancient cereals, pseudocereals and legumes on starch hydrolysis and antiradical activity of technologically viable blended breads

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Impact of ancient cereals, pseudocereals and legumes on starch hydrolysis and antiradical activity of technologically viable blended breads

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ABSTRACT

Wheat flour replacement from 22.5% up to 45% by incorporation of ternary blends of teff (T), green pea (GP) and buckwheat (BW) flours provided technologically viable and acceptable sensory rated multigrain breads with superior nutritional value compared to the 100% wheat flour (WT) counterparts. Blended breads exhibited superior nutritional composition, larger amounts of bioaccessible polyphenols, higher anti-radical activity, and lower and slower starch digestibility. Simultaneous lower rapidly digestible starch (57.1%) and higher slowly digestible starch (12.9%) and resistant starch (2.8%) contents (g per 100 g fresh bread), considered suitable nutritional trends for dietary starch fractions, were met by the blend formulated 7.5% T, 15% GP, 15% BK. The associated mixture that replaced 37.5% WT, showed a rather lower extent and slower rate of starch hydrolysis with medium-low values for C_∞, and H_{90}, and lowest k, and intermediate expected Glycaemic Index (86). All multigrain breads can be labelled as source of dietary fibre (≥3 g dietary fibre/100 g bread).

1. Introduction

Grains are basic, ubiquitous and healthy raw materials, good source of carbohydrates – mainly starch and dietary fibre – providing excellent vectors for diversity and innovation. It raises a great deal of recent interest that ancient crops (Angioloni & Collar, 2011a), pseudocereals (Collar & Angioloni, 2014a) and legumes (Angioloni & Collar, 2012), besides wheat, constitute nutrient-dense and healthy grains with explicited breadmaking applications.

A slow release and absorption of glucose may be generated in a food matrix according to the processing conditions and surrounding ingredients (Lehmann & Robin, 2007), encompassing beneficial effects in the management of diabetes and hyperlipidemia (Jenkins, 2007). Native cereal starches are ideal sources of slowly digestible starch (SDS) (>50%), and the slow progressive digestion property is realized by a layer-by-layer inside–outside (radial) digestion process (Zhang, Ao, & Hamaker, 2006a). Mechanical and thermal treatments change the structure and digestibility of starch. Thermal treatments such as the cooking process completely destroys the semicrystalline structure of native starch granules and causes the loss of SDS and resistant starch (RS) and increases rapid digestible starch (RDS) (Zhang, Venkatachalam, & Hamaker, 2006b). In cereal products, the starch gelatinization extent, which is mainly controlled by the moisture level and the cooking time and temperature influences the formation of SDS (Englyst, Vinory, Englyst, & Lang, 2003). In bread dough, although formation of resistant starch (RS3) may occur in the high water-containing parts during cooling, a large portion of starch is gelatinized during cooking and induces a rapid digestibility of starch (Bravo, Englyst, & Hudson, 1998). In extruded cooked cereal products such as breakfast cereals, in addition to the thermal treatment, the high pressure and shear forces destroy the starch granular structure and increase its gelatinization extent, making it more available to amylolytic enzymes (Le François, 1989). On the contrary, in pasta, a dense protein network is formed, which limits the accessibility of α-amylase to the starch and restricts the diffusion of water molecules to the starch granules. As a consequence, a reduction of the extent of starch gelatinization takes place (Englyst, Kingman, & Cummings, 1992). In some biscuits with very low moisture levels during the treatment, the extent of gelatinization is reduced and partially intact granules and gelatinized starch co-exist, resulting in a higher content of SDS compared to breakfast cereals and baked products (Englyst et al.,

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Impact of ancient cereals, pseudocereals and legumes on starch hydrolysis and antiradical activity of technologically viable blended breads

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Abstract

Wheat flour replacement from 22.5% up to 45% by incorporation of ternary blends of teff (T), green pea (GP) and buckwheat (BW) flours provided technologically viable and acceptable sensory rated multigrain breads with superior nutritional value compared to the 100% wheat flour (WT) counterparts. Blended breads exhibited superior nutritional composition, larger amounts of bioaccessible polyphenols, higher anti-radical activity, and lower and slower starch digestibility. Simultaneous lower rapidly digestible starch (57.1%) and higher slowly digestible starch (12.9%) and resistant starch (2.8%) contents (g per100 g fresh bread), considered suitable nutritional trends for dietary starch fractions, were met by the blend formulated 7.5% T, 15% GP, 15% BK. The associated mixture that replaced 37.5% WT, showed a rather lower extent and slower rate of starch hydrolysis with medium-low values for C_∞, and H_{90}, and lowest *k,* and intermediate expected Glycemic Index (86). All multigrain breads can be labelled as source of dietary fibre (≥3 g dietary fibre/100 g bread).

Keywords: bread, starch hydrolysis, ancient cereals, pseudocereals, legumes.

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Introduction

Grains are basic, ubiquitous and healthy raw materials, good source of carbohydrates – mainly starch and dietary fibre – providing excellent vectors for diversity and innovation. It raises a great deal of recent interest that ancient crops (Angioloni and Collar, 2011a), pseudocereals (Collar and Angioloni, 2014a) and legumes (Angioloni and Collar, 2012), besides wheat, constitute nutrient-dense and healthy grains with explicited breadmaking applications. A slow release and absorption of glucose may be generated in a food matrix according to the processing conditions and surrounding ingredients (Lehmann and Robin, 2007), encompassing beneficial effects in the management of diabetes and hyperlipidaemia (Jenkins, 2007). Native cereal starches are ideal sources of slowly digestible starch (SDS) (>50%), and the slow progressive digestion property is realized by a layer-by-layer inside-outside (radial) digestion process (Zhang, Ao, & Hamaker, 2006a). Mechanical and thermal treatments change the structure and digestibility of starch. Thermal treatments such as the cooking process completely destroys the semicrystalline structure of native starch granules and causes the loss of SDS and resistant starch (RS) and increases rapid digestible starch (RDS) (Zhang, Venkatachalam, & Hamaker, 2006b). In cereal products, the starch gelatinization extent, which is mainly controlled by the moisture level and the cooking time and temperature influences the formation of SDS (Englyst, Vinory, Englyst, & Lang, 2003). In bread dough, although formation of resistant starch (RS3) may occur in the high water-containing parts during cooling, a large portion of starch is gelatinized during cooking and induces a rapid digestibility of starch (Bravo, Englyst, & Hudson, 1998). In extruded cooked cereal products such as breakfast cereals, in addition to the thermal treatment, the high pressure and shear forces destroy the starch granular structure and increase its gelatinization extent, making it more available to amylolytic enzymes (Le François¸ 1989). On the contrary, in pasta, a dense protein network is formed, which limits the accessibility of α-amylase to the starch and restricts the diffusion

of water molecules to the starch granules. As a consequence, a reduction of the extent of starch gelatinization takes place (Englyst, Kingman, & Cummings, 1992). In some biscuits with very low moisture levels during the treatment, the extent of gelatinization is reduced and partially intact granules and gelatinized starch co-exist, resulting in a higher content of SDS compared to breakfast cereals and baked products (Englyst et al., 2003). In many plant sourced foods, such as legumes and minimally processed cereal grains, starch granules are trapped within the plant cell walls (e.g. whole grains), which retard their degradation (Würsch, Del Vedovo, & Koellreutter, 1986). Disruption of the granule structure as by milling can increase the susceptibility to enzymatic degradation. Legumes that are low glycemic index foods, which generate slow and moderate postprandial glucose and insulin response, have been shown to decrease blood glucose responses compared to other cereal based foods (Tovar, Granfeldt, & Bjorck, 1992) such as whole bread. The digestibility of legume starch is much lower than that of cereal starch (Madhusudhan and Tharanathan, 1996). Cooked legumes are prone to retrograde more quickly, thereby lowering the process of digestion. The higher content of amylose in legumes, which probably may lead to a higher RS content, may possibly account for their lower digestibility. Also, legumes contain more of proteins than cereals, and protein-starch interaction in legumes may equally contribute to their decreased glycemic responses (Geervani and Theophilus, 1981). Additionally, the presence of high amounts of dietary fibre and antinutritional factors such as phytates and amylase inhibitors may greatly influence the rate and extent of legume starch digestibility. The current proposal is aimed at exploring the competences and exploiting the suitability in mixed wheat matrices of non-breadmaking whole grains with unique nutritional components (teff, green pea and buckwheat flours), to obtain novel and healthy fermented baked goods meeting the functional and sensory restrictions of viscoelastic breadmaking systems. Bread functional and nutritional profiles were assessed in quaternary wheat blended matrices, and compared with the wheat flour

counterparts. Special emphasis will be placed on starch hydrolysis kinetics and relevant starch nutritional fractions in mixed grain matrices.

Materials and Methods

Materials

Commercial flours from refined common wheat *Triticum aestivum* (WT), and whole teff *Eragrostis tef* (T), green pea *Pisum sativum* (GP), and buckwheat *Fagopyrum esculentum* (BW) were purchased from the Spanish market. Refined WT (70% extraction rate) of 356×10^{-4} J energy of deformation *W*, 0.64 curve configuration ratio P/L, 95% Gluten Index, 62% water absorption in Brabender Farinograph, was used. Ireks Vollsauer sour dough was from Ireks (Spain); Novamyl 10,000 a maltogenic thermostable α-amylase from Novozymes (Denmark); and calcium propionate, from Sigma-Aldrich (USA).

Methods

Bread making of wheat and wheat-based blended flours

Doughs and breads were prepared for WT as control, and wheat-based blended flours (T, GP, BW) by WT replacement from 22.5% up to 45%, and incorporation of ternary blends of T, GP and BW flours according to a Multilevel Factorial Design with the following attributes: three experimental factors (T, GP and BW flours) at 2 levels, coded 0 (7.5% wheat flour replacement) and 1 (15% wheat flour replacement), and 5 error degrees of freedom. The model resulted in 8 randomized runs in 1 block. A 3 digit bread sample code was set referring to low (0) and high (1) wheat flour replacement by T (1st digit), GP (2nd digit), and BW (3rd digit) flours in sample formulation, as it follows: 010, 001, 011, 000, 111, 101, 100, 110. Blended flours (100 g), water (62%, flour basis), commercial compressed yeast (4%, flour basis), salt (1.5%, flour basis), vegetable fat-margarine (4%, flour basis), sugar (2%, flour basis), commercial sour dough (4%,

flour basis), milk powder (5%, flour basis), Novamyl 10,000 (7.5 mg, flour basis), and calcium propionate (0.5%, flour basis) were mixed in a 10 kg mixer at 60 revolutions min-1 for 10 min up to optimum dough development. Fermented doughs were obtained after bulk fermentation (10 min at 28 ºC), dividing (500 g), rounding, moulding, panning and proofing up to maximum volume increment (30 min at 28 ºC), and were baked at 200 ºC for 30 min to make control and blended breads.

Chemical and nutritional composition of flours and breads

Moisture, protein, ash and fat contents of commercial flours, control and blended breads were determined following the ICC methods (ICC, 1976–1996). Total, soluble and insoluble dietary fibre contents were determined according to the AOAC method 991.43 (AOAC, 1991). Three replicates were made for each analysis. Digestible carbohydrates were calculated by indirect determination as 100 − [Moisture + Protein + Fat + Ash + Dietary Fibre] (FAO, 2003). Resistant starch determination was performed according to AOAC Official Method 2002.02 (AOAC, 2000) by using Megazyme kit K-RSTAR 08/11. Bioaccessible phenol determination was carried out by conducting an "in vitro" digestive enzymatic mild extraction that mimics the conditions in the gastrointestinal tract according to the procedure of Glahn, Lee, Yeung, Goldman, and Miller (1998) and adapted by Angioloni and Collar (2011b) for breads. The stable 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical was used to measure the radical scavenging capacity of flour and bread samples according to the DPPH• method (Brand-Williams, Cuvelier, & Berset, 1995), modified by Sánchez-Moreno, Larrauri, and Saura-Calixto (1998) and adapted. 2 g of flour, and 3 g of French bread (freeze-dried and milled <0.5 mm) were placed in a centrifuge tube (50 mL) and 20 mL of acidic methanol/water (50:50 v/v, pH2) was added (10 mL for French bread). The tube was thoroughly shaken at room temperature for 1 h. The tube was centrifuged at 2500g for 10 min, and the supernatant was recovered. 20 mL of acetone/water (70:30, v/v) was added to the residue (10 mL for bread), and

shaking and centrifugation were repeated. Both methanolic and acetonic extracts were combined and adjusted to 25 for bread or 50 mL with methanol. After gentle shaking, aliquots of 0.1 mL were taken, and 3.9 ml of a solution of DPPH 0.050 g/L (equivalent to 0.1268 μmol/mL) was added. Tubes were gently shaken, and 4 mL of each tube were added to a 4 mL cuvettes, and A515 nm was read at 1 min and every 5–10 min until the plateau was reached. A cuvette containing 4 mL of DPPH 0.494 μmol in methanol was read at the same periods. A blank of methanol was used. Lectures were taken in duplicated samples. Plots of μmol DPPH *vs* time (min) were drawn, and calculations were made to know the antiradical activity (AR). $AR = [([DPPH]_{INITIAL} - [DPPH]_{PLATEAU}) \times 100]/[DPPH]_{INITIAL}$.

Bread measurements

Enzymatic/biochemical determinations. In vitro starch hydrolysis kinetics and relevant starch fractions in blended breads was determined following the AACC (2005) method 32–40, adapted as previously described (Angioloni and Collar, 2011c). Rapidly digestible starch (RDS) and slowly digestible starch (SDS) were measured after incubation for 20 min and 120 min, respectively, as stated by Englyst et al. (2003). Total digestible starch (DS) was determined in the supernatant after 16 h of incubation while resistant starch (RS) was determined in the pellet as the starch remaining after 16 h incubation. The digestion kinetics and expected glycemic index (eGI) of bread were calculated in accordance with the procedure followed by Chung, Liu, Pauls, Fan, and Yada (2008) based on the method established by Goñi, Garcia-Alonso, and Saura-Calixto (1997). A first order kinetic equation [$C = C_\infty (1 - e^{-kt})$] was applied to describe the kinetics of starch hydrolysis, where C, C_∞ and k were the hydrolysis degree at each time, the maximum hydrolysis extent and the kinetic constant, respectively. The hydrolysis index (HI) was calculated as the relation between the area under the hydrolysis curve (0–16 h) of blended bread samples and the area of standard material from white bread (control) (Chung et al., 2008). The expected glycemic index (eGI)

was calculated using the equation proposed by Granfeldt, Björck, Drews, and Tovar (1992): eGI = 8.198 + 0.862HI.

Physico-chemical and sensory determinations. Colour determinations were carried out on bread crumb using a Photoshop system according to the method previously described by Angioloni and Collar (2009), and results were expressed in accordance with the Hunter Lab colour space. The Photoshop (Ps Adobe Photoshop CS5 extended) system (L, a, b colour coordinates) was previously calibrated using colour sheets from Pantone® Formula Guide (Pantone, Inc., USA). Pantone colour sheets (for calibration) and bread slices (for colour measurement) images were acquired at 300 pixel resolution with a ScanJet II cx flatbed scanner (Hewlett-Packard, USA). The scanner was held in a black box in order to exclude the surrounding light. All measurements (three slices per sample) were made in triplicate. Hunter Lab colour space parameters from Minolta colorimeter were calculated from the calibration linear equation Colorimeter *vs* Photoshop (Angioloni and Collar, 2009). Parameters determined were L (L = 0 [black] and L = 100 [white]), a ($-a$ = greenness and $+a$ = redness), b ($-b$ = blueness and $+b$ = yellowness), ΔE – total colour difference, and WI – whiteness index (Collar and Angioloni, 2014b). Crumb grain characteristics were assessed in bread slices using a digital image analysis system. Images were previously acquired with a ScanJet II cx flatbed scanner (Hewlett-Packard, Palo Alto, CA, USA) supported by a Deskscan II software. The analysis was performed on 40 mm × 40 mm squares taken from the centre of the images. Data were processed using SigmaScan Pro 5 (Jandel Cor-poration, San Rafael, CA, USA). The crumb grain features evaluated were mean cell area, cells/cm^2, cell/total area ratio, wall/total area ratio and crumb area/total cell ratio (Collar, Bollaín, & Angioloni, 2005). In addition, area distribution and cell number distribution were counted, and percentages of cell were calculated according to pre-set cell size ranges: <0.4 mm^2, 0.4–1.0 mm^2, 1.0–10 mm^2, 10–20 mm^2. Sensory analysis of fresh breads was performed with a panel of eight trained judges (four males and four females aged 24–55) using semi structured scales, scored 1–10 in which extremes (lowest:

1; highest: 10) were described for each sensory attribute according to Setser (1996). Evaluated attributes were grouped into visual, textural and organoleptic characteristics (Collar et al., 2005). Bread primary and secondary mechanical characteristics (TPA in a double compression cycle) of fresh breads were recorded in a TA-XTplus texture analyser (Stable Micro Systems) using a 25 mm diameter probe, a 5 kg load cell, 50% penetration depth and a 30 s gap between compressions on slices of 25 mm width (Armero and Collar, 1998). For textural measurements, three slices of two freshly made breads were used for each sample.

Statistical analysis

Multivariate analysis of variance and non linear multiple regression analysis of data were performed by using Statgraphics V.7.1program (Bitstream, Cambridge, MN). Multiple range test (Fisher's least significant differences, LSD) for analytical variables was applied to know the difference between each pair of means.

Results and Discussion

Bread is a complex viscoelastic porous matrix, composed mainly of gluten/protein, starch, lipids and water, whose sensory, technological and nutritional final quality is multifactor dependent. The technological viability and sensory acceptability of blended bread matrices are explored first, prior to assess the "in vitro" starch hydrolysis kinetics, the relevant starch nutritional fractions and the anti-radical activity of blended breads *vs* wheat matrices.

Chemical and nutritional composition of single flours (WT, T, GP and BW) and quaternary blended breads

Single WT, T, GP and BW flours exhibited different chemical and nutritional profiles that resulted in quantitative different bread patterns regarding both chemical and nutritional composition (Table 1). Comparatively to wheat flour (T,

GP and BW *vs* WT, per 100 g flour basis, d.b.), non-wheat flours, accounted for much higher protein with the exception of teff (13.05%, 25.12%, 19.71% *vs* 14.13%), similar or higher fat (5.06%, 1.27%, 3.44% BW, *vs* 1.56%), and ash (2.05–2.58% *vs* 0.63%) contents, and much higher total dietary fibre (12.19–14.56% *vs* 1.4%), and significantly lower digestive carbohydrates (57–67% *vs* 82%). Data are compatible with a superior nutritional profile for ancient cereals (Angioloni and Collar, 2011a), pseudocereals (Collar and Angioloni, 2014a) and legumes (Angioloni and Collar, 2012), as reported earlier. Quaternary blended breads obtained by replacement of WT flour from 22.5% to 45% with mixed T, GP and BW flours, explicited (per 100 g fresh bread) compared to WT bread counterparts, similar protein (11.6–12.2% *vs* 11.1%) and fat (3.5–3.8% *vs* 3.4%) contents, but much higher total dietary fibre (2.9–4.3% *vs* 1.4%), insoluble (1.63–2.42% *vs* 0.83%) and soluble (1.2–1.9% *vs* 0.59%) dietary fibre sub-fractions, especially for bread samples with higher level of WT replacement (011, 111, and 110) by high-fibre non-wheat flours (Table 1). Blended bread samples contain about double to triple the fibre of the regular white bread, so that breads can be labelled as source of fibre (≥3 g DF/100 g food) according to Nutritional Claims for Dietary Fibre foods (Regulation (EC) No. 1924/2006).

Physical and sensory characteristics of blended breads vs wheat matrices

Bread crumb is a typical viscoelastic biopolymer foam system with cellular structure composed mainly of gluten/protein, starch, and water, and minor constituents such as lipids and non-starch polysaccharides in presence of other ingredients, additives and technological aids. Major breadmaking steps leading to bread from flour, significantly change dough viscoelasticity. In this research, 45% of WT replacement by combinations of non-gluten forming flours-T, GP, and BW was previously established as the maximum level of substitution that did not significantly hinder dough handling ability (data not shown) in terms of stickiness (<1 N), dough hardness (<85 N), cohesiveness (>0.3), adhesiveness (<160 N s) and springiness (>0.6). The simultaneous addition of T, GP and BW significantly

decreased the bread volume (from 3.1 mL/g to 1.9–2.3 ml/g for most samples except for the bread with lowest level of WT replacement (000) that develops similar volume (2.9 mL/g) to control bread (Table 2). With respect to refined WT flour bread types, lower volume blended breads encompassed harder (15.8–25.3 N *vs* 8.7 N) and low cohesive crumbs (0.499–0.630 *vs* 0.695) with poorer springiness (0.858–0.908 *vs* 0.955) particularly for medium-high replaced blends (Table 2). Blended breads are all visibly different from control WT breads ($\Delta E \geq 3$) in crumb colour features, characterized by lower lightness *L* and Whiteness Index, more red (*a* positive) and yellow (*b* positive) colour tri-stimulus values, with no significant differences among mixed samples. Crumb pore uniformity and crumb grain structure were not significantly affected, though in the non-wheat flour supplemented breads the crumb quality slightly decreased *vs* control breads in terms of lower average cell size for most samples (0.17–0.25 mm^2 *vs* 0.27 mm^2) and higher cell density (up to 150 cells/cm^2 *vs* 100 cells/cm^2) with variable cell to wall area ratio (25:75–33:67 *vs* 27:73). Cell area and cell number distribution evidenced that 42–64% of total cell area is occupied by alveoli sized 1.0–10 mm^2, while at about 90% of cells sized ≤0.4 mm^2 (Table 2). Blended breads were scored significantly higher than refined WT control breads in both taste and smell intensity, tactil smoothness, visual cell uniformity and round shaped cells, and biting firmness, adhesiveness, cohesiveness, and chewiness (Fig. 1). In addition, blended breads deserved similar ratings as compared to WT control breads concerning visual wall thickness, biting mouth-feel, and typical smell.

"In vitro" starch hydrolysis kinetics and anti-radical activity of blended breads vs wheat matrices

Taking into account the nutritional added value derived from non-wheat flour incorporation into wheat bread formulation, especially dietary fibre (Table 1), and considering that blended matrices were technologically viable (Table 2) and sensorially scored higher than wheat breads in most attributes (Fig. 1), in vitro

starch hydrolysis kinetics (Fig. 2) and relevant starch nutritional fractions (Tables 3–5), bioaccessible polyphenols, and anti-radical activity (Table 6) were determined.

