Characteristics and functional properties of green banana flour:

An opportunity for functional bread production

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Amir Amini Khoozani

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با لطف وجود آنهاست

“To my Family; Whom everything I had, have and will have is because of them”

Declaration

Most parts of this thesis have already been published in international peer review journals.

Publications

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2. AMINI KHOOZANI, A., BIRCH, J. & BEKHIT, A. E.-D. A. 2019. Production, application and health effects of banana pulp and peel flour in the food industry. *Journal of Food Science and Technology,* 56**,** 548-559.
3. AMINI KHOOZANI, A., BEKHIT, A. E.-D. A. & BIRCH, J. 2019. Effects of different drying conditions on the starch content, thermal properties and some of the physicochemical parameters of whole green banana flour. *International Journal of Biological Macromolecules,* 130**,** 938-946.
4. AMINI KHOOZANI, A., BIRCH, J. & BEKHIT, A. E.-D. A. 2020. Textural properties and characteristics of whole green banana flour produced by air-oven and freeze-drying processing. *Journal of Food Measurement and Characterization,* 14**,** 1533–1542.
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Awards

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Abstract

Characteristics and functional properties of green banana flour:

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By

Amir Amini Khoozani

The demand for functional food products has led to an increased interest in nutrients such as minerals, vitamins, bioactive compounds, fibre and prebiotics to be present in food formulations. Amongst the prebiotics, Resistant starch (RS) has gained more attention in recent years, due to its acknowledged health benefits such as prevention and control of colon cancer, diabetes, and obesity. Banana, the world's most favourite fruit, is one of the richest sources of RS at early stages of ripeness, when it is green (unripe). According to some estimates, more than 100 billion bananas are consumed globally each year, with an annual per capita consumption of 20 kg.

Green banana pulp is a rich source of essential phytonutrients, phenolic compounds, vitamin B group, ascorbic acid and tocopherols, while the green banana peel is a rich source of minerals, bioactive compounds and dietary fibre (DF) such as pectin, cellulose, hemicelluloses and lignin. Considering the nutritional value of both pulp and peel of green bananas, the production of green banana flour (GBF), which can be

obtained by proper drying techniques, provides a way to preserve the nutritional benefits and increase the shelf-life of banana nutrients.

White bread is the most popular bread type in the world, however, there is a growing research on fortifying bread with an array of different DF and functional compounds to take advantage of bread as a carrier of health benefiting compounds. Very few studies available that considered the effect of the GBF on technological properties, nutritional aspects and volatile fingerprint.

The physicochemical and thermal properties of GBF obtained from air oven drying (ODF) at three temperatures (50, 80 and 110 °C) and freeze-drying (FDF) were compared to white wheat flour (WF). Lightness and yellowness were negatively affected by the oven temperature increment. The FDF samples exhibited higher a* and L* values and had the closest browning index to WF (P-value < 0.05). Also, the ODF50 samples had the highest emulsion activity, whereas FDF had the highest emulsion stability (P-value < 0.05). The oil holding and water holding capacities of the FDF samples were significantly higher than all other samples (P-value < 0.05). A higher RS content was found in the FDF (46.72%) and ODF50 (44.58%) samples. Oven drying significantly increased the gelatinization temperature drastically for all GBF samples. Results from particle size separation indicated that drying at 50 °C generated smaller flour particles compared to ODF80 and ODF110 treatments. Freeze-dried flour samples had significantly higher bulk density, viscosity and firmness compared to the oven-dried samples and the reference sample, WF (P-value < 0.05), but the cohesiveness, consistency, compressibility and Hausner Ratio were not different from the ODF50 (P-value > 0.05). While the ODF110 presented the highest pasting temperature (81.23 °C) and breakdown viscosity (7118.67 cp) amongst the GBF samples, ODF50 were the only heat-treated samples that showed similar hold, final and setback viscosity values to those found in the FDF. In terms of mineral contents, all GBF samples had higher concentrations of K, Mg, Ca and Zn compared to the WF which makes GBF as a better source of these nutrients (P-value < 0.05). The overall results from both nutritional and technical aspects showed that amongst heat-treated GBF, ODF50 (ODF) was the best flour to be compared to FDF for fortification in bread. Three levels of FDF and ODF were used to substitute WF at three levels (10%, 20% and 30%) in bread formulation. At 30% fortification level, elasticity, loss modulus and complex viscosity of dough were significantly higher in the fortified samples compared to the 100% WF bread. At 20% fortification level, cohesion was significantly decreased in the fortified dough samples compared to control and FDF samples (P-value < 0.05). The use of GBF resulted in a denser, harder and chewier bread with increasing the fortification level. In terms of shelf life, the banana bread stored at -20 °C for one week had significantly lower firmness and water loss compared to 4 °C and 25 °C (P-value < 0.05).

A significant decrease in energy caloric value and an increase in moisture and total dietary fibre at > 20% fortification level was observed. The ODF-fortified samples had higher browning index compared to control and FDF ones. The addition of both GBF types improved macro minerals (Mg, Ca, Na, K and P) without a significant change in micro minerals (Fe, Zn, and Mn). The use of FDF in bread resulted in a marked increase in both resistant and slow digestible starch content in F30 compared to ODF-fortified samples at their comparable fortification levels.

GC-MS-based chemical fingerprinting successfully detected more than 100 volatile compounds in the GBF fortified bread samples. Chemometrics methods used to compare the effect of GBF type in bread (FDF and ODF-fortified-bread), fortification level (10%, 20% and 30%) and bread part (crumb and crust) on the formation of volatile compounds. Furan (furfural, 2-furanmethanol), Strecker aldehydes (2-methybutanal and 3-methylbutanal) and ketone (2-undecanone) were the most abundant volatiles in crust while alcohol (1-hexanol and 1-heptanol) and ester (ester butanoic acid ethyl) abundant in the breadcrumb. The level of fortification had a significant impact on the formation of 3-methyl-butanal (P-value < 0.05). Furthermore, bread made with freeze-dried GBF had more distinguished 'banana-like' flavour due to the presence of ethyl ester butanoic acid and 2-undecanone, while bread made with ODF represented more Maillard-related compounds which could signify a woody-malty aroma impression.

It can be concluded that fortification of bread with the GBF achieved from freeze-drying had a more desirable results from technological and nutritional points of view. Although between 10% and 20% fortification level there was no clear difference, the 30% bread samples showed a high value nutritious bread with distinctive volatile flavour. Overall, the type of the drying method of GBF preparation had an impact on developing discriminant volatiles compared to bread part and fortification level.

Keywords: Aroma volatiles, banana, bread, digestibility, drying, chemical fingerprinting, chemometrics, flour, green banana, GC-MS, nutritional, resistant starch, rheology, textural, thermal properties, volatile.

Prologue

To begin with, I would like to express my gratitude to my supervisors, Prof. Aladin Bekhit, Dr. Biniam Kebede and late Prof. John Birch (May his soul rest in peace). John was the reason of me being here, who made my dream come true. Before he resigns university due to his medical conditions, he was my primary supervisor who accepted my proposal and believed in my ideas. His continuous support and novel ideas kept motivating me to have thirst for knowing more every day. I will be forever thankful for the opportunity that I had to be his student and learn from him, God bless him.

After losing John, Aladin continued supporting me as my primary supervisor. Thank you for all the supports, caring, life lessons and sharing your experience which I value immensely. Without your continuous support, I couldn't finish what I initiated. Thank you Biniam for accepting to be a part of my project. You brought your skills and expanded the scope of my project.

My sincere thanks also go to all food science staff members, ranging from administrative ones to lab assistant: Joann Ayers, who gave me the very first feedback on my banana bread trial. Sarah Johnson, who allowed me to work during the night at laboratory number 2 when I was not getting the right result and always was there ready to hear the concerns. Michelle Petrie and Michelle Leus who were so supportive and generous about teaching everything I needed to know. Ian Ross, who was the first line of approach when I was facing a hurdle regarding materials, chemicals and equipment during my experiments. I am also grateful for my friends in Dunedin,

whom I call them my extended family. I knew no one here when I arrived but gradually, I adopted myself to new lifestyle, language, culture and expectation and could build strong bonds. Getting to know people from almost 40 different countries was truly a highlight in my life.

However, beyond all those support that facilitated this journey, there was numerous obstacles in addition to PhD challenges itself. This invisible mental pressure was with me along the way: In addition to carry the burden of the loss of my primary supervisor, whom I had planned everything in the beginning, homesickness, insufficient ability to communicate in English, the language I started to learn here, more dramatic occurring happened. As a Muslim, I was deeply affected by the terroristic attacks happened in Christchurch, just less than a week from losing John. Then, the revolutionary activities happened in my own home country. While people were being arrested and killed in streets of Tehran, I was worried sick about my family safety. Just a month later, the political tensions between Iran and the USA increased after the second most important Iranian military leader was assassinated and Iranian government's retaliation afterwards made me more concerned about my people, my family. With keeping all these to myself and dealing with PhD challenges, the year 2020 started and we all know that what happened. Suffice it to say that I had many plans to go to conferences for presenting my work, and everything was cancelled due to the COVID-19 pandemic, more mental pressure was added. In the meantime, because I was a collegiate community leader at Abbey College, the University of Otago post-grad college, I was responsible for taking care of the residents, from both sides of well-being and pastoral care. In fact, I had to paint a smile on my face, yet could not hide the tear trace. And utterly, here I am: Completed my research within its period despite everything happened. I learnt manifold things which I never take them for granted in my life. I stood against all the hardships, fought consistently and kept attaching to my believes and the one who's my faith is in. I can say, now, I am ready for the next chapter of my life.

Lastly, but not for least, surely, I dedicate this thesis to my family, my dad, mom and my sister who has just finished her PhD. Those who shaped my personality, guided me all the way through my hardships and life challenges. I would not have been here without them and I do anything just to bring the smile on their faces and make them proud.

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List of Abbreviations

AMG	amyloglucosidase
ANOVA	analysis of variance
a_w	water activity
BD	breakdown viscosity
BF	banana flour
BP	banana pulp
BPe	banana peel
DF	dietary fibre
DS	digestible starch
F	firmness
FDF	freeze-dried green banana flour
FAO	food and agriculture organization
FTIR	fourier-transform infrared spectroscopy
FV	final viscosity
G*	complex modulus
G′	storage modulus
G″	loss modulus
GBF	green banana flour
GBP	green banana pulp

GBPe	green banana peel
GBPeF	green banana peel flour
GBPF	green banana pulp flour
GC-MS	gas chromatography mass spectroscopy
GC-O	gas chromatography olfactometry
GF	gluten free
GOPOD	glucose oxidase peroxidase reagent
HDL	high density lipoprotein
HI	hydrolysis Index
HR	hausner ratio
HV	hold viscosity
HS-SPME	headspace-solid phase micro-extraction
ICP-MS	inductively coupled plasma mass spectrometry
LDL	low density lipoprotein
LV	latent variables
MC	moisture content
MVDA	multivariate statistical data analysis
η^*	complex viscosity
ODF	oven-dried green banana flour
OHC	oil holding capacity
PCA	principal component analysis

pGI	glycaemic Index
PLS-DA	partial least squares-discriminant analysis
PT	pasting temperature
PV	peak viscosity
PTFE	polytetrafluoroethylene
R	gelatinization range
RC	relative crystallinity
RDS	rapidly digested starch
RI	retention index
RS	resistant starch
RVA	rapid visco analyser
SB	setback viscosity
SDS	slow digestible starch
SD	standard deviation
SI	solubility index
SP	swelling power
SPME	solid-phase micro-extraction
SV	specific volume
T_0	onset temperature
tan δ	loss tangent
TDF	total dietary fibre

TIF	indigestible fraction
T_P	gelatinization/ peak temperature
TPA	texture profile analysis
TS	total starch
VID	variable identification coefficients
WB	wheat bread
WF	wheat flour
WHC	water holding capacity
XRD	x-ray diffraction
YBP	yellow banana pulp
YBPe	yellow banana peel
YBPeF	yellow banana peel flour
YBPF	yellow banana pulp flour
ΔH	transition enthalpy

CHAPTER 1

Introduction

Chapter aim:

This chapter discusses the importance, objectives and scope of the research carried out in this thesis.

1.1. The importance of project

Banana is one of the most consumed fruits in the world and the world's fourth most significant crop. The average global banana consumption has been reported to be 12 kg per capita. Banana is a universal term, comprising several species of this genus in the *Musaceae* family and is a tropical climacteric fruit. According to the latest Food and Agriculture Organization (FAO) statistics, Asia is the largest banana production region, with 54.4% of the world's banana production (FAOSTAT, 2017). In New Zealand, banana is an essential part of the diet, with consumption of more than 90,000 tons annually. Almost two-thirds of New Zealand's imported bananas come from Ecuador, one third from the Philippines, the two main banana exporters at the international level, and a small quantity (less than 2%) comes from Mexico (Edmunds, 2015). It is estimated that a total of 40% of green banana harvests are lost before exporting. Unsuitable handling, deformity of the banana, irregular shape and brown dots are some examples of the fruit rejection (De Gouveia and Zandonadi, 2013). Moreover, before bananas are treated by the ripening process in the destination country, there is another screening step to remove unwanted fruits which were affected by the transportation system (FAOSTAT, 2017). There are studies for use of this mass waste by extracting macromolecules, such as pectin, cellulose and dietary fibre (DF) for food products applications or animal feed (Viena et al., 2018, Sri Suryaningsih et al., 2018, Putra et al., 2018, Nikhila et al., 2018, Pathak et al., 2017, Chavan et al., 2013). Green banana pulp (BP) is a rich source of essential phytonutrients, including phenolic compounds and vitamins (niacin, pyridoxine, cobalamin, ascorbic acid and alpha-tocopherols). It also contains carotenoids, flavonoids, minerals and DF. It is also rich in resistant starch (RS) which has a positive impact on the colon's health, while ripe banana contains more digestible starch and monosaccharides. Banana peel (BPe) is a rich source of minerals, bioactive compounds, RS and fibres (Kurhade et al., 2016). With the aim of taking advantage of the nutritious compounds which are captivated within banana peel and pulp cell walls, drying is an alternative approach that has been introduced in recent years to prevent the waste before it happens (Vu et al., 2017, Musa et al., 2017, La Fuente and Tadini, 2017, Baptestini et al., 2017, Vaidya et al., 2016). Preserving the most important prebiotic in green banana, RS, requires a controlled-modified drying method.

Resistant starch is defined as the sum of starch and products of starch degradation that are not absorbed in the small intestine of healthy individuals (Goñi et al., 1996). It acts as a prebiotic and has a positive influence on the functioning of the digestive tract, reduces blood cholesterol levels and assists in the control of diabetes. In addition, to be an important source of bioactive compounds, green banana is one of the richest sources of resistant starch, which makes it a valuable natural resource for enrichment purposes in foods (Bello-Perez and Hoyos-Leyva, 2017).

1.2. Overview of project

There has been an increase in research towards the formulation of functional food products in the last decade; to develop products containing a wide range of beneficial nutrients ranging from micro-nutrient enriched foods to products that have probiotic functionality. The increased attention to health and wellbeing of consumers in the last decade, has led to an increased interest in vitamins, minerals, unsaturated fatty acids, bioactive compounds and fibres in food products. The utilization of by-products of fruits, especially banana, has recently become a trend, and many studies are underway to evaluate their effects on food properties (Chávez-Salazar et al., 2017).

Banana peel takes up approximately 40% of a banana weight, which is usually discarded. One of the aims of this study was to take advantage of the banana peel's high nutritional value by transforming it into flour together with the banana pulp. Doing so, can not only help sustainability and waste control, but also increases the production yield of the whole green banana flour (GBF). Furthermore, incorporating such banana flour into bread and investigating its effects on the technological, nutritional and volatile profile aspects of the bread were investigated.

Given the importance of a functional bread in a diet, and the potential use of banana peel as a rich source of bioactive compounds, especially RS, this thesis was designed to characterize the properties of green banana flour produced from different drying methods (freeze-drying and oven-drying). Thereafter, the quality properties, structural and textural attributes of dough and bread were assessed together with *in vitro* digestion tests in order to determine different starch types of the final fortified bread. Finally, a comparative volatile fingerprinting was performed in order to determine the key chemical compounds that distinguish a 100% wheat bread from fortified banana bread.

A review of the available literature is presented in chapter 2. Five experimental chapters are provided following a research design that is presented in chapter 3 in order to summarize research gaps and provide detailed objectives of this work. Each experimental chapter includes an introduction, method and materials, result and discussion and conclusion (chapter 4-8). Finally, the outcome, limitations and recommendations of this study are summarised in chapter 9. A schematic outline of how the present thesis is structured is shown in Fig 1.1.

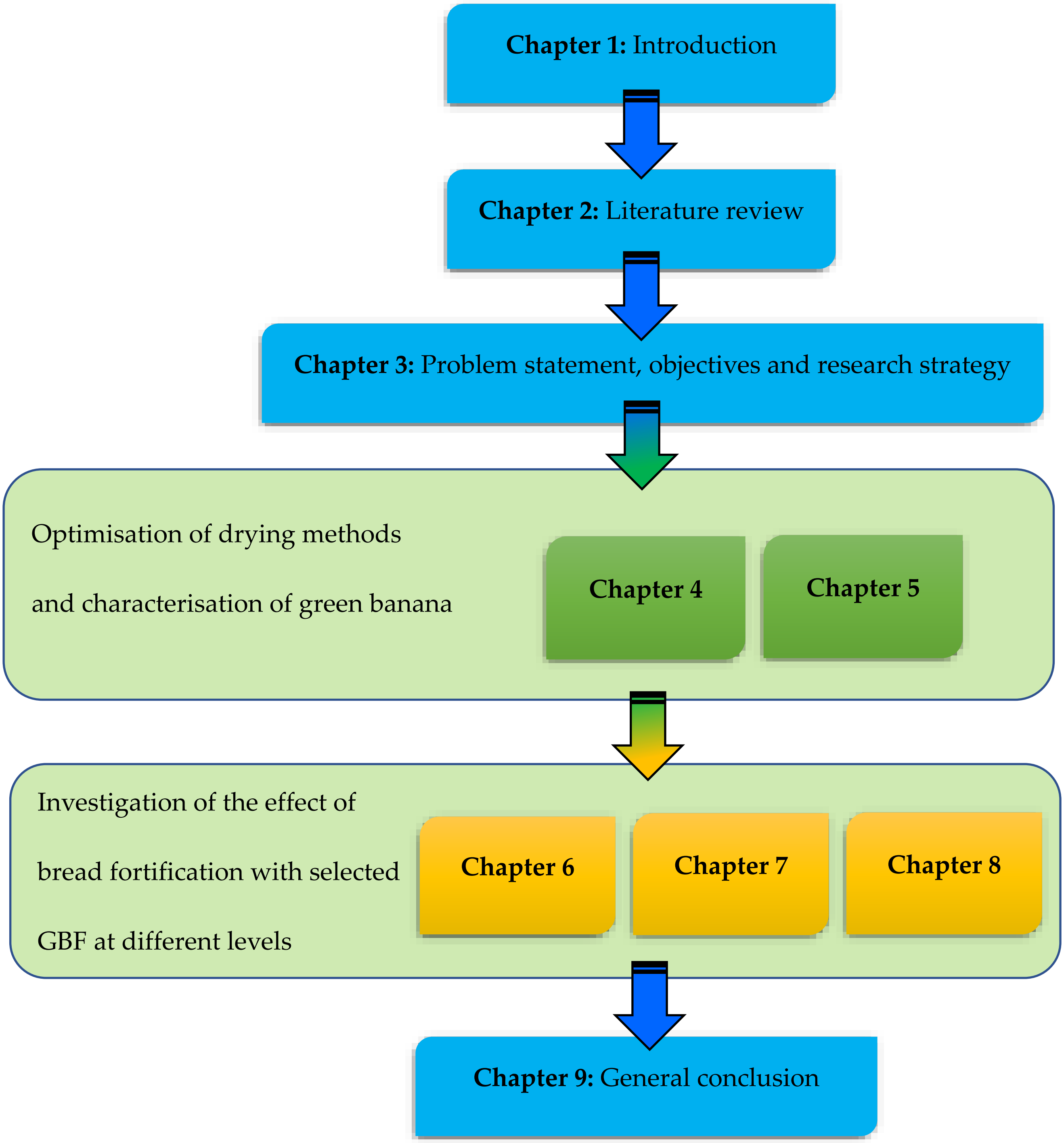

Figure 1.1. Schematic outline of thesis content

1.3. Thesis Format

Based on the guidelines of the University of Otago Graduate Research School, this thesis has been written in "hybrid format", whereby published materials are wholly inserted as chapters (**chapter 2, 4-7**) which has been approved by Board of Graduate Studies, 5 June 2014, revised on 5 June 2016. In order to maintain the coherency between the chapters, formatting was standardized (references, bibliography, page numbers, margins, etc) and duplicated material was deleted and cited where it was appropriate. Some changes have been made in published chapters in order to make the information more related amongst chapters. A bridging page has been implemented prior to where an experimental chapter begins. Points below were taken into consideration when writing this thesis:

1. New Zealand spelling were used in the text except the in-text citation and references.
2. Abbreviations or symbols were defined in appropriate places as far as being practical.
3. The international system of units (SI) was used in the whole thesis.
4. Harvard reference style were followed in citation and bibliography.

CHAPTER 2

Production, application and health effects of banana pulp and peel flour in the food industry

This chapter has been published as:

AMINI KHOOZANI, A., BIRCH, J. & BEKHIT, A. E.-D. A. 2019. Production, application and health effects of banana pulp and peel flour in the food industry. Journal of Food Science and Technology, 56, 548-559.

Chapter aim:

This chapter discusses the health benefits of banana bioactive compounds and utilization of different parts of the banana and flour produced at various ripeness stages in food applications. Of particular interest, various methods for producing banana flour are compared to highlight the effect of each one on physicochemical, nutritional and structural properties of flour properties

2.1. Introduction

Banana is a tropical climacteric fruit and universally comprises a number of species in the genus *Musa* of the family *Musaceae*. It is one of the most favoured fruits in the world and the fourth most important crop produced globally (Zhu et al., 2018). Nearly all of the identifiable banana cultivars are derived from two diploid species, *Musa acuminata* and *Musa balbisiana*, in which the *Cavendish* variety is the most common. Plantain is related to the hybrid triploid cultivars of banana and is longer, more angular and diverse in shape. In the mature state, plantain is firmer than Cavendish and thus it is less valued as a fresh product (Zhang et al., 2005). According to the latest Food and Agriculture Organization (FAO) statistics, Asia is the largest producer of banana with a share of 54.4% of the world's banana production. With an average banana consumption of 12 kg per capita, banana is amongst the world's major food crops, after rice, wheat and maize (FAOSTAT, 2017).

Banana fruit consists of two parts: peel and pulp. Banana peel (BPe), which is the main by-product of banana, is about 40% of total weight of the fruit. Until recently, BPe had no useful applications and was dumped as waste, contributing massive amounts of organic materials to be managed. Since researchers have begun to focus on studying the composition of BPe, several possible applications have emerged (Agama-Acevedo et al., 2016). Banana pulp (BP), which is the edible part of the fruit, has an abundant amount of nutrients. Studies conducted on BP have investigated different aspects ranging from its use as an ingredient for food enrichment to extraction and isolation of many health-beneficial components, such as different types of starch, cellulose and bioactive compounds (Singh et al., 2016). As stated by Kitts (1994), bioactive compounds are constituents with extra nutritional advantages that are naturally occurring in plants and foods in small amounts. They exert their beneficial biological effects by stimulating the probiotic growth and help in the prevention of cardiovascular disease and cancer (Kris-Etherton et al., 2002). Phenolics, carotenoids, flavonoids, biogenic amines, phytosterols and other phytochemicals can be found in BP and peel (Pereira and Maraschin, 2015). Due to the presence of these compounds, bananas have a higher antioxidant capacity than some berries, herbs and vegetables (Moongngarm et al., 2014). Bioactive compounds in different cultivars of banana and their health benefits were reviewed in details by Singh et al. (2016). They reviewed the content of phenolic compounds, carotenoids, biogenic amines, phytosterols, antioxidant activity and overall health benefits of banana based on different cultivars and ripening stage. They also reported the potential of banana pulp and peel usage as a functional food source against many chronic diseases.

The increasing attention to functional food products and health and wellbeing of consumers in the last decade has led to an increased interest in vitamins, minerals, unsaturated fatty acids, bioactive compounds and fibre in food products (Al-Sheraji et al., 2013). The utilization of by-products of fruits, especially banana, has become a trend as of late and many studies are in progress to evaluate their effects on food properties (Chávez-Salazar et al., 2017, Yu et al., 2018, Viena et al., 2018, Temesgen et al., 2018, Tan and So, 2018, Singh et al., 2018, Ren et al., 2018, Rahimi-Mohseni et al., 2018, Pereira et al., 2018). As approximately one-third of banana is lost due to the public tendency to consume only ripened fruit, utilization of different parts of the banana at different ripening stages has also gained interest over the past years (Sheikh et al., 2017).

This chapter discusses the health benefits of banana bioactive compounds and physicochemical composition of different parts of banana in all stages of ripeness. This is followed by a comparison between the available drying methods in terms of the effect of each one on various properties of the produced banana flour.

2.2. Composition of banana

Banana pulp is a rich source of essential phytonutrients, including phenolic compounds and vitamins (niacin, pyridoxine, cobalamin, ascorbic acid and alpha-tocopherols). It also contains carotenoids, flavonoids, amine compounds and dietary fibre (DF). Dietary fibres are indigestible carbohydrate polymers that are classified based on their water solubility into two types, soluble fibres (pectin and some hemicelluloses) and insoluble fibres (cellulose, lignin and resistant starch) (Alba et al., 2018). In general, it has been reported that BPe contains more DF than BP (Garcia-Amezquita et al., 2018). Extracting pectin from the peels could add value. In addition, BPe has high amount of lignin, cellulose and hemicelluloses fractions, which can be extracted as a formed complex substrate named lignocellulosic biomass which could be used to produce bioethanol (Happi Emaga et al., 2008). Also Khamsucharit et al.

(2018) signified that the extracted pectin from BPe could be an alternative source for commercial pectin.

One of the most important DF which has gained a lot of attention in recent years is resistant starch (RS). It is mainly composed of the linear part of starch (amylose) which is fermented by probiotics in the colon, specifically *Bifidobacterium* and *Lactobacillus* species (Kale et al., 2002). This brings about the production of short chain fatty acids, mainly butyric acid, which has a key role in prevention of colorectal cancer (Pérez-Burillo et al., 2018, Singh et al., 2016, Hu et al., 2015, Birt et al., 2013, Gratz et al., 2011, Perera et al., 2010). There are five types of RS introduced up to now: starch that is physically inaccessible in crop's cell walls (RS1), granular native starch with high crystalline structure (RS2), retrograded starch achieved by heating and cooling of starchy foods (RS3), chemically modified starch (RS4) and amylose-lipid complex (RS5). Fractionation of the DF and RS is different parts of banana in different levels of maturation is shown in Table 2.1.

Unripe banana is rich in RS2, which is beneficial to colon health, while ripe banana contains more digestible starch and protein (Singh et al., 2016). Banana peel is a rich source of minerals, bioactive compounds and DF (Kusuma et al., 2018). Several studies reported the use of banana peel flour (BPeF) as a functional food source (Ramli et al., 2009, Ramli et al., 2010, Türker et al., 2016, Agama-Acevedo et al., 2016). According to some reports, both pulp and peel have high antioxidant activity (González-Montelongo et al., 2010, Agama-Acevedo et al., 2016). As lipid oxidation in food components is one of the unwanted reactions causing rancidity, food producers rely on synthetic antioxidants to minimize lipid deterioration. Potential health risks, however, is a limiting factor of using these preservatives extensively in food products, especially staple ones (Pathak et al., 2017). Given that BPe extract has been found to be non-toxic to human cells, more information has become available on using it as an inexpensive fruit by-product source of antioxidants (Segundo et al., 2017b).

The amount of ash, protein, crude fibre and digestible starch of BPeF was reported to be significantly higher than that of pulp, which makes the BPeF more effective as a functional additive (Nasrin et al., 2015). For instance, the higher quantity of ash can be valuable in treating deficiencies of minerals caused by celiac disease. Additionally, several studies have shown the application of BPe as a low-cost precursor for producing materials such as anionic dye and heavy metal adsorbents (Vilardi et al., 2018, Singh et al., 2018, Oyewo et al., 2018, Munagapati et al., 2018, Mahindrakar and Rathod, 2018), recovering phenolic compounds (Vu et al., 2018), producing cellulose nanofibres (Tibolla et al., 2018, Harini et al., 2018, Costa et al., 2018), as well as bioethanol (Prakash et al., 2018, Berawi and Bimandama, 2018) and pectin extract (Khamsucharit et al., 2018). In following sections, some of the exclusive added value components in banana are introduced.

2.3. Health effects of banana bioactive compounds

Carotenoids are natural antioxidants that contribute to the stability of foods during storage. Previous studies documented the existence of various carotenoids in banana fruit (Davey et al., 2006). Although some suggested that the cultivars genotype specifies the quantity of carotenoids, they mostly concurred that the amount of trans-alpha and trans-beta carotene comprised the majority of pro-vitamin A compounds (Yan et al., 2016). Another significant carotenoid reported was lutein, which exhibited antioxidant properties and an inhibitory effect on the age-related macular degeneration. Interestingly, it has been identified that green banana peel (GBPe) has substantially higher carotenoids than the pulp (Davey et al., 2006).

Phytochemicals, especially phenolic acids, are the main bioactive compounds known for exerting health benefits. Unexpectedly, the percentage of phenolic compounds has been reported to be greater in the peel than the pulp (Kanazawa and Sakakibara, 2000). For example, it was reported that the quantity of gallocatechin in the peel was five times greater than the pulp. The banana peel extract was found to inhibit lipid oxidation better than pulp extract (Someya et al., 2002). Phytosterol compounds, such as cycloeucalenone, cycloeucalenone, cycloeucalenol, cycloartenol, stigmasterol, campesterol and b-sitosterol, were reported to be in the range of 2.8–12.4 g/kg dry weight base in GBP (Villaverde et al., 2013).

Recently, it has been shown that gallocatechin extracted from GBPe was effective in the healing of surgical wounds in rats (Von Atzingen et al., 2015). Correspondingly, a unique flavonoid named leucocyanidin was found in aqueous extract of unripe plantain pulp, which is now known to be effective in the treatment of gastric diseases (Lewis et al., 1999).

Biogenic amines play a key role in the prevention of depression. Catecholamines, dopamine, norepinephrine (noradrenaline) and epinephrine (adrenaline) are the best-known examples of these bioactive compounds which regulate hormones in glycogen metabolism (González-Montelongo et al., 2010). Results of dopamine levels in different ripening stages of banana revealed an inverse relation between its concentration and fruit's maturity, noting that BPe contained more dopamine than pulp (Kanazawa and Sakakibara, 2000).

There is a wide range of DF in banana fruit, including pectin, cellulose, lignin and hemicellulose which can be found naturally in banana flour (BF). Amongst them, RS is the most notable one which provides bioactive effects (Thebaudin et al., 1997). Resistant starch, which is mainly derived from retrograded amylose in cooked starchy food, is resistant to digestion in the small intestine after 2 h incubation (Homayouni et al., 2014). After it reaches the colon undigested, it will be fermented by membrane microbiota (mainly probiotics) and cause pH reduction. Therefore, the environment will be undesirable for the growth of pathogenic microbiota and formation of carcinogenic cells (Hung et al., 2016). A considerable number of studies has been published on the direct relationship between RS intake and reduction of the large bowel cancer risks either *in vitro* or *in vivo*; it seems that RS may be a major protective factor against colorectal cancer (Yin and Zhao, 2017, Panebianco et al., 2017).

In a randomized clinical trial on 22 healthy adults who were under 5 g per week of green banana pulp flour (GBPF) diet, decreased hunger and increased satiety were reported (Hoffmann Sardá et al., 2016). Also, Silva et al. (2016) reported a consumption of 25-70% of GBP could prevent oxidative damage in liver and kidney and improves biochemical parameters in type 1 over a 12-week diet. Arun et al. (2017) reported similar results in a GBPF-enriched diet, which resulted in a reduction of type 2 diabetes risk and associated cardiovascular diseases.

It has been shown that the amount of RS decreased from 8% to 2% with progression in banana peel ripeness (changing the colour from green to yellow). However, the percentage of total dietary fibre (TDF) slightly increased in yellow banana peel (YBPe) (Ramli et al., 2010). In a parallel report, the RS and TDF contents of GBPF of 49.9% and 7.2% were reported, respectively (Menezes et al., 2011). In general, it can be concluded that because approximately 70% (dry basis) of the peeled green banana comprises of RS2, the unripe pulp is a remarkable source of this bioactive compound (Wang et al., 2017a). It is well-known that phytosterols are immune system modulators and exert cholesterol-lowering and anticancer properties in the intestine (González-Montelongo et al., 2010). As reported by (Marangoni and Poli, 2010), a daily intake of phytosterols up to three grams/day significantly reduced total and low density lipoprotein (LDL). A study pointed to a high amount of phytosterols can be found in GBF, mainly beta-sitosterol, campesterol and stigmasterol (Bertolini et al., 2010). Although citric acid pre-treatment reduced phytosterol in flour obtained from the peel, no change was detected between phytosterols of acidified and control samples from the pulp (Bertolini et al., 2010). Consequently, the known properties of phytosterols suggest these flours could be used as functional food components. Recently, Falcomer et al. (2019) reviewed the latest studies on health benefits of green banana products' consumption with a major focused on *in vivo* works. Overall, the studies showed the health benefits can be obtained from green banana products consumption. Mainly, they have a positive impact on the gastrointestinal diseases, the glycaemic index, diabetes and weight control. However, more studies, *in vivo* or *in vitro*, are needed to investigate the changes in digestibility of fortified food products with GBF.

2.4. Methods for banana flour preparation

Due to the climacteric nature of the banana, it is highly perishable and requires drying during processing for preserving for a longer period of time. Banana flour is a product with high storability potential and long shelf life and can be readily applied to food products. The proximate composition of the flour also depends on the origin, variety, time of harvest, and drying procedure of bananas (Haslinda et al., 2009). Table 2.1 depicts a summary of the proximate composition of both green and yellow BF made from pulp and peel.

Table 2.1. Composition (g/ 100 g) of banana flour produced from different ripening stage

Banana Maturity	Flour base	Starch	RS	DF	Ash	Lipid	Protein	Reference
Green (stage 1-2)	Pulp	64-75	17.5-48	7.5-15	2.6-4.7	0.4-2.7	6.5-14.3	(Juarez-Garcia et al., 2006, Haslinda et al., 2009, Menezes et al., 2011)
	Peel	10.1-11.7	8.2-8.6	43-50	1.2-9.6	6.1-9	4.1-8.1	(Haslinda et al., 2009, Nasrin et al., 2015, Eshak, 2016, Agama-Acevedo et al., 2016)
Yellow (stage 6-7)	Pulp	56-63	11-17	17-18	3.3-6.9	0.6-1.2	3.76	(Da Mota et al., 2000, Aurore et al., 2009, Segundo et al., 2017b)
	Peel	3.5-6.3	2.3-2.5	47-53	9-11	3.8-11	5-8	(Ramli et al., 2009, Zhang et al., 2005, Kurhade et al., 2016)

RS, resistant starch. DF, dietary fibre.

Processing steps used for flour preparation from banana pulp and peel are similar, except for the heating procedure used. Pre-treatment process steps of BF preparation are summarized in Fig 2.1.

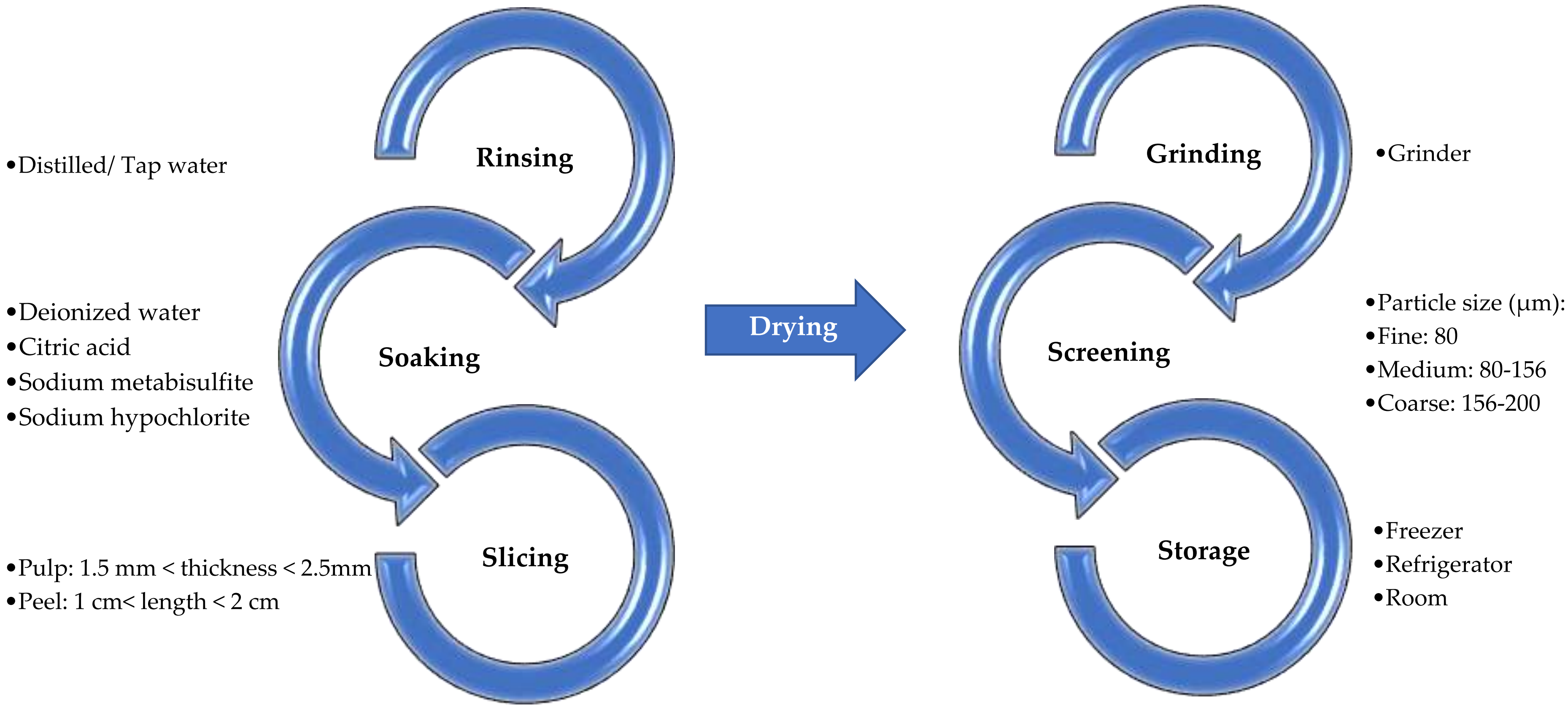

Figure 2.1. The process steps of banana flour preparation

While the most published studies applied oven drying for banana fruit (Nasrin et al., 2015, Türker et al., 2016, Kurhade et al., 2016, Gomes et al., 2016, Segundo et al., 2017a, Segundo et al., 2017b), spouted bed drying (Bezerra et al., 2013a, Bezerra et al., 2013b) and lyophilization (Da Mota et al., 2000, Wang et al., 2012, Türker et al., 2016). In order to minimize enzymatic browning, soaking in sodium metabisulfite, sodium hypochlorite or citric acid solutions was a general pre-treatment and considered the first step after rinsing bananas with water.

The composition of samples obtained by lyophilization and spouted drying techniques showed a significant increase in phenolic acid content, heat sensitive vitamins and minerals compared to traditional drying methods such as solar or hot air-oven drying. Using a spouted drier for producing GBF resulted in high DF and RS content with an average of 21.91% and 68.02% dry-weight base, respectively. This technique did not alter the RS content; however, this effect was consistently reported since a similar study reported lower values, 13.89% and 40.14% for DF and RS, respectively (Bezerra et al., 2013a).

Likewise, post-treatments could affect the composition of BF produced. For example, smaller particle sizes of GBPF (less than 80 μm diameter) had a higher amount of RS, while flour particles bigger than 156 μm had more TDF, ash, protein and phenolic compounds (Segundo et al., 2017a). Briefly, depending on the enrichment purpose, selecting a proper procedure is imperative. Table 2.2 demonstrates the effect of various drying process on produced banana flour properties regarding the type and part of banana used.

Table 2.2. The effect of different drying methods on banana flours

Method	Conditions	Banana flour type	Remarks	Reference
Room drying	23°C 6 d	GBPeF	- Minimum or no Maillard Reaction - Long preparation time	(Eshak, 2016)
Oven drying	40°C - 60°C 12h - 24h	GBPF	- No changes in phenolic acids and flavonols (thermally stable) - ↓ 50% of RS in final flour - ↓ Epicatechin (the most abundant flavan-3-ol in green banana)	(Juarez-Garcia et al., 2006, Alkarkhi et al., 2011, Segundo et al., 2017a, Pico et al., 2019b)
Air flow drying	55°C, 6h 1.4m/s	GBPF	- RS content remained constant between GBPF and banana paste - Low dispersibility and solubility in water	(Tribess et al., 2009)
Spouted bed with hot air flow	80°C 50 m^3/h	GBF GBPF	- No need for grinding process after drying - Maintaining RS to 35% in GBF and 42% in GBPF - RS content remained constant between GBPF and banana paste	(Bezerra et al., 2013b)

			- Low solubility under cold conditions	
Pulsed-fluidized bed agglomeration	95 °C, 0.3 m/s 10 Hz 1 m/s (air flow)	GBPF	- Lower wetting time - Dispersible in cold water - Higher level of RS achieved in GBPF	(Rayo et al., 2015)
Ultrasound and pulsed vacuum followed by air drier	20-25 min (ultrasound) 60 min under 50 KPa pressure (vacuum convective drier) 50 °C (air drying)	GBPF	- ↓ RS in ultrasound pre-treatment samples which followed by 60 °C heating in GBPF - No significant increase in RS amount	(La Fuente et al., 2017)
Microwave heating	960 W for 6 min	YBPeF	- ↓ Lightness more than other drying methods - Lowest drying time - Minimum loss of bioactive compounds, total phenolic and antioxidant activity - Maximum moisture content compared to other drying methods (but below the safe limit)	(Vu et al., 2017)

Freeze drying	-50 °C, 12 – 48 h	GBPeF GBPF GBPF YBPF	- Maintained the original colour regarding the least browning reaction - Highest value of total flavonoids reported in YBPeF and GBPF	(Da Mota et al., 2000, Wang et al., 2012, Türker et al., 2016, Vu et al., 2017, Pico et al., 2019b)
Spray drier	27000 rpm (atomizer rotation) 50% (biomass concentration) 30 °C (biomass temperature) 40 mL/min (biomass flow rate)	GBPF	- Moisture content of 9.17% - Fastest method after microwave - No analytical measurements	(Oi et al., 2013)
Dehumidified drier	60 °C, 13 h	YBPeF	- Highest flour lightness after freeze drying - Lowest phenolic compounds	(Vu et al., 2017)

GBPeF, green banana peel flour. GBPF, green banana pulp flour. YBPeF, yellow banana peel flour. YBPF, yellow banana pulp flour. RS, resistant starch.

