PHYSICAL AND SPECTROSCOPIC CHARACTERIZATION OF BAKERY PRODUCTS MADE FROM DIFFERENT FLOUR MIXTURES

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ABSTRACT

PHYSICAL AND SPECTROSCOPIC CHARACTERIZATION OF BAKERY PRODUCTS MADE FROM DIFFERENT FLOUR MIXTURES

In this study, it was aimed to show the effect of legume and nut flours in bread formulations in order to improve the nutritional quality and also sensorial appeal of gluten-free bakery products. Breads with and without yeast were prepared according to mixture designs, in which white bean and hazelnut flours were incorporated in rice flour-corn starch based recipes. Flour and bread samples were described by their chemical, technological properties and mid-infrared Fourier transform spectroscopic profiles. Analyses were finalized by starch digestion of the best bread formulations.

White bean flour was characterized by its high protein and water retention capacity, whereas hazelnut flour came forward for its highest fat and crude fiber content. Their spectroscopic profiles magnified the differences and confirmed the information gathered with chemical and physical analysis. Seven bread samples with and without yeast were prepared with flour mixtures containing bean and hazelnut flours between 0 and 30%. Doughs and breads were analyzed for their physical properties, and multivariate models were generated to differentiate samples of different gluten-free flour mixtures.

Yeast bread containing 15% hazelnut flour was found to have the closest physical properties to standard bread and the capacity to increase nutritional values. The best formulation for yeast-free bread was found to have 15% bean and 15% hazelnut flours w.r.t. criteria of softness, taste, and volume. Starch digestion showed that the inclusion of bean and hazelnut flours in the formulation reduced the rapidly digested starch content of breads.

ÖZET

FARKLI UN KARIŞIMLARINDAN ÜRETİLEN ÜRÜNLERİN FİZİKSEL VE SPEKTROSKOPİK KARAKTERİZASYONU

Bu çalışmada, glütensiz unlu mamullerin beslenme ve duyusal kalitesini artırmak için ekmek formülasyonlarında baklagil ve fındık unlarının etkisini göstermek amaçlanmıştır. Mayalı ve mayasız ekmekler, karışım deney tasarımına göre beyaz fasulye unu ve fındık unu ile pirinç unu ve mısır nişastası içeren un karışımları ile hazırlanmıştır. Un ve ekmek örnekleri, kimyasal, teknolojik özellikleri ve orta kızılötesi Fourier dönüşümü spektroskopik profilleri ile tanımlanmıştır. Analizler en iyi ekmek formülasyonlarının in vitro nişasta sindirim analizi ile sonuçlandırılmıştır.

Beyaz fasulye unu, yüksek protein ve su tutma kapasitesi ile karakterize edilirken, fındık unu en yüksek yağ ve ham lif içeriği ile öne çıkmıştır. Spektroskopik profiller, unlar arasındaki farklılıkları ortaya çıkarmış ve kimyasal ve fiziksel analizlerle toplanan bilgileri doğrulamıştır. Fasulye ve fındık unu içeren un karışımları (%0–30 arasında) ile mayalı ve mayasız olmak üzere yedişer ekmek formülasyonu hazırlanmıştır. Hamur numuneleri ve ekmekler, fiziksel, tekstürel özellikleri açısından analiz edilmiştir ve un, hamur ve ekmeklere ait spektroskopik verilerle çok değişkenli modeller üretilmiştir.

Glutensiz mayalı ekmeklerde, %15 fındık unu içeren karışım ile besin değerlerini yükseltme kapasitesine ek olarak standart glütensiz mayalı ekmeğe en yakın fiziksel özelliklere sahip ekmek üretilmiştir. Mayasız ekmeklere uygulanan karışım tasarımı ve duyusal analiz verileri, standart bir referans glütensiz ekmeğe kıyasla hacim, sertlik ve tat açısından en iyi formülasyonların %30 beyaz fasulye-fındık unu ve %30 fındık unu içerenler olduğunu göstermiştir. Nişasta sindirim analizi, mayasız formülasyona fasulye ve fındık unlarının dahil edilmesinin ekmeklerin hızlı sindirilen nişasta içeriğini azalttığını göstermiştir.

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LIST OF ABBREVIATIONS

C: Corn starch used in gluten-free yeast bread

R: Rice flour used in gluten-free yeast bread

I-C: Corn starch used in gluten-free and yeast-free bread

I-R: Rice flour used in gluten-free and yeast-free bread

B: White bean flour, used in both gluten-free bread formulations

H: Hazelnut flour, used in both gluten-free bread formulations

fm: flour mixture

GF: gluten-free

GF-YF: gluten-free and yeast-free

FT-IR: Fourier Transform Infrared Spectroscopy

WRC: Water retention capacity

OAC: Oil absorption capacity

EA: emulsion activity

ES: emulsion stability

FC: foaming capacity

FS: foam stability

MVA: Micro visco amylograph

SEM: Scanning Electron Microscopy

PCC: Pearson Correlation Coefficient

PCA: Principal Component Analysis

PC: Principal Component

CHAPTER 1

INTRODUCTION

Legumes, belonging to the Fabaceae family, are widely cultivated crops globally, consisting of approximately 16,000 species. They have played an essential role in human diets due to economic, environmental, nutritional, and health benefits while being overshadowed in history by other energy sources. Legumes possess physicochemical properties, such as water and oil retention capacity, making them suitable for food product development. The edible seeds of legumes are known as pulses and are rich in protein, complex carbohydrates, dietary fiber, and resistant starch. Due to delayed starch hydrolysis, the pulses offer a balanced amino acid profile and exhibit low-energy properties.

The presence of functional and bioactive compounds contributes to preventing chronic diseases. Due to increasing consumer demand, pulses and their flours have been used in conventional combinations, partially or fully replacing traditional ingredients. Legumes are also valuable in plant-based diets as a rich protein source. Their health benefits, nutritional properties, and gluten-free nature have driven their significance in food innovations and product development, including gluten-free foods. Beans dominate legume production in terms of food supply. India, Brazil, and Myanmar are leading bean producers worldwide. Turkey holds a significant share of common bean (*Phaseolus vulgaris* L.) production in Europe, although it is not among the top producers. Additionally, dry beans rank third in pulse species production in Turkey, after chickpeas and lentils.

The substitution of wheat flour with more nutritious alternatives in traditional bread recipes has gained attention due to the high glycemic index of wheat. Legume flours have been used as replacements, given their cost-effectiveness and accessibility. Various research has shown that using bean flours can lower the glycemic index of wheat bread and improve dough consistency, resulting in softer, higher-volume bread with enhanced sensory qualities. Similar investigations on legume breads highlighted the effects of

legumes on dough rheological properties and nutritional quality, volume, and specific volume of the breads, in addition to improved sensorial and functional properties.

Nuts, specifically tree nuts, are nutrient-rich foods with significant health benefits. They comprise monounsaturated and polyunsaturated fatty acids, proteins, soluble and insoluble fibers, vitamins, minerals, antioxidants, carotenoids, and phytosterols. Regular consumption of nuts has been associated with improved glycemic and lipid profiles along with increased satiety and thermogenesis. Studies suggest that nuts contribute to the prevention and management of undesirable health-related conditions such as hypertension, cardiovascular diseases, diabetes mellitus, and obesity while reducing oxidative stress, inflammation, visceral adiposity, and insulin resistance. Hazelnuts, the fifth most produced tree nut globally, are the most cultivated species in Turkey. With its abundant production and health-promoting effects, hazelnuts present an excellent opportunity for incorporation into various food products.

Hazelnuts are nutrient-rich, particularly in fats, proteins, and low in carbohydrates. They contain micronutrients like tocopherols, polyphenols, minerals, and B-complex vitamins. They are widely used in various food applications due to their flavor and texture enhancement. However, only a small portion of hazelnut production is available for direct consumption, with most being utilized in different fields of the food industry. A limited number of studies focus on hazelnut flour to enhance the nutritional value of food products. Some research has shown that incorporating hazelnut flour in bread can be an effective way to improve the nutritional properties and acceptability of the product. It has also been observed that hazelnut addition can improve dough and baking properties while also increasing dietary fiber content. The lack of studies on hazelnut flour in breads presents an opportunity for further research and food product development, particularly in Turkey, where hazelnuts are abundant. Hazelnut flour can be incorporated into various products, including bread, with or without yeast.

Bread is a staple food consumed worldwide, typically made from flour, water, salt, and yeast. Wheat flour is commonly used due to its gluten content and viscoelastic structure. However, the high glycemic index and gluten-related issues have recently started raising concerns. The production of yeast-free breads has been explored to improve efficiency and productivity in the bread industry. However, limited studies have focused on commercial-like yeast-free breads, but alternative methods have been used in unleavened bread production. These methods have shown promise in reducing bread density, hardness, and production time. Some studies have also used CO2 as a leavening

agent in wheat breads without yeast. Research opportunities exist for the production of gluten-free and yeast-free breads, which have received limited attention in the literature despite the growing interest in healthy and time-efficient food options.

Legumes, such as beans, can enhance the nutritional value of bread by increasing fiber, resistant starch, protein, mineral, vitamin contents, and bioactive compounds. Legume by-products, including husks, pods, and waste waters, have also been explored for their nutritional and environmental benefits. Various studies have investigated using legume fibers, hulls, and extracts to increase fiber and protein content in breads. Additionally, legume by-products like wastewater and okara flours have been used for their emulsifying properties and as thickeners. Flour-based approaches incorporating legumes in bread have been widely employed due to their overall benefits. Different studies have examined the effects of legume flour replacements on the technological and nutritional properties of bread, with some demonstrating improved quality and balanced properties. Furthermore, gluten-free bread faces challenges due to the lack of gluten protein, resulting in a grainy texture and decreased volume. Additives like hydrocolloids and modified starches are commonly used, but legume flours have shown promise in improving dough structure and carbon dioxide retention. Incorporating legumes in gluten-free bread formulations have the potential to address these challenges while providing nutritional enhancements.

The global food system's impact on greenhouse gas emissions, deforestation, and water usage presents a significant challenge in feeding the growing population while considering the planet's limitations. Protein consumption plays a significant role in water usage and greenhouse gas emissions. Humans require sufficient high-quality protein easily digestible and rich in essential amino acids to meet nutritional needs. Meat, poultry, fish, eggs, nuts, legumes, and dairy products are the primary sources of high-quality protein. However, meat consumption is increasing worldwide, leading to significant greenhouse gas emissions, mainly from ruminant livestock.

In contrast, legumes and nuts have the lowest greenhouse gas footprint and possess nitrogen-fixing capabilities, reducing the need for fertilizers and lowering emissions. The environmental impact can be reduced by replacing meat and dairy with legumes. Studies have shown that replacing livestock with legumes in diets can significantly decrease greenhouse gas emissions and land occupation. Incorporating legumes into bread production aligns with sustainable practices and vegan alternatives, contributing to reducing greenhouse gas emissions and environmental preservation.

As far as our knowledge goes, there is no study about the combined use of bean and hazelnut flours as alternative plant sources in vegan and gluten-free diets. No studies focused on gluten-free bread formulations using bean flour and hazelnut flour as the partial replacement for rice flour-starch base. Hence, this study aimed to investigate the effect of white bean and hazelnut flour addition to the rice flour corn starch gluten-free breads made of rice flour and corn starch with or without yeast. The replacement of the rice flour and corn starch base was studied using different levels at 15 and 30% according to a three-component extreme vertices mixture design. The primary (linear) and interaction (i.e., quadratic and special cubic) effects were simultaneously evaluated to identify the optimal gluten-free bread formulations. Bread samples were characterized by their textural and physical properties (fresh and 48-h-stored) to determine the best formulations. The gluten- and yeast-free samples were also analyzed by their spectroscopic profiles, sensorial properties, and starch fractions. Sensory, storage, and starch fraction analyses were carried out on the samples selected according to the mixture design results of the fresh gluten- and yeast-free breads.

The Results and Discussion part was divided into three parts. In Chapter 4, gluten-free yeast breads made at the Università degli Studi di Milano (within the scope of the Erasmus program) were discussed, whereas gluten-free and yeast-free breads (produced at Izmir Institute of Technology) were examined in Chapter 5. The sixth chapter was reserved for multivariate statistical analyses. In this chapter, it was investigated whether the FT-IR spectra of flours, gluten-free and yeast-free breads (i.e., dough, fresh bread, and stored samples) and the physical properties of yeast and yeast-free breads could be differentiated.

CHAPTER 2

LITERATURE REVIEW

2.1. Legumes

Legumes are defined as plants whose fruits are enclosed in pods and are among the prominent crops cultivated worldwide. They are from the Fabaceae (i.e., Leguminosae) family, with 16,000 species (Boukid et al. 2019). Since ancient times, legumes have taken part in the human diet as sources of starch (i.e., energy) and protein. Even though legumes have not been the "most important" energy sources due to the attention on potatoes, cereals, and animal-based foods, they are still essential in the diets of millions of people, mainly because of economic, environmental factors and the nutritional and health benefits (Erbersobler, Barth and Jahreis 2017; Martín-Cabrejas 2019).

The edible seeds of the legumes are called "pulses" (e.g., beans, chickpeas), and they are well known for their high protein and complex carbohydrate contents such as dietary fiber and resistant starch. Pulses are also important sources of vitamins and minerals such as potassium (K), iron (Fe), zinc (Zn), and calcium (Ca) (Sivakumar, Chaudhry and Paliwal 2022; Asif et al. 2013; Martín-Cabrejas 2019).

The protein content of pulses is between 20 and 40% on a dry basis, which is almost equal to the protein content of meats (18-25%), and they help maintain the essential amino acid balance (Bojňanská, Musilová and Vollmannová 2021; Erbersobler, Barth and Jahreis 2017; Chávez-Murillo et al. 2018). On the other hand, the starches contained in pulses have been associated with delayed starch hydrolysis (due to the presence of soluble and insoluble fiber), which results in a low digestion rate by amylolytic enzymes and higher thermal stability during pasting. Hence, pulses are considered "low-energy" products, providing low energy (318 ± 14 kcal on average, 301 – 359 kcal per 100 g range, on a dry basis). Overall, pulses are naturally rich in bioactive components and valuable enough to be considered to integrate into foodstuff-making (Boukid et al. 2019).

Due to their dietary fiber, legumes have physicochemical properties such as water and oil retention capacity and solubility that could positively affect their potential use in food products (Erkan et al. 2020; Keskin et al. 2022). Furthermore, the functional and bioactive compounds abundant in legumes may help prevent or reduce the risk of some chronic diseases such as cardiovascular diseases (CVD), diabetes, and some types of cancer (especially colorectal cancer) (Martín-Cabrejas 2019). Additionally, the seed matrix composition and molecular arrangement of pulses can affect the protein availabilities of the cooked flours, which might be influenced by the decrease in the predicted glycemic index (Chávez-Murillo et al. 2018). As the nutritional and health benefits of using pulses were determined in studies, there has been an increase in consumer demand for pulses, which led the researchers and producers to use pulses and their flours in conventional combinations by partially or fully replacing the traditional ingredients such as wheat flour (Sivakumar et al. 2022). Moreover, plant-based foods rich in proteins can supply vegan and vegetarian diets. Hence, pulse flours and products based on protein isolates of legumes started to participate in food technology (Erbersobler, Barth and Jahreis 2017). Because of their health benefits and nutritional properties (e.g., protein and fiber content, antioxidant properties), legumes have been gaining importance in food product developments and innovations. Besides, they are gluten-free; hence, they also take an extensive part as supplements in developing gluten-free products (Carbas et al. 2021).

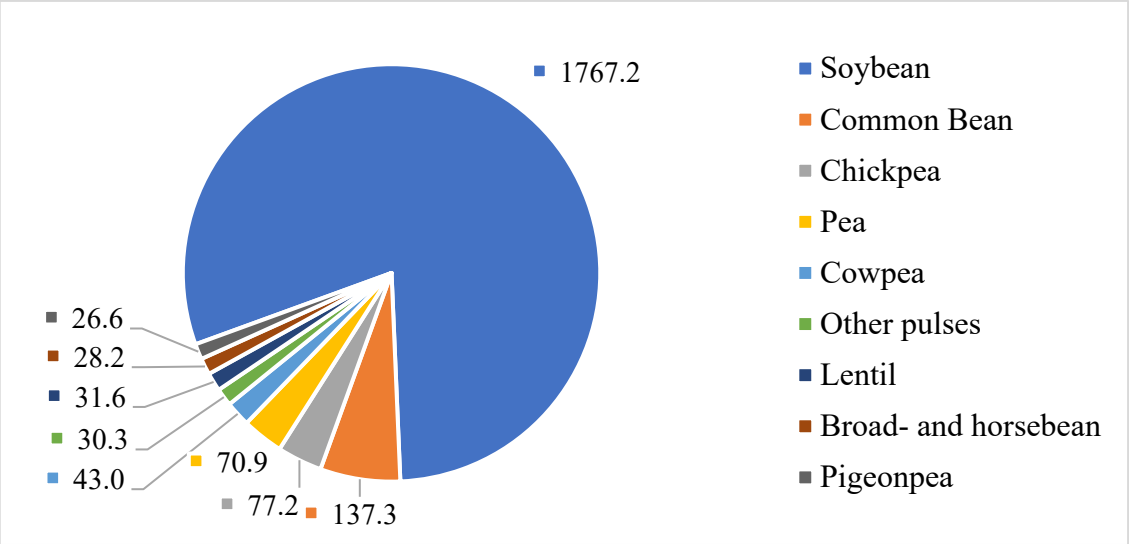

Figure 2.1. Worldwide legume production between 2017 and 2021, data are shown in million tons (data are from FAOSTAT 2023)

Figure 2.1 represents the production of specific legume groups, including soybean, bean, chickpea, pea, cowpea, lentil, broad bean, horsebean, pigeon bean, and other pulses. Even though soybean was significantly the most produced legume crop (1,767.2 million tons), only 3.2% of its total production is used in the food supply. Hence, beans are the most common crop among legumes, with 77.2 million tons between 2017 and 2021, corresponding to 70.9% of the total bean production worldwide in terms of food supply. Asia countries lead bean production by 43.7%, followed by the Americas (North and South, 27.4%), Africa (26.5%), Europe (1.9%), and Oceania (0.5%). India (29.5 million tons), Brazil (14.8 million tons), and Myanmar (13.4 million tons) are the top three producers, and they cultivated a total of 57.7 million tons (62.1% of the total bean production by the top ten producers) of beans between 2017 and 2021 (FAOSTAT 2023).

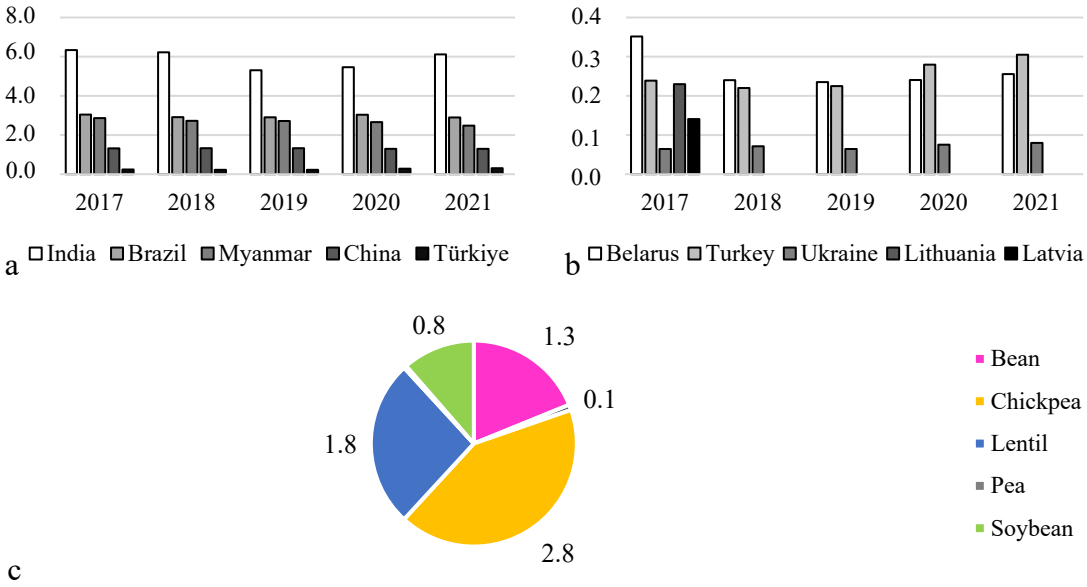

Figure 2.2. Bean production (a) worldwide and (b) in Europe, and (c) total legume production in Turkey between 2017 and 2021 (Data are from FAOSTAT 2023)

According to the current data covering 129 countries, Türkiye is among the first 25 countries (22nd largest) in total bean production in the last five years (available information from 2017 to 2021, worldwide total: 137.3 million tons; Türkiye: 1.3 million

tons). Türkiye is not among the top producers; however, common bean production in Türkiye corresponds to 33.1% of the total of Europe, where the closest productions belong to Belarus, Ukraine, and Lithuania (34.5, 9.3 and 6.0%, respectively). In addition, the dry bean is the third most produced pulse species after chickpea (2.8 million tons) and lentil (1.8 million tons) in Türkiye (Figure 2.2, data are from FAOSTAT 2023).

2.1.1. Use of Bean Flour in Breads

Replacement of wheat flour in traditional breads with more nutritious ingredients has been gaining attention since wheat has relatively high glycemic index values caused by the amount of rapidly digestible starches (Bo et al. 2017; Gao et al. 2018; Bojňanská, Musilová and Vollmannová 2021). In this context, legume flours were used since they are relatively cheaper and more easily accessible than processed ingredients. In a study by Udani et al. (2009), it was shown that a water extract product of white bean, in capsule and powder forms, could lower the glycemic index significantly, especially by adding the 3000 mg extract in powder form to the butter (5 g, total), which was served with or without the white bean extract.

Aguiar et al. (2022) investigated the relationship between instrumental and sensorial techniques to compare gluten-free breads prepared using rice flour (0, 50, 100%) and bean flour (0, 50, 100%) with different water levels (150, 175, 200%). They concluded that the rice and bean flours blend improved the dough consistency, which could be attributed to the increase in bread softness and volume, degree of liking of texture, flavor, appearance, and overall. Rizzelo et al. (2014) characterized the main nutritional, sensorial, and functional properties of wheat-legume breads, including wheat, chickpea, lentil, and bean flours, at 85:5:5:5 ratio. They determined that the use of wheat-legume sourdough bread maximized the investigated properties of wheat-legume breads. In another study (Xhabiri and Hoxha 2022), the effect of increasing white bean flour amount (0-25%) on the rheological, qualitative, and nutritional properties of wheat bread were investigated. Significant effects on the rheological properties (e.g., extensibility, resistance, and dough energy) were determined. Additionally, the breads had increased nutritional quality, yet volume and specific volume decreased as the bean flour percentage

increased. Olaoye et al. (2016) investigated the effects of replacing wheat flour with white bean flour at 5, 10, 15, and 20% on breads. They reported findings similar to those of Xhabiri and Hoxha, and also concluded that the replacement of wheat flour with bean flour up to 10% gave comparable results in terms of sensory attributes, whereas the breads prepared with a replacement level up to 20% were richer than whole wheat in terms of ash, protein, and fat.

2.2. Nuts

The term “nuts” mainly refers to the dry foods grown in trees with one seed and hardened ovary wall (i.e., tree nuts). They contain high levels of nutrients and have significant effects on numerous health conditions (Alasalvar and Shahidi 2008; Zec and Glibetic 2018). Due to these influences on humans, nuts are considered among the essential parts of nutritionally beneficial diets (Vadivel, CKunyanga and Biesalski 2012). Some of the most important compositional elements of nuts are monounsaturated and polyunsaturated fatty acids (MUFA and PUFA, respectively). In particular, a significant amount (up to 35-40%) of the energy intake in the Mediterranean diet is provided by nuts. Besides, the presence of protein, soluble and insoluble fibers, vitamins E and K, vitamin B (especially folate and thiamine), minerals (magnesium, Mg; copper, Cu; potassium, K; selenium, Se), and other substances such as antioxidants, carotenoids, and phytosterol compounds makes the nuts vital foods in human diet (De Souza et al. 2017; Vadivel, CKunyanga and Biesalski 2012). A vital relationship exists between health and a diet rich in nutrients, improving both glycemic and lipid profiles in human bodies (Udayarajan, Mohan and Nisha 2022; Amoah et al. 2022). In particular, nut ingestion can help control satiety and increase thermogenesis. Several studies have claimed that regular consumption of nuts results in a beneficial effect on human health conditions, including hypertension, cardiovascular diseases, diabetes mellitus, and obesity. Additionally, nut consumption may reduce the mediators of chronic diseases, such as oxidative stress, inflammation, visceral adiposity, and insulin resistance (De Souza et al. 2017; Zec and Glibetic 2018).

Hazelnut contains valuable nutrients with exceptionally high fat (approximately 62%), protein (approximately 16%) contents, and low carbohydrates (approximately 11%, most of the carbohydrates consist of fibers), among others. Moreover, the presence of micronutrients such as tocopherols, polyphenols, minerals, and B-complex vitamins contribute to the health benefits of hazelnuts (Guiné and Correia 2020). Hazelnuts also add flavor and texture to formulations in various industries, such as bakery, confectionery, cereal, dairy, salad, entrée, sauce, and desserts, due to their importance in human diets (Alasalvar et al. 2003; Turan, Çapanoğlu and Altay 2015). Most of the cultivated hazelnuts are transferred to industry. In general, only 10% of the total production is left available for direct consumption or other applications rather than chocolate (70%) or ice cream and pastries (20%) (Guiné and Correia 2020).

Production of tree nuts in the world has been increasing since 2010, and as shown in Figure 2.3, hazelnut was the fifth most produced tree nut crop between 2017 and 2021 with 5.1 million tons, following almond (18.0 million tons), cashew nut (18.0 million tons), walnut (16.1 million tons) and chestnut (11.0 million tons). According to the same data, Türkiye is known as the largest producer of hazelnuts worldwide (3.3 million tons), followed by Italy (0.6 million tons) and the USA (0.2 million tons) (Hernández-López et al. 2022; FAOSTAT 2023).

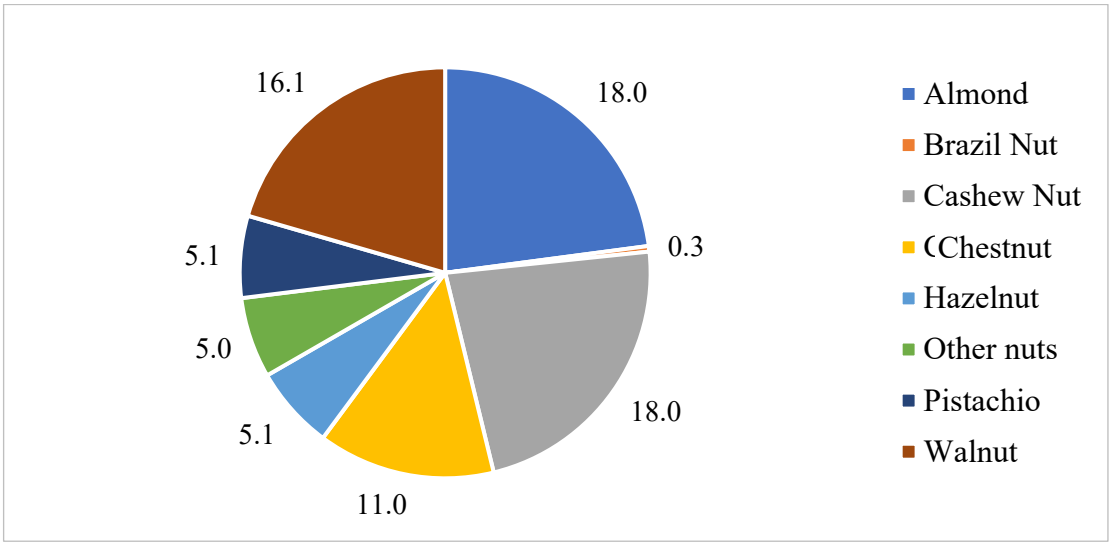

Figure 2.3. Worldwide tree nut production between 2017 and 2021, data are shown in million tons (data are from FAOSTAT 2023)

Hazelnuts are also the most produced species of tree nuts in Türkiye (Figure 2.4a), more than walnuts (1.3 million tons), pistachios (0.8 million tons), almonds (0.7 million tons), and chestnuts (0.4 million tons) between 2017 and 2021 (Hernández-López et al. 2022; FAOSTAT 2023).

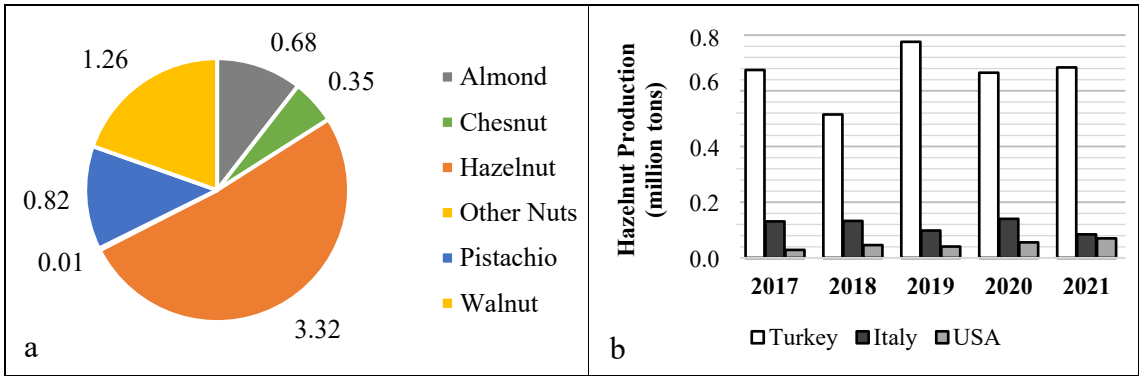

Figure 2.4. (a) Production of tree nuts in Türkiye) and (b) hazelnut production in the top three producers, 2017-2021 (data are from FAOSTAT 2023)

In fact, use of hazelnut as a raw material in common food products can provide an inevitable opportunity in terms of food development and enhancement, when its health-promoting benefits and production share of Türkiye are considered.

2.2.1. Use of Hazelnut Flour in Breads

Even though hazelnuts are among the most beneficial ingredients, the number of reports focusing on hazelnuts in flour form to enhance the nutritional values of food products is limited. In a research, Devi et al. (2016) found that bread enriched with hazelnuts (30 g per 120 g of bread) in different forms (sliced, semi-defatted, and their combination) was an acceptable and effective product. In another study, the effect of defatted hazelnut flour addition was examined by replacing rice flour with defatted hazelnut flour (DHF, containing 47.22% protein) at 5%, 10%, and 15% levels along with gum (locust bean, guar, or xanthan) addition (Tunç and Kahyaoğlu 2016). It was

concluded that hazelnut additions improved the gluten-free breads' dough properties, along with the nutritional and baking properties. With the purpose of increasing the dietary fiber content of the breads with wheat flour, Anil (2007) used hazelnut testa (skin) in fine and coarse forms, at 5% and 10% levels, and as hydrated or non-hydrated. He demonstrated that the ratio, particle size, and hydration process of the hazelnut testa had significant effects on the quality parameters of the doughs and breads. In a more recent study, the authors investigated the effects of hazelnut and walnut flour addition on the rheological properties of flour and wheat dough. They reported that nuts in fact weakened the dough structure while also deteriorating the rheological properties and decreasing the water absorption of the flours which negatively affects dough-proofing properties. However, they also noted the possible positive effects of hazelnut and walnut such as improved sensory, and health benefits (Pycia and Lesław 2022).

From a different point of view, Capuano et al. (2020) studied the effects of the incorporation of hazelnuts into bread matrices; and they found that hazelnut incorporation had a relatively small but significant effect on the hazelnut bolus particle size distribution.

In fact, studies on hazelnut flour being limited is actually an excellent opportunity for food development research, especially in Türkiye since it holds a significant share worldwide in hazelnut production. For example, it can be incorporated into many kinds of products, including bread, with or without yeast.