Starch hydrolysis kinetics

The in vitro determination of carbohydrate digestibility to predict the glycemic response of complex foods is of great interest since in vivo evaluations are invasive, labour-intensive and costly (Lehmann and Robin, 2007). In cereal products, the starch gelatinization extent, which is mainly controlled by the moisture level (Primo-Martin, Van Nicuwenhuijzen, Hamer, & Van Vliet, 2007) and the cooking time and temperature influences the formation of SDS (Englyst et al., 2003). In addition, amylose can complex with lipids hindering attack by hydrolytic enzymes more than is free carbohydrate (Nebesny, Rosicka, & Tkaczyk, 2004). Characteristics such as solubility and the presence of fibre, fat and protein all contribute to the rate of digestion (Dona, Pages, Gilbert, & Kuchel, 2011). Through the process of retrogradation, gelatinized or solubilized starch can be transformed from an unstructured into a more ordered or crystalline state. This large physical change causes heat processed starchy foods to harden or become stale as they spontaneously approach a metastable state of lower free energy. This has been reported to decrease the GI value, due to an increased resistance to amylase (Chung, Lim, & Lim, 2006). Starch hydrolysis that follows first order kinetics (Frei, Siddhuraju, & Becker, 2003), proceeded at different rate and extent for blended samples compared to the WT flour counterparts (Table 3). The steady state kinetic constant (k) of amylolysis that has been proposed as a reliable index of the inherent susceptibility of flour starches to amylase hydrolysis (Frei et al., 2003) ranged from 0.0477 (011) to 0.1106 (111) in blended samples *vs* 0.0720 in control breads, evidencing from slower to faster hydrolysis kinetics, respectively, depending on bread formulation. C_∞ that corresponds to the equilibrium percentage of starch hydrolysed after 16 h, varied from 65.7 (001) to 74–76 for all the remaining blended breads except for the highly replaced sample 111 that

showed intermediate values (71.2). Control breads underwent up to 81% of starch hydrolysis, so that all the non-wheat replaced samples showed a lower extent of starch hydrolysis despite at 90 min of reaction time, the equilibrium is already reached in almost all blended samples (Fig. 2) with similar values for C_∞ and H_{90} except for sample 011 (Table 3). Calculation of the samples hydrolysis indices (HI%), the proportion of flour starch that is theoretically digestible, by dividing the area under the hydrolysis curve of each blended sample by the corresponding area of the control sample (Table 3) pointed out the lowest value in sample 001 in good accordance with the lowest equilibrium percentage of starch hydrolysed C_∞, and hence leading to the lowest eGI (78). Multiple analysis of variance (MANOVA) provided information on the significant ($p < 0.05$) single and/or interactive effects of the rate (low and high) of wheat flour replacement by non-wheat flours T, GP and BW in blended breads on starch hydrolysis kinetics (Table 4). Increased doses of single T and BW led to opposite changes in starch hydrolysis kinetic parameters: single T encompassed higher C_∞ (71.9–74.5), k (0.0696–0.0780) and H_{90} (71.6–74.3), while BW lowered both C_∞ (74.7–71.7), and H_{90} (74.6–71.4) values, leading to faster and slower starch hydrolysis kinetics, respectively (Table 4). Simultaneous presence of both T and BW at lower (0) and higher (1) dose, respectively, slowed down hydrolysis kinetics giving the lowest k (0.0651) and H_{90} (69.4) values through a significant antagonistic effect (Table 5). The high protein content of BW flour *vs* T flour (Table 1) may obstruct enzyme attack by hindering enzyme accessibility due to protein-starch interactions in hydrated blended flours (Dona et al., 2010).

Relevant starch nutritional fractions

Categorized starch fractions based on the rate of glucose released and its absorption in the gastrointestinal tract include RDS, SDS and RS, defined here as the three consecutive nutritional fractions divided by reaction time when “in vitro” starch digestion takes place (Table 3, Fig. 2). RDS is the fraction of starch granules that cause a rapid increase in blood glucose concentration after ingestion

of carbohydrates. The fraction of starch that is said to be RDS in vitro is defined as the amount of starch digested in the first 20 min of a standard digestion reaction mixture (Englyst et al., 1992). Although RDS is defined by experimental analysis of digestion in vitro, it has been reported that the rate of starch conversion to sugar follows similar kinetics in the human digestive system (Dona, Pages, Gilbert, & Kuchel, 2010). Values for RDS (g/100 g bread, as is) were all lower in blended breads (from 54.3% – 110 to 62.5% – 100) than in control WT (68.5%) breads (Table 3). In fact, increased T dose provided significantly ($p < 0.05$) higher RDS values (57.2% – 0 to 59.1% – 1), while GP increased dose led to lower RDS fraction (59.2% – 0 to 57.1% – 1) (Table 4). Simultaneous presence of both flours provided significant interactions in such a way that at higher levels of WT replacement by T (15%), higher amounts of GP (15%) are needed to keep RDS fraction at about 57%. SDS is the fraction of starch that is digested slowly but completely in the human small intestine. From studies of in vitro digestion (Dona et al., 2010), it has been observed that there is a transition in the smoothness of the progress curves of reducing sugar production from RDS to SDS in good agreement with profiles in Fig. 2. SDS is defined as the starch that is digested after the RDS but in no longer than 120 min under standard conditions of substrate and enzyme concentration (Englyst et al., 1992). Blended breads explicited a wide range of SDS (g/100 g bread, as is) values ranging from 2.3% (111) to 17.5% (011), *vs* control breads that contained intermediate amounts (7.5%) (Table 3). Higher T presence decreased SDS formation (from 9.2% to 7.8%), while higher dose of BW favoured SDS accumulation (7.8–9.2%) (Table 4). Maximum SDS values 11.5–13% were achieved in breads when the pairs T/GP and/or T/BW do not exceed 22.5% of WT replacement in blended bread formulations (Table 5). The fraction of starch that escapes digestion in the small intestine, and may be subject to bacterial fermentation in the large intestine, is termed RS. Blended breads contained similar amounts of RS (g/100 g bread, as is), regardless the formulation (from 2.2% to 2.9%), and in general higher than the content found in control breads (1.8%) (Table 3). Increased dose of either T or GP

slightly decreased RS, while higher BW inclusion slightly increased RS (Table 4). The highest RS in blended breads was observed for the binary T/GP 00 and T/BW 01 including 7.5% T, 7.5% GP, and 15% BW (Table 5). Simultaneous lower rapidly digestible starch (57.1%) and higher slowly digestible starch (12.9%) and resistant starch (2.8%) contents (g per 100 g fresh bread), considered suitable nutritional trends for dietary starch fractions (Englyst et al., 2003), were met by the blend formulated 7.5% T, 15% GP, 15% BK (sample 011). The associated mixture that replaced 37.5% WT, showed a rather lower extent and slower rate of starch hydrolysis (Table 3, Fig. 2) with medium-low values for C_∞, and H_{90}, and lowest k, and intermediate expected Glycemic Index (86). The incorporation of non-wheat flours into wheat bread formulation seems to reduce starch hydrolysis, probably because of their lower starch and higher fibre and protein contents, especially for GP and BW flours (Table 1). The reduced rate and overall reduced starch digestibility of blended breads may be affected by the high content of viscous soluble dietary fibre components in legume matrices (Angioloni and Collar, 2012). In addition, high protein content particularly for GP flour (Table 1) can promote starch-protein interactions restricting enzyme attack as pointed out for lentils (Chung et al., 2008).

Bioaccessible polyphenols and anti-radical activity of blended breads vs wheat matrices

Bioaccessible polyphenol content (mg gallic acid/100 g flour, asis) of blended breads ranged from 416 mg to 482 mg, and were 1–16% larger ($p < 0.05$) than bioaccessible polyphenols determined in WT breads (414 mg), and higher with no exception than the content observed in flours (303–380 mg) (Table 6). Accumulation of bioaccessible polyphenols from flour to bread varied from 9% in control WT bread to 15–31% in blended breads (Table 6), in good accordance with previous results on multigrain blended breads (Angioloni and Collar, 2011b). Mechanical input during mixing and thermal treatment during baking may induce depolymerization of constituents, mainly fibre, and hence may favour bread

accessibility to enzyme attack and the subsequent release of fibre-associated polyphenols. In addition, Maillard reactions that occur during bread baking can result in the synthesis of substances with antioxidant properties (Vogrincic, Timoracka, Melichacova, Vollmannova, & Kreft, 2010).

Anti-radical activity was determined by the extent of the reduction of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical. Results expressed correspond to the remaining unreacted DPPH• amount when 0.494 μmol of the free radical are initially available to react with methanol/acetone/water extracts from 12 mg flour or freeze-dried bread. The plateau (steady state) was decided at 150 min of reaction in all cases. Higher anti-radical activity for flours (71–88%) than for breads (32–48%) was observed (Table 6). It should be noticed the high anti-radical activity of BW flours (88%) as pointed out earlier (Angioloni and Collar, 2011b) that resulted in a concomitant higher anti-radical activity in blended breads with 15% of BW replacement (001, 011, 111, 101) (Table 6). The observation, can be ascribed to the changes occurring over breadmaking steps in terms of (a) the lipoxygenase catalysed oxidation of polyunsaturated fatty acids that can lead to oxidation of phenolic compounds (particularly for the cinnamic acid derivatives) by coupled reaction due to substantial incorporation of oxygen in the dough during mixing (Eyoum et al., 2003), and (b) losses or degradation of phenolic compounds during baking (Angioloni and Collar, 2011b) as a result of the known susceptibility of phenolic acids and flavonoids to heat.

In addition, it has been stated that dietary fibre and other compounds of proven resistance to the action of digestive enzymes, such as resistant starch, resistant protein, Maillard compounds and other associated compounds, may reduce the bread phenol bioaccessibility (Saura-Calixto, García-Alonso, Goñi, & Bravo, 2000).

This is not the net result in this research, but analogous speculation can be applied to the loss of anti-radical activity from flours to breads, since non-wheat flours all have (Table 1) high dietary fibre content (>12%) and most of them, high protein content (>20%, GP and BW).

Conclusions

Wheat flour replacement from 22.5% up to 45% by incorporation of ternary blends of T, GP and BW flours provided technologically viable and acceptable sensory rated multigrain breads with superior nutritional value compared to the 100% wheat flour (WT) counterparts. Blended breads exhibited superior nutritional composition, larger amounts of bioaccessible polyphenols, higher anti-radical activity, and lower and slower starch digestibility, which extent was formulation dependent. Suitable nutritional trends for dietary starch fractions in terms of simultaneous low RDS (57.1%) and high SDS (12.9%) and RS (2.8%) contents (per 100 g fresh bread), were met by blends formulated 7.5% T, 15% GP, 15% BW, that replaced 37.5% WT. The associated breads showed a rather low extent and slower rate of starch hydrolysis with the medium low values for C_∞, and H_{90}, lowest k, and intermediate eGI (86). Low and slow starch digestibility can be ascribed to the high protein and dietary fibre contents of non-wheat flours (especially GP and BW) that favour starch-protein interactions and constitute a physical interference in bread matrices, respectively, obstructing and delaying enzyme attack and subsequent starch digestion. All multigrain breads can be labelled as source of dietary fibre (≥3 g dietary fibre/100 g bread). The formulation based on WT:T:GP:BW flours, 62.5:7.5:15:15 fulfilled from 25% (men) to 40% (women) of dietary fibre, and from 54% (men) to 66% (women) of protein daily requirements (Otten, Hellwig, & Meyers, 2006), when a daily consumption of 250 g of bread is accomplished, following the WHO bread intake recommendation.

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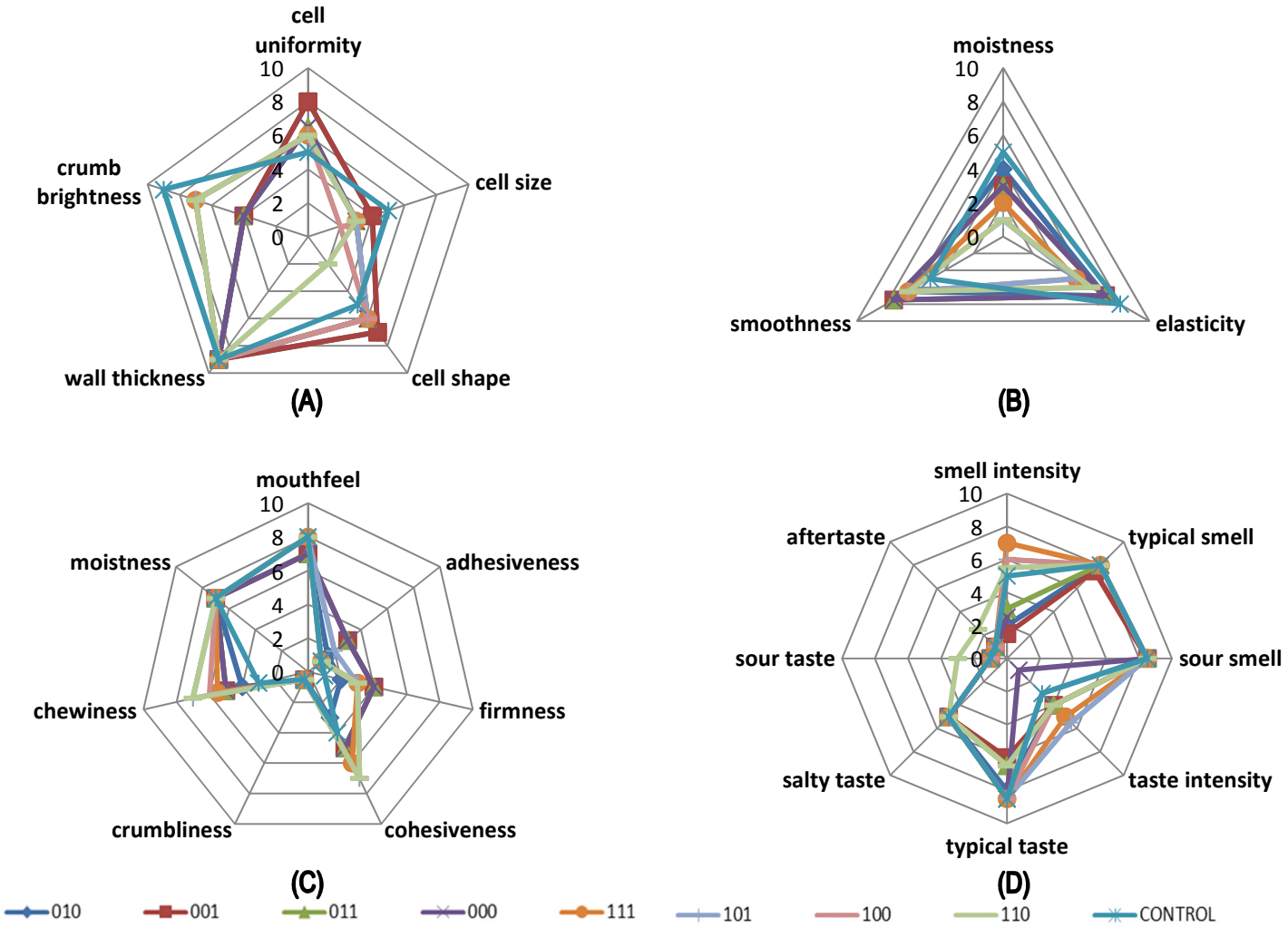

Fig. 1 Spider graphs of visual (A), tactil (B), biting (C), and flavour (D) sensory attributes of control and blended wheat-based breads. Three digit sample code refers to low (0) ang high (1) wheat flour replacement by teff: green pea: buckwheat flours in samples.

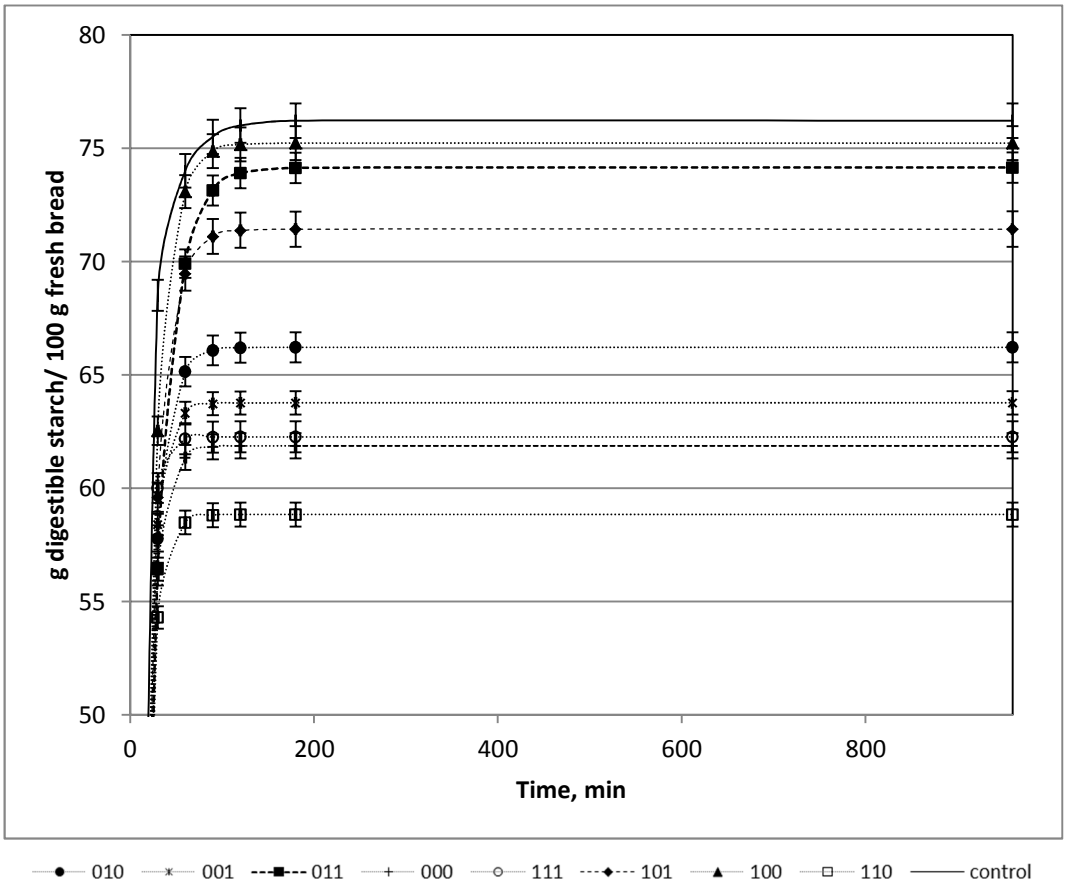

Fig. 2 Digestible starch hidrolysis kinetic curves (mean of three replicates) of control and blended wheat-based breads. A first order kinetic equation [$C = C_{\infty}(1\text{-}e^{-kt})$] was applied to describe the kinetics of digestible starch hydrolysis. Three digit sample code refers to low (0) ang high (1) wheat flour replacement by teff: green pea: buckwheat flours in sample formulation.

Table 1
Proximate chemical and nutritional composition[a] of flours (per 100 g flour, d.b.) and breads (per 100 g fresh blended bread).

Sample code[b]	Protein[e] (g)	Total dietary fibre (g)	Insoluble dietary fibre (g)	Soluble dietary fibre (g)	Fat (g)	Ash (g)	DC[c] (g)	Energy (kcal)[d]	Moisture (g)
Flours									
Wheat	14.13 ± 0.05b	2.19 ± 0.12a	1.20 ± 0.09a	0.99 ± 0.25a	1.56 ± 0.09a	0.63 ± 0.09a	81.70	−	14.32 ± 0.10c
Grren pea	25.12 ± 0.04d	14.56 ± 0.95d	8.50 ± 0.15d	6.05 ± 0.27c	1.27 ± 0.15b	2.58 ± 0.12c	56.63	−	8.17 ± 0.09a
Buckwheat	19.71 ± 0.06c	13.52 ± 0.38 c	6.58 ± 0.25b	6.93 ± 0.36d	3.44 ± 0.18c	2.05 ± 0.19b	61.16	−	11.70 ± 0.18b
Teff	13.05 ± 0.02a	12.19 ± 0.49b	7.40 ± 0.36c	4.80 ± 0.36b	5.06 ± 0.09d	2.21 ± 0.09b	66.97	−	11.90 ± 0.09b
Breads									
010	11.9 ± 0.1b	3.3 ±0.3b	1.9 ± 0.31b	1.42 ± 0.39b	3.5 ± 0.2a	−	47.8	277	33.4 ± 0.8c
001	11.7 ±0.2b	3.3 ± 0.2b	1.85 ± 0.34b	1.49 ± 0.30b	3.6 ± 0.4a	−	48.5	280	32.9 ± 0.6bc
011	12.3 ± 0.1c	3.9 ± 0.3c	2.18 ± 0.39b	1.72 ± 0.28b	3.6 ± 0.2a	−	47.7	281	32.5 ± 0.9b
000	11.6 ± 0.3b	2.9 ± 0.3b	1.63 ± 0.45b	1,24 ± 0.25b	3.6 ± 0.1a	−	49.6	283	32.3 ± 0.4bc
111	12.2 ± 0.2c	4.3 ± 0.5c	2.42 ± 0.39b	1.88 ± 0.44b	3.8 ± 0.3a	−	47.8	283	31.9 ± 0.3b
101	11.7 ± 0.1b	3.8 ± 0.2c	2.13 ±0.28b	1.67 ± 0.37b	3.8 ± 0.2a	−	51.4	295	29.3 ± 0.8a
100	11.7 ± 0.1b	3.3 ±0.2b	1.9 ± 0.24b	1.41 ± 0.31b	3.7 ± 0.4a	−	50.8	290	30.5 ± 0.9a
110	12.2 ± 0.2c	3.8 ± 0.1c	2.21 ± 0.41b	1.63 ± 0.45b	3.7 ± 0.1a	−	48.1	282	32.2 ± 0.4bc
Control	11.1 ± 0.1a	1.4 ± 0.2a	0.83 ± 0.36a	0.59 ± 0.32a	3.4 ± 0.2a	−	51.2	283	32.9 ± 0.6bc

[a]Mean values ± standard deviation. Within columns, values (mean of three replicates) with the same following letter do not differ significantly from each other ($p > 0.05$).
[b]Three digit bread sample code refers to low (0) and high (1) wheat flour replacement by teff: green pea: buckwheat flours in sample formulation.
[c]DC: digestible carbohydrates calculated by indirect determination: DC = 100 − [Moisture + Protein + Fat + Ash + Dietary Fibre].
[d]Energy conversion: protein × 4 kcal/g; fat × 9 kcal/g; digestible carbohydrates × 4 kcal/g; dietary fibre × 2 kcal/g.
[e]Conversion factor from *N* to protein = 6.25.

Table 2
Physical characteristics of blended breads.

Characteristic	Blended bread samples [a,b]								
	010	001	011	000	111	101	100	110	Control
Volume and Textural									
Specific volume, mL/g	1.9 ± 0.2a	2.0 ±0.1 a	2.0 ± 0.1 a	2.9 ±0.2b	2.1 ± 0.2a	2.3 ± 0.3a	2.1 ± 0.1a	2.1 ± 0.1a	3.1 ± 0.2b
Hardness, *N*	22.8 ± 2.7d	20.6 ±1.4d	25.0 ± 0.5e	15.8 ± 1.7ib	25.3 ± 0.1e	17.8 ± 2.1bc	18.7 ± 1.0cd	18.1 ±0.1c	8.7 ±0.2a
Cohesiveness	0.599 ±0.008cd	0.630 ±0.016e	0.521 ± 0.015a	0.609 ± 0.018de	0.499 ± 0.021a	0.592 ± 0.002c	0.635 ± 0.016e	0.541 ± 0.005b	0.695 ± 0.004f
Springiness	0.879±0.002b	0.896 ± 0.001d	0.842 ± 0.001a	0.908 ± 0.026e	0.898 ± 0.084abcde	0.878 ± 0.008bc	0.885 ± 0.001c	0.858 ± 0.024a	0.955 ± 0.001f
Colour									
L	71.9a	70.6a	69.6a	72.2a	68.6a	69.1a	71.5a	70.5a	74.6b
a	2.9b	3.1b	3.9c	2.7b	4.1c	3.3b	3.1b	3.3b	1.1a
b	15.3b	14.9b	16.0b	15.0b	16.1b	15.3b	15.3b	15.7b	12.0a
Whiteness Index	67.9b	66.9b	65.4a	68.3b	64.4a	65.3a	67.5b	66.4b	71.9c
ΔE	4.7	5.3	7.0	4.2	8.0	6.8	5.0	6.0	–
Crumb grain									
Mean cell area, mm^2	0.17a	0.21a	0.24b	0.24b	0.25b	0.31c	0.20a	0.21a	0.27c
Max area, mm^2	9.4a	20.0d	13.7b	7.3a	11.8b	12.9b	12.0b	9.1a	16.4c
Cell area distribution, %									
<0.4 mm^2	34	25	20	22	20	14	27	24	22
0.4-1.0 mm2	24	24	16	20	18	15	26	23	15
1.0-10 mm2	42	44	59	59	60	64	44	54	50
10-20 mm2	0	7	5	0	2	7	3	0	12
Cell number distribution, %									
<0.4 mm^2	90	87	88	86	87	84	87	87	88
0.4-1.0 mm^2	6	8	6	8	7	8	8	8	6
1.0-10 mm2	4	4	6	7	6	8	4	5	5
Cell density, cells/cm^2	151d	131c	136	115b	123c	105a	126c	135c	102a
Cell to total area ratio	25:75	28:72	33:67	28:72	31:69	33:67	25:75	29:71	27:73

[a]Mean values ± standard deviation. Within rows, values (mean of three replicates) with the same following letter do not differ significantly from each other ($p > 0.05$).
[b]Three digit bread sample code refers to low (0) and high (1) wheat flour replacement by teff: green pea: buckwheat flours in sample formulation.