2.5. Banana flour applications

The high DF content of banana and high levels of mentioned bioactive compounds have enabled the production of BF-fortified food products with remarkable functionalities. Moreover, discovering high nutrition value of BPeF represents a low-cost by-product for industrial application. As discussed earlier, depending on the ripening stage, there are four flour products produced from banana with different chemical composition (Table 2.1). Due to the structure of BF, cereal-based products have gained more attention than other food products. The main starch-based foods targeted for enrichment with BF products are listed in the following sections. In each food product section, the effect of BF addition has been reviewed into three categories: physicochemical, structural and nutritional. At the end of this section, Table 2.3 gives a summary of the finding reported in literature.

2.5.1. Bread

2.5.1.1. Physicochemical properties

In a study by Juarez-Garcia et al. (2006), GBF was obtained from a Mexican species (*Musa paradisiacal L.*) to develop a high gluten bread using 37% GBF in the formulation. Chemical composition analysis showed an increase in ash, protein, TDF and starch percentage of banana bread compared to wheat bread. Similarly, increased protein and TDF content were reported in leavened bread containing 20% and 30% fermented green banana slurry substituted with wheat flour (Adebayo-

Oyetoro et al., 2016). In another study, while TDF and ash content increased significantly in 20% fortification of bread made with GBF, protein content decreased by 5% due to the reduced amount of gluten (Gomes et al., 2016). Also, Gomes et al. (2016) reported that the moisture content (MC) and water activity (a_w) of the fortified bread increased significantly compared to a traditional bread made with no GBF fortification. This was explained by the increased capacity of DF which came from GBF. These findings were similar to a 10%-fortified balady bread with GBPeF: increased DF, MC and protein. Unfortunately, statistical analysis was not available in this study to understand the reason behind the increase in protein content (Eshak, 2016).

In terms of bread lightness changes (L*), Mohamed et al. (2010) findings on composite bread with 10% peeled yellow banana pseudo-stem flour fortification with wheat flour resulted in a darker bread compared to the control sample. The presence of reducing sugars in the BF was described as the reason of the Maillard Reactions. In another report, (Kurhade et al., 2016) found that with the fortification of yellow banana peel flour (YBPeF) at any ratio with wheat flour in chapatti bread, L* index was declined.

2.5.1.2. Structural (textural) properties

In a study on gluten-added bread, substitution of 25% of a commercial freeze-dried banana powder (maturity level was not mentioned) with wheat flour resulted in increased volume and viscosity of leavened bread. Regarding the evaluation of shelf life, while bread firmness increased in higher banana powder fortification level regardless of storage temperature (25°C, 4°C and -20°C), the stiffness of the control was higher than BF-fortified bread. By comparing storage temperature, it was stated that bread stored at -20°C up to seven days, experienced lower firmness compared to other samples (Mohamed et al., 2010). Similar findings are reported by Ho et al. (2013) who prepared a steam bread with 30% GBPF wheat flour substitution. However, the adverse impact of 30% GBPF with 80% added gluten caused an increase in hardness and adhesiveness in the final bread product. The cohesiveness, elasticity and chewiness of bread supplemented with 30% GBPF were decreased due to the lack of consistency in gluten structure. With reference to higher specific volume in banana bread samples containing 8% gluten, researchers explained that GBPF could affect the gluten network and attenuate the gas holding capability of the dough, which leads to low elasticity and expansion in leavened bread. Loong and Wong (2018) associated the increased hardness in Chinese steamed bread fortified with 15% GBPF with the presence of more DF, incomplete gelatinisation of starch, less gluten matrix and less expansion of gas cells. In order to improve the texture quality, a following study investigated the relation between gum type and BF effect on volume, in which the addition of 0.8% sodium carboxymethyl cellulose (NaCMC) improved specific volume (Ho et al., 2015). NaCMC, which is a soluble DF, acts like a sponge and absorbs water in the intestine. Therefore, it helps in mixing with the starchy food’s structure to form a dense structure that results in slowing down the rate of digestion. As a result, it can be a suitable additive together with peeled yellow banana pseudo-stem flour (Fig 2.2). These findings were in agreement with Steel et al. (2013) who researched on fortified pan bread with 20% of GBPF and also consistent with those who worked on yellow banana peel flour (YBPeF) and GBF, respectively (Kurhade et al., 2016, Gomes et al., 2016). The chapatti containing 10% YBPeF was found to be softer owing to a decrease in the tear force (Kurhade et al., 2016). In contrary to previous findings, Eshak (2016) also reported a higher amount of protein in banana bread (12.52%) which was ascribed to the slightly higher amount of protein in GBPeF (8.74%) compared to wheat flour (8.68%). Although the bread baking performance and textural analysis were not done in the study, the pictures showed an irregular shape (Fig 2.3).

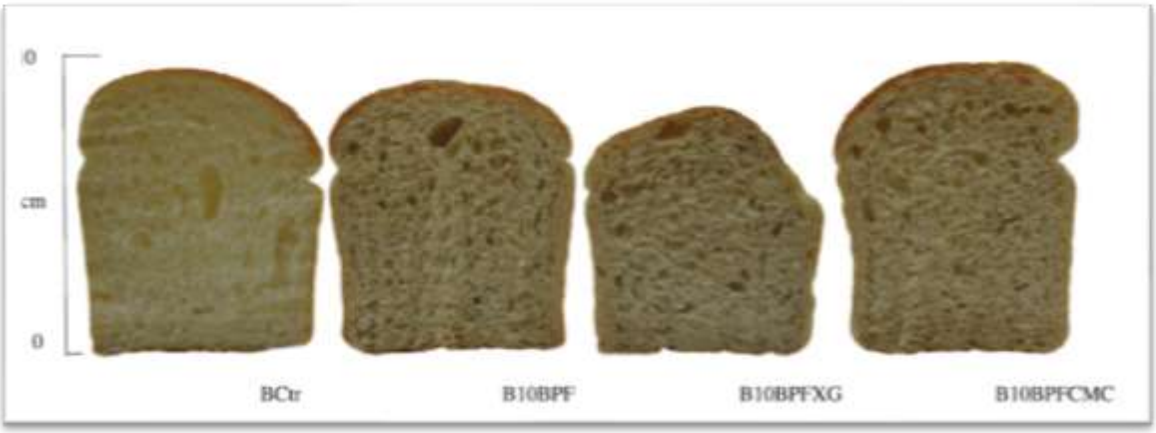

Figure 2.2. Bread crumb cross-sectional views (Ho et al., 2015)

BCt, control bread, B10BPF, fortified with 10% pseudo-stem flour, XG, xanthan, CMC, sodium carboxymethyl cellulose

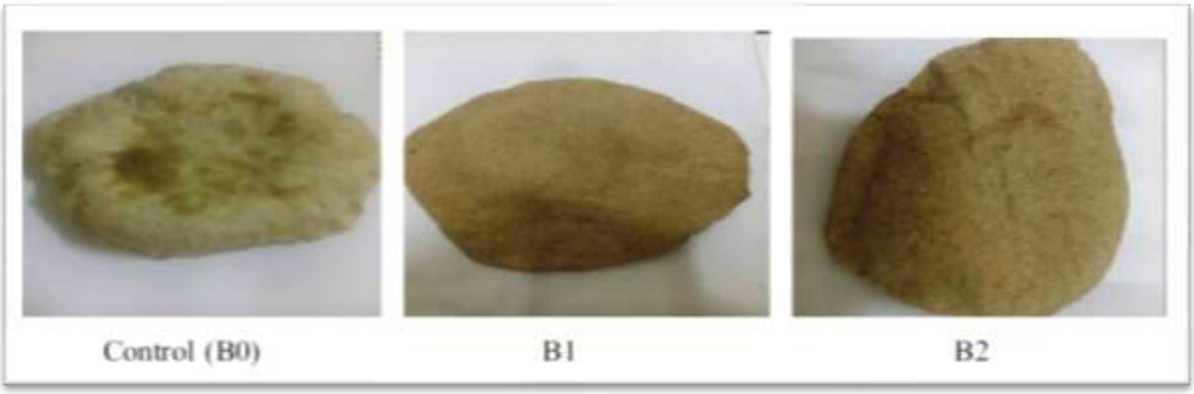

Figure 2.3. The balady bread (Eshak, 2016)

B0, control bread, B1, 5%-fortified sample, B2, 10%-fortified sample with GBPeF

2.5.1.3. Nutritional and sensory attributes

In a study conducted by Juarez-Garcia et al. (2006), even though RS content was decreased from 17.5% in GBPF to 6.7% in a 100% GBPF-fortified bread (with the addition of 20% gluten). The authors also reported a significant decrease in predicted Glycaemic Index (pGI) and Hydrolysis Index (HI) of the final product; a result that was in accordance with a higher value of total indigestible fraction, the main ingredients unavailable for digestion in the small intestine. A reduction in TDF level and a slight increase in RS level (2.6%) were observed in a freeze-dried tortilla bread containing 40%-GBPF substituted with corn flour. Moreover, higher values of pGI and HI than control showed that GBPF addition may not be a proper enrichment strategy for the purpose of producing a low-calorie tortilla bread (Aparicio-Saguilan et al., 2013). This could also be attributed to the high amount of RS2 in corn flour which could lead to a major increase in RS amount (Robles-

Ramírez et al., 2012). Total phenolic content, flavonoids, antioxidants and free radical scavenging activity were higher in a chapatti bread fortified with 10% YBPeF than the control sample, which justified the high concentration of these elements in YBPeF (Kurhade et al., 2016).

In another study on GBPF bread fortification by Ho et al. (2013), a significant increase was shown in a 10%-fortified bread with GBPF without the addition of hydrocolloids compared to the samples with added xanthan and sodium carboxy methyl cellulose. Also a significant increase in total phenolic content and TDF demonstrated the fact that peeled mature banana pseudo-stem flour could be effective in producing a functional composite bread (Ho et al., 2013). These findings were attributed to the high antioxidant activity of GBPF due to a higher level of phenolic compounds (Pico et al., 2019b). In a following study, researchers also found an interaction between gum type and BF effect on bread specific volume, in which the addition of 0.8% sodium carboxymethyl cellulose (NaCMC) improved minerals (Na, K, Mg and Ca) and RS content (14.98%) more than the same amount of xanthan (Ho et al., 2015).

The sensory evaluation on bread samples in the following studies was conducted using the seven-point hedonic scale. The GBF substitution with wheat flour resulted in lower sensory scores in 20% enriched-samples (Gomes et al., 2016). However, no significant difference was observed between 10%-fortified bread with GBPF and control bread sample, except for colour and softness in a sensory analysis (Ho et al., 2013). This was similar to a bread made with 10% YBPeF substitution with wheat flour which its overall acceptability score was not distinguished by panellists up to 10% of substitution (Kurhade et al., 2016). Nevertheless, when green banana peel flour (GBPeF) was added to a level of 10% in balady bread in another study, the control sample scored better in all sensory parameters, except taste and chewiness (Eshak, 2016). In contrast, the Chinese steamed bread fortified with 15% GBPF showed a higher overall acceptability compared to wheat bread (Loong and Wong, 2018). In accordance with the application of GBPeF in previous studies, sensory scores diminished for all treatments with more than 10% substitution of green banana slurry with wheat flour (Adebayo-Oyetoro et al., 2016). It can be concluded that the mixed findings in literature could be due to the hedonic test which is based on non-trained consumers' perception.

2.5.2. Pasta

2.5.2.1. Physicochemical properties

The properties of spaghetti enriched with different substitution ratios of GBPF with semolina flour was investigated in two studies. Following a similar pattern in most of the banana bread products, a reduction in lightness, protein and fat content of spaghetti containing 20, 35 and 40% GBPF was reported (Ovando-Martinez et al., 2009). In another study, increased water absorption led to an increased cooking loss in a 15%-GBPF fortified pasta compared to control samples (Krishnan and

Prabhasankar, 2010). Amylose leaching from starch structure during the cooking process was mentioned to be the reason behind this phenomenon.

Incorporation of green banana parts, individually, has also been considered in pasta products. A separate substitution of 10% green pulp and peel (obtained from two different varieties, *Cavendish* and *dream*) with wheat flour was conducted to produce darker noodles (the lowest a*and L* values for GBPeF-fortified samples) (Ramli et al., 2009). It was explained that an extension of the Maillard reaction was possibly associated with non-enzymatic browning reactions. In addition, polyphenol oxidase activity in banana peel could be contributed to enzymatic browning. In another study, banana flour was prepared from a commercial GBPF and added to tagliatelle pasta up to 30% substitution with wheat flour. Contrary to previous findings, there was no evidence of darker colour in banana pasta which possibly was due to the use of wheat flour instead of semolina flour (Zheng et al., 2016). However, Biernacka et al. (2020) explained the swelling of the wheat pasta and the conversion of pigments caused by cooking as the reasoned of darkening fortified pasta with commercial 5% GBPF.

2.5.2.2. Structural (textural) properties

Ovando-Martinez et al. (2009) study on fortified spaghetti with 20, 35 and 40% GBPF showed an increased adhesiveness and chewiness compared to the control, which was because of the release of amylose from starch granules during cooking. This also caused a rise in cooking loss (less than 7%) with increasing GBPF in the formulation. Since, regarding that a cooking loss below 8% is considered acceptable for semolina-based pasta products, it might not be considered as a negative effect. On account of the high amount of RS (12%) and incomplete gelatinization of starch granules, enriched samples showed lower values in vitro digestion tests. Krishnan and Prabhasankar (2010) findings showed a substantial decrease in pasta firmness in samples fortified with 15% GBPF compared to control. This result could be due to the insufficient gluten strength because of the substitution. Biernacka et al. (2020) reported only in lower fortification level of GBPF (3% substitution with wheat flour) cutting force can be increased significantly.

Whilst a 10%-GBPF fortified noodles exhibited higher elasticity, fortified samples with 10% GBPeF showed the lowest values. In discussion, it was stated that higher sugar content was responsible for high levels of total solid content which led to an increase in the density of the molecular structure (Ramli et al., 2009). Also, noodles prepared from GBPF contained high levels of starch which made them gradually become disintegrated over cooking. This could result in swelling of starch granules and more water absorption (Cleary and Brennan, 2006).

2.5.2.3. Nutritional and sensory attributes

Pasta products are foodstuffs with an important role in diets. In addition to being easily produced with a long shelf life, pasta products also have a lower predicted glycaemic index (pGI) in comparison with white bread or rice (Nilsson et al., 2008).

Hence, enrichment of pasta products with different DF-enriched flours and micronutrients has been considered in the last decade (Filipović et al., 2010). Since the GBPeF was higher in TDF but lower in RS content than the GBPF, the low pGI of 10%-fortified noodles with GBPeF was primarily because of its high TDF (Ramli et al., 2009). Also, digestion tests showed that 30% GBPF-fortified pasta is more resistant to enzyme digestion compared to wheat flour pasta (Zheng et al., 2016). The authors also reported that banana pasta presented higher ash, TDF and total phenolic content than the control, although, 15% substitution showed more ash content (Zheng et al., 2016).

In a study conducted by Agama-Acevedo et al. (2009), produced GBPF comprised of 42.54% RS2. Higher percentage of polyphenols and antioxidant capacity of banana pasta was reported in the spaghetti they made with 30% GBPF substituted with semolina. These results were confirmed by Krishnan and Prabhasankar (2010) findings a year later. Besides, they found a synergic relation between sprouted Ragi Flour and GBPF; in which a combination of 15% sprouted Ragi Flour and 15% GBPF reported to increase iron and zinc in pasta samples compared to control one. Moreover, it exhibited a higher antioxidant activity compared to 15%-GBPF fortified pasta sample. Because pasta is subjected to hydrothermal treatment prior to consumption, a decrease in the antioxidant activity of durum wheat pasta has been reported (Biernacka et al., 2020). Overall, the literature has many areas to investigate the impact of GBF on remaining RS content, especially in gluten free pasta products. Considering mixed results from textural properties of cooked pasta, the sole effect of GBF needs to be investigated.

2.5.3. Confectionaries

2.5.3.1. Physicochemical properties

Owing to its high sugar content, utilization of BF in confectionaries is increasing by the food industry, specifically cereal-based ones. The growth of pathogens in a cake premix substituted with 60% GBPF instead of wheat flour over four months of room storage was investigated. Despite the high sugar concentration, the pre-mixture remained significantly unaltered in pH and pathogenic growth, fungus or yeasts (Borges et al., 2010). In another study, foaming stability increased in the presence of 10% banana powder (maturity was not mentioned) in sponge cake (Park et al., 2010).

2.5.3.2. Structural (textural) properties

In a study by Park et al. (2010), it was reported that with the increase level of BPF to 20%, higher hardness was recorded in sponge cake, although, chewiness and adhesiveness were not significantly different amongst samples. Furthermore, all the fortified samples (even at 5% level) showed lower MC and specific volume compared to control. These results indicated the lower amount of MC in fortified samples had a negative impact on textural properties (Park et al., 2010). In terms of technological properties, sponge cakes were noticeably worsened with the presence of banana flour (lower specific volume, inferior sensory characteristics and higher hardness), which was diminished at the 15% ratio; except for cohesiveness that showed a dramatic decrease in all samples compared to the control. The authors accounted for different gelatinization and retrogradation behaviour of banana starch compared to wheat starch for textural changes and decreased sensory scores in banana cakes (Segundo et al., 2017a).

In concurrence with their previous work, increased hardness and decreased volume in sponge cake fortified with different levels of YBPF led to a more dense structure, especially in 40% banana cakes (Segundo et al., 2017b). The same behaviour was observed by Oliveira de Souza et al. (2018), even though they used a higher concentration of GBP puree instead of flour for pound cakes. Considering the correlation between volume and hardness was more significant in sponge than in layer cakes, the maturity of banana was not correlated to improvements of textural properties (Segundo et al., 2017b). Another research on a fortified biscuit, YBPeF was substituted up to 75% with wheat flour and caused a decline in hardness (Joshi, 2007). According to the discussions, prior treatments of mashed peels together with DF led to an increase in softness of the product. While a high level of consistency and crispiness is required in a biscuit product, reduced amount of gluten in higher concentrations of YBPeF led to insignificant decrease of these attributes in all enriched samples (Joshi, 2007). The findings reported by Carvalho and Conti-Silva

(2018), on fortified cereal bars with YBPeF exhibited a darker colour, more hardness and adhesiveness at 14% level.

2.5.3.3. Nutritional and sensory attributes

With the aim of increasing DF and RS in layer and sponge cakes, GBPF was added at different particle size, ranging from 80 μm (fine) to 200 μm (coarse) in diameter. Researchers showed that the fine flour comprised of 40% RS compared to 25% RS in the coarse flour. This fact specified higher RS content (about 3%) in 30% replacement samples with fine flours in both layer and sponge cakes. However, the percentage of TDF and phenolic compounds were higher in the coarse flour attributes (Segundo et al., 2017a). However, the flour with fine particle size showed significantly higher RS content compared to medium and coarse ones (more than 10%). The presence of particles with different size indicates differences in the hardness between different parts of the dried pulp. The coarse parts could come from the hardest fractions of the dried pulp, resulting in prolonged times inside the laboratory mill before escaping through the mesh and consequently initiating some damage at granular level (Segundo et al., 2017a). Similar results were reported in their following study on the selection of YBPF in 40% substitution with sugar in sponge and layer cakes. Both cake types depicted enhancement in DF, polyphenols and antioxidant capacity values (Segundo et al., 2017b). With focusing on sugar reduction in confectionaries, cookies containing 20% of YBPeF were rich in TDF and were categorized as a low-calorie product (Agama-Acevedo et al., 2012). The same pattern was reported by Elaveniya and Jayamuthunagai (2014) in which banana blossom powder was added at 5 g in 100 g of biscuit formulation. Park et al. (2010) reported that up to 10% fortification of sponge cake with BPF could bring reasonably high overall acceptability. In another study, consumer acceptance of biscuit samples for colour, crispiness and taste decreased with the addition of BPe, while it was acceptable at 5% substitution (Elaveniya and Jayamuthunagai, 2014). Organoleptic results were not significantly different in terms of colour, flavour, after taste and mouthfeel at even the 75% level of substitution with YBPF in biscuit (Joshi, 2007).

Overall, replacement of BF, regardless of ripeness degree, was feasible up to 15% for achieving optimal quality and maximum 30% for functional purposes in confectionaries (Carvalho and Conti-Silva, 2018). Still, quality control of produced different types of cakes with BPeF is needed to be investigated.

2.5.4. Gluten-free products

Because of the rise of the gluten related disorders, such as celiac disease and *dermatitis herpetiformis*, it has been essential to expand the gluten-free (GF) food market. Moreover, considering that untreated celiac disease contributes to intestinal cancer, nutrient deficiencies and oxidative stress, developing GF products with the consideration of additional nutritional values is highly important (Wang et al., 2017b). In this regard, bioactive compounds are a prominent ingredient in GF foods. As most of GF starchy products do not provide proper technological qualities, application of different types of BF has been considered recently (Torres et al., 2017).

2.5.4.1. Physicochemical properties

Zandonadi et al. (2012) reported a reduction in protein and increase in ash content in a 47% GBPF fortified-GF pasta. Also, cooking time increased significantly for GF-pasta samples. Researchers attributed this to more presence of RS in GF-samples, which had a higher gelatinization temperature, hence, needed more time for being fully cooked. An increase in ash, TDF and MC content was reported in a GF-layer cake made with 100% GBPF compared to 100% corn flour one (Segundo et al., 2020). Also, substitution of corn flour with GBPF improved the yellowness and lightness index in the samples.

2.5.4.2. Structural (textural) properties

By incorporation of 47% commercial GBPF in 100 g pasta formulation, Zandonadi et al. (2012) studied on a GF-pasta with an additional proportion of egg white and hydrocolloids. Regarding excess amount of water absorption during cooking, increased stickiness of the product led to a weakened structure with a higher cooking loss compared to standard pasta sample. However, Rachman et al. (2019) reported that optimum cooking time decreased in a GF-pasta made with 100% BF because of the poor structure in the absence of a gluten network which resulted in faster water penetration into the pasta structure. Likewise, a decrease in firmness was reported in Rachman et al. (2019) study which was associated to the use of egg white, guar and xanthan gums in GF-samples. Similar behaviour was reported in 100%-banana GF-pasta together with extensibility decrease (Rachman et al., 2019). The authors ascribed this due to the lower protein content of banana flour (maturity level was not mentioned). They showed with increasing protein content by the addition of egg white powder, firmness can be increased significantly.

Gluten free bread has also been produced by the use of BF. In a study conducted by Sarawong et al. (2014a), while crumb firmness increased in 15% of green plantain flour addition, bread volume improved by 25% addition at a lower baking temperature over a longer time. An elevated proportion of green plantain flour in GF-bread contributed to higher water binding capacity and a reduction in retrogradation owing to the presence of extra starch hydration. Also, breadcrumb started to become significantly darker at 5% addition level. In sweetened GF-bread, both green and yellow fortified bread samples showed lower volume, lesser height and darker colour, but, when black banana pulp flour was utilized at 20% of the formulation, those variables improved to a significant level (Seguchi et al., 2014).

The substitution of 15% of GBPeF was with rice flour in GF cake gave rise to a decrease in GF cake volume. Due to an increase in viscosity, the density of banana GF cakes rose (Türker et al., 2016). Higher water absorption and soluble DF in GBPF made the GF-sponge cake batter fortified with 30% GBPF show the highest storage modulus amongst all samples, even those made with corn flour (Segundo et al., 2020). They correlated the compacted structure in the layer cake batter made with 100% GBPF instead of corn flour to larger size of banana starch molecules compared to corn flour ones. Increased elastic properties (G′) also might lead into a decrease in gas expansion during baking of cake samples (Segundo et al., 2020).

2.5.4.3. Nutritional and sensory attributes

Radoi et al. (2015) reported a growth in total phenolic acids, cinnamic acids and minerals (mainly Fe, Cu, Zn, Mn, and Ni) with presence of 30-40% banana pasta. Also, Seguchi et al. (2014) showed an increase in RS amount could be achieved by 2.5% fortification of GF-bread. In terms of antioxidant activity, baking temperature could decrease the phenolic compounds. The addition of 28.5% of commercial GBPF to GF-muffins resulted in a reduction in the antioxidant activity. This was associated with the high temperature applied during baking which led to the degradation of essential compounds such as phenolics and anthocyanins (Radünz et al., 2020). Overall quality of GF-pasta fortified with 47% GBPF received better scores in sensory evaluation (Zandonadi et al., 2012). Similar acceptability index was reported in Radünz et al. (2020) study carried out on fortified GF-muffins with 28.5% of commercial GBPF.

The GF-food products should be nutritious enough in order to compensate the loss of high-protein flour which contains minerals, vitamins and dietary fibres. Besides, the texture undermines negative changes, especially in products which high porosity and specific volume is required. The insufficient work on textural and rheological aspects of GF-foods and contradictory results shows that still there is a need for more research in this area. Table 2.3 summarizes the detailed findings of the literature.

Table 2.3. banana flour application effect on different properties of food products

Food product	Type and level of banana fort.	Drying method	Physicochemical properties	Textural/ rheological properties	Volatile analysis	Storage quality	Ref.
Bread							
Chinese steamed bread	GBPF (10, 15%)	OD at 50 °C/ 18 h	↑ SV ↓ L*, b*	↑ Hardness ↓ Cohesiveness and adhesiveness	NA	NA	(Loong and Wong, 2018)
Non fermented gluten-added flat bread	GBPF (38%)	OD at 50 °C	↑ Ash, MC, RS, TDF in GBPF and fortified bread ↓ Lipid, HI and pGI	NA	NA	NA	(Juarez-Garcia et al., 2006)
Leavened gluten-added bread	Commercial BF (10-30%) (banana ripening stage	NA	↑ Loaf volume up at 10% with a reduction at higher levels ↓ Freezable water above 20% ↑ MC at 20%	↑ G′ in 30%-samples Bread firmness (staling): At -20, 4 and 25°C: ↓ Up to 10%	NA	↓ firmness at -20, 4 and 25 °C until the second day at 10%	(Mohamed et al., 2010)

	was not mentioned)		↓ L* in crumb and crust without a significant change in a* of crumb	then ↑ (from 20% was no significant) Only in -20°C firmness increasing wasn't significant after 2,5 and 7d		Equal firmness after 7 days storage at -20 °C for 30%-sample	
Steamed gluten-added bread	GBPF (30%)	OD at 60 °C/ 12 h	↑ WHC and OHC ↑ Volume ↓ L* ↑ RS, ash, TDF, fat ↑ K, Mg and Ca in both	↑ Hardness and adhesiveness ↓ Cohesiveness, elasticity and chewiness	NA	NA	(Noor Aziah et al., 2012)
Freeze-dried Tortilla bread (with corn flour)	Commercial GBPF (40%)	NA	↓ Prt and TDF ↑ RS, ash, fat and TS remained constant ↑ HI ↑ pGI in 0h, decreased after 1d	NA	NA	NA	(Aparicio-Saguilan et al., 2013)
Composite bread with	BPsF (10%)	OD at 60 °C/ 24 h	↓ SV, LV, LH ↑ SV in presence of NaCMC WL remained constant	NA	NA	NA	(Ho et al., 2013)

hydrocolloids			↑ ϱ ↑ Ash, MC, TDF ↓ Prt, fat, L* ↑ Phenolics, DPPH and FRAP values				
Unleavened Indian flat bread	YBPeF (5-20%)	35 °C/ unknown time	↑ Total phenolic content and flavonoid ↑ Antioxidant activity, free radical scavenging activity and flavonoid content ↑ WHC ↓ L* starting at 5%-samples	↑ Dough stickiness, strength, adhesion, rollability and kneading Sticky dough at 20% ↓ Extensibility and tear force	NA	NA	(Kurhade et al., 2016)
Balady flat bread	GBPeF (5%, 10%)	At room for 6 d	↑ Prt, TDF, WHC and fat ↑ WHC and OHC ↓ MC ↑ Ca in 5% but ↓ in 10%-sample ↑ Mg, Zn, Fe, K ↓ P	NA	NA	NA	(Eshak, 2016)

Leavened bread	GBPF (10-30%)	OD at 60 °C/ 24 h followed by a 24 h fermentation	↑ MC in 30% ↑ Swelling properties and ϱ in 30% Ash and fat, Zn and Fe remained constant in all samples ↑ TDF and prt ↓ L*	NA	NA	NA	(Adebayo -Oyetoro et al., 2016)
Leavened bread	GBF (10, 20%)	OD at 50 °C/ 72 h	↓ SV ↑ TDF and ash ↓ caloric value, prt ↓ L*	↑ Hardness in 20%	NA	NA	(Gomes et al., 2016)
			Pasta products				
Wheat pasta	GBPF (1-5%)	FD at 40 °C/ 12 h	↑ Cooking loss ↓ L* and b* ↓ Antioxidant activity Water absorption remained constant	↑ Firmness only up to 3%	NA	NA	(Biernacka et al., 2020)

Spaghetti	GBPF (15-45%)	50 °C/ unknown time	↑ Diameter of cooked spaghetti in 30% ↑ WBC (Only in 15%) ↓ L*	Hardness and elasticity remained constant ↑ Adhesiveness and chewiness	NA	NA	(Agama-Acevedo et al., 2009)
Spaghetti	GBPF (15-45%)	50 °C/ unknown time	↑ Cooking loss from 15% but constant in 30 and 45%-samples ↓ Prt and fat ↑ Ash and RS (42% in BF and 12% in 45%-sample) ↑ In vitro carbohydrate hydrolysis rate in control sample ↑ Polyphenols ↑ Antioxidant capacity	NA	NA	NA	(Ovando-Martinez et al., 2009)
Yellow alkaline noodles	GBPF GBPeF YBPF YBPeF	OD at 60 °C/ 12 h	pGI: Least pGI was observed in GBPeF RS: GBPF > GBPeF	↑ Elasticity in pulp-enriched samples	NA	NA	(Ramli et al., 2009)

	Prepared from 2 varieties (10%)		TDF: GBPF < GBPeF				
Semolina pasta	Commercial GBPF (10-30%)		↓ Prt and fat ↑ TDF ↑Antioxidant capacity ↑ Minerals ↑ Cooking loss ↓ L*	↓ Firmness	NA	NA	(Krishnan and Prabhasankar, 2010)
Pasta paste	Whole green banana paste (25-75%)	No drying and extrusion	↑ Ash, ↓ crude fibre, prt, MC ↓ Oxidative damage in rat kidneys	NA	NA	NA	(da Silva et al., 2016)
Tagliatelle pasta	GBPF 4:1 GBPeF (15, 30%)	OD at 60 °C/ 20 h	↑ Ash, fat, TDF MC and colour remained constant ↓ Prt	NA	NA	NA	(Castelo-Branco et al., 2017)

Confectionaries							
Cookie	GBPF (15-50%)	OD at 50 °C/ unknown time	↑Ash, MC, TDF and RS (from 2.3 to 8.37%) ↓ Prt and fat in 30 and 50%-samples ↓ HI and pGI in 30 and 50%-samples	NA	NA	NA	(Agama-Acevedo et al., 2012)
Fish cracker **Cassava cracker**	GBPF (5-25%) GBPF (10-50%)	FD at 50 °C/ 12 h	↑ Ash, TDF ↑ ϱ ↓ porosity ↓ L* ↑ b* ↑ K, Mg, P and Ca ↓ Fat ↑ Total polyphenol and superoxide radical scavenging capacity Water absorption remained constant	NA	NA	NA	(Wang et al., 2012)

Sponge cake **Layer cake**	YBPF (20, 40% substituted with sugar)	NA	↑ DF (<1%) ↑ Polyphenols	↑ Hardness ↓ Cohesiveness Constant springiness	NA	NA	(Segundo et al., 2017b)
Biscuits	YBPF (4%)	OD at 60 °C/ 12 h	↓ L* ↑ Spread ratio ↑ WHC and OHC ↑ Total phenolic and flavonoid content	NA	NA	NA	(Mahloko et al., 2019)
			Gluten-free products				
Pasta	Commercial GBPF (47%)	NA	↓ Fibre, prt, lipid ↑ WHC ↑ Volume ↑ Ash	↑ Stickiness ↓ Firmness	NA	NA	(Zandonadi et al., 2012)
Pasta	Commercial GBPF (47-80%)	NA	↓ Digestion rate in fortified samples	NA	NA	NA	(Zheng et al., 2016)
Bread	Commercial GBPF (5-35%)	NA	↓ L* at 5% ↑ SV up to 25% ↑ RS in all fortified samples up to 2.5%	↑ Firmness at 15%	NA	NA	(Sarawong et al., 2014a)

Bread	GBPF YBPF BBPF (22%)	FD (15 °C/70 h)	BBPF-fortified breads: ↑ Bread height ↑ SV ↓ L* in all samples	↓ μ in BBPF-fortified samples	NA	NA	(Seguchi et al., 2014)
Cake	GBPeF (5-20% substituted with rice flour)	FD (48 h)	↓ Vol ↑ ϱ ↓ Baking loss (15%, 20%)	NA	NA	NA	(Türker et al., 2016)
Other food products							
Jelly	BPeF (10-30%) (semi ripe)	FD (48 h)	↑ Ash, prt, fat, TDF, carbohydrates and pectin ↑ Total phenolic and flavonoids ↓ MC	↑ Hardness, chewiness and springiness with increasing BPeF compared to lower levels	NA	NA	(Lee et al., 2010)
Fermented synbiotic milk	GBPF (1-5%)	OD for 24 h (unknown T)	↓ pH after 14 days ↑ Post acidification, proteolysis and concentration of lactic and acetic acids up to 3% ↑ Probiotic growth after a week of storage	NA	↑ Hexanol and pentanol after 14	↑ Hardness after two weeks of storage	(Batista et al., 2017)

			↑ RS up to five times in 5%-samples ↓ RS after 21 days of storage		days of storage ↑ 2-nonanone in at least 3%-samples		
Ice cream	GBPF and GBPeF (1-2%)	OD at 60 °C/ 12 h	↓ Overrun value in all fortified samples ↑ K, Mg, P, Fe, Zn and Na in all peel-fortified samples compared to pulp ones ↓ Ca in both sample types ↓ L* more in peel-fortified samples	↑ μ in 2%-peel fortified samples ↓ μ in all pulp samples	NA	NA	(Yangilar, 2015)
Dried banana	YBPF	Heat pump drying for 24	NA	NA	↓ Ester, alcohol and	NA	(Saha et al., 2018)

		h (unkno wn T)			aldehyde compounds right after drying		
Banana powder	YBPF	Vacuum belt drying (1150 Pa) FD OD (75 °C)	NA	NA	Preserved 3-methylbutanoic acid 3-methylbutyl ester, 3-methylbutyl acetate in	NA	(Wang et al., 2007)

	FD- sample

BPsF, banana pseudo-stem flour. ϱ density, FDF, freeze-dried flour, FD, freeze-dried, GBF, whole green banana flour, GBPF, green banana pulp flour, GBPeF, green banana peel flour, YBPF, yellow banana pulp flour, YBPeF, yellow banana peel flour, BBPF, black banana pulp flour, ODF, oven-dried flour, OD, oven-dried, SV, specific volume, μ, viscosity, vol, loaf volume, ϱ, density, RS, resistant starch, WHC, water holding capacity, OHC, oil holding capacity, TDF, total dietary fibre, prt, protein, MC, moisture content, L*, lightness index, b*, yellowness index, HI, starch hydrolysis index, pGI, predicted glycaemic index, T, temperature.

2.6. Conclusion

It is desirable to find proper food applications for banana pulp and peel for achieving two goals: First, helping the environment through sustainability by utilizing secondary processing products and second, creating a new outlook for consumers and producers for generating value-added food products. Besides adding nutritional value to food products, GBF also stands out for not creating production waste, thus representing the complete use of the fruit, increasing the yield and reducing manpower costs due to peeling which is not required. In gluten-added starchy products, such as bread, cake and pasta, textural properties were not negatively affected by banana flour addition to a certain extent. As discussed in this chapter, there are several methods for producing banana flour. Comparing new drying technologies with existing methods and their effect on bioactive components of produced products would be an important subject for future research. This was one of the areas that has been covered in this thesis.

CHAPTER 3

Research strategy

Chapter aim:

This chapter shows the detailed objectives of this thesis.

3.1. Research problem statement

As discussed in the literature review, it is clear that over the past decade, a wealth of research has been done on the impact of different types of banana flour (BF) on various food quality attributes. What is applicable in food industry though, is to what extent this new food ingredient can improve nutritional and technological properties. Comparing the composition of different types of BF (yellow banana pulp flour, yellow banana peel flour, green banana pulp flour, green banana peel flour) showed higher nutritional value of GBPF and GBPeF. In addition to showing an overall significant difference from the technological point of view in food applications, the use of unripe fruit flour exhibited higher total dietary fibre (TDF), resistant starch (RS) and antioxidant activity in the final product compared to the application of ripe banana flour. However, the following topics were either missing from the literature or were not been thoroughly investigated.

3.1.1. Preparation of the whole green banana flour

Considering that 40% of green banana weight is in its nutritious peel, hence, this part was removed in most of the "green banana fortification" studies. The application of both peel and pulp was studied separately in some studies and compared with each other (Table 2.3). However, preparing a flour from the whole fruit and fortifying bread to then investigate its effect on different dough and bread properties was seldom studied. **Chapter 4** and **5** focuses on optimizing the drying methods (thermal and non-thermal techniques) with providing a full characterization of produced samples, ranging from technological to nutritional aspects.

3.1.2. Textural properties

The structure of bread particularly dependent on its high protein content (Segundo et al., 2017a). Gluten, is generally claimed to be a superior compared to other protein molecules in terms of creating a complex to bring elasticity in dough and optimum hardness in bread (Mancebo et al., 2017). However, use of other food ingredients, such as shortenings, hydrocolloids, milk powder, egg white powder has been commonly researched in fortified breads with other BF types instead of wheat flour (Ferrero, 2017, Curti et al., 2017). As observed in Table 2.3, the knowledge about textural changes after BF fortification is limited. Changes in textural properties of bread could be related to one step earlier, dough making. viscoelastic behaviour of dough [Storage modulus (G'), loss modulus (G''), loss tangent ($\tan \delta$), complex modulus (G^*) and complex viscosity ($|\eta^*|$)] together with large deformation mechanical alterations (hardness, adhesiveness, resilience, cohesiveness, springiness) after dough making, could show a better understanding of forthcoming changes in bread texture. **Chapter 6** focuses on examining these properties and also bread textural properties.

3.1.3. Starch analysis

Increasing prebiotic intake via food products has been an interest amongst food producers for decades. The importance of delivering effective dosage of these DFs confirms the necessary analysis of the fortified food products to quantify these components. In particular of interest, resistant starch (RS) is the dominant prebiotic in green banana, and it is vital to keep this DF intact until consumption. The review on literature showed that the studies conducted on RS remaining content of BF and the bread produced from it still needs more work. In order to understand the changes in starch from flour to bread, RS content was analysed in both flour and bread to monitor the loss of this prebiotic during the breadmaking process. Also, **Chapter 7** gives a comprehensive outlook to starch digestion showing the concentration of slow digestible and rapidly digestible starch.

3.1.3. Volatile compounds fingerprint

There is a lack of knowledge on the specific volatile compounds which are responsible for fortified bread with BF. While sensory evaluation was the main focus on many studies, analysis of volatile profile was neglected. Considering the mixed results in descriptive sensory analysis of different BF-bread products, **Chapter 8** aims to investigate the chemical compounds which are responsible for the distinctive fortified bread aroma.

3.2. Research strategy

For the first time in literature the whole green banana flour (GBF) was made with freeze-drying and its properties, from both technological and nutritional aspects, were compared to flour obtained from a modified drying method; an air-oven drying technique with reduced drying time. In addition, to make a fortified bread with acceptable structural properties, this study has provided a nutritious bread with the consideration of sustainability and increasing the resistant starch intake. Also, a comprehensive investigation on viscoelastic, textural and rheological properties of both dough and bread was fulfilled in this thesis. Another area which was not studied was the volatile profile of bread containing GBF. The last chapter of thesis assessed the feasibility of using an untargeted headspace gas chromatography mass spectroscopy fingerprinting and chemometrics to increase insight into the volatiles in different bread fortified with different levels and types of GBF. Detailed content of the objectives of this thesis is summarized in Table 3.1.

Table 3.1. The detailed content of the thesis experimental chapters

Experimental chapters	Objectives
Chapter 4: Effects of different drying conditions on the starch content, thermal properties and some of the physicochemical parameters of whole green banana flour	1. Green banana flour preparation (air oven-drying, freeze-drying) 2. Physicochemical properties of the GBF 3. Colour measurements 4. Oil and water holding properties 5. Relative crystallinity 6. Starch analysis (Resistant starch, total starch, amylose and amylopectin) 7. Diffraction scanning calorimetry

Chapter 5: Textural properties and characteristics of whole green banana flour produced by air-oven and freeze-drying processing	1. Sample preparation 2. Particle size distribution 3. Textural properties 4. Pasting/ rheology properties 5. Fourier-transform infrared spectroscopy 6. Minerals analysis
Chapter 6: Rheological, textural and structural changes in dough and bread partially substituted with whole green banana flour	1. Green banana flour preparation 2. Dough preparation 3. Viscoelastic properties 4. Large deformation mechanical properties of dough 5. Bread preparation 6. Bread characteristics 7. Bread baking performance

	8. Textural properties of fresh breadcrumb 9. Storage stability of bread
Chapter 7: The Effect of Bread Fortification with Whole Green Banana Flour on Its Physicochemical, Nutritional and In Vitro Digestibility	1. Bread preparation 2. Bread physicochemical properties 3. Bread crust and crumb colour 4. Mineral profile of bread samples 5. In vitro starch digestibility tests 6. Total starch analysis of bread 7. Resistant starch content of bread
Chapter 8: Evaluating volatile compounds in the fortified bread with the whole green banana flour: An integrated headspace GC-MS fingerprinting and chemometrics approach	1. Bread preparation 2. Headspace SPME GC-MS analysis 3. Data pre-processing of total ion chromatograms 4. Multivariate statistical data analysis

	5. The overall effect on crumb and crust 6. Effect of Crumb/ Crust per each fortification levels 7. Effect of fortification level in crumb and crust per processing 8. Effect of processing on crumb and crust

CHAPTER 4

Effects of different drying conditions on the starch content, thermal properties and physicochemical parameters of whole green banana flour

This chapter has been published as:

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Chapter aim:

The chapter's aim is to evaluate the effects of freeze-drying and air oven-drying at three different temperatures on the resistant starch content, thermal properties and some physicochemical parameters of whole green banana flour.