2.3. Bread

Bread is a special food that has been widely consumed in the world for thousands of years (Mollakhalili-meybodi et al. 2023; Graça, Raymundo and de Sousa 2021). The size, shape, and texture of the bread may change throughout the world; however, its ingredients are basically the same: flour, water, salt, and yeast. The flour used in bread is generally wheat because it has a viscoelastic structure that creates a network that allows the gases produced during breadmaking (especially fermentation) to be recovered due to its gluten content (Mollakhalili-meybodi et al. 2023; Borczak et al. 2018). In addition to its structural preferability and familiar taste, it is an important energy source for humans because it has a high amount of rapidly digestible starch, which is responsible for a high

glycemic index (Kurek et al. 2018; Graça, Raymundo and de Sousa 2021; Mollakhalili-meybodi et al. 2023). Unfortunately, a high glycemic index has been associated with substantial weight gain, which can lead to health problems (Borczak et al. 2018). Additionally, the presence of gluten in foods such as wheat bread may not benefit some people with gluten-related problems such as celiac disease, gluten sensitivity, and wheat or gluten allergy (Foschia et al. 2016; 2017; Kahraman et al. 2018).

Producing yeast-free breads was another point of this project since eliminating or shortening the proofing durations was previously suggested to increase the efficiency and productivity in the bread sector since they would decrease time and storage consumption (Srivastava et al. 2022; Ruttarattanamongkol, Wagner and Rizvi 2011). It was also claimed that replacing yeast with chemical bakery alternatives such as sodium bicarbonate would aid the production of CO_2, which is a well-known product of yeast fermentation that allows the dough to leaven (Srivastava et al. 2022). Unfortunately, studies on the production of commercial-like breads that were allowed to leaven using bakery alternatives such as baking powder or soda (i.e., non-yeasted or yeast-free bread) have been very limited in the last 13 years. In fact, there were not ~~any reliable~~ studies that used the methodology presented in this study for gluten-free and yeast-free bread. Still, during the literature review, some studies were found that tried the supercritical fluid extrusion (SCFX) method proposed by Hiçşaşmaz (2003) or similar approaches (Tursunbayeva et al. 2019) in unleavened breads.

Furthermore, Ruttarattanamongkol, Wagner and Rizvi (2011) and Kasih (2009) used this method in their studies to eliminate carbon dioxide deficiency during the production of unleavened bread. Ruttarattanamongkol, Wagner and Rizvi (2011) suggested and proved that combining vacuum and conventional baking under SCFX conditions reduces bread density and crumb hardness while reducing production time. Tursunbayeva et al. (2019), on the other hand, used a method called the “Accelerated Test,” in which they concluded that the use of this test significantly reduced the time consumed by the breadmaking process (i.e., reduction to 33-40 minutes, approximately three times less than a traditional method). More recently, (Srivastava et al. 2022) used CO_2 to leaven the wheat breads without using any yeast. They concluded that using a gas hydrate (CO_2) at 20% and 40% levels resulted in comparable volume and pore size to those of the standard formulation prepared with yeast. Additionally, studies that worked on gluten-free and yeast-free bread production can be found in the literature; however,

those mainly studied the doughs (Leray et al. 2010), or used animal-based ingredients (Shanina et al. 2019), which negatively affects the sustainability of the product.

In summary, the production of gluten-free and yeast-free bread is an area full of research opportunities. However, it has not received the attention it deserves even though the perspective on food consumption has become healthy and yeast-free breadmaking does not waste time as much as yeast bread.

2.3.1. Breads with Alternative Formulations Using Legumes

Replacement of traditional cereals in breads with legumes (e.g., bean) promises an increase in their nutritional values through a rise in their fiber, resistant starch, protein, mineral, vitamin contents, and bioactive compounds (Boukid et al. 2019; Bojňanská, Musilová and Vollmannová 2021).

One of the most common forms to increase the nutritional quality and sensorial acceptance is protein in concentrate or isolate forms, and legumes are considered to be appropriate for this purpose due to their low cost and high protein contents (Ladjal-Ettoumi et al. 2016). There are also some studies focused on using the legume parts that have recently started to gain attention, such as their by-products (e.g., husks, pods, wastewaters, seed coats, hulls, and pods) since processing them provides nutritional, economic, and environmental benefits (Nartea et al. 2023; Kumar et al. 2017). There are studies worked on fibers (Niño-Medina et al. 2019) and hulls (Kasprzak and Rzedzicki 2010; Ni et al. 2020) of by-products to increase the fiber content of breads. The effects of hulls (Kasprzak and Rzedzicki 2010; Ni et al. 2020) and pods (Fendri et al. 2016) were also noted for protein content. Niño-Medina et al. (2019) investigated the effect of chickpea fibers on the total phenolics contents of breads, whereas Chávez-Santoscoy et al. (2016) used black bean extracts with the same purpose with a specific focus on flavonoids and saponins. Huang et al. (2017) used the wastewater (e.g., soaking water) of legumes in gluten-free breads to evaluate their emulsifying properties. In another study, okara flours from soy and chickpea were used as thickeners, and it was reported that their use could increase the moisture content and dough viscosity of gluten-free breads (Lian et al. 2020). Another example of using okara from soybean was presented more recently

(Pešić et al. 2023), and the authors reported that the gluten-free bread formulated using buckwheat, rice, and millet enriched with 30% okara belonged to the “products with increased protein content” group.

Even though there are valuable studies that used legumes in various forms, such as protein concentrates and protein isolates, the flour-based approach is the most common method to include legumes in bakery products such as bread since it basically benefits from legumes as a whole. As such, Moreno-Araiza et al. (2023) investigated the influence of pre-treated green pea flour on the technological and nutritional properties of bread, between 10 and 50% replacement levels and reported that 10% improved nutritional quality as specific volume and texture properties were similar to the control, and 30% replacement resulted with balanced technological and nutritional properties. Similarly, Mastromatteo et al. (2015) worked on whole-meal bread, replacing 5% of semolina with old and modern pea flour. They found that this replacement level, along with 2% guar gum, resulted in better sensorial and textural properties, as well as having improved ash and total soluble fiber contents, while also reducing glycemic index. On the other hand, Agbara et al. (2022) studied several wheat composite flours (with root tuber and grain legumes) at a 30% replacement level and concluded that even though nutritional values of wheat-legume blends were higher in terms of protein, they were more unsuccessful in terms of bread volume than wheat-root tuber blends.

Moreover, Kahraman et al. (2022) used raw, dehulled, and roasted chickpea flours to observe their effects on the nutritional and technological properties of rice-based gluten-free bread. They reported that the replacement of rice flour at a 25% level showed generally improved nutritional and technological properties. Likewise, Aguilar et al. (2015) tried to benefit from the protein content of chickpeas and the lipid content of tiger nuts (and interactions of these compounds) by replacing the emulsifier and shortening agents totally or partially in gluten-free bread. In the end, they found that the chickpea and tiger nut mixture reduced hardness and concluded that those flours could be used as replacers for shortening and emulsifier agents without affecting crumb hardness.

2.3.2. GF Bread Disadvantages

The challenges of gluten-free baking can be related to several reasons, such as the lack of gluten protein in their flours, which provides the structure of traditional bread dough, and different nutritional profiles that may affect the final composition of the final product (Naqash et al. 2017). In gluten-free doughs, the retained air (during mixing) and the escape of the carbon dioxide produced by yeast (during fermentation) occurrence is relatively easier since there is not a gluten network to entrap them. This “escape” causes the gluten-free dough to become less elastic and cohesive, with a more liquid-like structure, resulting in the bread with a grainy texture and decreased volume. Additionally, gluten-free formulations generally require more water in order to provide the appropriate moisture in the crumbs (Melini et al. 2017; Stoin et al. 2021)., Some additives such as hydrocolloids (such as HPMC and gums), modified starches, and other stabilizers are used to avoid this problem (Zannini et al. 2012; Mastromatteo et al. 2015).

However, in some cases, the use of legume or nut flours can avoid this adverse effect in gluten-free breads, reducing or eliminating the need for such additives. For instance, raw, dehulled, and roasted chickpea flours were used in rice-flour-based blends to investigate their effects on the gluten-free dough (Kahraman et al. 2018). It was reported that the use of chickpea flour increased carbon dioxide retention, which was a problem in gluten-free doughs due to the absence of gluten. Additionally, as explained in the previous section, the use of composite flours for gluten-free breads, rather than alone, could provide more desired dough and bread structures along with nutritional improvements (Aguilar et al. 2015).

2.4. Sustainability of the vegan products

Food systems significantly contribute to global greenhouse gas (GHG) emissions. In particular, activities such as "deforestation and water use" to feed the world's population also contribute to significant environment-related problems as they take part in the depletion of the planet's natural resources more rapidly. Actually, protein (especially the sources) in the human diet now has been reported as the biggest effect on water use

and GHG emissions. To maintain growth and maximum health, humans need to consume enough high-quality dietary protein that is both highly digestible and rich in essential amino acids. Meat, poultry, fish, eggs, nuts, legumes, and dairy products including milk, yogurt, and cheese are the main sources of high-quality protein. Due to expanding populations and rising affluence, meat consumption is rising globally. In fact, ruminant livestock has the greatest GHG footprint among the major protein sources, followed by non-ruminant livestock and dairy. On the other hand, Legumes and nuts have the lowest GHG footprint (Semba et al. 2021; Tidåker et al. 2021).

In addition, legumes has the ability to fix nitrogen, which, in turn, reduces the demand for mineral fertilizers and consequently reduces the GHG emission. Besides, legumes can decrease the load on the lands and resources while also reducing the high GHG emissions caused by the livestock, when consumed instead of meat and dairy (Cusworth, Garnett and Lorimer 2021).

For instance, a study was carried out based on the assumption that consumption in Sweden decreased by half, and the lands used for the livestock were replaced with beans, lentils, and peas. The authors calculated the GHG emissions from a diet and land occupation to be reduced by 20% and 23%, respectively (Röös et al. 2018).

In summary, using legumes and hazelnuts in bread making would significantly contribute to the sustainability approach that can save the future by protecting the environment. At the same time, not using any products of animal origin (i.e., producing a vegan product) promises to be one of the crucial factors affecting the decrease in GHG.

CHAPTER 3

MATERIALS AND METHODS

This section presents formulations and analyses of gluten-free bread with compressed yeast and gluten-free and yeast-free bread, along with the characterization of flours. Gluten-free bread with compressed yeast was formulated and analyzed at the Department of Food, Environmental and Nutritional Sciences at the University of Milan during a study within the Erasmus framework.

3.1. Materials

For the gluten-free bread with compressed yeast breadmaking process, pre-cooked white bean flour (B; Naturelka, Aydin, Türkiye), rice flour (R; Il Molino Chiavazza, Casalgrasso, Italy, and I-R; Ingro, Karaman, Turkey), Hazelnut flour (H; Ingro, Karaman, Türkiye) and corn starch (C; Maizena, Roma, Italy, and I-C; Ingro, Karaman, Türkiye) were supplied (Figure 3.1). Other ingredients in addition to the rice flour and corn starch were purchased from the local markets: hydroxypropyl methylcellulose (HPMC, Benecel F4M, Ashland, Wilmington, DE, USA), compressed yeast (GS S.p.A., Milano, Italy), sugar (GS S.p.A., Milano, Italy), salt (GS S.p.A., Milano, Italy), and extra virgin olive oil (Farchioni Olii S.p.A., Gualdo Cattaneo, PG, Italy).

For the gluten-free and yeast-free breadmaking process, pre-cooked white bean flour (B; Naturelka, Aydin, Türkiye), raw hazelnut flour with skin (testa), rice flour, and corn starch (H, I-R and I-C; Ingro, Karaman, Türkiye) were used. Other ingredients were purchased from the local markets: baking powder, baking soda (Dr. Oetker, Izmir, Türkiye), olive oil, apple vinegar (Taris, Izmir, Türkiye), white sugar (Irmak, İşmen Gıda, Istanbul, Türkiye), salt (Billur, Izmir, Türkiye) and xanthan gum (Alfasol, Kimbiotek A.Ş., Istanbul, Türkiye).

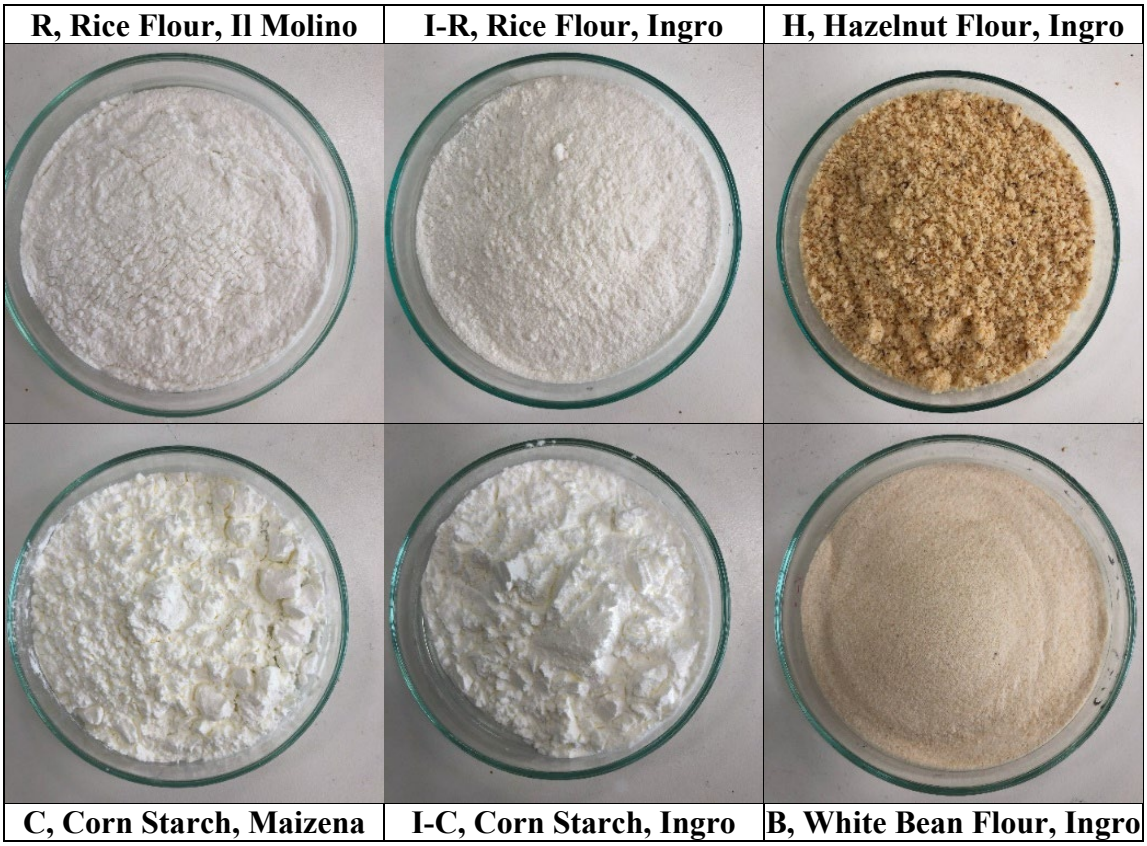

Figure 3.1. Flours used in the gluten-free bread formulations (R and C were used in yeast breads whereas I-R and I-C were used in yeast-free breads)

3.2. Methods

In this section, analyses performed on flours, gluten-free yeast and yeast-free bread samples are described.

3.2.1. Flour Properties

In this section, analyses carried out on the flour samples that were used in the gluten-free bread formulations are explained in detail.

3.2.1.1. Moisture Content

Moisture contents of the samples were determined according to the AACC Method 44-15A, One Stage Air Oven Method (2000), with slight modifications. First, the glass petri dishes were dried at 105 °C in the laboratory oven (Binder, ED53, Tuttlingen, Germany) for 2 hours and cooled to room temperature in a desiccator until the weights of the dishes were constant. Each petri dish was given a number using a permanent marker, and their tare weights were recorded using the laboratory scale (Precisa, XB220A, Switzerland). 2.5000 ± 0.01 g of samples were weighed in the dishes. Flour-added petri dishes were transferred to the laboratory oven. The same procedure was followed for the drying process of the petri dishes.

At the end, the dishes were weighed using the laboratory scale, petri dish tare was subtracted from the final weight to obtain the dried flour amount. The analysis was repeated three times for each sample. Moisture contents (M, %) of R, C, B and H were determined using the following formula:

$$\mathrm{M} = \frac{\text{weight of flour} - \text{weight of dried flour}}{\text{weight of flour}} \times 100$$

(Equation 1)

3.2.1.2. Crude Protein Content

Crude protein contents of samples were determined based on the AACC Method 46-10.1 (Improved Kjeldahl Method, 2000), with minor modifications, in three steps: mineralization, distillation, and titration.

0.40 ± 0.01 g, 1.00 ± 0.01 g, or 0.70 ± 0.01 g of sample was weighed in the Kjeldahl flasks; if the expected protein content was more than 15 g/100 g flour (B and H), less than 5 g/100 g flour or between 5 and 15 g/100 g flour, respectively. The flasks were placed in the mineralization unit after adding the catalyzer (Kjeldahl tablet) and sulfuric acid (H_2SO_4, 98% purity, 15 mL). During this part, H_2SO_4 reacted with the sample, which converted its nitrogen to ammonium sulfate ($(NH_4)_2SO_4$). Heating in this unit took approximately 1.5 hours, and samples were allowed to cool down for 30

minutes. For the distillation part of the analysis, a solution containing 10 mL boric acid (H_3BO_3, 3%), 40 mL distilled water, and six drops of indicator (Rusty indicator with 4.5 pH: 0.25 g red methylene, 0.075 g blue methylene, 150 mL ethyl alcohol, 100 mL H_2O) was prepared for each sample. The Kjeldahl and Erlenmeyer flasks were transferred to the distillation unit, and distillation started with pretreatment and a re-wash of 2 minutes. Then, NaOH addition started manually by pressing the "NaOH" button: 20 mL at the beginning and approximately 2 mL at each press until the solution became brown, indicating no reaction was occurring. While NaOH was added, $(NH_4)_2SO_4$ was neutralized since it was converted to ammonia (NH_3). Then, NH_3 was distilled into an Erlenmeyer flask containing H_3BO_3, forming ammonium borate ($(NH_4)_3BO_3$). Then, "aspiration" was applied to ensure that as little sample was left on the Kjeldahl flask as possible.

The flasks were placed on the magnetic stirrer under the burette containing 0.2 M HCl in the titration part. $(NH_4)_3BO_3$ was titrated with the HCl solution to determine the approximate total nitrogen content of the sample (Goulding, Fox and O'Mahony 2020; Jiang et al. 2014). Then, the amount of HCl used was recorded and the following formulas were used to determine nitrogen (N, g/100 g flour) and protein (P, g/100 g flour) contents of R, C, B and H:

$$N = \frac{\text{HCl (mL)} \times \text{Mo} \times 1.4 \left(\frac{\text{g}}{\text{mol}}\right)}{\text{sample (g)}}$$

(Equation 2)

$$P = N \times \text{Conversion Factor}$$

(Equation 3)

Where "HCl" stands for the amount of HCl added (mL), "Mo" was the molarity of the HCl solution (0.2 mol/mL), and "1.4" g/mol was the molecular weight of nitrogen. The conversion factor was 5.95 for rice flours and 6.25 for B, H, C and I-C.

Protein analysis using the Kjeldahl method was performed at the Department of Food, Environmental and Nutritional Sciences (DeFENS) at Università degli Studi di Milano, Milan, Italy.

3.2.1.3. Crude Fat Content

Crude fat contents of samples were determined according to the AOAC method 960.39 (Soxhlet Method, 1990). First, the fat extraction system was set up: 300 mL hexane was poured into a volumetric flask on a magnetic stirrer hot plate and the extraction chamber was connected to it. 4.000±0.01 g of flour was weighed in a porous thimble. Then, it was put into the extraction chamber, and the heating process was started. During the analysis, a continuous flow of water was provided, and when the water level reached the filling level, hexane with extracted fat was siphoned back into the flask. One siphoning operation represented one cycle. After several cycles, which depended on the expected fat content of the samples, hexane was evaporated at 50 °C for 12 hours, and the fat contents (F, g/100 g flour) of the samples were calculated using the amount of fat remained in the flask, with the following equation:

$$F = \frac{\text{The weight of the contents remained in the flask (g)}}{\text{Initial amount of sample (g)}} \times 100$$

(Equation 4)

A separate crude fat analysis was conducted for hazelnut flour to analyze the crude fiber content of hazelnut flour accurately. The fat content value obtained from this experiment was used to calculate the correct crude fiber content of hazelnut flour.

3.2.1.4. Total Ash Content

The total ash contents of the samples were determined following the official AOAC Method 923.03 (1990) with minor modifications due to the working principle of the muffle furnace (Protherm Furnaces, Alser Teknik Seramik A.Ş., Ankara, Türkiye). The crucibles were dried in the laboratory oven following the procedure explained in the Moisture Content method at 130 °C. The dried crucibles were then weighed, and the tares were recorded. 4.00±0.01 g R, C, B, and H were weighed in these crucibles. The samples were carefully placed in the muffle furnace.

Due to the working principle of the muffle furnace, the samples were first heated and kept at 105 °C for 12 minutes. The temperature was then increased from 105 °C to 250 °C in increments of 10 °C per minute and held at 250 °C for 30 minutes. The temperature was then raised to 575 °C in 20 °C increments per minute and kept at this temperature for 3 hours. The samples were cooled from 575 °C to 105 °C in the final step.

After incineration, samples were removed from the furnace and placed in a desiccator to cool down to room temperature. The final weights of the samples with crucibles were measured, and the Total Ash Content (TA, g/100 g sample) of each flour and corn starch was determined using the following formula on a wet basis:

$$TA = \frac{\text{Weight of the crucible with the ash-Weight of the dry crucible (g)}}{\text{Initial amount of sample (g)}} \times 100$$

(Equation 5)

3.2.1.5. Crude Fiber Content

Crude fiber contents of the samples were determined in two steps: boiling and incineration, following the official AOAC Methods 14.020 and 7.065 (1990) with slight modifications due to the working principle of the equipment used.

Before starting the analysis, 250 mL of 1.25% NaOH (base, w/V) and 250 mL of 1.25% H_2SO_4 (acid, w/V) solutions were prepared for each sample. 2.00 ± 0.01 g of samples were weighed and transferred to a 1000 mL beaker. 200 mL H_2SO_4 (1.25%) solution was poured into the beaker and the mixture was boiled precisely for 30 minutes while it was being rotated periodically to prevent the solid particles from adhering to the sides. After boiling, the beaker was removed from the heating pan and its contents were filtered through a cheesecloth. The beaker was rinsed with 50 mL of distilled H_2O and filtered thrice. The contents on the cheesecloth were transferred to the beaker by washing with NaOH (1.25%) solution, and the liquid was continued to be added until the total solution amount reached 200 mL. The new mixture was also boiled and filtered as described previously, but in the second filtering, the beaker was washed with 25 mL H_2SO_4 solution, 25 mL ethyl alcohol, and 50 mL distilled H_2O (three times). After that, all the contents accumulated on the cheesecloth were transferred to a crucible.

The crucibles containing the samples were dried in the laboratory oven at 130 °C for 2 hours and kept in a desiccator to cool down to room temperature (approximately 25 °C) for at least 30 minutes. The weights of the crucibles with samples were measured the following day and incineration was carried out as reported in the "Total Ash Content" method. The crude Fiber Content (Fi, g/100 g sample) of the ground sample was determined using the following formula:

$$\text{Weight of Ash (Final)} = \text{Weight of the crucible with Ash} - \text{Weight of the dry crucible}$$

(Equation 6)

$$\text{Fi} = \frac{\text{Weight of Dried Contents (g)} - \text{WoA (Final) (g)}}{\text{ground sample (g)}} \times 100$$

(Equation 7)

Hazelnut flour had to be defatted to perform crude fiber analysis since its high-fat content caused some problems in the crude fiber methodology.

3.2.1.6. Total Phenolics Content (TPC)

Solutions required to perform extraction and TPC analyses were prepared as follows:

- HCl-methanol (1%): To obtain a 1% HCl-methanol solution from a 37% HCl solution, it was diluted to 1% by mixing 2.70 mL HCl with 97.3 mL methanol for a 100 mL HCl-methanol solution.
- Na_2CO_3-H_2O (8%): To make a 10 mL Na_2CO_3-H_2O (8%) solution, 0.8 Na_2CO_3 was mixed with 9.2 mL distilled H_2O.
- Diluted Folin Ciocalteu Reagent (10x): For 100 mL of 10-fold diluted Folin Ciocalteu Reagent, 10 g of the reagent was mixed with 90 g distilled H_2O.

The samples were prepared for the TPC analysis by extracting the phenolics of the samples in accordance with Byanju, Evangelista and Lamsal (2021). 0.5000±0.005 g of flour was put in a 14-mL centrifuge tube and mixed with 7.5 mL of 1% HCl-methanol

solution. The mixture was kept in a dark environment for 2 hours to allow the reaction. After the time was up, the mixtures were transferred to the bench-top centrifuge (2-16 KC, Sigma, Osterode am Harz, Germany), which operated for 10 minutes at 2000 × g and 25 °C. Supernatants were collected in new 14-mL centrifuge tubes.

TPC determination was started immediately after the supernatants were transferred, as explained by do Socorro et al. (2010), with minor modifications. The supernatants were diluted 10-fold into micro-centrifuge tubes by mixing 0.1 mL sample and 0.9 mL distilled H_2O. Then, a 50 µL sample and 250 µ L 10-fold diluted Folin-Ciocalteu reagent were added to a different micro-centrifuge tube. The tubes were kept in the dark for 5 minutes to allow the reaction to occur. Then, 200 µl Na_2CO_3 (8%) was added, and the tubes were kept in the dark environment for 60 minutes. The samples were transferred to a multicell plate (96-cell, flat, 0.2 mL per cell), and their absorbances were measured at 760 nm. TPC of distilled H_2O was also determined as a reference (blank).

The following formula was used to obtain the TPC of the samples in terms of Gallic Acid Equivalent (mg GAE/L and mg GAE/g flour), where 10 was the dilution factor and 170.12 (g/mol) was the molecular weight of gallic acid:

$$\text{TPC}\left(\frac{\text{mg GAE}}{\text{L}}\right)=\frac{\text{Sample Absorbance}-\text{Blank Absorbance}}{0.0011}\times 10\times\frac{170.12}{1000}$$

(Equation 8)

$$\text{TPC}\left(\frac{\text{mg GAE}}{\text{g flour}}\right)=\text{TPC}\left(\frac{\text{mg GAE}}{\text{L}}\right)\times\frac{7.5}{1000}\times\frac{1}{0.5}$$

(Equation 9)

3.2.1.7. Total Carbohydrates (by Difference)

Total carbohydrates (TC, g/100 g sample) of the flours were expressed in grams per 100 g flour and calculated by the difference method as shown in the formula below (Maclean et al. 2003):

$$\text{TC} = 100 - (\text{M} + \text{P} + \text{F} + \text{TA})$$

(Equation 10)

3.2.1.8. Water Retention Capacity (WRC)

WRC (%) is defined as the amount of water held by the flour after centrifugation, and it is expressed as the percent of flour weight on a 14% moisture basis[1] (Niu et al. 2017). WRC was determined according to the AACC Method 56-11 (2000). The analysis was started by taring 50-mL centrifuge tubes and weighing 5.00±0.05 g flour with known moisture content into the tubes. 25.00 ± 0.05 g distilled H_2O was added. The tubes were shaken and kept on a tube rack for 20 minutes to suspend the flour or starch in water. The obtained slurries were shaken on the 5th, 10th, 15th, and 20th minutes before being transferred to the bench-top centrifuge (2-16 KC, Sigma, Osterode am Harz, Germany). Samples were centrifuged at 1000 g for 15 minutes at 25°C. The supernatants were removed from the tubes and the weights of the gels (consisting of flour and water it held) were measured. Experiments were repeated twice for each flour.

The following formula was used to determine the Water Retention Capacity (WRC%) of the samples:

$$WRC=\left(\frac{\text{gel weight (g)}}{\text{flour weight (g)}}\times\left(\frac{100\text{-}14}{100\text{-}M}\right)\text{-}1\right)\times 100$$

(Equation 11)

The amount of water added to gluten-free and yeast-free bread samples was approximated by the WRC of the flour combinations in the bread formulations.

3.2.1.9. Bulk Density

The bulk densities (BD, g/mL) of the samples were determined by following the procedure described in the literature (Narayana and Rao 1984; Turan, Çapanoğlu and Altay 2015) with slight modifications. First, a 25-mL graduated cylinder was tared and filled with flour or starch by gently tapping until the volume did not exceed the mark of

[1] 14% is claimed to be the reference moisture percentage of wheat flour.

10 mL. Then, the cylinder filled with flour or starch was weighed. The bulk densities of the samples were calculated using the following formula and expressed as grams per milliliter:

$$BD = \frac{\text{Weight of GC with sample-Weight of empty GC (g)}}{10\ (\text{mL})}$$

(Equation 12)

3.2.1.10. Oil Absorption Capacity

Oil absorption capacities of the samples were determined in accordance with the procedure reported in the literature (Falade and Okafor 2013). 1.00 ± 0.01 g sample was allowed to stand for 30 minutes to suspend the samples in the sunflower oil. Then the mixtures were centrifuged at 3000 g for 20 minutes at 25 °C. The supernatants were collected immediately after centrifugation and the weights of the supernatants (or the gels formed) were determined. Oil Absorption Capacity (OAC%) of each flour and starch were expressed as g of oil absorbed per gram of flour:

$$OAC\% = \frac{\text{Weight of Gel (g)-Ground Sample (g)}}{\text{Ground Sample (g)}} \times 100$$

(Equation 13)

3.2.1.11. Emulsion Activity and Stability

Analyses for emulsion activity and stability were carried out following the methods previously used in the literature (Turan, Çapanoğlu and Altay 2015; Zhao et al. 2018) with modifications because of the sample size and equipment capacity. 0.50±0.01 g of flour sample was homogenized with 25 mL of distilled H_2O at 12000 rpm for 30 seconds in 50-mL centrifuge tubes using a light-duty homogenizer equipped with a rotor for liquid media (1-250 mL capacity, ISOLAB, GmbH, Germany). Then, 25 mL of

sunflower oil was added to each tube and the homogenization process was repeated (mixing time was increased to 1 minute). The tubes were centrifuged at 1200 g for 5 minutes. The emulsions were then heated to 80 °C, kept at 80 °C for 30 minutes, and centrifuged again at 1200 g for 5 minutes. The volumes of the emulsions were recorded after each step. The Emulsion Activity (EA, %) and Emulsion Stability (ES, %) of each sample were calculated as follows:

$$EA=\frac{\text{Emulsion Volume After Centrifugation}}{\text{Emulsion Volume Before Centrifugation}}\times 100$$

(Equation 14)

$$ES=\frac{\text{Emulsion Volume After Heating and Centrifugation}}{\text{Emulsion Volume Before Heating}}\times 100$$

(Equation 15)

3.2.1.12. Foaming Capacity and Stability

Foaming properties such as foaming capacity (FC, %) and stability (FS, %) for the samples were determined using the method described by Turan, Çapanoğlu and Altay (2015), with minor modifications. 25 mL 3% (w/v) solution was prepared with distilled H_2O and 0.75±0.01 g flour or starch, in 50-mL centrifuge tubes. The solutions were homogenized at 12000 rpm for 2 minutes. Volumes of the samples were recorded both before and after homogenization. The solutions were then allowed to stand for 1 hour, recording the volume change at 5th, 10th, 20th, 30th and 60th minutes. FC% and FS% were determined using the following formula and expressed as foam formed due to stirring and foam remained after waiting, respectively:

$$FC=\frac{\text{Volume before homogenization (mL)}-\text{Volume after homogenization (mL)}}{\text{Volume before homogenization}}\times 100$$

(Equation 16)

$$FS = \frac{\text{Volume after homogenization (mL)} - \text{Volume at } 60^{\text{th}} \text{ minute (mL)}}{\text{Volume after homogenization}} \times 100$$

(Equation 17)

3.2.1.13. Color Measurements

The colors of the flours were determined by using a colorimeter (CR-400 Konica Minolta, Tokyo, Japan) with standard illuminant D65. L* (lightness; from black (0) to white (100)), a* (from green (-100) to red (+100)) and b* (from blue (-100) to yellow (+100)) were the terms used to express the color measurement results in the CIELAB space (Commission Internationale de l'Eclairage 2018).