Table 3
Starch Hydrolysis kinetics, expected Glycemic Index and relevant Starch Nutritional fractions of blended breads.

Characteristic	Blended bread samples [a,b]								
	010	001	011	000	111	101	100	110	Control
Starch Hydrolysis kinetics									
C_∞	74.3±1.3c	65.7±0.9a	74.1±0.9c	73.6±1.3c	71.2±1.2b	75.8±0.8c	75.2±0.7c	75.7±0.8c	81.0±0.9d
k	0.0686±0.0032c	0.0825±0.0091c	0.0477±0.0013a	0.0797±0.0062c	0.1106±0.0085d	0.0599±0.0031b	0.0593±0.0029b	0.0821±0.0079c	0.0720±0.0081c
H_{90}, %	74.2±1.1c	65.7±1.2a	73.1±0.8c	73.6±0.8c	71.2±0.6b	75.4±0.9c	74.9±0.9c	75.6±0.6c	81.0±1.1d
HI, %	90±3b	81±4a	92±4b	91±3b	88±2b	94±3b	93±3b	93±3b	100±1c
eGI	86±4b	78±2a	86±b	87±4b	84±3b	89±3b	88±3b	89±3b	94±1c
Starch Nutritional fractions (per 100 g bread, as is)									
RDS, g	57.8±0.9b	58.4±1.1b,c	56.4±1.0b	56.2±1.1a	60.0±1.2c	59.6±1.5b,c	62.5±1.4c	54.3±1.0a	68.5±1.1d
SDS, g	8.4±1.1c	5.4±0.7b	17.5±e	5.7±0.6b	2.3±0.6a	11.8±d	12.6±d	4.5±1.1b	7.5±1.0c
DS, g	66.2	63.8	73.9	61.9	62.3	71.4	75.2	58.8	76.0
RS, g	2.4±0.1b	2.7±0.3b	2.9±0.5b	2.8±0.4b	2.5±0.2b	2.3±0.2b	2.5±0.2b	2.2±0.2a,b	1.8±0.3a
TS, g	69	67	77	65	65	74	78	61	78

([a]) Mean values ± standard deviation. Within rows, values (mean of three replicates) with the same following letter do not differ significantly from each other ($p > 0.05$).
([b]) Three digit bread sample code refers to low (0) ang high (1) wheat flour replacement by teff: green pea: buckwheat flours in sample formulation. RDS: rapidly digestible starch, SDS: slowly digestibly starch, eGI: expected glycemic index, DS: digestible starch, RS: resistant starch, TS: total starch. C_∞: equilibrium concentration, *k*: kinetic constant, H_{90}: total starch hydrolysis at 90 min, *HI*: hydrolysis index. A first order kinetic equation [$C = C_\infty(1- e^{-kt})$] was applied to describe the kinetics of starch hydrolysis *C* concentration at t time; C_∞ equilibrium concentration; *k* kinetic constant; *t* time. TS= DS + RS; DS= RDS + SDS.

Table 4
Single significant effects (p<0.05) of rate of wheat flour replacement by low (0) and high (1) dose of teff, green pea and buckwheat on starch hydrolysis kinetic parameters and relevant starch nutritional fractions of blended breads (per 100 g bread, as is).

Parameter	Level	Overall mean	Teff	p<0.05	Green pea	p<0.05	Buckwheat	p<0.05
C_∞	0	73.2	71.9±1.8	a	ns		74.7±1.8	b
	1		74.5±1.8	b			71.7±1.8	a
k	0	0.0738	0.0696±0.0073	a	0.0704±0.0073	a	ns	
	1		0.0780±0.0073	b	0.0773±0.0073	b		
H_{90}	0	73.0	71.6±1.7	a	ns		74.6±1.7	b
	1		74.3±1.7	b			71.4±1.7	a
RDS (%)	0	58.2	57.2±1.7	a	59.2±1.7	b	ns	
	1		59.1±1.7	b	57.1±1.7	a		
SDS (%)	0	8.5	9.2±1.5	b	ns		7.8±1.5	a
	1		7.8±1.5	a			9.2±1.5	b
DS(%)	0	66.7	66.4±0.2	a	68.0±0.2	b	65.5±0.2	a
	1		66.9±0.2	b	65.3±0.2	a	67.8±0.2	b
RS (%)	0	2.6	2.7±0.0	b	2.6±0.0	b	2.5±0.0	a
	1		2.4±0.0	a	2.5±0.0	a	2.6±0.0	b

ns: non significant, p>0.05. For each parameter, within rows, values (mean of three replicates±standard error) with the same following letter do not differ significantly from each other (p > 0.05).

Table 5
Second order significant interactions (p<0.05) of rate of wheat flour replacement by low (0) and high (1) dose of teff (T), green pea (GP) and buckwheat (BW) – design factors on starch hydrolysis kinetic parameters and relevant starch nutritional fractions of blended breads (per 100 g bread, as is).

Parameter	Level	Overall mean	T x GP	p<0.05	T x BW	p<0.05	GP x BW	p<0.05
C_∞	00	73.2	69.7±1.8	a	ns		ns	
	01		74.2±1.8	bc				
	10		75.5±1.8	c				
	11		73.4±1.8	bc				
k	00	0.0738	0.0811±0.073	bc	0.0742±0.073	b	ns	
	01		0.0582±0.073	a	0.0651±0.073	a		
	10		0.0596±0.073	a	0.0707±0.073	ab		
	11		0.0964±0.073	c	0.0853±0.073	c		
H_{90}	00	73.0	69.6±1.7	a	73.9±1.7	b	ns	
	01		73.6±1.7	bc	69.4±1.7	a		
	10		75.2±1.7	bc	75.3±1.7	b		
	11		73.4±1.7	bc	73.3±1.7	b		
RDS (%)	00	58.2	57.3±1.7	a	ns		59.4±1.7	b
	01		57.11±1.7	a			59.0±1.7	b
	10		61.07±1.7	b			56.0±1.7	a
	11		57.15±1.7	a			58.2±1.7	b
SDS (%)	00	8.5	5.5±1.5	a	7.0±1.5	a	9.1±1.5	b
	01		13.0±1.5	b	11.4±1.5	b	8.6±1.5	ab
	10		12.2±1.5	b	8.6±1.5	a	6.5±1.5	a
	11		3.4±1.5	a	7.0±1.5	a	9.9±1.5	b
DS(%)	00	66.7	62.8±0.2	b	64.0±0.2	a	68.5±0.2	d
	01		70.1±0.2	c	68.8±0.2	c	67.6±0.2	b
	10		73.3±0.2	d	67.0±0.2	b	62.5±0.2	a
	11		60.6±0.2	a	66.8±0.2	b	68.1±0.2	c
RS (%)	00	2.6	2.8±0.02	b	2.6±0.0	c	2.6±0.0	c
	01		2.7±0.02	b	2.8±0.0	d	2.5±0.0	b
	10		2.4±0.02	a	2.3±0.0	a	2.3±0.0	a
	11		2.4±0.02	a	2.4±0.0	b	2.7±0.2	d

ns: non significant, p>0.05. For each parameter, within rows, values (mean of three replicates±standard error) with the same following letter do not differ significantly from each other (p > 0.05). RDS: rapidly digestible starch, SDS: slowly digestibly starch, eGI: expected glycemic index, DS: digestible starch, RS: resistant starch, C_∞: equilibrium concentration, k: kinetic constant, H_{90}: total starch hydrolysis at 90 min, HI: hydrolysis index. A first order kinetic equation [$C = C_\infty(1- e^{-kt})$] was applied to describe the kinetics of starch hydrolysis.

Table 6
Bioaccessible polyphenols and anti-radical activity of blended breads.

Sample	Bioaccessible polyphenols[a]			Anti-radical activity[c]	
	mg gallic acid/ 100 g flour, as is	Δ with respect to flour content, %	Δ with respect to wheat bread content, %	Remaining µmols DPPH at steady state	%
Flours					
Wheat	380±15c	-	-	0.1310±0.010b	74.2a
Teff	303±15a	-	-	0.1407±0.009b	72.3a
Green pea	343±17b	-	-	0.1451±0.010b	71.4a
Buckwheat	365±12b,c	-	-	0.0604±0.008a	88.1b
Breads[b]					
010	442±10b	20	7	0.3150±0.016b	37.9
001	464±11c	26	12	0.3009±0.018b	40.7
011	482±13c	31	16	0.2715±0.017a	46.5
000	466±15c	26	13	0.3102±0.015b	38.8
111	464±15c	28	12	0.2619±0.019a	48.4
101	478±18c	31	16	0.2655±0.020a	47.7
100	443±10b	30	7	0.2816±0.016a	44.5
110	416±12a	15	1	0.2876±0.018a	43.3
Control	414±15a	9	-	0.3427±0.013c	32.4

([a]) Mean values ± standard deviation. Within columns, values (mean of three replicates) with the same following letter do not differ significantly from each other ($p > 0.05$).
([b]) Three digit bread sample code refers to low (0) ang high (1) wheat flour replacement by teff: green pea: buckwheat flours in sample formulation.
([c]) Corresponding to 12 mg flour or freeze-dried bread that consumed DPPH when 0.494 µmols of the free radical are initially available to react. The plateau was decided at 150 min of reaction.

7 CHAPTER III

Significance of thermal transitions on starch digestibility and firming kinetics of restricted water mixed flour bread matrices

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Impact of ancient cereals, pseudocereals and legumes on starch hydrolysis and antiradical activity of technologically viable blended breads

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ABSTRACT

Wheat flour replacement from 22.5% up to 45% by incorporation of ternary blends of teff (T), green pea (GP) and buckwheat (BW) flours provided technologically viable and acceptable sensory rated multigrain breads with superior nutritional value compared to the 100% wheat flour (WT) counterparts. Blended breads exhibited superior nutritional composition, larger amounts of bioaccessible polyphenols, higher anti-radical activity, and lower and slower starch digestibility. Simultaneous lower rapidly digestible starch (57.1%) and higher slowly digestible starch (12.9%) and resistant starch (2.8%) contents (g per 100 g fresh bread), considered suitable nutritional trends for dietary starch fractions, were met by the blend formulated 7.5% T, 15% GP, 15% BK. The associated mixture that replaced 37.5% WT, showed a rather lower extent and slower rate of starch hydrolysis with medium-low values for C_∞, and H_{90}, and lowest k, and intermediate expected Glycaemic Index (86). All multigrain breads can be labelled as source of dietary fibre (≥3 g dietary fibre/100 g bread).

1. Introduction

Grains are basic, ubiquitous and healthy raw materials, good source of carbohydrates – mainly starch and dietary fibre – providing excellent vectors for diversity and innovation. It raises a great deal of recent interest that ancient crops (Angioloni & Collar, 2011a), pseudocereals (Collar & Angioloni, 2014a) and legumes (Angioloni & Collar, 2012), besides wheat, constitute nutrient-dense and healthy grains with explicited breadmaking applications.

A slow release and absorption of glucose may be generated in a food matrix according to the processing conditions and surrounding ingredients (Lehmann & Robin, 2007), encompassing beneficial effects in the management of diabetes and hyperlipidemia (Jenkins, 2007). Native cereal starches are ideal sources of slowly digestible starch (SDS) (>50%), and the slow progressive digestion property is realized by a layer-by-layer inside–outside (radial) digestion process (Zhang, Ao, & Hamaker, 2006a). Mechanical and thermal treatments change the structure and digestibility of starch. Thermal treatments such as the cooking process completely destroys the semicrystalline structure of native starch granules and causes the loss of SDS and resistant starch (RS) and increases rapid digestible starch (RDS) (Zhang, Venkatachalam, & Hamaker, 2006b). In cereal products, the starch gelatinization extent, which is mainly controlled by the moisture level and the cooking time and temperature influences the formation of SDS (Englyst, Vinory, Englyst, & Lang, 2003). In bread dough, although formation of resistant starch (RS3) may occur in the high water-containing parts during cooling, a large portion of starch is gelatinized during cooking and induces a rapid digestibility of starch (Bravo, Englyst, & Hudson, 1998). In extruded cooked cereal products such as breakfast cereals, in addition to the thermal treatment, the high pressure and shear forces destroy the starch granular structure and increase its gelatinization extent, making it more available to amylolytic enzymes (Le François, 1989). On the contrary, in pasta, a dense protein network is formed, which limits the accessibility of α-amylase to the starch and restricts the diffusion of water molecules to the starch granules. As a consequence, a reduction of the extent of starch gelatinization takes place (Englyst, Kingman, & Cummings, 1992). In some biscuits with very low moisture levels during the treatment, the extent of gelatinization is reduced and partially intact granules and gelatinized starch co-exist, resulting in a higher content of SDS compared to breakfast cereals and baked products (Englyst et al.,

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Significance of thermal transitions on starch digestibility and firming kinetics of restricted water mixed flour bread matrices

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Abstract

The impact of wheat (WT) flour replacement up to 45% (weight basis) by incorporation of ternary blends of teff (T), green pea (GP) and buckwheat (BW) flours on the thermal profiles of quaternary blended dough matrices have been investigated by simulating baking, cooling, and storage in differential scanning calorimeter (DSC) pans. Endothermal transitions related to suitable patterns for low and slow starch hydrolysis, softer crumb and retarded firming kinetics in blended breads include delayed temperatures for starch gelatinization, and for the dissociation of amylose-lipid complex. In addition, (a) higher stability for the amylose-lipid inclusion complex, (b) lower energy for starch gelatinization, (c) lower limiting melting enthalpy and (d) slower rate for amylopectin retrogradation meet thermal requirements for achieving suitable textural and starch digestibility features in blended breads, fulfilled by adding T/GP/BW to replace 45% of WT flour in blended dough formulations.

Keywords: bread, differential scanning calorimetry, thermal transitions, starch hydrolysis, ancient cereals, pseudocereals, legumes.

Introduction

Bread that explicits both a multicomponent and multiphase nature, can be viewed as a composite material where amylose amylopectin, and protein form separate phases due to thermodynamic immiscibility of the polymers in presence of surrounding ingredients (Hug-Iten, Escher, & Conde-Petit, 2003). The final structure of bread crumb described as a porous material with flexible elastic cell walls, is the result of a water-dependent thermal process related to the number and type of cross-links formed between the nearest neighboring chains of biopolymers (protein network and starch) present in the dough (Biliaderis, Page, Maurice, & Juliano, 1986). Starch gelatinization and protein coagulation induce bread crumb formation. After cooling, the higher final water content of the crumb (35–45%) is responsible for the rubbery behaviour, which gives structural mobility and smooth bread crumb behaviour, and explains the sensitiveness of starch to retrograde during storage (Cuq, Abecassis, & Guilbert, 2003). The extent of gelatinization and retrogradation are major determinants of the susceptibility of starch to enzymatic digestion and its functional properties for food processing such as stickiness, ability to absorb water and ageing (Wang and Copeland, 2013). The gelatinization degree of starch in baked products depends primarily on the water availability and the amount of heating (Shin, Kim, Ha, Lee, & Moon, 2005). Products (white bread, sugar cookies, pie crust, angel food cake, cake doughnuts and cinnamon rolls) can range from essentially completely gelatinized (97%) to almost native-like conditions (4%) (Lineback and Wongsrikasem, 1980). Other factors influencing gelatinization account for other components in the food matrix competing for water (e.g. sugar and proteins), heat transfer, the presence of lipid/starch complexes or other types of complexes, and they are usually negatively associated with extent of swelling, probably due to increased hydrophobicity. Modifications of water availability by the presence in dough matrices of hydrocolloids (Santos, Rosell, & Collar, 2008), low molecular weight dextrins (Miyazaki, Maeda, & Morita, 2004), blended starches of different sources

(Waterschoot, Gomand, Fierens, & Delcour, 2014) and high damaged starch flours retrogradation (León, Barrera, Pérez, Ribotta, & Rosell, 2006) among other factors changed the thermal behaviour of flour-water mixtures during gelatinization and retrogradation. Mechanisms involved relate restriction of enzyme-substrate contact, interference as a physical barrier to prevent amylopectin chain association during storage, and a viscosity effect that affects mobility within the stored system (Khanna and Tester, 2006). All these factors may limit the gelatinization degree constraining the swelling and breakdown of the starch granule structure, thus resulting in less digestible starch (Llorca et al., 2007). In general, any process or condition where the water availability or thermal energy is limited could generate the same effect, this is a lower degree of gelatinization encompassing a lower amorphous structure, and thus a lesser amount of digestible starch (Parada and Aguilera, 2011). In addition, granule size and surface characteristics (for example, pores, grooves or furrows, and surface-associated proteins and lipids), starch damage, amylose content, fine structure of amylopectin, degree of crystallinity and phosphorus content, can all affect digestibility (Wang and Copeland, 2013). Main studies focused on the effects of gelatinization and retrogradation at higher water content on starch digestibility, but there is scarce information on the effect of retrogradation at low water content on starch digestibility (Wang and Copeland, 2013). The amount of gelatinization, swelling and hydrolysis are intimately controlled by the water content of the system and the temperature, and moderated by the botanical origin and composition of starches in limiting water conditions. According to Tester and Sommerville (2000), gelatinization, swelling and hydrolysis are restricted where crystalline order is retained within starch granules. However, when the water content and temperature profile become sufficient to allow gelatinisation and starch hydrolysis by α-amylase to proceed, swelling may be constrained because starch ability to hydrate and expand is hindered as a consequence of the complex composition, particularly in starch blends of different botanical origin. Starch retrogradation involves reassociation of starch component molecules into a

partially crystalline, ordered structure. Amylopectin recrystallization requires several days. Because firming of bread also develops over several days, most staling models view the changes in amylopectin as the primary cause for crumb firming (Zobel and Kulp, 1996). The slow crystallization of amylopectin was referred to as a nucleation-limited growth process, which occurred above the glass transition in a mobile, viscoelastic, fringed-micelle network (Roos, 1995). Staling involves hardening of the crumb that is a complex phenomenon in which multiple mechanisms operate, all of them involving amylopectin retrogradation as the main player (Gray and BeMiller, 2003). Water plays a critical role in bread staling. When the retrogradation of amylopectin occurs, water molecules are incorporated into the crystallites and the distribution of water is shifted from gluten to starch/amylopectin, thereby changing the nature of the gluten network (Gray and BeMiller, 2003). Besides the molecular order of starch, water also plays an important role in crumb firmness due to its plasticizing effect on the crumb network (Hug-Iten et al., 2003). High wheat flour replacement by non-gluten forming flours from cereals, pseudocereals and legumes, particularly associated mixtures of teff, buckwheat and green pea have proven to provide technologically viable and acceptable sensory rated multigrain breads with superior nutritional value compared to the 100% wheat flour counterparts (Collar, Jiménez, Conte, & Fadda, 2014). Blended flours of different starch nature are expected to modify the mechanism of water mobility in bread crumb, and concomitantly its thermal properties during gelatinization and ageing due to water restrictions for swelling, gelatinization and starch hydrolysis. Starch digestibility kinetics and crumb firming evolution during storage of blended breads are both water-dependent processes. Thermal transitions of multicomponent bread matrices baked at restricted water conditions are not well known, and the possible relationships between thermal properties, textural behaviour and the susceptibility of starch to enzymatic digestion in those heterogeneous matrices lack. This paper is aimed (a) at investigating the thermal transitions that occur during starch gelatinization and retrogradation in complex grain flour matrices with restricted water availability,

(b) at knowing the impact of non-breadmaking whole grains (teff, green pea and buckwheat flours), highly replacing wheat-based matrices on the transition phases and (c) at exploring the relationships between thermal properties and starch digestibility and firming kinetics of technologically viable and sensorially accepted multigrain bread matrices.

Materials and Methods

Materials

Commercial flours from refined common wheat Triticum aestivum (WT), and whole teff *Eragrostis tef* (T), green pea *Pisum sativum* (GP), and buckwheat *Fagopyrum esculentum* (BW) were purchased from the Spanish market. Protein, dietary fibre and fat contents (% flour, dry basis) were 14.13%, 2.19%, 1.56 (WT); 25.12%, 14.56%, 1.27 (GP); 19.71%, 13.52%, 3.44% (BW), and 13.05%, 12.19%, 5.06% (T), respectively. Refined WT (70% extraction rate) of 356×10^{-4} J energy of deformation W, 0.64 curve configuration ratio P/L, 95% Gluten Index, 62% water absorption in Brabender Farinograph, was used. Ireks Vollsauer sour dough was from Ireks (Spain); Novamyl 10000 a maltogenic thermostable α-amylase of 10,000 Maltogenase Units (MANU) of activity, from Novozymes (Denmark); and calcium propionate, from Sigma-Aldrich (USA).

Methods

Bread making of wheat and wheat-based blended flours

Doughs and breads were prepared from WT as control, and wheat-based blended flours (T, GP, BW) by WT replacement from 22.5% up to 45%, and incorporation of ternary blends of T, GP and BW flours according to a Multilevel Factorial Design (Statgraphics Centurion XV, version 15.2.11, Statpoint Technologies, Inc. Warrenton, Virginia, USA) with the following attributes: three experimental factors (T, GP and BW flours) at two levels, coded 0 (7.5% wheat flour

replacement) and 1 (15% wheat flour replacement), and five error degrees of freedom. Levels of wheat flour replacement were chosen after performing preliminary trials to set the range of non-wheat flours to be incorporated in associated blends to the formulations in such a way that significant enhancement of bread nutritional properties was achieved without notable deterioration of sensory attributes (Collar et al., 2014). The model resulted in eight randomized runs in 1 block. A 3 digit bread sample code was set referring to low (0) and high (1) wheat flour replacement by T (1st digit), GP (2nd digit), and BW (3rd digit) flours in sample formulation, as it follows: 0 1 0, 0 0 1, 0 1 1, 0 0 0, 1 1 1, 1 0 1, 1 0 0, 1 1 0. Blended flours, water, commercial compressed yeast, salt, margarine, sugar, commercial sour dough, milk powder, Novamyl 10000, and calcium propionate were mixed, and used to make control and blended breads according to the quantitative formulations and bread making procedure described earlier (Collar et al., 2014). Bread samples were stored for 1, 3, 6, and 8 days to describe firming kinetics.

Bread measurements

Chemical and nutritional composition of breads

The chemical and nutritional composition of control and blended breads was fully determined in a previous paper (Collaret al., 2014), from where some selected bread characteristics are compiled in Table 1. Moisture, fat and protein contents were determined following the ICC methods (ICC, 2014). Total, soluble and insoluble dietary fibre contents were determined based on the AOAC method 991.43 (AOAC, 1991) as described by Megazyme International Ireland (2012) in the Total Dietary Fibre Assay Procedure (kit K-TDFR 05/12). Three replicates were made for each analysis. Resistant starch (RS) determination was performed according to AOAC Official Method 2002.02 (AOAC, 2000) and AACC Method 32-40 (AACC, 2005), as described by Megazyme International Ireland (2011) in the Resistant Starch Assay Procedure (kit K-RSTAR 08/11). In vitro starch hydrolysis kinetics and relevant starch fractions in freeze-dried and ground fresh

blended breads was determined following the AACC (2005) method 32–40, adapted by Angioloni and Collar (2011) as previously described (Collar et al., 2014). Rapidly Digestible Starch (RDS) and Slowly Digestible Starch (SDS) were measured after incubation for 20 min and 120 min, respectively (Englyst, Vinory, Englyst, & Lang, 2003). Digestible starch (DS) was calculated by the sum of RDS and SDS. Total starch (TS) was calculated by the sum of DS and RS. A first order kinetic equation [$C = C_\infty (1 - e^{-kt})$] was applied to describe the kinetics of starch hydrolysis, where C, C_∞ and k were the hydrolysis degree at each time, the maximum hydrolysis extent and the kinetic constant, respectively.