4.1. Introduction

The increased demand for functional food products has led to an increased interest in nutrients such as minerals, vitamins, bioactive compounds, fibre and prebiotics to be present in food formulations (Coleman et al., 2014). Amongst the prebiotics, resistant starch (RS) has gained a lot of attention in recent years, due to its acknowledged health benefits such as prevention and control of colon cancer, diabetes, and obesity (Birt et al., 2013). Resistant starch is present in grains, legumes and seeds (RS1), raw starchy plants (RS2), retrograded starchy foods (RS3), chemically modified starch (RS4) and in amylose-lipid complexes (RS5) that are considered to be physically inaccessible to digestion (Chávez-Salazar et al., 2017). Banana, the world's most favourite fruit, is one of the richest sources of RS2 at early stages of ripeness (Campuzano et al., 2018).

Globally, banana is reported to be the fourth most demanded food after rice, wheat and corn (Silva et al., 2015). According to FAO, banana (*Musa Cavendish, AAA group*) is an essential part of New Zealanders diet with a per capita consumption on average of 20 kg of banana per year, which is reported to be more than the worldwide average consumption (FAOSTAT, 2018). However, banana is the second most discarded food in New Zealand after bread, contributing to 3% of the total food waste (Edmunds, 2015). Banana is a perishable food commodity and is also sensitive to postharvest defects including bruising during transportation and browning because of inappropriate ripening environment (Maunahan et al., 2018).

Unripe banana is rich in RS which is recognized to have positive effects on colon health (Moongngarm et al., 2014), while ripe banana contains more digestible starch and protein (Ramli et al., 2009). Banana consists of two parts, the banana pulp (BP) and the banana peel (BPe). The pulp is a rich source of essential phytonutrients, phenolic compounds, B group vitamins, ascorbic acid and tocopherols, while the BPe is a rich source of minerals, bioactive compounds and dietary fibre (DF) such as pectin, cellulose, hemicelluloses and lignin (Zhu et al., 2018).

Considering the nutritional value of both pulp and peel of green bananas, the production of banana flour (BF) obtained by drying, provides a way of preserving the nutritional benefits and increasing the shelf-life of banana material (Wang et al., 2017a). The drying conditions used, such as different temperatures and humidity levels, can have a profound influence on both functional and technological characteristics of the obtained BF.

The effects of room drying (Eshak, 2016), air oven-drying (Yangilar, 2015, Segundo et al., 2017a), spouted bed with hot air flow (Bezerra et al., 2013b), pulsed-fluidized bed agglomeration (Rayo et al., 2015), ultrasound and pulsed-vacuum followed by air drying (La Fuente et al., 2017), microwave heating (Vu et al., 2017), freeze-drying (Türker et al., 2016, Vu et al., 2017) and spray drying (Oi et al., 2013) on some of the attributes of different green banana parts have been discussed in chapter 2. However, the collective information available comparing different air oven-drying temperatures with non-heat treatment processes on properties of the whole green banana flour (GBF), especially on RS content and hydration properties, have scarcely been reported in the literature. Additionally, the effect of freeze drying, which is considered to be one of the best dehydration methods for heat-sensitive materials, on thermal properties and starch content of GBF has not yet been investigated.

Compared to other methods of drying, freeze-drying is considered to be the best dehydration method for heat-sensitive materials to enable achievement of the highest possible quality, minimal comprising of colour, structure, nutrients and flavour (Ahmed et al., 2020). As most of the leading banana producers are located in developing countries, a simple and efficient drying method would be preferable for production of GBF with the highest possible yield and quality. Consequently, from the feasibility point of view in the food industry and the minimum effect on green banana nutrients, air oven-drying and freeze-drying (followed by blast freezing) were chosen for analysis in the present chapter, to investigate the effect of banana drying process on the properties of the produced flour. This chapter aims to evaluate the effect of freeze-drying and of three different air oven-drying methods on whole GBF, with examination of the effects of these methods on the starch content, functional behaviour and physicochemical properties.

4.2. Materials and Methods

4.2.1. Sample preparation

A total of 20 kg green banana (*Musa Cavendish* spp AAA) was supplied by a local food supply company in Dunedin, New Zealand between February and March 2018. The bananas were harvested approximately 100 days after anthesis and were not exposed to any maturation treatments. Bananas were processed on arrival and were used for the measurement of pH, firmness, total soluble solids and titratable acidity. The production steps used to produce the GBF are shown in Fig. 4.1. Wheat flour (WF) was provided by the same local food supply company in Dunedin New Zealand and was used as a reference sample.

4.2.2. Method optimisation

The process of selection and optimisation of the proper drying method took around four months based on the availability of the equipment at University of Otago, feasibility of applied methods in industry and novelty in literature (Table A1, appendix). For achieving the best comparison, drying methods was applied in two types: thermal and non-thermal. The selection of a thermal heat treating amongst dehumidified drying, spouted bed with hot air flow, microwave, spray drying, ultrasound and pulsed-vacuum followed and air-oven drying was based on these factors: literature study, availability of the equipment for a large scale sample preparation in a specific time period, the cost of renting the equipment from other departments, feasibility in food industry and how common a method is at the moment. The selection of the temperatures was based on the literature in the area of drying fruits. The 50 °C was selected as the lowest (based on the constant weight measurement method) and the 110 °C was selected as the maximum temperature in regards of the minimum drying time. The 80 °C was selected as the median one.

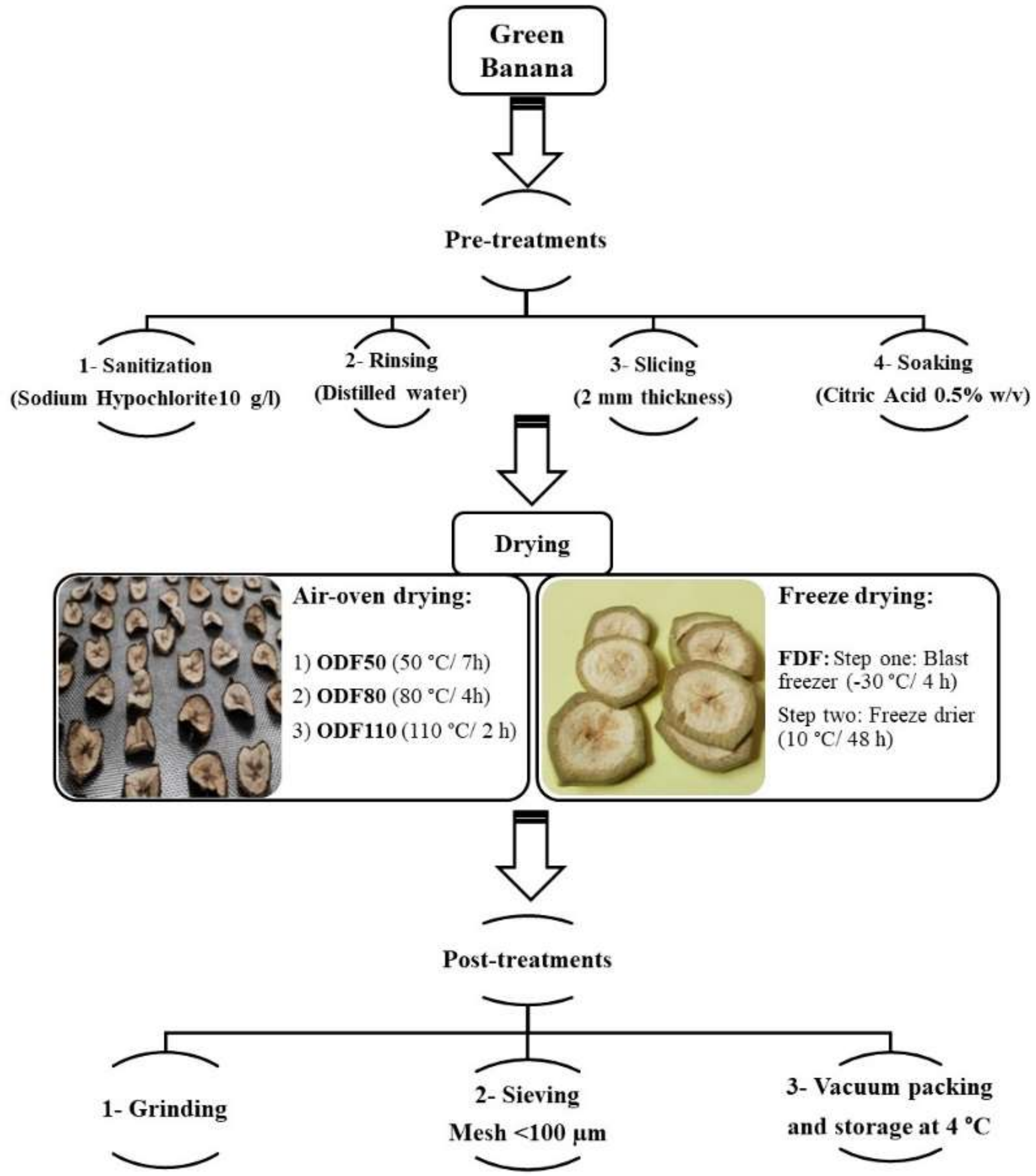

Figure 4.1. Flow chart of green banana flour production by freeze-drying and oven-drying

4.2.3. Sample characterization

The pH of processed banana samples was measured using a pH meter (HANNA Instruments, Woonsocket, USA). Titratable acidity was determined according to the official method 942.15 AOAC (AOAC, 2005) by titration with 0.1 N sodium hydroxide until a phenolphthalein indicator was just changed into a light pink colour. A hand-held refractometer (ATAGO N1, California, USA) was used to determine the total soluble solids with correction for acidity and temperature values according to Kar et al. (2003). Total solids were assessed by gravimetric determination after drying at 60 °C under a pressure of 60 mmHg for 16 hours.

4.2.4. Green banana flour production

The procedure was performed based on Yangilar (2015) method with modifications. After rinsing the supplied green bananas with distilled water containing sodium hypochlorite (10 g/l), the bananas were immersed in 0.5% (w/v) citric acid solution for 15 min and then drained, in order to minimize the enzymatic browning. The unpeeled green bananas were then cut into slices with two mm thickness and then dipped in 0.5% (w/v) citric acid solution for 15 min and then drained well. The treated sliced bananas were dried using either an air oven-drying method or freeze-drying method. The optimum condition of the drying methods was achieved based on literature and preliminary tests (Fig B1-B4, appendix).

4.2.4.1. Air oven-drying method

The sliced bananas were distributed on screened trays and transferred into a pre-heated forced-air oven-dryer (Steridium, Marrickville, NSW, Australia) at either 50 °C, 80 °C, or 110 °C for 7, 4 or 2 h, respectively, until no further change in weight was achieved.

4.2.4.2. Freeze-drying method

The banana samples were placed on stainless steel trays in a blast freezer (IRINOX, Treviso, Italy) at -30 °C for 4 h and then transferred to a freeze dryer (SP Scientific, New York, USA) for 48 h until the water activity reached below 0.4. The dried green banana slices that were subjected to either of the drying methods were then grounded in a home coffee grinder for 30 seconds per batch and the powder was passed through a 100 mesh (150 mm) screen, then vacuum packed, and stored at 4 °C until used for analysis.

4.2.5. Physicochemical properties of the banana flour

Some of the physicochemical properties, such as water activity (a_w), moisture content, ash, protein, lipid and carbohydrate contents were determined according to the AOAC 2000 standard methods (AOAC, 2005). The a_w was measured by an Aqualab device (Decagon Devices, Washington DC, USA) at 24 °C. The moisture content of the samples was determined using a vacuum oven dryer (Thermoline, Australian Marketing Group, Marrickville, NSW, Australia) at 60 °C for 16 hours (Method 925.40; AOAC 2000). The ash content was determined using a furnace at 550 °C for 4 h (Method 923.03; AOAC 2000). The Kjeldahl method was used to determine the protein content (%N×6.25) of samples using Method 923.03; AOAC 2000 based on the digestion of samples with concentrated H_2SO_4, distillation with NaOH 35% and titration with 0.01 HCl. The lipid content was determined by the Soxtec machine based on extraction by the petroleum ether (Method 920.39; AOAC 2000) (AOAC, 2005). The carbohydrate content was calculated by difference using the following equation:

Carbohydrate (g/ 100 g) = 100 - [moisture content (g) + ash (g) + protein (g) + lipid (g)]

4.2.5.1. Colour measurements

The colour of banana samples was assessed by using a Hunterlab Spectrocolorimeter (Hunter Lab Mini Scan Plus Colorimetric, USA). The colour parameters were defined using the CIE L*a*b* system, where L^* ($L^* = 0$ is complete black and $L^* = 100$ is perfect white), a^* ($-a^*$ = greenness and $+a^*$ = redness) and b^* ($-b^*$ = blueness and $+b^*$ = yellowness) (Nasrin et al., 2015).

Chroma values (C) that were near zero were interpreted to indicate subdued colour, whereas high chroma values were interpreted to specify a more vibrant colour. Browning index and chroma were measured by the following formula (Cornejo and Rosell, 2015).

$$\text{Chroma} = \sqrt{a^2 + b^2} \quad (4.1)$$

Browning index = $\frac{100(x-0.31)}{0.17}$, where x is obtained using the formula 4.2:

$$x = \frac{a^* + 1.75L^*}{5.645L^* + a^* - 3.012b^*} \quad (4.2)$$

4.2.5.2. Oil and water holding properties

The oil holding capacity (OHC), water holding capacity (WHC), solubility index (SI) and swelling power (SP) were determined according to the methods of Nasrin et al. (2015). One g aliquots of banana sample were dispersed in either 50 mL of distilled water to determine the WHC, or 50 mL of canola oil to measure the OHC in previously weighed centrifuge tubes (Nalgene, Rochester, USA.). The tubes were capped and placed in a water bath at various temperatures (40 °C, 60 °C and 80 °C) for 30 min with intermittent stirring. The tubes were then cooled to room temperature and then centrifuged at 1500 *g* for 20 min. The OHC was calculated as g of oil per g of dried flour. The supernatant generated from the centrifugation step in the WHC measurement was dried at 105 °C for 5 h to determine the SI (g/ 100g). The paste was weighed and then dried for 5 h at 100 °C. The WHC (g/ g) and SP (g/ g) were determined according to the following formula (Abbas et al., 2009):

WHC = $(W_3 - W_4)/ W_4$, where W3 and W4 are the weights of wet residue and dry residue, respectively.

$SI = \frac{W_2}{W_1} \times 100$, where W_1 and W_2 are the weights of the dry sample itself and the dry sample in supernatant, respectively.

$$SP = \frac{W_3}{W_4} \quad (4.3)$$

For determination of emulsion activity (EA), a mixture consisting of 10 mL of distilled water, 10 mL of soybean oil and one g of the sample was transferred into a calibrated centrifuge tube after mixing for two minutes. After centrifugation at 1500 g for 5 min, the ratio of the height of emulsion layer to the total height of the mixture in the tube was recorded as EA percentage (Rodríguez-Ambriz et al., 2008)

After keeping the emulsion at 80 °C for 30 min in a heated water-bath, the tubes were cooled down under running cold tap water for five minutes until it reaches to the ambient temperature and then centrifuged at 1500 g for 15 min. The emulsion stability was calculated as the ratio of the height of the emulsified layer compared to the total height of the mixture in the tube (Rodríguez-Ambriz et al., 2008)

4.2.6. Relative Crystallinity

An X-Ray diffractometer (Bruker Model D8 Discover, Billerica, USA) was set up with the following parameters; 30 mA and 40 kV, a diffraction angle (2θ) range of 5– 40°, a 0.03° step size and measuring time of 15 s, according to the method reported by Campuzano et al. (2018). The relative crystallinity (RC) was calculated according to the following equation:

RC (%) = $\left(\frac{Ac}{Ac+Aa}\right) \times 100$, where Aa is the amorphous area and Ac is the crystalline area obtained from the graph provided by the device. These areas were quantified using the software Origin® 2017 (OriginLab Corporation, Northampton, USA).

4.2.7. Starch analysis

4.2.7.1. Resistant starch

The RS content was determined according to the methodology of AOAC 2002.02, using a Megazyme kit (Resistant starch assay kit; Megazyme International, Wicklow, Ireland) according to the manufacturer guidelines. The samples were incubated in a shaking water bath with two enzymes, pancreatic α-amylase and amyloglucosidase (AMG) for 16 h at 37 °C in order to achieve complete hydrolysis of the non-resistant starch. After centrifugation at 1500 g for 15 min at room temperature, the pellet was washed with ethanol (50% v/v) and the supernatant was decanted. The RS in the pellet was dissolved in 2 M KOH by vigorously stirring in an ice-water bath for 20 min. The starch was quantitatively hydrolysed to glucose with AMG and then the glucose determined using a glucose oxidase/peroxidase reagent (GOPOD) and RS was calculated as mg of glucose × 0.9 (as the conversion factor).

4.2.7.2. Total Starch

The total starch content was assessed according to the AOAC method 996.11 using a kit (total starch assay kit; Megazyme International, Wicklow, Ireland). The principle of the method involves the hydrolysis of starch using a thermostable α-amylase into soluble branched and unbranched maltodextrins, according to the method of Moongngarm et al. (2014). Digestible starch (DS) was determined by pooling and washing the supernatant, adjusting the volume to 100 mL, and measuring the D-glucose content with GOPOD. Resistant starch was subtracted from TS to determine DS.

4.2.7.3. Amylose and amylopectin content

The amylose content was quantified using a commercial kit (amylose/amylopectin kit; Megazyme International, Wicklow, Ireland) based on the separation of amylose and amylopectin and precipitation of amylopectin with the addition of concanavalin-A and removing it by centrifugation at 1500 g for 5 min at room temperature.

4.2.8. Diffraction scanning calorimetry

Starch gelatinization temperature was examined by differential scanning calorimetry (Diamond DSC, Perkin Elmer, Connecticut, USA), using an instrument equipped with an intercooler unit (Perkin Elmer, Model 2P, USA.), with nitrogen as the purge gas. Based on the method of Tribess et al. (2009), approximately two mg (db) of the sample was accurately weighed in an aluminium pan and 7 μL of deionized water was added. After tightly sealing the pans and maintaining them at room temperature for an hour, the samples were exposed to a temperature gradient from 24 °C to 120 °C at a rate of 10 °C min^{-1}. The gelatinization/ peak temperature (T_P), onset temperature (T_0) and transition enthalpy (ΔH) and gelatinization range (R) were obtained directly from the data curves using the Pyris® manager software.

4.2.9. Statistical analysis

Statistical analysis was performed by Minitab® program version 16 (Minitab, Australia). After confirming that the data were normally distributed, the one-way ANOVA test was applied to assess the significance of the differences among the treatments for each variable. The correlation between the variables was determined using Pearson's correlation. The differences among the mean values were resolved using Tukey's test. The significance level was set at P-value < 0.05, with a confidence interval of 95%. All the tests were performed in triplicates.

4.3. Results and discussion

4.3.1. Physicochemical properties

The physicochemical characteristics and firmness of green bananas are presented in Table 4.1 compared to data reported by Tribess et al. (2009) and Abbas et al.

(2009). The results suggest that the banana samples used for GBF production was at the first stage of ripeness.

Table 4.1. Characterisation of banana samples maturity using pH, total solid, firmness and total acidity of green banana used in the present study compared to literature findings

Banana characteristics	Data from literature*		Current findings
	First ripening stage	Second ripening stage	
pH	5.3 ± 0.1	4.8 ± 0.1	5.3 ± 0.1
Soluble solids (°Brix)	5 ± 2	7 ± 1	4.2 ± 1.0
Total solids (g/ 100 g)	NR	NR	30.7 ± 2.3
Firmness (N)	26 ± 6	14 ± 2	26.8 ± 3.0
Titratable acidity	0.24 ± 0.03	0.44 ± 0.03	0.32 ± 0.07

Mean values ± SD of triplicate determinations. NR, not reported

* Tribess et al. (2009), Abbas et al. (2009)

4.3.1.1. Proximate composition

The composition of GBF flour samples was compared to the reference control (WF) and the results are shown in Table 4.2. Compared to WF, all GBF samples regardless of the drying method showed significantly lower moisture content (MC), a_w, protein content and lipid content values, and significantly higher ash and carbohydrate contents (P-value < 0.05). This behaviour was also reported in a report on green banana pulp flour (GBPF) (Bezerra et al., 2013a). The lower water content in all GBF samples indicated better stability and longer shelf life due to the lower potential for microbial growth (a_w < 0.4) and better stability against physical and chemical reactions (Menezes et al., 2011). Amongst the GBF samples, as the drying temperature increased above 50 °C, a substantial change in water content was observed (P-value < 0.05), relating to the higher rate of drying compared to others (Menezes et al., 2011). The FDF samples had the same carbohydrates, lipid, protein and ash contents found in oven-dried samples. However, the FDF samples had higher moisture content than the ODF80 and ODF110 (P-value < 0.05) and was not different from ODF50 (Table 4.2). This could be due to the smaller particle size and more porous structure of FDF.

As green banana have been considered as a valuable fruit due to its abundance in minerals, taking advantage of the minerals in the BPe also results in higher amount of ash compared to peeled green banana flour (Wang et al., 2017a). The results indicated that carbohydrate content was over 80% of GBF (g/100 g db), which was similarly found by Bezerra et al. (2013a) who investigated on GBPF. The inclusion of the peel in addition to the pulp could lead to a greater amount of RS2 and RS3. Overall, the effect of drying methods on the composition of GBF was not statistically significant (P-value > 0.05).

Table 4.2. The composition of green banana and wheat flour samples (g/ 100 g)

Sample	Moisture Content	Ash	Protein	Lipid	Carbohydrate
WF	9.47±0.17 a	2.83±0.11 a	9.78±0.03 a	1.24±0.01 a	76.68±0.43 b
FDF	5.27±0.11 b	5.21±0.01 bc	3.97±0.13 b	0.92±0.01 b	84.61±0.71 a
ODF50	5.09±0.01 bc	5.19±0.06 b	4.17±0.12 b	0.93±0.01 b	84.62±0.12 a
ODF80	4.56±0.21 d	5.21±0.08 bc	4.13±0.1 b	0.93±0.01 b	85.14±0.32 a
ODF110	4.46±0.19 d	5.24±0.10 bc	4.14±0.04 b	0.93±0.01 b	85.26±0.62 a

Mean ± standard deviation values of triplicate determinations. Values with different letters in the same column are significantly different (P-value < 0.05). WF, wheat flour, FDF, freeze-dried flour, ODF50, oven-dried flour at 50 °C, ODF80, oven-dried flour at 80 °C, ODF110, oven-dried flour at 110 °C.

4.3.1.2. Colour

The differences in colour parameters of GBF samples and WF are shown in Table 4.3. The results showed that FDF had a light green colour flour; with the highest values for lightness, yellowness and chroma amongst the GBF samples were found in that treatment. Increasing the temperature resulted in a darker colour amongst oven-dried samples (P-value < 0.05). The same trend was observed by previous researchers in GBPF (Sarawong et al., 2014b, Andrade et al., 2018). The degradation of chlorophyll, which is sensitive to oxygen, heat and pH, produces pheophytin and pheophorbide which accounts for the green–brown colour (Andrade et al., 2018). Moreover, the high amount of starch and protein along with the high temperatures may have resulted in the partial Maillard reaction (Falade and Oyeyinka, 2015). Similarly, Yangilar (2015) stated that FDF had a greener colour than spray dried banana pulp flour. The degree of colour saturation (chroma) was notably higher in FDF compared to oven-dried GBF and WF (P-value < 0.05) corresponding with Aziah and Komathi (2009) findings on unpeeled pumpkin four. The GBF obtained from freeze-drying and all air oven-drying methods were darker in colour compared to spouted bed drying method (Bezerra et al., 2013b). Also, it was reported that GBF made from BPe had shown a darker colour when produced by microwave drying and freeze-drying, respectively (Türker et al., 2016, Vu et al., 2017). According to Jeet et al. (2015), the bright colour of flour could be more acceptable for the consumer. Yet, the incorporation of flour as an ingredient into food products could eventually affect the colour of the flour. In general, it seems that more starch would be preserved in FDF and ODF50 compared to other samples.

Table 4.3. Colour analyses of green banana and wheat flour samples

Sample	L*	a*	b*	Browning Index	Chroma
WF	66.69±0.65 e	0.87±0.03 b	11.82±0.12 a	20.05±0.42 a	11.85±0.11 a
FDF	59.72±0.19 d	-0.75±0.05 a	15.55±0.13 d	28.45±0.30 b	15.57±0.12 e
ODF50	51.46±0.58 c	2.08±0.04 c	14.32±0.83 c	33.67±0.85 c	14.47±0.13 d
ODF80	46.55±0.77 b	2.55±0.10 d	12.13±0.41 b	34.93±0.97 cd	12.40±0.09 b
ODF110	42.42±0.23 a	2.97±0.04 de	13.63±0.26 c	42.90±0.27 d	13.94±0.53 c

Mean ± standard deviation values of triplicate determinations. Values with different letters in the same column are significantly different (P-value < 0.05). WF, wheat flour, FDF, freeze-dried flour, ODF50, oven-dried flour at 50 °C, ODF80, oven-dried flour at 80 °C, ODF110, oven-dried flour at 110 °C.

4.3.2. Hydration properties

As shown in Table 4.4, as the incubation temperature increased, the mean value of WHC increased significantly in all GBF and control samples (P-value < 0.05). At 40 °C, all GBF samples absorbed more water than WF, and the FDF samples showed the highest significant value (P-value < 0.05). Similar trends among the samples were observed at higher temperatures with the ODF110 values had lower values than WF at 60 °C and 80 °C. While WHC could be related to the physical status of amylose (Happi Emaga et al., 2007), the amount of insoluble dietary fibres, protein and particle porosity of the flour could also play an important role in water absorption (Nasrin et al., 2015, Yangilar, 2015).

Table 4.4. Hydration properties of green banana and wheat flour samples

Sample	WF	FDF	ODF50	ODF80	ODF110
WHC40	1.97±0.19 c	3.58±0.32 c	3.01±0.2 a	3.00±0.75 ba	3.06±0.82 a
WHC60	4.71±0.63 b	5.23±0.74 b	4.310.1 cb	4.86±0.11 dc	3.90±0.88 c
WHC80	7.87±0.81 ba	9.62±0.33 a	7.61±0.55 b	7.64±0.75 c	7.07±0.12 c
OHC40	2.74±0.21 cb	5.81±0.72 b	2.77±0.22 cb	2.70±0.21 dc	2.49±0.68 c
OHC60	2.54±0.4 c	5.91±0.88 b	2.77±0.22 cb	2.76±0.22 dc	2.53±0.33 c
OHC80	2.34±0.55 c	5.9±0.7 a	2.92±0.21 a	2.74±0.57 dc	2.47±0.32 c
SI40	0.06±0.45 c	0.12±0.21 c	0.14±0.56 b	0.19±0.44 cb	0.18±0.21 ba
SI60	0.08±0.12 c	0.13±0.56 c	0.14±0.45 b	0.19±0.18 cb	0.18±0.75 ba
SI80	0.16±0.23 c	0.14±0.2 d	0.17±0.66 cb	0.20±0.56 b	0.20±0.78 a
SP40	3.28±0.74 c	5.01±0.21 cb	4.64±0.58 b	4.65±0.79 c	4.58±0.45 b
SP60	6.24±0.78 cb	5.96±0.56 d	6.02±0.89 cb	6.77±0.56 b	5.70±0.42 b
SP80	10.73±0.64 b	11.75±0.2 b	10.31±0.17 cb	10.63±0.6 c	9.93±0.71 c

Mean ± standard deviation values of triplicate determinations. Values with different letters in the same row are significantly different (P-value < 0.05). WHC, water holding capacity. OHC, oil holding capacity. SI, solubility index. SP, swelling power. WF, wheat flour, FDF, freeze-dried flour, ODF50, oven-dried flour at 50 °C, ODF80, oven-dried flour at 80 °C, ODF110, oven-dried flour at 110 °C.

Increasing the temperature results in protein denaturation and starch gelatinization. This may lead to greater water absorption due to the availability of hydroxyl (OH) groups (Agama-Acevedo et al., 2016). According to Alkarkhi et al. (2011), the release of amylose into water during heating, occurs more in pulp flour than peel flour. Compared to other findings, the current results showed higher

WHC for FDF than those reported by previous findings (Ramli et al., 2009, Yangilar, 2015). High WHC indicates the capability of FDF to be used as a thickener in semi-liquid and liquid foods (Abbas et al., 2009). Also, Grigelmo-Miguel et al. (1999) stated the syneresis in food can also be controlled by adding flour with high WHC due to the increase in expansibility of starch matrix.

The OHC of flour relates to the hydrophobic character of starch: the higher the OHC, the higher the EA (Rodríguez-Ambriz et al., 2008). In other words, stabilization of food systems with high fat content, such as in bakery products, can be easily achieved by emulsifiers (Gómez et al., 2013). By promoting the formation of starch-protein complex and strengthening the gluten-gluten interaction, emulsifiers can improve the aeration in bakery products (Aziah and Komathi, 2009). The results of this chapter specified a substantial increase in OHC for FDF sample at all temperatures compared to the control and other GBF (P-value < 0.05). Comparing results at 80 °C showed a significant increase in OHC of ODF50, which means it can be an emulsifier compared to the control and other GBF samples. However, ODF50 showed significantly lower values for OHC compared to FDF. The current results for FDF were higher than those reported for GBPF (Rodríguez-Ambriz et al., 2008, Yangilar, 2015), mango peel flour (Larrauri et al., 1996) and citrus peel flour (Chau and Huang, 2003). The OHC found in FDF is almost equals to the OHC content of Balady flat bread which contained GBPeF (Eshak, 2016) and unripe plantain peel flour (Agama-Acevedo et al., 2016).

It has been previously stated that the amylose-lipid complex formation, which can be formed during heating at above gelatinization temperatures, produces RS5 which is resistant to hydrolysis formulation. As a result, FDF could increase the RS content, and also be used as an alternative emulsifying agent in food. The results of the emulsifying properties were correlated with OHC properties ($r > 0.92$, P-value < 0.05). The ES of different samples ranged between 30.54% and 36.47%, where ODF110 had the lowest stability and ODF50 showed the highest stability (P-value < 0.05) as shown in Table 4.5. However, the ES values varied from 9.69% to 28.99% for WF and FDF, respectively. Consequently, FDF appears to act as an emulsifier as it has the highest stability amongst whole green banana flours as well as WF.

The SI is related to the soluble solid contents in dried flour, while the SP is an index that relates to the strength of the starch granule to stay intact during high cooking temperatures (Bezerra et al., 2013a). The SI and SP specify the range of interaction within the amorphous (amylopectin) and crystalline (amylose) areas of starch molecule together with the length of branches, degree of branching, and formation of the molecules (Nasrin et al., 2015). As mentioned earlier, an increase in SI and SP brings about gelatinization, which is the basis for making pre-cooked starchy foods, especially in bakeries (Oliveira de Souza et al., 2018). The obtained results showed that all GBF samples had higher SI compared to WF, and this increase was significant for ODF80 and ODF110 (P-value < 0.05).

Table 4.5. Emulsifying properties of green banana and wheat flour samples

Sample	Emulsion Activity (%)	Emulsion Stability (%)
WF	32.61±0.17 b	9.69±0.08 e
FDF	32.59±0.16 b	28.99±0.15 a
ODF50	36.47±0.22 a	19.21±0.09 d
ODF80	32.65±0.09 b	23.15±0.10 c
ODF110	30.54±0.22 c	25.36±0.08 b

Mean ± standard deviation values of triplicate determinations. Values with different letters in the same column are significantly different (P-value < 0.05). WF, wheat flour, FDF, freeze-dried flour, ODF50, oven-dried flour at 50 °C, ODF80, oven-dried flour at 80 °C, ODF110, oven-dried flour at 110 °C.

However, the increment in SI was not as high as GBPF (de la Torre-Gutiérrez et al., 2008, Bezerra et al., 2013a, Campuzano et al., 2018), but greater than in green banana peel flour (GBPeF) (Ratnayake et al., 2002, Nasrin et al., 2015). The same pattern was observed for SP as well; considering FDF had a more stable starch structure than other GBFs as temperature increases. As Campuzano et al. (2018) reported, the ripening stage is positively correlated with SI. By increasing the temperature over the gelatinization temperature (more than 65 °C), starch degradation and sugar release lead to an increase in both SI and SP.

The distribution of amylopectin and amylose in starch granule was suggested as a factor that influenced the SI value by Seguchi et al. (2014). As amylose plays a key role in maintaining the starch structure, the higher amylose content could eventually create a more compact starch granule. At temperatures rose above 65 °C, both the SI and SP were dramatically increased (P-value < 0.05). As the hydrogen bonds break, the water molecules bind to the exposed OH groups and the granule expands and exudes amylose, meaning it would be more difficult for starch to overflow outside of the granules (de la Torre-Gutiérrez et al., 2008). According to Ratnayake et al. (2002), green banana starches compared to those of corn, chayote and cassava, swell more slowly, which may signify that a strong micellar arrangement needs to be broken. In consequence, incorporation of the peel into the pulp flour has improved the SI and SP of the whole GBF.

4.3.3. Starch analysis

Starch is classified into two groups based on its digestibility: digestible starch (comprises of slow and rapidly digestible starches) and resistant starch (Englyst and Cummings, 1987). In the whole green banana, 70-80% of the dry weight contributes to starch, considering that the peel contains up to 12% of the total amount of starch (Campuzano et al., 2018). It has been previously described that the RS type in green banana is type two, which is resistant to hydrolysis (Bi et al., 2017). However, during the production of GBF the amount of RS2 decreases due to gelatinization, especially in higher temperature (Kar et al., 2003). That fact signifies the importance of choosing the right processing condition together with proper pre-treatments.

The present results corroborate the previous findings about the destructive effect of drying at higher temperatures (Fig 4.2). Interestingly, ODF50 displayed similar values to FDF, which had the highest amount of RS. Although the difference between FDF and ODF50 samples was significant (P-value < 0.05), the RS content was well preserved during the ODF50 heat-treated flour compared to ODF80 and ODF110.

More than half of the total starch in FDF and ODF50 was RS. While the digestible starch represented a range between 17% and 36% dry weight base (db) in GBF samples, the WF contained the highest amount of available starch. Also, the results indicated that drying temperatures over 80 °C did not have a significant effect on RS content reduction (P-value > 0.05).

Drying conditions at temperatures below 60 °C, the RS content of FDF and ODF50 were higher than the reported values for GBPF (Menezes et al., 2011, Bezerra et al., 2013b, Segundo et al., 2017a) and only lower than the findings of Surendra Babu et al. (2014), who also worked on GBPF, is because of the different variety (*Monthan*) used in their study. Considering the dramatic increase in the levels of digestible starch in both ODF80 and ODF110, it can be suggested that retrogradation could be a reason for the disruption of the starch molecule, precisely amylose.

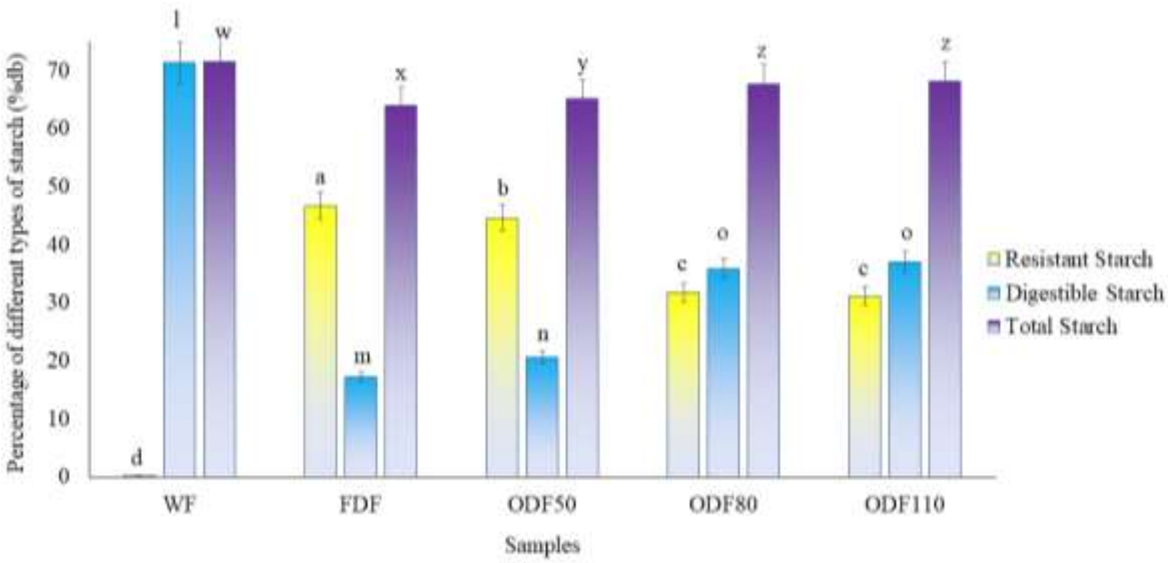

Figure 4.2. Starch analysis of green banana and wheat flour samples

Bars showing mean and standard deviation for each starch type that have different letters are significantly different (P-value < 0.05).

As shown in Fig 4.3, the amount of amylose was decreased dramatically by the application of heat treatment. Contrarily, during the freeze-drying process the amylose crystallites remained nearer to the double amount compared to other GBF samples and WF. The linear structure of amylose (α-1→4) is responsible for the RS2 structure, whereas amylopectin is branched and its chains are connected through (α-1→6) bonds to form a composite structure. Multiple significant correlations were found between amylose and RS (r > 0.91, P-value < 0.05) as well as TS (r > 0.89, p = 0.05), and WHC (r > 0.90 p = 0.05) According to Eshak (2016), higher amount of amylose led to a higher OHC, which was seen in the current chapter. This brings about the possibility of amylose-lipid complex that has been shown is resistant against α-amylase (RS5). The FDF results were higher than those found in GBPF reported by Bi et al. (2017) but almost the same for ODF50 (Zhang et al., 2005,

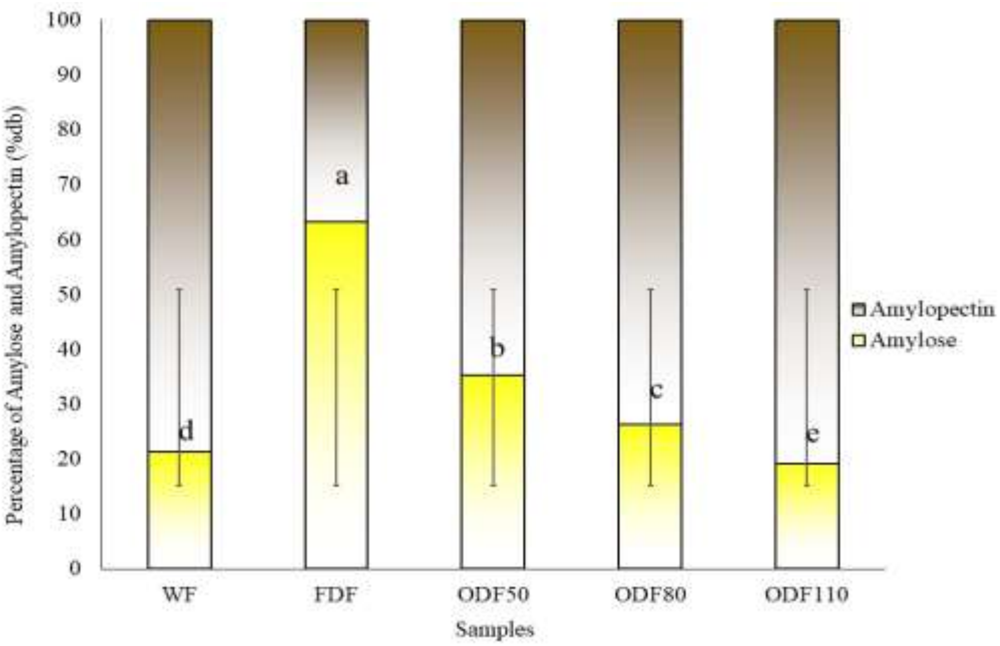

Figure 4.3. Amylose and Amylopectin content of green banana and wheat flour samples

Bars showing mean and standard deviation for amylose variable that have different letters are significantly different (P-value < 0.05).

Sarawong et al., 2014b) and even more than culled plantain starch (Nasrin et al., 2015). As the amount of RS found in FDF and OFD50 were higher than other samples, it may be advisable for nutritional purposes to use RS as a functional ingredient, as it contributes to the prevention the colorectal cancer (Malcomson et al., 2015, Birt et al., 2013, Gratz et al., 2011, Perera et al., 2010).

4.3.4. Relative Crystallinity

The XRD patterns of starches are categorized into three types: A, B and C. A-type starches possess surface pores and channels which are slowly digestible, while C-type can be easily lost in foods due to the hydrothermal procedures such as boiling, cooking and baking bringing about complete or partial starch gelatinization (Cahyana et al., 2019). However, B-type starches do not have pores in their structure and are inherently resistant to digestion (Van Hung et al., 2012). In green banana, the abundance of B-type makes it valuable in terms of health benefits (Zhang et al., 2005).

The XRD patterns of GBF different samples and WF are shown in Fig 3.4. Three prominent peaks were detected by diffractogram within the diffraction angles of 15° and 25°, which relates to B-type crystallinity (Van Hung et al., 2012). While WF showed the least amount of crystallinity, the increased drying temperature resulted in the disruption of the starch crystalline section.

The FDF samples had 58.34% crystallinity, which was the greatest amongst the GBF samples (P-value < 0.05). These results explained the existing significant correlation between the obtained RS amount and the effect of temperature ($r > 0.91$, P-value = 0.05). These results also confirmed that destroying the granular crystalline structure of starch was associated with decreasing in the amount of RS. This relation was presented by Zhang and Hamaker (2012) who investigated the starch properties in green banana. The current findings suggest a greater crystallinity in FDF compared to some studies on GBPF obtained from different cultivars (Bi et al., 2017). Furthermore, Wang et al. (2017a) reported the effect of cultivar on the percentage of crystallinity in this order: *Pisan Awak* > *Bluggoe* > *Cavendish* > *Pisang*. Most of the RS found in GBF is type two (Silva et al., 2015). The crystalline area is structurally tight and orderly in a way that makes it hard for it to be eroded by acids and enzymes, whereas the non-crystalline area (amorphous part) can be easily degraded (Rodríguez-Ambriz et al., 2008, Tribess et al., 2009). In this chapter, all heat-treated flours showed that crystallinity decreased along with the amylose content.

Thermal properties of the whole GBF and WF are presented in Table 4.6. All of the thermal variables, except for gelatinization range, were significantly higher in GBF samples than the control (P-value < 0.05). Owing to the presence of RS in the GBF samples, a higher T_P than WF was found, ranging from 74.99 °C to 75.9 °C. This significant difference could be related to the high amount of DF in pulp, and especially peel (Silva et al., 2015). The values of T_P found in this work were higher than those found in corn starch (67.88 °C) (Han et al., 2009), potato (64.48 °C) (Zaidul et al., 2008) and cassava (71.08 °C) (Hong et al., 2016). This trend was similar in T_0.