3.2.1.14. Pasting Properties of the Flours

The pasting properties of flours and their mixtures depending on the bread formulation (Table 3.1), with known moisture contents, were determined using a Micro-Visco Amylograph (MVA, Brabender OHG, Duisburg, Germany). The analysis was carried out by preparing slurries containing 13.5 g flour or starch in 90 mL distilled H2O, with arrangements in sample and water amount due to moisture contents of the flours and their mixtures on a 14 g/100 g wheat flour moisture basis. Each analysis started at 30 °C; the slurries were first heated to 95 °C in 3 °C increments per minute, held at this temperature for 30 minutes, then cooled to 30 °C in 3 °C decrements per minute. At the end of the analyses, gelatinization temperature (GT, °C); peak viscosity (PV, Brabender Units, BU); breakdown (BD, BU); setback (SB, BU), and final viscosity (FV, BU) were determined for each flour, starch, and mixture (Cappa, Lucisano and Mariotti., 2013).

3.2.1.15. Fourier Transform Mid Infrared Spectroscopy (FT-IR) Analysis of Flours

FT-IR spectra of the flour samples were analyzed using a Perkin Elmer FT-IR spectrometer (Spectrum 100, Perkin Elmer, Massachusetts, USA) in KBr pellets (flour:KBr, 3:97, w/w). To prepare the pellets, 4.5 mg of flour or starch and 145.5 mg KBr powder were mixed thoroughly in a mortar while grinding with the pestle (50-mm mortar and pestle, P/N 161-5050, Pike Technologies, Wisconsin, USA). The mixture was then transferred to a pellet die (for 13 mm pellets, Pike Technologies, Wisconsin, USA). The die was placed in a hydraulic press (Wir Sas, Camilla '95, Germany) and pressed at 200 bar for 3 minutes. The newly formed pellet was cautiously removed from the die and placed into the magnetic pellet holder (Perkin Elmer, Middlesex County, Massachusetts, USA).

Five pellets were prepared for each flour or starch, and spectra were collected five times per pellet at room temperature, in the wavenumber range of 4000-450 cm^{-1} with a 4 cm^{-1} resolution and 128 scans. The background was also taken under the same conditions before each measurement.

3.2.1.16. Scanning Electron Microscopy (SEM)

The morphology of flour and starch samples were analyzed using a scanning electron microscope (SEM; Quanta 250 FEG, FEI, Oregon, USA) equipped with Everhart-Thornley Detector (ETD) in the Center for Materials Research (CMR) at Izmir Institute of Technology.

Approximately 4.00 ± 0.01 g of flour was weighed and dried in glass petri dishes, as explained in the "Moisture Content" section. The dried flours were then scanned without any coating at different magnifications: 250, 500, 1000, 2500, 5000, and 10000× under a voltage of 2.00 kV (HV) with a working distance (WD) of 10.00 mm.

Rice flour and corn starch used in gluten-free bread with compressed yeast were scanned with a gold-palladium coating at different magnifications (250, 500, 1000, 2500,

5000, and 10000×) as well; however, the scanning was performed under a voltage of 15.00 kV and using a large-field detector (LFD).

3.2.2. Mixture Design for Bread Formulations

The formulations for the gluten-free breads (with and without yeast) were determined using a three-component mixture design, which created seven formulated including a reference gluten-free bread formulation denoted as "STD" with only rice flour and corn starch as the flour component. Details of the mixture design are presented in Table 3.1.

Table 3.1. Gluten-free bread sample codes and the flour mixture (fm) according to the three-component extreme vertices design

Description	B (g/100 g fm)	H (g/100 g fm)	RC (g/100 g fm)
STD	0.0	0.0	100.0
B30	30.0	0.0	70.0
H30	0.0	30.0	70.0
H15	0.0	15.0	85.0
B15	15.0	0.0	85.0
BH30	15.0	15.0	70.0
BH15	7.5	7.5	85.0

RC: equally mixed Rice flour (R) and corn starch (C); B: White bean flour; H: Hazelnut flour.
Sample codes: STD, reference gluten-free bread formulation containing RC; 15 and 30, percentages of B, H, or their equally mixed amounts in flour composite.

Since B and H could not provide any profiles for the pasting properties, a maximum level for the addition of these flours to the STD mixture was determined based on the preliminary experiments conducted with these flours using a Micro-Visco Amylograph (MVA, Brabender OHG, Duisburg, Germany).

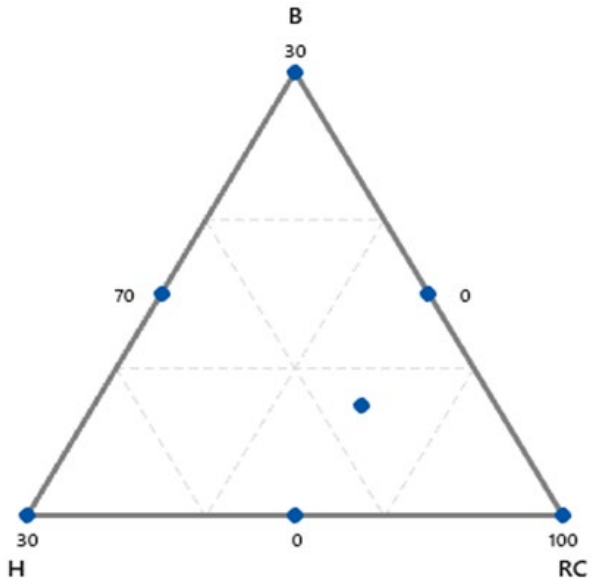

Figure 3.2. The design space with 7 experimental points according to the extreme vertices design as given in Table 3.1

Overall, the three components of the mixture design were hazelnut flour (H), bean flour (B), and the mixture containing R and C equally (RC). H, B, and RC changed in the range of 0-30 g/100 fm, 0-30 g/100 g fm, and 70-100 g/100 g fm, respectively (Figure 3.2).

3.2.3. Gluten-Free Bread with Compressed Yeast Formulation

The standard (STD) formulation was defined as expressed by Cappa et al. (2016), with slight modifications due to the difference in the ingredients of the decided formula and the one described in the study. The general formulation excluding water is shown in Table 3.2, where the total flour consisted of 100 g RC per 100 g fm (STD); 70 g RC per 100 g fm, and 30 g B, H, or BH per 100 g fm (B30, H30 or BH30, respectively); 85 g RC per 100 g fm and 15 g B, H or BH per 100 g fm (B15, H15 or BH15, respectively).

Table 3.2. Gluten-free yeast bread base formulation in terms of grams per 100 grams of dough (except water*) and grams per 100 grams of the flour mixture (fm)

Ingredient	Amount (g/100 g dough*)	Amount (g/ 100 g fm)
Flour	83.5	100
HPMC	1.5	1.8
Olive Oil	6.0	7.2
Sugar	4.0	4.8
Salt	2.0	2.4
Compressed Yeast	3.0	3.6

*Water was not included in the formula initially because the amount was decided after the farinograph trials and differed for each flour combination.

3.2.4. Preparation of Gluten-Free Bread with Compressed Yeast

The gluten-free yeast breadmaking process is given in Figure 3.3. In brief, flour mixture (as given in Table 3.1), HPMC, white sugar, and salt were mixed in the Brabender® farinograph (Brabender OHG, Duisburg, Germany) equipped with a 300-g bowl and set at 30°C. After being mixed for approximately 5 minutes, olive oil and compressed yeast, mixed with some water from the farinograph, were added to the dry mixture (Figure 3.3).

The water addition started immediately and continued until the dough consistency reached 200±10 BU. Then, the addition stopped, and the dough was kneaded for 15 minutes. Following mixing and kneading, the added water amount was recorded. 60.1, 70.2, 80.5, 51.0, 43.9, 60.2, and 62.5 g water/100 g dough were added to STD, B15, B30, H15, H30, BH15, and BH30, respectively.

The dough was divided into six portions of 60 g weight and placed into oiled metal molds with 10.0 × 6.0 × 4.5 cm dimensions. These portions were placed in a multifunction oven (mod. AMW698/IXL, Whirlpool, EMEA S.p.A., Biandronno, VA, Italy) and fermented using its leavening function at 35 °C for 45 minutes.

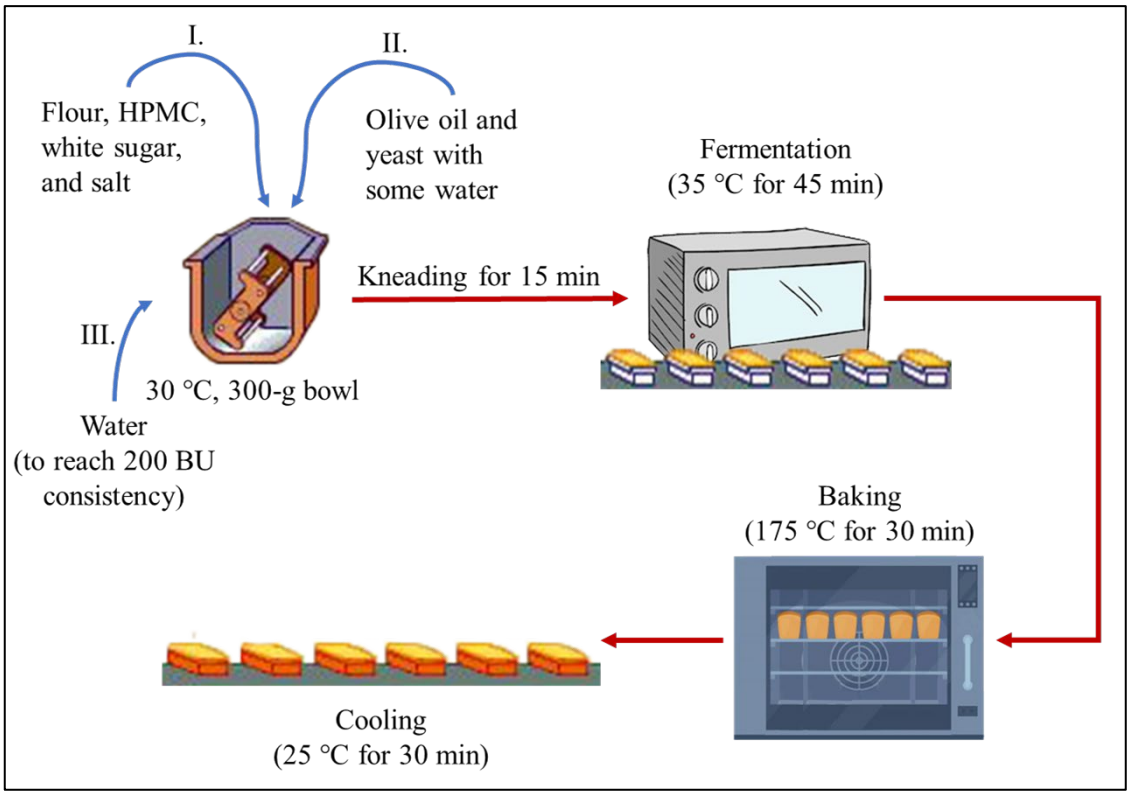

Figure 3.3. Breadmaking process of the gluten-free yeast bread (bread and bowl icons are from Encyclopædia Britannica 2023; oven icons are from Freepik 2023)

Leavened dough samples were transferred to a pre-heated electric static oven (mod. G2551MF816A, Whirlpool, EMEA, S.p.A., Biandronno, VA, Italy) and baked for 30 minutes at 175 °C. The bread loaves were then cooled for 30 min at room temperature before being removed from the molds and characterized.

The breads were then separated for analysis immediately (t_0, fresh bread); after 24 hours (t_{24}) and 48 hours (t_{48}) of storage.

3.2.5. Dough Properties of Gluten-Free Yeast Bread

This section covers the analyses applied to the gluten-free and yeast-free dough samples: leavening and Fourier Transform Infrared Spectroscopy.

3.2.5.1. Leavening Properties of GF Yeast Bread Dough

Dough leavening properties of gluten-free yeast bread were determined by following the image analysis explained by Cappa, Lucisano and Mariotti (2013), with minor modifications. After kneading, three portions of 10 grams were taken from each dough, which was recovered from the farinograph. These portions were shaped spherically using a spoon and placed into petri dishes. The samples were then leavened in an incubator (Memmert UFE500, Schwabach, Germany) at 35 °C for 60 minutes. Before starting the leavening and every 15 minutes during leavening (at t=0, 15, 30, 45, and 60 minutes), the images of the petri dishes were scanned full scale in 256 grey levels using a flatbed scanner (Epson Perfection V850pro scanner, Seiko Epson Corporation, Suwa, Japan) at 300 dpi. Images were saved in TIFF format and processed using Image Pro-Plus software (v. 7.0, Media Cybernetics Inc., Rockville, MD, USA). The increase in the dough area during leavening (Area%) was calculated as follows:

$$\text{Area\%} = \frac{\text{Area}_{t=0} - \text{Area}_{t=0,\ 15,\ 30,\ 45,60}}{\text{Area}_{t=0}} \times 100$$

(Equation 18)

3.2.5.2. FT-IR Spectra of GF Yeast Bread Dough

A Vertex 70 spectrometer (Bruker Optics, Milan, Italy) equipped with a Germanium multiple reflection ATR cell was used to collect the FT-IR spectra of the dough samples immediately after dough preparation before allowing the dough to be leavened. Each dough was spread on the cell surface cautiously to avoid gaps. Two portions of samples were collected from each dough, and the absorbances were measured in duplicates in the wavenumber range of 4000–800 cm^{-1} at room temperature with a 4 cm^{-1} resolution and 32 scans. Backgrounds were also collected under the same conditions. The data were acquired using Opus software (v.6; Bruker Optics, Ettlingen, Germany). The same software also managed instrument control. Results were obtained in terms of absorbance units measured at each wavenumber.

3.2.6. The Properties of Gluten-Free Yeast Bread

Aanalyses applied to the frest gluten-free yeast bread samples are explained in this section.

3.2.6.1. Height (h), Weight (W), and Baking Loss (BL)

The maximum height (mm) and weight of each bread loaf (including the ones that were going to be separated for storage, a total of 6 loaves per formulation) were measured using a caliper and laboratory scale, respectively.

Baking loss (BL, %) was calculated using the fresh bread weight (g, after cooling) and dough weight (g, before leavening) as follows:

$$BL=\frac{W_{dough}-W_{bread,\ t_0}}{W_{dough}}\times 100$$

(Equation 19)

3.2.6.2. Specific Volume

Specific Volume (SV, mL/g) was determined using the seed displacement method (AACC method 10-05.01 2000) using rapeseeds and expressed in terms of volume (mL) per gram of bread (g).

First, the specific volume of the seeds (SV_{seed}) was determined using a 100-mL graduated cylinder:

$$SV_{seed}=\frac{100\ mL}{W_{seed\ in\ the\ graduated\ cylinder}\ (g)}$$

(Equation 20)

Then, the volume of seed in the container ($V_{container}$, mL), bread volume (V_{bread}, mL), and bread specific volume (SV_{bread}, mL/g) of each loaf was calculated using the following formulas:

$$V_{container}=W_{seed\ in\ container}\times SV_{seed}$$

(Equation 21)

$$V_{bread} = V_{container} - (W_{bread+seed} - W_{bread}) \times SV_{seed}$$

(Equation 22)

$$SV_{bread} = \frac{V_{bread}}{W_{bread}}$$

(Equation 23)

3.2.6.3. Crust and Crumb Color

The crust and crumb colors of each loaf were measured using a tristimulus colorimeter (Chroma Meter II, Minolta, Osaka, Japan) with standard illuminant C. L* (lightness; from black (0) to white (100)), a* (from green (-100) to red (+100)) and b* (from blue (-100) to yellow (+100)) were the terms used to express the color measurement results in the CIELAB space (CIE, 2018). Based on the CIE L*a*b* coordinates obtained from the fresh or stored bread samples, color differences (ΔE), between the standard (STD) and the rest of the formulations (B15, B30, H15, H30, BH15, BH30), and browning index (BI) were determined using the following equations (Wronkowska, Haros and Soral-Śmietana 2013; Pathare et al. 2013):

$$\Delta E=\sqrt{\left(L^*_{STD}-L^*\right)^2+\left(a^*_{STD}-a^*\right)^2+\left(b^*_{STD}-b^*\right)^2}$$

(Equation 24)

$$BI = \frac{100 \times (x - 0.31)}{0.172}$$

(Equation 25)

where,

$$x = \frac{a^* + 1.75 \times L^*}{5.645 \times L^* + a^* - 3.012 \times b^*}$$

(Equation 26)

3.2.6.4. Crumb Water Activity, Slice and Crumb Moisture Content

The water activity of each bread crumb was measured using an AquaLab Series CX-3 device (Decagon Devices Inc. Pullman, WA, USA).

The moisture contents of each slice and crumb (MC_{slice}, MC_{crumb}, %) were determined following the AACC method 44-15A (2000). Half slices (approximately 5–8 g) were used for slice moisture, where two crumbs per bread loaf were cut cylindrically (13 mm diameter, 20 mm height, approximately 1–2 g). Prepared samples were put in a laboratory oven and dried at 105 °C, as explained in section 2.2.1.1.

$$MC_{crumb/slice} = \frac{W_{crumb/slice} - W_{crumb/slice,\ dry}}{W_{crumb/slice}} \times 100$$

(Equation 27)

3.2.6.5. Crumb Porosity

Crumb porosity was determined using an image analysis method: a central crumb portion (approximately 70%) was selected from each bread slice previously scanned in 256 grey scale levels and 600 dpi resolution using a scanner (Epson Perfection V850pro scanner, Seiko Epson Corporation, Suwa, Japan). Images were processed using the Image Pro-Plus software (v. 7.0; Media Cybernetics Inc., Rockville, MD, USA). Holes were identified, counted, and classified into five classes based on their size: (1) $0.05 \leq x < 0.2$ mm^2, (2) $0.2 \leq x < 0.5$ mm^2, (3) $0.5 \leq x < 1$ mm^2, (4) $1 \leq x < 5$ mm^2 and (5) $5 \leq x < 10$ mm^2. The proportion of holes in each class (%; expressed as the percentage of holes

having a selected size with respect to the total number of holes in the image crop) and the crumb porosities (%; expressed as the percentage of the total hole area in the crop with respect to the total crop area) were calculated (Cappa, Lucisano and Mariotti 2013).

3.2.6.6. Bread Crumb Hardness

A dynamometer (mod. 3365, Instron Division of ITW Test and Measurement Italia S.r.l., Pianezza, TO, Italy) equipped with a 100 N load cell was used to measure the bread crumb hardness (Alamprese et al. 2002). Each bread was sliced into a thickness of 20 mm using a standard knife. Penetration with a 0.098 N trigger force of up to 40% was applied to each slice at a compression rate of 1 mm/s using a cylindrical probe with a 13-mm diameter. The measurement process was controlled using the BlueHill software (v. 2.9, Instron Corporation, USA). Based on the results evaluated, crumb resistance to 30% penetration was selected to indicate crumb hardness (H, N). Four slices were analyzed for each enriched bread, whereas three measurements were taken for STD.

3.2.6.7. Stored Bread

Loaves labeled t24 and t48 were stored at 25 °C and 60% relative humidity in unsealed hand-folded paper bags to simulate a domestic shelf-life (Mariotti et al. 2006; 2013).

All fresh bread analyses except height, specific volume, and crumb porosity were carried out for the loaves at t_{24} and t_{48} as well.

Weight losses during storage (Storage Loss, SL, g/100 g) were determined using the following formula:

$$SL_{t_{24/48}} = \frac{W_{bread,t_0} - W_{bread,t_{24/48}}}{W_{bread,\ t_0}} \times 100$$

(Equation 28)

The rate of staling (RoS, %) was also calculated as follows for samples t_{24} and t_{48} (Kahraman et al. 2022):

$$RoS_{t_{24/48}} = \frac{H_{t_{24/48}} - H_{t_0}}{H_{t_0}}$$

(Equation 29)

Where H_{t0} is crumb hardness at t=0, $H_{t24/48}$ is the crumb hardness at t=24 and 48 h, respectively.

3.2.7. Gluten-Free and Yeast-Free Bread Formulation

GF-YF bread was also formulated as explained in the "Gluten-free Yeast Bread Formulation" section and as described by Cappa et al. (2016) with minor modifications based on the results obtained in the preliminary experiments.

Table 3.3. Gluten-free and yeast-free bread base formulation in terms of grams per 100 gram dough and grams per 100 gram flour mixture (fm), excluding water

Ingredient	Amount (g/100 g dough)	Amount (g/100 g fm)
Flour	77.2	100.0
Xanthan Gum	1.4	1.8
Olive Oil	5.6	7.2
Sugar	7.4	4.8
Salt	2.3	3.0
Baking Powder	3.1	4.0
Baking Soda	0.8	1.0
Vinegar	2.3	3.0
Water	Determined w.r.t WRC of each flour mix	

The general formula is summarized in Table 3.3, where the total flour amount consisted of 100 g RC per 100 g fm (STD), 70 g RC per 100 g fm, and 30 g B, H, or BH per 100 g fm (B30, H30 or BH30, respectively); 85 g RC per 100 g fm and 15 g B, H or BH per 100 g fm (B15, H15 or BH15, respectively).

The water retention capacities (WRC) of flour mixtures were determined to approximate the amount of water to be added in dough preparation. After some preliminary experiments to obtain acceptable gluten-free and yeast-free breads, the following water contents were added: For STD, B15, B30, H15, H30, BH15, and BH30 formulations, 92, 106, 118, 88, 86, 103, and 108 g water per 100 g of the flour mixture, respectively. Each bread formulation was replicated twice (n=2).

3.2.8. Preparation of Gluten-Free and Yeast-Free Bread

The dry ingredients (flour, xanthan gum, white sugar, salt, baking powder, and baking soda) were added to the mixer bowl (KitchenAid, Artisan Stand Mixer, 5KSM125, Whirlpool EMEA, USA) and premixed for 2 minutes using a silicone spatula. The bowl was placed in the mixer, and the dough was kneaded for 15 minutes using the dough hook at speed 2. Then, olive oil, vinegar, and water were slowly added while mixing (Figure 3.4).

After the kneading was completed, the dough was divided into two portions where each portion weighed 200 g and placed into molds lined with oiled baking paper. Then, by spreading approximately 2 g water on the doughs with a silicone brush, they were baked in an industrial oven (mod. CMK-04, Senox, Izmir, Türkiye) at 180 °C for 45 minutes. The breads prepared according to the mixture design were immediately analyzed after they cooled down to room temperature (approximately 1 hour, 25 °C). The dimensions of baking tins were 15 cm × 6.5 cm × 6.5 cm (Dr. Oetker, Izmir, Türkiye).

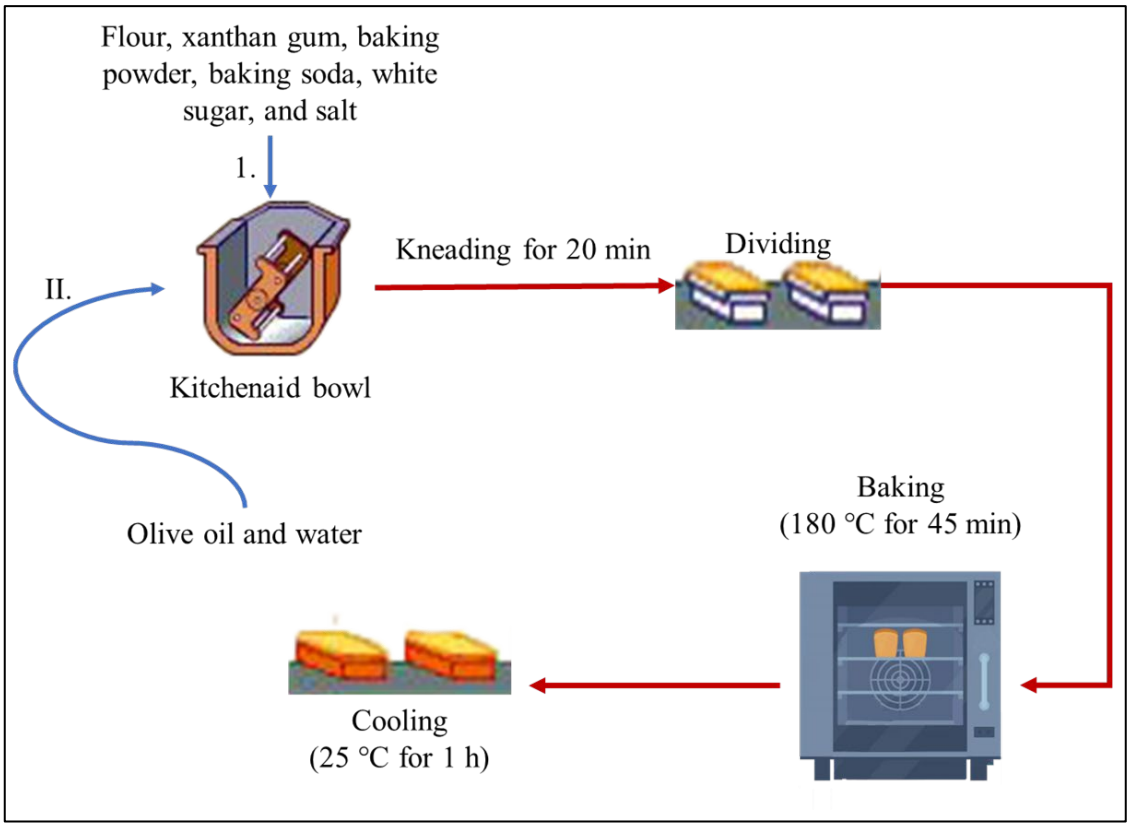

Figure 3.4. Breadmaking process of gluten-free and yeast-free (GF-YF) bread (bread and bowl icons are from Encyclopædia Britannica 2023; oven icon is from Freepik 2023)

H30, BH30, and STD formulations were selected for storage experiments based on the results obtained from mixture design analysis and reference. They were analyzed immediately (t0, fresh bred) and after 48 hours (t48) of storage at room temperature and 90% relative humidity in a desiccator containing specifically prepared NaCl-H_2O (36:100 w/w) solution.

3.2.9. Dough Properties of Gluten-Free and Yeast-Free Bread

In this section, analyses applied to the gluten-free and yeast-free bread dough samples are explained: back extrusion and Fourier Transform Infrared Spectroscopy.

3.2.9.1. Rheological Properties of GF-YF Bread Dough

Rheological properties of the GF-YF bread doughs were analyzed using the backward extrusion technique (Ronda, Pérez-Quirce and Villanueva 2017; Nasaruddin et al. 2012) using a texture analyzer (TA-XT2i, Stable Microsystems, UK) equipped with a back extrusion rig (mod.A/BE) and 25-mm cylinder probe (P/25). The probe approached (2 mm/s, pre-test speed), penetrated 20 mm into the sample (3 mm/s, test speed), and returned (10 mm/s, post-test speed) to its starting position. Approximately 80 g of dough (approximately 30-35 mm in height) was placed into a standard-size container with a 50-mm diameter.

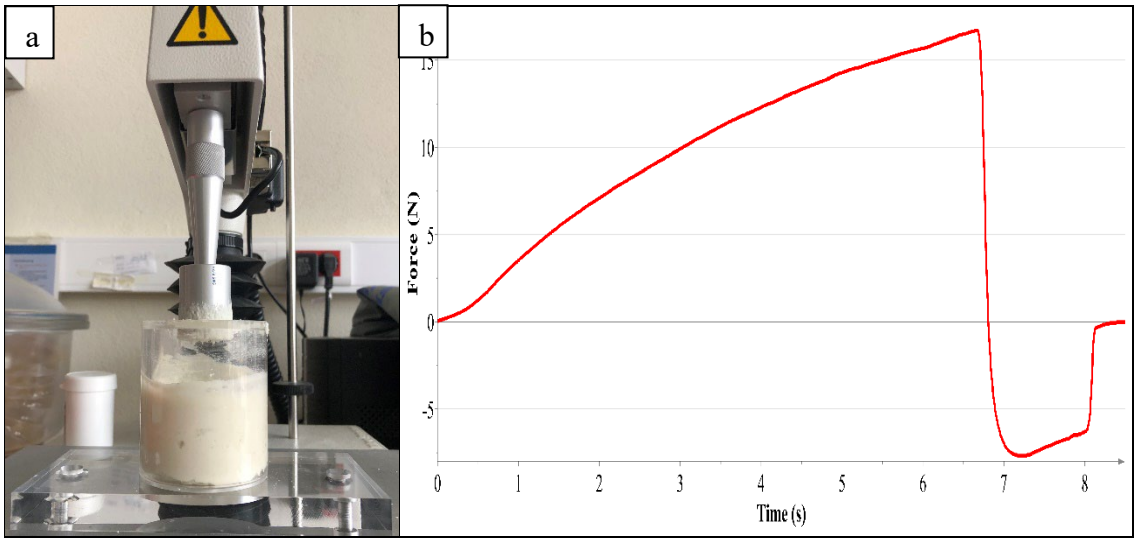

Figure 3.5. Measurement (a) and a representative profile (b) of back extrusion

At the end of each measurement, firmness (maximum force on the positive side of the curve, N), consistency (area under the positive side of the curve, N.sec), viscosity index (maximum force during return, N), and cohesiveness (area under the negative side of the curve, N.sec) were recorded. The data were collected and processed using Exponent software (v.6.1.9, Stable Micro Systems, UK) and Microsoft Excel (Microsoft Inc., Washington, USA), respectively. A representative figure for the back extrusion profile and its measurement setup is presented in Figure 3.5.

3.2.9.2. FT-IR Spectra of Gluten-Free and Yeast-Free Bread Dough

FT-IR spectra were collected after dough preparation using a Perkin Elmer FTIR spectrometer (Spectrum 100, Perkin Elmer, Massachusetts, USA) equipped with a 45° Zinc-Selenium Attenuated Total Reflectance (ATR) cell. Each dough was spread on the cell surface cautiously to avoid empty spaces. Spectra were collected on five dough aliquots, in the wavenumber range 4000–650 cm^{-1} with 96 scans and 4 cm^{-1} resolution. The background was also taken under the same conditions. The data were collected using Spectrum software (v.6.0.1, Perkin Elmer, Massachusetts, USA) and processed using SpectraGryph (Optical Spectroscopy Software, v.1.2.16, Oberstdorf, Germany), Microsoft Excel (Microsoft Inc., Washington, USA), and SIMCA (version 14.1, MKS Umetrics, Malmo, Sweden) software. Results were obtained in terms of absorbance units measured at each wavenumber.

3.2.10. Properties of Gluten-Free and Yeast-Free Bread

For each formulation, two replications were performed. At each replicate, two breads were produced. All results were given as the average and standard deviation of the replicates.

3.2.10.1. Height (h), Weight (W), and Baking Loss (BL)

The height and weight of each bread loaf were determined as explained in section 3.2.6.1., with slight modifications. In brief, height measurements were made at the same three points (distance between top and bottom of left and right edges and center) for dough and bread. The average of these measurements was recorded as the height of the dough and bread, respectively. For the height change (h%) calculation, the following equation was used:

$$h\% = \frac{h_{dough} - h_{bread}}{h_{dough}} \times 100$$

(Equation 30)

Baking loss (BL%) was calculated using Equation 19.

3.2.10.2. Specific Volume

The specific Volume (SV, mL/g) of each bread was determined, as explained in section 3.2.6.2., by only changing the graduated cylinder and container volumes. A 50-mL graduated cylinder and a 2000-mL beaker were used as the container for the specific volume measurements. Equations 20, 21, 22, and 23 were used to determine seed specific volume (SV_{seed}, mL/g), beaker volume ($V_{container}$, mL), bread volume (V_{bread}, mL), and bread specific volume (SV_{bread}, mL/g), respectively.

3.2.10.3. Crust and Crumb Color

Flour colors were determined by using a colorimeter (CR-400 Konica Minolta, Tokyo, Japan) with standard illuminant D65. Calculations were made using equations 24, 25, and 26.