Physical determinations

Bread texture and firming kinetics

Bread mechanical characteristics (TPA in a double compression cycle) of fresh and stored breads were recorded in a TA-XTplus texture analyser (Stable Micro Systems, Surrey, UK) using a 25 mm diameter cylindrical aluminium probe, a 5 kg load cell, 50% penetration depth at a running speed of 1 mm/s, and a 30 s gap between compressions on crust-free slices of 25 mm width (Armero and Collar, 1998). For textural measurements, three slices of two breads were used for each sample. The obtained firming curves during bread storage were modelled using the Avrami equation, and model factors were estimated by fitting experimental data of hardness to the non linear regression equation $\vartheta = \frac{T_\infty - T_t}{T_\infty - T_0} = e^{-ktn}$ where ϑ is the fraction of the recrystallisation still to occur; T_0, T_∞ and T_t are crumb firmness at time zero, ∞ and time t, respectively, k is a rate constant, and n is the Avrami exponent.

Thermal measurements and retrogradation kinetics

Thermal properties regarding starch gelatinization, retrogradation, and amylose-lipid complexation of control and blended samples were assessed in a Differential Scanning Calorimeter Perkin-Elmer DSC-7 (Norwalk, USA) according to the

method of León, Durán, and Benedito de Barber (1997), with some modifications as previously reported by Santos et al. (2008).

Starch gelatinization

Dough samples were prepared by mixing flour blends and the remaining ingredients with 62% of water. For DSC analysis, 50–70 mg of dough samples were weighed in large volume pre-weighed, sealed stainless-steel pans. An empty pan was used as a reference. Simulation of the temperature profile in the centre of the bread crumb during baking was performed in the calorimeter under the following scanning conditions: samples were kept at 30 ºC for 2 min, then heated from 30 ºC to 110 ºC at a rate of 11.7 ºC /min, kept at 110 ºC for 5 min, and finally cooled from 110º to 30 ºC at a rate of 50 ºC /min. Gelatinized samples were stored at 22 ºC for 0, 1, 3, 6, and 8 days. Thermal transitions of starch samples were defined as T_o (onset), T_p (peak of gelatinization) and T_e (end); the enthalpy associated with starch gelatinization was defined as ΔH_g, and was calculated from the area under the curve defined after scanning, and expressed in J/g of dry sample. The gelatinization temperature range (R) was computed as ($T_e - T_o$), as described by Vasanthan and Bhatty (1996).

Starch retrogradation and amylose–lipid complexation

Stored gelatinized dough samples were submitted to a second DSC scan to analyse starch retrogradation and amylose–lipid complexation at the different storage periods. Scanning conditions included keeping sample pans at 25 ºC for 1 min, and then heating from 25 to 130 ºC at a rate of 10 ºC/min. The enthalpy of amylopectin retrogradation (ΔH_r) and the enthalpy for amylose-lipid complex dissociation (ΔH_d) were calculated, and T_o, T_p, and T_e for the different thermal transitions, identified. All samples were analyzed in duplicate. Modeling of crystallization data was carried out using the Avrami equation, and model factors were estimated by fitting experimental data for melting enthalpies to the non linear regression equation (Jouppila, Kansikas, & Roos, 1998) $\vartheta = \frac{H_\infty - H_t}{H_\infty - H_0} = e^{-ktn}$

where ϑ is crystallinity, H_∞ is the leveling-off value of melting enthalpy at which the extent of crystallization in starch stoped, H_t is the melting enthalpy at time t, and H_o is the melting enthalpy at initial time, t is time of crystallization, k is a rate constant, and n is the Avrami exponent. The values of the constants k and n were used to calculate the value of half-life, $t_{1/2}$, for starch crystallization and bread firming. Half-life was taken as the time required to achieve 50% of the leveling-off extent of crumb crystallinity or firmness as defined as $t_{1/2} = (-\ln 0.5/k)^{1/n}$ by Jouppila et al. (1998) and reported by Ronda and Roos (2011). The shorter the half-life is, the quicker is the amylopectin recrystallization or the hardening of the bread crumb.

Statistical analysis

Multivariate analysis of variance, correlation matrix and non linear multiple regression analysis of data were performed by using Statgraphics V.7.1 program (Bitstream, Cambridge, MN). Multiple range test (Fisher's least significant differences, LSD) for analytical variables was applied to know the difference between each pair of means.

Results and discussion

The thermal transitions (Figs. 1 and Fig. 2) and textural behaviour (Fig. 3) during storage of composite breads are explored first and compared to those of WF flour counterparts (Table 2), prior to assess the single and interactive effects of the non-wheat flours T, GP and BW on the molecular and macroscopic quality picture of breads (Table 3 and Table 4), and to correlate the thermal parameters with the firming and the starch hydrolysis kinetics of blended breads (Table 5).

Thermal and textural parameters of composite breads: Effects of non-wheat flours on the calorimeter transitions and staling kinetics

The parameters characterizing endothermal transitions – starch gelatinization, amylose-lipid complex dissociation and melting of amylopectin and staling

kinetics – crumb firming and starch retrogradation - of the different blended doughs and breads thereof are presented in Table 2. The multiple analysis of variance (MANOVA) provided information on the significant ($p < 0.05$) single (Table 3) and/or interactive effects (Table 4) of the rate (lowand high) of WT replacement by non-wheat flours T, GP and BW in blended breads on thermal transitions and firming (Fig. 2) and starch retrogradation kinetics (Fig. 3).

Starch gelatinization

During gelatinization, DSC thermograms exhibited a biphasic endotherm (Fig. 1) associated to water-restricted starch-based systems (Wang and Copeland, 2013). According to Donovan (1979), at high water concentration (>66 wt% or water/starch ratio >1.5), a single symmetrical endothermic transition appears in a temperature range of 60–80 ºC in the DSC profiles (called endotherm *G*). As the water/starch ratio is decreased, the magnitude of this endothermic transition decreases progressively, with a concomitant development of a second high temperature endothermic transition (referred to as endotherm *M1*). In the present research dough samples contain intermediate water concentration (34 wt%), and two peaks were defined during the first DSC scan (Fig. 1) named peak 1 (*G*) and peak 2 (*M1*), respectively. The thermal transitions (Table 2) for peak 1 were more energetic (ΔH_g: 2.844–4.232J/g flour), occurred at lower and close temperatures (T_p: 75.55–76.96 ºC) and exhibited a broader gelatinization temperature range (R: 23.67–33.44 ºC) than those for peak 2 (ΔH_g: 0.821–1.867J/g flour; T_p: 90.78–93.61 ºC; R: 8.62–13.88 ºC). The swelling-driven melting theory (Donovan, 1979), the crystallite stability model (Evans and Haisman, 1982), the sequential phase transition model (Nakazawa et al., 1984; Slade and Levine, 1987), the three-stage phase transition model (Biliaderis et al., 1986) and smectic-nematic/isotropic-helix-coil transitions (Waigh, Gidley, Komanshek, & Donald, 2000) have been proposed so far to interpret experimental observations and the nature of the biphasic endothermic *G* and *M1* transitions of starch-water systems at medium water content (34–66 wt%). According to the different proposed

models to explain the biphasic gelatinization endotherm compiled recently (Wang and Copeland, 2013), the first *G* endotherm (peak 1) can be suggested to result from (a) plasticization in amorphous regions (b) swelling-driven crystalline disruption and/or (c) melting of the less stable crystallites in sufficient water, and/or (d) associated with the smectic–nematic/isotropic transition; while the *M1* endotherm (peak 2) represents the melting of the remaining less hydrated and more stable crystallites, encompassing a fast helix-coil transition. In general, the endothermal transitions peak 1 and peak 2 for gelatinization of blended doughs encompassed higher values for both temperatures and enthalpies but narrower temperature range R than did the control WT doughs, except for ΔH_g (peak 2) that showed the opposite trend (Table 2). When the water concentration is limited, complete gelatinization will not occur at the usual gelatinization temperature range, but the transition temperature as well as the enthalpy of transition increases with decreasing water concentration for a given starch type (Parada and Aguilera, 2011). The higher *R* values of WT doughs suggest the presence of flour components of varying stability within the different structure domains (amylose/amylopectin ratio) of its starch granules. The differences in gelatinization temperatures among doughs may be attributed mainly to differences in size, form and distribution of starch granules in the blended flours, and to the internal arrangement of starch fractions within the granule, as stated earlier for legume flours (Kaur and Singh, 2005). In fact, starches in the flour blends are composed of granules differing in size, from small to large: pea (wrinkled) 5–34 μm, small wheat granules 2–3 μm (Zhou, Hoover, & Liu, 2004), large wheat granules 22–36 μm, pseudocereals <2 μm (Pérez and Bertoft, 2010), teff 2–6 μm (Bultosa and Taylor, 2004). Regarding the complex flour composition, WT flour replacement by T from low (0) to high (1) doses did not affect gelatinization parameters (Table 3), but T significantly interacted with GP and/or BW at different doses (Table 4), modifying in a small extent the thermal parameters. A significant increase on the gelatinization temperature (1–3 ºC) of peak 1 and peak 2 were respectively provided by increasing doses of GP and BW (Table 3). In

addition, higher doses of GP resulted in a small decrease of ΔH_{g1}. Significant 2nd order interactions GP × BW were denoted (Table 4), leading to the highest values for T_{01} (69.82◦C) and the lowest for ΔH_{g1} (3.22 J/g flour) when both flours replaced WT flour at 15%, respectively, in good accordance with the experimental values (Table 2) recorded for samples 011 (T_{01}: 69.62 ºC, ΔH_{g1}: 3.374 J/g flour) and 111 (T_{01}: 69.45 ºC, ΔH_{g1}: 2.844 J/g flour).

Amylose–lipid complex dissociation

Amylose–lipid complexes can be naturally present in starch or formed upon gelatinization of starch in the presence of lipids (Putseys, Lamberts, & Delcour, 2010). Blended breads used in the present work account for 3.4–3.8 g/100 g fresh bread of lipids (Table 1). Stored gelatinized control and blended dough samples when submitted to a second DSC scan defined a peak corresponding to an endothermal transition phase with T_p ranging from 100.78 ºC to 106.87 ºC, and ascribed to the dissociation of amylose-lipid inclusion complex (Table 2). In agreement with previous results (Russell, 1983), no significant change in the magnitude of the endotherm was observed with ageing. Two thermally distinct forms of amylose-lipid complexes have been identified: an amorphous structure with a random distribution of aggregated helices (termed Form I) with an endothermic transition in the DSC near 100 ºC, and crystalline structures with DSC transitions at about 115 ºC (Form IIa) and 125 ºC (Form IIb) (Copeland, Blazek, Salman, & Tang, 2009). Both Forms I and II of amylose-lipid complexes may be present in processed starch-based foods depending on the method of processing, and the length of the available fatty acid to complex. Despite at high moisture levels, melting was highly cooperative and thus a single endothermic transition was shown, some amylose-lipid complexes always exhibited a single transition even at intermediate or low water contents (Biliaderis, Page, Slade & Sirett, 1985) as it can be the case of blended samples (34wt%). The observed values for ΔH_d (0.317–0.621 J/g flour) are low as described earlier for water contents lower than 50%, and probably reflect the composite effect of several

processes such as crystallite melting, helix-coil transition, and recrystallization phenomena that occur simultaneously (Biliaderis et al., 1985). Only BW dose has a significant single effect on the temperatures for the thermal transition as the degree of WT replacement goes from 7.5 to 15% (Table 3), denoted by a decrease in T_0 (−1.5 ºC), T_p (−1.3 ºC), and T_e (−2 ºC). Changes may be ascribed to the formation of less thermostable amorphous/crystalline structures, at low moisture contents. This explanation can also apply to blended samples with the pair T/GP at high/high doses and the pair GP/BW at low/high doses, respectively (Table 4).

Starch retrogradation

Extent of retrogradation is very sensitive to water content of starch gels (Zeleznak and Hoseney, 1986). Crystallization during aging depends on the water content occurring only in gels with starch content of 10–80% with the maximum crystallization taking place in gels with 50–60% starch (Eliasson, 1985). Recrystallization increases with increasing water content up to 45–50% because of progressively more effective plasticization (increased molecular mobility) (Slade and Levine, 1987). In this research, a proximate range for starch concentration was 70–76 g/100 g of flour blend (including control) with a concomitant water concentration of 34% in control and blended doughs.

DSC thermograms of amylopectin retrogradation for gelatinized blended doughs at any time of storage showed a similar qualitative shape regardless the quantitative flour composition in the blends (plots not shown). Changes in the ΔH thermal profile for up to 8 days of storage of gelatinized blended doughs were defined according to the flour blend composition (Fig. 2). The kinetics of amylopectin recrystallization on aging of blended doughs were modeled using the Avrami equation as reported previously (Davidou, Le Meste, Debever, & Bekaert, 1996; Santos et al., 2008). Results on the model factors ΔH_0, ΔH_∞, n, and k for the enthalpy of amylopectin retrogradation are compiled in Table 2. Compared to control WT doughs, and with some exceptions, blended doughs exhibited lower (ΔH_∞: 0.451–3.860 J/g flour *vs* 1.302 J/g flour) and variable rate (n_r: 0.7316–

2.5068 *vs* 0.9075) of retrogradation kinetics along storage, with in some cases much longer half-life $t_{1/2}$ (17 days for 101, 15 days for 100 *vs* 1.6 days for control WT breads), endorsing slower amylopectin recrystallization (Fig. 2). Increasing WT flour replacement from 7.5% to 15% by single T, GP and/or BW significantly modified amylopectin retrogradation kinetics (Table 3), in terms of a common decrease in the constant of proportion particularly relevant for BW (k_r: 74%). Moreover, main changes were provided by the pair T × GP added at different doses (Table 4, Fig. 2). Suitable trends for achieving the lower and slower retrogradation kinetics were fulfilled by adding T/GP/BW, each at higher dose (15%), to replace 45% of WT flour in blended dough formulations (Fig. 2D). The corresponding composite bread (111) showed the following values of the Avrami model factors for amylopectin retrogradation kinetics: ΔH_0: 0.000, ΔH_∞: 0.451, n: 2.5068, and k: 0.0759.

Crumb firming

At macroscopic level, during storage, fresh blended and control breads aged in a variable extent following different firming kinetics (Table 2, Fig. 3). As expected, control WT breads were initially softer (T_0: 900 g *vs* 1608–2645 g) and staled less (T_∞: 5908 g *vs* 4685–15136 g) and at lower rate (n_f: 0.52 *vs* 0.50–2.18) than non-wheat flour formulated breads did (Fig. 3). Blended breads with the high dose of GP aged in a higher extent than those replaced by the low dose, means values for T_∞ varying from 9741 g to 6509 g (Table 3). The crumb hardening effect of non-wheat flours was particularly relevant in the staling rate k_f by the simultaneous presence at high/low and low/low doses of T/GP and T/BW (Table 4). The staling rate can be minimized when the pairs replaced 30% (T/GP) and 22.5% (T/BW) of WT flour, respectively.

Relationships between thermal parameters, starch hydrolysis and firming kinetics of composite breads

The retrogradation of amylose in processed foods is considered to contribute to properties relating to stickiness, ability to absorb water, and digestibility, whereas

retrogradation of amylopectin is probably a more important determinant in the staling of bread and cakes (Wang and Copeland, 2013). Cooking and processing increase the susceptibility of starch to enzymatic digestion, which is a function of the degree of starch gelatinization, as shown in several in vitro and in vivo studies. Breads are processed in limited water conditions, in which starch is only partially gelatinized (Lineback and Wongsrikasem, 1980). Using Pearson correlation analysis, a range of correlation coefficients (r) (from 0.35 to 0.88) was obtained for the relationships between thermal properties and starch hydrolysis and firming kinetic parameters of multigrain bread matrices (Table 5). Despite r values were discrete, significant ($0.01 < p < 0.05$) interdependences ($0.3600 < r < 0.7773$) between starch gelatinization (T_{pg}, T_{eg}), amylopectin retrogradation (ΔH_{∞}, n_r, $t_{1/2r}$), amylose–lipid complex dissociation parameters (T_{0d}, ΔH_d), and starch digestibility (k, C_{∞}, H_{90}, starch nutritional fractions) were found (Table 5). After cooking, differences in susceptibility of starch to enzymic attack are related more to the products of gelatinization and, more critically, retrogradation (Copeland et al., 2009). In these food systems such as breads, the physico-chemical behaviour (especially swelling and gelatinization) of starch is restricted and subsequently restricts the ease and extent of hydrolysis with amylases during digestion. In fact, the higher the T_{eg} was, the lower the RDS ($r = -0.4056$) and the slower the starch hydrolysis kinetics ($r = -0.4524$) were. As well, both the leveling-off value of melting enthalpy at which the extent of crystallization in starch stoped (ΔH_{∞}) and the half-life time ($t_{1/2r}$) positively correlated with the starch hydrolysis extent C_{∞} ($r = 0.5385$, 0.4196) and with the relevant starch nutritional fractions DS ($r = 0.7773$, 0.7566), and negatively with the starch hydrolysis rate k ($r = -0.6707$, -0.5371). In model systems starch-fatty acid, it has been observed that the hydrolysis rate of the gelatinized starch can be reduced by the complex formation between amylose and fatty acids (Kawai, Takato, Sasaki, & Kajiwara, 2012). In this work, the higher the enthalpy of dissociation of amylose-lipid complex ΔH_d, associated to a more stable inclusion complex, the slower the rate (k) of the starch digestibility ($r = -0.5765$), and the higher the amounts of SDS ($r = 0.6395$) and

RS (r = 0.5893). The presence of some non-starchy substances in breads such as proteins and lipids (Table 1) over the granule surface may also limit surface accessibility and subsequently the rate and/or extent of enzymatic hydrolysis by blocking the adsorption sites, and therefore influencing enzyme binding (Oates, 1997). Despite kinetic patterns for crumb firming (Fig. 3) and starch retrogradation (Fig. 2) to assess bread staling/aging at macroscopic and molecular levels, respectively, strongly differed in shape, some correspondence for highly replaced wheat flour breads can be observed in agreement with some results reported in the literature for wheat flour (Russell, 1983; Zobel and Kulp, 1996) and gluten-free matrices (Ronda and Roos, 2011). Significant correlations between T_{eg} *vs* T_0 (r = −0.8043), ΔH_g *vs* n_f (r = −0.6061) and n_r *vs* T_0 (0.8828) were found (Table 5). This means that softer breads correspond to delayed temperatures and lower associated enthalpy for starch gelatinization and slower rate for amylopectin retrogradation. Solid relationships between parameters characterizing the stability of the amylose–lipid complex and crumb firming kinetics were established in terms of delayed temperatures (T_{0d}, T_{pd}, T_{ed}) for the dissociation of amylose-lipid complex associated to lower initial crumb firmness T_0 (r = −0.5554 to 0.7364) and slower staling rate n_f (−0.6391 to 0.7150) during storage. The presence of lipids during hydrothermal treatments can decrease the swelling capacity of the starch granules, and complex formation has been shown in many studies to retard retrogradation (Wang and Copeland, 2013).

Conclusions

WT flour replacement from 22.5% up to 45% by incorporation of ternary blends of teff (T), green pea (GP) and buckwheat (BW) flours significantly modify the qualitative and quantitative thermal profile of starch gelatinization and amylopectin retrogradation kinetics during storage, and impact less on the dissociation of the amylose-lipid complex of the resulting hydrated flour blends assessed by DSC. Non-wheat flour incorporation of ternary blends of teff (T), green pea (GP) and buckwheat (BW) from 22.5% up to 45% into restricted water-

WT flour systems delayed endothermic transition temperatures for the biphasic gelatinization, and provided variable associated endothermic enthalpies for both gelatinization and retrogradation phenomena. Restricted or delayed swelling of starch granules as a result of the presence of associated flours in limiting water, delayed the T_o for gelatinization in hydrated flour mixtures. Upon melting, unavailable water for the remaining ungelatinized granules, force them to melt at higher temperatures and encompass variable energy to disorganize its structure. With some exceptions, blended doughs exhibited lower and variable rate of retrogradation kinetics along storage, with in some cases much longer half-life endorsing slower amylopectin recrystallization, compared to control WT doughs counterparts. Physical interferences by the presence of proteins and insoluble amylose-lipid complexes in limited water systems can explain hindrance for starch crystallization in blended breads. Suitable trends for achieving the lower and slower retrogradation kinetics were fulfilled by adding T/GP/BW, each at higher dose (15%), to replace 45% of WT flour in blended dough formulations. Trends for thermal transitions related to suitable patterns for (a) low and slow starch hydrolysis, (b) initial softer crumb firmness and (c) retarded firming kinetics in blended breads include (i) delayed temperatures for starch gelatinization (lower RDS contents, slower starch hydrolysis kinetics, and softer bread crumbs), and (ii) for the dissociation of amylose–lipid complex (lower initial crumb firmness and slower firming rate), (iii) higher enthalpies for the amylose-lipid inclusion complex dissociation (slower rate for starch digestibility, higher amounts of SDS and RS), but (iv) lower enthalpy for starch gelatinization (softer bread crumbs), (v) lower leveling-off value of melting enthalpy at which the extent of starch crystallization stoped (lower starch hydrolysis extent and lower total DS) and (vi) slower rate for amylopectin retrogradation (softer bread crumbs).

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2014.12.083.

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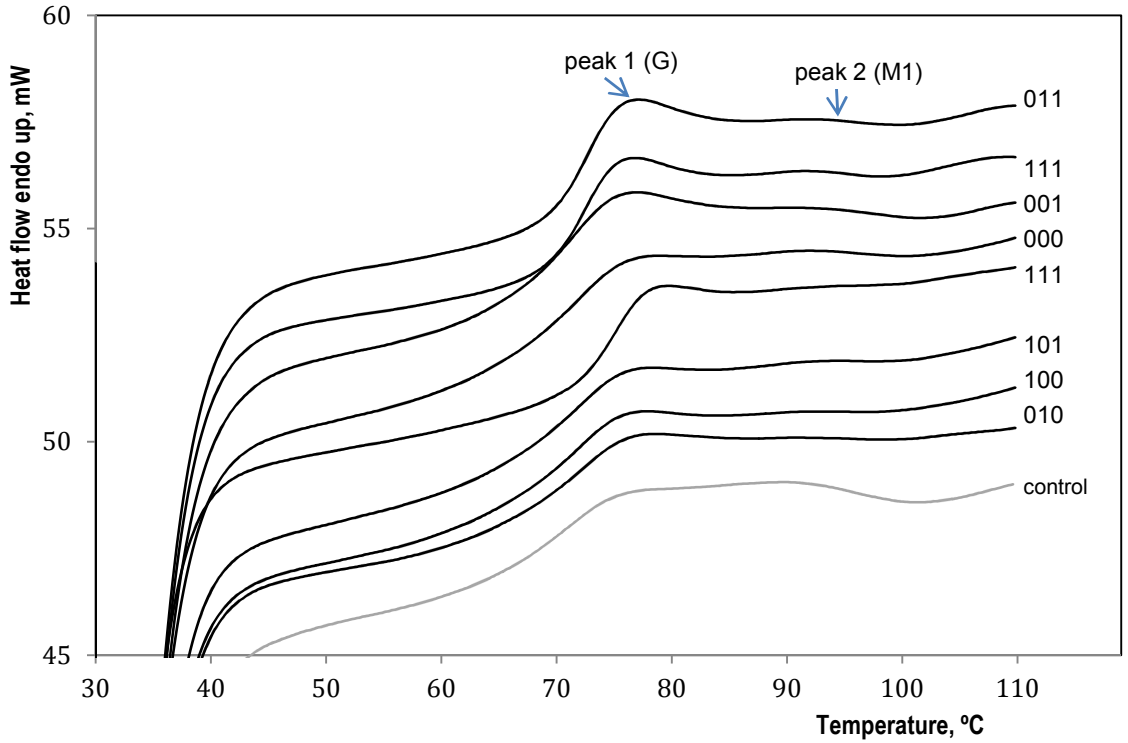

Fig. 1 DSC thermograms of wheat-based mixed doughs formulated with teff (T), greenpea (GP) and buckwheat (BW) flours during starch gelatinization. Three digit code refers to low (0) ang high (1) wheat flour replacement by T:GP:BW flours in sample formulation.