Sample	Crystallinity	SEM
WF	41.16 e	1.23
FDF	58.34 a	1.34
ODF50	48.39 b	1.19
ODF80	45.64 c	1.41
ODF110	43.37 d	1.49

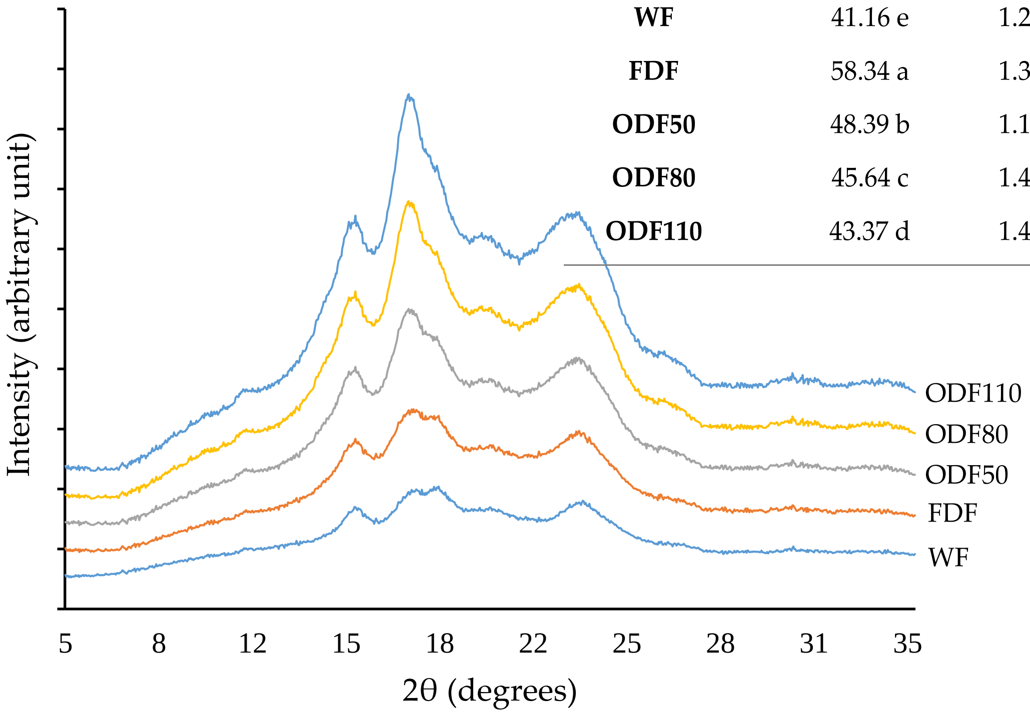

Figure 4.4. X-Ray diffraction patterns of green banana and wheat flour samples

Almost the same results were reported in previous studies with different banana varieties but same drying conditions (Waliszewski et al., 2003, Pelissari et al., 2012), except for *Musa paradisiaca Macho*, which showed a T_P value of 77 °C (Bello-Pérez et al., 2000); and the freeze-drying method exhibited a higher T_P than other methods such as ultrasound treatment (Rayo et al., 2015) and pulsed-fluidized bed agglomeration (Tribess et al., 2009). The required energy for breaking the molecular interactions within the starch structure during gelatinization defines the ΔH (Bello-Pérez et al., 2000). Lower values of ΔH would indicate either a fractional melting of amylopectin branches or a possible variance in the crystals' stability related to the size of the starch granules (Pelissari et al., 2012).

Table 4.6. Thermal properties of green banana flour samples and wheat flour

Sample	Start (°C)	Maximum (°C)	Stop (°C)	Delta H (J/ g)	R (°C)
WF	54.70±0.20 d	62.62±0.10 c	69.42±0.13 d	5.41±0.07 e	11.95±0.03 a
FDF	64.18±0.16 c	74.99±0.11 b	90.64±0.32 a	23.16±0.2 a	8.02±0.09 b
ODF50	67.73±0.24 ab	75.9±0.06 a	85.51±0.16 a	19.48±0.24 b	4.69±0.64 d
ODF80	66.69±0.12 b	740.6±0.19 b	81.61±0.01 c	10.31±0.2 d	6.88±0.50 c
ODF110	68.54±0.43 a	75.86±0.05 a	84.37±0.36 b	11.70±0.2 c	5.92±0.16 cd

Mean ± standard deviation values of triplicate determinations. Values with different letters in the same column are significantly different (P-value < 0.05). WF, wheat flour, FDF, freeze-dried flour, ODF50, oven-dried flour at 50 °C, ODF80, oven-dried flour at 80 °C, ODF110, oven-dried flour at 110 °C.

For that reason, the control samples had the least amount of ΔH (5.41 J/g), while FDF and ODF50 showed a dramatically higher temperature amongst GBF samples. Also, as seen in Table 4.6, the R value of WF was the highest (11.95 °C), while ODF50 represented the smallest gelatinization range amongst all GBF samples. This is explained by the presence of the large amount of amylose per unit mass in flour. Compared to the results acquired from the oven drying for producing GBPF and GBPeF (Zaidul et al., 2008, Kaur et al., 2016), the freeze-dried sample and ODF50 presented higher values which means that these flours can potentially be useful in products which delayed pasting is favourable. Also, the current results suggest that the incorporation of peel flour into pulp flour may increase the gelatinization temperature positively.

4.4. Conclusion

The world largest banana producers are developing countries and thermal processing is the most common preservation treatment. As a result, optimizing a feasible drying method that has the least negative effects on green banana components, especially its RS2 content, is very useful for streamlining production of banana flour. Furthermore, banana peel is rich in dietary fibres, minerals and RS2. Producing GBF from whole green banana can add nutritional and economic incentives to process. The present chapter investigated the effects of drying conditions on the properties of whole green banana flour.

Colour analysis showed that FDF had bright greenish-like flour with the closest browning index to wheat flour and the ODF50 treatment may contribute to a better consumer acceptancy amongst heat-treated green banana samples. The emulsifying properties showed that FDF and ODF50 can act as an emulsifier in oil-containing food products and they are better than ODF80 and ODF110 samples. Additionally, the high WHC of all GBF samples, especially the FDF, suggests that they can be applied in food products as a thickening agent.

High drying temperatures decreased the percentage of RS starch and increased the percentage of digestible starch. These findings, which were in accordance to high crystallinity and gelatinization temperature of starch, showed that 50 °C is the best temperature for hot-air oven drying that can be applied with the least possible effect on RS content. Further, the ODF50 samples were the closest properties to the non-heat-treated sample (i.e. FDF). Future studies should focus on the particle size separation, starch granule microstructure and rheological properties of the whole green banana flour to understand their potential behaviour during packaging, transportation and use in food systems.

4.4. Limitations

There were some limitations which should be considered for designing future studies. In order to better understand the changes in water absorption of green banana flour, farinograph test, which was not available at the time, can be used to estimate the amount of water required to make dough, evaluate the effects of ingredients on mixing properties, evaluate flour blending requirements and to check flour uniformity.

CHAPTER 5

Textural properties and characteristics of whole green banana flour produced by air-oven and freeze-drying

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Chapter aim:

In the previous chapter, the effects of oven-drying and freeze-drying on the starch content, thermal properties and physicochemical properties of GBF were investigated. This chapter aims to investigate the effects of three different heat treatments (50, 80 and 110 °C) on the physical, nutritional and rheological properties of the produced whole green banana flours as well as their chemical compositions compared to GBF made from freeze-drying. Wheat flour was used as a reference.

5.1. Introduction

Banana is a tropical fruit that follows rice, wheat and maize in terms of popularity as a food crop (Aurore et al., 2009). *Musa Cavendishi* is the main commercial banana cultivar. As a climacteric fruit, maturation of the banana continues after the harvest in seven stages. According to Aurore et al. (2009), each stage represents a dramatic change in physicochemical characteristics and fruit firmness. At early stages of ripeness, BP is considered a rich source of dietary fibre (DF), resistant starch type 2 (RS2), phenolic compounds, phytonutrients and vitamins (B groups, ascorbic acid and tocopherols) (Singh et al., 2016). Banana peel, which comprises about 40% of total weight of the fruit, has no useful applications and is usually dumped as waste. Recent studies on the compositional properties of banana peel (BPe) reported high RS2, micronutrients and DF contents compared to pulp (BP) (Homayouni et al., 2014). Extraction of nutrients from green banana and evaluation of its technological benefits as a functional ingredient in the form of flour in food formulations have attracted the attention of researchers (Agama-Acevedo et al., 2016, Biernacka et al., 2020, Pico et al., 2019b, Falcomer et al., 2019). Nevertheless, information comparing different air-oven drying temperatures with freeze-drying processes on properties of whole green banana flour (GBF), especially rheological properties, have scarcely been reported in the literature.

Compared to other methods of drying, freeze-drying has been considered the best dehydration method for heat-sensitive materials to obtain the highest possible quality in terms of colour, structure, nutrients and flavour (Pathak et al., 2017). The crystallization of water molecules into ice crystals with subsequent sublimation, results in a high-quality porous dried banana peel product (Vu et al., 2017). However, the effect of freeze-drying on textural properties of GBF has not been thoroughly investigated yet.

From technological and financial points of view, high cost impedes the application of the freeze-drying method for drying purposes, especially in developing countries where banana production is mainly found. Moreover, leading countries in banana production face losses during and after harvest-time. Due to the lack of proper infrastructure and poor transportation, about one-fifth of all harvested banana becomes unusable (Izidoro et al., 2011). Therefore, making flour from green banana requires a feasible drying method with the least effect on both nutritional and technological properties of the produced flour.

In the previous chapter, the effects of oven-drying and freeze-drying on the starch content, thermal properties and physicochemical parameters of GBF were investigated. To gain a complete understanding of the produced flour utility, the rheological and nutritional aspects need to be elucidated. The aims of this study were twofold: first, assessing the effect of the three different heat treatments (50, 80 and 110 °C) on the physical, nutritional and rheological properties of the produced whole green banana flour samples. Secondly, investigating the chemical compositions and comparing the results with the GBF obtained from freeze-drying while wheat flour was used as a reference.

5.2. Material and Methods

5.2.1. Green banana flour preparation

Samples (air-oven-dried green banana flour and freeze-dried one) were produced based on the method that discussed in chapter 4, section 4.2.1.

5.2.2. Particle size separation

The particle size of the samples was determined according to AOAC (2000) using a sieve shaker equipment (Endecotts MINOR 200, New town, USA) with sizes of 35, 48, 60, 80, 100 and 200 Mesh (0.500, 0.354, 0.250, 0.177, 0.149 and 0.074 mm, respectively) (Bezerra et al., 2013a).

5.2.3. Bulking properties

Samples were put into 25 ml measuring cylinders up to the 5 ml line. The measuring cylinder was then tapped 100 times continuously on a table until a constant volume was obtained. The bulk density of the flour was calculated as the weight divided by the bulk volume and expressed as g/cm^3 using the formula 5.1 (Falade and Oyeyinka, 2015).

$$\text{Bulk Density} = \frac{\text{Weight of the sample}}{\text{Volume of the sample after tapping}} \qquad (5.1)$$

Compressibility Index (CI) and Hausner Ratio (HR) show the propensity of the flour to flow and define the interactions among the flour particles that influence the bulking properties. The CI and HR were measured using the formula 5.2 and 5.3 (Rayo et al., 2015):

$$\text{Compressibility Index} = 100 \times [(V_0\text{-}V_f)/\ V_0] \qquad (5.2)$$

$$\text{Hausner Ratio} = \frac{V_0}{V_f} \qquad (5.3)$$

Where V_0 and V_f are unsettled apparent volume and final tapped volume, respectively.

5.2.4. Textural properties

A TA-XTplus Texture Analyzer (Stable Micro Systems, Surrey, U.K.) was used to analyse the texture of the flour slurry (10% w/v) as described by Segundo et al. (2017b). Backward extrusion tests were conducted with a disc with a diameter of 45 mm, setting the probe travel distance at 30 mm. The test was performed with a test speed of 5 mm/s, a trigger force of 5×*g*, a crosshead speed and recording speed of 5 mm/s and force in compression mode. The test was performed in the room temperature.

5.2.5. Pasting properties

Pasting properties were determined according to Li et al. (2011) with a Rapid Visco Analyzer (RVA Perten Starch Master Instruments, Hägersten, Sweden). About 3.5 g of dried sample and 25 ml of deionized water were put into a canister. The RVA settings were holding at 50 °C for one min, then heating up gradually to 95 °C at 12.2 °C min^{-1}, holding at 95 °C for 2.5 min and cooling down to 50 °C at 11.8 °C min^{-1}. The starting rotational speed of the paddle was 960 rpm for 10 s and was kept at 160 rpm until the l completion of the test. Various parameters such as pasting temperature (PT), peak viscosity (PV), hold viscosity (HV), final viscosity (FV), setback (SB) and breakdown (BD) were recorded by the RVA software.

5.2.6. FTIR spectroscopy

The mid-infrared spectra were collected using an infrared spectrometer (Bruker Alpha, Fishers, USA) equipped with a deuterated triglycine sulphate detector and a single reflectance attenuated total reflection (ATR) cell with a ZnSe (Zinc Selenide) crystal. A total number of 24 scans were acquired for each spectrum at a resolution of 4 cm^{-1} and co-added. All flour measurements were recorded at ambient temperature in a wavenumber range of 600-4000 cm^{-1}. The acquired spectrum was generated by the standard direct mode where the background was recorded with no sample in the ATR cell (Meziani et al., 2011). Data analysis was carried out using the OPUS 7.2 Bruker software (Bruker Alpha, Fishers, USA).

5.2.7. Mineral analysis

An Agilent 7500ce quadrupole ICP-MS mass spectrometer was used for the analysis of minerals in the flour samples. Dried flour samples (two mg) were weighed into a MARSXpress (CEM Corporation, Matthews, USA) digestion tube, followed by the addition of 10 mL of concentrated quartz distilled HNO_3. The samples were digested in a CEM MARS6 programmable 2450 MHz microwave reaction system with a selectable operator output of 0 – 1600 W (CEM Corporation, Matthews, USA) using the manufacturer's directions.

Dilutions of the sample digests and blanks in 2% (v/v) HNO_3 were spiked offline with a cocktail of seven reference elements to compensate for any drift or possible matrix effects. Calibration standards were prepared by serial dilution of a SPEX CertiPrep multi-element standard (Spex Certiprep, Metuchen, USA). In order to minimize the instrumental drift and interferences, the ICP-MS was tuned according to the manufacturer's recommendations. The accuracy of the measurement was established with a corn meal reference material, supplied High Purity Standard North Charleston, USA (Anyasi et al., 2018).

5.2.8. Statistical analysis

Statistical analysis was carried out by Minitab® software version 16. After confirming that the data were normally distributed, the one-way ANOVA test was used to evaluate the significance of the differences between the treatments for each measured parameter. The correlation between the variables was determined using Pearson’s correlation. The differences among the means were resolved using

Tukey's test. The significant level was set at P-value < 0.05, with a confidence interval of 95%.

5.3. Results and discussion

5.3.1. Particle size separation

The uniformity of particle size is correlated to particle density and can influence the thermal properties and mixing behaviour of the flour during dough making (Musha et al., 2013). The flour size values are shown in Table 5.1. As evidenced by the significant differences in the size separation of the flours, all the oven-dried GBF had non-uniform particle size distribution. The flour obtained from ODF110 had the highest percentage of large particle size (500 mm). While the majority of ODF80 particles were around 250 mm, ODF50 had the smallest particle sizes amongst the oven-dried samples and the closest particle size values to the FDF, even though the mean values were different between the two treatment groups (P-value < 0.05). Interestingly, GBF obtained from freeze-drying exhibited the same particle sizes of the WF (P-value > 0.05). Bezerra et al. (2013a) investigated unpeeled green banana dried by spouted bed dryer at 80 °C and reported smaller particles sizes than those found in the FDF and ODF50 in the current study. This behaviour can be attributed to the accumulation of fibres and starch on the surface of the banana slices during drying. With increasing the rate of drying, the possibility of case hardening phenomenon increases, especially in the presence of peel which contains more DF than pulp (Tarvainen et al., 2006). Another explanation could be the collapse of starch granules which results in more dense structure and more resistance to the mechanical grinding (Turchiuli et al., 2005).

Table 5.1. Particle size separation of green banana and wheat flour samples

Sample	Mesh size (μm)					
	> 500	**354**	**250**	**177**	**149**	**74 >**
WF	0.01±0.07 d	1.01±0.09 d	6.13±2.23 d	32.66±2.13 bc	47.42±3.15 a	9.75±1.85 a
FDF	0.05±0.09 d	1.45±0.31 d	6.82±3.54 d	33.91±6.18 b	47.23±4.61 a	9.14±1.54 a
ODF50	2.71±0.11 c	8.31±2.61 c	10.22±4.12 c	37.06±8.17 a	31.71±5.76 b	5.46±1.61 b
ODF80	17.11±1.65 b	31.01±6.73 b	32.14±3.88 a	6.61±1.64 d	9.12±1.53 c	4.73±1.27 c
ODF110	40.10±6.02 a	38.21±7.62 a	25.99±4.02 b	12.45±2.71 c	6.61±1.02 d	3.64±0.97 d

Mean ± standard deviation values of triplicate determinations. Values with different letters in the same column are significantly different (P-value < 0.05). WF, wheat flour, FDF, freeze-dried flour, ODF50, oven-dried flour at 50 °C, ODF80, oven-dried flour at 80 °C, ODF110, oven-dried flour at 110 °C.

The application of freeze-drying causes instant sublimation of ice crystals and produces a porous structure that eventually results in easier grinding. In heat-treated banana slices, reducing the drying temperature allows proper evaporation of water through the structure, so less shrinkage occur which leads to easier grinding afterwards. This explains the smaller particles size of ODF50 compared to ODF80 and ODF110. It has been reported that smaller particle size may lead to better water/ oil absorption and might improve hydration properties of the flour (Aparicio-Saguilán et al., 2015).

It can be concluded that the use of the oven drying method at 50 °C brings about the best flour particles compared to the other higher temperatures and produces the closest flour particle size to non-heat treatment.

5.3.2. Bulking properties

The bulk density is an indicator of the flour heaviness and is important property for the transportation and packaging of materials (Elaveniya and Jayamuthunagai, 2014). As shown in Table 5.2, FDF had the lowest bulk density value compared to all heat-treated GBF samples and WF (P-value < 0.05). The ODF50 presented almost the same bulk density found in the reference samples (P-value > 0.05), while ODF80 and ODF110 treatment groups showed the highest bulk density values. Rayo et al. (2015) produced green banana pulp flour (GBPF) at 55 °C followed by pulsed-fluidized bed agglomeration, which resulted in a higher bulk density than the FDF and ODF50 in the present work.

The Hausner Ratio and Compressibility Index are used for assessing the tendency of flour, the ability to settle down and flow behaviour (Rayo et al., 2015). Flour samples with $HR < 1.2$ are categorized as free-flowing, flour with $1.2 < HR < 1.4$ is classified as intermediate flowing powder and at $HR > 1.4$ flour is considered a non-flowing or very cohesive powder (Turchiuli et al., 2005). Amongst all air-oven-dried GBF samples, ODF50 showed the highest CI, which was statistically similar to the freeze-dried one (P-value > 0.05). Considering the strong correlation between bulk density and CI (r > 0.92, P-value < 0.05), it can be concluded that ODF110 samples will have more flowability than the other samples. Similarly, HR results were in accordance with the CI results (r > 0.73, P-value < 0.05). It can be observed in Table 5.2 that while FDF, ODF50 and WF contained HR > 1.4 (very cohesive), ODF80 is classified as intermediate flowing flour and ODF110 is considered a free flowing one.

Table 5.2. Bulk properties of green banana and wheat flour samples

Sample	Bulk Density (g/m^3)	% Compressibility index	% Hausner Ratio
WF	0.42±0.01 c	38.46±0.15 a	1.62±0.30 a
FDF	0.13±0.01 d	35.70±0.32 b	1.55±0.01 b
ODF50	0.43±0.02 c	35.24±0.30 b	1.55±0.01 b
ODF80	0.53±0.01 b	21.56±0.48 c	1.28±0.02 c
ODF110	0.66±0.01 a	9.78±0.18 d	1.10±0.02 d

Mean ± standard deviation values of triplicate determinations. Values with different letters in the same column are significantly different (P-value < 0.05). WF, wheat flour, FDF, freeze-dried flour, ODF50, oven-dried flour at 50 °C, ODF80, oven-dried flour at 80 °C, ODF110, oven-dried flour at 110 °C.

Adebayo-Oyetoro et al. (2016) reported that fine particles with HR > 1.4 display a strong agglomeration with an inclination to create a better flour slurry in presence of water. Moreover, Abdullah and Geldart (1999) affirmed that free-flowing flours have lesser consolidation and take longer to collapse in a container. Therefore, higher CI and HR in FDF and ODF50 samples could markedly improve pasting and textural properties of the flours.

5.3.3. Textural properties

Textural properties of banana flour are noteworthy as they can specify the type of food product for successful GBF incorporation. Firmness, consistency, cohesiveness and viscosity index are the determining factors for textural properties. It was noted that thermal processing caused a significant reduction in the firmness of banana slurry compared to FDF and WF (P-value < 0.05) (Table 5.3). Wheat flour showed the highest resistance to the maximum applied force (0.42 N), while ODF110 displayed the least force (0.14 N). According to Abbas et al. (2009), the firmness of the banana slurry signifies the gelation property of the flour. By increasing the drying temperature, larger flour particle size was generated, which leads to low ability to absorb water/rehydration and results in a lower ability to create a firm slurry. According to Segundo et al. (2017b), flour slurry exhibits more resistance against the instrument probe when its pulled out of the sample as the extrusion energy becomes higher. This phenomenon indicates that viscosity is related to the resistance to flow and also to cohesiveness and consistency of the sample (Segundo et al., 2017b). Cohesiveness, which is related to fibre content, is the extent to which a material can be deformed before disruption (Toledo De Camargo Ranzani et al., 1996). Abbas et al. (2009) reported that the high viscosity of flours is attributed to the protein content and carbohydrates (Wang et al., 2017a). This explains the significantly higher viscosity, cohesiveness and consistency of WF compared to all GBF samples (P-value < 0.05).

Table 5.3. Textural properties of green banana and wheat flour samples

Sample	Firmness (N)	Consistency (N)	Cohesiveness (N)	Viscosity Index
WF	0.42±0.13 a	3.80±0.21 a	0.12±0.69 a	0.31±0.82 a
FDF	0.29±0.06 b	1.03±1.01 b	0.09±0.14 b	0.12±0.42 b
ODF50	0.25±0.41 c	1.01±0.30 b	0.08±0.20 bc	0.10±0.83 c
ODF80	0.25±0.41 c	0.94±0.80 bc	0.07±0.09 c	0.09±0.97 cd
ODF110	0.14±0.40 d	0.64±1.01 c	0.04±0.72 d	0.07±0.45 d

Mean ± standard deviation values of triplicate determinations. Values with different letters in the same column are significantly different (P-value < 0.05). WF, wheat flour, FDF, freeze-dried flour, ODF50, oven-dried flour at 50 °C, ODF80, oven-dried flour at 80 °C, ODF110, oven-dried flour at 110 °C.

As shown before, small particle sizes contributed to higher compressibility and HR index in FDF and ODF50. The correlation between HR index and textural parameters (r = 0.87, P-value < 0.05) confirmed that the drying method could exert substantial effect on GBF textural attributes. Consequently, these results show that physical differences in GBF may have certain implications on the textural properties of dough made from these flours. However, information on textural properties of GBF is scarce in the literature and more studies are required to ensure a better understanding of the behaviour of banana flours during the baking process (Abbas et al., 2009).

5.3.4. Pasting properties

Wang et al. (2017a) highlighted that the functionality of starch is dependent on its pasting properties. They described that after heating the banana flour, amylose leaches out of the swollen starch molecule and eventually leads to a rapid increase in viscosity with increasing temperature until PV is reached. Due to the high temperature (95 °C) and the mechanical shear stress, more amylose leaches out of the flour granules and affects the HV and BD indexes that are influenced by temperature, nature of the starch and the applied shear stress. As the temperature of the mixture drops to around 60 °C, starch molecules become retrograded and form a viscose gel network (final viscosity).

When starch is gelatinized after being heated in the presence of water and cooled, the disrupted amylose and amylopectin chains can progressively re-associate into an ordered structure in a process called retrogradation (Bertolini et al., 2010). Starch retrogradation is often accompanied with increased viscosity, gel formation and increased degree of crystallinity (Chockchaisawasdee et al., 2010). Amylose arrange a network after starch gelatinization, which results in swollen granules and changes in gel characteristics (Wang et al., 2017a). Pasting properties of GBF and WF are presented in Table 5.4. Pasting temperature represents the least temperature needed for cooking the samples (Bertolini et al., 2010). WF had a PT at 66.63 °C while

ODF110 showed the highest temperature amongst GBF samples with 81.23 °C (P-value < 0.05). The remarkable increase was found when the drying temperature had been increased from 50 °C onwards (P-value < 0.05). Compared to the WF samples, the higher RS content of GBF might be the reason for the increment in PT as the gelatinization temperature of RS is higher than normal starch (Singh et al., 2016), which indicates higher temperature needs for food products containing ODF80 and ODF110 to be fully cooked compared to other GBF samples. Ng et al. (2014) explained that the higher PT of ripe banana flour compared to WF was due to the higher sugar content. Further, the increment in PT has also been attributed to the increase in the crystalline part (granular structure) of the starch (Cahyana et al., 2019). Considering all GBF samples showed significantly higher PT than WF (P-value < 0.05), the high percentage of DF in GBF makes its starch more preserved during thermal processing (Aparicio-Saguilán et al., 2015).

The water binding capacity of the flour and ability to swell during cooking are indicated by PV. High PV value shows a highly viscose gel formation and a stable gel (Haslinda et al., 2009). The PV values for GBF samples ranged from 2523 to 5390 cp, with the ODF80 samples having the lowest PV, whereas the highest value was found in the ODF50 samples. Considering all the GBF samples showed significantly higher PV compared to WF (P-value < 0.05), the higher amylose content in GBF samples may be a reason for higher viscosity (Bertolini et al., 2010). The stability of starch gel in the ODF50 samples was statistically similar to FDF (P-value > 0.05).

The HV and FV as well as the SB decreased dramatically with the application of high drying temperatures to produce GBF. Hold viscosity measures the viscosity when the swollen starch was disrupted due to shearing and heating (Cahyana et al., 2019). Starch granules become progressively susceptible to shear stress when they are swollen, especially in starches with lower amylose content (e.g., ODF80 and ODF110).

Table 5.4. Pasting properties of green banana and wheat flour samples

Sample	PT (°C)	PV (cp)	HV (cp)	FV (cp)	SB (cp)	BD (cp)
WF	66.63±0.25 d	3768±245 c	5768±159 a	6258±332 a	2489±256 a	3891±106 d
FDF	77.10±0.10 c	4989±137 b	4589±302 bc	5659±274 b	2386±291 a	5675±152 b
ODF50	77.06±0.15 c	5390±201 a	4390±172 c	5442±140 b	2269±267 ab	5275±157 c
ODF80	79.06±0.15 b	2523±249 d	2723±364 cd	4751±264 c	1225±223 b	7252±227 a
ODF110	81.23±0.35 a	2735±297 d	2535±317 d	4674±137 c	1098±214 b	7118±151 a

Mean ± standard deviation values of triplicate determinations. Values with different letters in the same column are significantly different (P-value < 0.05). PT, pasting temperature. PV, peak viscosity. HV, hold viscosity. FV, final viscosity. SB, setback. BD, breakdown. WF, wheat flour, FDF, freeze-dried flour, ODF50, oven-dried flour at 50 °C, ODF80, oven-dried flour at 80 °C, ODF110, oven-dried flour at 110 °C.

While drying banana at temperatures higher than starch gelatinization temperature results in disruption of granular sections (Shafie et al., 2016), ODF50 samples were the only heat-treated GBF that showed no significant difference compared to the freeze-dried samples (P-value > 0.05).

Final viscosity provides information about the specific quality of the starch and reflects the stability and ability of the cooked gel to form a viscous paste after cooling, and SB is an index for syneresis of starch (Khamsucharit et al., 2018). As these two parameters are correlated with syneresis, especially during freeze-thaw cycles, it can be concluded that FDF and ODF50 samples are more likely to generate more stable products during storage. Furthermore, the SB indicates starch retrogradation tendency during storage; a lower SB shows less tendency for retrogradation (Shafie et al., 2016). Adding FDF and ODF50 in formulations of food products may increase the content of resistant starch type 3 (RS3) by enhancing retrogradation after cooling down. This trend was also observed in another study on ripe banana flour (Cahyana et al., 2019).

Breakdown viscosity specifies the susceptibility of cooked starch granules to be disintegrated by mixing at elevated temperatures. As shown in Table 5.4, FDF was the most stable GBF to shear and heat treatment during the pasting process. On the other hand, ODF50 had significantly the highest resistance against shearing amongst the heat-treated samples (P-value < 0.05). Considering previous results, the ability to form a cohesive gel (FDF and ODF50) might be correlated with the stability of flour paste during mixing at high temperature in food processing. Cahyana et al. (2019) reported similar results for GBPF and WF. Conversely, Nasrin et al. (2015) reported reduced viscosity in peel flour compared to the pulp one. The result of this study showed that with the incorporation of peel into the banana pulp flour, the overall viscosity properties were improved.

5.3.5. FTIR spectroscopy

The chemical structures of GBF and WF samples were characterized by FTIR and the results are presented in Table 5.5. The information obtained from the spectra is associated with short range order in the starch granule (Khamsucharit et al., 2018). In order to understand the behaviour of the starch upon heat treatment or the changes during storage, the analysis of the information of FTIR spectrogram is important (Meziani et al., 2011). As shown in Fig 5.1, the FTIR spectra shows the functional groups on the flours. By matching the band transmittance on FTIR spectra with the vibrational modes of various chemical bonds based on experimental data, the differences in peak intensity might be used to predict the occurrence of conformational changes in starch granules (Ng et al., 2014).

The stretching absorption band centred at 3277.52 cm^{-1} represents the OH groups (carboxylic acids) in all GBF samples (Table 5.5). Similar findings were found in a study on GBPeF (Pathak et al., 2017). The band observed at around 2900 cm^{-1} contributed to stretching vibration of methyl and methylene groups for all samples. Except for the FDF sample, all the other samples showed a peak at around 1630 cm^{-1}, which signifies that the amide I bond was broken during the freeze-drying process. Meziani et al. (2011) suggested that the β-plated sheet and α-helix structures are partially unfolded under freezing. This explains the shifting of a new range of formed β-sheets to a less strongly hydrogen-bonded structure. Due to the presence of more protein, a sharp shift around 1640 cm^{-1} in the WF samples indicated stronger intra-molecular bonds compared to GBF.

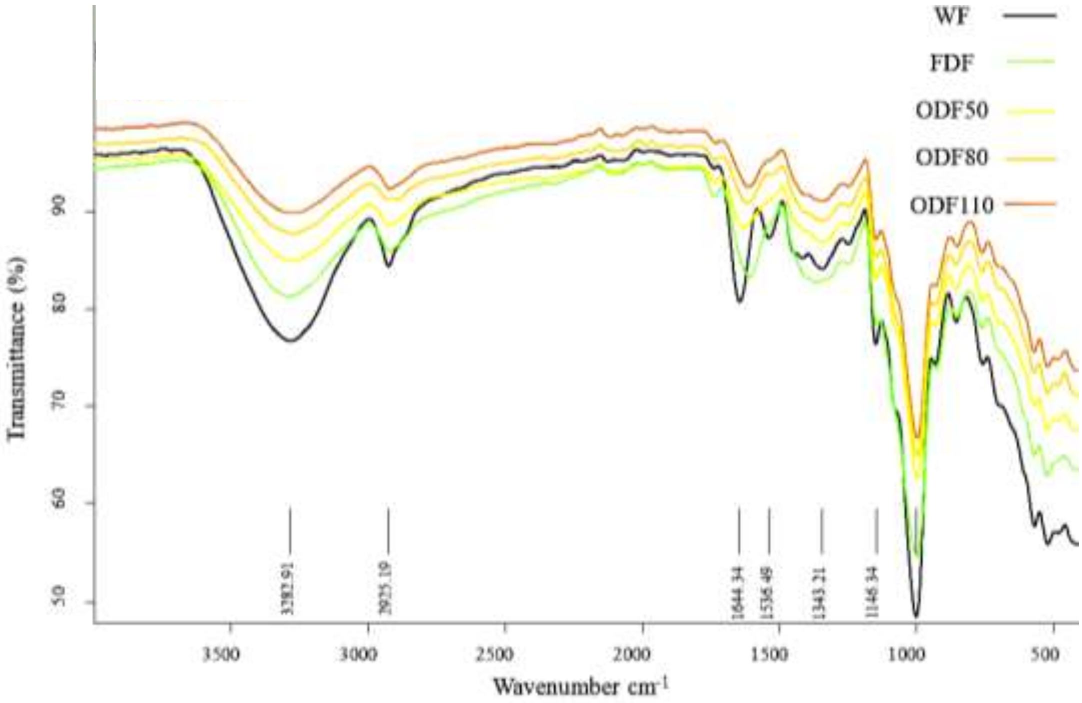

Figure 5.1. FTIR spectrum of green banana and wheat flour samples

WF, wheat flour, FDF, freeze-dried flour, ODF50, oven-dried flour at 50 °C, ODF80, oven-dried flour at 80 °C, ODF110, oven-dried flour at 110 °C.

The presence of high CHO groups in WF samples caused a significant drop in transmittance around 1100 cm^{-1} compared to heat-treated flours which showed less stretching of C-O-C, C-O and C-C bonds. Although there was no trace of notable nucleic acids, phosphorylated proteins, phospholipids in GBF samples, the WF samples showed a band assignment of asymmetric phosphodiesters at 1000 cm^{-1}. These results confirmed the previous findings about sensitivity of β-sheet, α-helix and amide structures to the freezing-drying process (Nasrin et al., 2015). a-molecular bonds compared to GBF.

Table 5.5. FTIR peaks for green banana and wheat flour samples

Wavenumber (cm^{-1})					Band assignments	Primary contributing biomolecules
WF	**FDF**	**ODF50**	**ODF80**	**ODF110**		
3282.91	3281.52	3277.79	3271.98	3272.09	ν(=C–H) OH groups	Unsaturated lipids
2925.19	2922.4	2924.39	2892.58	2919.47	ν_{as}(C–H) of CH_3 OH groups and CH_2 deformations	Saturated lipids
1644.34	-	1631.27	1615.49	1611.32	amide I	Protein
1536.49	-	-	-	-	amide II	
1343.21	1368.06	1339	1338.55	1337.34	amide III	
1146.34	998.06	995.49	995.83	996.24	ν(C–O–C), ν(C–O), ν(C–C), ν(C–O–P), ν(P–O–P)	Carbohydrates, nucleic acids, phospholipids and proteins
1000.02	-	-	-	-	ν_{as}(P=O) of phosphodiesters (PO_2^-) C–O (Aliphatic amines)	Nucleic acids, phosphorylated proteins, phospholipids

ν, stretching, s, symmetric, as, asymmetric, WF, wheat flour, FDF, freeze-dried flour, ODF50, oven-dried flour at 50 °C, ODF80, oven-dried flour at 80 °C, ODF110, oven-dried flour at 110 °C.

5.3.6. Mineral content

It is well known that ripe banana pulp is known as a rich source of potassium and magnesium in the diet, while ripe banana peel do not contain much of these minerals (Segundo et al., 2017b). There is no information available on the mineral content in whole green banana flour. As reported by Sulaiman et al. (2011) in a study on different species of fresh banana, the peel showed almost three times more K content compared to the pulp. In the present work, the average potassium and magnesium content of GBF samples was at least 11 and three times more than those found in the WF, respectively. Considering that the type of the heat treatment used for GBF production did not show significant difference amongst the samples (P-value > 0.05), it can be concluded that 100 g of GBF contributes 42% of the Dietary Reference Intake (DRI) for potassium (Monsen, 2000). Furthermore, the marked increase in copper content in all GBF samples indicated that this flour is a rich source of this essential trace mineral (Table 5.6). The average Mg content of all GBF samples (145.33 mg/100 g) was significantly higher than WF (37.5 mg/100 g) (P-value < 0.05). The DRI for Mg has been reported as 420 mg for male adults and 320 mg for female adults (Monsen, 2000). The Mg content of GBF produced in this study, from either of the drying methods, can provide around 34% (males) to 45% (females) of the DRI for Mg.

This trend was similar in terms of Ca, Mn, Fe and Zn content of GBF samples. The DRI for calcium is 1000 mg for adults (Monsen, 2000). The GBF obtained in this work would supply about 8% of the DRI for Ca. As Haslinda et al. (2009) showed in his comparative study on GBF and GBPF (*awak ABB Muasa Acuminate*), the amount of minerals increases when peel is incorporated into pulp flour. The current results mostly agree with the previous reports for Cu and Mg obtained from GBF dried at 60 °C (Toledo De Camargo Ranzani et al., 1996, Pereira and Maraschin, 2015). However, the average values for Ca, P, Fe, K, Zn and Na are higher than those reported by Aurore et al. (2009) and Haslinda et al. (2009). This might be due to the differences in cultivars, genus and also environmental factors (Pereira and Maraschin, 2015).

Table 5.6. Mineral composition of green banana and wheat flour samples (mg/100 g)

Minerals	WF	FDF	ODF50	ODF80	ODF110
Na	13.01±0.20 b	17.01±0.10 a	17.50±0.1 a	17.30±0.1 a	17.60±0.10 a
Mg	110.50±11.5 b	143.33±8.9 a	144.66±13.07 a	143.03±11.05 a	143.66±9.12 a
P	335.01±2.71 a	293.33±3.23 b	292.3±2.18 b	292.02±2.61 b	291.90±3.62 b
K	377.33±3.10 b	2001.30±2.10 a	2002.66±2.11 a	2007.66±2.17 a	2002±3.71 a
Ca	23.73±0.10 b	85.53±0.53 a	84.60±0.23 a	83.23±0.31 a	83.33±0.36 a
Mn	1.43±0.01 b	2.49±0.05 a	2.62±0.06 a	2.60±0.04 a	2.52±0.05 a
Fe	1.25±0.10 b	3.41±0.05 a	3.99±0.06 a	3.39±0.07 a	3.46±0.10 a
Cu	0.20±0.04 b	0.40±0.01 a	0.39±0.03 a	0.34±0.02 a	0.35±0.01 a
Zn	1.90±0.01 b	3.09±0.02 a	3.14±0.02 a	3.04±0.02 a	3.04±0.01 a

Mean ± standard deviation values of triplicate determinations. Values with different letters in the same column are significantly different (P-value < 0.05). WF, wheat flour, FDF, freeze-dried flour, ODF50, oven-dried flour at 50 °C, ODF80, oven-dried flour at 80 °C, ODF110, oven-dried flour at 110 °C.

5.4. Conclusion

The particle size results suggested that ODF50 treatment group had the highest percentage of uniform flour compared to other higher drying temperatures and was the nearest to the non-heat treatment method. This behaviour was correlated to density properties, where ODF50 samples showed the same heaviness to FDF samples, while higher drying temperature (i.e., ODF80 and ODF110 treatment groups) resulted in flour that is easier for transportation and packaging. As the Hausner ratio and compressibility index show, FDF and ODF50 displayed a strong agglomeration with propensity to create a better slurry in the presence of water. These results were correlated with textural properties, which indicated the ODF50 treatment resulted in a flour that was viscose cohesive with higher consistency compared to the other heat-treated samples. These results, together with the pasting behaviour of GBF samples, indicated that the functionality of GBF was significantly affected by treatment at temperature higher than 50 °C. Consequently, due to the lower setback parameter, syneresis phenomena are more likely to happen in ODF80 and ODF110 samples. Likewise, the findings from this study confirmed that the incorporation of peel into pulp flour improved the stability of flour paste and increased resistance during mixing. Likewise, FDF can act as a good thickener and stabilizer in the food matrix. Furthermore, FDF and ODF50 samples are remarkable sources of potassium, magnesium and calcium. However, further studies are required to understand the behaviour of these GBF samples in different food products.

5.5. Limitations

More textural attributes could have been done with different probes (Light Knife Blade, Kieffer dough & gluten extensibility rig, Chen-Hoseney dough stickiness rig and Warburtons dough stickiness system) which were not available. Textural tests such as extrusion, tensile and adhesion could have provided more information on the green banana flour characteristics for potential application in other food products. Also, use of the amylograph test could have provided information about alpha amylase activity, gelatinisation of starch and its relation to the observed rheological properties.

CHAPTER 6

Rheological, textural and structural changes in dough and bread partially substituted with whole green banana flour

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Chapter aim:

The nutritional properties of the green banana flours produced in chapter 4 were evaluated in chapter 5. To gain more insights on the utility of these flours the rheological properties of the flours need to be studied. The present chapter investigates the textural and storage properties of a bread made at different fortification levels of whole green banana flour produced by oven or freeze-drying techniques to examine their impact on the technological properties of dough and bread in relation to fortification level.

6.1. Introduction

Bread is the most consumed staple food around the world, especially in developing countries, and is mainly composed of flour, water, yeast and salt (Mancebo et al., 2017). Bread is considered an unstable solid foam formed by crust and crumb with a dispersion of starch molecules in a continuous protein matrix (Arp et al., 2018). White bread is the most popular bread type in the world, however, there is a growing research on fortifying it with an array of dietary fibres (DF) and functional compounds to make bread as a carrier of health benefiting compounds. In recent years, substitution of wheat flour with bran, prebiotics, vitamins, minerals, and others functional ingredients has been proposed (Al-Sheraji et al., 2013).

Considering the global high amount of banana loss along the production chain, the utilization at early stage can be a feasible solution to reduce banana waste. Drying and converting the whole green banana into a flour has been proposed as a potential process to produce a long-lasting food ingredient. The effect of different drying treatments on green banana has been reported in the previous chapters.

The structural quality of bread emanates from the gluten-starch matrix, and substitution of wheat flour with other ingredients could result in poor technological properties. Researchers have attempted to predict bread quality by dough properties analysis (Al-Sheraji et al., 2013). However, those studies were limited to banana pulp flour only. Others investigated banana flour (BF) in pasta, noodles and gluten-added bread with the focus on nutritional and digestive attributes of the products more than the technological aspects (Anyasi et al., 2018, Alkarkhi et al., 2011, Vu et al., 2018, Almeida-Junior et al., 2017).

In the previous chapters, the characteristics of four types of prepared GBF were examined. The GBF prepared at 50° C (ODF50) and by freeze drying (FDF) were selected to be incorporated in bread model to examine the effects of the flour samples on the nutritional properties of the fortified bread (chapter 5). The present study investigates the textural and storage properties of this fortified bread made at three different fortification levels to examine the impact on the technological properties of dough and bread.