3.2.10.4. Slice and Crumb Moisture Content

The moisture content of each crumb and slice were determined using the AACC method 44-15A (2000), with a slight modification: instead of 105 °C, the temperature was set to 135 °C since breads were denser and heavier than the GF yeast bread samples. Half

slices (approximately 17 g) and rectangular prism-shaped crumb pieces (approximately 5 g) were prepared, and Equation 27 was used to determine the moisture contents.

3.2.10.5. Bread Crumb Texture Properties

Textural properties (texture profile, double compression) were determined using a texture analyzer (TA-XT2, Stable Microsystems, Godalming, UK) equipped with a 5 kg load cell. In brief, bread crumbs were cut cubically (2.5×2.5×2.5 cm^3 dimensions) and placed in the texture instrument. For the texture profile analysis (TPA), 40% compression was applied twice at 1 mm/s for pre-test, test, and post-test speeds. Texture profiles were evaluated using Exponent software (v.6.1.9, Stable Micro Systems, UK) and Microsoft Excel (Microsoft Inc., Washington, USA). The measurement system and an example texture profile are presented in Figure 3.6.

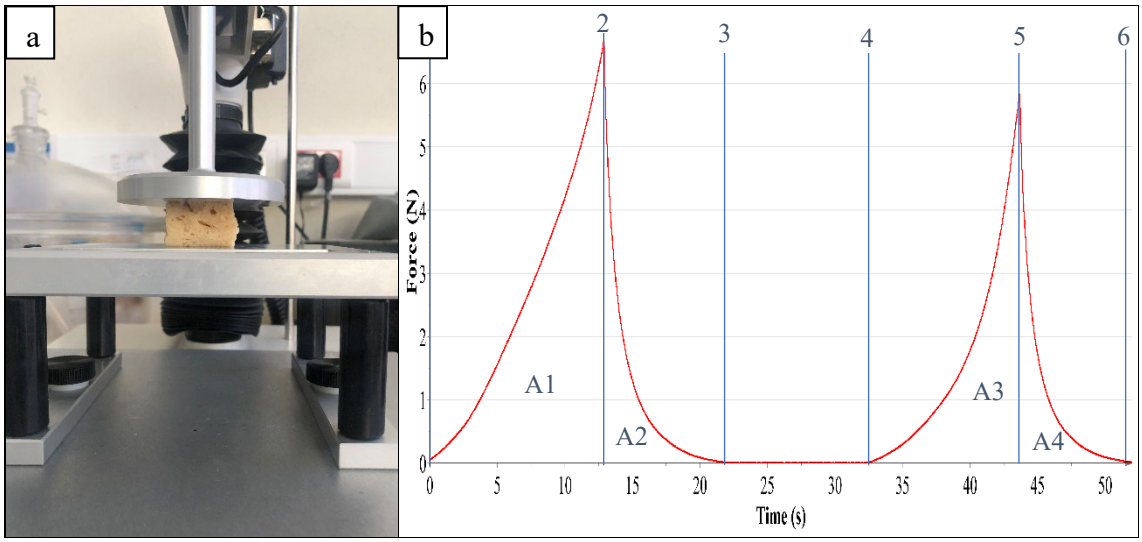

Figure 3.6. GF-YF texture measurement (a) and a representative TPA profile (b) with anchor locations (1, 2, 3, 4, 5, 6) and area (A1, A2, A3, A4) specifications

The hardness (HA, N) value was obtained directly from the profile data (maximum force during the first compression period). Additionally, cohesiveness (CO, related to the internal resistance of bread), springiness (SP, basically the elasticity of bread crumb), and chewiness (CH, energy required for chewing a solid food) were determined using the following equations (Kahraman, 2016):

$$CO = \frac{A4+A5\ N\times s}{A1 + A2\ N\times s}$$

(Equation 31)

$$SP = \frac{\text{Distance between 4 \& 5}}{\text{Distance between 1 \& 2}}$$

(Equation 32)

$$CH = \frac{HA \times (A4+A5) \times (\text{Distance between 4 \&5})}{(A1+A2) \times (\text{Distance between 1 \&2})}$$

(Equation 33)

3.2.10.6. Stored Gluten-Free Bread

Samples to be stored were selected based on preliminary results such as specific volume, crumb hardness, and appearance. In addition to the analyses described between sections 3.2.10.1 and 3.2.10.5, storage loss and rate of staling were calculated using Equations 28 and 29, respectively.

3.2.10.7. Sensory Evaluation of GF-YF Breads

Based on the interpretation of the physical properties of the breads, H30 and BH30 were selected to be the subjects of the sensory evaluation, which was performed with the participation of 32 untrained panelists (11 males and 21 females) with an average age of 28.5.

The panelists were asked to score samples according to their color, odor, texture, taste, and overall liking on a 1–7 hedonic scale (where 1 was the lowest and 7 was the highest score). The sensory study was approved by the Izmir Institute of Technology Scientific Research and Publication Ethics Committee (Number: 19.09.2022-E.96273). The score sheet given to the participants is shown in Figure 3.7.

Female Male Age:

Please evaluate the samples between 1 and 7 scale. **1**: The lowest 7: The highest

Please make sure that you evaluated all samples for all attributes.

Samples include white bean flour, hazelnut flour, rice flour and corn starch.
Samples do not include any animal products.

Sample	Color	Flavor	Texture	Taste	Overall liking
429					
571					
836					

Any comments:

Figure 3.7. Sensory evaluation score sheet for STD (571), H30 (836), and BH30 (429)

3.2.11. In Vitro Starch Digestion

Starch fractions of STD, H30, and BH30 were determined based on the method developed by Englyst et al. (1996; 1999; 2000; 2018) and modified by Ozel-Tasci et al. (2020), with slight adjustments. Wheat bread and distilled water were used as the positive and negative control samples, PC and NC, respectively. A regular wheat bread purchased from a market was used to check whether the enzyme solutions were working.

3.2.11.1. List of Solutions

- Gastric Enzyme Solution: 0.5 g pepsin and 0.5 g guar gum mixed in a 50 mL 0.05 M HCl solution.
- Intestinal Enzyme Solution: 3 g pancreatin (EC 232-468-9) was weighed in a 50-mL centrifuge tube. It was centrifuged at 4500 rpm for 10 minutes at room temperature (25 °C). 15 mL of the supernatant was then transferred to another tube, mixed with 0.666 mL amyloglucosidase and 1 mL (10 mg/mL) invertase.

- 0.25 M Sodium Acetate (NaOAc, using Sodium acetate trihydrate powder)
- 7M potassium hydroxide (KOH) solution
- 0.5 M Acetic Acid
- 1 M NaOAc (pH 4.5)
- Glucose Oxidase Peroxidase Kit (GAGO20, Sigma-Aldrich, Mannheim, Germany): The reagent was dissolved in 39.2 mL ultra-pure H2O. It was stored at +4 °C until further analysis (at –20 °C for the longer waiting periods).

3.2.11.2. Rapidly and Slowly Digestible Starch Fractions

Preparation procedure for free sugar glucose (FSG), and starch fractions at t=0 (gastric phase, G0), t=20 min (intestinal phase, G20) and t=120 min (intestinal phase, G120) is summarized in Figure 3.8. In brief, bread samples were sliced into 1 cm thickness to increase the surface area and mimic mechanical digestion. The slices were then minced using a glass blender (mod. K-8020, Arçelik, Istanbul, Türkiye) at the highest speed until the particles (crust and crumb) could no longer get any smaller. 0.25 g of each sample (the amount was decided after preliminary experiments) was then measured in 50-mL centrifuge tubes.

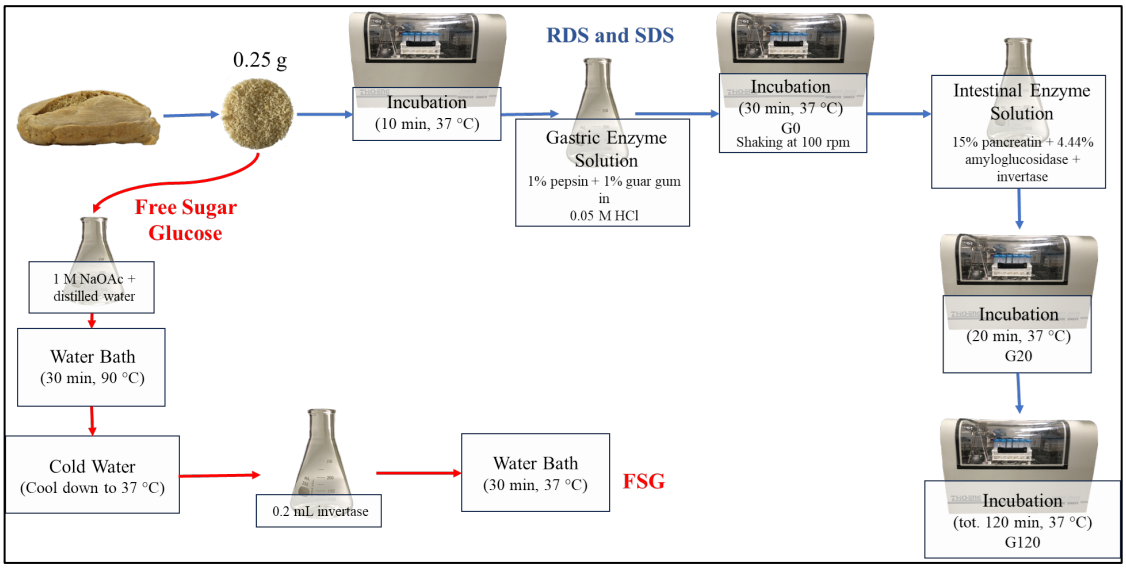

Figure 3.8. Starch digestion procedure for free sugar glucose (FSG), and fractions at t=0 (gastric phase, G0), t=20 min (intestinal phase, G20) and t=120 min (intestinal phase, G120)

They were kept in an incubator (mod. Zhwy-200B, Zhicheng, Shanghai, China) at 37°C for 5 minutes to reach human body temperature (i.e., approximately 37 °C, initial phase). 5 mL of gastric enzyme solution was added to each tube and vortex mixed. Five glass balls were added to each tube to aid the mechanical disruption during incubation. Then, the tubes were placed in a shaking incubator at 37°C and 150 rpm for 30 minutes to simulate gastric digestion. Samples in the incubator during initial, gastric, and intestinal phases are shown in Figure 3.9.

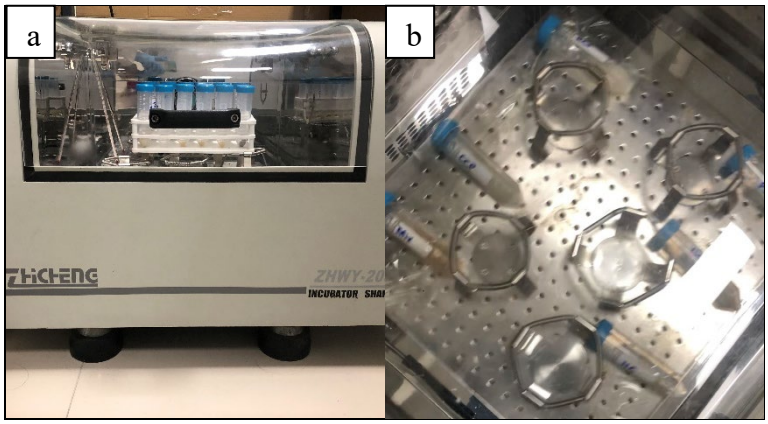

Figure 3.9. Samples in the incubator during (a) initial, (b) gastric, and intestinal phases

Immediately after incubation, 0.1 mL of sample was collected from each tube, and the enzymes were inactivated at 95 °C for 5 minutes (G0). Then, 5 mL of 0.25 M sodium acetate (NaOAc) and 2.5 mL of intestinal enzyme solution were poured into each tube and vortex mixed. The tubes were again placed in a shaking incubator at 37 °C and 150 rpm. Hence, intestinal digestion started. Then, 0.1 mL of samples were collected at 20th (G20) and 120th (G120) minutes to calculate RDS and SDS fractions.

3.2.11.3. Free Sugar Glucose (FSG) Analysis

0.25 g of STD, H30, BH30, positive control (PC), and negative control (NC) were weighed in 50-mL centrifuge tubes and prepared as explained in the “Rapidly and Slowly Digestible Starch Fractions” section. 0.25 mL of 1 M NaOAc (pH 4.5) and 20 mL distilled

H_2O were added to each sample tube. The tubes were vortex mixed and placed into a water bath at 90 °C for 30 minutes. Then, the tubes were vortex mixed again, cooled down to 37 °C, and 0.2 mL invertase was added to each tube. Tubes were placed in a water bath at 37 °C for 30 minutes. 0.1 mL of each sample was also transferred to 1.5-mL centrifuge tubes at the end of G0, G20, and G120. The transfers were followed by heat inactivation at 95 ºC for 5 minutes.

3.2.11.4. Determination of Starch Fractions

Determination of starch fractions following the sample collections is summarized in Figure 3.10. All tubes were centrifuged at 900 g for 5 minutes at 25 °C to avoid any precipitate before the determination of the glucose contents.

Procedure for starch fraction determination started as soon as the 50 µL of the sample was poured into the well plate cell (a 96-cell well plate was used). The pouring was followed by 100 µL glucose oxidase peroxidase addition. The plate was then incubated in a laboratory oven for 30 minutes at 37 °C.

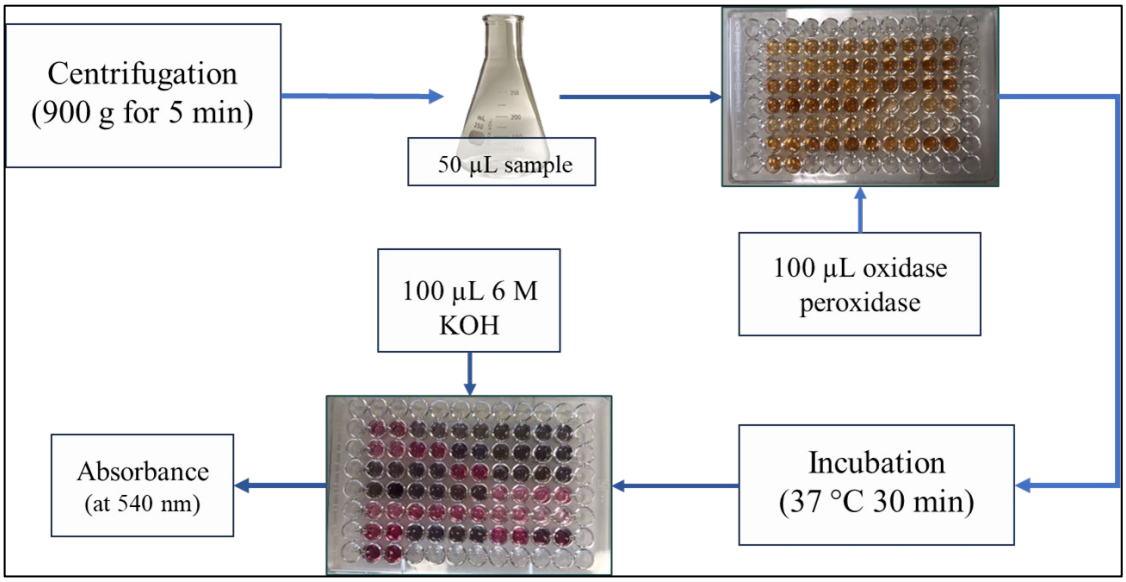

Figure 3.10. Procedure for determination of the starch fractions: FSG, G0, G20 and G120.

Immediately after the incubation, 100 μL of 6 M H_2SO_4 was added to each cell. Absorbances of the plates were measured at 540 nm and 25 °C using a microplate reader (MultiskanTM GO, Thermo Fisher, MA, USA). RDS, SDS, and their change from STD were calculated using the following equations:

$$\text{D-glucose } \left(\frac{\mu g}{mL}\right) = \frac{\text{Sample Absorbance (SA)} - \text{Blank Absorbance (BA)}}{0.013}$$

(Equation 34)

$$\text{D-glucose } \left(\frac{\mu g}{mg}\right) = \text{D-glucose } \left(\frac{\mu g}{mL}\right) \times \text{Sample Volume (mL)}$$

(Equation 35)

Sample volume was 5, 12.4, 12.3, and 20.45 mL for G0, G20, G120, and FSG, respectively.

$$\text{D-glucose } \left(\text{Sample } \frac{\mu g}{mg}\right) = \text{D-glucose } \left(\frac{\mu g}{mg}\right) - \text{ D-glucose } \left(\frac{\mu g}{mg}\right)_{NC}$$

(Equation 36)

Where NC was the “negative control” sample, prepared with water, followed the same procedure.

$$RDS = 0.9 \times (G20 - FSG)$$

(Equation 37)

$$SDS = 0.9 \times (G120 - G20)$$

(Equation 38)

$$\%Change_{RDS/SDS} = \frac{RDS/SDS_{H30/BH30} - RDS/SDS_{STD}}{RDS/SDS_{STD}} \times 100$$

(Equation 39)

3.2.12. Statistical Analysis

Analyses were carried out in duplicates on flours and in triplicates on doughs and breads unless specified otherwise. Tables were prepared in the following form: mean ± standard deviation.

Data were analyzed using the Minitab statistical software program (v.19.1, Minitab Inc., Pennsylvania, USA). One-Way ANOVA and Tukey's Multiple Range Test were performed on the data at $p<0.05$ to determine any significant differences between the flours regarding their proximate compositions and functional properties. Pairwise Pearson correlation analysis was applied to the results specified in their respective sections. Mixture Design (extreme vertices design) was used in the determination of design points. The models of bread data were evaluated in terms of the p-values and R^2 values.

Collected FT-IR spectra containing gluten-free and yeast-free dough, bread, and stored bread data, in addition to physical properties of the breads and their doughs (if necessary), were analyzed by applying Principal Component Analysis (PCA) on SIMCA software (version 14.1, MKS Umetrics, Malmo, Sweden).

CHAPTER 4

RESULTS AND DISCUSSION PART I: GLUTEN-FREE YEAST BREAD

In this chapter, the characterization of gluten-free flour mixtures used in yeast bread formulations is given. The physical and spectroscopic properties of bread dough and the characterization of fresh and stored loaves of gluten-free (GF) yeast bread are discussed. The optimum formulation based on the data analysis is determined. The results of this chapter was published (Tuna et al. 2023).

4.1. Flour Samples Used in GF Yeast Breads

The proximate compositions of the flours and their functional properties are essential for the development of a nutritionally balanced bread recipe with the desired texture and structure. Rice (R), corn starch (C), white bean (B), and hazelnut (H) flours used in the gluten-free yeast bread in this study were characterized in terms of chemical and functional properties (Table 4.1 and Table 4.2).

4.1.1. Proximate Composition and Total Phenolics Content (TPC)

As represented in Table 4.1, rice flour (R) contains the highest moisture among the other flours, whereas hazelnut flour (H) has the least amount. The moisture contents of all the flours (except H) are within the ranges reported in the literature: 10.9 – 14.0 for rice flour (R) (Mugalavai et al. 2021; Cannas et al. 2020), 11.05 – 12.4 for corn starch (C) (Cappa, Lucisano and Mariotti 2013; Sanchez, Osella and De La Torre 2002), 7.0 – 11.4

for bean flour (B) (Choe et al. 2022; Guldiken et al. 2022a) and 3.4 – 8.1 g water per 100 g flour for hazelnut flour (H) (Turan, Çapanoğlu and Altay 2015).

Regarding crude protein content, B (18.77 g/100 g flour) and H (15.60 g/100 g) stand out, as expected. Protein contents of R, B, and H are in agreement with the literature with protein content ranges of 5.9 – 9.6, 18.8 – 30.5, and 14.7 – 18.2 g/100 g flour, respectively (Park et al. 2021; Zhu et al. 2020; Sanfilippo et al. 2023; Guldiken et al. 2022a; Turan, Çapanoğlu and Altay 2015). The protein content of C was reported in the literature as "non-detectible" or as little as 0.2 g/100 g (Cappa, Lucisano and Mariotti 2013; Cappa et al. 2016; Sanchez, Osella and De La Torre 2002). Even though the protein content of the B, a pre-cooked flour, was found to be within the reported range, most of the protein content reports for the common bean flour were above 20 g/100 g (Nosworthy et al. 2018; Nwadike et al. 2018; Romero and Zhang 2019; Salazar, Rodas and Aranibia 2020; Choe et al. 2022; Guldiken et al. 2022b). The reason for the relatively lower protein content of B can be the reduction in its protein content (probably due to protein denaturation) during the pre-cooking process (Alajaji and El-Adawy 2006; Güzel and Sayar 2012; Choe et al. 2022; Güldiken et al. 2022; Kumar, Sadiq and Anal 2022). In addition, it is known that flours of the different cultivars of common beans can differ in terms of their compositional elements based on several conditions (Marquezi et al. 2016; Wani et al. 2017; Carbas et al. 2020).

Table 4.1. Chemical composition of rice flour (R), corn starch (C), white bean (B), and hazelnut flour (H)

Flour	Moisture (g/100 g)	Proteins (g/100 g)	Fat (g/100 g)	Carb. (g/100 g)	Total Ash (g/100 g)	Crude Fiber (g/100 g)	TPC (mg GAE/g)
R	12.54 ± 0.03^{A}	6.03 ± 0.06^{B}	1.09 ± 0.12^{A}	79.73	0.61 ± 0.03^{B}	1.51 ± 0.02^{B}	0.22 ± 0.02^{A}
C	11.04 ± 0.05^{B}	0.52 ± 0.01^{A}	0.54 ± 0.03^{A}	87.74	0.16 ± 0.02^{A}	0.98 ± 0.03^{A}	0.15 ± 0.02^{A}
B	7.89 ± 0.05^{C}	18.77 ± 0.02^{D}	2.08 ± 0.33^{A}	68.15	3.11 ± 0.02^{D}	3.71 ± 0.19^{C}	0.38 ± 0.08^{A}
H	1.84 ± 0.02^{D}	15.60 ± 0.03^{C}	66.38 ± 1.60^{B}	14.19	1.99 ± 0.04^{C}	13.43 ± 0.06^{D}	2.05 ± 0.18^{B}

$^{A-D}$, mean values in the same column with different superscript letters are significantly different ($p \leq 0.05$)

H contains significantly higher crude fat (66.38 g/100 g flour), in line with the values reported in the literature: 57.7 – 69.4 (Turan, Çapanoğlu and Altay 2015).

According to the fatty acid profile of hazelnut oil, most of its fat consists of monounsaturated fatty acids, which can be regarded as "healthy product". The dominant fatty acid in its structure is oleic acid (C18:1), which has been reported to prevent cholesterol-induced cardiovascular disease by increasing the number of high-density lipoproteins (HDL) and reducing the amount of low-density lipoproteins (LDL) (Turan 2018; Tüfekçi and Karataş 2018; Karaosmanoğlu and Üstün 2019). B, R, and C are also in agreement with the literature, even though they had much lower crude fat contents than H: 1.2 – 2.3, 0.2 – 1.3, 0.0 – 0.7 g/100 g flour, respectively (Park et al. 2021; Mugalavi et al. 2021; Tabasum et al. 2019). There were some problems faced due to the high fat content of hazelnut flour; therefore, crude fiber content and spectroscopic profile of hazelnut flour was determined using the defatted samples that were obtained for crude fat analysis.

Total ash content is generally defined as a precursor for the total amount of minerals since it demonstrates the total inorganic compounds in the foods (Twinomuhwezi, Godswill Awuchi and Rachael. 2020). The highest amount of total ash was detected in B (3.11 g/100 g flour), followed by H, R, and C (1.99, 0.61, and 0.16 g/100 g flour, respectively), indicating that B and H contain more minerals than R and C. Total ash contents of B, H, and R are within the ranges reported in the literature: 1.1 – 4.9; 2.05 – 2.6; 0.1 – 1.1 g/100 g flour (Salazar, Rodas and Aranibia 2020; Romero and Zhang 2019; Turan, Çapanoğlu and Altay 2015). Total ash content of corn starch was not generally reported in the literature.

Fiber takes part in the human body by promoting satiety, inhibiting constipation, and intervening the metabolism of lipids and carbohydrates. It is among the components that helps defining a product as “functional food”, due to its known positive effects on human health (i.e., it has therapeutic implications on diabetes, hyperlipidemia, and obesity), in addition to being preventive against other diseases such as hypertension, prostate cancer, coronary heart disease and high cholesterol (Slavin 2013; Afifi, Hashim and Altubji 2017). In terms of crude fiber, H stands out (13.4 g/100 g flour), followed by B, R and C (Table 4.1). Crude fiber of whole hazelnut flour was not reported in literature before, but it has been reported that hazelnuts contain insoluble dietary fiber between 10.67 – 17.21 g/100 g (Alasalvar et al. 2003; Tunçil, 2020). Even though B did not differ significantly from R and C, it still has a relatively higher range for crude fiber (2.9 – 6.8 g/100 g) in literature (Gamboa-Gómez et al. 2016; Mora-Avilés et al. 2007). Fiber contents of R and C were not reported in literature.

Phenolic compounds are known to have a positive effect on health-related considerations as well as different parameters such as taste and color of the final product (Camelo-Méndez et al. 2017; Herrero et al. 2023). More specifically, phenolics have been found to interact with starch and inhibit the digestive enzymes, which may lead to a decrease in glycemic response in vivo (Camelo-Méndez et al. 2016; Camelo-Méndez et al. 2017; Barros, Awika and Rooney 2012). Even though they were present in relatively small amounts (0.15 – 2.05 mg GAE/g flour), TPC values of the flours should still be considered important, since they are also defined as plant constituents with redox properties that are responsible for antioxidant activity (Aryal et al. 2019). The highest TPC belonged to hazelnut flour (2.05 mg GAE/g flour), which was abundant in literature with a huge range, because of the different applications on the nut: 1.00 – 91.40 mg GAE/g pomace (Li and Parry, 2011). R, C, and B did not significantly differ from each other.

4.1.2. Technological Properties

One of the essential technological properties of flour is water retention capacity (WRC) since gluten-free dough highly depends on the ability of the flour to absorb and bind water to form a dough network. WRC values as g/100 g flour are presented in Table 4.2 for B, R, and C. As proven by several studies, leguminous flours can generally retain more water than their weight (Kohajdová, Karovicova, and Magala 2011; Kohajdová, Karovicova, and Magala 2013; Liu et al. 2018). On the other hand, due to the high-fat content of hazelnut flour, its WRC value could not be determined. Turan, Çapanoğlu and Altay (2015) also could not evaluate the WRC of raw hazelnut flour. It has been reported that R and C can hold almost as much water as their own weight.

Bulk density changes based on the particle size, and it basically indicates the heaviness of the sample (Shafi et al. 2016). Among the flours of GF yeast bread, B was the most dense flour (0.83 g/mL) while H was the lightest one in the same volume (0.49 g/mL). The low bulk density of hazelnut flour was probably caused by its high fat content and larger particles. Even lower bulk densities for hazelnut flour (0.22 – 0.31 g/mL) were reported by Turan, Çapanoğlu and Altay (2015), which also showed that roasting

decreased the density of hazelnut flours significantly. Most of the studies carried out for properties of hazelnut flour were performed on fully or partially defatted ones, so no information about bulk density range for H could be evaluated. On the other hand, hazelnut flour is expected to lose density because of the defatting process due to its particles being too large (Appendix A and Figure 4.2). 0.5 – 0.8 g/mL are the bulk density ranges previously reported in the literature for B (Siddiq et al. 2010; Du et al. 2014). The bulk densities of R and C are consistent with values of 0.6-0.9 and 0.4-0.6 reported in the literature (Mahapatra 2011; Jan et al. 2020; Téllez-Morales et al. 2020; Jan, Panesar and Singh 2017).

Table 4.2. Technological properties of rice (R), corn starch (C), bean (B), and hazelnut (H) flours

Code	Water Retention Capacity (g/100 g)	Bulk Density (g/mL)	Oil Absorption Capacity (g/100 g)	Emulsion Activity (mL/100 mL)	Emulsion Stability (mL/100 mL)	Foaming Capacity (mL/100 mL)	Foaming Stability (mL/100 mL)
R	131.71 ± 2.85^{B}	0.68 ± 0.01^{CD}	125.71 ± 5.40^{AB}	53.06 ± 0.00^{AB}	96.15 ± 0.00^{A}	8.50 ± 2.12^{A}	$0.00 \pm 0.00^{-}$
C	82.65 ± 2.09^{C}	0.59 ± 0.01^{D}	95.36 ± 1.12^{BC}	50.00 ± 0.00^{B}	100.00 ± 0.00^{A}	$0.00 \pm 0.00^{-}$	$0.00 \pm 0.00^{-}$
B	261.97 ± 2.85^{A}	0.83 ± 0.03^{B}	114.48 ± 0.88^{BC}	59.00 ± 1.41^{A}	83.91 ± 0.81^{A}	8.92 ± 1.53^{A}	1.49 ± 0.72^{A}
H	N.A.$^{-}$	0.49 ± 0.00^{E}	158.50 ± 20.20^{A}	54.01 ± 4.23^{AB}	87.47 ± 12.06^{A}	12.00 ± 0.00^{A}	2.00 ± 2.83^{A}

$^{A-C}$, mean values in the same row with different superscript letters are significantly different ($p \leq 0.05$)

Oil absorption capacity (OAC) is known to enhance mouthfeel while keeping the flavor of the food product. Fundamentally, it is the total amount of fat bound by the non-polar side chain of proteins, which makes this value in the products high in protein content (Iwe, Onyeukwu and Agiriga 2016), such as B and H. There was not any information regarding the relationship between oil absorption and fat content; however, it was previously stated that surface polarity and hydrophobicity are responsible for affecting the OAC (Awuchi, Igwe and Echeta 2019). It was also claimed that presence of fiber could increase the oil absorption capacity due to its hydrophobic nature (Cui and Roberts 2009; Adeloye, Osho and Idris 2020). In addition, the amount of fat present in hazelnut flour makes it structure highly hydrophobic, which might be another reason for making its oil absorption capacity higher than B. On the other hand, the presence of the monounsaturated fatty acids (MUFA, approximately 98.2%) in H causes it to have

relatively lower OAC than its defatted or partly-defatted versions (Parcerisa et al. 1997; Tatar, Tunç and Kahyaoğlu 2015; Aditya, Liu and Sathe 2015). That is probably because the MUFA in hazelnut flour would also compete with the added oil to bind to the proteins. Overall, hazelnut flour could absorb the highest amount of oil (158.50 g/100 g) due to presence of high amounts of protein and crude fiber, even though it contains the most crude fat among the flours used in this study (Table 4.1). Similar results were also stated in other studies regarding hazelnut flour (Aditya, Liu and Sathe 2015; Turan, Çapanoğlu and Altay 2015; Tunç and Kahyaoğlu 2015). OAC of H and B was followed by R and B, which also could absorb oil higher than their weights.

The ability and capacity of a protein to assist emulsion formation and stabilize the slurry is defined by the terms "emulsion activity (EA)" and "emulsion stability (ES)" (Sreerama et al. 2012; Chandra, Singh and Kumari 2015). Basically, it is suggested that proteins can stabilize and form emulsions by creating electrostatic repulsion on oil droplet surfaces (Chandra, Singh and Kumari 2015). The highest difference in emulsion activities was observed between B (59.0 mL/100 mL) and C (50.0 mL/100 mL). Emulsion activity of H (54.01 mL/100 mL) is within the range reported for raw and roasted hazelnut flour samples: 32 – 63 mL/100 mL (Turan, Çapanoğlu and Altay 2015), whereas EA of B is slightly lower than the literature values: 63.8 – 88.9 mL/100 mL (Du et al. 2014). R, on the other hand, showed higher emulsion activity than the range reported previously: 21.6-35.2 mL/100 mL (Marcoa and Rosell, 2008; Jan et al. 2020). In terms of stabilities, no flour stands out at a 95% confidence level. R is within the range in terms of emulsion stability: 28.61 – 98.1% (Jan et al. 2020; Marcoa and Rosell, 2008). Yet, emulsion stability of B falls slightly out of the range reported: 84.2 – 96.9% (Du et al. 2014), whereas ES of H is higher even than the values reported for both raw and roasted hazelnut flours: 34-76% (Turan, Çapanoğlu and Altay 2015).