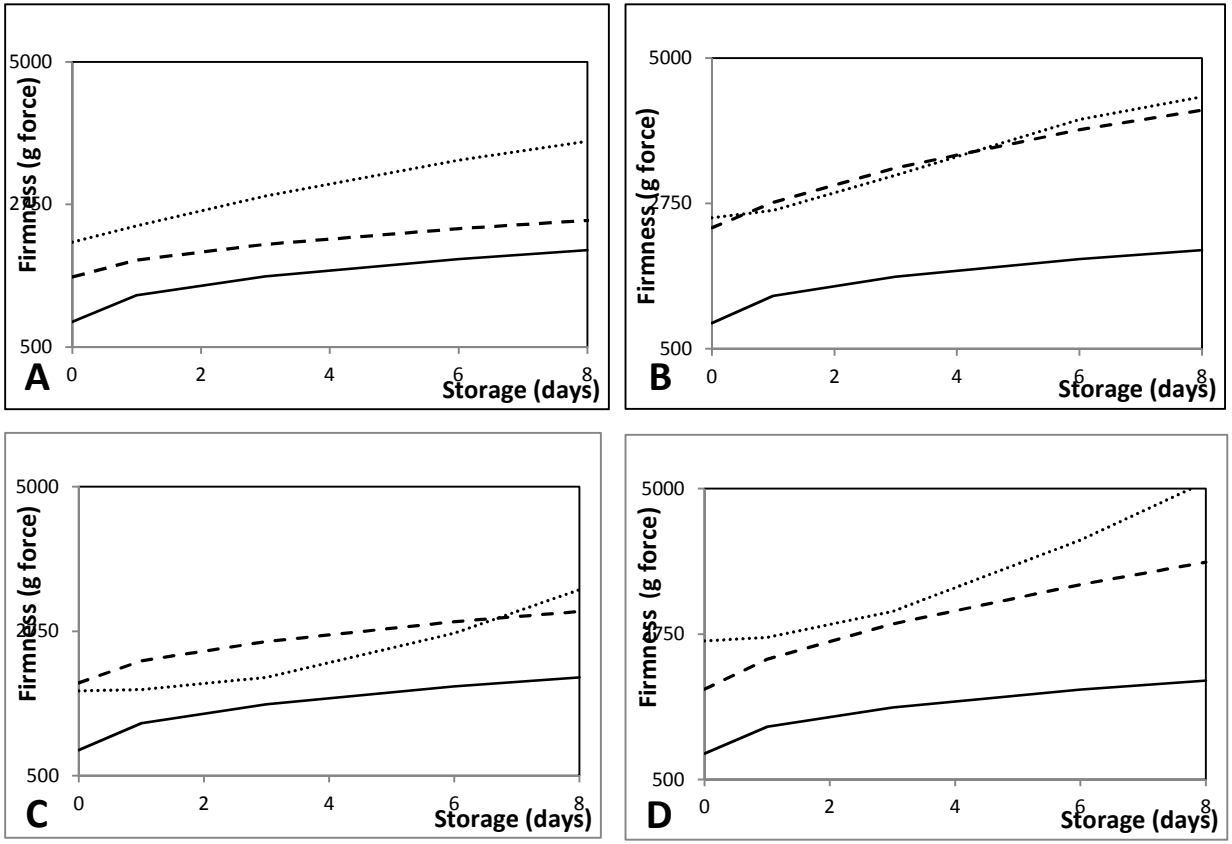

Fig. 2 Evolution of crumb firming during storage of wheat-based mixed breads formulated with teff (T), greenpea (GP) and buckwheat (BW) flours. A: 7.5% T, 7.5% GP; B: 7.5% T, 15% GP; C: 15% T, 7.5% GP; D: 15% T, 15% GP. Control wheat breads (----), 7.5% BW (- - -), 15% BW (….).

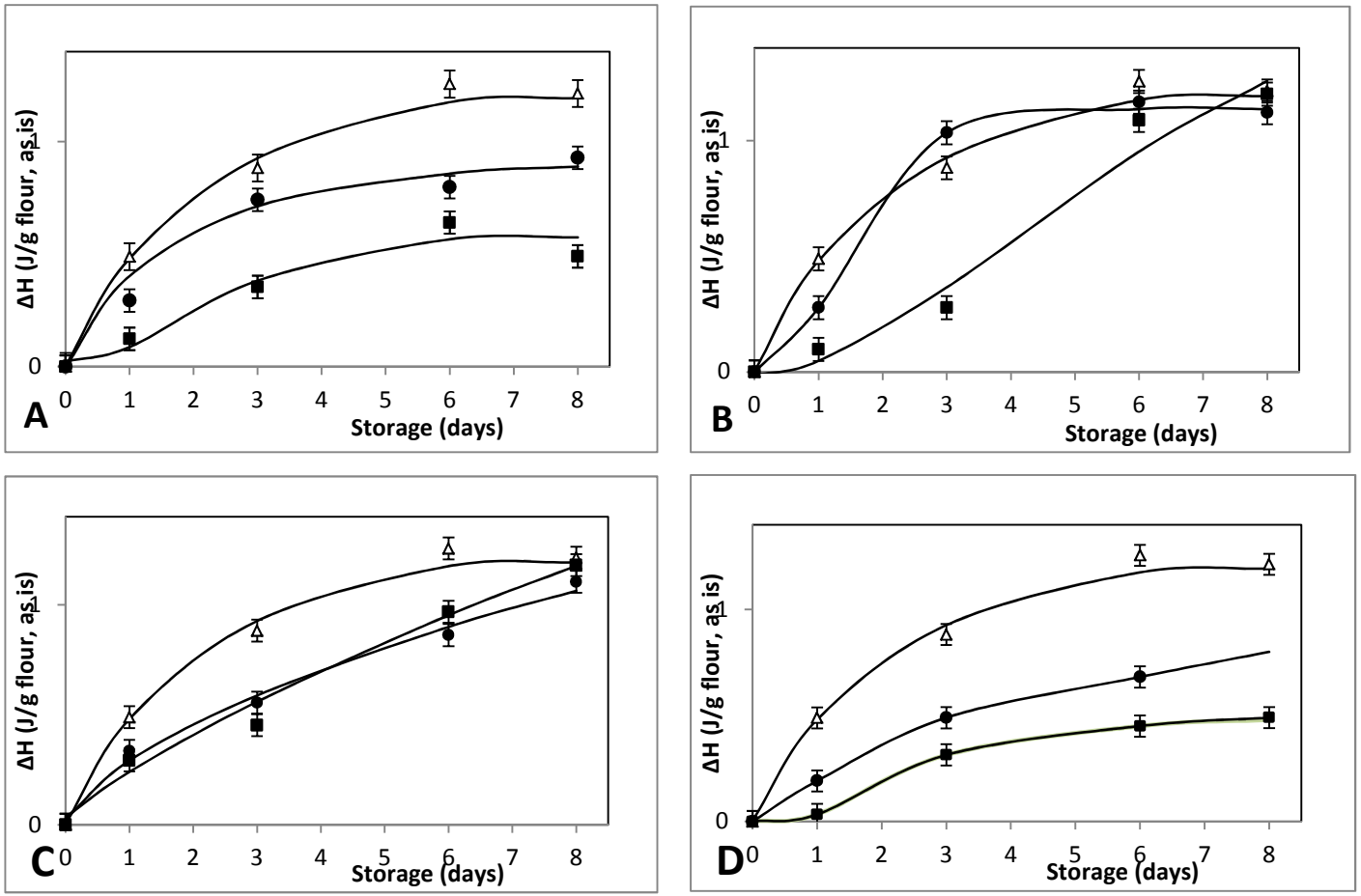

Fig. 3 Evolution of retrogradation enthalpy during storage of wheat-based mixed breads formulated with teff (T), greenpea (GP) and buckwheat (BW) flours. A: 7.5% T, 7.5% GP; B: 7.5% T, 15% GP; C: 15% T, 7.5% GP; D: 15% T, 15% GP. Experimental data: control wheat breads (Δ), 7.5% BW (●), 15% BW (■); adjusted data: black lines.

Table 1
Proximate chemical and nutritional composition of composite breads (per 100 g fresh blended bread).

Characteristic	Blended bread samples [a,b]								
	010	001	011	000	111	101	100	110	Control
Moisture, g	33.4±0.8c	32.9±0.6bc	32.5±0.9b	32.3±0.4bc	31.9±0.3b	29.3±0.8a	30.5±0.9a	32.2±0.4bc	32.9±0.6bc
Fat, g	3.5±0.2a	3.6±0.4a	3.6±0.2a	3.6±0.1a	3.8±0.3a	3.8±0.2a	3.7±0.4a	3.7±0.1a	3.4±0.2a
Protein[c], g	11.9±0.1b	11.7±0.2b	12.3±0.1c	11.6±0.3b	12.2±0.2c	11.7±0.1b	11.7±0.1b	12.2±0.2c	11.1±0.1a
TDF, g	3.3±0.3b	3.3±0.2b	3.9±0.3c	2.9±0.3b	4.3±0.5c	3.8±0.2c	3.3±0.2b	3.8±0.1c	1.4±0.2a
RS, g	2.4±0.1b	2.7±0.3b	2.9±0.5b	2.8±0.4b	2.5±0.2b	2.3±0.2b	2.5±0.2b	2.2±0.2a,b	1.8±0.3a
RDS, g	57.8±0.9b	58.4±1.1b,c	56.4±1.0b	56.2±1.1a	60.0±1.2c	59.6±1.5b,c	62.5±1.4c	54.3±1.0a	68.5±1.1d
SDS, g	8.4±1.1c	5.4±0.7b	17.5±e	5.7±0.6b	2.3±0.6a	11.8±d	12.6±d	4.5±1.1b	7.5±1.0c
C_∞	74.3±1.3c	65.7±0.9a	74.1±0.9c	73.6±1.3c	71.2±1.2b	75.8±0.8c	75.2±0.7c	75.7±0.8c	81.0±0.9d
k	0.0686± 0.0032c	0.0825± 0.0091c	0.0477± 0.0013a	0.0797± 0.0062c	0.1106± 0.0085d	0.0599± 0.0031b	0.0593± 0.0029b	0.0821± 0.0079c	0.0720± 0.0081c

TDF: total dietary fibre, RS: resistant starch, RDS: rapidly digestible starch, SDS: slowly digestible starch. C_∞ maximum starch hydrolysis extent, k kinetic constant for starch hydrolysis.
([a]) Mean values ± standard deviation. Within columns, values (mean of three replicates) with the same following letter do not differ significantly from each other ($p > 0.05$).
([b]) Three digit bread sample code refers to low (0) ang high (1) wheat flour replacement by teff: green pea: buckwheat flours in sample formulation.
([c])Conversion Factor from N to protein = 6.25.

Table 2
Thermal and textural parameters of composite breads.

Characteristic	Units	Blended bread samples [a,b]								
		010	001	011	000	111	101	100	110	Control
Gelatinisation Peak 1										
T_o	ºC	66.99±0.35ab	66.08±1.06ab	69.62±2.74d	67.53±2.16c	69.45±2.08d	66.39±0.93ab	65.84±0.87ab	65.18±1.20a	66.30±0.57abc
T_p	ºC	76.65±0.45cd	75.65±0.56a	76.96±1.41d	76.70±0.95cd	76.65±1.95bcd	76.17±0.53abcd	75.74±0.76ab	76.45±1.34abc	75.55±0.40a
T_e	ºC	96.60±1.76ab	95.07±3.47ab	96.21±4.71ab	98.94±1.31ab	93.12±5.31a	99.83±0.83b	96.16±4.03ab	98.79±4.41ab	98.61±1.48ab
ΔH_g	J/g flour	3.337±0.270abc	4.232±0.233c	3.374±0.564ab	3.482±0.761abc	2.844±0.772a	3.445±0.565abc	4.007±0.696bc	4.145±0.739abc	3.497±0.571abc
R	ºC	29.61	28.99	26.59	31.41	23.67	33.44	30.32	31.89	32.31
Gelatinisation Peak 2										
T_o	ºC	88.08±1.21d	87.83±0.96bc	87.69±0.60d	86.19±1.14b	87.46±0.67cd	86.75±0.50bcd	86.68±0.52bcd	87.25±3.13bcd	83.96±1.10a
T_p	ºC	91.81±2.51ab	92.77±0.87ab	93.61±0.63b	91.84±1.14a	93.64±0.90b	92.05±0.68ab	91.10±0.49a	92.42±5.49ab	90.78±0.45a
T_e	ºC	96.70±0.76a	98.96±0.90bc	99.43±2.09cd	98.10±1.12abc	99.06±2.28bcd	99.52±0.76cd	97.03±1.49a	100.58±2.00d	97.84±1.21ab
ΔH_g	J/g flour	0.821±0.146a	1.561±0.371bcd	1.138±0.450ab	1.495±0.530bcd	1.258±0.251abc	1.514±0.278bcd	1.203±0.339abc	1.285±0.764cd	1.867±0.765d
R	ºC	8.62	11.13	11.74	11.91	11.60	12.77	10.35	13.33	13.88
Amylose-lipid complex dissociation										
T_o	ºC	95.66±0.78b	93.45±0.21a	91.47±2.03a	98.10±1.13b	94.18±1.74a	94.88±2.62b	96.21±0.07b	96.64±0.79b	96.38±1.07b
T_p	ºC	106.28±1.06bc	104.12±2.71a	100.78±1.77a	105.26±1.67bc	102.70±0.00a	104.26±2.06c	106.60±0.09bc	104.45±0.59ab	106.87±0.47bc
T_e	ºC	114.90±3.35d	110.48±2.75ab	105.50±4.29a	113.15±0.68bc	108.03±0.71ab	111.48±2.16bcd	114.18±0.92bcd	113.95±1.30bcd	116.53±0.85cd
ΔH_d	J/g	0.531±0.229a	0.455±0.258a	0.621±0.315a	0.330±0.206a	0.317±0.107a	0.335±0.143a	0.385±0.103a	0.289±0.100a	0.411±0.075a
Retrogradation kinetics										
ΔH_∞	J/g flour	1.136±0.121c	0.575±0.096a	1.672±0.195e	0.915±0.123c	0.451±0.096a	3.860±0.452g	2.980±0.325f	0.754±0.065bc	1.302±0.025d
k_r		0.2798±0.052d	0.1218±0.0094c	0.0379±0.0090a	0.5812±0.0720e	0.0759±0.0082b	0.0552±0.0152a	0.0945±0.0156b	0.2924±0.0458d	0.4578±0.0589e
n_r		1.9609±0.257bc	1.9618±0.324bc	1.7364±0.239b	0.8696±0.0956a	2.5068±0.435c	0.8945±0.0998a	0.7316±0.0965a	1.1611±0.226a	0.9075±0.102a
ΔH_0	J/g flour	0.000	0.023	-0.015	-0.002	0.000	0.034	0.025	0.000	0.003
R^2		99.82	91.34	96.41	95.19	98.36	98.29	98.7	96.22	98.94
$t_{1/2}$	days	1.60±0.90a	2.46±1.02a	5.36±1.59b	1.26±0.92a	2.46±1.02a	16.96±2.96c	15.26±3.56c	2.16±1.00a	1.60±0.85a
Firming kinetics										
T_∞	g force	6174±350b	5131±149a	4685±324a	5050±469a	8921±950c	8280±625c	12472±1900d	15136±1600d	5908±965ab
k_f		0,109±0.031e	0,091±0.006d	0,053±0.010c	0,08±0.009d	0,009±0.002b	0,003±0.000a	0,034±0.009c	0,036±0.010c	0,087±0.008d
n_f		0,86±0.09c	1,03±0.08d	1,75±0.01e	0,64±0.01b	1,93±0.06f	2,18±0.10g	0,50±0.06a	0,72±0.09b	0,52±0.08a
T_0	g force	2374±298c	2156±245c	2529±210c	1608±220b	2645±250c	1822±254b	1945±200b	1899±225b	901±125a
R^2		86,06	91,98	98,15	89,32	95,75	94,89	86,52	94,06	94,1
$t_{1/2}$	days	9±2b	7±2b	4±1a	30±4c	9±2b	12±3b	187±15f	60±9e	55±8d

([a]) Mean values ± standard deviation. ([b]) Three digit bread sample code refers to low (0) ang high (1) wheat flour replacement by teff: green pea: buckwheat flours in sample formulation. T_o: onset temperature, T_p peak temperature, T_e end temperature, ΔH_g gelatinization enthalpy, ΔH_d enthalpy of dissociation of amylose-lipid complex, ΔH_∞, ΔH_0 retrogradation enthalpy at ∞ and 0 time, respectively, R gelatinization temperature range, k_r constant of proportion of retrogradation kinetics, k_f constant of proportion of firming kinetics, n_r Avrami exponent of retrogradation kinetics, n_f Avrami exponent of firming kinetics, $t_{1/2r}$, $t_{1/2f}$ half-life for retrogradation and firming, respectively.

Table 3
Significant single effects of design factors (teff, green pea and buckwheat flours) on the gelatinization and amylose-lipid complex dissociation thermal parameters, and on Avrami kinetic parameters for crumb firming and starch retrogradation during storage of composite breads. Levels of design factors were: 0 (7.5 g/100 g flour) and 1 (15 g/ 100 g flour).

Parameter	Unit	Level	Overall mean	Teff	*p*<0.05	Green pea	*p*<0.05	Buckwheat	*p*<0.05
Gelatinisation Peak 1									
T_o	ºC	0	67.47	ns		66.44±2.00	a	66.92±2.00	a
		1				68.51±2.02	b	68.02±2.01	b
T_p	ºC	0	76.47	ns		76.09±1.05	a	ns	
		1				76.85±1.06	b		
T_e	ºC	0	96.80	ns		ns		ns	
		1							
ΔH_g	J/g	0	3.62	ns		3.79±0.54	b	ns	
		1				3.44±0.54	a		
Gelatinisation Peak 2									
T_o	ºC	0	86.81	ns		86.37±1.20	a	ns	
0		1				87.25±1.20	b		
1	ºC	0	92.16	ns		ns		91.28±1.61	a
0		1						93.02±1.61	b
1	ºC	0	98.50	ns		ns		97.81±1.63	a
0		1						99.19±1.61	b
1	J/q	0	1.35	ns		ns		ns	
0		1							
1									
T_p	ºC	0	95.80	ns		ns		96.58±1.40	b
0		1						95.03±1.40	a
1	ºC	0	104.99	ns		ns		105.64±1.34	b
0		1						104.35±1.34	a
1	ºC	0	112.33	ns		ns		113.32±1.87	b
0		1						111.33±1.87	a
1	J/g	0	0.379	ns		ns		ns	
0		1							
1									
T_∞	g force	0	8125	ns		6509±1499	a	ns	
		1				9741±1585	b		
k_f		0	0.0535	ns		ns		ns	
		1							
n_f		0	1.2008	ns		ns		ns	
		1							
T_0	g force	0	2124	ns		ns		ns	
		1							
$t_{1/2f}$	days	0	40	ns		16±3	a	ns	
		1				64±6	b		
Retrogradation kinetics									
ΔH_∞	J/g	0	1.54	1.07±0.14	a	2.08±0.14	b	ns	
		1		2.01±0.14	b	1.00±0.14	a	ns	
k_r		0	0.19240	0.2552±0.0274	b	0.2132±0.0274	b	0.3120±0.0274	b
		1		0.1295±0.0274	a	0.1715±0.0274	a	0.0727±0.0274	a
n_r		0	1.4778	1.6322±0.1733	b	1.1144±0.1733	a	1.1808±0.1733	a
		1		1.3235±0.1733	a	1.8413±0.1733	b	1.7749±0.1733	b
ΔH_{0r}	J/g	0	0.008	ns		ns		ns	
		1							
$t_{1/2r}$	days	0	6	ns		4±1	a	ns	
		1				8±2	b		

T_o: onset temperature, T_p peak temperature, T_e end temperature, ΔH_g gelatinization enthalpy, ΔH_d enthalpy of dissociation of amylose-lipid complex, T_∞, T_0 crumb firmness at ∞ and 0 time, respectively, k_f constant of proportion of firming kinetics, n_f Avrami exponent of firming kinetics, ΔH_∞ , ΔH_{0r} retrogradation enthalpy at ∞ and 0 time, respectively, k_r constant of proportion of retrogradation kinetics, n_r Avrami exponent of retrogradation kinetics, $t_{1/2f}$, $t_{1/2r}$ half-life for firming and retrogradation, respectively.

Table 4
Second order significant interactions (p<0.05) of rate of wheat flour replacement by low (1) and high (2) dose of teff (T), green pea (GP) and buckwheat (BW) –design factors–on the gelatinization and amylose-lipid complex dissociation thermal parameters, and on Avrami kinetic parameters for crumb firming and starch retrogradation during storage of composite breads. Levels of design factors were: 0 (7.5 g/100 g flour) and 1 (15 g/ 100 g flour).

Parameter	Unit	Level	Overall mean	T x GP	p<0.05	T x BW	p<0.05	GP x BW	p<0.05
Gelatinisation peak 1									
T_o	ºC	00	67.47	ns		ns		66.65±2.00	a
		01						66.23±1.99	a
		10						67.19±2.00	a
		11						69.82±2.02	b
ΔH_g	J/g	00	3.62	ns		3.38±0.54	a	3.72±0.54	b
		01				3.83±0.54	b	3.85±0.54	b
		10				4.01±0.54	b	3.66±0.54	b
		11				3.24±0.54	a	3.22±0.54	a
Gelatinisation peak 2									
T_e	ºC	00	98.5	98.53±1.62	ab	ns		ns	
		01		97.79±1.62	a				
		10		98.24±1.62	ab				
		11		99.45±1.62	b				
ΔH_g	J/g	00	1.35	1.46±0.46	b	ns		ns	
		01		1.00±0.46	a				
		10		1.38±0.46	ab				
		11		1.53±0.46	b				
Amylose-lipid complex dissociation									
T_p	ºC	00	104.99	104.81±1.34	a	ns		106.08±1.34	c
		01		105.01±1.34	ab			103.74±1.34	a
		10		105.70±1.34	b			105.20±1.34	bc
		11		104.45±1.34	a			104.95±1.34	b
T_e	ºC	00	112.33	ns		113.72±1.87	c	ns	
		01				110.77±1.87	a		
		10				112.93±1.87	bc		
		11				111.89±1.87	ab		
Firming kinetics									
k_f		00	0.0535	0.0527±0.0240	ab	0.0795±0.0240	b	ns	
		01		0.0720±0.0240	b	0.0452±0.0240	ab		
		10		0.0668±0.0178	b	0.0358±0.0170	a		
		11		0.0224±0.0205	a	0.0533±0.0213	b		
Retrogradation kinetics									
ΔH_∞	J/g flour	00	1.54	0.74±0.20	a	ns		ns	
		01		1.40±0.20	b				
		10		3.42±0.20	c				
		11		0.60±0.20	a				
k_r		00	0.1924	0.35±0.04	c	0.43±0.04	c	ns	
		01		0.16±0.039	b	0.08±0.04	a		
		10		0.07±0.04	a	0.19±0.04	b		
		11		0.18±0.04	b	0.07±0.04	a		
n_r		00	1.4778	1.42±0.25	bc	ns		ns	
		01		1.85±0.25	c				
		10		0.81±0.25	a				
		11		1.83±0.25	c				
$t_{1/2r}$	days	00	5.89	4.92±1.23	b	ns		ns	
		01		4.71±1.23	b				
		10		2.52±1.13	a				
		11		11.40±1.61	c				

T_o: onset temperature, T_p peak temperature, T_e end temperature, ΔH_g gelatinization enthalpy, k_f constant of proportion of firming kinetics, ΔH_∞ , retrogradation enthalpy at 0 time, k_r constant of proportion of retrogradation kinetics, n_r Avrami exponent of retrogradation kinetics, $t_{1/2r}$ half-life for retrogradation.

Table 5
Significant Pearson correlations (p<0.05 *, p<0.01 **) between thermal and starch hydrolysis and firming kinetic parameters of composite breads.