6.2. Material and Methods

6.2.1. Green banana flour preparation

The flour samples (air-oven-dried and freeze-dried green banana flours) were produced as described in chapter 4, section 4.2.1.

6.2.2. Breadmaking process

Preliminary tests were conducted to determine the maximum quantity of green banana flour (GBF) to prepare a bread with at least specific volume of 4 L/kg (Sluimer, 2005). Maximum GBF incorporation to be used in formulation was 30%. The dough was formulated with a mixture of wheat flour (WF) or three substitution levels of freeze-dried green banana flour (FDF) and oven-dried green banana flour

(ODF) (10%, 20% and 30% w/w) and labelled as F10, F20, F30 and O10, O20 and O30. Above 30% of substitution resulted in poor technological and sensory properties. Only the breads with specific volume greater than 4 L/kg were selected to continue the study because lower values than that negatively affect the quality of the breads (Sluimer, 2005). A control bread was baked with 100% wheat flour and labelled as WB (wheat bread).

The formulation for making 100 g control dough sample was as follows: 53.2 g of WF, 16 ml of water, 16 ml of standard milk (3% fat), 0.5 g of sodium chloride, 5 g of honey, 8.8 g of butter and 0.5 g of activated dried yeast. Treatments were made with 10%, 20% and 30% substitution of GBF replacing the WF. Water addition was kept constant in all formulations in order to examine the true effect of fortification on bread dough. The dough was made using a bread maker-mixer (Breville, Sydney, Australia). All dry ingredients were mixed for 3 min at a low speed followed by addition of liquid ingredients and mixed for another 3 min. The mixture was kneaded for 12 min at a medium speed (Arp et al., 2017). This fresh dough was used for the assessment of viscoelastic and mechanical properties.

6.2.3.1. Viscoelastic properties

The fundamental rheological analysis of the dough samples was performed by oscillation tests based on Mancebo et al. (2017) method. A Haake RheoStress rheometer (Thermo Fisher Scientific, Schwerte, Germany) equipped with a Phoenix

II P1-C25P water bath at 25 °C. The rheometer was installed with a 60 mm titanium serrated plate-PP60 Ti in parallel plate-plate geometry with a 3-mm gap. The dough was rested for 300 s prior the test. Firstly, a strain sweep test was performed at 25 °C with a stress range of 0.1 - 10 % at the frequencies of 0.1, 1, 10 and 100 Hz to identify the linear viscoelastic region. Based on the obtained results, a strain value of 0.1% in the linear region was selected (part of the plot stress versus strain where is linear). Finally, 1 Pa was chosen for all dough samples. Storage modulus (G′), loss modulus (G″), loss tangent (tan δ), complex modulus (G*) and complex viscosity (|η*|) responses were collected a HAAKE RheoWin Data Manager ver. 3.50.0012 (Thermo Fisher Scientific, Schwerte, Germany).

6.2.3.2 Large deformation mechanical properties of dough

The texture profile analysis (TPA) was used to determine hardness, adhesiveness, resilience, cohesiveness, springiness and chewiness. The test was completed using a TA-XT2 texture analyser (Stable Micro Systems, Surrey, UK) using a 50 mm diameter aluminium probe at speed of 1.0 mm/s, 75 s delay between first and second compression and a 60% compression of dough original height (Sanz-Penella et al., 2010). Approximately 20 g of dough was freshly made (without yeast) in a spherical shape. To prevent moisture loss and the distortion induced by the negative peak of adhesiveness, a plastic film was applied on the dough. A second

TPA was made without plastic film for measuring the dough adhesiveness (Sanchez et al., 2014). The test was performed at the room temperature.

6.2.4. Bread characteristics

Bread samples (n = 3) were prepared according to a straight dough procedure with the same bread-maker (Breville, Sydney, Australia). After the first dough proofing for 90 min, it was kneaded for 5 min for deaeration. Followed by the final proofing (30 min), baking was completed within 180 °C for 25 min in the same bread maker-mixer. Bread samples were cooled at room temperature for one hour before packing in polypropylene zip bags. Except for textural analysis, breadcrumb samples were grounded, freeze-dried and vacuum packed for the further analysis.

6.2.4.1 Bread baking performance

Baking performance parameters were carried out on the fresh bread loaves. The bread mass was determined by the average value of a direct measurement of three bread loaves. The apparent volume was measured by the rapeseed's displacement method (AACC 10-05 method) as described by previous researchers (Gomes et al., 2016, Altuna et al., 2016, Ho et al., 2013). The seeds were poured into an empty container to measure its volume. Then, the rapeseeds in the container were measured in a graduated cylinder and marked as V_1. Afterwards, a bread loaf sample was placed into the same container and rapeseeds were poured in until the test bread sample was covered. The rapeseeds were measured again in a graduated cylinder and labelled as V_2.

Bread apparent volume = $V_1 - V_2$ (6.1)

Bread density was calculated by dividing bread mass to bread volume. The baking loss and specific volume (SV) were calculated as described in equations below (Ho et al., 2013, Gujral et al., 2018):

$$\% \text{ Baking loss} = \frac{\text{Weight of the dough - Weight of baked bread}}{\text{Weight of the dough}} \quad (6.2)$$

$$\text{Bread specific volume } (cm^3/g) = \frac{\text{Bread apparent volume } (cm^3)}{\text{Bread mass (g)}} \quad (6.3)$$

6.2.4.2. Textural properties of fresh breadcrumb

Texture profile analysis was performed by the texture analyser using a 50 kg load cell and a 50 mm aluminium cylindrical probe. The pre-test and post-test speed was 1.0 mm/s and the test speed was 4.0 mm/s with one second interval time between the first and second cycles (Liu et al., 2018). The texture profile analysis software (TPA) was used to calculate hardness, resilience, springiness and chewiness.

6.2.4.3. Storage stability of bread

In order to understand the changes in bread structure during the storage time, firmness (F) and moisture content (MC) were analysed under different time and temperature values. The AACC (2000) method number 74-09 was employed as modified by Mohamed et al. (2010). A bread slice with 25 mm in thickness was undergone in the TPA using a 50 kg load cell equipped with a 50 mm aluminium cylindrical probe. The analysis was performed on bread slices after 0, 2, 4 and 7 days of storage at 25 °C, 4 °C and -20 °C.

6.2.6. Data analysis

The statistical analysis of data was performed using Minitab® program version 16 (Minitab, Australia). Data were analysed using one-way ANOVA test to determine the effect of GBF type and fortification levels on the measured parameters. The correlation amongst parameters was assessed using Pearson's correlation. Post-hoc Tukey test was used to determine the differences amongst the means at P-value < 0.05. The means of three replicates expressed as mean ± standard deviation.

6.3. Results and Discussion

6.3.1. Dough rheological properties

Frequency sweep results showed that all dough samples had higher G′ than G″, signifying a viscoelastic behaviour of dough (Table 6.1). Compared to the low GBF fortification levels (10% and 20%), WB showed significantly higher G′ in dough samples regardless of the GBF type (P-value < 0.05). This could be as a result of gluten network loss in the GBF samples (Zhang et al., 2019). In the F30 and O30 samples, the effect of low content of gluten owing to replacing the WF with GBF, might be compensated by the presence of high percentage of GBF.

Higher percentage of soluble DF in green banana peel together with higher total starch content has also been considered factors that can increase G* value (Kurhade et al., 2016). This can be seen in Table 6.1, where F30 and O30 showed the highest complex modulus which indicated those dough samples contained the greatest rigidity amongst other ones (P-value < 0.05).

Table 6.1. Viscoelastic properties of bread dough determined at frequency of 1 Hz

Sample	G′ (Pa)	G′′ (Pa)	tan (δ)	ǀG*ǀ (Pa)	ǀɳ*ǀ (Pas.s)
WB	2571±100.90 b	732±48.80 b	0.28±0.01 d	2665±89.80 c	425±19.70 b
F10	1392±100.01 cd	499±50 cd	0.36±0.01 a	1475±104.40 de	235±19.60 cd
F20	1605±98.70 c	573±50.50 c	0.36±0.01 a	1705±98.50 d	272±19.90 c
F30	2844±120.70 ab	936±50.80 a	0.33±0.01 c	3180±107.20 b	473±21.20 ab
O10	1172±93.20 d	429±50.60 d	0.36±0.01 a	1249±98.80 e	198±20.10 d
O20	1774±103.70 c	625±46.50 c	0.33±0.01 b	1860±99.30 bc	264±20.20 c
O30	3075±162.70 a	1038±48.40 a	0.32±0.01 c	3262±89.30 a	524±15.70 a

Mean ± standard deviation values of triplicate determinations. Values with different letters in the same column are significantly different (P-value < 0.05). G′, storage modulus, G′, loss modulus, tan δ, loss tangent, G*, complex modulus, ǀɳ*ǀ, complex viscosity, WB, wheat bread, F10, F20 and F30, substitution levels of freeze-dried banana flour with wheat flour. O10, O20 and O30, substitution levels of air oven-dried banana flour with wheat flour.

Moreover, a lower tan (δ) value was found in WB samples compared to all fortified bread samples. Han et al. (2019) reported that increasing starch content can be associated with weakening the strength of the gluten strength and results in impaired dough viscoelasticity. Furthermore, presence of DF in the bread dough could reduce starch swelling which might decrease the bread volume (Blanco Canalis et al., 2019). The same increasing trend for G* and η* in 30% fortified bread samples resulted in lower bread volume compared to 20% and 10% treatments (P-value < 0.05). These results might indicate the dough ability to resist the expansion caused by yeasts during fermentation (Mirsaeedghazi et al., 2008).

6.3.2. Dough mechanical properties

The hardness of the dough represents the peak force measured during the first compression in TPA. Considering the high amount of DF and lower gluten in GBF compared to WF, an increase in hardness with the increasing in fortification level was recorded (Table 6.2). Higher water holding capacity in GBF, especially FDF, promotes partial dehydration of gluten, starch incomplete gelatinisation and trans-conformational change in dough structure (Loong and Wong, 2018). These changes make the fibres to act as a barrier against water migration and protected starch granules from swelling (Steglich et al., 2014). Current findings concurred with those found in a high-fibre steamed bread made with high amylose maize starch (Lin et al., 2012) and also in a bread fortified with 10% bran (Bock et al., 2013).

It has been reported that gums and soluble DF can increase the adhesiveness of the dough because of the hydrophilic sites (Altuna et al., 2015). In support, Guo et al. (2003) reported that high amylose content is another factor for increasing dough adhesion. The GBF used in the current study showed almost three times more amylose than WF (Fig 4.3). In fact, a suitable dough for Breadmaking should not be too sticky (Loong and Wong, 2018). As it can be seen in Table 6.2, fortified dough samples exhibited lower adhesion compared to control, without any significant difference amongst GBF samples. Overall, it can be concluded that FDF had slightly improved dough characteristics compared to ODF.

Cohesion showed a significant decrease above 10% fortified dough samples compared to control ones (Table 6.2). Also, dough made with ODF exhibited lower cohesiveness values in comparison to FDF-fortified bread samples (P-value < 0.05). These results were similar to those reported by Arp et al. (2017) who suggests the presence of gluten is vital for cohesiveness. This phenomenon can also explain the lower adhesiveness values in all fortified doughs compared to the wheat dough. Steglich et al. (2014) reported that DF can prohibit water migration and inhibit the starch swelling, which results in the reduction of adhesiveness.

Elasticity or springiness refers to the tendency of the deformed dough to return to its undeformed condition after removal of force. Springiness showed no significant difference amongst treatments; however, lower values were recorded in control dough (P-value < 0.05) which was similar to the findings found in dough enriched with RS (Altuna et al., 2015).

Table 6.2. Large mechanical properties of bread dough samples

Sample	Hardness (N)	Adhesiveness (N.sec)	Resilience (%)	Cohesiveness	Springiness (%)
WB	0.82±0.28 c	0.24±0.01 a	0.08±0.01 cd	0.67±0.02 a	0.47±0.01 b
F10	0.98±0.44 abc	0.59±0.02 ab	0.11±0.01 a	0.61±0.02 ab	0.78±0.01 a
F20	0.99±0.83 abc	0.91±0.01 b	0.09±0.01 ab	0.6±0.01 b	0.69±0.01 a
F30	1.05±0.73 ab	0.69±0.05 b	0.07±0.01 d	0.47±0.01 c	0.63±0.01 ab
O10	0.8±0.12 c	0.59±0.01 ab	0.09±0.01 bc	0.59±0.03 ab	0.68±0.01 a
O20	0.86±0.24 bc	0.81±0.06 b	0.08±0.01 cd	0.58±0.02 bc	0.68±0.01 a
O30	1.08±0.63 a	0.83±0.02 b	0.08±0.01 cd	0.4±0.02 d	0.66±0.01 a

Mean ± standard deviation values of triplicate determinations. Values with different letters in the same column are significantly different (P-value < 0.05). WB, wheat bread, F10, F20 and F30, substitution levels of freeze-dried banana flour with wheat flour. O10, O20 and O30, substitution levels of air oven-dried banana flour with wheat flour.

6.3.4. Bread baking performance

A significant decreasing trend was observed in bread height and SV with more incorporation of GBF regardless of its drying method (P-value < 0.05). As the GBF percentage increased in the bread, a denser structure with a significant higher SV was recorded (Table 6.3). Higher loaf volume achieved in a bread fortified with the flour made from banana pseudo-stem pulp only when it was accompanied with carboxyl methylcellulose (Ho et al., 2013). Partial substitution of WF with rye, oat, barley and sweet potato resulted in a significant increment in bread density (Al-Sheraji et al., 2013). This observation could be related to the reduction in glutenin, which causes the reduced ability of bread to hold the expanded structure (Lazaridou et al., 2007).

It is well-known that gluten plays a key role in bread structure and the maintenance of moisture during the baking process. Baking loss is an indicator of water loss from dough during baking (Arp et al., 2017). To reduce moisture loss during baking, partial substitution of WF with starch, flour improvers, hydrocolloids and hydrophilic groups have been suggested (Silvas García et al., 2013). The ability of gluten-starch matrix to hold water in the texture during baking is directly related to the percentage of GBF added, and a lower baking loss percentage was evident at higher level of fortifications. The substitution at 30% FDF or ODF significantly reduced the moisture loss (P-value < 0.05), which could be because of an increase in hydrophilic bonds of total starch content in fortified bread samples. Our previous findings on the same samples showed at least 5% more total starch content in 30% fortified samples compared to control bread samples (Fig 4.2). These findings also were similar to those found in banana bread made with 10% banana pseudo-stem pulp with added carboxy methyl cellulose as a texture improver (Ho et al., 2013, Altuna et al., 2015, Gujral et al., 2018). Considering the baking loss values, it can be concluded that there was no significant difference between the types of the GBF used in bread in 30% fortification level compared to control.

Table 6.3. Baking performance of bread samples

Sample	Bread Height (cm)	SV (cm^3/g)	Density (g/cm^3)	Baking Loss (%)	Dough mass (g)	Bread mass (g)	Apparent volume (cm^3)
WB	9.10±0.01 b	4.50±0.03 b	0.22±0.01 e	6.80±0.26 d	586±7.81 ab	546±6.51 ab	2500±12.7 b
F10	9.90±0.05 a	4.80±0.01 a	0.21±0.02 f	8.78±0.52 b	587±3.86 ab	536±3.31 bc	2596±5.69 a
F20	8.90±0.05 c	4.46±0.03 c	0.23±0.01 d	8.60±0.62 bc	591±0.47 ab	540±3.31 abc	2396±5.29 c
F30	8.17±0.05 f	4.12±0.01 d	0.25±0.03 a	7.40±0.16 cd	592±0.93a	549±2.56 a	2164±15.11 f
O10	9.20±0.10 b	4.50±0.07 bc	0.23±0.01 c	10.22±0.01 a	590±3.08 ab	533±4.24 c	2433±13.01 c
O20	8.60±0.1 d	4.20±0.05 d	0.22±0.01 de	8.91±0.63 b	591±1.59 ab	538±2.31 bc	2278±30.63 d
O30	8.40±0.010 e	4.10±0.50 d	0.24±0.02 b	7.5±0.42 cd	580±4.44 b	536±4.36 bc	2227±14.01 e

Mean ± standard deviation values of triplicate determinations. Values with different letters in the same column are significantly different (P-value < 0.05). WB, wheat bread, F10, F20 and F30, substitution levels of freeze-dried banana flour with wheat flour. O10, O20 and O30, substitution levels of air oven-dried banana flour with wheat flour. SV, Specific volume.

6.3.5. Textural analysis of breadcrumb

The texture of breadcrumb is a vital factor which can be regarded as a freshness indicator by consumers (Hernández-Aguirre et al., 2019). Despite of the type of GBF, the increase in GBF levels in bread samples increased hardness and chewiness (Fig 6.1, P-value < 0.05). Since the baking performance of fresh bread exhibited a denser structure in fortified samples, consequently, it affected resilience and springiness in a converse manner (P-value < 0.05). This phenomenon could be due to the presence of the high solid components, incomplete gelatinisation of starch, partial retrogradation after baking, less gluten matrix, and less expansion of gas cells (Loong and Wong, 2018, Agama-Acevedo et al., 2019). Moreover, significant differences were observed due to the GBF type where chewiness values were higher in ODF-fortified bread samples compared to FDF ones (Fig 6.1). Our previous results, using the approach under the same experimental conditions, showed a higher porous structure in FDF compared to the ODF, which led to higher water holding capacity and oil holding capacity compared to the ODF (Table 4.4 and 5.2). The lower water binding capacity and the coarse structure of ODF compared to FDF could be the reasons for a denser structure and higher chewiness in the former treatment.

Interactions between protein, DF, starch and lipid are important for structural changes occurring during breadmaking (Liu et al., 2018). For instance, incorporation of 20% shortenings decreases hardness due to the softening effect of lipids and water entrapment within of the bread structure (Mancebo et al., 2017). Further, high amylose maize starch increased the springiness of bread when it substituted WF at 30% (Arp et al., 2018). The hardness values were in accordance to the findings of Gomes et al. (2016) for bread contained 20% green banana pulp flour together with protein and flour improver. Soto-Maldonado et al. (2018) findings on muffin made with partial substitution of over ripen banana flour exhibited a lower hardness compared to control, which suggests that the lower DF in ripe banana compared to green one affects the texture of the product.

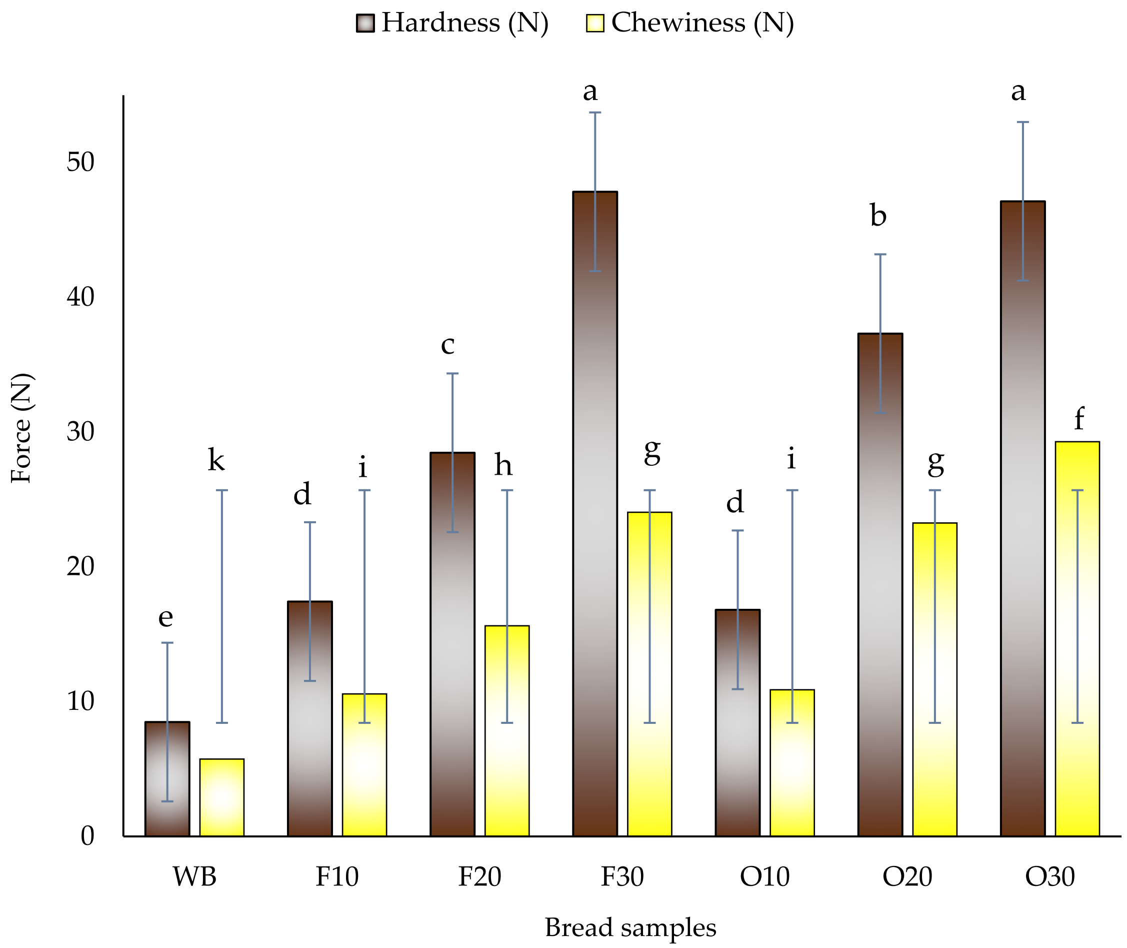

Figure 6.1. Textural properties of fresh bread crumb samples

Mean ± standard deviation values of triplicate determinations. Bars with the different letters in the same variable are significantly different (P-value < 0.05) (a to e for hardness and f to k for chewiness). WB, wheat bread, F10, F20 and F30, substitution levels of freeze-dried banana flour with wheat flour. O10, O20 and O30, substitution levels of air oven-dried banana flour with wheat flour.

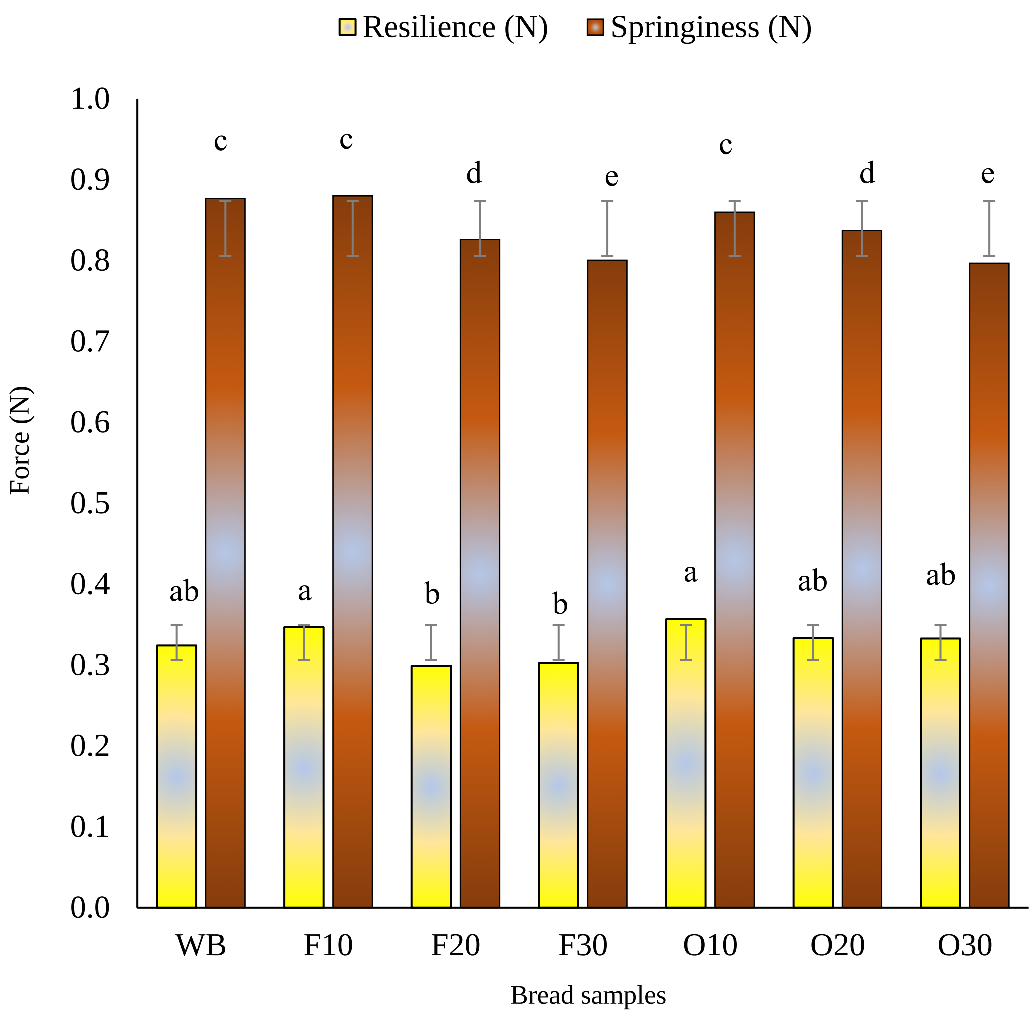

Figure 6.2. Textural properties of fresh bread crumb samples

Mean ± standard deviation values of triplicate determinations. Bars with the different letters in the same variable are significantly different (P-value < 0.05) (a to b for resilience and c to e for springiness). WB, wheat bread, F10, F20 and F30, substitution levels of freeze-dried banana flour with wheat flour. O10, O20 and O30, substitution levels of air oven-dried banana flour with wheat flour.

6.3.6. Bread staling during storage

Starch gelatinisation occurs during baking, implicating the loss of double helix structure of amylopectin and leaching amylose out of starch granule. Retrogradation of starch is a phenomenon that stems from slow recrystallization of amylose and amylopectin. Retrogradation results in bread staling which contributes to increasing the firmness of the breadcrumbs and thus it becomes less desirable by consumers (Curti et al., 2014). These changes are directly influenced by the temperature and time of storage as well as the ingredients used in the making of the bread. This can affect consumer acceptability due to the changes in firmness and moisture loss. As a result, measuring these variables are considered indications for bread staling (Salinas and Puppo, 2018).

Overall, the lowest MC was recorded in O30 samples stored at 25 °C after seven days of storage (Table 6.4). According to the results, the highest water retention was found in control samples stored and 10% fortified ones stored at 25 °C after two days of storage. Results for firmness indicated the highest value in O30 samples after seven days of storage in the fridge and at room temperature, while F10 samples depicted the least firmness under freezing condition after two days of storage (5.2 N) (Table 6.5).

Regardless of the storage conditions, firmness of bread fortified with ODF was at least similar or higher than FD-fortified ones and WB (P-value < 0.05). Considering the fact that MC level was significantly higher in WB compared to the fortified samples throughout the storage, it can be suggested that loss of moisture can affect the rate of starch retrogradation, especially in the 4 °C samples. These findings were in accordance with the findings of Mohamed et al. (2010), who reported higher banana pulp flour fortification lead to high staling rate after two days of storage. The authors suggested that a lower gluten content and a higher insoluble fibres content can increase firmness. This behaviour was also seen in inulin-fortified bread stored at ambient temperature after the third day of storage (Salinas and Puppo, 2018). In terms of storage temperature, storage at -20 °C showed the lowest firmness (P-value < 0.05) independent from the type of GBF in fortified bread samples. The F10 treatment exhibited the nearest firmness to WB after a week of storage at -20 °C. Regarding fortification level, due to the increment in coarse DF amounts and lower moisture content in the fortified bread, there was an inverse relationship between GBF fortification level and firmness at all storage conditions. Al-Mahsaneh et al. (2018) showed that elevated amount of sugar has a direct influence on the migration of water from gelatinised starch, gluten and soluble fibres, which eventually causing bread stiffness. Current findings showed that the amount of carbohydrate was higher in FDF and ODF compared to WF. The Tables 6.4 and 6.5 show the results of firmness and MC changes during different storage conditions.

Table 6.4. The moisture content of bread samples during different storage conditions

Sample	Days	0	2	4	7
	T (°C)	**MC (%)**	**MC (%)**	**MC (%)**	**MC (%)**
WB	25	38.5±2.7 a	37.7±1.9 b	34.9±1.7 d	33.7±2.1 f
	4		34.6±1.7 de	32.3±1.5 g	29.5±1.9 h
	-20		35.5±1.6 c	34.5±1.6 de	34.1±1.5 ef
F10	25	35.8±2.3 a	35.2±1.9 b	34.8±1.6 bc	33.2±1.6 e
	4		34.2±1.8 d	32.5±1.7 f	30.7±1.8 g
	-20		34.9±1.9 bc	34.9±1.9 bc	34.6±1.8 cd
F20	25	35.3±2.4 a	34.8±0.9 ab	34.3±1.2 c	31.3±0.7 e
	4		34.5±1.9 bc	31.8±1.8 d	30.3±0.8 f
	-20		34.9±0.9 ab	34.6±1.3 bc	34.3±1.1 c
F30	25	34.8±2.9 a	32.5±1.1 c	31.5±1.3 d	29.1±1.8 f
	4		33.7±1.9 b	30.7±1.5 e	29.5±1.7 f
	-20		34.8±1.5 a	34.4±1.2 a	34.2±1.5 ab
O10	25	35.4±2.7 a	35.2±1.6 ab	34.4±1.7 c	33.6±1.6 d
	4		34.6±1.5 bc	32.4±1.9 e	30.5±1.9 f
	-20		34.6±1.6 bc	34.4±1.0 c	34.2±1.8 cd
O20	25	35.9±2.7 a	34.5±2.1 bc	34.1±2.6 c	33.5±2.3 d
	4		34.3±2.1 bc	31.6±2.3 e	30.4±2.2 f
	-20		34.7±2.2 b	34.7±2.1 bc	34.5±2.5 bc
O30	25	34.4±2.6 a	34.2±2.7 ab	31.2±2.8 d	29.0±2.8 e
	4		32.9±2.6 c	31.1±2.1 d	29.9±2.9 e
	-20		33.8±2.7 b	33.74±2.6 b	32.9±2.5 c

Mean ± standard deviation values of triplicate determinations. Values with different letters for each bread sample are significantly different (P-value < 0.05). T, temperature, WB, wheat bread, F10, F20 and F30, substitution levels of freeze-dried banana flour with wheat flour. O10, O20 and O30, substitution levels of air oven-dried banana flour with wheat flour. SV, Specific volume.

Table 6.5. The firmness of bread samples during different storage conditions

Sample	Days	0	2	4	7
	T (°C)	F (N)	F (N)	F (N)	F (N)
WB	25	4.2±1.9 j	12.5±2.7 f	19.9±2.4 e	38.9±4.7 a
	4		35.3±4.1 b	23.3±3.9 d	32.4±4.1 c
	-20		4.90±1.1 i	7.20±1.9 h	10.7±2.7 g
F10	25	4.2±1.7 i	19.1±2.5 f	23.7±4.5 e	40.2±6.9 c
	4		34.1±3.8 d	45.4±6.4 b	46.3±5.4 a
	-20		5.2±1.4 i	7.1±1.3 h	10.2±1.7 g
F20	25	8.7±2.1 j	30.7±6.1 f	44.3±6.8 d	46.1±6.4 c
	4		35.1±5.4 e	47.6±5.2 b	50.4±5.9 a
	-20		10.2±1.4 i	11.2±1.4 h	15.2±1.9 g
F30	25	12.2±2.5 h	39.2±5.7 c	48.1±5.8 b	49.9±6.1 a
	4		35.8±5.2 d	49.7±5.1 a	50.2±6.1 a
	-20		14.7±1.8 g	20.6±3.7 f	28.1±3.1 e
O10	25	5.3±1.1 g	20.1±2.6 e	37.8±3.8 bc	41.1±3.5 b
	4		35.3±3.5 cd	41.1±3.7 b	46.5±3.6 a
	-20		10.4±1.7 f	33.1±2.1 d	32.2±3.3 d
O20	25	16.1±2.9 g	35.4±3.7 d	46.3±5.1 c	47.1±5.1 b
	4		35.6±4.6 d	45.9±6.1 c	50.1±5.7 a
	-20		21.5±3.3 f	33.4±6.5 e	33.5±6.1 e
O30	25	19.6±2.8 i	39.3±3.2 d	49.3±3.1 c	52.9±5.1 a
	4		35.7±5.1 e	49.9±5.3 b	52.8±4.9 a
	-20		32.7±3.2 h	33.8±3.6 g	34.9±4.1 f

Mean ± standard deviation values of triplicate determinations. Values with different letters for each bread sample are significantly different (P-value < 0.05). T, temperature, WB, wheat bread, F10, F20 and F30, substitution levels of freeze-dried banana flour with wheat flour. O10, O20 and O30, substitution levels of air oven-dried banana flour with wheat flour.

6.4. Conclusion

In view of the importance of the technological aspects of fortified foods, this study aimed to determine the effect of two types of GBF on structural properties of the fortified bread. Dough viscoelastic properties indicated a resistant dough at 30% fortification, regardless of the GBF type, without losing considerable stretching modulus compared to the control wheat dough. This study also showed that the drying method used for GBF production had an impact on bread structural properties. Utilization of FDF caused a significant decrease in moisture loss during baking, while ODF incorporated bread samples showed lower bread height and specific volume in all fortification levels. Although that both types of fortified bread samples exhibited harder and chewier crumbs compared to wheat bread, even at 10%, they did not change the springiness and resilience of breadcrumbs. The effect of storage conditions on bread staling depicted that the loss of moisture and freshness during storage could be controlled by keeping fortified bread samples in -20 °C. In this regard, changes in firmness were not significantly different from control sample up to 20% of fortification with the use of FDF. More studies are needed for the evaluation of microstructural changes in GBF bread mainly with FDF as it has shown less textural damaging effect on bread slices. Also, sensory evaluation and consumer acceptance are suggested for further studies in this area.

6.5. Limitations

In order to make a correlation between machinery results and human senses in terms of textural properties, a descriptive sensory panel test would have been beneficial which have not been done in this study due to the insufficient budget and time limitation. Furthermore, for better understanding the correlation between bread firmness and bread mechanical properties, the determination of air-bubble parameters (such as mean bubble area, mean bubble perimeter, number of bubbles and bubble area ratio) needed the flatbed image scanner which was not available.

CHAPTER 7

The effect of bread fortification with whole green banana flour on its physicochemical, nutritional and *in vitro* digestibility

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Chapter aim:

Previous chapters investigated the technological and nutritional aspects of the green banana flour (GBF) samples to obtain background information the potential of GBF obtained from different processing regimes. In order to investigate the nutritional aspects of including the GBF in bread, this chapter is designed to determine the effect of both heat treatment used in drying and fortification levels of whole green banana flour on physicochemical and nutritional properties as well as the digestibility of the fortified bread.

7.1. Introduction

Banana is one of the most favourite fruits in the world and contains a high nutritional value. Banana contains higher flavonoids, dietary fibre (DF) and resistant starch (RS) at its early stage of ripeness than when it is ripe (Vu et al., 2018). One of the important features of RS is not being digested within 2 h in the intestine and it enhances the growth of probiotics in the large intestine, which may have a positive indirect effect on colorectal cancer (Nascimento-Souza et al., 2018, Malcomson et al., 2015, Hu et al., 2015, Birt et al., 2013, Sánchez-Pardo et al., 2007). A whole green banana, including the peel, is considered a rich source of RS (41-59%) (Perera et al., 2010). Considering the undesirable taste of green banana, green banana flour (GBF) production has been recommended to add value to banana crops in terms of reducing waste and loss during production chain, improve sustainability and capture some of its nutritional benefits that gets lost with ripening (Hernández-Aguirre et al., 2019).

It has been reported that the type of heating process used in GBF preparation has an impact on both physicochemical and technological properties of the flour (chapter 5). For instance, the RS content in banana flour can vary from 20% to 59% based on banana species, stage of ripeness and most importantly, the drying treatment applied (Tribess et al., 2009). In order to deliver additional health benefits using this functional ingredient, a mixture of banana flour and other alternative flours has been proposed in mostly starch-based food products, such as pasta (Agama-Acevedo et al., 2009), bread (Gomes et al., 2016, Kurhade et al., 2016, Eshak, 2016), cake (Borges et al., 2010, Segundo et al., 2017a, Oliveira de Souza et al., 2018) and gluten-free products (Türker et al., 2016, Radoi et al., 2015, Seguchi et al., 2014). Amongst those, bread has gained more attention in this regard. It is extensively consumed all over the world as it contributes to the intake of proteins, lipid and carbohydrates especially in developing countries. Thus, there is a good opportunity to use banana flour to fortify bread and increase its nutritional value (De Souza Viana et al., 2018).

Even though a wide range of bread types are available in the market, white bread is still the first choice of many consumers due to its sensory attributes. However, white bread is considered a high glycaemic index food due to its high percentage of rapidly digested starch (RDS), which is positively correlated with *in vivo* predicted glycaemic index (pGI) after 20 min of digestion (Martínez et al., 2018).

On the other hand, slow digestible starch (SDS) is accounted for starch digested between 20 to 120 min according to the definition provided by Englyst et al. (1992). As recent studies have shown notably higher content of SDS and RS compared to RDS in green banana pulp flour, utilization of it in bread has gained attention as of late (Menezes et al., 2011, Nasrin et al., 2015, Eshak, 2016, Agama-Acevedo et al., 2016). The slow rate of SDS and RS digestion can improve the insulin response which controls metabolic syndrome and contributes to the risk reduction of obesity, diabetes and cardio-related diseases (Agama-Acevedo et al., 2019).

Although there are few reports on the effect of replacement of wheat flour with banana flour on bread's technological properties (Aurore et al., 2009), *in vitro* studies investigating the digestibility of starch in this model is scarce. Furthermore, the available information on the effect of GBF preparation method on bread properties is scarce. In order to maximize the yield of green banana flour and taking advantage of its high nutritional value, the use of both peel and pulp is proposed (Qamar and Shaikh, 2018). The objectives of this study were to determine the effect of both heat treatment used in drying and fortification levels of the GBF on physicochemical and nutritional properties as well as the digestibility of a bread fortified with different GBF levels.

7.2. Materials and Methods

Green banana (*Musa Cavendish spp AAA*), wheat flour and all the ingredients required for Breadmaking (baking powder, commercial dry yeast, butter, salt, milk and honey) were purchased from a local store (Dunedin, New Zealand).

7.2.1. Green banana flour preparation

The flour samples (air-oven-dried and freeze-dried green banana flours) were produced as described in chapter 4, section 4.2.1.

7.2.2. Breadmaking process

Bread samples were prepared as described in chapter 6, section 6.2.3.

7.2.3. Bread chemical composition

All the physicochemical properties of bread samples were determined using the approved AOAC 2000 standard methods (AOAC, 2005). The moisture content was assayed using a vacuum oven dryer (Thermoline, Australian Marketing Group, Marrickville, NSW, Australia) at 60 °C for 16 h. The water activity was measured by an Aqualab device (Decagon Devices, Washington DC, USA) at 25 °C (Method 925.40; AOAC 2000). Ash content was measured by using a furnace according to the method 923.03 (AOAC 2000). Total fat content was measured using the Soxhlet extraction method (Method 920.39; AOAC 2000). Protein content was conducted using Kjeldahl method to determine total nitrogen and a conversion factor of 6.25 was used (Method 923.03; AOAC 2000). The carbohydrate and energy values were calculated via the following formula:

$$\text{Carbohydrate (g)} = 100 - [\text{moisture content (g)} + \text{ash (g)} + \text{protein (g)} + \text{lipid (g)}] \quad (7.1)$$

$$\text{Energy value (Kcal/100 g)} = 4 \times \text{protein (\%)} + 9 \times \text{lipid (\%)} + 4 \times \text{carbohydrate (\%)} \quad (7.2)$$

The total dietary fibre (TDF) was determined using Megazyme kit (K-TDF; Megazyme International, Wicklow, Ireland) based on AOAC Method 991.43 and AACC Method 32-07.01. Briefly, one gram of ground dried bread was added to 50 mL phosphate buffer (pH 6, 0.5 M) and then subjected to heat stable α-amylase at 95-100 °C initial heating in a water bath for 30 min. In order to hydrolyse the protein, the solution was cooled to room temperature and pH was adjusted to 7.5. Afterwards, an incubation step at 60 °C for 30 min with *Bacillus licheniformis* protease was followed by amyloglucosidase addition at pH 4-4.6 at the same time and temperature for starch hydrolysis. After hydrolysis of proteins and starch, the sample was precipitated with ethyl alcohol 95% at 60 °C and cooled for an hour at room temperature. The mixture was filtered by a Whatman 40, and the wet residue was washed with warm distilled water and ethanol (95%), sequentially, it was dried at 103 °C in an oven overnight. Two portions of the residue were used for determination of protein and ash. The TDF value was corrected for protein and ash contents.

Total dietary fibre = weight (residue) – weight (ash + protein) (7.3)

7.2.4. Bread crust and crumb colour

The colour of the breads' crust and crumb was measured by a Hunterlab Spectrocolorimeter (Hunter Lab MiniScan Plus Colorimetric, Virginia, USA). Colour was measured at three points of the fresh bread samples in triplicates. The results were determined using the CIE L* a* b* system, where L* (from pitch black, 0, to perfect white, 100), a* (from –a* greenness to +a* redness) and b* (from –b* blueness to +b* yellowness) parameters. Chroma, browning index and ΔE were calculated by the following formulas (Cornejo and Rosell, 2015).

$$\text{Chroma}=\sqrt{a^{*2}+b^{*2}} \quad (7.4)$$

Browning index= $\frac{100\,(x - 0.31)}{0.17}$, where x was obtained by the following formulas:

$$x = \frac{a^{*} + 1.75\,L^{*}}{5.645\,L^{*} + a^{*} - 3.012\,b^{*}} \quad (7.5)$$

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad (7.6)$$

7.2.5. Mineral profile

The analysis of minerals was performed by an Agilent 7500ce quadrupole ICP-MS mass spectrometer connected to a CEM MARS6 programmable 2450 MHz microwave reaction system with a selectable operator output of 0–1600 W (CEM Corporation, Matthews, USA). Approximately two mg of dried bred sample were weighed in a MARSX press (CEM Corporation, Matthews, USA) digestion tube with the addition of 10 mL of concentrated quartz distilled nitric acid (HNO_3) subsequently. After digestion of the samples in microwave, they were spiked offline with a cocktail of seven reference elements to compensate for any drift or possible matrix effects. Serial dilutions of a SPEX CertiPrep multi-element were prepared for calibration standards (Spex Certiprep, Metuchen, USA). The ICP-MS was tuned according to the manufacturer's recommendations for minimizing the instrumental interferences. The accuracy of the measurement was established with a corn meal reference material, supplied High Purity Standard North Charleston, USA, following the method of Anyasi et al. (2018).