Foaming Capacity is a functional property that can be affected by the flour concentration in the water and total whipping volume. Even though the flour-to-water ratio in this study was at 3%, almost no considerable foam formation could be observed. In fact, no foam occurred in the slurry prepared with C. Consequently, foaming stabilities of R, C, B, and H did not differ significantly. However, some studies reported foaming capacity ranges of 13.4 – 25.4 and 34 – 76 percent for B and H, with an average stability range of 5-10% after 1 hour, respectively (Gupta et al. 2018; Turan, Çapanoğlu and Altay 2015). In addition, rice and corn are not well-known for their foaming abilities due to their low protein contents; thus, they were not expected to form any foam in the slurry.

L*, a*, and b* values of R, C, B, and H are presented in Table 4.3. H was significantly darker than other samples, with the lowest L*. R and C are the lightest samples, in addition to being the least red (+a*) and yellow (+b*).

Table 4.3. Color properties of R, C, B, and H

Flour	L*	a*	b*
R	97.72 ± 0.04^{A}	-1.69 ± 0.03^{C}	14.07 ± 0.03^{C}
C	96.67 ± 0.31^{AB}	-2.24 ± 0.04^{C}	11.78 ± 0.05^{D}
B	93.60 ± 1.28^{B}	0.95 ± 0.05^{B}	22.13 ± 0.09^{B}
H	71.46 ± 4.37^{C}	3.25 ± 2.49^{A}	31.14 ± 0.80^{A}

$^{A-D}$, mean values in the same column with different superscript letters are significantly different ($p \leq 0.05$)

Even though color values might change depending on its cultivar, hazelnuts are generally known for their kernels being high values of a* and b* and relatively low lightness (L*) (Ercisli et al. 2011). The presence of skin in hazelnut flour probably caused it to be darker with a more red-like and yellow color (Özdemir et al. Romero-Aroca, et al. 2021). Bean flour, on the other hand, is also more red-like and yellow than R and C but lighter than hazelnut; it was previously reported to make the end products darker and more yellow (Anton, Fulcher and Arntfield 2009; Siddiq et al. 2010; Romano et al. 2015).

4.1.3. FT-IR Spectra of the Flours

Due to its high-fat content, the pellets of hazelnut flour were prepared with defatted H samples. Hence, its FT-IR spectra were found to be slightly different than expected based on the analysis of its proximate components in terms of its fat content.

FT-IR spectra of R, C, B, and defatted H samples showed major and minor peaks at the wavenumber ranges of 3700 – 3000 cm^{-1}, 2950 – 2800 cm^{-1}, 2800 – 2750 cm^{-1}, 1800-1730 cm^{-1}, 1700 – 1400 cm^{-1}, and 1250-800 cm^{-1}. Besides, continuous decreases in the transmittances were observed within the wavenumber ranges of 1450 - 1000 cm^{-1} and 800 – 450 cm^{-1} (Figure 4.1).

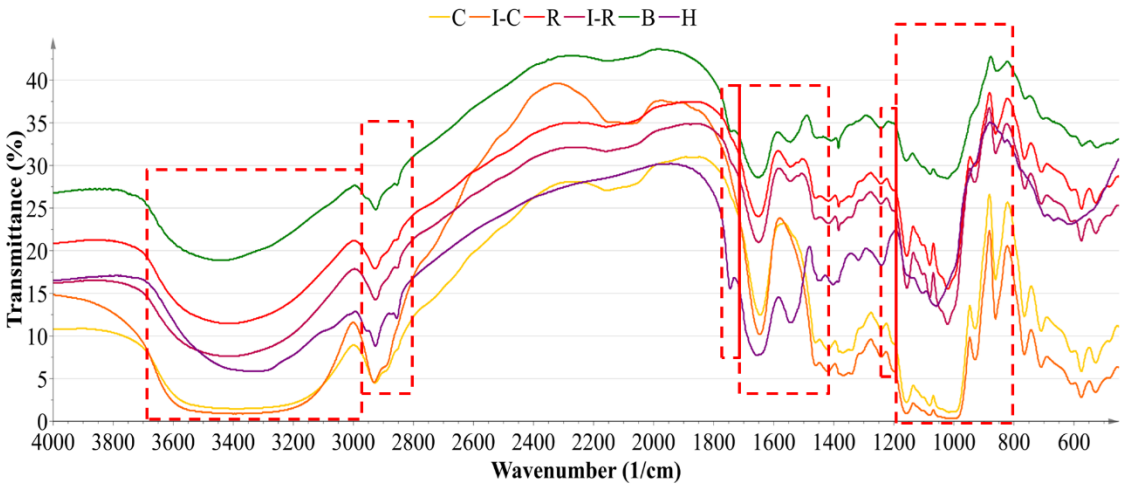

Figure 4.1. FT-IR spectra of C, R, B, and defatted H between wavenumbers 4000 and 450 cm^{-1}

The major broad region observed between 3700 and 3000 cm^{-1} is attributed to O-H stretching, demonstrating the presence of water. It also represents the hydroxyl groups of the aliphatic and phenolic structures (Casiraghi et al. 2008; Xu et al. 2023). The minor peaks following that at 3000 – 2800 cm^{-1} are attributed to C-H stretching of the side chains' methyl and methylene groups, indicating the presence of carbonyl groups which are mainly related to the lipid content. The peak around 1750 cm^{-1} shows a C=O stretching associated with the presence of the ester fatty acid groups (e.g., fat triglyceride ester linkages). The peaks around 1620, 1500, and 1300 cm^{-1} are associated with the presence of Amide I (C=O stretching), Amide II (N-H bending and C-N stretching), and Amide III (C-N) bonds, respectively. The peak around 1450 cm-1 represents the asymmetric C-H bending from the methoxyl groups.

The region between 1200 and 1000 cm^{-1} is generally called the “fingerprint region” (Saxton and McDougal 2021). In other words, the peaks within this region are the characteristic peaks for the polysaccharides in the samples. The bands between 1050 and 1200 cm^{-1} are associated with the stretching vibration of C–O and C–C in the C–O–H groups. The peaks at 1040 and 1020 cm^{-1} are attributed to the crystalline structure and amorphous region of starch, respectively (Sinelli, Casiraghi, and Downey 2008; Căpraru et al. 2009; Skendi,Papageorgiou and Papastergiadis 2021).

Considering the differences in environmental conditions and structural properties of the samples, it does not seem possible to make a comparison between flours over the

transmittance values. Distinctive differences have been observed, but these differences are insufficient to make the expected distinction on the gross composition (e.g., moisture, fat, protein, fiber, carbohydrate) using only the spectra. Hence, a multivariate statistical analysis was performed to investigate if the FT-IR spectra of the flours were able to distinguish the samples in Chapter 6.

4.1.4. Scanning Electron Microscopy (SEM) Analysis

Figure 4.2. shows the SEM images of R, C, B, and H at a magnification of 5000×. According to the observations made on the other magnification levels (Appendix A) as well as at 500×, H has the largest particles, whereas C has the lowest. When other magnifications were investigated, it was determined that almost all C particles were smaller than 20 µm, as reported in their study by Singh et al. (2003), where the particles of the flour samples (R, B, and H) were larger than 20 µm.

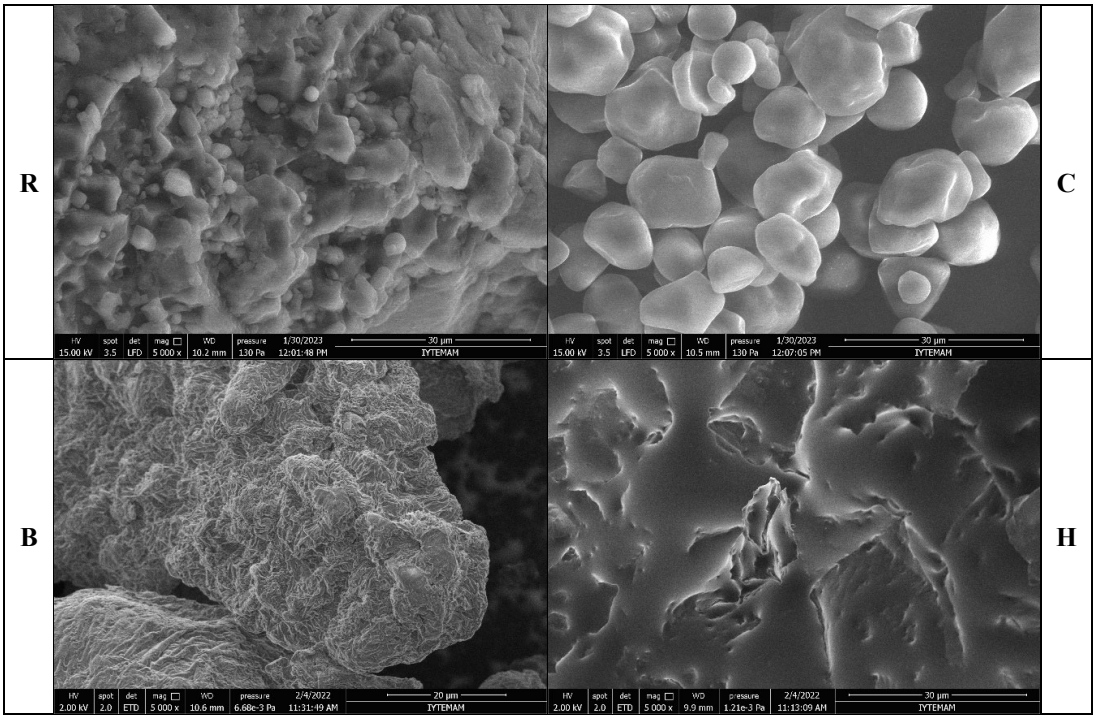

Figure 4.2. SEM images of R, C, B, and H at 5000× magnification

In line with the literature, corn starch has the smoothest and most homogeneous structure with no aggregates and almost circular (due to angles being not too sharp and a slightly polygonal geometry) shapes (Singh et al. 2003; Rodrigues et al. 2020). Particles of R, on the other hand, had a sharper and more irregular shape, with a particle size distribution between 50-100 μm, as found in the literature (Lapčíková et al. 2021). Additionally, R, B, and H showed complex structures due to the protein (more in B and H), fiber, and fat fractions (more in H) surrounding the starch granules according to the SEM images, as mentioned in several studies (Mitrus et al. 2020; Bala et al. 2020).

4.1.5. Micro Visco Analysis (MVA)

MVA is a reliable tool for assessing the gelatinization and retrogradation properties of flours and starchy substances in water through some controlled cycles of heating and cooling. In the preliminary experiments, the use of B or H alone did not result in reliable pasting properties (Appendix B). Hence, pasting properties such as gelatinization temperatures and viscosities at different phases during these heating and cooling periods in Brabender Units (BU) are given for flour mixtures formulated in this study (Figure 4.3 and Table 4.4).

Gelatinization temperature (GT, °C) is the temperature at which an initial increase in viscosity occurs. Peak viscosity (PV, Brabender Units, BU) is defined as the maximum paste viscosity achieved during heating. It represents the thickening power of the starch. Final viscosity (FV, BU) is the paste viscosity at the end of the cooling period, and it is associated with the starch molecules and their aggregation. The breakdown viscosity is the disintegration degree of the swollen starch granules during heating. In other words, the viscosity decrease index is calculated as the difference between peak viscosity and the viscosity at the end of the holding period at 95°C. The lower breakdown viscosity indicates higher resistance to heat and degradation. Finally, the setback viscosity is the viscosity index increase during cooling, which can be related to retrogradation of the gelatinized starch and be used as an indicator for rate of staling (Barrera et al. 2013; Cappa, Lucisano and Mariotti 2013; Liu et al. 2018; Pasqualone et al. 2020).

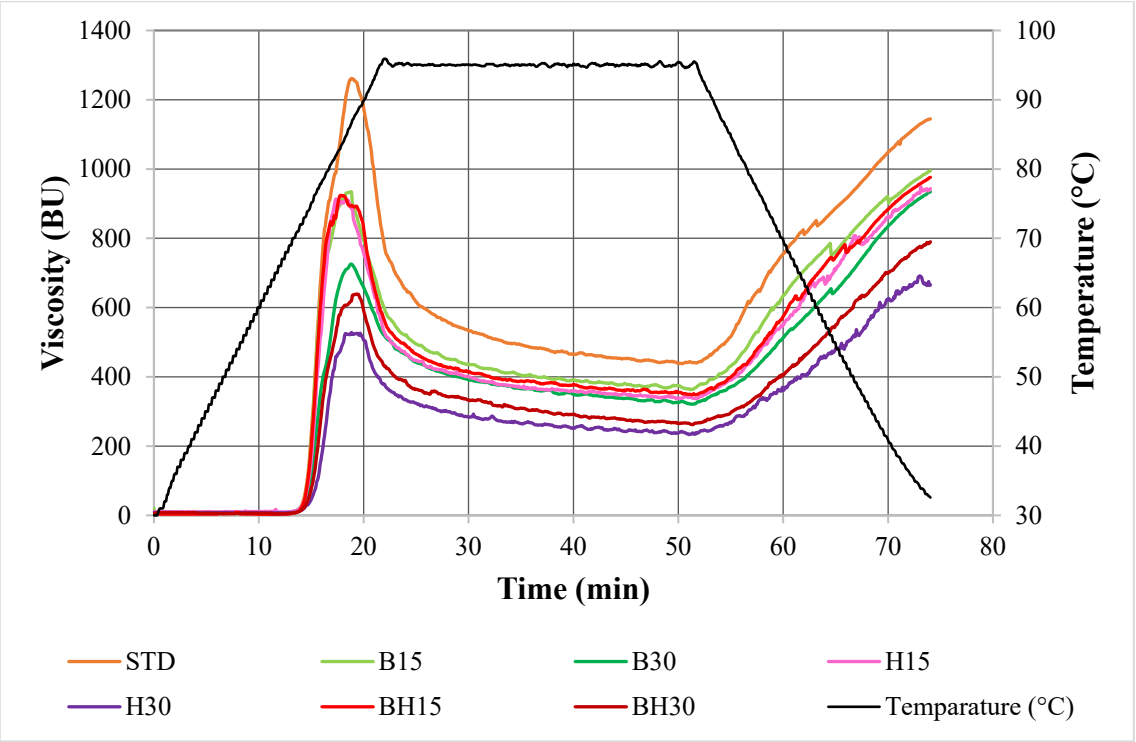

Figure 4.3. Pasting profiles of the flour mixtures of gluten-free yeast bread formulations

Due to their starch contents, C had the highest viscosity profile, followed by R. However, in B and H, there are macromolecules other than starch in high amounts, affecting the pasting properties, such as lipids, fibers, and proteins. These macromolecules are also affected by heat and interact with each other and water, making the phenomena taking place during the heating and cooling process more complex. For instance, the presence of fiber and proteins intervenes in the starch reorganization during the cooling phase and determines intense competitions during the initial hydration phase (Cappa, Lucisano and Mariotti 2013; Cappa, Kelly and Ng 2018; Pasqualone et al. 2020). Therefore, the inclusion of B and H reduced the peak and final viscosities and increased the gelatinization temperatures due to the lower starch and higher fiber, fat, and protein contents (Figure 4.3, Table 4.4). Similar findings were reported in studies comparing legume flours, rice flour, and starch (Di Cairano et al. 2020; Al-Attar et al. 2022). For example, Webb et al. (2023) found that the addition of 20% legume flour resulted in significantly higher temperature requirements to develop peak viscosity. It was due to the increase in the amylose content in the starch molecules, which is high in legume flours (Aguiar et al. 2022). This characteristic of the legume flours can also be explained by the

lower accessibility of starch granules by α-amylase, which can lead to a low glycemic index (Zhu et al. 2011; Gularte, Gómez and Rosell 2012).

Table 4.4. Pasting properties of the flour mixtures

Sample	Gelatinization temperature (°C)	Peak viscosity (BU)	Breakdown (BU)	Setback (BU)	Final Viscosity (BU)
STD	71.5 ± 0.1^{C}	1276 ± 2^{E}	835 ± 3^{E}	687 ± 4^{D}	1145 ± 2^{E}
B15	71.3 ± 0.1^{BC}	937 ± 19^{C}	572 ± 22^{C}	630 ± 17^{C}	995 ± 14^{D}
B30	71.6 ± 0.1^{C}	726 ± 29^{B}	404 ± 13^{B}	613 ± 1^{C}	934 ± 17^{C}
H15	71.9 ± 0.1^{CD}	948 ± 17^{C}	611 ± 11^{CD}	607 ± 15^{C}	944 ± 9^{C}
H30	72.9 ± 0.4^{E}	536 ± 40^{A}	301 ± 33^{A}	431 ± 8^{A}	666 ± 14^{A}
BH15	71.7 ± 0.1^{C}	937 ± 26^{C}	586 ± 21^{C}	625 ± 8^{C}	976 ± 13^{CD}
BH30	72.5 ± 0.2^{DE}	641 ± 24^{AB}	378 ± 16^{AB}	527 ± 1^{B}	790 ± 9^{B}

$^{A-E}$, mean values in the same column with different superscript letters are significantly different ($p \leq 0.05$)

The highest breakdown and setback values were obtained from STD, suggesting that this formulation was more susceptible to staling, where reduction in RC amount by adding H and B (H15, H30, BH15, and BH30) significantly reduced breakdown, setback, and final viscosities. The lowest setback values obtained by H30 and BH30 (431 and 527 BU, respectively) propose that H addition can retard the starch retrogradation. On the other hand, adding B to the formulations (i.e., B15 and B30) resulted in intermediate peak and setback viscosities due to its relatively lower starch content.

4.2. Dough Analysis

In this section, dough properties such as farinographic water absorption (that determined the water added (g) to the dough based on decided dough consistency), dough leavening (the change of dough area during fermentation, starting from a constant dough weight, 10 g), and FT-IR analysis (specific component regions and differences in

absorbances at those specified regions) were discussed. Bread formulations are given in Table 4.5.

Table 4.5. Exact formulations used to prepare GF yeast breads (g/g total dough)

Ingredient	STD	B30	B30	H15	H30	BH15	BH30
Flour (total)	250.5	250.5	250.5	250.5	250.5	250.5	250.5
R	*125.3*	*106.5*	*87.7*	*106.5*	*87.7*	*106.5*	*87.7*
C	*125.3*	*106.5*	*87.7*	*106.5*	*87.7*	*106.5*	*87.7*
B	-	*37.6*	*75.2*	-	-	*18.8*	*37.6*
H	-	-	-	*37.6*	*75.2*	*18.8*	*37.6*
HPMC	4.5	4.5	4.5	4.5	4.5	4.5	4.5
Olive Oil	18.0	18.0	18.0	18.0	18.0	18.0	18.0
Sugar	12.0	12.0	12.0	12.0	12.0	12.0	12.0
Salt	6.0	6.0	6.0	6.0	6.0	6.0	6.0
Compressed Yeast	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Water	180.3	210.6	241.5	153	131.7	180.6	187.5
Total Dough	***480.3***	***510.6***	***541.5***	***453.0***	***431.8***	***480.6***	***487.5***

4.2.1. Farinographic Water Absorption

Water absorption capacities (i.e., Farinographic Water Absorption) of the doughs were determined for 200 Brabender® Units (BU) during kneading, after all ingredients were added (Table 4.6). 200 BU was selected since it gave the dough a liquid-like consistency, as previously claimed to be preferable in gluten-free breads with similar formulations (Tufaro, Bassoli and Cappa 2022; Cappa, Lucisano and Mariotti 2013). The water absorption capacities of the doughs, which resulted the highest in B- and lowest in H-containing formulations, were highly dependent on WRC of flours.

Table 4.6. Farinographic water absorption capacities of the gluten-free yeast bread formulations according to 200 BU dough consistency

Sample	STD	B15	B30	H15	H30	BH15	BH30
Water Absorption (%)	60.1	70.2	80.5	51.0	43.9	60.2	62.5

4.2.2. Gluten-Free Yeast Bread Dough Leavening Properties

Observation of the dough leavening behavior through image acquisition is an alternative and a less time-consuming method. It helps evaluate the dough development of different formulations during the leavening process through dough area increase (Cappa, Lucisano and Mariotti 2013; Hager and Arendt, 2013). Images and leavening profiles of the samples are given in Figures 4.4 and 4.5, numerical data are in Appendix C.

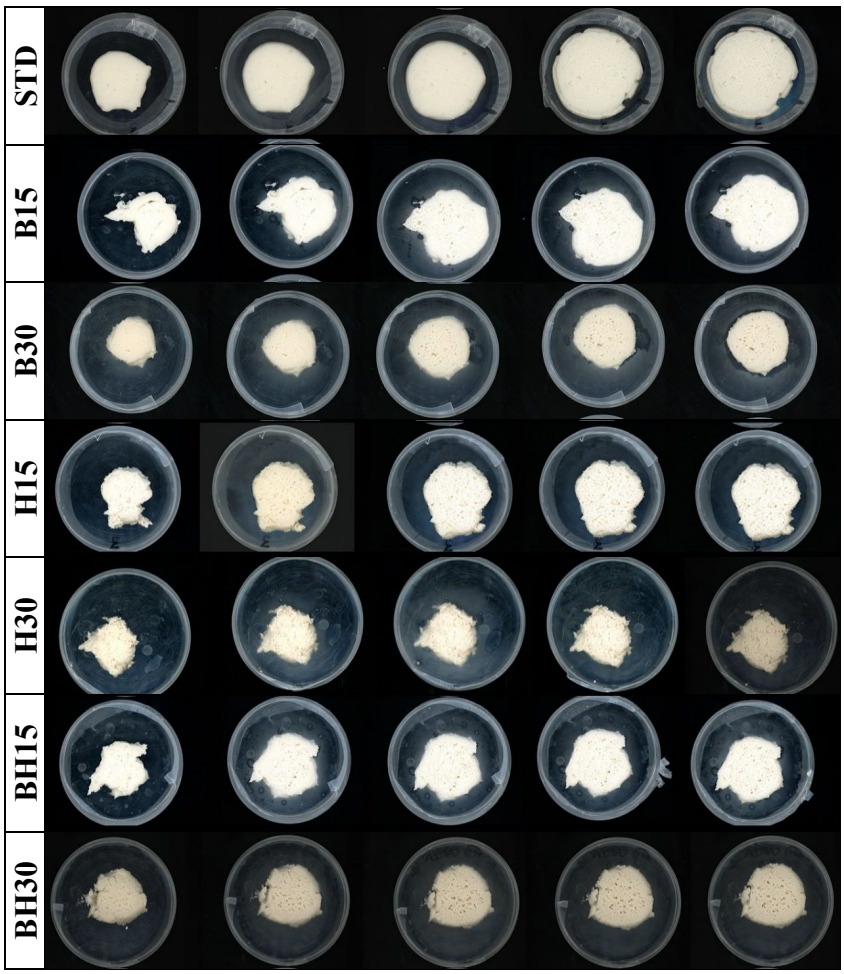

Figure 4.4. Leavening images of gluten-free yeast bread dough samples from 0^{th} to 60^{th} minutes of fermentation

The results suggest that the inclusion of H in the formulations caused the dough network to be weaker, affecting the network more than B. This can mainly be associated with H having the largest particles among the flours used in this study (Figure 4.2, from section 4.1.4). It is widely known that particle size significantly affects the final product structure. Similarly, in some cases, the use of coarse powders resulted in less-developed gluten-free products such as thinner cookies (Cappa et al., 2020) and bread with low volume (Qin et al., 2021) were reported.

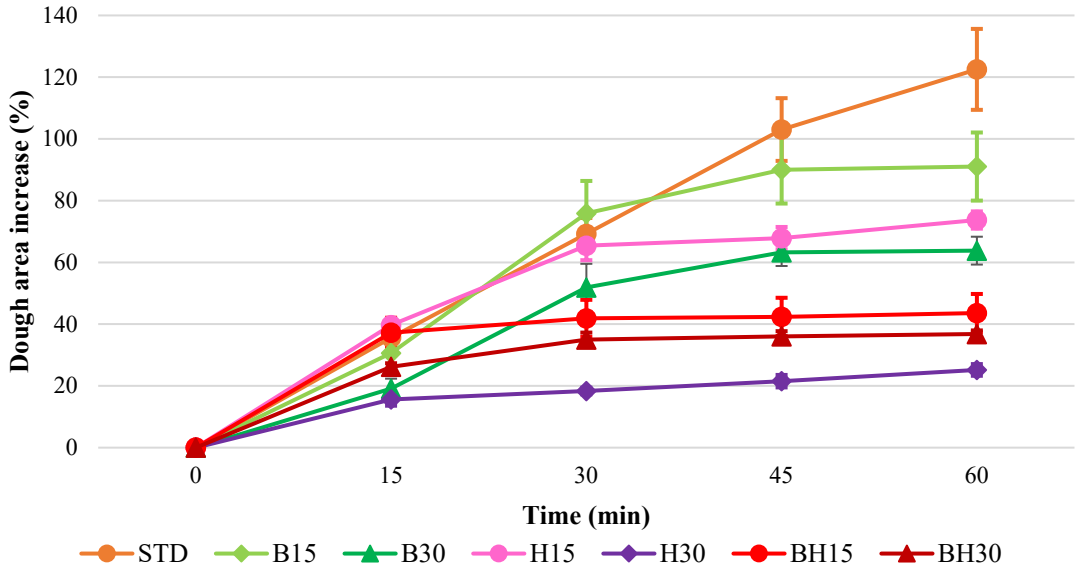

Figure 4.5. Leavening profiles of gluten-free yeast bread dough samples from 0th to 60th minute of fermentation

Overall, the replacement of RC at a 30% level by H, B, or the combination of them (BH) resulted in a lower dough area during leavening, on the other hand, a reduction in replacement percentage (i.e., replacing 15% of RC with H, B, or BH) caused the dough area to approach that of STD. Relatively, in several studies, it was found that the addition of fibrous material (Anil 2007) or legume flours (Bojňanská, Musilová and Vollmannová 2021; Kotsiou et al. 2022) negatively affects the ability of the dough to rise. These results can be concluded by claiming that the addition of B and H, because of their fiber contents being higher than rice flour and corn starch, limited dough extensibility and expansion capacity by altering the dough strength.

4.2.3. Gluten-Free Yeast Bread Dough FT-IR Analysis

FT-IR spectra of the gluten-free bread dough samples showed major and minor peaks at 3700-3000 cm^{-1}, 3000-2900 cm^{-1}, 2900-2850 cm^{-1}, 1770-1730 cm^{-1}, 1700-1590 cm^{-1}, 1580-1490 cm^{-1}, 1455 cm^{-1}, 1240 cm^{-1}, 1190-1082 cm^{-1}, 1046 cm^{-1} and 1021 cm^{-1} (Figure 4.6).

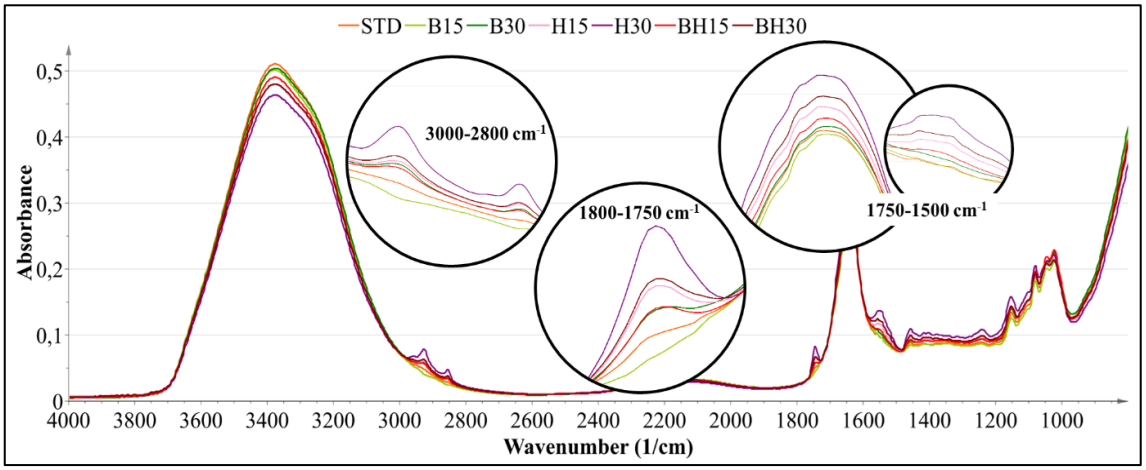

Figure 4.6. FT-IR spectra of the gluten-free yeast bread doughs: STD, B15, B30, H15, H30, BH15, and BH30 between wavenumbers 4000 and 800 cm^{-1}

The first major peak observed between 3700 and 3000 cm^{-1} is attributed to O-H stretching, which demonstrates the presence of water. It may also show the hydroxyl groups in the aliphatic and phenolic structures (Casiraghi et al. 2008; Xu et al. 2023). The minor peaks following that at 3000 – 2850 cm^{-1} are attributed to C-H stretching of the side chains' methyl and methylene groups, representing the presence of carbonyl groups. The peak around 1750 cm^{-1} shows a C=O stretching attributed to the fat triglyceride ester linkage (e.g., the presence of the ester fatty acid group). The peaks around 1650, 1550, and 1240 cm^{-1} are associated with the presence of Amide I (80% C=O stretching, 10% C-N stretch), Amide II (60% N-H bending, 30% C-N stretching, and 10% C-C stretching), and Amide III (C-N) bonds, respectively (Kotsiou et al. 2021).

The region between 1200 and 1000 cm^{-1} is labeled as the fingerprint region (Saxton and McDougal 2021). In other words, the peaks within this region are the

characteristic peaks for the polysaccharides. The peak around 1450 cm^{-1} shows the asymmetric C-H bending from the methoxyl groups, The bands between 1100 and 1190 cm^{-1} are associated with the stretching vibration of C-O and C-C in the C-O-H groups. The peaks at 1050 and 1020 cm^{-1} are attributed to the crystalline structure and amorphous region of starch, respectively (Sinelli, Casiraghi, and Downey 2008; Căpraru et al. 2009; Skendi,Papageorgiou and Papastergiadis 2021).

A minimum amount of water was added to H30 dough formulation, which caused lower absorption between wavenumbers 3000-2800 cm^{-1}. Around 1750 cm^{-1}, it also differed from the spectra of the other samples due to its higher fat content. The same trend was also observed for the wavenumber range 1650 – 1000 cm^{-1}, which can be associated with the high protein and fiber content of H. Higher absorption at the peaks showing the protein and fiber contents was also observed for the formulations containing B and H, especially BH30 and BH15.

4.3. Bread Analysis

During baking, the weights of the GF yeast bread samples significantly decreased according to baking loss results, representing partial water removal. The most reduction occurred in STD bread, whereas the lowest occurred in the samples containing H flour (in increasing order: H30, BH30, BH15, and H15). Low baking loss of those made with H flour can be related to relatively lower water addition levels, which were mainly affected by the water absorption values that varied, primarily because of the fat, fiber, and protein contents of the flours (Table 4.2 from section 4.1, and Table 4.7). Consequently, the moisture contents of the slices and the crumbs were strongly related to the water absorption capacities of the doughs (for 200 BU dough consistency) with correlation scores of 0.920 and 0.986, respectively (Pairwise Pearson correlation). Moisture contents were lower for the H-containing loaves. On the contrary, the use of B significantly increased the slice and crumb moisture since the amount of water added during kneading was considerably higher in the loaves that contained only B than the ones where RC composite was replaced with only H (70.2 – 80.5 g/100 g and 43.9 – 51.0 g/100 g, respectively). Another water-related measurement, water activity, indicates the difference

in the number of soluble component contents of the bread crumbs and the amount of water initially added (Osella et al. 2005; Rybicka, Doba and Bińczak 2019). Hence, water activity was higher in STD bread and the B-containing samples than the water activity of H breads.