	T_{0g}	T_{pg}	T_{eg}	ΔH_g	T_{0d}	T_{pd}	T_{ed}	ΔH_d	ΔH_∞	k_r	n_r	ΔH_{0r}	$t_{1/2r}$
C_∞		0.3799 *	0.6052 **		0.3755 *				0.5385 **		-0.5857 **		0.4196 *
k			-0.4524 **					-0.5765 **	-0.6707 **		0.5144 **		-0.5371 **
H_{90}		0.36 *	0.6179 **		0.4508 **		0.3862 *		0.5087 **		-0.5915 **		0.395 *
RDS		-0.5573 **	-0.4056 *						0.5181 **	-0.5292 **		0.6262 **	0.6557 **
SDS					-0.4423 *			0.6395 **	0.6684 **	-0.4248 *			0.5736 **
DS					-0.4076 *			0.5017 **	0.7773 **	-0.5797 **			0.7566 **
RS	0.4431 *				-0.4102 *	-0.4522 **	-0.567 **	0.5893 **				-0.4094 *	
TS					-0.4207 *			0.5213 **	0.7593 **	-0.5699 **			0.7368 **
T_∞				0.3691 *	0.3981 *		0.4296 *	-0.5991 **			-0.3526 *		
k_f								0.5253 **	-0.4733 **	0.4981 **			-0.5841 **
n_f	0.4968 **			-0.6061 **	-0.6391 **	-0.6857 **	-0.715 **			-0.636 **	0.3537 *		
T_0	0.6588 **		-0.8043 **	-0.506 **	-0.7364 **	-0.5554 **	-0.6249 **	0.5441 **		-0.5647 **	0.8828 **	-0.4406 *	

T_o: onset temperature, T_p peak temperature, T_e end temperature, ΔH_g gelatinization enthalpy, ΔH_d enthalpy of dissociation of amylose-lipid complex, T_∞, T_0 crumb firmness at ∞ and 0 time, respectively, k_f constant of proportion of firming kinetics, n_f Avrami exponent of firming kinetics, ΔH_∞, ΔH_{0r} retrogradation enthalpy at ∞ and 0 time, respectively, k_r constant of proportion of retrogradation kinetics, n_r Avrami exponent of retrogradation kinetics, $t_{1/2r}$ half-life for retrogradation, C_∞ maximum starch hydrolysis extent, k kinetic constant for starch hydrolysis, H_{90} starch hydrolysis extent at 90 min, RDS rapidly digestible starch, SDS slowly digestible starch, DS digestible starch, RS resistant starch, TS total starch.

8 CHAPTER IV

Gluten-free dough-making of specialty breads: significance of blended starches, flours and additives on dough behaviour

Article

FOOD SCIENCE AND TECHNOLOGY INTERNATIONAL

Gluten-free dough-making of specialty breads: Significance of blended starches, flours and additives on dough behaviour

Concha Collar[1], Paola Conte[1,2], Costantino Fadda[2] and Antonio Piga[2]

Abstract

The capability of different gluten-free (GF) basic formulations made of flour (rice, amaranth and chickpea) and starch (corn and cassava) blends, to make machinable and viscoelastic GF-doughs in absence/presence of single hydrocolloids (guar gum, locust bean and psyllium fibre), proteins (milk and egg white) and surfactants (neutral, anionic and vegetable oil) have been investigated. Macroscopic (high deformation) and macromolecular (small deformation) mechanical, viscometric (gelatinization, pasting, gelling) and thermal (gelatinization, melting, retrogradation) approaches were performed on the different matrices in order to (a) identify similarities and differences in GF-doughs in terms of a small number of rheological and thermal analytical parameters according to the formulations and (b) to assess single and interactive effects of basic ingredients and additives on GF-dough performance to achieve GF-flat breads. Larger values for the static and dynamic mechanical characteristics and higher viscometric profiles during both cooking and cooling corresponded to doughs formulated with guar gum and Psyllium fibre added to rice flour/starch and rice flour/corn starch/chickpea flour, while surfactant- and protein-formulated GF-doughs added to rice flour/starch/amaranth flour based GF-doughs exhibited intermediate and lower values for the mechanical parameters and poorer viscometric profiles. In addition, additive-free formulations exhibited higher values for the temperature of both gelatinization and retrogradation and lower enthalpies for the thermal transitions. Single addition of 10% of either chickpea flour or amaranth flour to rice flour/starch blends provided a large GF-dough hardening effect in presence of corn starch and an intermediate effect in presence of cassava starch (chickpea), and an intermediate reinforcement of GF-dough regardless the source of starch (amaranth). At macromolecular level, both chickpea and amaranth flours, singly added, determined higher values of the storage modulus, being strengthening effects more pronounced in presence of corn starch and cassava starch, respectively.

Keywords

Gluten-free, dough, starch, flour, additive, viscoelasticity

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INTRODUCTION

Research, development and innovation in gluten-free (GF) products constitute areas of increasing interest to meet cereal-based goods requirements of coeliac and wheat intolerant patients. Flat breads are the

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Gluten-free dough-making of specialty breads: significance of blended starches, flours and additives on dough behaviour

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The capability of different gluten-free (GF) basic formulations made of flour (rice, amaranth and chickpea) and starch (corn and cassava) blends, to make machinable and viscoelastic GF-doughs in absence/presence of single hydrocolloids (guar gum, locust bean and psyllium fibre), proteins (milk and egg white) and surfactants (neutral, anionic and vegetable oil) have been investigated. Macroscopic (high deformation) and macromolecular (small deformation) mechanical, viscometric (gelatinization, pasting, gelling) and thermal (gelatinization, melting, retrogradation) approaches were performed on the different matrices in order to (a) identify similarities and differences in GF-doughs in terms of a small number of rheological and thermal analytical parameters according to the formulations and (b) to assess single and interactive effects of basic ingredients and additives on GF-dough performance to achieve GF-flat breads. Larger values for the static and dynamic mechanical characteristics and higher viscometric profiles during both cooking and cooling corresponded to doughs formulated with guar gum and Psyllium fibre added to rice flour/starch and rice flour/corn starch/chickpea flour, while surfactant- and

protein-formulated GF-doughs added to rice flour/starch/amaranth flour based GF-doughs exhibited intermediate and lower values for the mechanical parameters and poorer viscometric profiles. In addition, additive-free formulations exhibited higher values for the temperature of both gelatinization and retrogradation and lower enthalpies for the thermal transitions. Single addition of 10% of either chickpea flour or amaranth flour to rice flour/starch blends provided a large GF-dough hardening effect in presence of corn starch and an intermediate effect in presence of cassava starch (chickpea), and an intermediate reinforcement of GF-dough regardless the source of starch (amaranth). At macromolecular level, both chickpea and amaranth flours, singly added, determined higher values of the storage modulus, being strengthening effects more pronounced in presence of corn starch and cassava starch, respectively.

Keywords: gluten-free, dough, starch, flour, additive, viscoelasticity.

Introduction

Research, development and innovation in gluten-free (GF) products constitute areas of increasing interest to meet cereal-based goods requirements of coeliac and wheat intolerant patients. Flat breads are the oldest and most well-known bread type worldwide (*pita*, *arepa*, *tortilla*, *chapati*, *roti*, *injera*), made from either gluten-forming (wheat) or non-gluten-forming (corn, sorghum, teff) cereals in regions of Central America, South Europe, Scandinavia, South Africa, the Middle East and part of China (Mohammadi et al., 2014). In some Mediterranean regions, flat breads are made of durum wheat to provide specialty baked goods like *spianata* in Sardinia, a major Mediterranean island. Durum wheat breads are not compatible with gluten-intolerant patients, and Sardinia has a significant prevalence of coeliac disease (124 per 100,000) over the population (Sardu et al., 2012). Proper replacement of gluten-forming cereals by non gluten-forming systems in baked goods is still a major challenge particularly in the achievement of sensory and nutritionally balanced leavened baked goods, despite the

accumulating knowledge on physical, chemical and technological principles of GF-matrices (Schober, 2009). Complex formulations involving the incorporation of starches of different origin, dairy proteins, other non-gluten proteins, gums, hydrocolloids and their combinations, into a GF flour base (mostly rice and corn flour) are often used to simulate the viscoelastic properties of lacking gluten (Mariotti et al., 2009), and may result in variable success regarding structure, mouthfeel, acceptability and shelf-life of the finished GF-products. The incorporation of dairy and egg proteins has long been established in the baking industry, and has proven to significantly affect viscoelasticity of GF-systems (Ronda et al., 2014). Legumes can also be a good supplement for cereal based foods added either in flour or concentrated/isolated forms since they substantially increase the protein content and complement the nutritional value of cereal proteins (Angioloni and Collar, 2012). Pseudocereals such as buckwheat, quinoa and amaranth can also be useful for nutritional improvement of breads with no significant impairment of the final bread quality when added at low amounts (Collar and Angioloni, 2014). Gums and hydrocolloids are either a good source of soluble dietary fibre (Angioloni and Collar, 2011) or essential structuring ingredients in GF bread formulations for improving the texture, the volume and the keepability of the final products (Ronda et al., 2013).

In breadmaking applications, a careful selection of structural ingredients with suitable physico-chemical properties preventing permanent disruption of the protein matrix that encompasses excessive weakening of the protein/starch networks is a pre-requisite to obtain processable doughs, particularly for GF systems lacking the endogenous viscoelastic biopolymer. To date, the main approach for the development of GF breads has been the addition of structural macropolymers such as hydroxypropylmethylcellulose to mimic gluten viscoelastic properties (Ahlborn et al., 2005). Other hydrocolloids of vegetal origin such as galactomannans and high ester pectin (Angioloni and Collar, 2008), and more recently, Psyllium fibre (Mariotti et al., 2009) have shown to provide either a reinforced hydrated flour-fibre structure with promoted values for storage

and loss moduli (locust bean (LB) gum), or an enhancement of the physical properties of the doughs due to the film-like structure that it was able to form (psyllium fibre). In addition, a health promoting effect associated to the cholesterol-lowering effect and insulin sensitivity improvement capacity of Psyllium fibre (You et al., 2003) has been stated.

This study is aimed at exploring the capability of different GF-basic formulations made of different flour (rice, amaranth and chickpea) and starch (corn and cassava) blends, to make processable and viscoelastic GF-doughs in absence/presence of single hydrocolloids (guar gum (GG), LB and psyllium fibre), proteins (milk and egg white) and surfactants (neutral, anionic, and vegetable oil). Macroscopic (high deformation) and macromolecular (small deformation) mechanical, and viscometric (gelatinization, pasting, gelling) and thermal (gelatinization, melting, retrogradation) approaches were performed on the different matrices in order to (a) identify similarities and differences in GF-doughs in terms of a small number of rheological and thermal analytical parameters according to the formulations, and (b) to assess single and interactive effects of basic ingredients and additives on GF-dough performance to achieve GF-flat breads.

Materials and Methods

Materials

Commercial flours, starches, proteins, dietary fibres, surfactants and oils were used. Rice flour (RF), corn starch (CS), cassava starch (CaS), milk proteins (MP), GG, diacetyl tartaric acid ester of mono- and diglycerides (DATA), psyllium fibre (PF) and LB gum were from Chimab Campodarsego (PD, Italy). Amaranth flour (AF), egg white proteins (EP), and chickpea flour (CF) were from Molini Bongiovanni S.p.A. – Cambiano (TO, Italy). Sodium stearoyl-2-lactylate (SSL) was from DuPont™ Danisco®, and sunflower oil (SF) was from Carapelli Firenze (Italy).

Methods

Dough making of GF-samples. GF-doughs were prepared by using six different basic formulations coded A–F according to the following qualitative and quantitative composition on a 100 g solid basis: A – RF (50%) + CS (50%), B – RF (50%) + CaS (50%), C – RF (45%) + CS (45%) + CF (10%), D – RF (45%) + CaS (45%) + CF (10%), E – RF (30%) + CS (30%) + AF (40%), F – RF (30%) + CaS (30%) + AF (40%). Individual/single proteins, dietary fibres, surfactants and oils were added to each basic formulation (g/100 g solid basis) at two levels of addition (low/high) as it follows: GG (1/2), LB (1/2), PF (1/2), MP (5/10), EP (5/10), DATA (0.5/1.0), SSL (0.5/1.0) and SF (4/8).
A total of 102 different GF-doughs resulted from basic and 2 level additive-containing formulations. Solids (100 g), and water (70% for A and B, 61% for C and D, 58% for E and F basis) optimized according experimental trials to obtain non-sticky non-slack doughs, were mixed using a Kitchen-Aid Artisan mixer (5KSM150PS, Kitchen Aid, St. Joseph, MI) with a dough hook (K45DH) for 2 min at speed 2, and 2 min at speed 4.

Chemical and nutritional composition of GF ingredients. Chemical and nutritional composition of flours, starches, hydrocolloids, proteins and surfactants were provided by the manufacturers (Table 1). Amylose/amylopectin ratio (Megazyme kit K-AMYL 07/11) was estimated by using a modification of a Con A method developed by Yun and Matheson (1990) that uses an ethanol pre-treatment step to remove lipids prior to analysis.

Dough rheological measurements

a. Large-deformation mechanical tests

Dough machinability was assessed by texture profile analysis (TPA) in a TA-XTplus texture analyser (Stable Micro Systems, Godalming, UK) using a 5 cm diameter probe, a 75 s waiting period and 60% compression as described

previously (Collar et al., 1999). The resistance to penetration was assessed with penetration tests according to Sciarini et al. (2012). Dough was compressed until the probe (P/5.5mm diameter) disrupted the dough surface structure, penetrating into the sample, at 15 mm/s. The force value corresponding to the intersection of the two straight lines defined in the curve was set as the penetration force. Stress relaxation tests were accomplished according to Singh et al. (2006), and modified by Fois et al. (2012). % relaxation was calculated as the force registered after 35 s, divided by the maximum registered force in percentage.

b. Small-deformation tests

Fundamental dough rheology of GF-doughs was assessed by dynamic oscillation tests on an RS1 controlled stress rheometer equipped with a Phoenix II circulating bath (Haake, Karlsruhe, Germany) using a 60 mm serrated plate–plate geometry with a 1 mm gap between plates (Angioloni and Collar, 2009). The upper plate was lowered and the excess of sample was trimmed off. The exposed surface was covered with a thin layer of mineral oil to prevent moisture loss during testing. Samples were rested for 10 min after loading prior to testing, to allow sample relaxation. Strain sweep tests were run to identify the linear viscoelastic region. Oscillatory measurements of storage modulus (G′), loss modulus (G″) and phase angle (δ) were performed at 25 °C within a frequency range from 0.1 to 10 Hz. All measurements were made in triplicate. Values for dynamic moduli were registered at $\lambda = 1$ Hz and quoted G'_1 and G''_1.

Viscometric properties. Pasting profiles (gelatinisation, pasting and setback properties) of formulated flour/starch blends were obtained with a Rapid Visco Analyser (RVA-4, Newport Scientific, Warriewood, Australia) using ICC Standard method 162. The pasting temperature (in °C; when viscosity first increases by at least 25 cP over a 20-s period), peak time (when peak viscosity occurred), peak viscosity (maximum hot paste viscosity), holding strength or trough viscosity (minimum hot paste viscosity), breakdown (peak viscosity minus

holding strength or trough viscosity), viscosity at 95 °C, viscosity at the end of the 95 °C holding period, viscosity at 50 °C, final viscosity (end of test after cooling to 50 °C and holding at this temperature), setback (final viscosity minus peak viscosity) and total setback (final viscosity minus holding strength) were calculated from the pasting curve using Thermocline v. 2.2 software (Collar, 2003). For each viscometric measurement, two replicates were made.

Thermal properties. Thermal properties regarding starch gelatinization and retrogradation of formulated GF-doughs containing the higher level of the different additives were assessed in a differential scanning calorimeter Perkin-Elmer DSC-7 according to the method of León et al. (1997), with some modifications as previously reported by Andreu et al. (1999) and Santos et al. (2008).

Starch gelatinization. Dough samples were prepared by mixing all solid ingredients and 70% of water. For DSC analysis, 50–70mg samples were weighed in large volume pre-weighed, sealed stainless-steel pans. An empty pan was used as a reference. Simulation of the temperature profile in the centre of the bread crumb during baking was done in the calorimeter under the following scanning conditions: samples were kept at 30 °C for 2 min, then heated from 30 to 110 °C at a rate of 11.7 °C/min, kept at 110 °C for 5 min, and finally cooled from 110 to 30 °C at a rate of 50 °C/min. Gelatinized samples were stored at 22 °C for 6 days. Thermal transitions of starch samples were defined as T_o (onset), T_p (peak of gelatinization) and T_c (conclusion); the enthalpy associated with starch gelatinization was defined as ΔH_g.

Starch retrogradation. Stored gelatinized dough samples were submitted to a second DSC scan to analyse starch retrogradation. Scanning conditions included keeping sample pans at 25 °C for 1 min, and then heating from 25 to 130 °C at a rate of 10 °C/min. The enthalpy of amylopectin retrogradation (ΔH_r) was

calculated. All samples were analysed in duplicate. Enthalpies were calculated from the area under the curves defined after scanning. Gelatinization and retrogradation enthalpies (ΔH) were expressed in J/g of dry sample. Each formulation was analysed twice and an average value was calculated.

Statistical analysis

Multivariate analysis of variance and factor analysis were applied to data by using Statgraphics V.7.1 program (Bitstream, Cambridge, MN). Multiple range test (Fisher's least significant differences, LSDs) for analytical variables was applied to know the difference between each pair of means.

Results and Discussion

GF-sample classification

Classification of GF-samples on the basis of their distinctive and significant responses in terms of dynamic and static rheological performance, viscometric profile and thermal behaviour was achieved by means of multivariate data handling. A total of 30 functional variables were measured in the different GF-doughs. The purpose of the analysis is to obtain a small number of factors, which account for most of the variability in the 30 variables. Factor analysis grouped GF-dough functional parameters into four different factors that explained 84.62% of the cumulative variance (VE), since four factors had eigenvalues greater than or equal to 1.0. The first three factors explained 76.28% of the variability of the results (Table 2). Factor 1 (36.18% VE) included dynamic and static rheological properties, while factor 2 (23.62% VE) grouped flour pasting and gelling characteristics, and factor 3 (16.48% VE) accounted for the thermal features during gelatinization and retrogradation (Table 2). Factor 1 correlated positively with storage modulus, loss modulus, penetration force, % of stress relaxation, hardness, cohesiveness, resilience and springiness. Factor 2 correlated positively with the viscometric characteristics during cooking – peak viscosity and holding

strength – and cooling – viscosity at 50 °C and total setback. Factor 3 showed positive dependence of T_p retrogradation and T_p gelatinization, while depended negatively on ΔH of both gelatinization and retrogradation thermal processes (Table 2). Plots of scores of factor 1 versus factor 2 and factor 1 versus factor 3 illustrating sample location in the scatterplot are depicted in Figure 1. Separation of samples along the x axis was observed according to factor 1, allowing to clearly differentiate GF-doughs formulated with hydrocolloids, that located in the positive zone of the x axis, from the rest of the samples (Figure 1).

These samples exhibited higher values for the static and dynamic mechanical characteristics in terms of higher mechanical spectra (G′ and G″), texture profile, resistance to penetration and % of residual stress. In a descending order, surfactant- and protein-formulated GF-doughs with intermediate and lower values of the already mentioned characteristics, respectively, locate in the middle and in the negative zone of the x axis. Highest values for variables in factor 1 were observed for doughs formulated with GG and PF and bases E and F that contain AF, while lowest values corresponded to doughs with MP and EP and bases A and B containing RF and starch. Classification of samples according to factor 2 differentiated matrices with different basic formulation in such a way that A, C and B bases showing higher viscometric profiles during both cooking and cooling located in the positive zone of the y axis, while D, E and F based GF-doughs exhibiting poorer viscometric profiles were placed in the negative zone of the y axis of the sample scatterplot (Figure 1). Factor 3 clearly discriminated additive-free GF-doughs that accounted for the higher temperatures and lower enthalpies for both gelatinization and retrogradation thermal transitions.

Fundamental and empirical rheological properties of formulated GF-doughs

It has been widely recognised that dough should convene certain mechanical requests to produce good quality bread. Those requirements concern a proper combination of small and large rheological properties and viscometric and thermal

response during breadmaking steps. Suitable rheological trends to perform high-quality baked goods have been closely linked to dough formula. Changes in dough technological properties by using non-wheat/non-gluten raw materials may result in different processing performance and associated production problems linked with slack or excessively stiff dough, leading to bread of poorer quality (Collar, 2008). In dynamic oscillation tests, the frequency sweep shows how the viscous and elastic behaviour of the material changes with the rate of application of strain or stress, while the amplitude of the signal is held constant.

Mechanical spectra of GF-doughs (plots not shown) significantly depended on both the basic formulation (flours/starches) (Table 3) and the presence and dose of main tested additives (Table 4). For major formulations in the whole range of frequencies, G' was greater than G'' giving to dynamic mechanical loss tangent (tan $\delta = G''/G'$) values smaller than unity suggesting a solid elastic-like behaviour of the GF-doughs as found earlier by others (Lazaridou et al., 2007; Mariotti et al., 2009; Samutsri and Suphantharika, 2012). Effect of basic formulation on dynamic moduli and loss tangent (Table 4) evidenced significant changes in G' and tan δ according to flour(s)/starch(es) composition.

High G'_1 generally reflects a more rigid and stiff material whose tan δ is small. The presence of CF (C, D vs. A, B) and AF (E, F vs. A, B) in the basic recipe determined higher values of G'_1 and lower values of tan δ_1. Strengthening effects were more pronounced for CF in presence of CS (G'_1 = 59,243 Pa) and for AF in presence of CaS (G'_1 = 36,820 Pa). Replacement of CS by CaS in a basic formula (B *vs* A) significantly weakened the dough giving the highest values for tan δ_1 (0.750 *vs* 0.496). Additive incorporation into basic formulas provided significant effects in both elastic and viscous components of GF-samples, particularly for hydrocolloids and proteins, effects being opposite and concentration dependent (Table 4). An increase in both G'_1 and G''_1 was observed for GG, LB and PF formulated GF-doughs, especially for PF containing matrices as found earlier (Mariotti et al., 2009), and probably associated to a synergistic interaction between starch and hydrocolloid polymer molecules to form a co-polymer

network (Chen et al., 2009). Protein incorporation strongly decreased the values of dynamic moduli, the extent being dependent on the protein concentration, and greater for G' than for G''_1 (Table 4). As a result, tan δ_1 values tend to increase. In a previous work (Ronda et al., 2014), doughs enriched with albumin at 5% and 10% of addition exhibited a lower mechanical spectra profiles than unsupplemented protein-samples, regardless the dose of addition and the absence/presence of acid. With few exceptions, effects of basic formulation followed a similar pattern on static mechanical properties (Table 4). Basic formulations flour/starch A and B exhibited the poorest textural quality in terms of resistance to penetration (0.16–0.18 N), residual stress after compression (8.13–6.30 N), resistance to indentation (2.34–2.60 N) and cohesiveness (0.081–0.087), irrespective of the starch source (CS in A, CaS in B). Addition of 10% CF to RF/CS blends provided a large GF-dough strengthening effect in presence of CS (C) and an intermediate structuring effect in presence of CaS (D). AF encompassed similar intermediate reinforcement of GF-dough regardless the source of starch (E, F) (Table 3). Effects of different additives (data not shown) were significant in some cases but of very small extent, especially when compared to the effect of basic dough formulation.