7.2.6. *In vitro* starch digestibility of bread samples

Based on its digestion, starch has been classified into rapidly digestible starch (RDS, amount of released glucose after 20 min); slowly digestible starch (SDS, amount of released glucose between 20 and 120 min) and RS (starch remaining undigested after

180 min) (Gujral et al., 2018). All starch determinations were analysed using Megazyme kits (Megazyme International, Wicklow, Ireland) based on the official method AOAC modified by Sharma et al. (2017). The released glucose was measured at 0, 20 and 120 min of small intestine digestion. Firstly, bread crumb samples (100 mg, d.b) were subjected to 0.2 ml artificial porcine salivary amylase solution (one g amylase in 10 ml of buffer KCl–HCl solution in 0.02 M phosphate buffer at pH 1.5). Then incubation of the samples was carried out at 37 °C for 30 min after the addition of pepsin (1 mg/ml) enzyme prepared in 0.02 M HCl (40 mg α- amylase A-3176; Sigma, Madrid, Spain) to mimic the gastric phase Lastly, in order to mimic the intestinal phase, samples were incubated at 37 °C for 2 h in presence of pancreatin (5 mL of 2.5% solution in 0.1 M sodium maleate buffer pH=6), bile salts at a concentration of 10 mM, and $CaCl_2$ at a concentration of 0.3 mM at pH 7) and amyloglucosidase (0.1 ml) prepared in 0.2 M sodium acetate buffer (pH=6) were added and the released glucose was measured using glucose oxidase-peroxidase reagent (GOPOD) (Goñi et al., 1997): (7.7)

$$\text{Digestible starch (g/ 100 g d.b)} = \frac{0.9 \times \text{glucose concentration after 2h digestion of small intestine} \times \text{volume of digesta (ml)}}{\text{Sample weight (g)} \times \text{starch (g)}}$$

The total starch was measured according to the method of Moongngarm et al. (2014) which was based on AOAC Method 996.11 using the Megazyme total starch assay kit (Megazyme International Ireland Ltd, Bray, Ireland). A 100 mg of dried samples were mixed with 10 ml of sodium acetate buffer, pH 5 and 0.1 ml of thermostable α-amylase (280 U) at 100 °C for 15 min in sealed tubes with intermittently vortex. With the addition of 0.1 ml amyloglucosidase (AMG) 3300 U/ml after cooling the solution to 50 °C, the suspension was centrifugated at 3000 *g* at room temperature for 5 min. The total starch content om the solution was measured as the released glucose with glucose oxidase/peroxidase reagent assay and was determined at 510 nm. The different digestible starches were calculated according to the equations provided by Liu et al. (2019):

$$RDS\ (\%) = (G20 - FG) \times 0.9 \times 100 \quad (7.8)$$

$$SDS\ (\%) = (G120 - G20) \times 0.9 \times 100 \quad (7.9)$$

where G20 and G120 are the amount of glucose released after 20 min and 120 min, respectively, and FG is the amount of free glucose in the undigested samples.

For resistant starch determination, bread crumb samples (100 mg, d.b) was incubated with 3.5 ml maleate buffer (50 mM, pH 6) and the mixture of pancreatic α-amylase (40 KU/g) and AMG (17 KU/g) at 37 °C for 4 h. After stopping the starch hydrolysis reaction by adding ethanol and centrifugation at 1500 *g* for 5 min at room temperature, the pellet was dissolved in KOH 4 M by vigorous stirring in an ice-water bath over a magnetic stirrer. Digested pellet and supernatant were incubated separately with AMG (14 ml of 3300 U/ml amyloglucosidase on soluble starch) at 50 °C for 20 min in a water bath. Finally, the absorbance of the released glucose was measured by GOPOD assay at 510 nm against the reagent blank. Resistant starch content was calculated as mg of glucose × 0.9 based on the method described by Menezes et al. (2011).

7.2.7. Statistical analysis

Data were analysed using one-way ANOVA to determine the effects of GBF type, the addition percentage on the measured parameters. The statistical analysis was performed by Minitab® program version 16. Post-hoc Tukey test was used to determine the differences amongst the means at 95% of confidence level. The results are the means of three replicate experiments and expressed as mean ± standard deviation.

7.3. Results and discussion

The results shown in Table 7.1 present the proximate chemical composition of bread samples. The fortification above 10% caused a significant increase in water activity (a_w) of the bread samples compared to the control ones. The MC and TDF content increased (P-value < 0.05) with increasing GBF fortification level in bread. This suggests the presence of more hydrophilic chains that resulted in higher water absorption capacity (Ho et al., 2013). These findings were in agreement with the results obtained by Eshak (2016) and Gomes et al. (2016) who used hydrocolloids as water binders. Protein and fat contents decreased with the increased addition of GBF regardless of its preparation method (P-value < 0.05). This was expected since the protein content was lower in the GBF (approximately 36% less in F30 and O30) compared to the WF (chapter 6). This is in agreement with the findings reported by Gomes et al. (2016) who utilised GBF and Loong and Wong (2018) who used green banana pulp flour (GBPF).

High ash content in banana bread samples indicates the presence of high mineral contents. The higher ash content in GBF compared to WF improved the ash level in fortified-bread samples and resulted in almost 2.5 times increase in the ash content for F30 and O30 samples (Table 7.1).

Table 7.1. Chemical composition properties of bread samples (g/ 100 g)

Sample	MC	Ash	Fat	Protein	Carbohydrate	TDF	EV (Kcal/100 g)
WB	31.2±0.09 g	1.21±0.01 f	3.32±0.01 a	5.50±0.02 a	58.6 ±0.03a	3.31±0.03 g	286.82±0.42 a
F10	34.2±0.05 e	2.21±0.01 e	3.60±0.02 cd	5.21±0.01 b	54.7±0.03 c	3.60±0.03 e	272.40±0.32 c
F20	35.81±0.05 c	2.62±0.01 c	3.81±0.01 b	4.73±0.05 d	53.01±0.01 d	3.82±0.01 c	265.10±0.10 e
F30	38.52±0.40 a	3.01±0.01 a	4.01±0.05 a	4.26±0.01 f	50.1±0.04 e	4.13±0.04 a	253.91±0.51 g
O10	33.50±0.14 f	2.21±0.01 e	3.60±0.01 d	5.16±0.03 c	55.6±0.03 b	3.61±0.03 f	274.95±0.10 b
O20	35.10±0.02 d	2.63±0.01 d	3.73±0.02 c	4.64±0.01 e	53.9±0.02 c	3.72±0.02 d	267.62±0.40 d
O30	36.53±0.39 b	2.94±0.01 b	3.86±0.02 b	4.01±0.01 g	52.6±0.01 d	3.90±0.03 b	261.10±0.40 f

Mean ± standard deviation values of triplicate determinations. Values with different letters in the same column are significantly different (P-value < 0.05). TDF, total dietary fibre. EV, energy value. WB, wheat bread, F10, F20 and F30, fortification levels of freeze-dried banana flour. O10, O20 and O30, fortification levels of air oven-dried banana flour.

From the results obtained from TDF and EV, it can be seen that higher content of TDF gave rise to lesser EV of the final product. Bread samples made with FDF displayed higher TDF compared to ones made with ODF in all fortification levels (P-value < 0.05). This result may be explained by the fact that fibres were preserved more during freeze-drying compared to air oven-drying. Also, surface furrowing and cracking stems from heat treatment can be the cause of lower TDF in all ODF and eventually in the bread (Chang and Morris, 1990). The findings of chemical composition indicated that GBF-fortified bread samples had superior nutritional value than the control bread with a decrease in protein content.

7.3.2. Bread colour analysis

The control sample showed the L* value of 66.6 and 72.5 which indicates the whiteness of crust and crumb bread slice, respectively (Table 7.2). In relation to browning index of bread crust, a significant increasing trend was observed in oven-dried fortified bread samples compared to freeze-dried ones. Although this trend was similar in breads fortified with FDF as well, breads with freeze-dried flour showed lower browning index, yellowness and redness (Table 7.2). Previous findings have shown that chlorophyll was more preserved in FDF compared to ODF (Oliveira et al., 2015, Yang et al., 2009). Crust colour saturation exhibited a declining trend with more GBF content levels in bread. Martínez-Castaño et al. (2019) also reported the brownest colour in crust for gluten free bread made with partial banana flour substitution (15%). Control samples exhibited higher crumb yellowness and a more intense crust browning compared to all FDF-fortified bread samples (P-value < 0.05). The presence of different xanthophylls and phenolic compounds might have been associated with the yellowish-brownish colour of banana bread samples (Kurhade et al., 2016). Also, Mohamed et al. (2010) mentioned that for fruits like banana that contains high levels of polyphenol oxidase, enzymatic oxidation of mono-phenolic compounds and releasing

Table 7.2. Colour parameters of bread crust and crumb

Sample	L*	a*	b*	Browning index	Chroma	ΔE
Crust colour						
WB	66.6±0.4 a	13.1±0.1 b	37.7±0.5 a	94.5±0.9 d	41.1±0.1 a	-
F10	37.0±0.1 e	7.6±0.1 c	19.8±0.4 d	69.9±0.4 f	21.2±0.2 d	35±0.9 c
F20	34.4±0.6 f	6.2±0.2 cd	16.8±0.2 e	78.3±0.1 ef	17.9±0.1 e	38.9±0.9 b
F30	33.2±0.1 g	5.6±0.4 d	14.7±0.3 f	89.2±1.0 de	15.8±0.1 e	41.2±0.8 a
O10	52.3±0.1 b	15.7±0.2 a	37.9±0.3 a	110.4±1.1 c	41.0±0.2 a	14.6±1.1 f
O20	47.7±0.9 c	15.7±0.2 a	34.7±0.1 b	138.2±3.0 b	38.1±0.2 b	23.4±0.9 d
O30	43.5±0.6 d	11.4±0.2 b	29.8±0.1 c	162.1±1.1 a	31.9±0.1 c	20.5±1.1 e
Crumb colour						
WB	72.5±0.1 a	4.2±0.1 f	28.6±0.3 a	53.1±0.9 f	29.2±0.1 a	-
F10	43.1±0.1 b	3.7±0.1 g	15.2±0.1 g	49.1±0.4 f	15.7±0.1 g	32.2±0.1 d
F20	41.1±0.1 d	5.2±0.1 e	18.5±0.1 f	67.4±0.1 e	19.2±0.1 f	33.0±0.1 cd
F30	38.6±1.6 f	7.1±0.2 c	20.1±0.1 e	85.1±0.1 d	21.4±0.1 e	35.1±0.2 b
O10	42.5±0.4 c	5.6±0.1 d	25.4±0.9 d	95.9±5.1 c	25.9±1.0 d	30.8±0.3 e
O20	39.4±1.6 e	7.7±0.2 b	27.3±0.1 b	122.1±1.8 b	28.3±0.1 b	33.3±0.1 c
O30	36.6±0.1 g	8.2±0.2 a	26.6±0.6 c	133.5±9.5 a	27.9±0.6 c	36.2±0.3 a

Mean ± standard deviation values of triplicate determinations. Values with different letters in the same column for the same bread part (crumb or crust) are significantly different (P-value < 0.05). WB, wheat bread, F10, F20 and F30, fortification levels of freeze-dried banana flour. O10, O20 and O30, fortification levels of air oven-dried banana flour.

o-diphenols and o-quinones is likely to happen. The colour differences between control and fortified samples increased (P-value < 0.05) with increasing GBF in bread. The higher carbohydrate content in GBF also may have resulted in Maillard reaction especially in OD-breadcrumb samples similar to what was found in literature (Tribess et al., 2009, Loong and Wong, 2018, Agama-Acevedo et al., 2012).

7.3.3. Mineral profile

Minerals play a key role in enzyme systems and facilitate the functioning of certain organs (Wang et al., 2012). Some minerals are essential in larger amounts (macro minerals) and other in smaller (micro minerals) quantities. As more GBF was incorporated into the bread, the mineral content was increased, especially the macro elements (Fig 7.1). All the 20% and 30% bread samples contained significantly higher K, P, Na, Ca and Mg compared to control (P-value > 0.05). Considering recommended daily intake of minerals (Fayet-Moore et al., 2018), each 100 g of F30 sample can provide 18.6% K, 18.16% P, 4.91% Na, 8.07% Ca and 14.74% Mg of the recommended daily intake. Similarly, all oven-dried fortified bread samples showed a higher percentage of Fe, Zn and Cu (O20 and O30) compared to control bread (Fig 7.1b).

This significant increase (P-value < 0.05) could be due to the high ash content of GBF (Anyasi et al., 2018). These findings can be beneficial in particular for the products which are made for celiac disease (Hernández-Aguirre et al., 2019). The same observation was reported in bread made with fermented banana flour (Adebayo-Oyetoro et al., 2016).

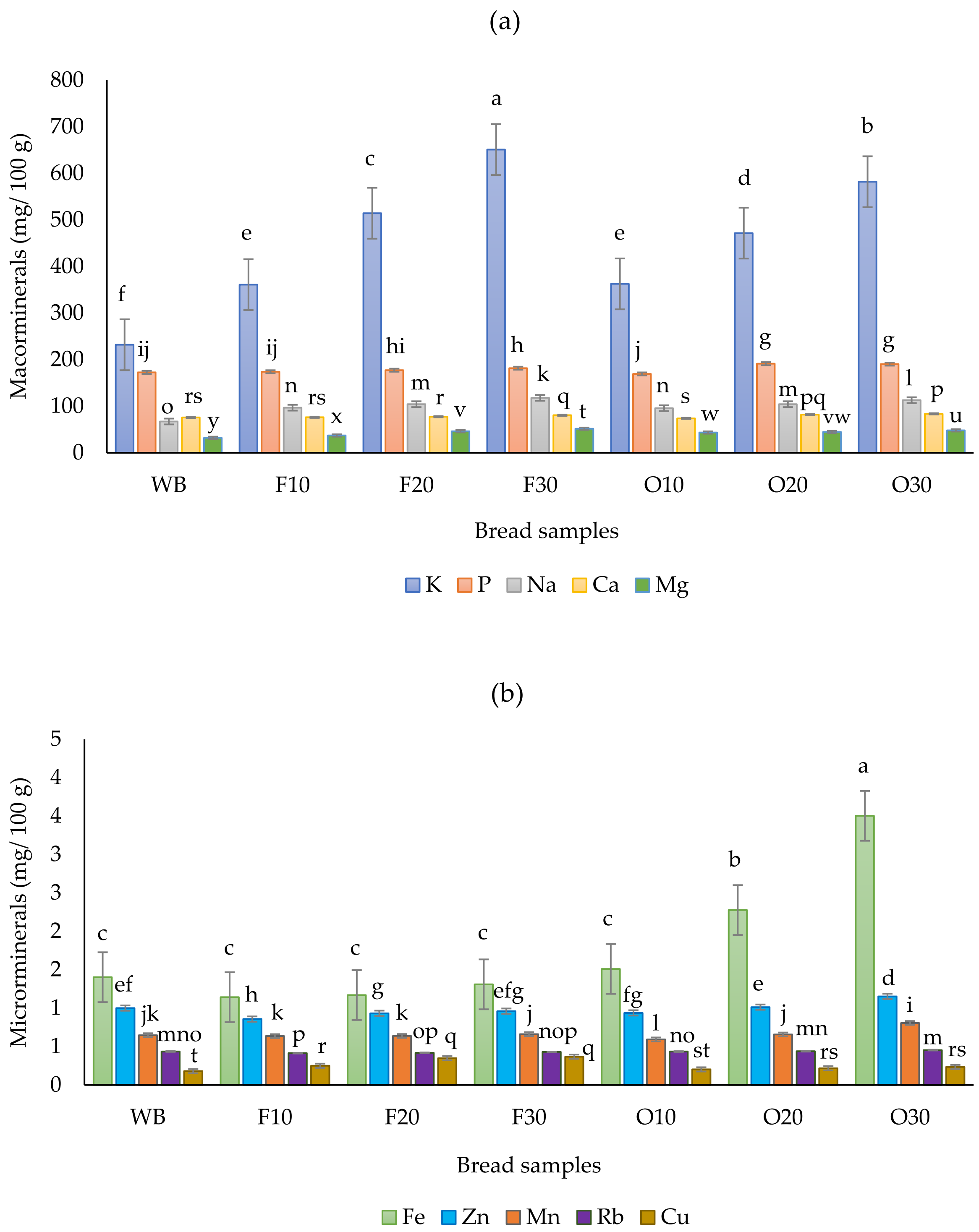


Figure 7.1. a) Macro minerals in bread samples b) Microminerals in bread samples

Bars showing mean and standard deviation for each of the individual minerals that have different letters are significantly different (P-value < 0.05).

Correspondingly, Ho et al. (2015) reported an increment in Na, K, Mg and Ca contents in bread made with 10% peeled yellow banana pseudo-steam flour in presence of sodium carboxy methyl cellulose. Apart from these studies, there are no similar works in this area to compare the results. These results indicated that addition of GBF, especially that produced by freeze-drying, could be utilized as a supplement to improve the essential minerals in bread products.

7.3.4. Starch digestibility analysis

It is well known that food products with high level of RS and SDS do not cause postprandial hyperglycaemic and hyperinsulinemic spikes associated with the RDS (Aparicio-Saguilan et al., 2013). It can be seen from Table 7.3 that control bread displayed low RS content (2.17%), which agrees with results from studies on wheat breads (Roman et al., 2019, Ho et al., 2015). As the amount of GBF increased in bread, the RS percentage increased significantly, with F30 samples (8.55%) exhibiting the highest percentage (P-value < 0.05). Lower RS content in O10, O20 and O30 samples was possibly due to the lesser amount in the ODF compared to FDF (Fig 4.2). Consumption of 100 g of F30 and O30 bread can supplement the recommended daily intake of 6-12g/d of RS for prebiotic effects (Peres, 2014, Moongngarm et al., 2014). Retrogradation process which occurs after cooling the bread samples and the formation of the amylo-lipid bonds creates RS3 and RS5, respectively (Martínez-Castaño et al., 2019).

The presence of more DF in banana bread samples, specially above 20% level, resulted in a change in viscosity, which acts as a physical barrier on the starch surface and prevents it from being subjected by digestive enzymes (Liu et al., 2018). As can be seen in Table 7.3, the amount of SDS increased significantly in O30 and F30 compared to other treatments. It has been mentioned that RS has a "*sponge role*" and is capable of absorbing water in the big intestine and helps in mixing with the foodstuff in a tangled network form, and thereby can slow down the rate of digestion (Juarez-Garcia et al., 2006).

Table 7.3. Starch digestibility of bread samples (g/ 100 g d.b)

Sample	RS (%) *	TS (%) *	SDS (%) **	RDS (%) **	DS (%) **
WB	2.17±0.06 g	71.60±0.64 a	10.21±0.06 c	59.20±0.53 a	71.61±0.65 ab
F10	3.20±0.06 e	72.34±1.02 a	13.91±0.15 bc	55.20±0.86 b	72.70±1.02 a
F20	4.33±0.01 c	77.14±1.01 a	15.60±0.21 b	57.10±0.79 ab	69.10±1.01 abc
F30	8.55±0.06 a	76.43±1.44 a	17.80±0.50 a	50.11±0.92 d	67.90±0.06 bc
O10	2.61±0.02 f	68.10±1.71 b	10.42±0.22 c	54.90±1.48 bc	70.63±0.02 ab
O20	3.52±0.4 d	74.12±2.67 a	13.42±0.45 bc	57.11±2.22 ab	65.44±0.04 c
O30	5.92±0.03 b	75.70±1.73 ab	15.90±0.55 b	53.90±1.16 c	69.80±0.03 ab

Mean ± standard deviation values of triplicate determinations. Values with different letters in the same column are significantly different (P-value < 0.05). RS, resistant starch, SDS, slow digestible starch, RDS, rapidly digestible starch, DS, digestible starch, TS, total starch, WB, wheat bread, F10, F20 and F30, substitution levels of freeze-dried banana flour with wheat flour. O10, O20 and O30, substitution levels of air oven-dried banana flour with wheat flour.

* Measured chemically according to the AOAC method 2002.02.

** Measured indirectly by calculation through total starch analysis by formulas 7.8 and 7.9.

Roman et al. (2019) reported the remaining RS content cannot be beyond 2% w/w in bread made with 20% banana starch. Likewise, De Souza Viana et al. (2018) reported that 25% of substitution of WF with GBPF led to an increase in RS content up to 3.2% in the final bread. In a study on a gluten-added bread made with 60% GBPF, a low RS (6.7%) was reported, which was explained by the low level of RS in the prepared flour (17%) (Juarez-Garcia et al., 2006). In a recent work conducted by Agama-Acevedo et al. (2019), bread crust was analysed for the remaining RS and it was found that no more 2.5% was available. Similarly, Hernández-Aguirre et al. (2019) reported 3.5% of RS in a gluten free bread but without reporting the RS content of GBPF. It can be concluded that the use of whole banana for making flour could have an effect on remaining RS content in the final bread. More studies are needed to compare the bread fortified with different green banana flour sources: peel only, pulp only and the whole fruit.

7.4. Conclusion

It can be concluded that the use of whole green banana flour (GBF) up to 30% in substitution for wheat flour significantly increased total dietary fibre, ash and mineral content in bread. Additionally, bread samples made with air-over dried GBF exhibited darker colour in both crumb and crust, with similar yellowness compared to the control bread sample in 10% level of fortification. The marked increase in macronutrients, P, K, Mg, Na and Ca in particular, with a slight increase in micro minerals in all GBF bread samples, specially above 10% fortification, showed an improvement in nutritional value of all fortified bread samples. The addition of 20% and 30% of GBF in the bread resulted in a product with the pronounced percentage of RS and SDS. As FDF-bread samples showed lower RDS and DS compared to OD-bread samples, it might be concluded that the 30% GBF addition can be considered as a functional bread containing prebiotic dosage. Current findings are also expected to help the sustainability of food systems by the use of whole green banana and prevent losses during maturation, transportation and storage. Also, the study on the synergistic effect of hydrocolloids with resistant starch can be studied on bread formulation.

7.5. Limitations

The human-involved digestion test (*in vivo*) was not carried out. Also, total phenolic content and antioxidant activity, which required 2,2-bipyridyl, 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH), was not performed due to the time limitation and limited access to the lab due to the COVID-19 pandemic condition. These additional tests can show the nutritional impact of GBF fortification more.

CHAPTER 8

A chemometrics approach to investigate the volatile compounds of wheat bread fortified with whole green banana flour

This chapter is currently under review as an original article up to this date.

Chapter aim:

The aim of the present study was to compare the volatile profile of the crust and crumb of bread made with different fortification levels of green banana flour produced using different drying methods (oven-drying and freeze-drying). The volatile compounds were studied using an untargeted headspace solid-phase micro-extraction GC-MS fingerprinting procedure. Advanced chemometrics and feature selection were applied to classify the samples, explore the trends and identify discriminant volatile compounds.

8.1. Introduction

Banana is one of the most consumed fruits in the world and it is also the second largest fruit crop after citrus contributing to 16% of the global fruit production (Ji and Srzednicki, 2015). *Musa Cavendish*, a triploid cultivar, is the most appreciated and cultivated one. The popularity of banana relates to its nutritional value, aroma and flavour (Berhal et al., 2017). Also, based on the level of maturation, banana's nutritional value varies. While the yellow fruit (ripeness stage 6-7) are high in digestible fibres, ash and carbohydrates, green banana has high antioxidant activity, phenolic compounds, dietary fibres (DF) and resistant starch (RS) (Agama-Acevedo et al., 2016, Kurhade et al., 2016). The banana peel represents about 30-40% of its total weight, resulting in a large amount of undesired waste generated from the consumption and processing of this crop. The peels are highly perishable, seasonal and often disposed of improperly, and when it is green, the peel contains almost 50% DF (Sharma et al., 2016). Since the limitation on the consumption of green banana relates to the undesirable taste and aroma, several studies have shown the possibility of producing green banana flour (from pulp, peel or both) as a functional ingredient by the use of different drying techniques which were reviewed in chapter 2. Thermal air-oven drying, and non-thermal freeze-drying methods have been introduced in chapter 4 to obtain flour from the whole fruit, i.e., peel and pulp together (GBF), yet, they have different impacts on the properties of the final fortified product as discussed in Chapters 6 and 7.

As a basic foodstuff, bread is considered one of the most consumed food products around the world, and it is also considered a major source of protein and carbohydrate in developing countries. Thus, bread can be a valuable carrier for delivering additional health benefits by fortifying it with functional ingredients (Gomes et al., 2016), such as inulin (Rubel et al., 2015), probiotics (Pepe et al., 2013), oligosaccharides (Damen et al., 2012), fruit flour (Rolim et al., 2011), rye bran (Karppinen, 2003) and organic salts (Salinas et al., 2016). Studies on the effect of different yellow and green banana flour fortification on technological and nutritional properties on bread have been reviewed in Chapter 2. However, no comprehensive reports were available on the changes in bread aroma profile when green banana flour is incorporated.

Bread aroma is one of the most imperative sensory characteristics which is perceived just before consumption. It is produced during mixing, fermentation, kneading, baking and is also affected by additional ingredients (Pico et al., 2016). The aroma of bread is composed of volatile compounds including aldehydes, alcohols, esters, ethers, carboxylic acids, ketones, furans, sulphur compounds, hydrocarbons, pyrrolines and pyrazines. Onishi et al. (2011) and Pico et al. (2018a) reported that Maillard reaction, caramelisation and other thermally-induced degradation (i.e. lipid oxidation) are responsible for the formation of bread volatile aroma compounds. Several studies on the representative volatile compounds of bread have already been presented (Pico et al., 2018a, Jourdren et al., 2017, Jensen et al., 2011, Hussein and Ibrahim, 2019, Birch et al., 2014, Rannou et al., 2008, Pico et al., 2018b). On the other side, studies on green banana volatile profile is divided into two categories: fruit and dried fruit/ flour, mainly made from pulp.

Qamar and Shaikh (2018) reported that the majority of volatile aroma compounds in green banana fruit derived from terpenes and alcohols, which aligns with the results achieved by Zhu et al. (2018) and Aurore et al. (2009). These compounds could vary in terms of species, fruit ripeness, climate conditions, postharvest storage and biosynthetic pathways (Zhu et al., 2018, Saha et al., 2018, Berhal et al., 2017). In terms of banana flour (BF), it was reported that the type of drying method had an impact on volatile compounds (Wang et al., 2007). They found that freeze-drying (FD) and vacuum belt drying of banana resulted in the highest retention of volatiles in banana powder, mainly esters and alcohols. The banana fruity odour was reported to be related to butanoic acid 3-methylbutyl ester and 3-methylbutanoic acid 3-methylbutyl ester. These findings were supported by Mui et al. (2002) who associated the effectiveness of FD with microregion entrapment theory. During the first stage of FD process, freezing, rapid crystallization of water molecules forms the microregions which contains concentrated carbohydrates and banana aroma compounds. In the next stage, when sublimation occurs and entrapped moisture content level decreases in the microregions, association of carbohydrates molecules begins via hydrogen bonds and keeps the volatiles inside of the texture.

On the other hand, regarding thermal drying, banana chips dried in a combination of air-oven drying at 70 °C and vacuum microwave drying, resulted in a better retention of volatile compounds compared to microwave only (Mui et al., 2002). In another study conducted on the effect of drying process on banana's volatile compounds, Boudhrioua et al. (2003) reported a total loss of aroma content of banana fruit during high temperature air drying (80 °C for 24 h) and could be followed by an increase in elemicine levels. The profile changes in BF volatiles (prepared from a fruit of stage five ripeness) during low temperature air-oven drying showed that ester and aldehyde levels decreased, followed by an increase towards the end of drying, whilst high molecular weight compounds such as eugenol and elemicine were not affected significantly (Saha et al., 2018). Considering the literature, there is still limited understanding into the effect of different drying method of whole green banana fruit on volatile compounds of the produced flour and the retention of them in bread.

This study is the first time to systematically integrate untargeted GC-MS-based chemical fingerprinting, which is a non-discriminatory detection of as many volatile compounds as possible present in the bread samples headspace fraction, and modern chemometrics data analysis to evaluate volatiles in bread fortified with different levels and types of the GBF. The aim of the present study was to compare the volatile profile of bread crust and crumb with different fortification levels of GBF, to investigate the impact of processing used for GBF preparation and to identify discriminant compounds that can segregate the treated samples.

8.2. Materials and Methods

Green banana (*Musa Cavendish* spp AAA), wheat flour, and the ingredients essential for Breadmaking (activated dried yeast, butter, milk, baking powder, salt and honey) were purchased from a local food supplier (Dunedin, New Zealand).

8.2.1. Green banana flour preparation

The flour samples (air-oven-dried and freeze-dried green banana flours) were produced as described in chapter 4, section 4.2.1.

8.2.2. Breadmaking process

Bread samples were prepared as described in chapter 6, section 6.2.3.

8.2.3. Sample preparation

After slicing each freshly baked bread loaf with a kitchen electric bread automatic cutter, bread crust was removed from the crumb by precisely cutting one centimetre from the edge of the bread slice with a stainless-steel knife, followed by gently cutting into minimum 2×2×2 mm pieces (Fig 8.1). Then, one gram (±0.050 g) of each bread sample (crumb and crust, separately) was weighed in a 20 mL vial and tightly sealed with magnetic PTFE/ silicone septum and crimp caps (Fig 8.3).

8.2.4. Headspace SPME GC-MS analysis

Volatile compounds were extracted by the headspace-solid phase micro-extraction (HS-SPME) method according to Arcena et al. (2020b) and examined using the Agilent 6890N GC system connected to an Agilent MSD 5975 VL. This technique permits the extraction of the volatile compounds from the solid and liquid matrices in a simple and time effective way. Equilibration, extraction and desorption of volatile compounds were automated using an autosampler (Santa Clara, California, United States). With the aim of detecting an extensive range of volatile compounds, the HS-SPME-GC-MS method of analysis was optimised in advance for an untargeted fingerprinting (Arcena et al., 2020a). The optimisation included SPME fibre incubation, extraction conditions, GC-MS analysis parameters and sample weight. An SPME fibre with a balanced polarity [50/30 μm divinylbenzene/ carboxen/ polydimethylsiloxane (DVB/CAR/PDMS)] was selected because of its capacity to capture a wide range of volatile compounds from varied chemical classes (Kebede et al., 2013). The SPME fibres were conditioned and regenerated according to the manufacturer's guidelines. The vials were equilibrated at 50 °C for 5 min with agitation prior to headspace SPME extraction at 50 °C for 30 min. After volatile extraction, the SPME fibre was inserted into the GC injection port, where the volatile compounds were thermally desorbed at 230 °C for 5 min in a splitless mode. Using helium as the carrier gas, the volatile compounds were separated through the ZB-Wax column (60 m × 0.32 mm inner diameter × 0.5 μm film thickness, Phenomenex,

Figure 8.1. Examples of the typical breadcrumbs for GC-MS analysis

(a) Breadcrumb made from oven-dried green banana flour

(b) Breadcrumb made from white wheat flour with 0% fortification (control)

(c) Breadcrumb made from freeze-dried green banana flour

California, United States) at a constant 1 ml/ min flow rate. This column is excellent for separating polar complex mixtures and is widely used for profiling and fingerprinting purposes (Kebede et al., 2013, Arcena et al., 2020b, Arcena et al., 2020a). The GC-MS oven temperature was initially held at 50 °C for 5 min, followed by heating at 5 °C/min to reach 230 °C, followed by a further temperature ramp at 10 °C /min to reach the final 240 °C, and kept for another 5 min before cooling to 50 °C. Electron ionisation (EI mode) at 70 eV was used to obtain the mass spectra with a scanning range of 35 to 400 m/z (mass to charge ratio). The MS ion source and MS quadrupole temperature was set at 230 °C and 150 °C, respectively. Also, the GBF samples were analysed according to the same method (Fig. 8.4). Six independents SPME extraction were performed for each sample.

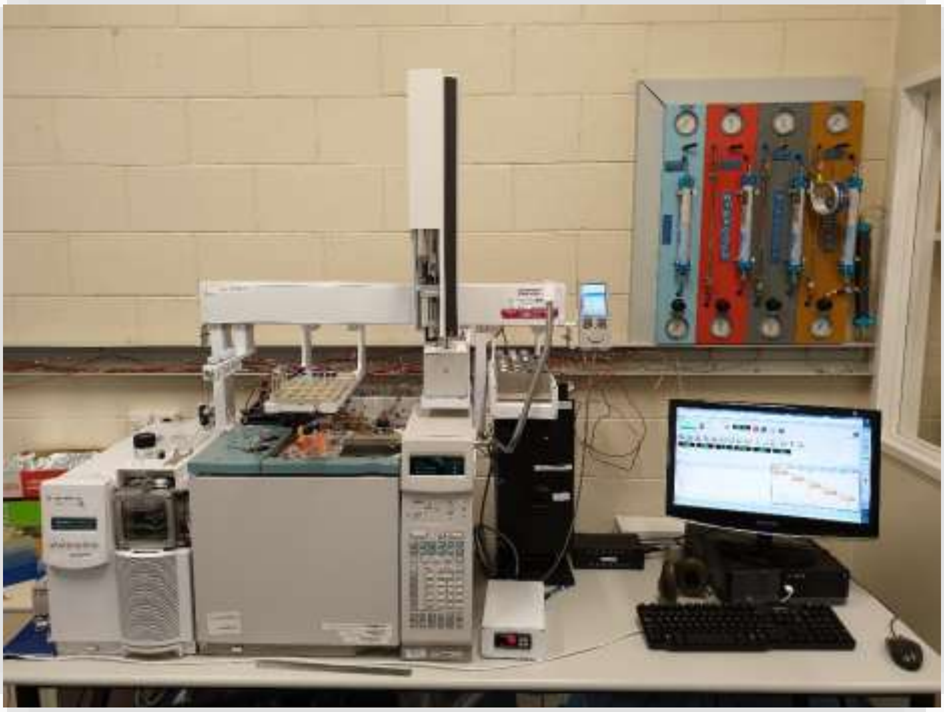


Figure 8.2. Gas chromatography mass spectroscopy setup

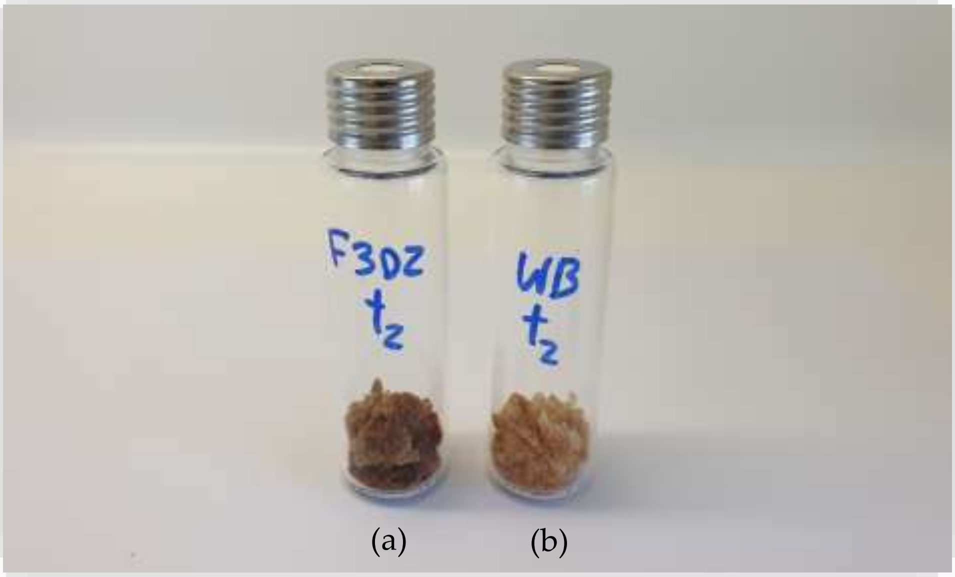


Figure 8.3. Examples of the typical breadcrust in vials for GC-MS analysis

(a) Breadcrust made from freeze-dried green banana flour at 30% level of fortification

(b) Breadcrust made from wheat bread crust with 0% fortification (control)

8.2.5. Data pre-processing of total ion chromatograms

The data pre-processing was performed according to the method reported by Kebede et al. (2013) and Arcena et al. (2020a). The GC-MS total ion chromatograms, usually loaded with co-eluting peaks, were analysed using an automated mass spectral deconvolution and identification system (AMDIS, version 2.72, 2014) to deconvolute and extract pure component spectra. This aids in acquiring a more accurate identification of the peaks using NIST spectral library (NIST14, version 2.2, National Institute of Standards and Technology). The deconvoluted spectra was analysed by Mass Profiler Professional (MPP) software (Version 14.9.1, 2017, Agilent Technologies) to filter the irregular, non-reproducible, non-related sample or very small peaks and align the similar peaks, producing the data table containing each peaks quantity level expressed as peak areas.

8.2.6. Chemometrics

The data matrix was auto scaled, which includes mean-centring and standardization. Then, in order to evaluate the data for outliers and groupings, the data was explored using unsupervised principal component analysis (PCA). The multivariate data was further analysed by a regression-based supervised classification technique, partial least squares-discriminant analysis (PLS-DA) to increase insight into the volatile differences and identify discriminant compounds. In this analysis, the optimum number of latent variables (LV) was selected with a criterion to explain the maximum variance/information in the data while maintaining the room mean squared error to a minimum (via cross-validation). In the present work, a venetian blind cross validation with 6 data splits was used. This helps limits the risk of over fitting. Based on the PLS-DA model. Bi-plots were constructed based on the PLS-DA model to examine the differences amongst different samples.

Finally, to identify metabolites significantly affected by GBF addition (potential discriminant volatiles), variable identification coefficients (VID) were calculated. The VID values are the corresponding correlation coefficients between X-variables (attributes) and predicted Y-variable (samples). Attributes with an absolute VID value of at least 0.650 were selected as a potential discriminating compound. These compounds were then identified and linked to associated reaction pathways. A bar plot was constructed for representative discriminant compound to clearly demonstrate the differences and Tukey's HSD test at $p < 0.05$ confidence level were used to evaluate the statistical differences between samples. In the current study, three criteria were employed to increase the power of volatile identification: (a) matching retention time and spectra with the control bread sample; (b) comparison of the experimental retention index (RI) with RI obtained from literature; (c) match and reverse match with the NIST library of 90% certainty.

8.3. Result and discussion

The volatile fractions of wheat flour (WF), freeze-dried green banana flour (FDF) and oven-dried green banana flour (ODF) were analysed using the HS-SPME-GC-MS fingerprinting method. The total ion chromatograms (TIC) of flour samples are shown in Fig 8.4, 8.5, 8.6. From visual comparison, one can see that the FDF and ODF samples seem to have a comparable volatile profile, whereas WF had a clearly different profile. The chromatogram of the WF had a total of 45 compounds mainly composed of alcohols (e.g., 1-hexanol, 1-nonanol, 1-pentanol and ethanol), alkane (e.g., 2,2-dimethyl-undecane, 2-methyl-octane, and heptadecane) and aldehydes (e.g., hexanal, and pentanal) (Fig 8.4).

The headspace fraction of the FDF samples was composed of ketones (e.g., 2-butanone, and 2-heptanone), alcohols (e.g., 2-methyl-1-undecanol, 1-octen-3-ol, 1-pentanol and 2-penten-1-ol), aldehydes (e.g., butanal, pentanal, 2-methyl-2-pentenal, hexanal, heptanal and 2-hexenal), Strecker aldehydes (e.g., benzaldehyde, and 2-methyl-butanal) and alkanes (e.g., heptane, octane, dodecane and hexane) (Fig 8.5). Comparable volatile compounds were detected in the ODF samples including aldehydes (e.g., butanal, propanal, 2-hexenal, heptanal, pentanal and nonanal), Strecker aldehydes (e.g. 2-methyl-butanal, and 3-methyl-butanal), ketones (e.g. 2-butanone and 2-heptanone), alcohols (e.g. 1-pentanol, 1-butanol and 1-octen-3-ol), furans (e.g. 3-methyl-furan, 2-ethyl-furan and 2-pentyl-furan) and alkanes (e.g. nonane, dodecane, hexadecane, pentadecane, heptane, undecane and 3-methyl-undecane) (Fig 8.6). Next, the volatile profile of the bread samples was characterized using the GC-MS-based chemical fingerprinting method. A total number of 102 volatile compounds from various chemical classes were identified in each bread sample, ranging from alcohol and ester in crumb to furan, ketone and aldehyde in crust. Visually, the volatile profiles appear to be different among the bread samples. Multivariate data analysis was used to investigate the impact of three factors: (i) bread part (crumb and crust); (ii) GBF fortification level (10%, 20% and 30%) and (iii) drying method used for GBF preparation (freeze drying and oven drying) on the volatile compounds. As discussed in sections 8.2.5 and 8.2.6, the raw chromatograms were pre-processed with AMDIS and MPP before applying the multivariate data analysis (PCA and PLSDA).