Specific volume, which affects consumer choice, is one of the technological properties to determine a preferable bread since it gives foresight about its physical characteristics (Monteiro et al. 2021). As can be interpreted from the increase in the dough area during leavening, STD had significantly higher volume and, consequently, higher specific volume than other samples (Table 4.7). It was also slightly greater than the study where a standard bread with a similar formulation (with the addition of psyllium and pea protein, 4 mL/g) and breadmaking conditions (Tufaro, Bassoli and Cappa 2022), and the specific volume significantly decreased as replacement amount of B, H or both increased. Xhabiri and Hoxha (2022) also observed a decrease in the specific volumes of bread loaves as the proportion of white bean flour increased (up to 25%). Similar observations were made by Sahagún and Gómez (2018) in GF bread enriched with pea proteins and by Azeez et al. (2022) in conventional bread with the addition of cashew nut proteins. In contrast, however, Kahraman et al. (2022) reported a specific volume higher than 2.5 mL/g in GF breads prepared with differently treated chickpea flours (i.e., raw, roasted, and dehulled) where they replaced 25% of rice flour in the formulation. Overall, their results suggested that the amount of added flour, its composition (i.e., fiber and protein content), and physical properties (i.e., foaming capacity and flour particle size) affect bread development. Specific volume greater than 2.5 mL/g was also observed in the formulations of this study that replaced 15% of RC with B, H, or both. In fact, there is a vast range of specific volume (1.3 – 7.58 mL/g) in literature (Hager & Arendt, 2013; Mariotti et al. 2013; Cappa, Lucisano and Mariotti 2013; Tufaro, Bassoli and Cappa 2022; Belorio and Gómez 2020). Moreover, it was stated by Monteiro et al. (2021) that bread specific volume above 3.5 mL/g could be counted as a threshold for GF bread quality, which was met only by H15 and STD.

As expected, the STD formulation had a greater porosity percentage than other formulations, with a value of 27.9%. A closer porosity ratio was also observed in a study that used a similar formulation with pea protein and psyllium addition (Tufaro, Bassoli and Cappa 2022) and another one that used 200 BU as the dough consistency (Cappa, Lucisano and Mariotti 2013).

Table 4.7. Physical and technological properties of GF yeast bread samples

Properties	STD	B15	B30	H15	H30	BH15	BH30
Baking Loss (%)	23.7 ± 0.8^{E}	19.3 ± 0.7^{CD}	20.0 ± 0.5^{D}	19.3 ± 0.8^{CD}	13.2 ± 0.3^{A}	17.7 ± 1.3^{BC}	17.5 ± 0.9^{B}
Crumb Moisture (g/100 g)	41.1 ± 0.7^{C}	46.4 ± 0.2^{E}	50.4 ± 0.2^{F}	39.4 ± 0.3^{B}	36.3 ± 0.4^{A}	43.3 ± 0.2^{D}	44.3 ± 0.2^{D}
Slice Moisture (g/100 g)	27.8 ± 0.6^{A}	34.5 ± 0.4^{C}	39.4 ± 0.3^{D}	27.5 ± 0.1^{A}	27.4 ± 0.4^{A}	32.2 ± 0.6^{B}	32.7 ± 0.1^{B}
Crumb Water Activity	0.985 ± 0.001^{D}	0.985 ± 0.006^{D}	0.979 ± 0.001^{CD}	0.965 ± 0.003^{B}	0.951 ± 0.002^{A}	0.977 ± 0.001^{CD}	0.971 ± 0.003^{BC}
Specific Volume (mL/g)	7.0 ± 0.2^{E}	2.7 ± 0.1^{C}	1.9 ± 0.1^{AB}	3.8 ± 0.1^{D}	1.7 ± 0.1^{A}	2.7 ± 0.1^{C}	2.4 ± 0.1^{BC}
Crumb Porosity (%)	27.9 ± 6.1^{B}	18.0 ± 1.0^{A}	19.9 ± 1.7^{A}	17.8 ± 1.5^{A}	17.5 ± 1.7^{A}	18.8 ± 1.3^{A}	20.9 ± 3.2^{A}
Class 1	9.3 ± 2.8^{BC}	7.5 ± 1.1^{C}	8.6 ± 1.4^{BC}	11.2 ± 1.8^{B}	16.9 ± 1.6^{A}	8.1 ± 1.2^{C}	8.3 ± 1.5^{C}
Class 2	10.1 ± 2.9^{C}	12.4 ± 1.9B^{C}	14.9 ± 1.9^{BC}	16.5 ± 1.9^{B}	22.7 ± 4.7^{A}	13.1 ± 1.2B^{C}	13.5 ± 2.1^{BC}
Class 3	12.5 ± 2.1^{B}	18.2 ± 3.0^{AB}	21.9 ± 3.9^{A}	20.3 ± 4.2^{A}	23.7 ± 4.4^{A}	18.4 ± 2.1^{AB}	18.1 ± 1.9^{AB}
Class 4	28.0 ± 5.2^{C}	49.5 ± 5.3^{A}	49.5 ± 5.4^{A}	41.9 ± 4.5^{AB}	36.7 ± 2.5^{BC}	48.2 ± 5.4^{A}	49.3 ± 3.3^{A}
Class 5	40.2 ± 5.7^{A}	12.4 ± 8.7^{B}	5.2 ± 4.9^{BC}	10.1 ± 8.3^{BC}	0.0 ± 0.0^{C}	12.1 ± 6.0^{B}	10.8 ± 5.6^{BC}
Crumb hardness (N)	0.43 ± 0.04^{A}	5.27 ± 0.37^{BC}	4.92 ± 0.33^{B}	1.59 ± 0.21^{A}	14.18 ± 1.08^{E}	7.97 ± 0.64^{D}	7.15 ± 0.48^{CD}
Crust Color							
*L**	77.6 ± 0.7^{A}	68.1 ± 0.8^{BC}	72.6 ± 3.3^{AB}	64.9 ± 0.8^{C}	67.6 ± 0.3^{BC}	69.0 ± 0.5^{BC}	66.1 ± 0.2^{C}
*a**	-0.5 ± 0.3^{C}	-0.4 ± 0.0^{C}	-0.6 ± 0.0^{C}	5.0 ± 0.1^{A}	0.9 ± 0.0^{B}	-0.6 ± 0.4^{C}	0.2 ± 0.2^{BC}
*b**	29.1 ± 0.3^{A}	30.3 ± 0.5^{A}	28.7 ± 4.5^{A}	33.1 ± 1.0^{A}	28.2 ± 0.6^{A}	28.8 ± 0.7^{A}	28.7 ± 0.5^{A}
ΔE	-	9.5 ± 0.0^{AB}	6.1 ± 2.3^{A}	14.4 ± 1.0^{C}	10.1 ± 0.4^{ABC}	8.6 ± 0.5^{AB}	11.6 ± 0.2^{BC}
Browning Index	44.6 ± 1.4^{B}	56.2 ± 0.4^{AB}	48.7 ± 12.5^{B}	74.4 ± 4.1^{A}	53.3 ± 1.1^{B}	51.7 ± 2.6^{B}	55.2 ± 1.7^{AB}
Crumb Color							
*L**	84.6 ± 0.2^{A}	75.6 ± 0.0^{C}	75.9 ± 0.3^{C}	79.6 ± 0.8^{B}	67.6 ± 1.2^{E}	71.8 ± 0.6^{D}	72.5 ± 0.1^{D}
*a**	-2.6 ± 0.1^{CDE}	-3.0 ± 0.1^{E}	-2.8 ± 0.1^{DE}	-1.9 ± 0.1^{BC}	-0.9 ± 0.4^{A}	-2.2 ± 0.1^{BCD}	-1.8 ± 0.2^{B}
*b**	5.2 ± 0.4^{F}	10.0 ± 0.5^{E}	12.4 ± 0.0^{C}	10.6 ± 0.0^{DE}	17.3 ± 0.7^{A}	12.2 ± 0.2^{CD}	14.4 ± 0.5^{B}
ΔE	-	10.2 ± 0.3^{B}	11.3 ± 0.2^{B}	7.4 ± 0.5^{A}	21.0 ± 0.6^{D}	14.4 ± 0.5^{C}	15.2 ± 0.2^{C}
Browning Index	4.0 ± 0.4^{E}	10.9 ± 0.6^{D}	14.6 ± 0.1^{C}	12.1 ± 0.0^{D}	27.8 ± 0.4^{A}	15.2 ± 0.1^{C}	19.7 ± 0.5^{B}

$^{A-F}$, mean values in the same row with different superscript letters are significantly different ($p \leq 0.05$)

Crumb porosity was found to be strongly correlated to specific volume (Pearson correlation coefficient = 0.836, Appendix D). At the same time, it had weaker affiliations with baking loss, dough leavening, and crumb hardness with correlation coefficients of 0.682, 0.622, and -0.514, respectively (any negative value indicates an inverse relationship between properties). Other formulations did not significantly differ from each other in terms of total crumb porosity. However, some discrimination could be made based on their pore sizes. As explained in Chapter 3, holes of the crumbs were divided into 5 classes depending on their sizes: (1) $0.05 \leq x < 0.2$ mm^2, (2) $0.2 \leq x < 0.5$ mm^2, (3) $0.5 \leq x < 1$ mm2, (4) $1 \leq x < 5$ mm^2 and (5) $5 \leq x < 10$ mm^2 (x: pore size, mm^2). Differences between the crumb porosities of the GF yeast bread samples can also be observed in Figure 4.7.

STD had majorly larger pores than other formulations (40.2% of its pores were larger than 5 mm^2), whereas H30 did not have any pores larger than 5 mm^2 (Table 4.7). Additionally, the largest pores of other formulations (except H30) had high standard deviations, indicating that the large pores were inconsistent. However, H30 had a considerably higher number of small pores. In fact, 63.3% of its pores were smaller than 1 mm2, while other formulations mostly had pores greater than 1 mm^2 (≥50.0%). It was also observed in the porosity results that an increased amount of B or H addition decreased the percentage of larger pore sizes (i.e., the number of large pores was higher in B15 and H15 than in B30 and H30, respectively).

Crumb hardness (N) values of the formulations were found to be related to specific volumes and crumb porosities (Appendix D, Pearson pairwise correlation: 0.716 and 0.514, respectively), and STD bread was significantly softer than other samples. The negative effect of RC replacement with increasing amounts of B, H, or both on bread porosity and specific volume also affected the crumb hardness of the samples. Similar findings were reported by Pycia and Ivanosova (2020), in which the effect of walnut and hazelnut flours in wheat bread were investigated. Collar and Angioloni (2017) also produced high-legume wheat-based breads with high hardness and low specific volume. An increase in hardness values was also observed in sourdough wheat bread mixed with legume flours depending on the amount of legume replacement (Rizzello et al. 2014). However, it was also suggested recently that softer crumbs could be obtained by reducing the dough consistency to 125 BU instead of preparing doughs with a consistency of 200 BU (Kahraman et al. 2022).

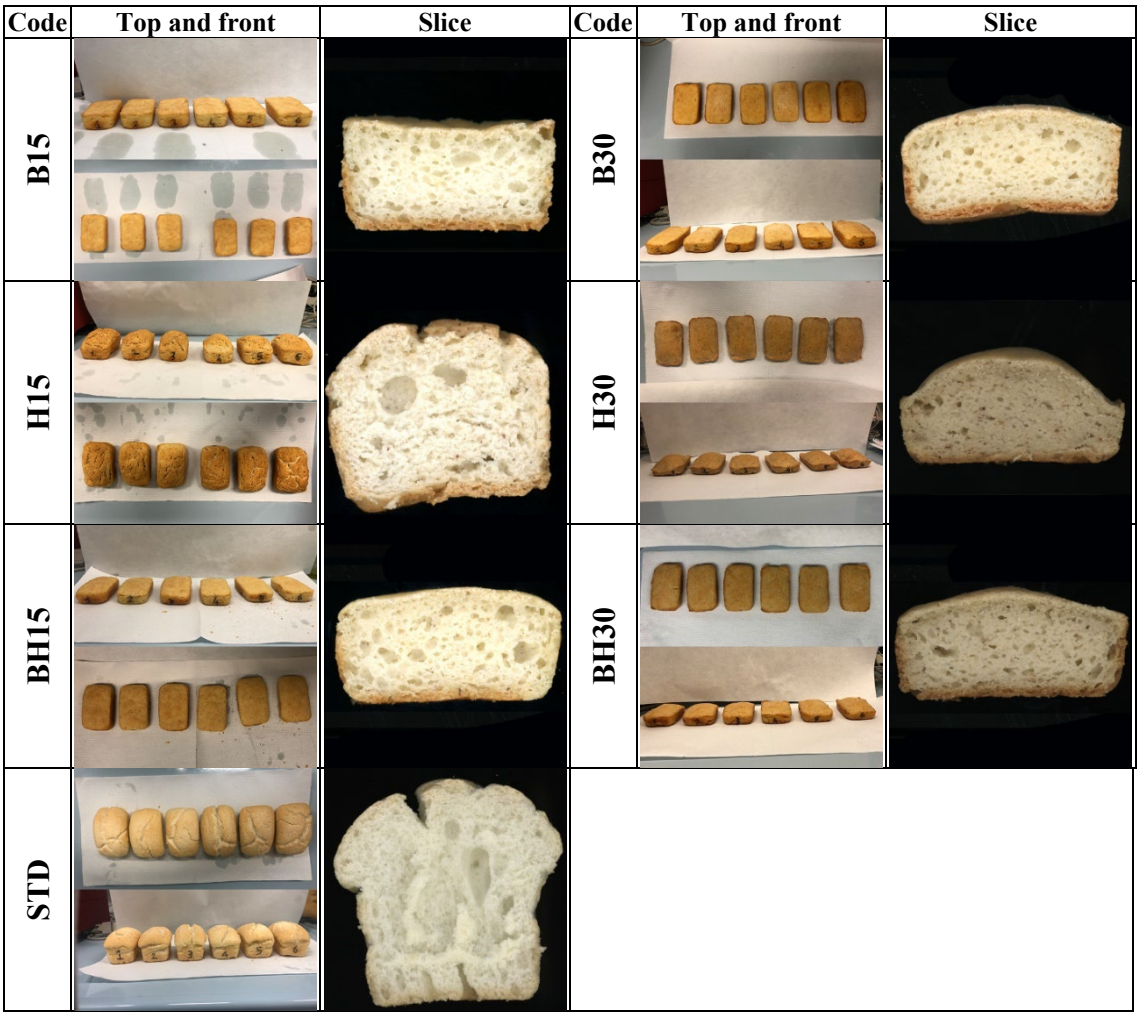

Figure 4.7. Top and front view images and slice scans of the GF yeast bread formulations

The colors of the crust and crumb are known to be among the properties that influence consumer preference (Arslan et al. 2019). The color of a final product depends on the color values of its raw materials and their physicochemical characteristics, such as amino acid and moisture content, pH, and reducing sugars. For instance, most of the GF yeast bread samples of this part had L*, a*, and b* values that reflected the color of the flours used in the formulations. The color of a final product is also related to baking conditions (e.g., temperature, heat transfer mode, and relative humidity) (Esteller and Lannes, 2008). More specifically, sugar caramelization and Maillard reaction affect crust color, but crumb color is not influenced that much. It is because the outer part of the dough hardens with the effect of heat during baking and forms the crust; hence, the bread crumbs are not exposed to the high temperature as the crust (Phimolsiripol et al. 2012; Arslan et

al. 2019). Phimolsiripol et al. (2012) also reported that using HPMC had an effect on both lightness (L*, increase) and redness (a*, decrease).

The changes in crust and crumb color values of the GF yeast bread samples are presented in Table 4.7. The lightest crusts and crumbs belonged to STD, as its flours (R and C) were determined to be the lightest ones. STD also had the lowest a* (redness) and b* (yellowness) values and, consequently, the least browning index (BI) among the samples. The darkest color was observed in the crust of H15, while H30 had the darkest crumb. Besides, the crumb color of H15 was the lightest after STD. Hazelnut is a well-known fiber source, mainly due to its testa, and its addition made crumbs darker. In fact, a decrease in the crumb lightness because of the addition of nut flour or other fiber sources was reported previously (Kurek and Wyrwisz 2015; Pycia and Ivanišová 2020). The reddest crust and crumb were H15 and H30, respectively, and samples containing B had reduced degrees of redness. Hazelnut testa is known for its red-like color, which in case, is expected to affect the color of the final product significantly. In their respective studies, Anil (2007) and Velioğlu et al. (2017) also observed darker and more red breads as the hazelnut testa amount increased in their breads. Hence, in terms of redness, it can be claimed that the presence of hazelnut made the red color dominate over green, as a* values was above 0 only in those containing H (except BH15) (Popov-Raljić et al. 2013). On the other hand, crust yellowness (b*) did not differ among the samples, while b* of the crumb was highest in H30 and lowest in STD. The addition of bean flour also increased the yellowness of the crumbs.

In the color difference (ΔE) calculations, average L*, a*, and b* of the STD were accepted as the standard reference values. Hence, the overall difference between STD and breads enriched with B, H, or both, was evaluated. ΔE of the crust was highest in H15, while the overall crust color of B30 resembles STD the most. The Crumb colors of the samples differed from STD more than the crusts. H15 was the sample the most similar to STD in terms of crumb color, whereas H30 differed the most. The correlation of ΔE of crust and crumb with lightness (L*) of crusts and crumbs was solid and negative (Pearson correlation coefficients= –0.969 and –0.999), while yellowness (b*) of the crumb was also firmly yet positively correlated (PCC=0.952) to ΔE. On the other hand, redness (a*) of crust and crumb, and crust yellowness (b*) showed weaker and positive correlations (PCC=0.569, 0.618, and 0.410), respectively.

The browning index basically indicates the purity of the brown color, and it is among the most common parameters that demonstrate browning in food products

(Pathare et al. 2013). It was previously explained that heat during baking did not affect crumbs as much as the crusts. Hence, browning indexes of crumbs being less than crusts were also expected. In fact, BI of STD and H15 were the lowest values, whereas H30 was the highest, due to the flour being used with testa and its high fiber content. BI of crust and crumb were strongly correlated to redness (a*, Pearson correlation coefficients=0.990 and 0.964, respectively), while lightness (L*, PCE= -0.567 and -0.479) and yellowness (b*, PCE= 0.652 and 0.534) had weaker negative and positive correlations, respectively.

4.4. GF Yeast Bread Storage Analysis

Bread samples stored for 24 and 48 h were evaluated for some quality properties (Figures 4.8 and 4.9). Similar to the baking loss of fresh bread loaves, moisture loss (storage loss) of the stored breads were sorted in decreasing order: STD, B30, B15, H15, BH15, BH30, and H30. This can be explained by the difference in the water addition amounts and the ability of R and C to retain less water, while B and H can retain more due to their higher protein and fiber contents, respectively.

As expected, the slice and crumb moisture of B30 was the highest, while STD and H15 had the least moisture among all formulations after both 24 and 48 hours of storage. The softest crumbs belonged to STD breads after both 24 and 48 hours of storage, while H30 crumbs were the hardest. Nevertheless, the change ratio in crumb hardness values (i.e., rate of staling) was higher in STD than in other formulations (Appendix E). Likewise, STD had a more drastic decrease in terms of water activity than other formulations after 48 hours of storage (from 0.984 at t0 to 0.887 at t48, Appendix E). The lowest water activity was observed for H15 (aw 0.883) at the end of 48 hours of storage.

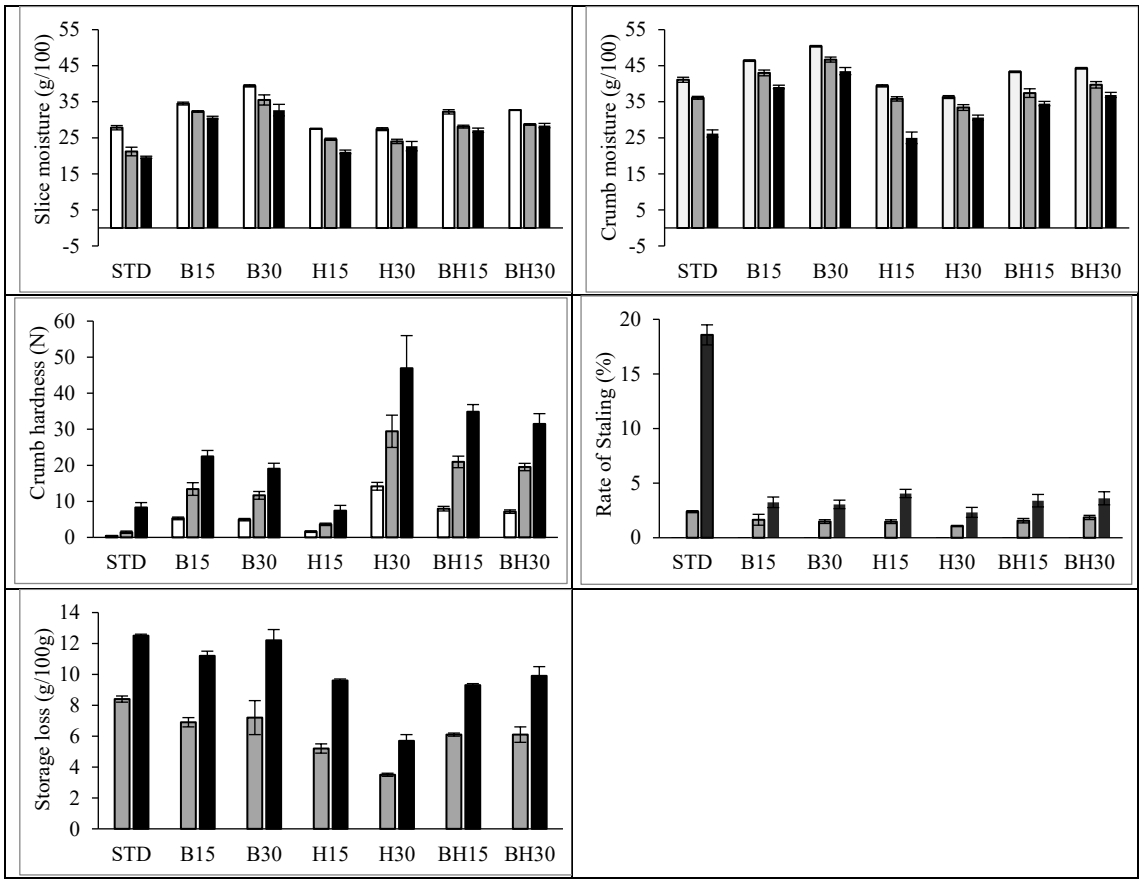

Figure 4.8. Change in the physical properties of GF yeast breads during storage where white, gray, and dark gray colors indicate breads at t=0, t=24 h, and t=48 h, respectively

Crust and crumb color parameters also showed similar patterns to those of fresh breads, with STD being the lightest (i.e., highest L*) while the ones containing H were the darkest. On the other hand, the breads containing H had higher a* values, indicating the degree of their redness as a result of hazelnut skin presence in H flour. The parameter showing the yellowness (b*) of the breads was highest in the breads containing B for the crust and H for the crumb.

Even more reliable investigation regarding the color properties of the crusts and crumbs of the breads can be made by evaluating ΔE and BI since these values provide the opportunity to compare the colors of different formulations with a single value. In particular, ΔE of crusts and crumbs showed that there was indeed a change in at least one of the bread samples, with the deviation from STD (fresh, avg) being more noticeable in crumbs (especially at t=48) than crusts.

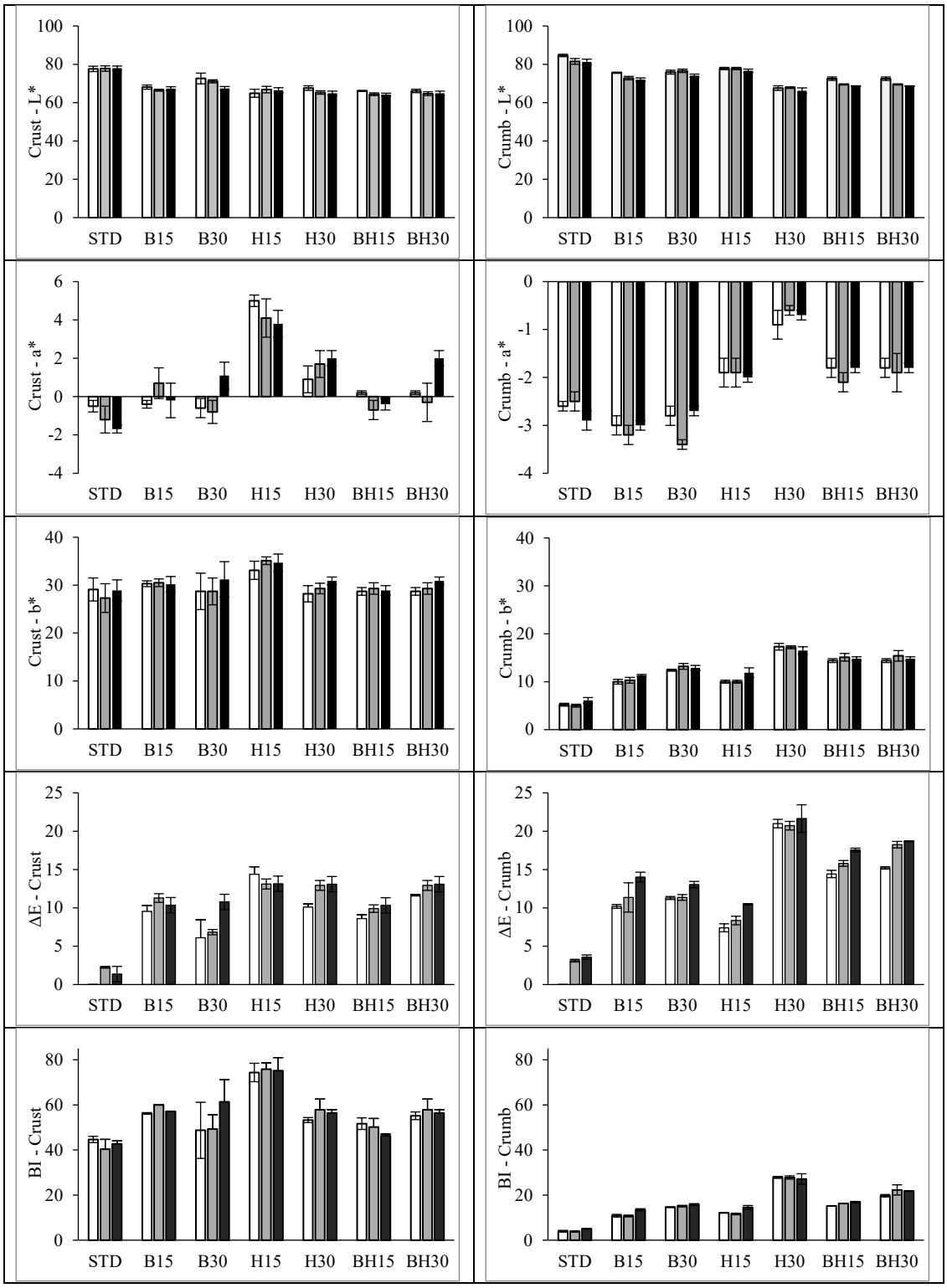

Figure 4.9. Color-related properties of fresh (white), and stored (t = 24 h, gray; t = 48 h, dark gray) GF yeast breads

4.5. Results of Mixture Design

Table 4.8 presents the ANOVA results of mixture design along with the coefficient terms and their signs. In brief, models for all parameters were significant except crust a*, b*, ΔE, browning index, and crumb hardness. Among the insignificant parameters, crust a*, crust b*, and browning index along with crumb hardness had adjusted R^2 values smaller than 0.75. Even though the model created for crumb hardness was not significant, quadratic interactions of RC with B and H were relatively more effective than the linear terms. The quadratic term HxRC was influential in specific volume, crumb porosity (in addition to its 4^{th} and 5^{th} classes), crumb hardness, and crust color (except the browning index). BxRC interaction term, on the other hand, was effective in baking loss, specific volume, crumb porosity (along with 4^{th} and 5^{th} classes), crumb hardness, crust L*, and crust browning index. BxH interacted with the parameters the least, as it was only found in 2^{nd}, 3^{rd}, 4^{th} and 5^{th} classes of crumb porosity and crust lightness (L*). The cubic interaction term (BxHxRC) was insignificant in all models except 1^{st} class of crumb porosity, where it showed that the interactions between B, H and RC decreased the percentage of 1^{st} class pores among the total number of holes.

Overall, the analyses gave significant results regarding mixture design with the exceptional parameters described in the previous paragraph. Replacement of RC with B, H, or both was found to be effective according to the mixture design models. The models successfully explain the data with adjusted R2 values above 0.80 (except crumb hardness, crust a*, b* and browning index, and crumb L* and ΔE.

In gluten-free breads, high specific volume and low crumb hardness are considered among the quality parameters (Monteiro et al. 2021). These properties also affect consumer preference and satisfaction. As expected, STD bread had the highest specific volume and lowest crumb hardness.

Table 4.8. Mixture design model results of GF yeast bread parameters

Parameter	p-value	R^2	Adj. R^2	Model Terms	
				Linear	*Quadratic*
Baking Loss (g/100 g)	<0.05	0.95	0.90	+B, -H, +RC	-BxRC
Crumb Moisture (g/100 g)	<0.01	0.99	0.98	+B, +H, +RC	-
Slice Moisture (g/100 g)	<0.01	0.98	0.96	+B, +H, +RC	-
Crumb Water Activity	<0.01	0.94	0.91	+B, +H, +RC	-
Specific Volume (mL/g)	<0.10	0.98	0.94	+B, +H, +RC	-BxRC and -HxRC
Crumb Porosity (%)	<0.01	0.95	0.86	+B, +H, +RC	-BxRC and -HxRC
Class 1 (%)	<0.05	0.94	0.87	+B, +H, +RC	-BxHxRC
Class 2 (%)	<0.01	>0.99	>0.99	+B, +H, +RC	-BxH
Class 3 (%)	<0.05	0.93	0.86	+B, +H, +RC	-BxH
Class 4 (%)	<0.10	>0.99	0.99	-B, -H, +RC	+BxH, +BxRC and +HxRC
Class 5 (%)	≤0.05	>0.99	0.99	+B, +H, +RC	+BxH, -BxRC and -HxRC
Crumb hardness (N)	>0.10	0.85	0.55	-B, +H, +RC	+BxRC and -HxRC
Crust Color					
*L**	<0.10	>0.99	0.99	+B, +H, +RC	-BxH, -BxRC and -HxRC
*a**	>0.10	0.86	0.72	-B,-H, -RC	+HxRC
*b**	>0.10	0.65	0.29	+B,-H, +RC	+HxRC
ΔE	>0.10	0.94	0.82	-B, -H, 0*RC	+BxRC and +HxRC
Browning Index	>0.10	0.14	0.00	+B, +H, +RC	-
Crumb Color					
*L**	<0.10	0.71	0.56	+B, +H, +RC	-
*a**	<0.05	0.90	0.85	-B, +H, -RC	-
*b**	<0.10	0.81	0.72	+B, +H, +RC	-
ΔE	<0.10	0.76	0.64	+B, +H, +RC	-
Browning Index	<0.01	0.93	0.90	+B, +H, +RC	-

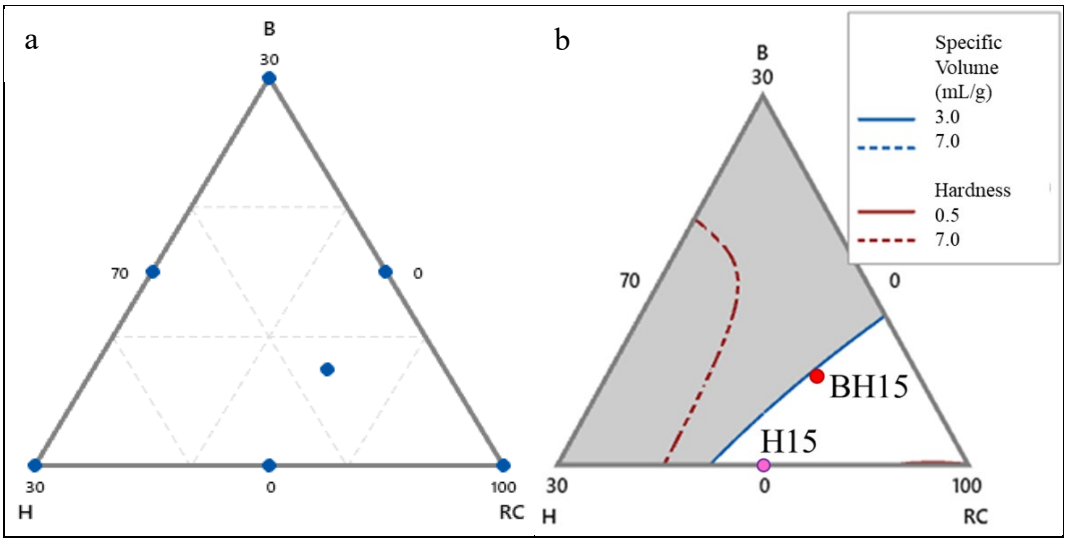

Figure 4.10. (a) Experimental design space with the seven formulations studied for GF yeast bread (component amounts in percentage) and (b) overlay contour plot for specific volume (mL/g) and crumb hardness (N) where the white area represents the optimal experimental space

The objective of this part of the study was to improve GF yeast bread nutritionally, specifically in terms of protein and fiber content, as well as to develop a formulation that keeps the crumb hardness and specific volume parameters as close as possible to those of STD bread. Therefore, a contour plot for bread optimization was created with those parameters, and the intermediate values of experimental observations were considered (Figure 4.10). The optimization plot was generated with the following constraints: 0.5 – 7 N for hardness and 3 – 7 mL/g for the specific volume. The ranges were selected since 7 N and 3 mL/g were the upper and lower limits that differed from STD by only one letter (i.e., closest values). The white area in Figure 4.10b represents the possible formulations with low hardness (less than 7 N) and relatively high specific volumes (greater than 3.0 mL/g), which are the closest ones (statistically) to the STD bread. H15 formulation, which gave similar results to STD, within this optimized region. Another formulation was BH15, which also had similar specific volume and crumb hardness, to the reference STD bread.