Viscometric and thermal properties of formulated GF-doughs

In starch blends, both additive and non-additive viscometric and thermal behaviours have been described according to intrinsic properties such as gelatinization temperature, swelling power, carbohydrate leaching during swelling and granule size of the individual starches in the blend (Waterschoot et al., 2014b). In more heterogeneous matrices such as flour/starch blends from different sources in absence/presence of single dietary fibres, proteins and surfactants, single (Tables 3–5) and interactive effects (Figure 2) were both observed regarding viscometric and termal properties. RVA viscometric profiles of single and associated basic ingredients and additive-formulated GF-doughs are depicted in Figure 2 for bases A and F. Single effects of qualitative levels (A-F) of basic

formula (Table 3) and quantitative additive levels (Table 4) were identified.
During gelatinization and pasting, higher RVA profiles were reached in base A, intermediate viscosity values were observed in B, C and D bases, while the lower values were attained in E and F bases (Table 3). This means that replacement of CS by CaS and/or partial replacement of any of both starches by either CF or AF hinders blended starch granules swelling during the process of gelatinization due to water competition, and composite starch polymer molecules (primarily amylose molecules) easily leach from the swollen granules (Shi et al., 1991), and thus, lower peak viscosity was reached. The process of pasting that follows gelatinization occurs with continued heating of starch granules in the presence of excess water and involves considerable continued granule swelling and leaching of starch polymer (primarily amylose) molecules. During the 95 °C hold, the more fragile swollen granules easily disintegrate under the shear conditions of the instrument, and the viscosity decreases to a lower holding strength (Table 3), being the degree of fragmentation dependent on the shear rate, shear time and nature of the starch granules. Single effects of additives on the cooking cycle viscosities (Table 4) revealed a general concentration dependent increase in peak viscosity, holding strength and viscosity of hot paste provided by hydrocolloids, EP and SSL, and some decrease in the pasting temperature particularly for LB, PF, DATA and SF. During gelling/cooling, hot pastes, especially of amylose-containing starches, begin to cool, and become more elastic developing different solid properties, i.e. gelation occurs (BeMiller, 2011). The transition from a viscous liquid to a gel is called setback; the molecular process that produces setback is known as retrogradation (Atwell et al., 1988), that is a non-equilibrium, polymer crystallization process. At higher amylose concentrations, which are the case in this study (amylose/amylopectin ratio: 17/83 CS, 7/93 CaS), a gel formation takes place. The first (short-term) phase of retrogradation occurs as the paste cools and involves network formation (entanglements and/or junction zone formation) between amylose molecules (Silverio et al., 1996), forming an elastic gel. Some amylopectin entanglements may be involved, but primarily

retrogradation of amylopectin is a much slower process that may proceed for several weeks (Silverio et al., 1996), depending on the storage temperature. In this work, effects on gelling viscometric properties of the different bases (Table 3) were much more prominent than those provided by additives (Table 4). Bases A and C exhibited the highest gelling profiles, while B and E showed intermediate behaviour, and D and F provided the lowest viscosity values during gelling (Table 3). CaS instead of CS decreased moderately the extent of retrogradation of the blend, of the same order that AF did in presence of CS. CF and AF significantly decreased retrogradation in presence of CaS. A relatively high cold paste viscosity can result from increased interactions between leached molecules and/or swollen granules of the different starches (Puncha-arnon et al., 2008), whereas a relatively low cold paste viscosity can be explained by a reduction in swelling power and thus carbohydrate leaching of one starch by the other (Waterschoot et al., 2014b). Concerning effects of additives, all the tested hydrocolloids, proteins and surfactants except SF promoted the RVA viscosity profiles during cooling, being effects concentration dependent (Table 4). It has been alluded that the addition of a hydrocolloid to a starch paste or gel makes an already complex system even more complex. It can be assumed that cooked starch–hydrocolloid systems are systems of various particles originating from swollen starch granules suspended in mixed polymer solutions or polymer networks of varying rheological properties and that the contributions of the dispersed and continuous phases to the properties of the overall system vary with factors such as relative concentrations of starch and hydrocolloid, preparation conditions, and interactions between and/or compatibilities of the various polymer molecules present (BeMiller, 2011). Similar or even higher complexity can be applied to other additives such as surfactants or ingredients like proteins, when added to a blended starches and/or composite flour/starch systems. In fact, interactive effects base x additive were observed for many viscometric measurements. Figure 2 illustrates RVA profiles of GF-doughs formulated with bases A (a) and F (b) containing hydrocolloids (GG, LB and PF), proteins (MP, EP), and surfactants (DATA, SSL, SF) at low (0)

and high (1) level of addition. As it can be seen, in general, effects of additives were significant in promoting viscosity levels for the base A (RF + CS) exhibiting a high RVA curve, particularly for hydrocolloids and proteins, while poor effects were provided by the same additives/doses when added to base F (RF + CaS + AF) showing a lower RVA profile. Exceptions accounted for LB, EP and SSL that moderately increased RVA curves during both pasting and gelling with increased concentration. For all other bases (data not shown), B, C and bases with intermediate RVA profile behaved like base A, while E base with low RVA profile did like base F. An aspect of the use of additives in this study that should be considered is, that apart from the complexity of flour composition, dietary fibres contain, in addition to the 81–88% polysaccharide, 2.5–5% protein which could influence behaviours of the starch-based matrix with which it is used (Table 1). Analogously, proteins from egg and milk (79–84%) contain 7.6–9.3% carbohydrates and up to 5.3% fat. DSC thermal profiles of single and associated basic ingredients and additive-formulated GF-doughs at higher dose of addition were performed. Since effects of additives were not significant ($p > 0.05$) in any of the thermal parameter determined, effects of individual basic ingredients (flours and starches) and qualitative levels (A–F) of basic formulations were studied (Table 5). Heating starch in excess water (>1:2 starch:water) above the gelatinisation temperature disrupts the molecular order of the granules and melts the crystallites, but when relatively less water (<1:2 starch:water) is available, gelatinisation is partly postponed to higher temperatures (Delcour and Hoseney, 2010), and a biphasic thermal transition takes place (Andreu et al., 1999). The main endotherm occurs essentially at constant temperature but a progressive shift of the second endotherm temperature towards higher values occurs when the water content decreases. The second endotherm represents that portion of the sample that did not gelatinize during the first heating, and the shift of the peak temperature is attributed to the heterogeneity of the starch granules (Biliaderis et al., 1980). Simulation of the baking process in calorimeter pans led to a biphasic endotherm for starch gelatinization as a consequence of the limited water content

of GFdoughs (41%). The first endotherm, corresponding to the gelatinization of the amorphous phase of the starch appeared between 71.09 °C (CaS) and 87.08 °C (RF) and had an enthalpy of 2.94–7.95 J/g dry weight (d.wt.). The second endotherm, corresponding to melting of the more stable crystalline structures was quantitative only in CF, CS and CaS, appeared at 87.86–98.39 °C with enthalpies ranging from 1.84 to 5.23 J/g d.wt. Gelatinisation onset (T_o), peak (T_p) and conclusion (T_c) temperatures of the different starches and flours used in the different basic formulations in restricted water (1:0.7 starch/flour:water) followed a general decreasing order: RF> AF> CF> CS> CaS, while gelatinization enthalpies (ΔH) were AF> CS>CaS> RF> CF (Table 5). For RF and AF, T_0 and T_c for gelatinization defined a wide interval for gelatinization (23–24 °C) and a high T_p, suggesting overlapping of gelatinization and melting in only one broad peak. Retrogradation is the process of crystallisation of AP molecules in a starch paste (Delcour and Hoseney, 2010). Besides storage temperature, also the starch-to-water ratio has an important effect on retrogradation. Water content should neither be too high (> 80%) nor too low (< 30%) to allow retrogradation (Zeleznak and Hoseney, 1986). After 6 days of storage of gelatinized samples, retrogradation was detected only in RF, CF and CaS, with melting of amylopectin crystals at T_p 59–65 °C and at melting enthalpy at 2.3–6.4 J/g (Table 5). As pointed out very recently (Waterschoot et al., 2014b), limited research has been done on the gelatinization properties of blends in concentrated starch–water systems (35–65% water content) although such systems are of particular practical relevance. Contrary to the behaviour in excess water, in limited water conditions, the starch granules from starch and flour compete for the available water. In this study, blended flour/starch bases A–F followed a general behaviour regarding the temperatures of thermal transitions (Table 5). Higher values of T_0, T_p and T_c of gelatinization, melting and retrogradation were observed in bases E and F, while lower values were provided by base B, and intermediate values were assigned to bases A, C and D. This means that CaS significantly decreased the temperature of thermal transitions in presence of RF when compared with CS. Results are in line

with the lower T_0, T_p and T_c of gelatinization stated for Ca when compared to CS (Gomand et al., 2010). In blended starches, the one with the lowest gelatinization temperature gelatinizes first and leaves less water for gelatinization of the other starch, resulting that gelatinization of the latter occurs at higher temperatures (Liu and Lelièvre, 1992). However, probably not only differences in gelatinization temperature, but also in granule size and rate of water absorption impact the gelatinization properties. In other studies, CS and CaS starches have been described to have granules with somewhat similar dimensions (5–20 μm for maize starch and 3–32 μm for CaS), but CaS has round or truncated granules while maize starch granules are polygonal (Jane et al., 1994). In this study, the water solubility index is greater for CaS (11.78%) than for CS (0.4%), leaching more amylose and amylopectin outside the granules (Waterschoot et al., 2014a). Moreover, addition of CF increased the transition temperatures in blends RF–CaS, and did not affect those of RF–CS. The presence of AF significantly promoted the temperature at which gelatinization, melting and retrogradation take place, regardless the nature of the starch blended with RF. Enthalpies of gelatinization – peak 1 and peak 2 – and retrogradation ranged 1.78–2.74 J/g, 2.01–3.80 J/g and 3.55–4.06 J/g, respectively (Table 5), and no relevant differences (even statistically significant) within bases were observed. For RF and AF, T_0 and T_c for gelatinization defined a wide interval for gelatinization (23–24 °C) and a high T_p, suggesting overlapping of gelatinization and melting in only one broad peak.

Conclusions

The ability of RF-based GF formulations to provide machinable and viscoelastic GF-doughs to make specialty flat breads, depended primarily on both the type of starch (corn and cassava) and the additional flour (amaranth and chickpea) of the basic blends, and in second place on the additional ingredients – proteins (milk and egg white) – and additives – hydrocolloids (GG, LB and psyllium fibre). Basic formulations RF/starch exhibited the poorest textural quality in terms of

macroscopic mechanical properties but the higher viscometric profile, irrespective of the starch source. Single addition of 10% of either CF or AF to RF/starch blends provided a large GF-dough strengthening effect in presence of CS and an intermediate structuring effect in presence of CaS (chickpea), and an intermediate reinforcement of GF-dough regardless the source of starch (amaranth). At macromolecular level, both chickpea and AFs, singly added, determined higher values of the storage modulus, being strengthening effects more pronounced in presence of CS and CaS, respectively. Replacement of CS by CaS in a basic formula significantly weakened the dough, whereas an increase in both dynamic moduli as an indicator of the fluid nature of the composite was observed for hydrocolloid formulated GF-doughs, especially for psyllium fibre containing GF-doughs, probably associated to a synergistic interaction between starch and hydrocolloid polymer molecules to form a co-polymer network. Protein incorporation strongly decreased the values of dynamic moduli, the extent being dependent on the protein concentration. During gelatinization and pasting, replacement of CS by CaS and/or partial replacement of any of both starches by either chickpea or AF hinders blended starch granules swelling during the process of gelatinization due to water competition, and lower peak viscosity and extent of retrogradation were reached. CaS significantly decreased the temperature of thermal transitions in presence of RF when compared with CS. The presence of AF significantly promoted the temperature at which gelatinization, melting and retrogradation take place, regardless the nature of the starch blended with RF. According to obtained results, a proper balance of viscoelastic, viscometric and thermal GF-dough properties is reached by matrices formulated with bases A – RF (50%) + CS (50%) – and C – RF (45%) + CS (45%) + CF (10%) – in presence of 2% of hydrocolloids, particularly Psyllium fibre. This formulation is encouraged to make GF breads with promoted protein and fibre contents, from machinable and moderately viscoelastic doughs.

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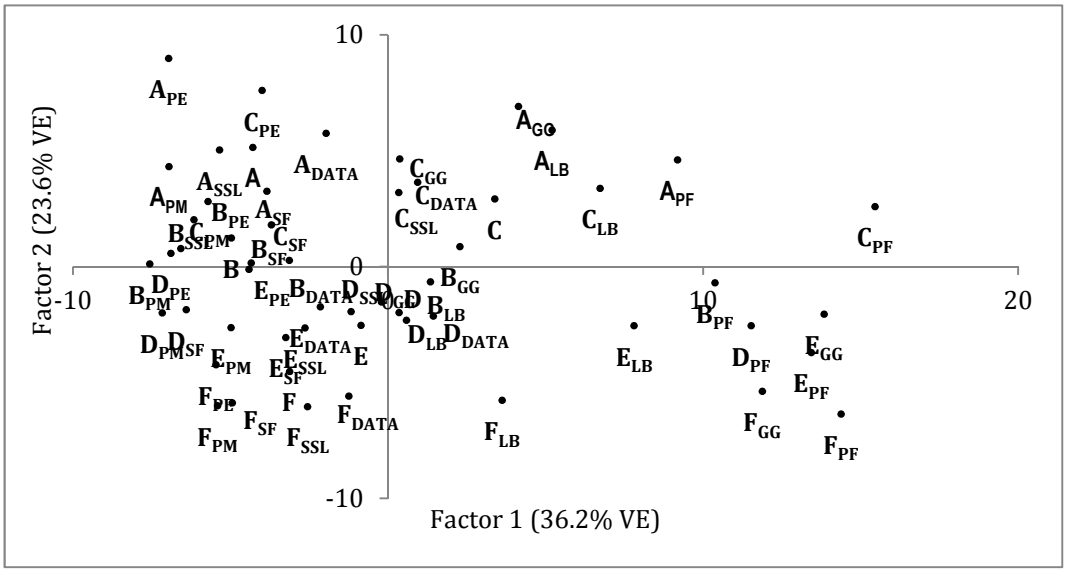
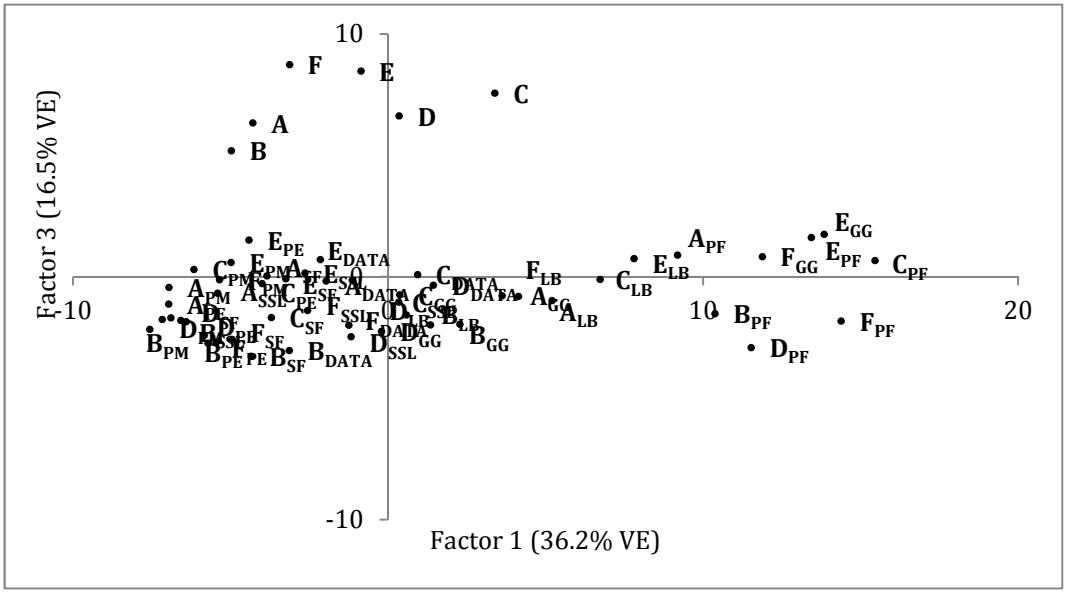


(a)



(b)

Fig. 1 Scatterplots of scores of factor 1 *vs* factor 2 (a) and factor 1 *vs* factor 3 (b) of GF-doughs formulated with bases A to F containing hydrocolloids (GG: guar gum, LB: locust bean gum and PF: psyllium fibre), proteins (MP: milk, EP: egg white), and surfactants (DATA: diacetyl tartaric acid ester of mono- and diglycerides, SSL: sodium stearoyl-2-lactylate, SF sunflower oil) at high level of addition.

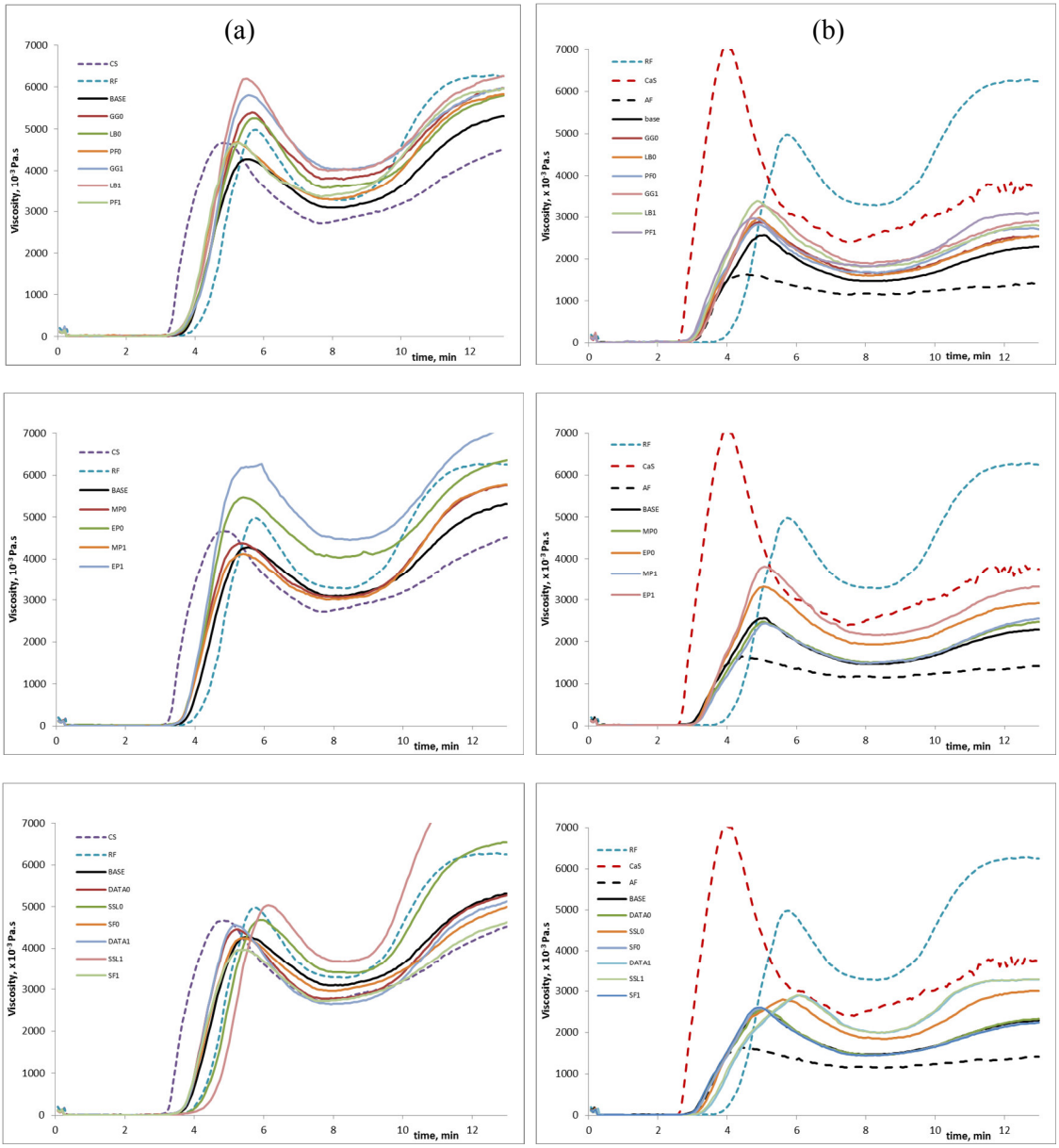


Fig. 2 RVA curves of GF-doughs formulated with bases A (a) and F (b) containing hydrocolloids (GG: guar gum, LB: locust bean gum and PF: psyllium fibre), proteins (MP: milk, EP: egg white), and surfactants (DATA: diacetyl tartaric acid ester of mono- and diglycerides, SSL: sodium stearoyl-2-lactylate, SF: sunflower oil) at low (0) and high (1) level of addition.

Table 1
Proximate chemical and nutritional composition of gluten-free ingredients.

Ingredient	Moisture	Protein	Fat	Ash	Digestible Carbohydrates	Total Dietary Fibre
	(g/ per 100 g ingredient, as is)					
Flours						
Rice	14	7.1	1.3	0.8	76.5	0.22
Amaranth	14.5	14.5	6.5	2.4	51	15
Chickpea	9.8	23	6.6	2.8	48.7	15
Starches						
Corn	12	0.3	0	0	88	0
Cassava	12.6	0.5	0.5	0.2	86	0.5
Proteins						
Egg white	2.73	84.39	0.1	3.47	9.31	0
Milk	4.8	79.2	5.3	3.2	7.6	0
Dietary Fibres						
Guar gum	7	5	0	1	0	88
Locust bean gum	10.0	5	1	1.1	0	83
Psyllium fibre	10	2.5	0.5	2	4	81[1]
Surfactants						
DATA	2.3	0	100[2]	0.3	0	0
SSL	0.6	0	100[2]	9.7	0	0
Sunflower oil	0	0	92[3]	0	0	0

DATA diacetyl tartaric acid ester of mono- and diglycerides, SSL sodium stearoyl-2-lactylate.
(1) 44 soluble fibre, 36 insoluble fibre.
(2) 98% saturated fat.
(3) 11.1% saturated fat.

Table 2
Factor Loading Matrix After Varimax Rotation in Factor Analysis.

	Factor 1 (36.18%VE)	**Factor 2** (23.62%VE)	**Factor 3** (16.48%VE)	**Factor 4** (8.34%VE)
Storage modulus, λ=1 Hz	0.9124	0.0425	0.0399	0.1337
Loss modulus, λ=1 Hz	0.9180	-0.0133	-0.0035	0.0985
Penetration force	0.8706	-0.0450	0.1383	0.0867
Stress Relaxation	0.8053	-0.0500	0.1236	0.0723
Hardness	0.9253	-0.1045	-0.0001	-0.0266
Cohesiveness	0.9516	-0.1010	-0.0357	0.0499
Resilience	0.8969	0.0212	-0.0406	0.0308
Springiness	0.8234	-0.0328	-0.1479	-0.0937
PastingTemperature	0.1046	0.2980	0.1618	0.8860
Peak Viscosity	-0.1484	0.9147	-0.1278	-0.2378
Holding Strength	-0.0721	0.9763	-0.0212	0.0398
Viscosity at 95ºC	-0.0907	-0.0575	-0.1287	-0.9358
Viscosity at 50ºC	-0.0345	0.8721	-0.0469	0.4019
Total Setback	0.0535	0.8358	-0.0468	0.4612
$T_{p\ \text{gelatinization}}$	-0.0766	-0.2586	0.8710	0.1872
$\Delta H_{\text{gelatinization}}$	0.03486	-0.5961	-0.5352	-0.1546
$T_{p\ \text{retrogradation}}$	-0.0192	0.0615	0.9616	-0.0620
$\Delta H_{\text{retrogradation}}$	-0.1324	0.0064	-0.8430	-0.1385

Table 3
Single significant effects (p<0.05) of qualitative levels (A-F) of basic formula on selected dynamic, textural, and viscometric gluten-free doughs properties.

Parameter	Unit	Overall mean	Level					
			A	B	C	D	E	F
Storage modulus G'$_1$	Pa	36668	31690b	20943a	59243d	31815b	39498c	36820c
Loss modulus G"$_1$	Pa	15706			ns			
Tan δ_1		0.471	0.496c	0.750d	0.265a	0.494c	0.398b	0.427b
Penetration force	N	0.338	0.164a	0.182a	0.618c	0.372b	0.369b	0.321b
Stress Relaxation	%	11.97	8.13a	6.30a	20.39c	13.91b	11.96b	11.12b
Hardness	N	3.377	2.34a	2.60a	4.04b	3.54b	3.97b	3.77b
Cohesiveness		0.095	0.087a	0.081a	0.099b	0.091a	0.105b	0.107b
Resilience		0.043			ns			
Springiness		0.136			ns			
Pasting Tre.	ºC	75.52			ns			
Peak viscosity	cP	5927	7913c	6183b	6569b	6271b	4195a	4432a
Holding strength	cP	3491	4891d	3002b	3830c	3761c	2707a	2753a
Viscosity at 95ºC	cP	2700	1886b	3106d	1435a	5578	1789b	2407c
Viscosity at 50ºC	cP	5363	8187f	5474d	6899e	2846a	4641c	4127b
Total Setback	cP	2904	4073d	2750c	4103d	2243b	2433b	1824a

Within rows, values with the same following letter do not differ significantly from each other (p> 0.05). ns: non significant.