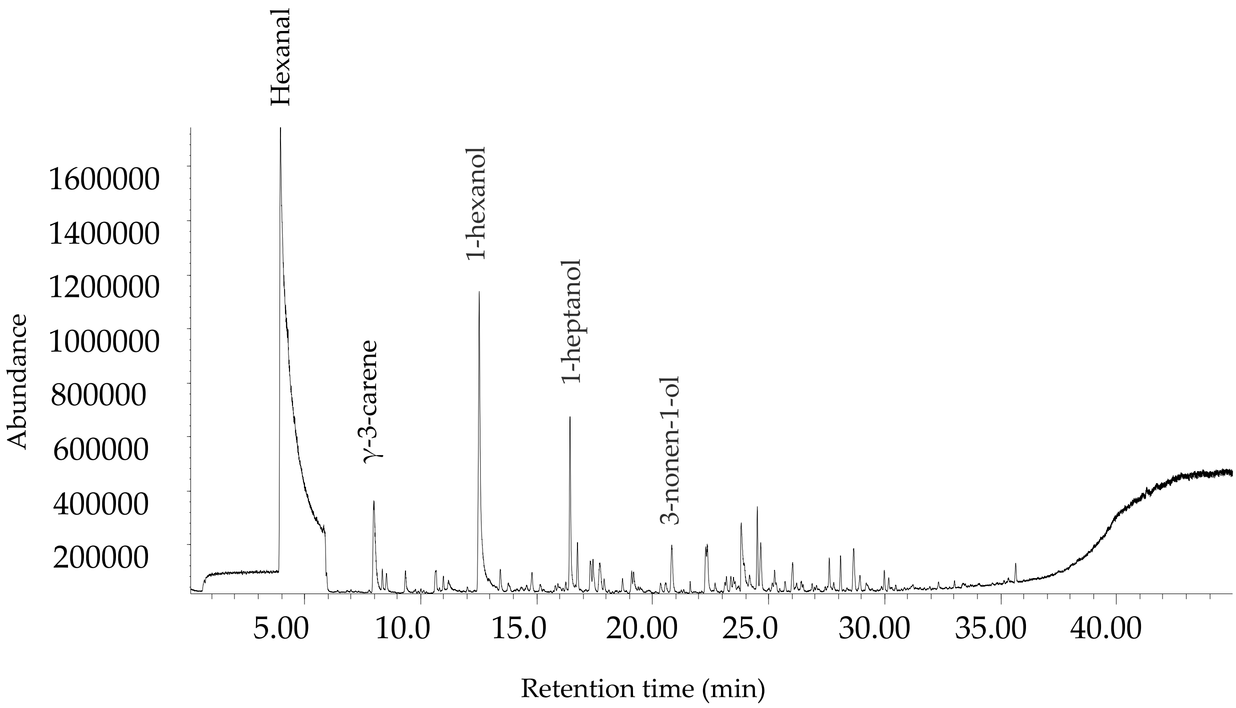


Figure 8.4. Total ion chromatogram of wheat flour

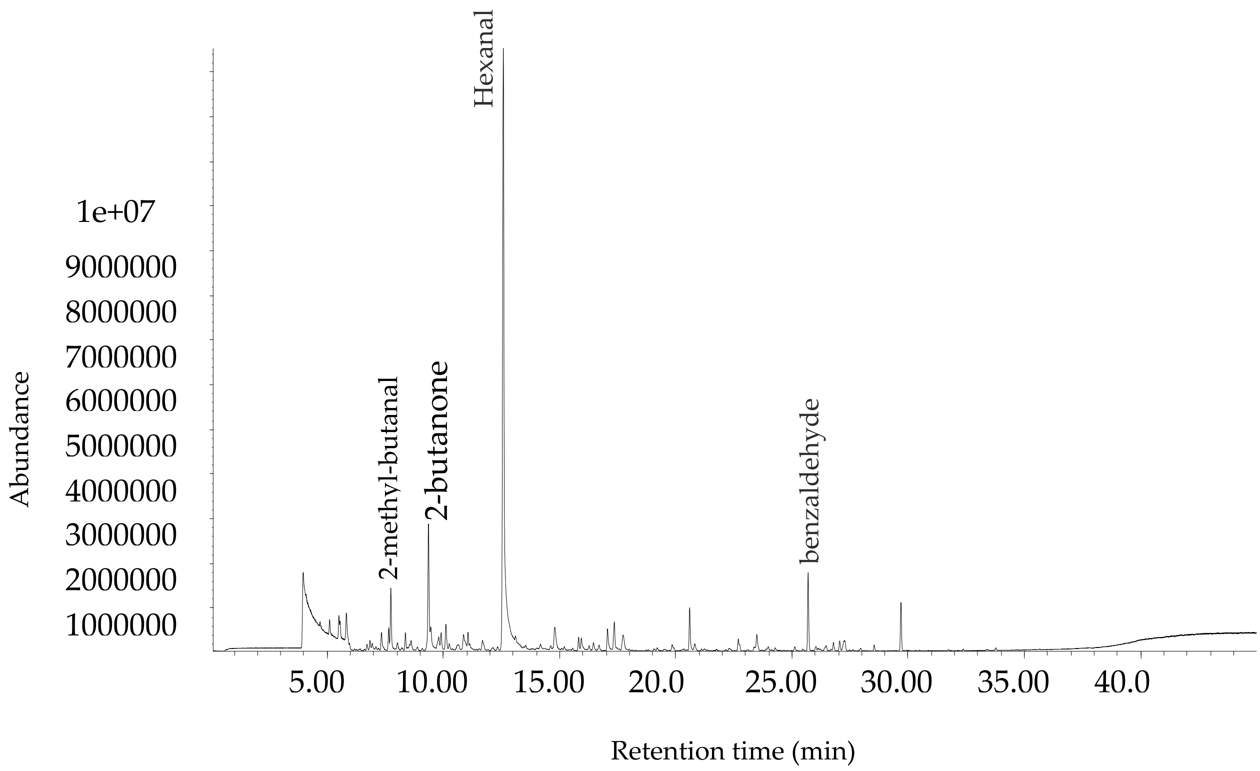


Figure 8.5. Total ion chromatogram of freeze-dried green banana flour

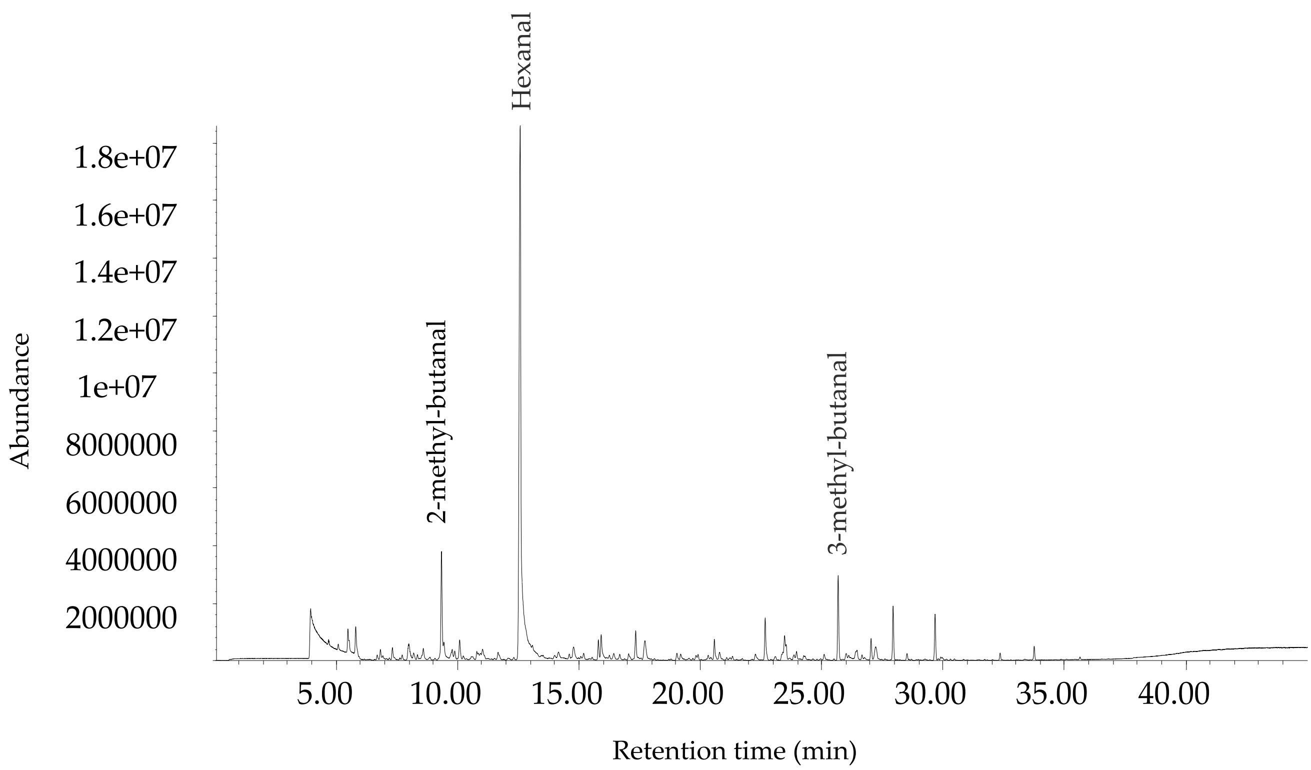


Figure 8.6. Total ion chromatogram of oven-dried green banana flour

Multivariate data analysis was used to investigate the impact of three factors on the volatile compounds:(i) bread part (crumb and crust); (ii) GBF fortification level (10%, 20% and 30%) and (iii) drying method used for GBF preparation (freeze drying and oven drying). The results of these analyses will be discussed step by step, starting with the analysis of the overall effect of all factors (section 8.3.1) to determine the most dominating trend. Then, the impact of each factor will be discussed, and discriminant volatile compounds will be selected (section 8.3.2, 8.3.3 and 8.3.4).

8.3.1. Investigating the overall trend in all 14 bread samples: Including the effects of bread part, GBF fortification and drying method

A PCA model was performed including all 14 bread samples, to identify the most dominant one out of the three factors: bread type, GBF fortification and drying method. PCA is an unsupervised explorative technique where only the volatile information (X-variable) is taken into account, without considering the sample information into the model. Figure 8.9 shows a PCA bi-plot constructed using the first two principal components (PCs). PCA is a powerful explorative technique useful to see any trends and patterns in the data and detect potential outliers. A clear difference between breadcrumb and crust volatile profiles was observed (Fig 8.9). This variation is represented by PC1. Next to the bread part (crust and crumb), the bi-plot also shows the partial effect of fortification level represented by the second PC. From the plot, the effect of the drying methods on the volatile fraction seems limited.

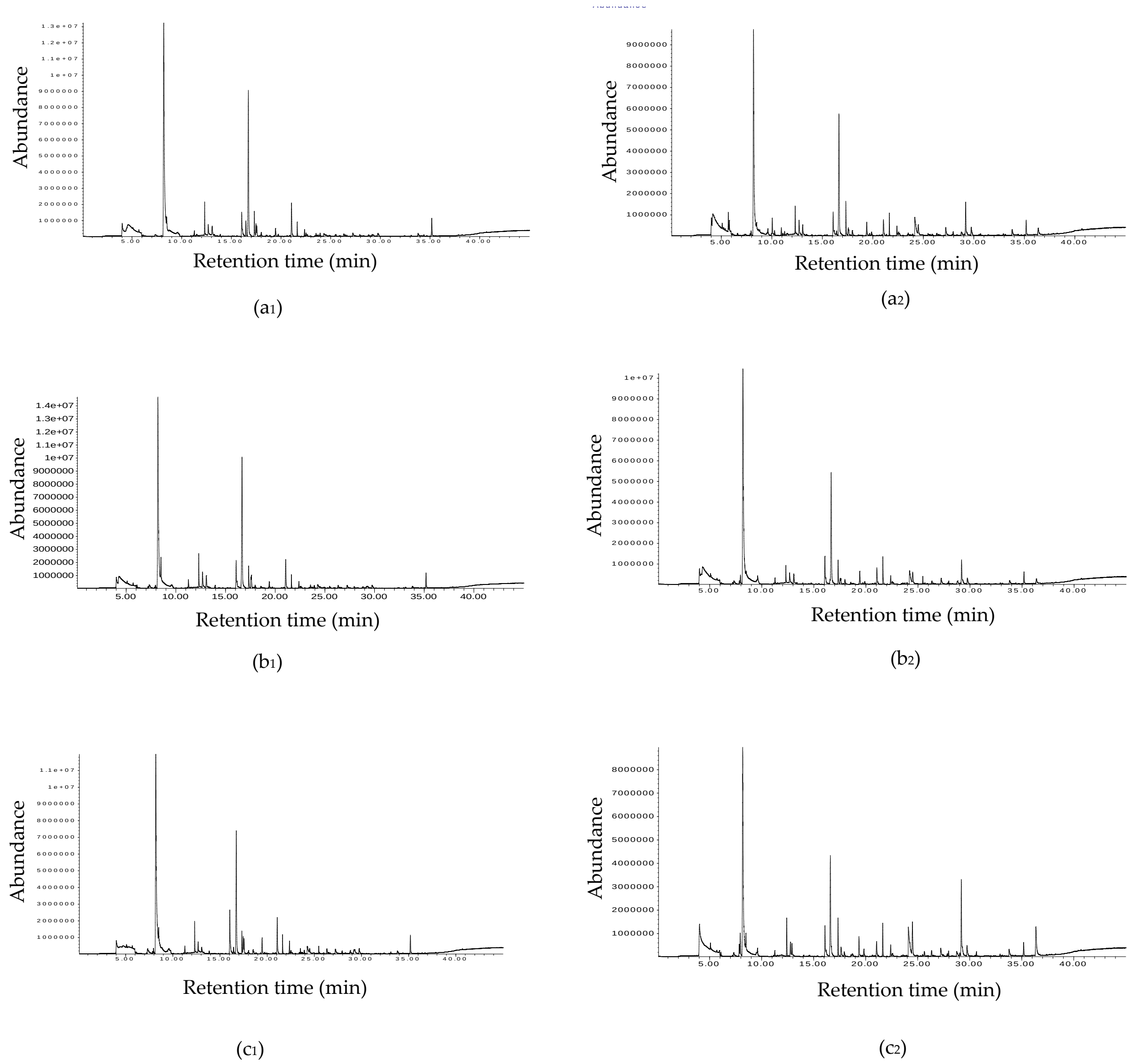


Figure 8.7. Total ion chromatograms of (a$_1$) O10B, (a$_2$) O10T, (b$_1$) O20B, (b$_2$) O20T, (c$_1$) O30B, (c$_2$) O30T obtained with the headspace GC-MS fingerprinting method

O10B, O20B, O30B, oven-dried-fortified breadcrumb at 10%, 20%, 30% substitution with wheat flour

O10T, O20T, O30T, oven-dried-fortified breadcrust at 10%, 20%, 30% substitution with wheat flour.

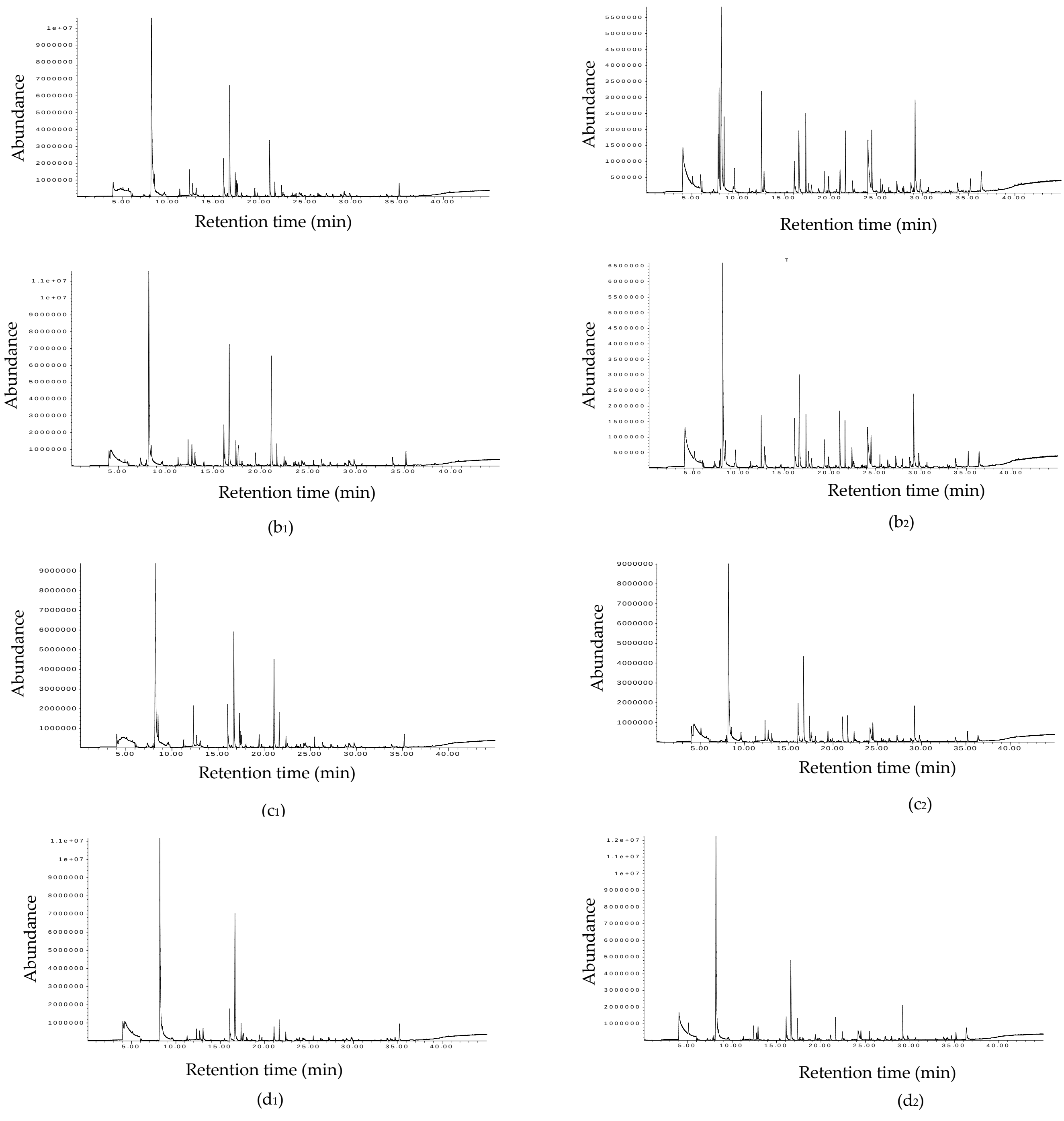


Figure 8.8. Total ion chromatograms of (a1) F10B, (a2) F10T, (b_1) F20B, (b_2) F20T, (c_1) F30B, (c_2) F30T, (d_1) WB, (d_2) WT obtained with the headspace GC-MS fingerprinting method

F10B, F20B, F30B, freeze-dried-fortified bread crumb at 10%, 20%, 30% substitution with wheat flour

F10T, F20T, F30T, freeze-dried-fortified bread crumb at 10%, 20%, 30% substitution with wheat flour

WB, wheat bread crumb, WT, wheat bread crust.

In summary, the PCA analysis displayed that the most dominant factor in the data is bread part (crust and crumb), mainly explained by the PC1, followed by effect of fortification and lastly the effect of banana drying process. In the next sections, each of these factors will be further investigated using a supervised PLS-DA method starting from the comparison between crumb and crust at each fortification level.

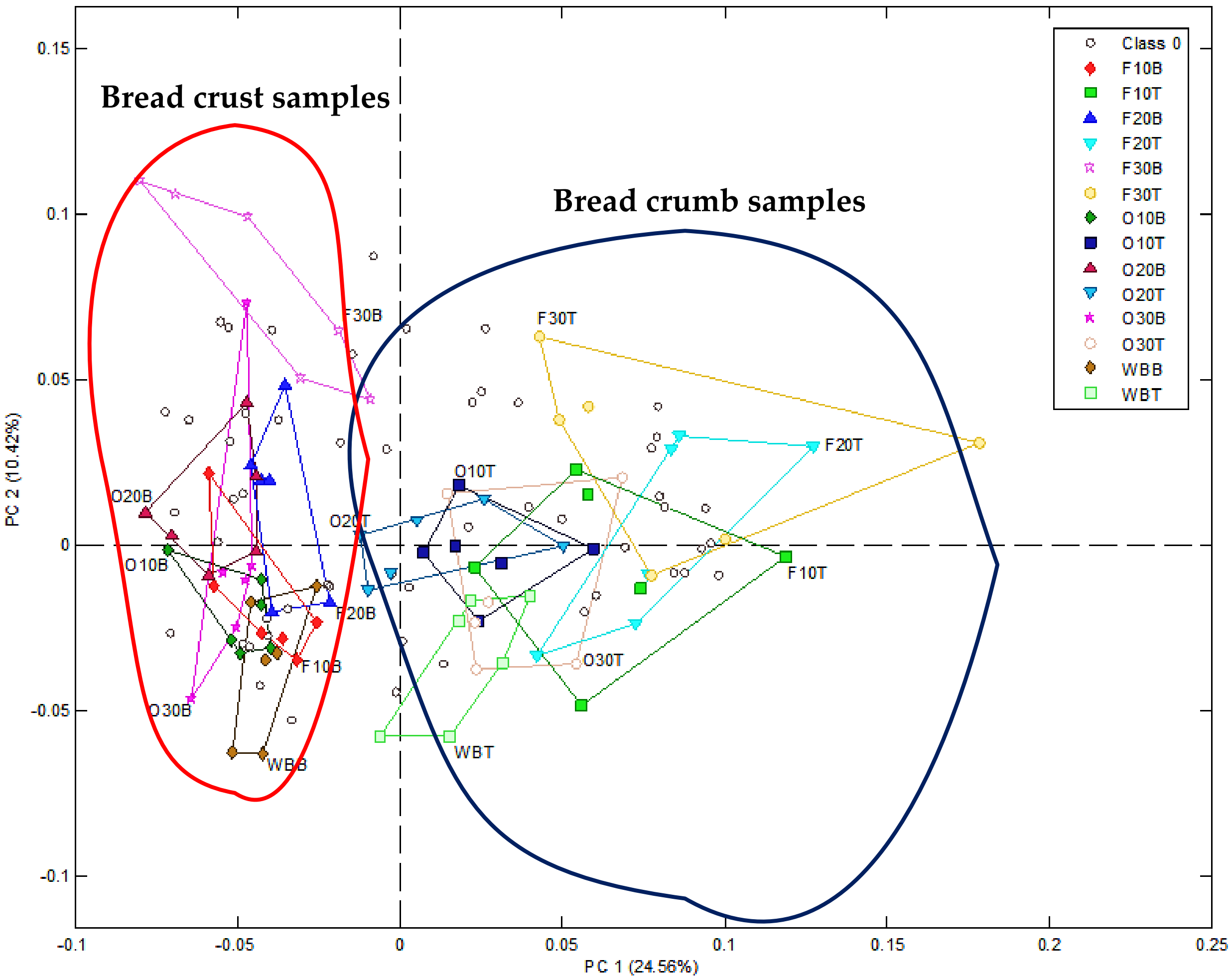


Figure 8.9. The Principal component biplot of bread samples, including the bread type, level of fortification and drying methods

F10B, F20B, F30B, freeze-dried-fortified breadcrumb at 10%, 20% and 30% substitution with wheat flour
F10T, F20T, F30T, freeze-dried-fortified breadcrust at 10%, 20% and 30% substitution with wheat flour
O10B, O20B, O30B, oven -dried-fortified breadcrumb at 10%, 20% and 30% substitution with wheat flour
O10T, O20T, O30T, oven-dried-fortified breadcrust at 10%, 20% and 30% substitution with wheat flour
WB, wheat breadcrumb, WT, wheat breadcrust

8.3.2. Comparison of the volatile profiles between breadcrumb and breadcrust at 10%, 20% and 30% fortification levels

A PLS-DA model was performed to compare the volatile profiles of crumb and crust samples at three fortification levels (10, 20 and 30%). For the model, the volatiles were considered as X-variables and crust and crumb as categorical Y-variables. Three latent variables (LVs) were selected as optimum based on venetian blind cross validation. As can be seen on the PLS-DA bi-plots, there is a clear difference between crumb and crust volatile profiles. This pattern appears to be comparable for all fortification levels (Fig 8.10 a, b, c). The volatiles discriminating the crust from the crumb samples, at each fortification level, were selected using the VID feature selection method (> 0.650 VID coefficient value) (Table 8.1, 8.2 and 8.3).

The VID feature selection method revealed the abundance of alcohol and ester in breadcrumb and aldehyde and furan compounds in the crust. Specifically, the volatile markers selected with higher amounts in the crumb were from alcohol (1-hexanol, 1-heptanol, phenylethyl alcohol) and ester (ethyl ester butanoic acid, octanoic acid) chemical classes, while furanic compounds (furfural, 2-furanmethanol), ketones (2-undecanone, 2-tridecanone) and Strecker aldehydes (2-methyl-butanal, 3-methyl-butanal) were the key ones detected in higher amounts in the crust.

It is worth mentioning that volatile compounds commonly associated with the Maillard reactions, Strecker degradation and high temperature processing, such as furfural, 2-methyl-butanal and 3-methyl-butanal, are detected at higher amounts in the crust samples (Rahaie et al., 2012). This makes sense as the thermal load during baking is much higher on the crust compared to the crumb. These compounds are commonly reported as indicators of high temperature processing in several processed food products (Drakula et al., 2019). Furfural is also a well-known indicator of non-enzymatic browning of a wide range of food products (Gibson et al., 2018). It is noteworthy that some of these compounds (2-methyl-butanal, 3-methyl-butanal) were already detected in ODF samples. Therefore, it can be hypothesized that these compounds are formed due to high temperature processing, starting from the drying of the GBF, and then increasingly formed, mainly in the crust, during Breadmaking.

On the other hand, the breadcrumb showed high contents of esters, ketones and alcohols. Esters have a fruity note (Yan et al., 2019, Pico et al., 2017, Salim Ur and Awan, 2011), whilst ketones are associated with the floral note of the ripening aroma (Qamar and Shaikh, 2018, Aurore et al., 2009) and alcohols are known for woody or musty flavour (Saha et al., 2018, Pico et al., 2017).

In contrast, the bread crust had high amounts of furans and Strecker aldehydes which can be linked to the high thermal load during baking. The breadcrumb, on the other hand, seemed to have higher contents of ester and alcohol compounds. According to Rohleder et al. (2019), the presence of alcohol and ester chemical classes might be more related to grassy green and fatty note. On the other side, aldehyde and furan compounds were mainly associated with Maillard reactions and were noted more in bread crust samples and have been reported to create caramel-like note (Boeswetter et al., 2019). However, comparing volatile compounds in Table 8.1, 8.2 and 8.3 shows that with increasing GBF fortification level in bread, different aromatic compounds were observed (showing a possible effect of fortification). Hence, in the next section, the effect of fortification level on producing volatiles in relation to the bread part will be discussed.

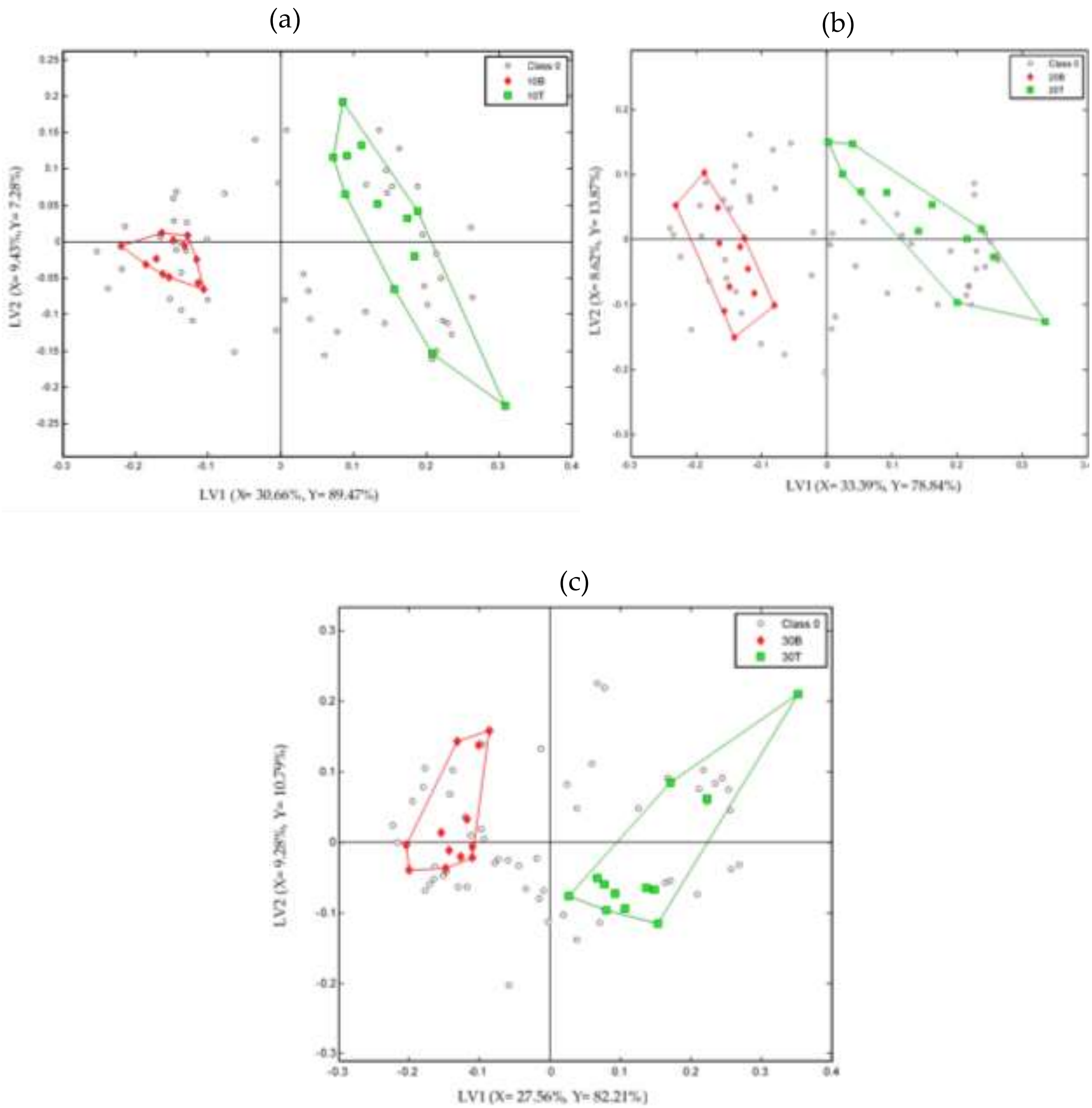


Figure 8.10. The partial least squares-discriminant analysis biplot of bread samples, showing a clear difference between the volatile profiles of the bread crust and crust samples at each fortification levels.

(a) 10% fortified-bread crumb samples (10B) vs. 10% fortified-bread crust (10T)

(b) 20% fortified-bread crumb samples (20B) vs. 20% fortified-bread crust (20T)

(c) 30% fortified-bread crumb samples (30B) vs. 30% fortified-bread crust (30T).

Table 8.1. Volatile compounds selected based on variable identification (VID) discriminating the bread crust and crust samples for 10% fortification level. The calculated retention index (RI) and one retrieved from literature (LRI) for volatile compounds are listed. The chemical class for each compound is also listed.

	VID	ID	Chemical class	CRI	LRI
	0.877	1-octyn-3-ol	alcohol	1297	1300
	0.788	nonanal	aldehyde	1391	1391
	0.725	toluene	alkane	1040	1042
	-0.653	2-undecanone	ketone	1595	1598
10B	-0.658	2-propoxy-ethanol	alcohol	1293	1326
	-0.679	1-(2-furanyl)-ethanone	furan	1505	1499
	-0.707	hexanoic acid, ethyl ester	straight-chain saturated fatty acid	1227	1233
	-0.738	2-octenal	aldehyde	1431	1429
	-0.912	1-hexanol	alcohol	1340	1355
	0.912	2,4-decadienal	medium-chain aldehyde	1815	1797
	0.870	2-tridecanone	ketone	1806	1809
	0.738	3-hydroxy-2-butanone	methyl ketone	1282	1284
	0.716	furfural	aldehyde	1462	1461
	0.706	hexanoic acid, ethyl ester	straight chain saturated fatty acid	1227	1233
10T	0.679	3-methyl-1-butanol	alcohol	1195	1209
	0.673	1-hydroxy-2-propanone	ketone	1297	1303
	0.658	heptyl ester	ester	1441	1453
	-0.725	maltol	pyrone	1967.2	1969
	-0.859	1-hexanol	alcohol	1339.7	1355
	-0.877	2-propanol	alcohol	1513.5	NA

10B, 10% fortified-breadcrumb samples, 10T, 10% fortified-bread crust.

Table 8.2. Volatile compounds selected based on variable identification (VID) discriminating the bread crust and crust samples for 20% fortification level. The calculated retention index (RI) and one retrieved from literature (LRI) for volatile compounds are listed. The chemical class for each compound is also listed.

	VID	ID	Chemical class	CRI	LRI
	0.807	ethyl ester butanoic acid	ester	1033	1035
	0.805	2,4-decadienal	aldehyde	1815	1797
	0.805	phenylethyl alcohol	alcohol	1911	1906
	0.786	1-hexanol	alcohol	1340	1355
	0.677	heptyl ester	ester	1441	1453
	0.668	2-propanol	alcohol	1518	1570
20B	-0.665	3-methyl-butanal	Strecker aldehyde	917	918
	-0.755	2-undecanone	ketone	1595	1598
	-0.800	2-methyl-propanoic acid	carboxylic acid	1566	1570
	-0.830	carbon sulphide	sulphur compound	732	135
	-0.850	2-tridecanone	ketone	1806	1809
	-0.851	3-furanmethanol	furan	1646	1670
	-0.879	furfural	aldehyde	1462	1461
	0.879	furfural	aldehyde	1462	14.61
	0.851	2-furanmethanol	furan	1646	1660
	0.850	2-tridecanone	ketone	1806	1809
	0.826	maltol	pyrone	1967	1973
	0.800	2-methyl-propanoic acid	carboxylic acid	1566	1570
	0.789	1-(2-furanyl)-ethanone	furan	1505	1499
20T	0.755	2-undecanone	ketone	1595	1598
	0.665	3-methyl-butanal	Strecker aldehyde	917	918
	0.656	pentanal	aldehyde	978	979
	-0.677	1-heptanol	alcohol	1441	1453
	-0.786	1-hexanol	alcohol	1340	1355
	-0.805	phenylethyl alcohol	alcohol	1911	1906
	-0.807	ethyl ester butanoic acid	ester	1033	1035

20B, 20% fortified-breadcrumb samples, 20T, 20% fortified-bread crust.

Table 8.3. Volatile compounds selected based on variable identification (VID) discriminating the bread crust and crust samples for 30% fortification level. The calculated retention index (RI) and one retrieved from literature (LRI) for volatile compounds are listed. The chemical class for each compound is also listed.

	VID	ID	Chemical class	CRI	LRI
30B	0.828	phenylethyl alcohol	alcohol	1911	1906
	0.739	ethyl ester butanoic acid	ester	1033	1035
	0.686	toluene	alkane	1040	1042
	0.657	octanoic acid, ethyl ester	ester	1428	1435
	-0.652	3-methyl-butanal	Strecker aldehyde	917	918
	-0.652	2-methyl-butanal	Strecker aldehyde	914	914
	-0.711	ethanone	ketone	1967	1973
	-0.728	pentanal	aldehyde	978	979
	-0.741	furfural	aldehyde	1462	1461
	-0.742	2-methyl-propanoic acid	carboxylic acid	1566	1570
	-0.785	1-(2-furanyl)-ethanone	furan	1505	1499
	-0.824	2-undecanone	ketone	1595	1598
	-0.902	2-tridecanone	ketone	1806	1809
	-0.939	2-furanmethanol	furan	1646	1660
30T	0.939	2-furanmethanol	furan	1646	1660
	0.902	2-tridecanone	ketone	1805	1809
	0.824	2-undecanone	ketone	1595	1598
	0.785	1-(2-furanyl)-ethanone	furan	1504	1499
	0.742	2-methyl-propanoic acid	carboxylic acid	1566	1570
	0.741	furfural	aldehyde	1462	1461
	0.728	pentanal	aldehyde	978	979
	0.725	maltol	pyrone	1967	1969
	0.652	2-methyl-butanal	aldehyde	914	914
	0.652	3-methyl-butanal	aldehyde	917	918
	-0.657	octanoic acid, ethyl ester	ester	1427	1435
	-0.739	ethyl ester butanoic acid	ester	1033	1035
	-0.828	phenylethyl alcohol	alcohol	1910	1906

30B, 30% fortified-breadcrumb samples, 30T, 30% fortified-bread crust.

8.3.3. Effect of fortification level on the volatile fraction of crumb and crust in each GBF type

PLS-DA model was used to investigate the effect of GBF fortification level on the volatile fraction of the crumb and crust (in each processing level). For the supervised PLS-DA model, the volatile compounds were used as X-variables and the fortification level as categorical Y-variable. As can be seen from the bi-plots (Fig 8.11), the volatile fraction of samples at different fortification levels was different. This shows that the volatile fraction of the bread samples is modified by the different levels of GBF fortification. It can be seen from Table 8.4 that increasing the fortification level in bread resulted in a higher number of discriminant volatile compounds. This trend was the same for both FDF and ODF-fortified bread samples.

For the freeze-dried breadcrumb (FB), furans (i.e., 2-pentenyl furan), aldehydes and alcohols (i.e. (E)-2-nonenal, 3-methyl-1-butanol) and ester (hexanoic acid ethyl ester) volatile compounds were detected at higher amounts in 30% compared to 10 and 20% level of fortification.

The breadcrumb samples made from oven-dried GBF (OB), had high levels of 3-methyl-butanal, benzaldehyde and furfural in 30% level of fortification. Similarly, for the oven-dried bread crust (OT), furans, Strecker aldehydes and pyrrolines were detected in O30T samples (Table 8.4). The formation of furfural and benzaldehyde in O30T samples indicates the effect of high thermal load in making these compounds (Göğüş et al., 2009). Benzaldehyde was observed in 30%, both crumb and crust, but only in OD-fortified bread samples. This Strecker aldehyde has been considered as a burnt sugar descriptor (Bugaud and Alter, 2016).

In summary, the level of fortification has a clear effect on the volatile compounds in both breadcrumb and crust. Specifically, furans and Strecker aldehyde volatiles were significantly increased at the highest level of fortification. Based on literature, the formation of these compounds is associated with thermally induced reactions, such as the Maillard reaction, Strecker degradation and lipid oxidation (Majcher et al., 2019, Pico et al., 2018a, Birch et al., 2014, Kebede et al., 2013). It can be hypothesised that the level of the fortification is influencing the concentration of precursors, such as carbohydrates and amino acids, which is available for the Maillard and lipid oxidative reactions. This hypothesis can be confirmed by monitoring and quantifying the number of sugars, amino acids and other precursors in the GBF and bread samples (this is outside the scope of this work).

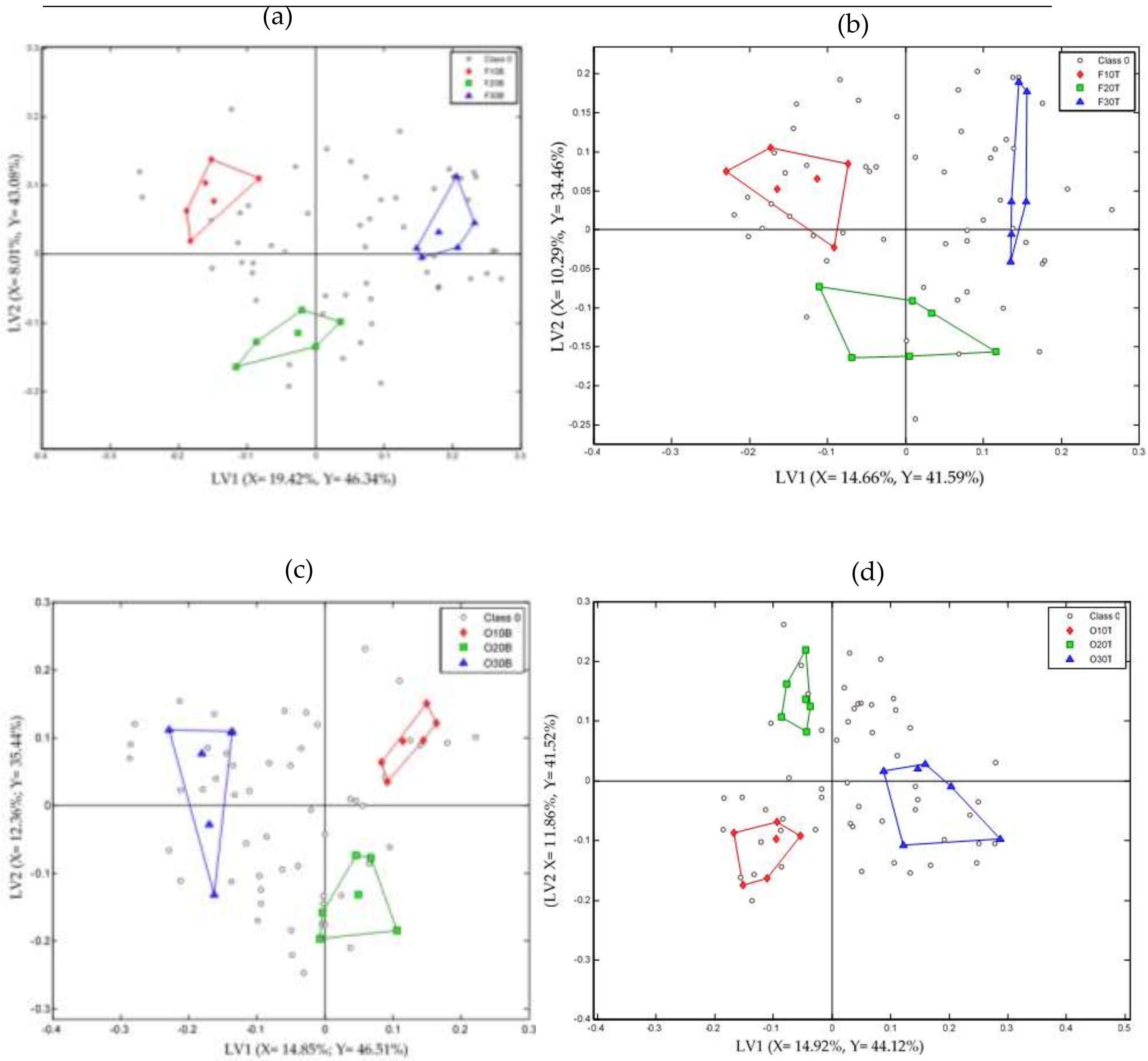


Figure 8.11. The partial least squares-discriminant analysis biplot of bread samples. There is clear effect of the level of GBF fortification on the final bread volatile profile.

(a) freeze-dried fortified breadcrumb samples, (b) freeze-dried fortified breadcrust samples, (c) oven-dried fortified breadcrumb samples (d), (c) oven-dried fortified breadcrust samples

F10B, F20B, F30B, freeze-dried-fortified breadcrumb at 10%, 20%, 30% substitution with wheat flour

F10T, F20T, F30T, freeze-dried-fortified breadcrust at 10%, 20%, 30% substitution with wheat flour

O10B, O20B, O30B, oven-dried-fortified breadcrumb at 10%, 20% and 30% substitution with wheat flour.

O10T, O20T, O30T, oven-dried-fortified breadcrust at 10%, 20% and 30% substitution with wheat flour.

WB, wheat bread crumb, WT, wheat bread crust

Table 8.4. Discriminant volatiles based on highest absolute variable identification coefficients (VID) coefficients for breadcrumb and crust samples through PLS-DA analysis. The calculated retention index (RI) and one retrieved from literature (LRI) for volatile compounds are listed.

	VID	ID	Chemical class	CRI	LRI
		FDB			
F10B	0.877	5-butyldihydro-2(3h)-furanone	alkane hydrocarbon	2044	1910
	0.720	2-propoxy-ethanol	alcohol	1293	1326
	-0.656	1-hepten-3-one	alkane	1297	1196
	-0.674	toluene	alkane	1040	1042
	-0.674	3-hydroxy-2-butanone	methyl ketone	1282	1284
	-0.747	1-hexanol	alcohol	1340	1355
F30B	0.828	2-pentenyl furan	furan	1297	NA
	0.779	3-hydroxy-2-butanone	methyl ketone	1284	1284
	0.765	hexanoic acid ethyl ester	ester	1846	1846
	0.731	1-hexanol	alcohol	1340	1355
	0.726	ethyl ester butanoic acid	ester	1033	1035
	0.722	3-methyl-1-butanol	alcohol	1195	1209
	0.716	(E)-2-nonenal	medium-chain aldehydes	1537	1534
	-0.689	2-propoxy-ethanol	alcohol	1293	1293
		FDT			
F30T	0.702	2-undecanone	ketone	1595	1598
		ODB			
O10B	0.766	2-propanol	alcohol	1514	1532
O30B	0.886	3-methyl-butanal	Strecker aldehyde	917	918
	0.879	benzaldehyde	Strecker aldehyde	1531	1520

	0.865	furfural	aldehyde	1462	1461
		ODT			
O10T	0.664	2-methyl-propanoic acid	short chain fatty acid	1566	1570
	-0.701	benzaldehyde	Strecker aldehyde	1531	1520
O20T	0.812	ethanol	alcohol	926	932
O30T	0.872	furfural	aldehyde	1462	1461
	0.809	benzaldehyde	Strecker aldehyde	1531	1520
	0.794	1-(2-furanyl)-ethanone	furan	1505	1499
	0.783	2-acetyl-1-pyrroline	pyrrolines	1967	1973

FDB, freeze-dried fortified breadcrumb, FDT, freeze-dried fortified breadcrust.