CHAPTER 5

RESULTS AND DISCUSSION PART II: GLUTEN-FREE AND YEAST-FREE BREAD

Some preliminary experiments were conducted to evaluate the exact formulation of gluten-free and yeast-free bread (GF-YF). In order to keep the basic flour mixtures and formulation as similar as possible to the gluten-free bread with yeast, proportions were kept the same unless necessary. The proportion of sugar decreased since there was no yeast to consume it. Consequently, salt was reduced to level/balance the taste. In the absence of yeast (3.6 g/100 g fm), baking powder (4.0 g/100 g fm), baking soda (1.0 g/100 g fm), and acidity enhancer (vinegar, 3.0 g/100 g fm) were added to make the dough rise. The same B and H flours as in the GF-yeast bread experiments; however, different R and C supplied from Türkiye were used. The proximate and some technological properties were given in Tables 5.1 and 5.2, as in Chapter 4. Detailed discussion were given in section 4.1. In this chapter, the flour section was summarized.

5.1. Flour samples used in GF-YF Breads

The chemical compositions and functional properties of the rice (I-R), corn starch (I-C), bean (B), and hazelnut (H) flours used in the gluten-free yeast-free (GF-YF) bread part of this study were determined.

All proximate and TPC results of the flours used in GF-YF breads were similar to those used in GF yeast bread since B and H flours were the same. Some differences between rice flour samples (R versus I-R) and corn starch samples (C and I-C) were observed since they were supplied from different brands (Sections 5.1.1 and 5.1.2). Hence, only the results of I-R and I-C were discussed in this section, specifically to investigate if there was a difference between RC mixtures of GF yeast and GF-YF breads.

5.1.1. Proximate Composition and TPC

I-R and I-C do not differ from R and C, in terms of chemical composition ($p>0.1$, according to the two-sample t-tests), except their moisture ($p = 0.002$ and 0.000 according to two-sample t-tests) and crude fiber contents ($p = 0.006$ and 0.013). More specifically, R and C contain higher moisture (12.4 and 11.5 g/100 g, respectively) and higher fiber (1.5 and 1.0 g/100 g, respectively.) than I-R and I-C (Tables 4.1 and 5.1). In addition, the protein content of I-R is slightly less than R (6.03 g/100 g).

Table 5.1. Chemical composition and total phenolics contents of GF-YF bread flours

Flour	Moisture (g/100 g)	Crude protein (g/100 g)	Crude fat (g/100 g)	Total ash (g/100 g)	Crude fiber (g/100 g)	Carb. (g/100 g)	TPC (mg GAE/g)
I-R	8.76 ± 0.27^{C}	5.87 ± 0.05^{D}	1.14 ± 0.06^{B}	0.89 ± 0.01^{C}	0.13 ± 0.01^{E}	83.34	0.18 ± 0.01^{C}
I-C	7.66 ± 0.08^{D}	0.60 ± 0.00^{E}	0.38 ± 0.01^{B}	0.09 ± 0.02^{D}	0.06 ± 0.00^{E}	91.27	0.11 ± 0.07^{C}
B	7.89 ± 0.05^{D}	18.77 ± 0.02^{A}	2.08 ± 0.33^{B}	3.25 ± 0.21^{A}	3.71 ± 0.05^{B}	68.01	0.34 ± 0.09^{BC}
H	1.84 ± 0.02^{E}	15.60 ± 0.03^{B}	66.38 ± 1.60^{A}	2.01 ± 0.07^{B}	13.43 ± 0.07^{A}	14.17	2.13 ± 0.19^{A}

$^{A-D}$, mean values in the same column with different superscript letters are significantly different ($p \leq 0.05$)

5.1.2. Technological Properties

I-R and I-C do not differ from R and C, in terms of chemical composition ($p>0.1$, according to the two-sample t-tests), except I-R, which is significantly denser than R ($p= 0.046$ according to a two-sample t-test, Tables 4.2 and 5.2). Emulsion activities and foaming capacities did not differ for R, C, I-R, or I-C. As discussed in the previous chapter, C could not form foam.

Table 5.2. Technological Properties of I-R, I-C, B and H

Flour	Water Retention Capacity (g/100 g)	Bulk Density (g/mL)	Oil Absorption Capacity (g/100 g)	Emulsion Activity (mL/100 mL)	Emulsion Stability (mL/100 mL)	Foaming Capacity (mL/100 mL)	Foaming Stability (mL/100 mL)
I-R	113.86 ± 5.04^{C}	0.93 ± 0.03^{CD}	110.38 ± 10.73^{AB}	50.00 ± 0.00^{AB}	97.00 ± 1.41^{A}	10.00 ± 0.00^{A}	1.00 ± 1.41^{A}
I-C	74.80 ± 3.01^{E}	0.73 ± 0.04^{D}	79.04 ± 13.04^{BC}	51.00 ± 1.41^{B}	97.04 ± 1.47^{A}	$0.00 \pm 0.00^{-}$	$0.00 \pm 0.00^{-}$
B	261.97 ± 2.85^{A}	0.83 ± 0.03^{B}	114.48 ± 0.88^{BC}	59.00 ± 1.41^{A}	83.91 ± 0.81^{A}	8.92 ± 1.53^{A}	1.49 ± 0.72^{A}
H	$0.00 \pm 0.00^{-}$	0.49 ± 0.00^{E}	158.50 ± 20.20^{A}	54.01 ± 4.23^{AB}	87.47 ± 12.06^{A}	12.00 ± 0.00^{A}	2.00 ± 2.83^{A}

$^{A-D}$, mean values in the same column with different superscript letters are significantly different ($p \leq 0.05$)

In the color properties of the flours, lightness (L*) and redness (a*) values of R, C, I-R, and C did not differ significantly ($p > 0.1$) (Tables 4.3 and 5.3). In fact, there was only one difference between GF yeast and GF-YF bread flours: b* of I-C was significantly higher than C ($p < 0.001$).

Table 5.3. Color properties of I-R, I-C, B, and H

Flour	L*	a*	b*
I-R	99.02 ± 0.50^{A}	-1.36 ± 0.02^{C}	13.82 ± 0.05^{C}
I-C	100.26 ± 0.07^{A}	-2.50 ± 0.06^{C}	12.53 ± 0.07^{D}
B	93.60 ± 1.28^{B}	0.95 ± 0.05^{B}	22.13 ± 0.09^{B}
H	71.46 ± 4.37^{C}	3.25 ± 2.49^{A}	31.14 ± 0.80^{A}

$^{A-D}$, mean values in the same column with different superscript letters are significantly different ($p \leq 0.05$)

5.1.3. FT-IR Spectra of GF-YF Flours

Because of its high fat content, the hazelnut flour pellets could not be formed before defatting the sample. Therefore, it should be noted that the defatting hazelnut flour increased the proportion of other components in its chemical structure, affecting the transmittances at specific wavenumbers (i.e., water, fat, protein, and starch). That is, while the transmittance in the protein, water, and starch regions were lower than expected, the value in the wavenumbers that suggests presence of fatty acids higher.

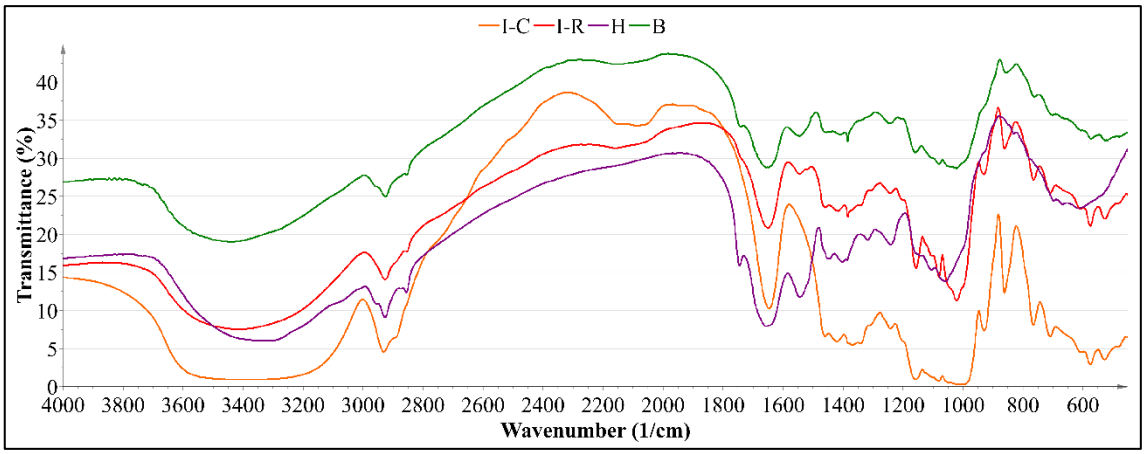

Figure 5.1. FT-IR spectra of I-C, I-R, B, and defatted H between wavenumbers 4000 and 450 cm^{-1}

FT-IR spectra of I-R, I-C, B, and defatted H showed major and minor peaks at the wavenumber ranges of 3700 – 3000 cm^{-1}, 3000 – 2800 cm^{-1}, 2800 – 2750 cm^{-1}, 1800-1730 cm^{-1}, 1700 – 1400 cm^{-1}. In addition, continuous decreases in the transmittances were observed in the wavenumber ranges of 1450 - 1000 cm^{-1} and 800 – 450 cm^{-1} (Figure 5.1). The spectra of I-R and I-C samples were similar to those given in section 4.1.3.

5.1.4. SEM Analysis

Figure 5.2 shows the SEM images of I-R, I-C, B, and H at a magnification of 5000×. The smallest particles belong to I-C (smaller than 20 µm), in a polygonal geometry and with no aggregates, while H has the largest particles. The only difference between C and I-C is that the interparticle space is greater in C than in I-C. The rice flour (I-R), on the other hand, has smaller but more dispersed particles than R in terms of size. Overall, the shapes, sizes and distributions of I-R and I-C particles are in agreement with the definitions given in SEM analyses in the literature (Section 4.1.4, Singh et al. 2003; Rodrigues et al. 2020; Lapčíková et al. 2021), since there are many varieties of those samples that have been studied previously.

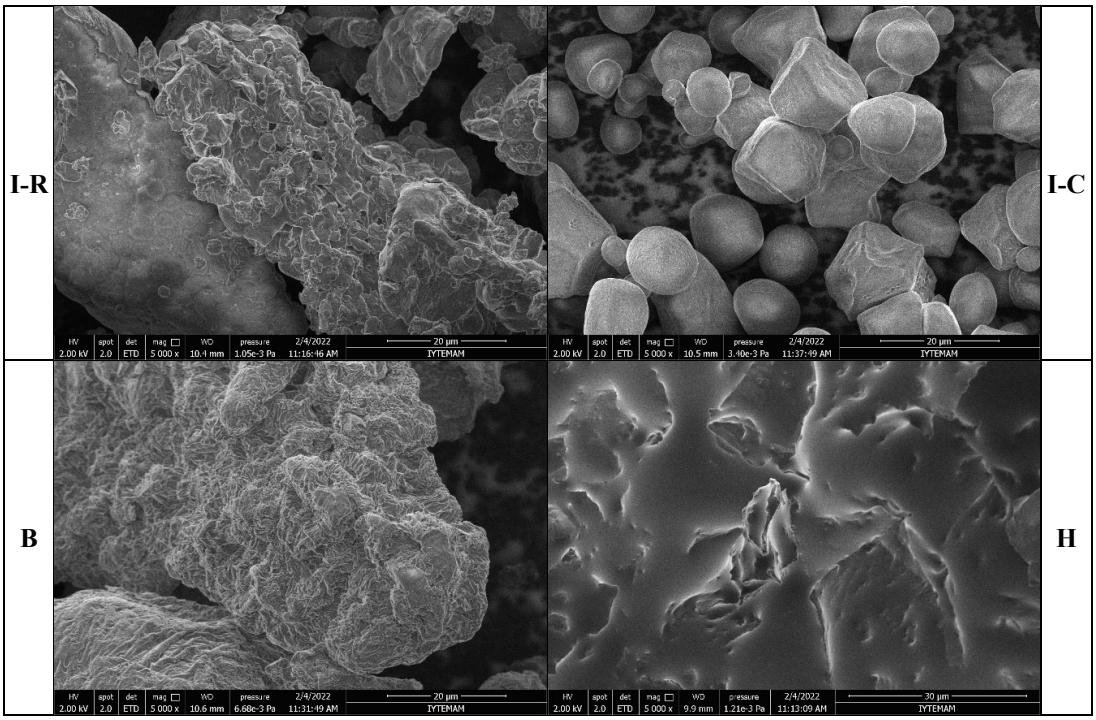

Figure 5.2. SEM images of GF-YF flours: I-R, I-C, B, and H at 5000× magnification

5.2. Dough analysis

Dough analysis results are presented in this section. The recipes of the bread formulations used in the analyses are given in Table 5.4. In brief, 400 g of the dough obtained was reserved for bread baking (200 g for each loaf), while the remainder was used for back extrusion and FT-IR analysis. The total dough amount changed between 646.8 and 742.8 g depending on the water added (g) to the formulations, based on the water retention capacities of the flour mixtures.

Table 5.4. Exact recipes used to prepare GF-YF breads for g/300 g fm

Ingredient	STD	B30	B30	H15	H30	BH15	BH30
Flour (total)	300.0	300.0	300.0	300.0	300.0	300.0	300.0
I-R	*150.0*	*127.5*	*105.0*	*127.5*	*105.0*	*127.5*	*105.0*
I-C	*150.0*	*127.5*	*105.0*	*127.5*	*105.0*	*127.5*	*105.0*
B	*0.0*	*45.0*	*90.0*	*0.0*	*0.0*	*22.5*	*45.0*
H	*0.0*	*0.0*	*0.0*	*45.0*	*90.0*	*22.5*	*45.0*
Xanthan Gum	5.4	5.4	5.4	5.4	5.4	5.4	5.4
Olive Oil	21.6	21.6	21.6	21.6	21.6	21.6	21.6
Sugar	28.8	28.8	28.8	28.8	28.8	28.8	28.8
Salt	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Baking Powder	12.0	12.0	12.0	12.0	12.0	12.0	12.0
Baking Soda	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Vinegar	9.0	9.0	9.0	9.0	9.0	9.0	9.0
Water	276.0	318.0	354.0	264.0	258.0	309.0	324.0
Total Dough	***664.8***	***706.8***	***742.8***	***652.8***	***646.8***	***697.8***	***712.8***

5.2.1. Water Retention Capacity (WRC) of The Flour Mixtures and Back Extrusion Analysis of the GF-YF Doughs

The amount of water added in each GF-YF bread formulation was adjusted with respect to the water retention capacities (WRC) of the flour mixtures. At the same time, the back extrusion results of bread in terms of consistency were checked to produce consistency values between 18 and 23 N.s, approximately (Table 5.5). The corresponding index of viscosity values were recorded between 3.7 and 5.4 N.s. The consistency values were decided after several trials and errors based on the convenient dough handling properties.

The highest WRC of the mixtures belonged to the B30 (118.5 g water/100 g flour), followed by BH30, B15, BH15, STD, H15, and H30. Even though there might be some differences due to the interactions between the flour components, bean and hazelnut flours significantly affected the WRC of the mixtures when present (Table 5.5).

Table 5.5. Back extrusion properties of the doughs and WRC of flour mixtures

Sample	Dough Firmness (N)	Cohesiveness (N)	Consistency (N.sec)	Viscosity Index (N.sec)	WRC (g/100 g)
B15	4.86 ± 0.01^{AB}	-2.99 ± 0.02^{AB}	18.93 ± 0.20^{AB}	-4.48 ± 0.07^{AB}	105.75 ± 0.29^{C}
B30	5.70 ± 0.44^{A}	-3.55 ± 0.30^{B}	22.63 ± 1.22^{A}	-5.38 ± 0.45^{B}	118.45 ± 0.94^{A}
BH15	4.62 ± 0.75^{AB}	-2.60 ± 0.20^{A}	17.65 ± 2.33^{B}	-4.08 ± 0.27^{A}	103.46 ± 0.61^{D}
BH30	4.23 ± 0.27^{B}	-2.50 ± 0.11^{A}	16.85 ± 1.18^{B}	-3.70 ± 0.19^{A}	108.41 ± 0.66^{B}
H15	4.50 ± 0.32^{B}	-2.88 ± 0.21^{AB}	17.75 ± 1.48^{B}	-4.13 ± 0.30^{AB}	88.00 ± 0.11^{F}
H30	4.54 ± 0.14^{B}	-2.83 ± 0.20^{A}	18.11 ± 1.11^{B}	-3.91 ± 0.40^{A}	86.39 ± 0.39^{F}
STD	4.39 ± 0.32^{B}	-2.47 ± 0.29^{A}	16.77 ± 0.83^{B}	-3.87 ± 0.50^{A}	91.97 ± 0.49^{E}

$^{A-F}$, mean values in the same column with different superscript letters are significantly different ($p \leq 0.05$)

Back extrusion (BE) was carried out using the remaining freshly prepared dough that would not be baked. According to the results obtained from the BE analyses, the most convenient bread doughs were obtained when the amount of water added was the same as WRC. When more water was added than the results obtained with WRC, some structural problems (e.g., the final product being under-baked) were encountered, although the dough firmness and consistency decreased, which resulted in softer dough (data in Appendix F).

B30 had the firmest and the most consistent dough (5.70 N and 22.63 N.s, respectively), with higher (in terms of magnitude) cohesiveness and viscosity index values (-3.55 N and -5.38 N.s, respectively). Other samples did not significantly differ from each other ($p \leq 0.05$).

5.2.2. FT-IR Analysis of the GF-YF Doughs

The FTIR profiles of dough samples of 7 different flour mixtures are shown in Figure 5.3. An unexpected peak order was observed in the 3800-3000 cm^{-1} wavenumber range (water region). While it was expected for B30 to give the highest absorbance due to water added, it was, in fact, STD. The following peaks were in line with the proximate

compositions of the flours: lipid (3000-2800 and 1800-1750 cm^{-1}) and protein (amide bands, 1750-1500 cm^{-1}) regions.

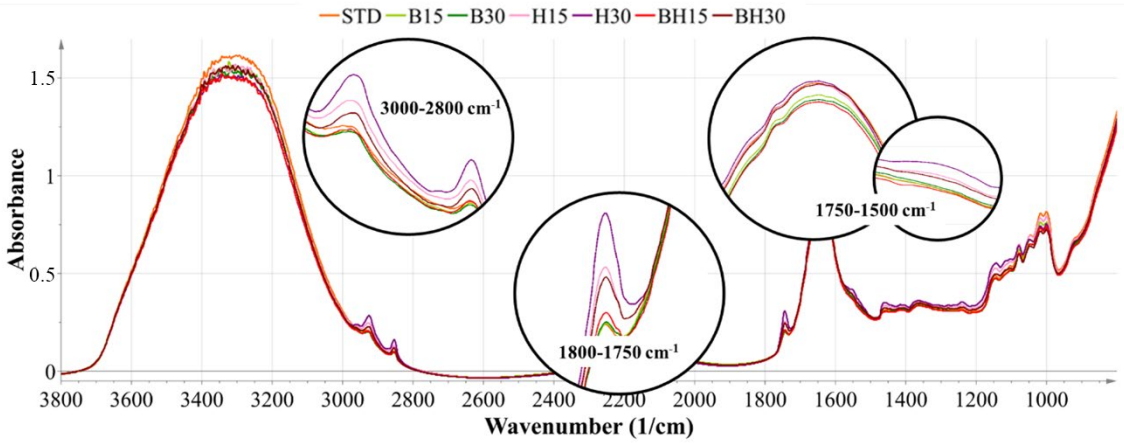

Figure 5.3. FT-IR spectra of the GF-YF bread doughs

The peak around 1550 cm^{-1} (Amide II) is more noticeable in GF-YF bread dough than it was in GF yeast bread dough spectra. However, the spectra of the GF yeast and GF-YF bread doughs could not be thoroughly compared due to the measurements being performed using FT-IR equipment from different brands. On the other hand, it is safe to claim that both bread types (i.e., yeast and yeast-free) had similar peaks around the same wavenumbers.

5.3. GF-YF Bread Physical Properties

GF-YF breads were evaluated based on their technological properties. Seven formulations were prepared and baked with respect to an extreme vertices mixture design (Section 3.2.2). The loaves and slices are presented in Figure 5.4.

The highest amount of water was added to the B30 formulation (Table 5.6). According to ANOVA test results followed by Tukey's comparison test ($p<0.05$), baking loss values of the formulations did not differ significantly. Similarly, bread height and change in height (from dough to bread) did not vary considerably. On the other hand,

specific volumes of BH30 (1.51 mL/g) and BH15 (1.50 mL/g) were significantly higher than other formulations, while specific volumes of B15, H30, and B30 were the lowest. Although the specific volumes of this part of the study are lower than the data reported in the literature for GF yeast bread (as mentioned in section 4.3), it should be considered that in this part, the breads were not leavened using yeast, but with baking powder and baking soda. Hence, it must also be noted that most of the volume of the yeast bread was gained during fermentation, not baking.

Table 5.6. Physical properties of fresh GF-YF breads

Sample	Baking Loss (g/100 g)	Bread Height (mm)	Height Change (%)	Specific Volume (mL/g)	Slice Moisture (g/100 g)	Crumb Moisture (g/100 g)	Water Added (g/100 g)
STD	18.03 ± 1.03^{A}	40.16 ± 0.79^{A}	70.0 ± 4.9^{A}	1.43 ± 0.02^{AB}	33.25 ± 0.34^{AB}	48.53 ± 0.32^{BC}	92.0
B15	20.14 ± 0.72^{A}	40.50 ± 5.89^{A}	70.4 ± 21.6^{A}	1.32 ± 0.01^{C}	35.49 ± 0.21^{A}	51.81 ± 0.02^{A}	106.0
B30	20.66 ± 1.96^{A}	36.29 ± 0.01^{A}	53.2 ± 1.3^{A}	1.27 ± 0.01^{C}	31.06 ± 0.90^{AB}	52.59 ± 2.11^{A}	118.0
H15	18.49 ± 0.91^{A}	41.52 ± 2.44^{A}	64.3 ± 0.7^{A}	1.33 ± 0.03^{BC}	28.41 ± 0.37^{B}	47.02 ± 0.12^{C}	88.0
H30	18.38 ± 0.01^{A}	39.10 ± 1.31^{A}	62.4 ± 1.3^{A}	1.29 ± 0.04^{C}	30.49 ± 0.22^{AB}	45.74 ± 0.04^{C}	86.0
BH15	18.67 ± 0.09^{A}	38.99 ± 0.41^{A}	64.1 ± 3.5^{A}	1.50 ± 0.04^{A}	33.35 ± 0.09^{AB}	50.89 ± 0.24^{AB}	103.0
BH30	19.78 ± 1.21^{A}	41.06 ± 3.38^{A}	73.1 ± 11.4^{A}	1.51 ± 0.02^{A}	32.91 ± 3.28^{AB}	51.04 ± 0.02^{AB}	108.0

$^{A-C}$, mean values in the same column with different superscript letters are significantly different ($p \leq 0.05$)

Regarding slice moisture, only two formulations resulted in significantly different values: B15 (highest, 35.49 g/100 g) and H15 (lowest, 28.41 g/100 g). The crumb moisture contents of the samples differed slightly more. As expected, H15 and H30 had the least moisture among other formulations, whereas B15 and B30 had the most. BH15 and BH30, on the other hand, had similar moisture to STD. More specifically, the use of bean flour increased baking loss, whereas the incorporation of hazelnut flour reduced it. On the other hand, both B and H (except their combination) decreased the specific volume while using bean flour increased the crumb moisture. Even though the results seem highly related to water added level, it only showed a considerably positive correlation with baking loss and crumb moisture (Appendix G, PCC=0.703 and 0.932, respectively). Furthermore, among the physical properties, only crumb moisture data showed considerable correlations with slice moisture (PCC=0.560) and baking loss (PCC=0.528).

In addition, specific volume showed a correlation with only the textural characteristics: dough firmness (PCC=-0.0559), consistency (PCC=-0.667), viscosity index (PCC=0.652), cohesiveness (PCC = 0.822) and crumb cohesiveness (PCC = 0.576) and springiness (PCC =0.681). Results with higher absolute values in dough texture produced breads with lower specific volumes, as confirmed by the correlation results. Accordingly, it can be interpreted that the dough rheology in terms of back extrusion method can actually give an idea about the end product characteristics that concerns the consumer the most (i.e., specific volume, hardness).

Unfortunately, a reliable comparison with the literature in terms of physical properties of gluten-free and yeast-free bread is not possible at the moment, since the studies that focus on eliminating the yeast in the gluten-free combinations are very limited. In fact, as explained in Chapter 2, no studies utilizing leavening agents such as baking soda and baking powder instead of yeast was found. On the other hand. The results of the GF-YF section of this study are in agreement with previous studies (eg GF yeast breads) in terms of the effect of flour addition levels on observed changes in physical properties (Section 4.3).

Side and top views of the breads along with the pictures of their slices are given in Figure 5.4. Samples with higher moisture contents in the crumbs (B15 and B30) had more wet appearances, causing them to look underbaked. Even though exact values could not be evaluated, the differences in lightness and redness of the samples depending on the replacement flour can also be observed in the pictures. In other words, it can be clearly seen that the samples with hazelnut flour have more red-like crumbs and that STD had the lightest crust along with B15 and B30. Some additional comments can also be made. For instance, in all breads, crack formation on the crust was observed. Moreover, the bean-added breads (except BH30) were more compact than hazelnut-added samples. Furthermore, the testa of hazelnut flour, which was previously discussed to have and give red-like color, was clearly visible in the breads with hazelnut inclusion of 15% and 30% (i.e., H15, H30, and BH30). The most desirable porous structure was observed in BH30, even though its pores were relatively smaller than those of STD and B15.

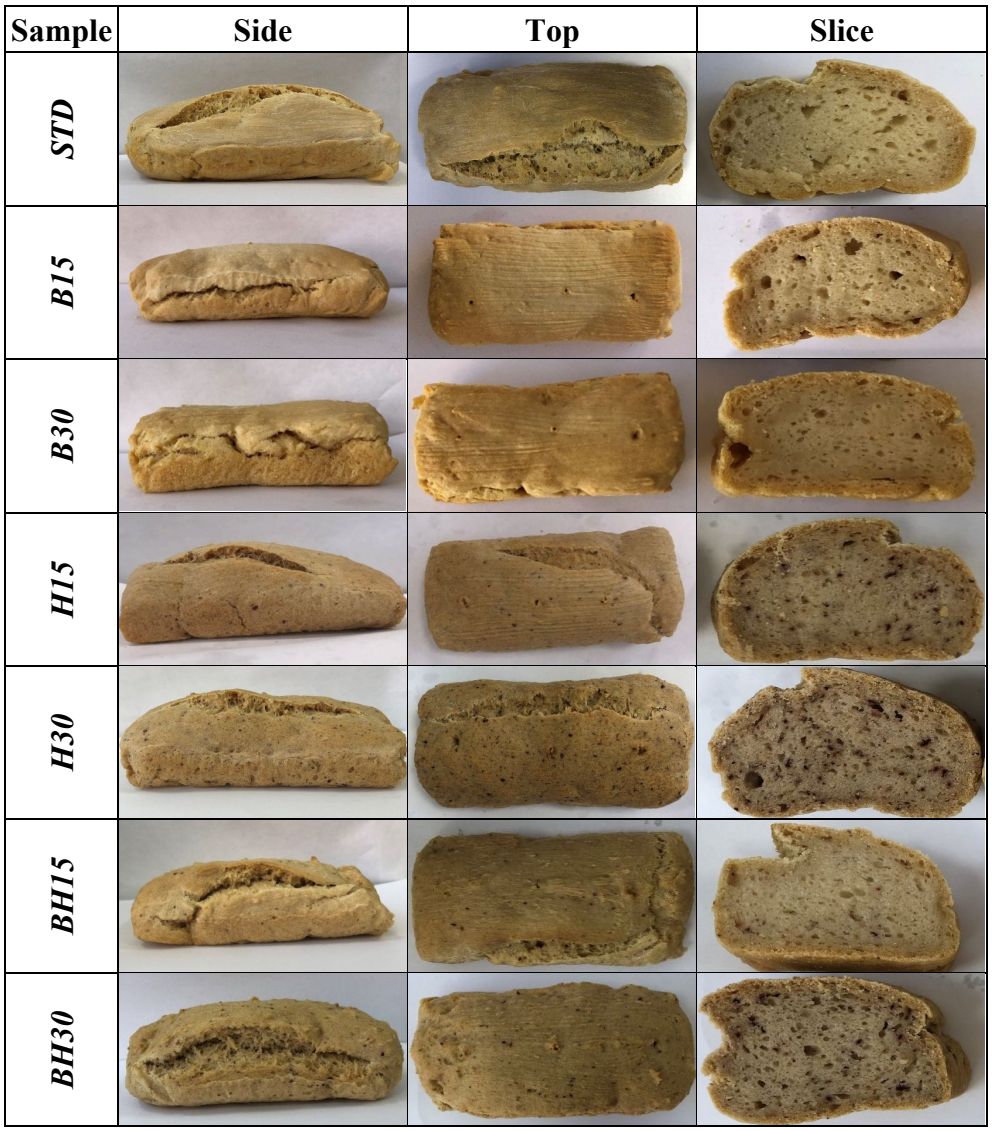

Figure 5.4. Side, top views and slices of the GF-YF breads

Texture properties of a GF bread are among the essential parameters that affect consumer preferences (Kiumarsi et al. 2019; Kahraman et al. 2022). In this study, crumbs of the GF-YF breads were subjected to a texture profile analysis with double compression to simulate mouth chewing, where hardness (N), cohesiveness, springiness, and chewiness (N.mm) were evaluated.

Hardness corresponds to the highest force applied to the crumbs during the first compression, and its values did not show a significant difference among the samples mainly due to standard deviation of B15 covering the total hardness range (9.04 – 22.73 N). However, it can still be interpreted that addition of bean flour increased the crumb hardness of GF-YF bread, whereas hazelnut inclusion decreased it. In most of the studies

present in literature, in which flour of a standard formulation was replaced with legume or nut flours, hardness was observed to be increased (Rizzello et al. 2014; Angioloni, 2017; Pycia and Ivanosova, 2020). However, this was not the case in this study, especially with the breads containing hazelnut flour since the softest crumb was obtained in the formulation where 30% of RC was replaced with 15% H and 15% B flour (i.e., BH30). In fact, the softness being lower in the crumb of BH30 can be explained by the specific volume and height change data (PCC = -0.549 and -0.732, respectively), as it was found to be considerably correlated to crumb hardness. In addition, hardness can also be associated with dough textural properties: firmness, cohesiveness, consistency, and viscosity index (PCC= 0.576, -0.602, 0.545, and -0.654, respectively). Crumb hardness was also used as an indicator for bread staling (Section 5.6).