Table 4
Single significant effects (p<0.05) of additives on selected dynamic and viscometric gluten-free doughs properties.

Parameter	Unit	Level	Factors							
			Guar gum	Locust bean	Psyllium	Milk	Egg	DATA	SSL	Sunflower oil
G'_1	Pa	0	34074a	32395a	28868a	41410c	41211c	ns	ns	40744b
		1	32535a	45051b	74131b	1325b	4192b			6397a
		2	79723b	92382c	116212c	880a	1003a			5806a
G''_1	Pa	0	9507a	9312a	8915a	11900b	11817c	ns	ns	11652b
		1	11525b	13070b	17578b	1061a	2240b			2755a
		2	26086c	27466c	28920c	666a	721a			2683a
Tan δ'_1		0	ns	ns	ns	0.287a	0.287a			0.286a
		1				0.801b	0.534b			0.431b
		2				0.756b	0.718c			0.462b
Pasting	ºC	0	ns	76.21c	76.70c	ns	ns	75.41b	73.50	75.80b
temperature		1		75.41b	75.28b			75.88b	75.79	75.60b
		2		74.94a	74.59a			75.28a	77.28	75.17a
Peak		0	5369a	5293a	5674a	6046c	5210a	5918a	5479a	ns
viscosity	cP	1	5943b	5907b	5995b	5914b	5966b	5867a	6002b	
		2	6469c	6582c	6112b	5821a	6605c	5997b	6301c	
Holding		0	3218a	3224a	3368a	3539b	3078a	3606b	3092a	3601b
strength	cP	1	3512b	3485b	3505b	3467a	3553b	3435a	3492b	3467a
		2	3741c	3763c	3598b	3465a	3840c	3431a	3888c	3403a
Viscosity at 95ºC	cP	0	2563a	2427a	2308a	2653a	2432a	2672a	2786b	2627a
		1	2700b	2656b	2777b	2742b	2747b	2661a	2617a	2707b
		2	2837c	3016c	3015c	2704b	2921c	2766b	2697a	2766c
Viscosity at 50ºC	cP	0	5033a	5013a	4899a	5283a	4883a	ns	4557a	5497c
		1	5367b	5324b	5419b	5396b	5420b		5313b	5319b
		2	5687c	5750c	5770c	5409c	5784c		6217c	5271a
Total Setback	cP	0	2831a	2825a	2680a	2716a	2704a	2807a	2264a	2946b
		1	2898b	2896b	2954b	2968b	2890b	2917b	2846b	2891a
		2	2984c	2992c	3079c	3029c	3119c	2988c	3603c	2875a

For each variable, within columns, values with the same following letter do not differ significantly from each other (p> 0.05). Levels: 0 (absence), 1 (low addition), 2 (high addition). Ns: non significant.

Table 5
Significant effects (p<0.05) of basic ingredients and qualitative bases of gluten-free basic formula on dough thermal properties.

	Ingredients					Bases					
Thermal transition	RF	CF	AF	CS	CaS	A	B	C	D	E	F
Gelatinization, peak 1											
T_0 (ºC)	78.11±0.13e	70.37±0.4c4	73.27±0.62d	68.65±0.74b	64.01±1.09a	69.2±0.37b	65.78±0.12a	71.2±0.65c	68.26±0.90b	73.01±0.58d	70.96±0.08c
Tp (ºC)	87.08±0.41e	78.89±0.69c	82.01±0.41d	74.89±0.83b	71.09±0.69a	74.99±0.52b	72.65±0.14a	77.72±0.41c	75.87±1.66b	80.25±0.97d	81.03±1.79d
Tc (ºC)	101.52±0.52e	87.4±0.19c	97.69±0.38d	83.47±0.48b	78.39±0.14a	81.89±0.43	77.84±0.04a	82.42±0.07c	81.12±0.07b	87.57±0.00e	84.22±0.03d
ΔH (J/g, d.b.)	5.07±0.12	2.94±0.03	7.95±0.08	7.15±0.31	6.46±0.3	2.38±0.16c	2.11±0.04b	2.15±0.01b	1.94±0.32a	2.74±0.21c	1.78±0.06a
Gelatinization, peak 2											
T_0 (ºC)	nd	87.40±0.19c	nd	83.47±0.48b	78.39±0.14a	81.89±0.43b	77.84±0.04a	82.42±0.07c	81.12±0.07b	87.57±0.00e	84.22±0.03d
Tp (ºC)		98.39±0.42c		91.76±0.69b	87.86±1.24a	93.61±0.82	92.83±0.00a	95.27±0.14b	94.78±1.10b	95.37±0.28b	94.59±0.55b
Tc (ºC)		105.89±0.26c		100.8±0.11b	97.26±0.21a	103.33±0.06	103.96±0.39a	104.34±0.29a	106.21±0.38b	104.28±0.02a	103.85±0.97a
ΔH (J/g, d.b.)		1.84±0.01a		3.17±0.07b	5.23±0.001c	2.75±0.14	3.80±0.01d	2.94±0.10b	3.22±0.10c	2.01±0.01a	2.05±0.44a
Retrogradation											
T_0 (ºC)	44.01±0.62a	48.43±0.09c	nd	nd	46.47±0.16b	nd	43.19±1.08a	45.24±0.92b	46.18±0.62b	47.87±1.2b	46.97±0.33b
Tp (ºC)	62.18±0.02b	64.7±0.24c			58.78±0.59a		56.18±1.16a	57.53±0.71a	58.7±0.71a,b	59.02±0.21b	58.95±0.59b
Tc (ºC)	77.09±0.10b	80.73±3.26c			72.05±1.61a		74.42±0.02b	75.49±0.31b	75.11±0.68b	74.74±0.22b	73.1±0.65a
ΔH (J/g, d.b.)	5.41±0.16b	2.31±0.02a			6.24±0.20c		4.06±0.001b	3.55±0.08a	3.67±0.03a	3.69±0.13a	3.58±0.01a

For each variable, within rows, values with the same following letter do not differ significantly from each other (p> 0.05). nd: non detected.

9 CONCLUSIONS

In the first study – *Techno-functional and nutritional performance of commercial breads available in Europe* – a comprehensive and detailed market overview was conducted as part of this thesis, including 20 commercial breads consisting of gluten (n=10) and gluten-free (n=10) samples from different European countries. Characterization of the different commercial breads evidenced that the main distinction, as it was expected, was between gluten and gluten-free breads.

Classification of samples on the basis of their distinctive and significant responses in terms of crumb and crust colour features, rheological behaviour, relevant nutritional fractions, bioactive components, and sensory ratings also evidenced that the extent of variability was formulation dependent on both gluten-containing and gluten-free breads, although the latters have shown a great variability in terms of overall quality. Highest values for the most significant variables (including protein and bioaccessible polyphenol contents, sensory parameters, mechanical properties and starch hydrolysis parameters) were observed for breads characterized by rich formulations in terms of presence of other and alternative flours (rye, buckwheat, quinoa, millet, durum wheat semolina, and whole wheat), grains (soybean) and seeds (flaxseed, sunflower, and sesame). Among these samples, the best overall behaviour was observed for gluten breads (1206, 3209, 2202 and 3208 particularly) but, intermediate values were also found for gluten-free samples 1112, 3220 and 2213. Conversely, lowest values were found in gluten-free breads characterized by poorer formulations in terms of absence or presence of low percentages of the above-mentioned ingredients (1211, 1115, and 1119). Although the efforts of research and bakery industry are moving in the right direction to develop and produce gluten and gluten-free high-quality products, data obtained in this study confirm that, from a nutritional point of view, there is still a substantial room for improvement on both of areas. In particular, most of the gluten-free breads continue to show common problems and none of these has yet equaled the quality of gluten-containing counterparts not only in terms of nutritional value but also for both textural and sensory aspects.

In the second study – *Impact of ancient cereals, pseudocereals and legumes on starch hydrolysis and antiradical activity of technologically viable blended breads* – it was evaluated the impact of wheat flour replacement from 22.5% up to 45% (weight basis) by incorporation of nutrience-dense raw materials (teff, green pea and buckwheat flours) in improving wheat bread quality. Wheat flour replacement provided dough systems with variable mechanical, viscometric and viscoelastic profiles (data not shown) with no significant hindrance of either dough machinability or gassing power ability during fermentation.

After baking, multigrain breads exhibited acceptable physico-chemical, technological and sensory features, and superior nutritional composition compared to the 100% wheat flour counterparts, in terms of larger amounts of bioaccessible polyphenols, higher anti-radical activity, and lower and slower starch hydrolysis, which extent was formulation dependent. In particular, the simultaneous low RDS (57.1%) and high SDS (12.9%) and RS (2.8%) contents, which are considered suitable nutritional trends for dietary starch fractions, were observed in blends formulated 7.5% T, 15% GP, 15% BW, that replaced 37.5% WT. The multigrain breads showed a rather low extent and slower rate of starch hydrolysis with the medium low values for C_∞, and H_{90}, lowest k, and intermediate eGI (86). Low and slow starch digestibility can be ascribed to the high protein and dietary fibre contents of non-wheat flours (especially GP and BW) that favour starch–protein interactions and constitute a physical interference in bread matrices, respectively, obstructing and delaying enzyme attack and subsequent starch digestion. All multigrain breads can be labelled as source of dietary fibre with values greater than 3 g of dietary fibre/100 g bread. The formulation based on WT:T:GP:BW flours, 62.5:7.5:15:15 fulfilled from 25% (men) to 40% (women) of dietary fibre, and from 54% (men) to 66% (women) of protein daily requirements, when a daily consumption of 250 g of bread is accomplished, following the WHO bread intake recommendation.

On the basis of the results obtained in the third study - *Significance of thermal transitions on starch digestibility and firming kinetics of restricted water mixed*

flour bread matrices – it can be said that WT flour replacement by incorporation of ternary blends of teff (T), green pea (GP) and buckwheat (BW) flours significantly modify the qualitative and quantitative thermal profile of starch gelatinization and amylopectin retrogradation kinetics during storage, and impact less on the dissociation of the amylose–lipid complex of the resulting hydrated flour blends assessed by Differential Scanning Calorimetry. Non-wheat flour incorporation of ternary blends of teff (T), green pea (GP) and buckwheat (BW) from 22.5% up to 45% into restricted water-WT flour systems delayed endothermic transition temperatures for the biphasic gelatinization, and provided variable associated endothermic enthalpies for both gelatinization and retrogradation phenomena. Restricted or delayed swelling of starch granules as a result of the presence of associated flours in limiting water, delayed the T_o for gelatinization in hydrated flour mixtures. Upon melting, unavailable water for the remaining ungelatinized granules, force them to melt at higher temperatures and encompass variable energy to disorganize its structure. With some exceptions, blended doughs exhibited lower and variable rate of retrogradation kinetics along storage, with in some cases much longer half-life endorsing slower amylopectin recrystallization, compared to control WT doughs counterparts. Physical interferences by the presence of proteins and insoluble amylose–lipid complexes in limited water systems can explain hindrance for starch crystallization in blended breads. Suitable trends for achieving the lower and slower retrogradation kinetics were fulfilled by adding T/GP/BW, each at higher dose (15%), to replace 45% of WT flour in blended dough formulations. Trends for thermal transitions related to suitable patterns for (a) low and slow starch hydrolysis, (b) initial softer crumb firmness and (c) retarded firming kinetics in blended breads include (i) delayed temperatures for starch gelatinization (lower RDS contents, slower starch hydrolysis kinetics, and softer bread crumbs), and (ii) for the dissociation of amylose–lipid complex (lower initial crumb firmness and slower firming rate), (iii) higher enthalpies for the amylose–lipid inclusion complex dissociation (slower rate for starch digestibility, higher amounts of SDS and RS), but (iv)

lower enthalpy for starch gelatinization (softer bread crumbs), (v) lower levelling-off value of melting enthalpy at which the extent of starch crystallization stopped (lower starch hydrolysis extent and lower total DS) and (vi) slower rate for amylopectin retrogradation (softer bread crumbs).

In the fourth study – *Gluten-free dough-making of specialty breads: Significance of blended starches, flours and additives on dough behaviour* – the capability of different gluten-free (GF) basic formulations made of flour (rice, amaranth and chickpea) and starch (corn and cassava) blends, to make machinable and viscoelastic GF-doughs in absence/presence of single hydrocolloids (guar gum, locust bean and psyllium fibre), proteins (milk and egg white) and surfactants (neutral, anionic and vegetable oil) have been investigated in order to select the most promising formulations for producing the so called *Spianata,* a typical and widely consumed Sardinian flat bread.

The ability of rice flour (RF) based GF formulations to provide machinable and viscoelastic GF-doughs, depended primarily on both the type of starch (corn and cassava) and the additional flour (amaranth and chickpea) of the basic blends, and in second place on the additional ingredients – proteins (milk and egg white) – and additives – hydrocolloids (guar gum, locust bean and psyllium fibre). Basic formulations RF/starch exhibited the poorest textural quality in terms of macroscopic mechanical properties but the higher viscometric profile, independently of the starch source. Single addition of 10% of either chickpea (CF) or amaranth flour (AF) to RF/starch blends provided a large GF-dough strengthening effect in presence of corn starch (CS) and an intermediate structuring effect in presence of cassava starch (CaS) (chickpea), and an intermediate reinforcement of GF-dough regardless the source of starch (amaranth). At macromolecular level, both chickpea and AFs, singly added, determined higher values of the storage modulus, being strengthening effects more pronounced in presence of CS and CaS, respectively.

Replacement of CS by CaS in a basic formula significantly weakened the dough,

whereas an increase in both dynamic moduli as an indicator of the fluid nature of the composite was observed for hydrocolloid formulated GF-doughs, especially for psyllium fibre containing GF-doughs, probably associated to a synergistic interaction between starch and hydrocolloid polymer molecules to form a co-polymer network. Protein incorporation strongly decreased the values of dynamic moduli, which extent was dependent on the protein concentration. During gelatinization and pasting, replacement of CS by CaS and/or partial replacement of any of both starches by either chickpea or AF hinders blended starch granules swelling during the process of gelatinization due to water competition, and lower peak viscosity and extent of retrogradation were reached. CaS significantly decreased the temperature of thermal transitions in presence of RF when compared with CS. The presence of AF significantly promoted the temperature at which gelatinization, melting and retrogradation take place, regardless the nature of the starch blended with RF. According to obtained results, a proper balance of viscoelastic, viscometric and thermal GF-dough properties is reached by matrices formulated with bases A – RF (50%) + CS (50%) – and C – RF (45%) + CS (45%) + CF (10%) – in presence of 2% of hydrocolloids, particularly Psyllium fibre. This formulation is encouraged to make GF breads with promoted protein and fibre contents, from machinable and moderately viscoelastic doughs.

In conclusion it can be said that bread, once again, has proven to be a highly versatile baked-product that lends itself to research and development of new products able to satisfy the needs of consumers more and more interested in a healthy diet.

In the second part of this work, satisfactory results have been obtained with regard to the improvement of nutritional value of wheat breads. Utilisation of pseudocereals, ancient cereals and legumes, added as part of a composite formulation, led to an improvement of the quality of the resulting enhanced-wheat breads, which may be placed into the growing market of cereal-based baked products having health-promoting and/or disease-preventing properties.

Research and development of gluten-free breads is undoubtedly more complex and less conclusive. In the third part of this thesis related to the gluten-free dough making, encouraging results are reached in obtaining machinable and moderately viscoelastic gluten-free doughs to achieve specialty flat breads with promoted protein and fibre contents. However, further studies are needed to produce the *Spianata* flatbread from the most promising selected formulations in order to evaluate the impact of the use of the selected ingredients on the technological, nutritional and sensory properties of the end products.

10 OTHER WORKS

Oral and poster presentations

10.1 ORAL PRESENTATIONS

Adding value to wheat flour-based breadmaking matrices: impact of ancient cereals, pseudocereals and legumes.

Conte, P.

13th EYCSTW European Young Cereal Scientist and Technologist Workshop.
14-16th May 2014, Freising, Germany

Adding value to wheat flour-based breadmaking matrices: impact of ancient cereals, pseudocereals and legumes

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Cereals are basic, ubiquitous and healthy raw materials, providing excellent vectors for diversity and innovation. It raises a great deal of recent interest that minor cereals, ancient crops, pseudocereals and legumes, besides wheat, constitute nutrient-dense and healthy grains with potential breadmaking applications. The current proposal is aimed at exploring the competences and exploiting the suitability of non-breadmaking whole grains (teff, buckwheat and green pea flours) with unique nutritional components, to be simultaneously included in mixed wheat matrices, to obtain novel and healthy fermented baked goods meeting the functional and sensory restrictions of viscoelastic breadmaking systems. Wheat flour replacement from 22.5% up to 45% by incorporation of ternary blends of teff, buckwheat and green pea flours provided dough systems with variable mechanical, viscometric and viscoelastic profiles with no significant hindrance of either dough machinability, or gassing power ability during fermentation. After baking, multigrain breads exhibited acceptable physico-chemical, technological and sensory features, and superior nutritional value compared to the 100% wheat flour counterparts, in terms of larger amounts of bioaccessible polyphenols, lower and slower starch hydrolysis, higher anti-radical activity and superior nutritional composition. All multigrain breads can be labeled as source of dietary fibre (≥3 g dietary fibre/100 g bread). The formulation based on wheat: teff:green pea:buckwheat flours, 55:15:15:15 fulfilled from 28% (men) to 43% (women) of dietary fibre, and from 54% (men) to 66 % (women) of protein daily requirements, when a daily consumption of 250 g of bread (WHO bread intake recommendation) is accomplished. This fact encompasses a substantial nutritional benefit with respect to the refined wheat flour bread that delivers when eaten daily at an amount of 250 g, from 9% to 14% of dietary fibre, and from 50% to 60% of protein daily requirements for men and women, respectively.

Creating value-added cereal-based baked products: marketplace offer, laboratory-designed goods, and revisited local products.

Conte, P.

XX Workshop on the Developments in the Italian PhD Research on Food Science Technology and Biotechnology.

September 23rd-25th, 2015, Perugia, Italy

XX Workshop on the Developments in the Italian PhD Research on Food Science, Technology and Biotechnology
Perugia, September 23rd-25th, 2015

Creating value-added cereal-based baked products: marketplace offer, laboratory-designed goods, and revisited local products

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The overall objective of this PhD research project is of creating cereal-based baked products with added value with respect to those currently available on the European market, satisfying the consumers' needs in terms of both sensory acceptability and superior nutritional profile. Firstly, 20 European commercial breads (gluten and gluten-free) have been assessed by physical-chemical, technological, nutritional, and sensory approaches. Secondly, multigrain breads in which the wheat flour was replaced from 22.5 % to 45% by simultaneous incorporation of teff, green pea and buckwheat flours have been produced and characterized. Finally, the "Spianata" (a typical Sardinian flat bread) for people suffering from coeliac disease will be produced and characterized.

Valore aggiunto di prodotti a base di cereali: offerta di mercato, prodotti disegnati in laboratorio e prodotti locali rivisitati

L'obiettivo generale di questa tesi di dottorato è quello di creare prodotti a base di cereali che possiedano un valore aggiunto rispetto a quelli attualmente presenti sul mercato europeo, in modo da soddisfare le esigenze del consumatore sia in termini di accettabilità sensoriale sia di superiore profilo nutrizionale. Un totale di 20 pani commerciali europei (con e senza glutine) sono stati caratterizzati da un punto di vista fisico-chimico, tecnologico, nutrizionale e sensoriale. Si è prodotto e caratterizzato un pane multicereale nel quale la farina di frumento è stata sostituita con farina di teff, grano saraceno e pisello. Infine, si è prodotto e caratterizzato un tipico pane sardo, la Spianata, destinato alle persone intolleranti al glutine.

Key words: Bread, pseudocereals, legumes, nutritional quality, polyphenol composition, starch hydrolysis kinetic.

Paola Conte, *Creating value-added cereal-based baked products: marketplace offer, laboratory-designed goods, and revisited local products.* Tesi di dottorato in "Scienze e Biotecnologie dei Sistemi Agrari e Forestali e delle Produzioni Alimentari" - Indirizzo "Biotecnologie Microbiche Agroalimentari" Università degli Studi di Sassari.

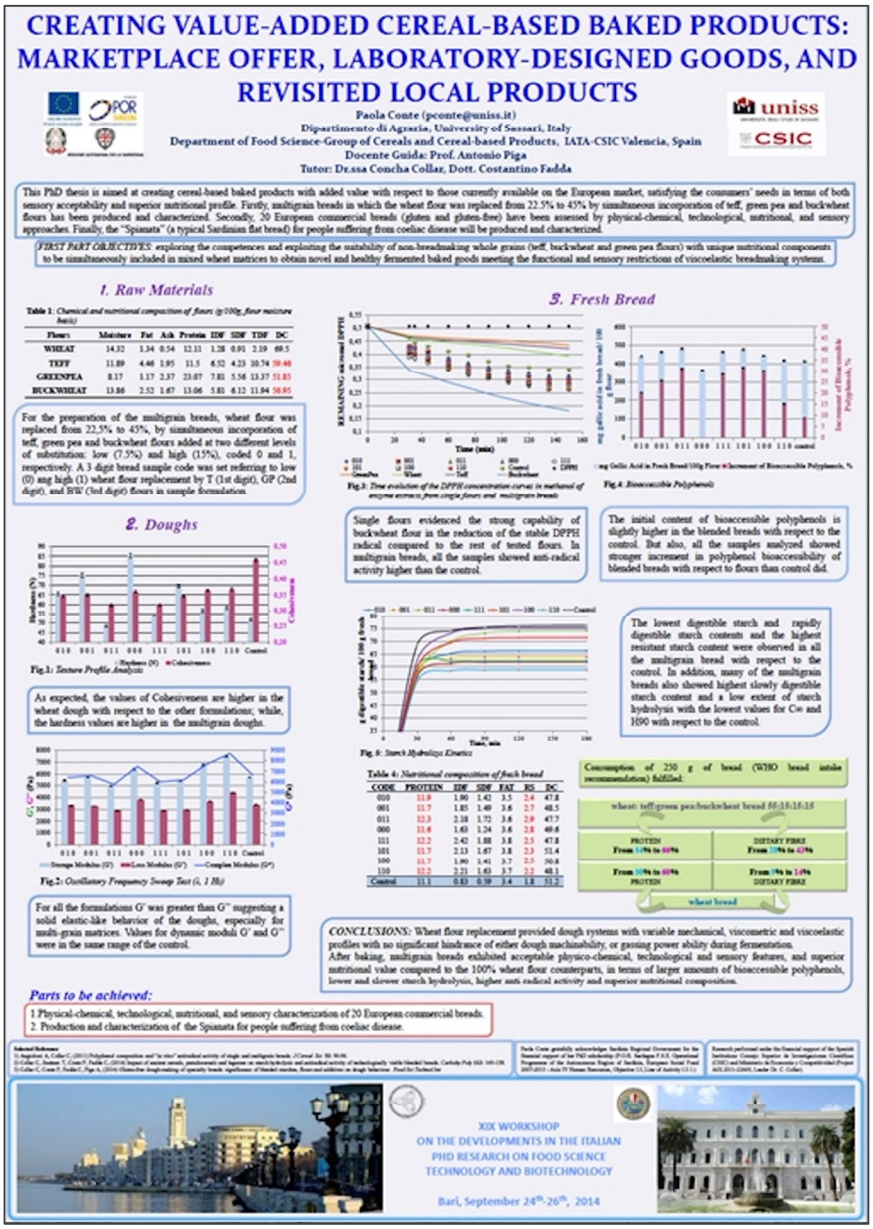
10.2 POSTER PRESENTATION

Creating value-added cereal-based baked products: marketplace offer, laboratory-designed goods, and revisited local products

Conte, P.

XIX Workshop on the Developments in The Italian Phd Research on Food Science Technology And Biotechnology

24th-26th September 2014, Bari, Italy



Paola Conte, *Creating value-added cereal-based baked products: marketplace offer, laboratory-designed goods, and revisited local products.* Tesi di dottorato in "Scienze e Biotecnologie dei Sistemi Agrari e Forestali e delle Produzioni Alimentari" - Indirizzo "Biotecnologie Microbiche Agroalimentari" Università degli Studi di Sassari.

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