ODB, oven-dried fortified breadcrumb, ODT, oven-dried fortified breadcrust.

F10B, F30B, freeze-dried-fortified breadcrumb at 10% and 30% substitution with wheat flour.

F10T, freeze-dried-fortified breadcrust at 10% substitution with wheat flour.

O10B, O30B, oven-dried-fortified breadcrumb at 10% and 30% substitution with wheat flour.

O10T, O20T, O30T, oven-dried-fortified breadcrust at 10%, 20% and 30% substitution with wheat flour.

8.3.4. Effect of drying method on volatile fraction of GBF-fortified bread

PLS-DA model was applied to compare the two drying methods. From the bi-plots in Fig. 8.12, there is less clear separation amongst samples regards to the drying method used for GBF preparation. This is in line with the observation found in the PCA bi-plot. In line with that, the feature selection managed to determine only few discriminant volatile compounds.

The FD-processing resulted in (E)-2-nonenal and 1-hexanol formation in breadcrumb and pentanal, 2-heptenal, 2-undecanone and furfural in bread crust. There were no notable volatiles for OT samples, while a range of compounds were observed for FT samples. This might be due to the decomposition of browning reactions' precursors during ODF preparation and higher surface temperature in crust during the baking process.

(a) (b)

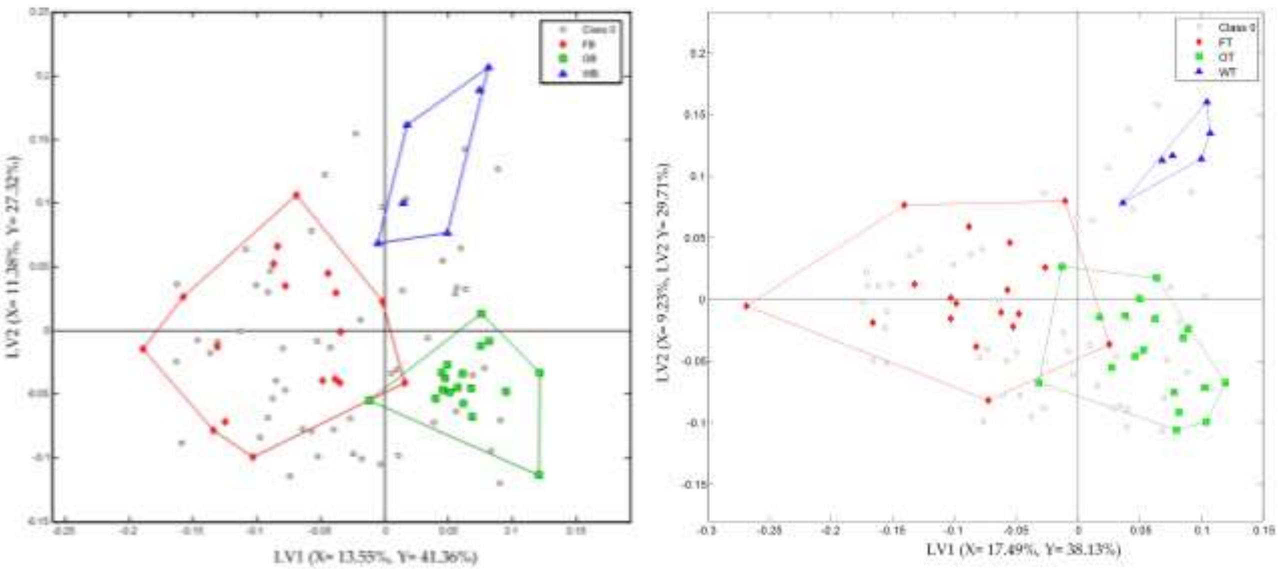


Figure 8.12. A PLS-DA bi-plot showing the difference in volatile profile of breadcrumb and breadcrust based on the drying process

(a) FB, fortified-bread crumb samples with freeze-dried green banana flour, OB, fortified-bread crumb samples with oven-dried green banana flour, WB, wheat bread crumb. (b) FT, fortified-bread crust samples with freeze-dried green banana flour, OT, fortified-bread crust samples with oven-dried green banana flour, WT, wheat bread crust.

Table 8.5. Discriminant volatiles based on highest absolute variable identification coefficients (VID) coefficients for breadcrumb and crust samples through PLS-DA analysis. The calculated retention index (RI) and one retrieved from literature (LRI) for volatile compounds are listed.

	VID	ID	Chemical class	CRI	LRI
FB	0.766	(E)-2-nonenal	medium-chain aldehyde	1537	1534
	0.757	1-hexanol	alcohol	1340	1355
	0.684	pentanal	aldehyde	978	979
OB	0.658	3-methyl-butanal	Strecker aldehyde	917	918
WB	0.719	octaethylene glycol monododecyl ether	ethylene glycol	1779	NA
	0.684	phenylethyl alcohol	alcohol	1911	1906
	0.665	2-phenylethanol	alcohol	1935	1915
FT	0.798	pentanal	aldehyde	978	979
	0.752	2-heptenal	alkyl aldehyde	1325	1322
	0.702	2-undecanone	ketone	1595	1598
	0.689	furfural	aldehyde	1462	1461
	0.664	1-hydroxy-2-propanone	ketone	1297	1300
WT	0.778	2-propoxy-ethanol	alcohol	1293	1326
	0.651	carbon disulphide	sulphur compound	732	735

FB, fortified-breadcrumb samples with freeze-dried green banana flour, OB, fortified-breadcrumb samples with oven-dried green banana flour, WB, wheat breadcrumb. FT, fortified-bread crust samples with freeze-dried green banana flour, WT, wheat bread crust.

8.4. Changes of key volatile compounds in bread samples

From what has been discussed so far, it can be concluded that the volatile profile of the bread crust is evidently different from that of the crumb. Furthermore, green banana flour fortification level also showed a clear effect on modifying the volatile profile in both breadcrumb and crust. Lastly, the processing method of GBF preparation had an impact on the fortified bread volatile profile (but relatively lower impact compared to the other factors). In summary, volatile compounds that were significantly affected by the bread part, level of fortification and processing can be grouped into:

(i) furanic compounds (furfural and 2-furanmethanol)

(ii) Strecker aldehydes (3-methyl-butanal)

(iii) esters (ethyl ester butanoic acid)

(iv) ketones (2-undecanone)

Each of these chemical classes were discussed in relation to the relevant chemical reactions' pathways and odour relevance. One should note that there are very limited studies investigating the impact of different variables in a bread made with BF. To clearly show the trend, a bar graph is prepared for these representative compounds in order to compare and interpret the significant difference amongst all the seven bread samples.

8.4.1. Furanic compounds

8.4.1.1. Furfural

As can be seen from Fig 8.13, the bread crust contained higher furfural compared to crumb in all the samples. The increasing quantity in furfural content in conjunction with an increase in fortification level is another important trend of Fig 8.13. However, this level of fortification effect was not statistically significant (P-value > 0.05) in breadcrumb or crust samples (except from O30T compared to other OT samples). Drying method used for GBF preparation had a notable impact on the production of furfural, in which the amount of furfural was higher in the FD samples compared to the OD samples. This might be due to the uncaramelized sugars and Maillard precursors (e.g., 5-hydroxymethylfurfural) which possibly remained intact during FD processing (Pico et al., 2017). According to Pico et al. (2018a), 5-hydroxymethylfurfural could be converted to furfural by Maillard reactions and caramelisation during cooking process. This hypothesis can be confirmed by designing a targeted experiment to quantify the concentration of 5-hydroxymethylfurfural and other intermediate products, however, this was outside the scope of this study. Furfural is considered as an indicator for non-enzymatic browning and is the distinguished aldehyde in bread crust. It could be formed by the heat-induced complex Maillard reaction primarily occurring on the dough surface during baking (Perez-Locas and Yaylayan, 2010).

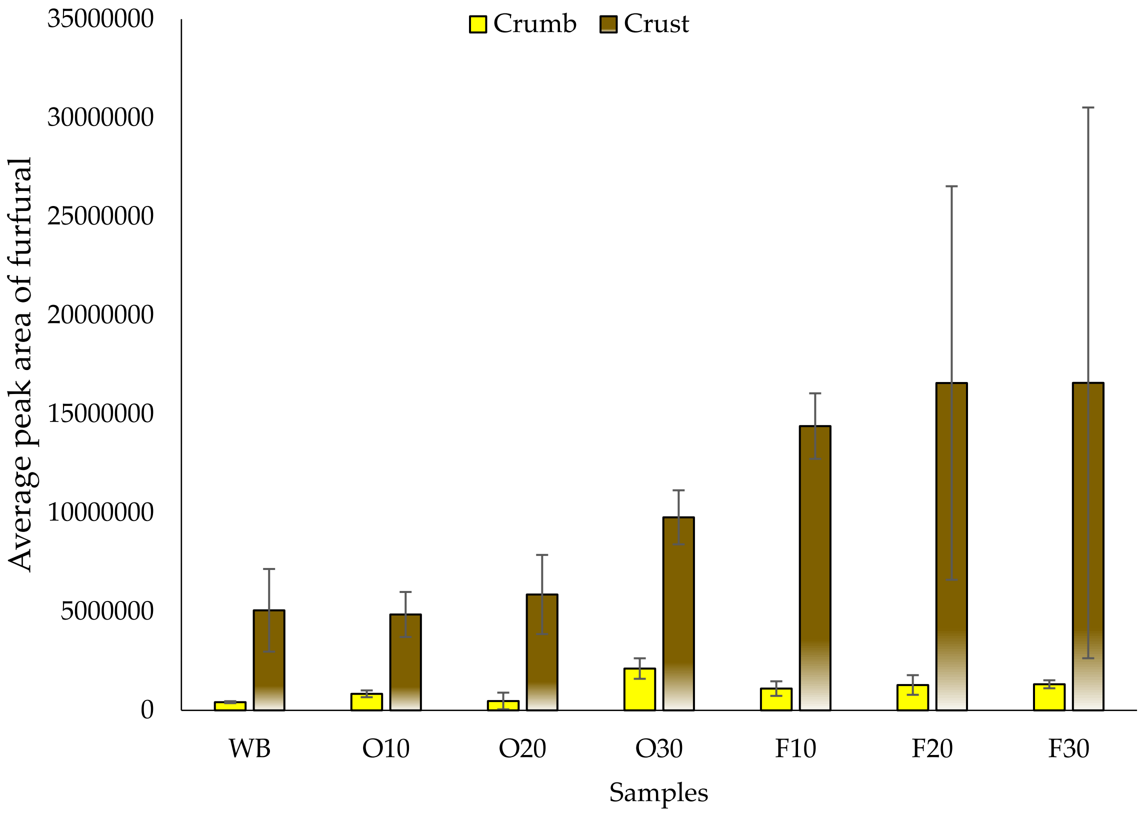


Figure 8.13. Furfural content in all bread samples

WB, wheat bread, O10, 10% oven-dried-fortified bread, O20, 20% oven-dried-fortified bread, O30, 30% oven-dried-fortified bread, F10, 10% oven freeze-dried-fortified bread, F20, 20% oven freeze-dried-fortified bread, F30, 30% oven freeze-dried-fortified bread. Bars for each bread part (column colour) depict mean and standard deviation values of determinations (n = 6).

This reaction occurs between carbonyls (most often reducing sugars) and a free amino, peptide, or protein, which results in the formation of brown nitrogenous polymers (Danehy, 1986). Due to its low odour threshold, furfural could be very important to the overall bread volatile profile. Furfural has been reported to exert caramel note (Conte et al., 2020).

8.4.1.2. 2-Furanmethanol

An increase was recorded in 2-furanmethanol content in all bread crust samples compared to crumbs (Fig 8.14). Furanmethanol or furfuryl alcohol is a cyclic furanic compound which has been reported to polymerise in acidic pH to aliphatic polymers, which causes a higher browning index to bread (Pico et al., 2018a). Pico et al. (2019a) signified furfuryl alcohol as one of the compounds responsible for darker colour of crust compared to crumb. They also reported that 2-furanmethanol can be generated by caramelisation processes. According to Conte et al. (2020) findings, 2-furanmethanol was reported as a reduction product from furfural in gluten-free bread. The appearance of 2-furanmethanol mainly in bread crust samples could contribute to the nutty, roasted aroma (Aguilar et al., 2015, Birch et al., 2014, Salim Ur and Awan, 2011). However, considering the non-significant difference amongst banana bread samples, this hypothesis needs to be confirmed using GC-olfactometry in future studies.

Overall, it can be said that furanic compounds were the discriminant volatiles in bread crust. All three selected furanic compounds were higher in FDF-fortified bread samples, which could be due to the presence of higher amounts of Maillard reactions precursors in FDF compared to ODF. Furthermore, fortification level showed a slight increase within each bread type which was only significant in O30T samples for 3-methyl butanal. Moreover, considering the non-significant difference amongst banana bread samples and fortification levels, this compound might not be related to GBF addition to bread.

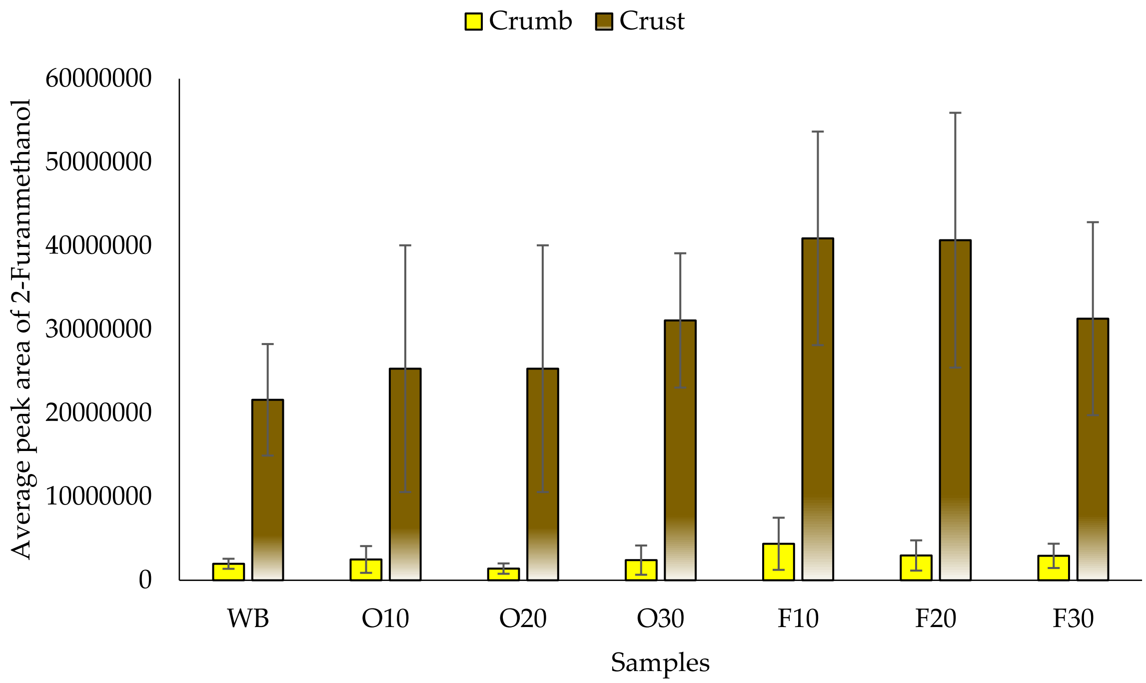


Figure 8.14. 2-Furanmethanol content in all bread samples

WB, wheat bread, O10, 10% oven-dried-fortified bread, O20, 20% oven-dried-fortified bread, O30, 30% oven-dried-fortified bread, F10, 10% oven freeze-dried-fortified bread, F20, 20% oven freeze-dried-fortified bread, F30, 30% oven freeze-dried-fortified bread. Bars for each bread part (column colour) depict mean and standard deviation values of determinations (n = 6).

8.4.2. Strecker aldehydes

From Fig 8.15, it can be seen that bread crust contained higher 3-methyl-butanal compared to breadcrumb samples. The drying method also had an effect on the formation of 3-methyl-butanal. 3-Methyl-butanal is formed through a Strecker degradation reaction, which is one of the side reactions of the Maillard reaction. In this reaction, amino acids react with dehydroreductones to produce aldehydes, in particular, 3-methyl-butanal from leucine (Barbarisi et al., 2019). As FDF was prepared by non-thermal drying (Chapter 4), higher 3-methyl-butanal content in F20T and F30T samples might be due to the presence of leucine which was kept in the FDF until the baking process. Its pungent malty aroma is reported to be easily detectable as it has a low flavour threshold (Pico et al., 2016). Overall, a combined effect of bread part, fortification level and drying method could be seen for 3-methyl-butanal.

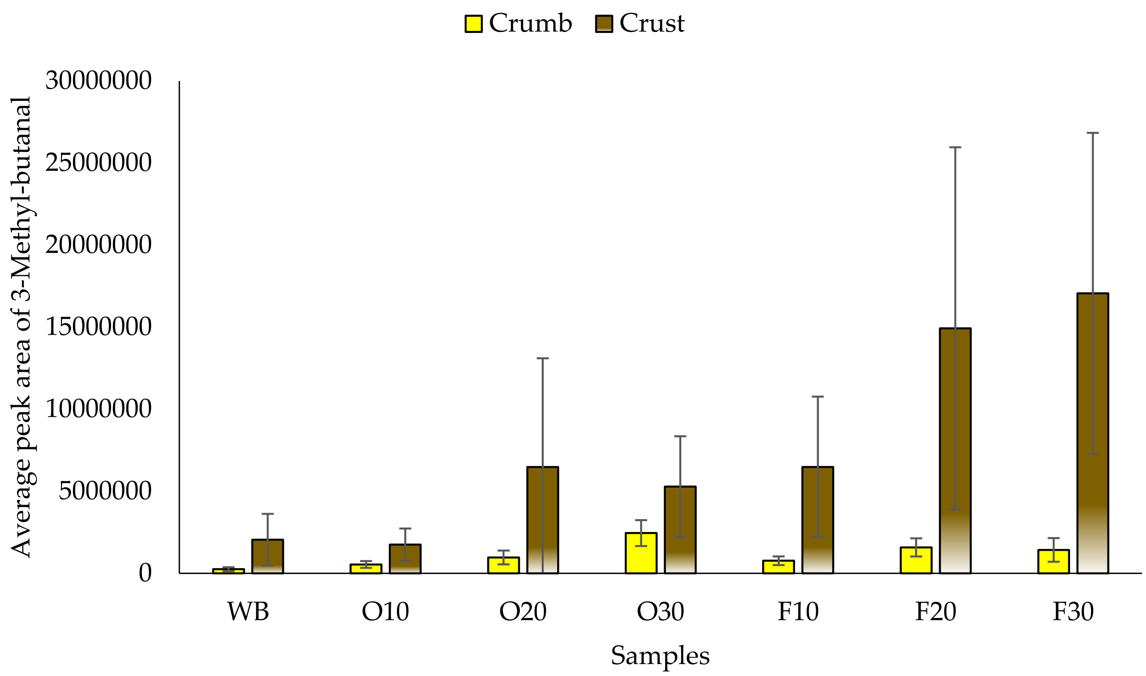


Figure 8.15. 3-Methyl-butanal content in all bread samples

WB, wheat bread, O10, 10% oven-dried-fortified bread, O20, 20% oven-dried-fortified bread, O30, 30% oven-dried-fortified bread, F10, 10% oven freeze-dried-fortified bread, F20, 20% oven freeze-dried-fortified bread, F30, 30% oven freeze-dried-fortified bread. Bars for each bread part (column colour) depict mean and standard deviation values of determinations (n = 6).

8.4.3. Esters

Contrary to furanic compounds, esters seemed to be found more in breadcrumb samples compared to crusts (Fig 8.16). The abundance of ethyl ester butanoic acid in breadcrumb was already reported in previous sections (Table 8.3). Regarding fortification level, no significant changes could be seen amongst bread samples (P-value > 0.05), however, above 10% level, FB samples showed a clear difference between crumb and crust. Amongst the ethyl acetate group (butanoate, octanoate, hexanoate, propanoate and 2-hydroxypropanoate), ethyl ester butanoic acid is also recognized as a discriminant compound in banana fruit (Zhu et al., 2018, Qamar and Shaikh, 2018). Also, it has been reported that can be generated in wheat breadcrumb as well (Birch et al., 2013a). This ester, which could enhance the sweet impression of sucrose, is generated by esterification of alcohols and acyl-CoA derived from both fatty acid and amino acid metabolism (Birch et al., 2013b).

The study of Seymour (1993) on different ripe banana varieties showed that amyl esters are responsible for the distinctive banana-like flavour, while fruity note is mainly attributed to butyl esters. Esters constitute the major class of compounds present in the volatile profile of the ripe banana, such as isoamyl acetate, ethyl acetate, butyl acetate, isoamyl isobutanoate and butyl butanoate (Zhu et al., 2018). According to Vermeir et al. (2009) findings, the predominant ester in semi-ripe banana was 3-methyl-butyl-butanoate. Overall, it can be concluded that although the processing approach taken to produce GBF had an impact on producing different volatile compounds, bread part played the dominant role. However, the FD-fortified bread samples might produce a more pronounced "banana like" aroma. The difference in volatile compounds found in fortified bread samples, in both crust and crumb, showed the method of GBF preparation prior to the Breadmaking could have an impact on the bread's flavour.

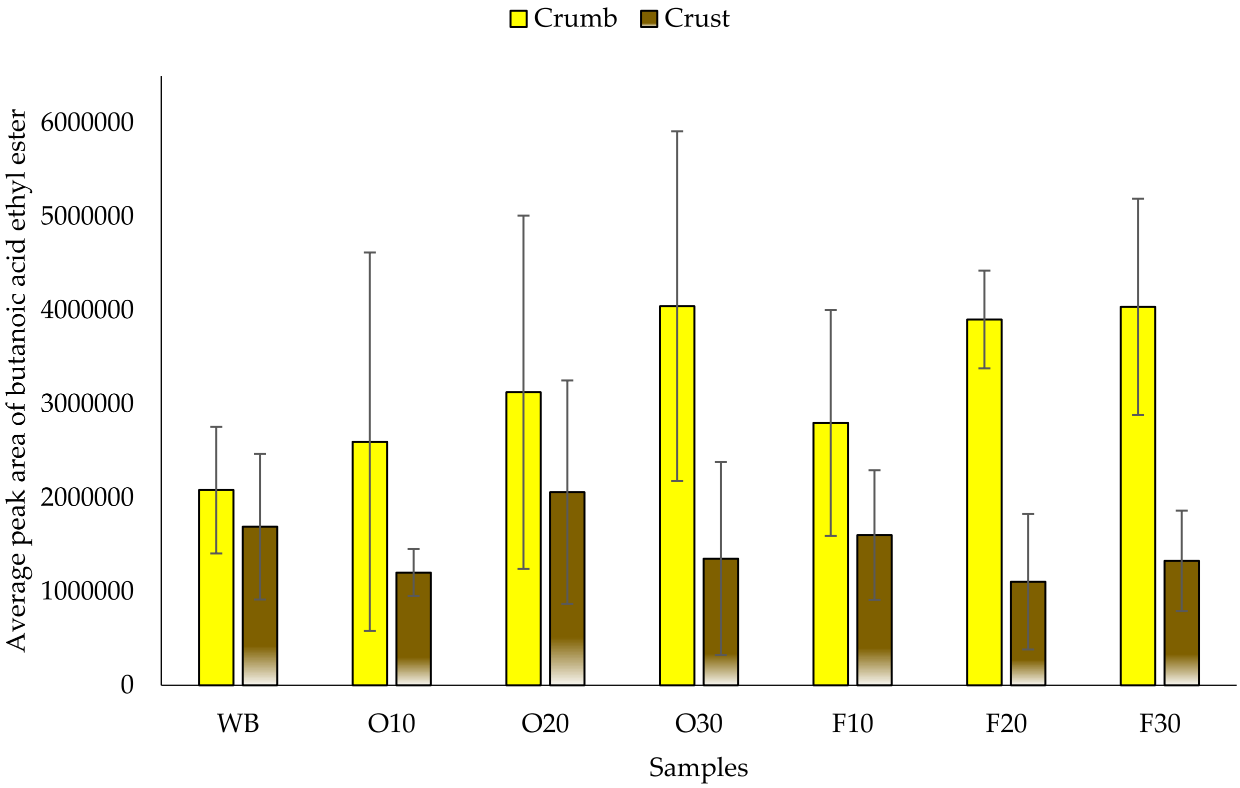


Figure 8.16. Butanoic acid ethyl ester content in all bread samples

WB, wheat bread, O10, 10% oven-dried-fortified bread, O20, 20% oven-dried-fortified bread, O30, 30% oven-dried-fortified bread, F10, 10% oven freeze-dried-fortified bread, F20, 20% oven freeze-dried-fortified bread, F30, 30% oven freeze-dried-fortified bread. Bars for each bread part (column colour) depict mean and standard deviation values of determinations (n = 6).

8.4.4. Ketones

In Fig 8.17, a discriminant compound trend from ketone chemical class is shown. A gradual increase in the peak area of 2-undecanone by adding more GBF was observed in bread crust samples. Similar to furanic compounds, 2-undecanone appeared more notably in crust compared to crumb. The gradual increase in 2-undecanone content was not significant in OD-fortified samples, however, increasing fortification level had an increasing impact on FDT samples. It was also shown that in higher levels of fortification, this volatile was detected more, particularly in F30T samples (Table 8.5). Studies on banana fruit volatiles have indicated that this compound is one of the aromatic compounds in the early ripening stage of green bananas (Zhu et al., 2018, Vermeir et al., 2009, Wang et al., 2007).

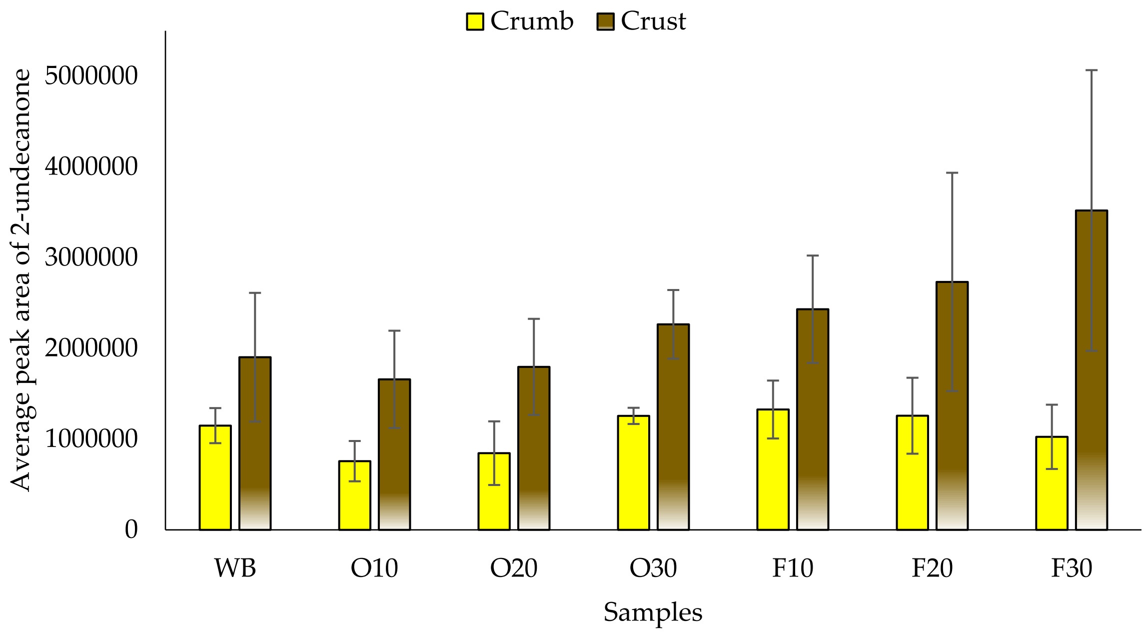


Figure 8.17. 2-Undecanone content in all bread samples

WB, wheat bread, O10, 10% oven-dried-fortified bread, O20, 20% oven-dried-fortified bread, O30, 30% oven-dried-fortified bread, F10, 10% oven freeze-dried-fortified bread, F20, 20% oven freeze-dried-fortified bread, F30, 30% oven freeze-dried-fortified bread. Bars for each bread part (column colour) depict mean and standard deviation values of determinations (n = 6).

Regarding the notable effect of drying method, the explanation might be due to the presence of this ketone in FDF considering non-thermal GBF preparation.

8.4. Conclusion

This study has provided an overview of the volatile compounds of wheat bread fortified with different levels of two types of green banana flour, using an integrated headspace fingerprinting and chemometrics. This was a successful approach which led to the detection of an increased number of volatile compounds from a wide range of chemical classes in two bread parts, crumb and crust. The implemented chemometrics modelling such as PCA and PLS-DA gave a detailed insight into three factors: bread part, fortification level and processing method used for GBF preparation.

From the three main factors, the volatile compounds were significantly different between the bread parts, i.e., crumb and crust. Maillard reactions and Strecker degradation were the most dominant reactions responsible for the development of 2-furanmethanol, furfural, 3-methyl-butanal and benzaldehyde in bread crust. The breadcrumb had a higher amount of ester and alcohol compounds. The second important effect was the level of GBF fortification in bread, which causes more volatile generation in 30% level and no significant changes between 10% and 20%. Alcohols (3-methyl-1-butanol and 1-hexanol) and esters (hexanoic and butanoic acid ethyl ester) in crumb and Strecker aldehydes (benzaldehyde and 3-methyl-butanal) and aldehydes (furfural) in crust were the result of lipid oxidation and browning reactions, respectively. Furthermore, the banana drying method used for GBF preparation had an impact on the generation of different key volatile compounds both in bread crust and crumb. The FDF-fortified samples yielded more distinctive volatile profile compounds, mainly Strecker aldehydes.

This study successfully demonstrated the potential of GC-MS-based fingerprinting and chemometrics to increase insight into the volatile changes and identify discriminant volatile compounds. However, headspace fingerprinting only allowed tentative identification and relative quantification of selected volatiles. In the future, there is a need for identity confirmation and absolute quantification of the selected discriminant components using pure standards. Furthermore, characterising headspace components with respect to reducing sugar and amino acid profile of GBF and the bread could contribute to more detailed information about the differences between the GBF preparation techniques. In addition, Gas Chromatography Olfactometry (GC-O) can provide a correlation between selected compounds and the odour activity. A follow-up descriptive sensory analysis can also be used to investigate how the volatile profile changes will be perceived.

8.5. Limitations

Although this study provides a broad perspective into the volatile profile of the GBF-fortified bread, the identification approach was a tentative identification. The identification of the selected compounds needs to be confirmed by injecting pure standards. Also, in order to understand the bread aroma profile, a descriptive sensory analysis would be advised.

CHAPTER 9

General conclusion and recommendations for future studies

Chapter aim:

In this chapter, the key findings of the study are discussed. Then, future possible follow-up studies and recommendations are summarized.

9.1. Introduction

The main goal of this study was to select the best feasible drying method for producing a functional ingredient for fortification of bread. Thereafter, the quality attributes and textural properties of dough and bread were assessed together with *in vitro* digestion in order to determine resistant starch content of the final fortified bread. Finally, a comparative volatile fingerprinting was performed in order to find the discriminant volatile compounds that distinguish different bread samples.

In this thesis, two drying techniques were applied in four conditions to investigate the effect of each one on resistant starch content, changes in thermal properties of the flour and physicochemical parameters of the whole green banana flour (Chapter 3). In order to find out the capability of the flour for application in bread formulation, textural, rheological, physical and nutritional properties of the produced flour was also investigated (Chapter 4). Based on the findings, two of the best treatments were selected for fortification of bread: oven-dried GBF obtained at 50 °C (ODF) and freeze-dried flour (FDF). The subsequent experimental chapters (5-7) focused on the effect of type of produced GBF and the fortification level in bread while comparing the viscoelastic, rheological and textural properties and finally the volatile profile of both dough and bread to a control sample made with 0% GBF.

9.2. General conclusion

The obtained results support the use of whole green banana to produce an added value food ingredient for fortification purposes. Freeze-dried green banana flour is a greenish-like powder with high water holding capacity and emulsifying attributes. It contains the highest possible percentage of resistant starch remained after drying processing. It also can generate more stable gels during storage due to the high setback viscosity. The application of this flour in bread structure resulted in a food product with a considerable amount of resistant starch, slow digestible starch and minerals compared to the control sample without GBF. The bread fortification with 10% of this flour resulted in a bread with a high specific volume. Continuing substitution up to 30%, resulted in a drop in bread springiness and resilience, while it increased the chewiness and hardness. This could suggest fortification at 30% level would be more suitable for other flat bread types. Overall, the bread made with maximum 30% freeze-dried green banana flour resulted in a bread with distinctive volatile compounds from both technological and nutritional aspects.

Comparing the results achieved from thermal treatment of green banana showed that although it is possible to produce GBF with only two hours at 110 ˚C of drying time, samples obtained after 7.5 h at 50 ˚C treatment had more similar properties to the non-thermal treated ones. The dough made with the latter one showed the lowest breakdown viscosity amongst heat treated samples. Considering that Maillard reactions and Strecker degradation produce more browning index colour in bread, it caused more generation of discriminant volatiles in bread crust.

9.3. Key outcomes

The key outcomes of this research can be summarised as follows:

9.3.1. Producing a gluten-free vegan flour from the whole banana fruit

Bananas are among the most traded fruits in the world. Representing almost 20% of yearly global banana production, 22.7 million tonnes of bananas are traded. Almost half of the annual banana production is wasted before maturation (around 40 million tonnes). Inappropriate handling during the harvest in farmlands, having an unacceptable appearance, shape or size from the market point of view and inappropriate transportation are some of the reasons behind this high rejection rate. The first important achievement of this study was the green banana flour which was produced from the whole fruit parts. Without separating the peel from the pulp, the aim of taking advantage of all the nutrients entrapped in the peel was achieved.

9.3.2. Introducing new drying methods

Another important achievement of this study was introducing new optimised thermal and non-thermal drying methods for producing GBF in the most efficient way. Effective oven drying at 110 °C with continuous air flow was achieved within two hours. Nutritional results reported in chapters 4 and 5 showed that heat-treated GBF samples could be considered as a good source of minerals. Freeze-drying successfully granted dried samples with maximum a_w of 0.24 in total 52 h, which was the least processing time reported in the literature. Sample preparation (slicing the whole fruit into 2-mm thickness circular shapes) helped minimizing the processing time, thermal conduction and sublimation of moisture from banana fruit. Freeze-drying can be an expensive drying technique, however, the characteristics of the flour obtained at 50 °C with oven-drying showed that this GBF had the most similar rheological, textural and nutritional properties amongst other drying conditions. This showed the possibility of using this treatment as an alternative low-cost technique compared to freeze drying, especially in developing countries which are the leading banana producers.

9.3.3 Full characterization of green banana flour, dough and bread

The green banana flour samples made from two methods in four conditions was examined to understand the effect of the banana drying process on its various properties. Resistant starch analysis showed a significant amount of available RS in freeze-dried and oven-dried samples, especially those dried at 50 °C which was slightly less than the freeze-dried sample. In chapter 7, it was shown that 20% GBF substitution with wheat flour resulted in at least 1.5 times more RS in bread, regardless of the type of GBF. However, for obtaining the highest possible intake of RS, 30% FDF addition showed a remarkable increase in RS content within the range of prebiotic dosage. This showed the importance of RS analysis in both GBF and bread to observe the processing effect on the remaining of RS. In terms of textural attributes, the strong correlation between fresh dough hardness and bread hardness (P-value < 0.05) showed the importance of textural analysis of dough for being able to predict the final product's properties.

9.3.4. Changes of bread quality during storage

Storage results showed that retrogradation reactions happened quicker in fortified bread samples compared to whole wheat bread (chapter 6). Also, higher levels of GBF increased the firmness after a week of bread storage. Although recrystallization of starch postponed at -20 °C, due to the increased amount of sugar in fortified samples, it resulted in greater stiffness. Moisture loss, however, was not significant amongst fortified bread which shows the effect of high-water holding capacity of GBF stored in freezer.

9.3.5. Increased amount of resistant starch in bread

The *in vitro* digestibility analysis showed that the 30% fortified bread can be considered as a prebiotic bread (chapter 6). The remaining RS and SDS content in the final bread was in the range of recommended daily intake of RS (6-12 g/d), which makes it a valuable source for increasing the intake of this nutritive DF. Considering that this amount was lesser in OD-fortified bread, it can be concluded that fortification with ODF at minimum of 30% can be as close as the FDF. As a result, these results could provide an opportunity in industries who are not capable of establishing freeze drying equipment. Considering results reported for ODF50 in chapter 4, the use of ODF50 as a functional ingredient in other food products can be further explored.

9.3.6. Increased the availability of macro and micro minerals in bread

Comparing the results from ICP-MS mass spectrometer revealed a significant rise in potassium, magnesium, iron, copper and zinc in all fortified bread samples, particularly at 30% level. This can play a part in fulfilling the daily intake advised for these elements. Regardless of the type of GBF, this study showed that for the purpose of increasing minerals in diet, the type of processing technique has the least effect on the remaining percentage of the elements.

9.4. Future recommendations

1) Future studies should focus on the particle size distribution, starch granule microstructure and rheological properties of the whole green banana flour to understand their potential behaviour during packaging, transportation and use in food systems.

2) Green banana flour produced with FD and dried at 50 °C generated a high viscose slurry in reaction with water molecules. In this regard, it can be added to other food products and act as a thickener, gelling agent, reinforcing agent and filler. Studies are required to understand the behaviour of these GBF samples in other food products.

3) More studies are needed for the evaluation of microstructural changes in GBF bread mainly with FDF as it has shown less textural damaging effect on bread slices. Also, sensory evaluation and consumer acceptance are suggested for further studies in this area.

4) Some previous studies have shown the synergic effect of hydrocolloids with resistant starch content which can be used for the future studies on bread formulation.

5) There is a need for identifying confirmation and quantification of the selected discriminant components by the use of pure standard samples. Furthermore, characterising headspace components with respect to reducing sugar and amino acid profile of GBF and the bread could provide more detailed information about the differences between the GBF production techniques.

6) Gas Chromatography Olfactometry (GC-O) can provide a correlation between specific chemicals and the odour concentration by the senses of trained panellists.

7) A follow-up descriptive sensory analysis or consumer acceptance testing should be carried out to improve the impact of these selected discriminant components.

APPENDIX

Table A1. The observations of the effect of different trial processes on green banana samples

Whole green Banana preparation	Before drying	After freeze-drying	After oven drying
Chopped and mixed in a mixer	Enzymatic browning started	Samples was not fully frozen after 6 hours (time consuming)	Browning after an hour
Cut the fruit in half (from length)	Difficult to split exactly in half due to the curvy shape of fruits	Incomplete drying after 48 h	Case hardening occurred with incomplete core drying
Green banana slices with a thickness range of 10 - 2 mm	Difficult handling for one person	Not efficient drying in slices with > 2 mm	- Needed more drying time - Not appropriate for temperature > 80 °C

Figure B 1. Fresh green banana slices (left) and dried ones (right) by oven drying

Figure B 2. Green banana slices immersion in 0.5% (w/v) citric acid

Figure B 3. Green banana flour prepared at 110 °C (left), 80 °C (middle) and 50 °C (right)

Figure B 4. Fresh frozen green banana slices (left) and freeze-dried slices (right)

Figure B 5. Freeze-dried green banana flour (left) and wheat flour (right)

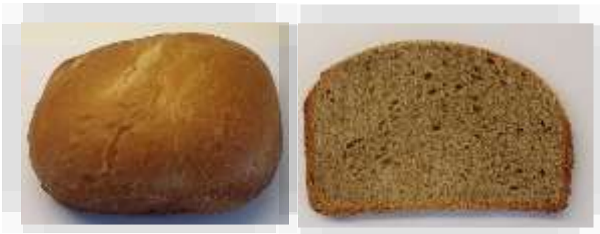
Figure B 6. Freeze-dried green banana flour (left) and oven-dried one at 50 °C (right)

Figure B 7. First trial of bread dough making

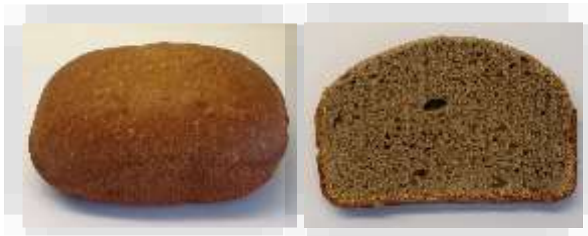
Figure B 8. First trial of non-fermented pan bread making

Figure B 9. The first trial for green banana fortified bread at 30% level (left) and wheat bread (right)

Figure B 10. Trials for other bakery product (cookie dough, left and baked one, right)

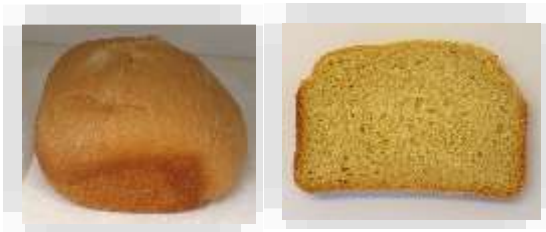


F10

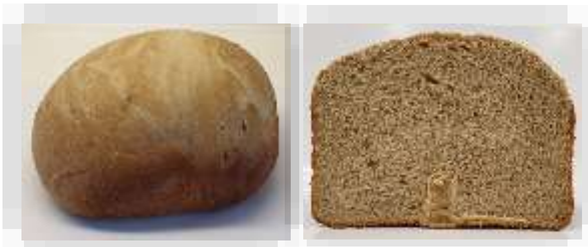


F20

F30



WB



O10

O20

O30

Figure B 11. All the prepared bread samples

* WB, wheat bread, F10, F20 and F30, substitution levels of freeze-dried banana flour with wheat flour.

* O10, O20 and O30, substitution levels of air oven-dried banana flour with wheat flour.

Figure B 12. Oven-dried banana flour (left) vs. freeze-dried samples (right)

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