Cohesiveness is defined as the internal resistance of the product to external pressure (Nishinari, Fang and Rosenthal 2019), and it was calculated by the ratio between the areas of the second and first compression curves. A higher level of cohesiveness indicates less crumbling, which occurs in the mouth (Onyango et al. 2011), and it is preferable. The highest cohesiveness was obtained in STD, whereas the lowest was calculated for B15 and B30. Additionally, samples containing H did not differ from STD or B-only samples in terms of cohesiveness. Dough cohesiveness (PCC=0.555) and consistency (PCC=-0.570), along with specific volume (PCC=0.576) and height change (PCC=0.571), were moderately correlated to crumb cohesiveness. Findings of studies that enriched the samples using proteins also support the reduction in legume-based replacements' crumb cohesiveness (Shevkani et al. 2015).

Springiness is described as the ability of a product to return to its initial shape, after being deformed (Novaković and Tomašević 2017). No samples showed a significant difference in terms of springiness; however, the ability of STD crumbs to regain their shape after being compressed was slightly higher than other samples, whereas H30 had the lowest springiness. Dough cohesiveness, consistency, specific volume, height change, and slice moisture had moderate correlations (PCC= 0.584, -0.527, 0.681, 0.599 and 0.556) with springiness, whereas it was more strongly associated with crumb cohesiveness (PCC=0.815).

Chewiness of a product interprets if bread can be easily broken in the mouth (Kahraman et al. 2022). In other words, it reflects the energy required to chew food until it is ready to be swallowed (Novaković and Tomašević 2017). Chewiness is obtained by multiplying hardness, cohesiveness, and springiness; hence it was expected to be highly

correlated to those results. However, it was found to be only positively correlated to hardness (PCC=0.939), while it was almost unrelated to cohesiveness and springiness (PCC=-0.044 and -0.076, respectively). In addition, the chewiness of the breads showed no significant difference even though the addition of B, H or both seemed to reduce the chewiness values.

Table 5.7. Texture properties of the GF-YF samples

Sample	Hardness (N)	Cohesiveness	Springiness	Chewiness (N.mm)
STD	17.96 ± 0.59^{A}	0.77 ± 0.00^{A}	0.94 ± 0.00^{A}	13.03 ± 0.40^{A}
B15	17.84 ± 7.46^{A}	0.69 ± 0.03^{B}	0.91 ± 0.02^{A}	11.06 ± 3.91^{A}
B30	22.73 ± 2.52^{A}	0.65 ± 0.03^{B}	0.87 ± 0.01^{A}	12.94 ± 0.73^{A}
H15	16.50 ± 4.38^{A}	0.71 ± 0.02^{AB}	0.89 ± 0.00^{A}	10.38 ± 2.52^{A}
H30	16.03 ± 0.96^{A}	0.67 ± 0.02^{B}	0.86 ± 0.04^{A}	9.21 ± 0.22^{A}
BH15	14.85 ± 2.07^{A}	0.71 ± 0.00^{AB}	0.93 ± 0.02^{A}	9.81 ± 1.23^{A}
BH30	9.04 ± 2.06^{A}	0.71 ± 0.01^{AB}	0.91 ± 0.02^{A}	5.79 ± 1.11^{A}

$^{A-B}$, mean values in the same column with different superscript letters are significantly different ($p \leq 0.05$)

The definition and importance of color properties were described in Section 4.3. The color, color difference, and browning index of the GF-YF samples were given in Tables 5.8 and 5.9. The colors of crusts and crumbs differed more than GF yeast breads. In fact, significant discrimination could be made between samples in terms of all parameters.

The darkest crumbs and crusts belonged to the H-containing samples, whereas the crusts of B-containing ones were as light as STD. Lightness decrease depending on the addition of nut flour (Kurek and Wyrwisz 2015), hazelnut testa (Anil 2007; Velioğlu et al. 2017), and other fiber sources (Pycia and Ivanišová 2020) were explained in the previous chapter. Additionally, the only crumb that was darker than expected was B30. Its relatively lower lightness (L*) can be related to its crumb looking like it was underbaked (i.e., the moist structure of its crumb). Red color (a*) was dominant in hazelnut breads except the crumb of BH15. The crust of B30 was also more red-like than expected, probably due to its browning. An unexpected result in yellowness (b*) was obtained for STD in both its crust and crumb. Its b* was higher even than B15 and B30, which were expected to have yellow as the dominating tone, as the b* of bean flour was

higher than others. Overall, the increase of H and B in the formulations significantly decreased the yellowness degree of the crumbs. However, in such a case, the influence of the chemical reactions and temperature described in the previous section should be considered. In addition, the presence of other ingredients, such as xanthan gum and vinegar, although they were added significantly less than flours, may have affected the color of the final product because their prominent colors were yellow.

Table 5.8. Color properties of GF-YF breads

Sample	Crust Color			Crumb Color		
	L*	a*	b*	L*	a*	b*
STD	69.43 ± 0.46^{A}	-0.98 ± 0.10^{F}	33.11 ± 0.02^{A}	71.53 ± 0.34^{A}	-4.14 ± 0.06^{G}	24.46 ± 0.11^{A}
B15	70.16 ± 0.30^{A}	-0.69 ± 0.06^{E}	32.70 ± 0.01^{AB}	69.83 ± 0.32^{B}	-3.48 ± 0.05^{F}	24.40 ± 0.12^{AB}
B30	69.73 ± 0.35^{A}	0.26 ± 0.04^{D}	32.70 ± 0.08^{AB}	64.87 ± 0.17^{C}	-2.98 ± 0.00^{E}	23.49 ± 0.01^{C}
H15	64.34 ± 0.08^{C}	1.42 ± 0.02^{B}	31.24 ± 0.26^{C}	56.60 ± 0.05^{E}	1.96 ± 0.04^{B}	23.83 ± 0.00^{BC}
H30	60.54 ± 0.24^{D}	2.45 ± 0.03^{A}	29.94 ± 0.02^{D}	54.31 ± 0.25^{F}	2.40 ± 0.04^{A}	22.89 ± 0.08^{D}
BH15	66.14 ± 0.19^{B}	0.63 ± 0.01^{C}	31.24 ± 0.71^{C}	65.15 ± 0.05^{C}	-1.84 ± 0.01^{D}	24.26 ± 0.03^{AB}
BH30	64.91 ± 0.12^{C}	1.38 ± 0.01^{B}	31.96 ± 0.02^{BC}	62.10 ± 0.81^{D}	-0.85 ± 0.06^{C}	22.85 ± 0.35^{D}

$^{A-G}$, mean values in the same column with different superscript letters are significantly different ($p \leq 0.05$)

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The differences between the average color of STD and other samples were evaluated as the STD was accepted to be the “reference bread” for color difference (ΔE) evaluation. In line with the expectations, the color of crust and crumb color of H30 differed from STD, whereas B15 and B30 were the samples that looked slightly more like STD in terms of crust and crumb color. BH15 also had a lower ΔE value in its crumb. The correlation of ΔE of crust and crumb with lightness (L*) was solid and negative (Pearson correlation coefficients= –0.988 and –1.000), while redness (a*) of crust and crumb and crust yellowness (b*) were also strongly correlated (PCC=0.961, 0.981 and -0.944) to ΔE. On the other hand, crumb yellowness (b*) showed a slightly weaker and negative correlation (PCC= -0.712).

It was previously explained in Section 4.3 that crumbs were not affected by heat during baking as much as the bread crusts. Hence, browning indexes of crumbs being less than crusts were expected. In fact, BI of STD, B15 and B30 were the lowest values,

whereas H15 and H30 were the highest due to the H containing testa and being high in fiber.

Table 5.9. Color difference (ΔE) and browning index of GF-YF breads

Sample	Crust Color		Crumb Color	
	ΔE	*Browning Index*	*ΔE*	*Browning Index*
STD	-	61.08 ± 0.72^{C}	-	36.06 ± 0.05^{F}
B15	0.90 ± 0.22^{E}	59.58 ± 0.42^{C}	1.82 ± 0.32^{E}	37.79 ± 0.02^{E}
B30	1.37 ± 0.07^{E}	61.17 ± 0.65^{C}	6.83 ± 0.17^{D}	39.99 ± 0.17^{D}
H15	5.94 ± 0.01^{B}	65.56 ± 0.86^{AB}	16.14 ± 0.03^{B}	55.60 ± 0.01^{B}
H30	10.04 ± 0.19^{A}	68.66 ± 0.38^{A}	18.48 ± 0.21^{A}	56.44 ± 0.51^{A}
BH15	4.15 ± 0.17^{D}	62.23 ± 2.11^{BC}	6.78 ± 0.05^{D}	42.95 ± 0.02^{C}
BH30	5.23 ± 0.10^{C}	66.71 ± 0.22^{A}	10.12 ± 0.83^{C}	43.46 ± 0.08^{C}

$^{A-F}$, mean values in the same column with different superscript letters are significantly different ($p \leq 0.05$)

BI of crust and crumb were strongly correlated to lightness (L*, Pearson correlation coefficients = -0.937 and -0.966, respectively) and redness (a*, PCC=0.917 and 0.990, respectively), while crust and crumb yellowness (b*, PCC= -0.737 and -0.537, respectively) had weaker and negative correlations.

5.4. Mixture Design Results of GF-YF Breads

ANOVA results of mixture design results were presented in Table 5.10. In brief, all models were significant except for height change. Even though a significant model for hardness could be created, and the quadratic interaction between RC and B was determined to be more effective than the linear terms, its adjusted R2 was only 0.56. Other relatively unreliable models (due to their adjusted R^2 being below 0.7) were firmness, consistency, baking loss, slice moisture, and bread texture properties, except for bread cohesiveness. In most of the models, the interaction term BxH was influential, suggesting that the inclusion of B and H together was effective in the majority of the bread characteristics. The most important proof for that was the results of the texture properties,

where BH30 had unexpectedly lower hardness, cohesiveness, springiness, and chewiness. Besides, interactions between RC and B or H were effective mainly in the color parameters, where H was mostly interactive in redness-related values and B was in those related to lightness and yellowness.

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Table 5.10. Mixture design model results of GF-YF bread parameters

Parameter	p-value	R^2	Adj. R^2	Model Terms	
				Linear	*Quadratic*
Dough Texture					
Firmness (N)	<0.01	0,69	0.60	+B, +H, +RC	-BxH
Cohesiveness (N)	<0.01	0.83	0.78	-B, -H, -RC	+BxHxRC
Consistency (N.sec)	<0.01	0.76	0.68	+B, +H, +RC	-BxH
Viscosity Index (N.sec)	<0.01	0.83	0.78	-B, -H, -RC	+BxHxRC
Bread Physical Properties					
Baking Loss (g/100 g)	<0.05	0.56	0.48	+B, +H, +RC	-
Crumb Moisture (g/100 g)	<0.01	0.93	0.90	+B, +H, +RC	+BxRC and +BxHxRC
Slice Moisture (g/100 g)	<0.05	0.73	0.61	-B, +H, +RC	+BxRC and -HxRC
Height Change (%)	>0.10	0.32	0.12	+B, +H, +RC	-
Specific Volume (mL/g)	<0.01	0.94	0.92	+B, +H, +RC	-BxH and +BxHxRC
Bread Texture					
Hardness (N)	<0.05	0.66	0.56	+B, +H, +RC	-BxH
Cohesiveness	<0.01	0.84	0.79	+B, +H, +RC	+BxH
Springiness	<0.01	0.73	0.65	+B, +H, +RC	+BxH
Chewiness	<0.01	0.69	0.60	+B, +H, +RC	-BxH
Crust Color					
*L**	<0.01	0.99	0.98	+B, +H, +RC	+BxH and -BxHxRC
*a**	<0.01	0.99	0.99	+B, -H, -RC	-BxRC and +HxRC
*b**	<0.01	0.95	0.93	+B, +H, +RC	+BxH and -BxHxRC
ΔE	<0.01	0.99	0.99	+B, -H, +RC	+HxRC
Browning Index	<0.01	0.92	0.90	+B, +H, +RC	-BxRC
Crumb Color					
*L**	<0.01	0.99	0.99	-B, +H, +RC	+BxH, +BxRC and -HxRC
*a**	<0.01	0.99	0.98	-B, -H, -RC	-BxH and +HxRC
*b**	<0.01	0.92	0.90	+B, +H, +RC	+BxRC
ΔE	<0.01	0.99	0.99	+B, -H, +RC	BxH, BxRC and HxRC
Browning Index	<0.01	0.99	0.98	+B, -H, +RC	-BxH and +HxRC

Additionally, the presence of the special cubic term in dough cohesiveness, viscosity index, crumb moisture, specific volume, crust lightness (L*) and crust yellowness (b*) suggests that its blending among the results was either additive or nonlinear (synergistic or antagonistic) binary.

In summary, almost all models created significant models. They showed quadratic or special cubic interactions between the parameters and the mixture design elements (i.e., B, H, and RC). The results of GF-YF breads were more significant than GF yeast breads in terms of mixture design analysis.

Specific volume and crumb hardness was selected to optimize the bread formulations, just as they were chosen in Section 4.5. In the same chapter, a specific volume threshold was reported to be 3.5 m L/g. However, the specific volumes of GF-YF breads (including STD, which would typically be expected to have a high volume) were below this limit. Therefore, considering the highest specific volume and the lowest crumb hardness possible since they are among the quality parameters affecting consumer preferences (Monteiro et al. 2021), an optimization plot was created (Figure 5.5a). The optimization plot was generated with the following constraints: 5 – 12 N for the hardness and 1.40 – 1.55 mL/g for the specific volume.

Hardness was selected so that the range of the softest crumb (BH30) was covered, whereas specific volume had the upper and lower limits of lightest breads (BH30 and STD). STD was not included in hardness since its value and texture turned out to be undesirable. The white area in Figure 5.5b represents the possible formulations with low hardness (6.98-11.10 N) and relatively high specific volumes (1.41-1.54 mL/g), which are closest ones to the STD bread. When the overlay table was read, it was determined that BH30 confidently meets the requirements to be the “optimum” formulation. In addition, H30 was also considered, since it had the lowest texture results after BH30, and the most-liked sample according to sensory analysis.

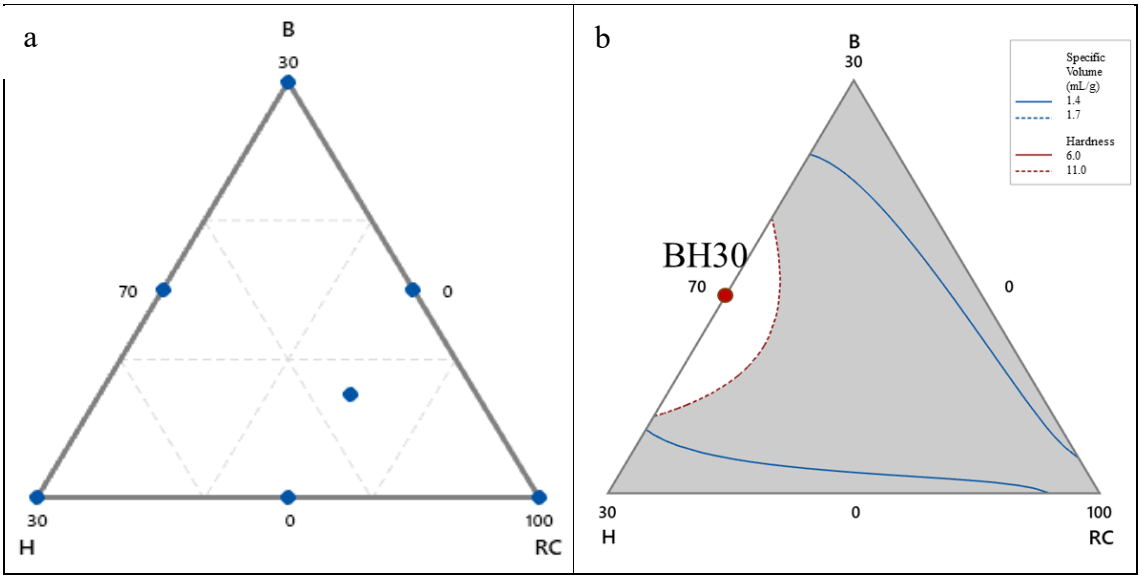

Figure 5.5. (a) Experimental design space with the seven formulations studied for GF-YF breads (component amounts in percentage) (b) Overlay contour plot for specific volume (mL/g) and crumb hardness (N): the white area represents the optimal experimental space.

5.5. Sensory Evaluation of GF-YF Breads

H30 and BH30, the samples that gave the most acceptable results in physical properties and mixture design, were compared to STD bread. Data for physical properties were analyzed, and the least hardness and the highest specific volumes were identified with H30 and BH30 formulations. The participants were presented with three samples STD, H30 and BH30, as shown in Figure 5.6, and they were asked to evaluate the samples according to their color, flavor, texture, taste, and their overall liking of the breads. The results of the sensory evaluation are given in the radar chart in Figure 5.7.

32 participants scored the breads according to the specified parameters, and the scores of the samples were evaluated using Microsoft Excel and Minitab software. The most-liked sample turned out to be H30 (5.9 ± 0.9), as expected due to previous tastings and analyses. It had higher scores than BH30 and STD in terms of color, flavor, texture, taste, and overall liking.

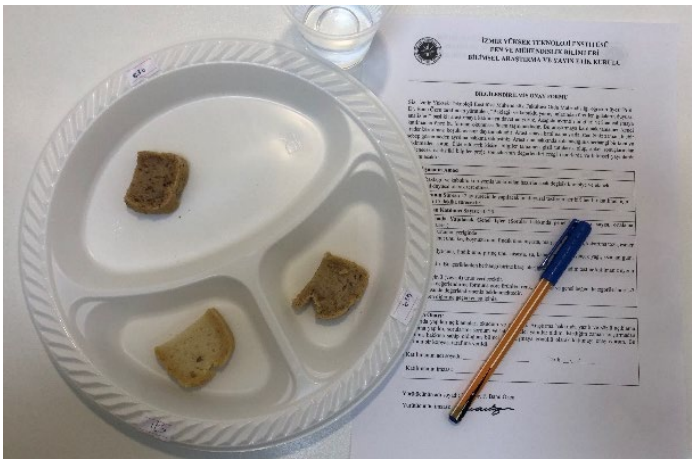

Figure 5.6. A plate and informed consent form used for sensory evaluation.

It was actually expected for STD to obtain the highest score in color, as its color was the lightest and normally breads were supposed to be (5.1 ± 1.4). Probably due to the dominant aroma of hazelnut, H30 and BH30 were prominent in flavor (6.0 ± 1.1 and 5.7 ± 1.2) and taste (5.4 ± 1.1 and 5.0 ± 1.2).

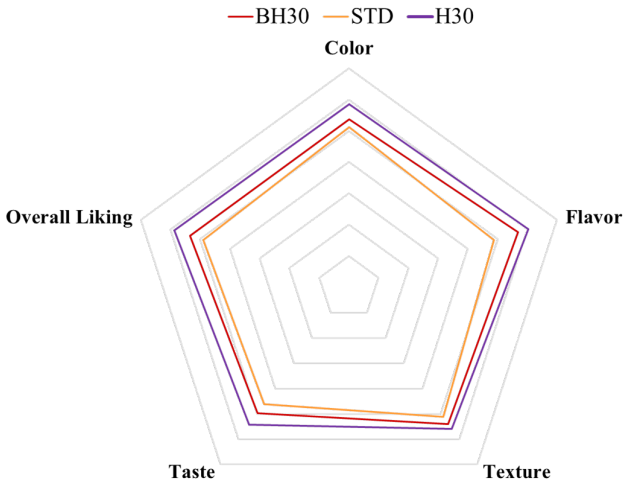

Figure 5.7. Sensory scores in radar chart for STD (orange), H30 (purple) and BH30 (dark red)

In texture, the scores were tighter; however, H30 had the highest score even though crumb of BH30 was found to be softer (5.6 ± 1.0 and 5.4 ± 1.3). This was probably

because of the texture in the sensory analysis including mouthfeel in addition to crust and crumb hardness.

5.6. Technological Properties of Stored GF-YF Breads

Bread formulations H30 and BH30 were chosen for storage experiments along with STD to evaluate the changes in their characteristics during 48 hours of storage. Samples for storage were selected based on their physical properties and mixture design findings. Results of the storage experiments can be found in Appendix H.

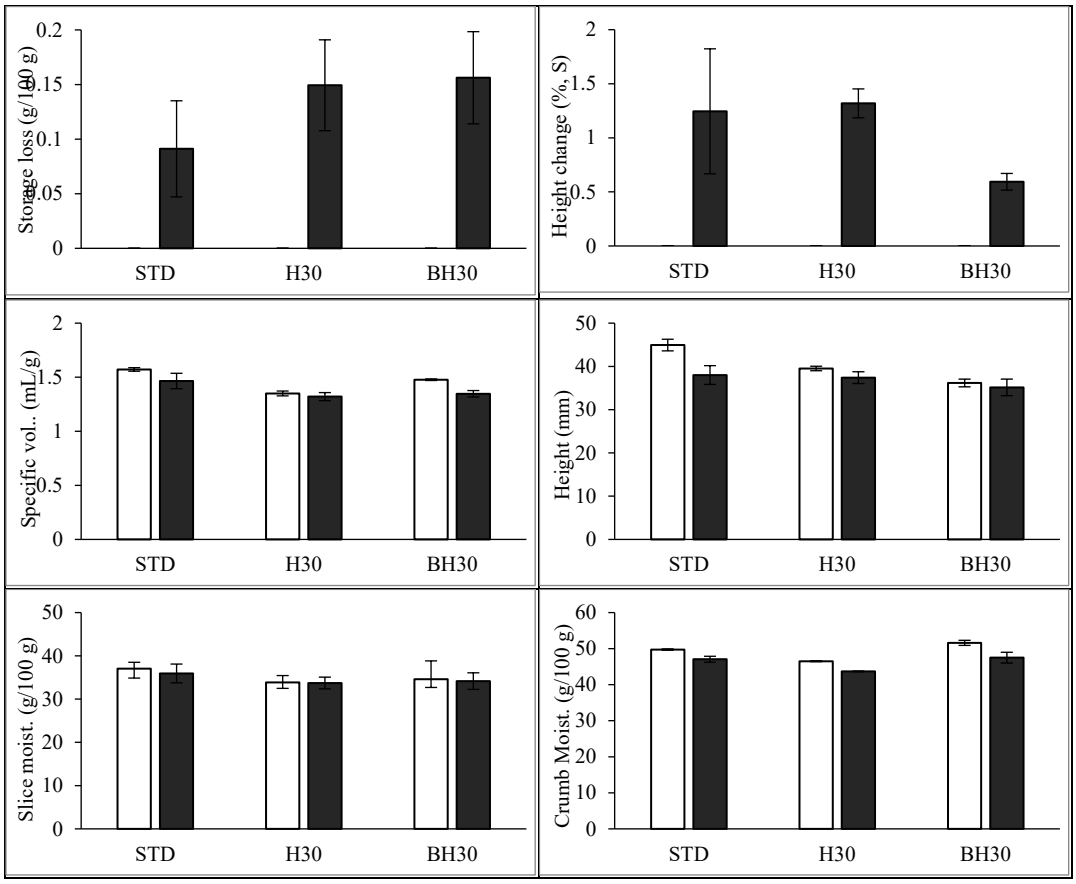

Figure 5.8. Change in the physical properties of GF-YF breads during storage (t = 0, white; t = 48 h, black)

Samples did not lose a considerable amount of water (approximately 15%) during storage (Figure 5.8). However, they did differ from each other significantly. They were not expected to lose weight since they were wrapped in plastic bags and kept in desiccators.

On the other hand, the samples lost approximately 1.5% of their heights, which was correlated to their storage loss. Since the breads did not change significantly, their moisture contents were not expected to change considerably, as well. Overall, the breads did not undergo any conditions that would interfere with their physical properties.

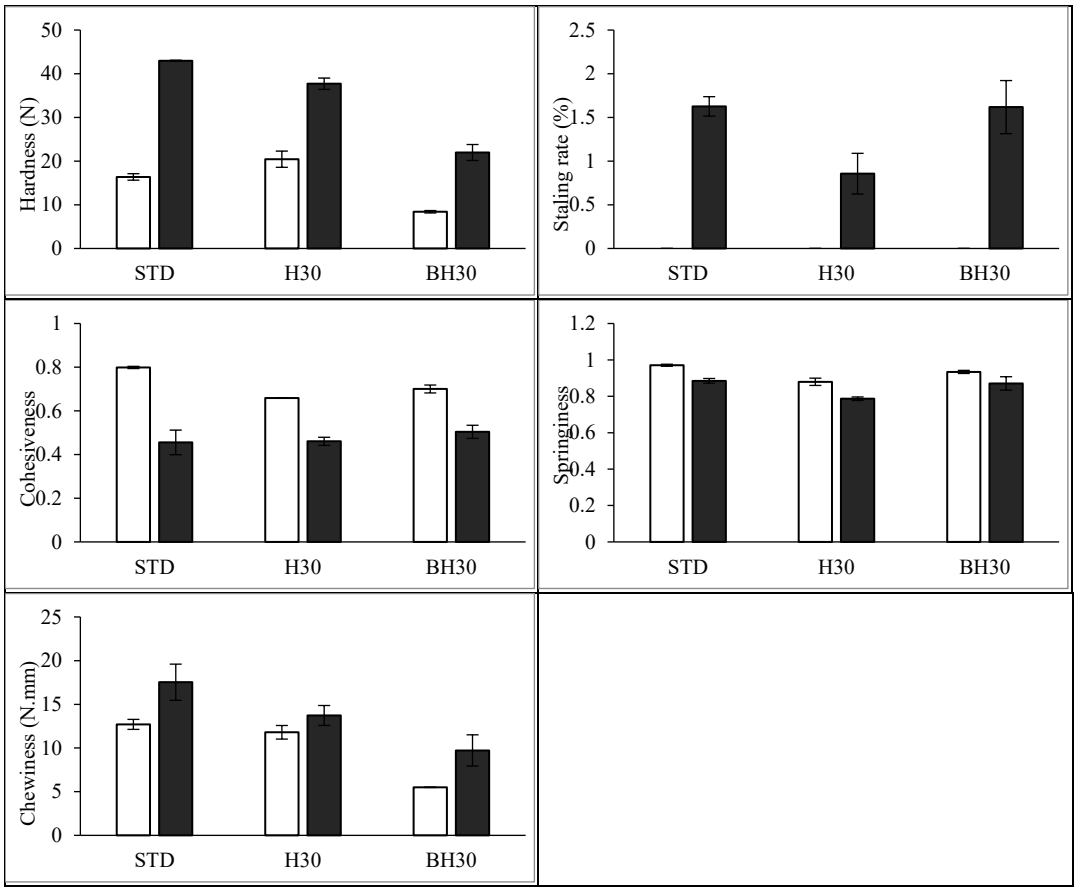

Figure 5.9. Change in the textural properties of GF-YF breads during storage (t=0, white; t=48 h, black)

In terms of texture, the most essential parameter was hardness, as it was directly effective in staling rate and chewiness, and relatively influential for cohesiveness and springiness. Bread staling is described as the increase in crumb hardness, and it is a result

of two occurrences: moisture loss from the crumb and starch retrogradation (Roman et al. 2020). Gluten-free breads are known to be susceptible to staling, as they primarily consist of starch since the formulations are based on starchy ingredients such as rice flour and corn starch (Kahraman et al. 2022).

Replacement of RC with H and BH probably reduced the amount of starch significantly, which in turn, decreased the rate of staling. In fact, the hardness of H30 and BH30 followed the same trend as their respective fresh versions, where the crumb of BH30 was the softest. On the other hand, the crumb of STD became the hardest, even though it was softer than H30 at t=0, probably due to starch retrogradation.

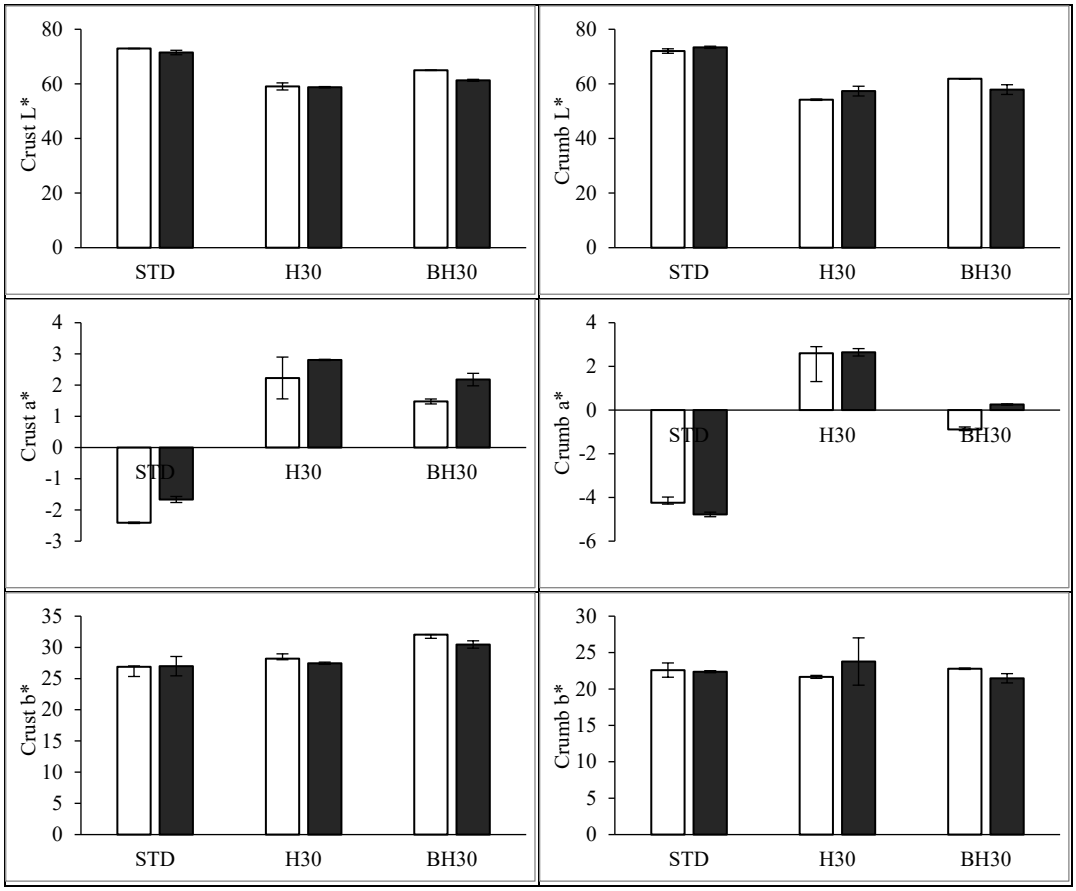

Figure 5.10. Change in the color properties of GF-YF breads during storage (t = 0, white; t = 48 h, black)

In particular, the main factor in the staling of GF-YF samples were expected to be starch retrogradation, as storage loss (i.e., moisture loss during storage) was under

control. The staling rate of H30 was the lowest among samples, whereas crumbs of STD and BH30 staled in similar rates. Interestingly, crumb cohesiveness became almost equal in all samples, whereas H30 was the least cohesive at t=0. The ability of returning to its starting shape significantly decreased for all samples, making crumb of H30 the least springy (elastic) one (Figure 5.9). H30 also kept its chewiness, as it changed the least. On the other hand, STD became harder to chew. Overall, BH30 also surpassed the stored H30 and STD, as its fresh breads.

The L*, a* and b* values of the samples did not change significantly, although the color values seemed to tend to decrease (Figure 5.10). The only exception was the redness (a*) of the crusts and BH30 crumb, in addition to yellowness (b*) of H30 crumb. In fact, BH30 had a significant change in its redness, as the value became positive, indicating that the dominating tone became red.

5.7. FT-IR Analysis of the Fresh GF-YF Breads

Spectroscopic analyses for the crumbs of the bread samples were performed using FTIR-ATR. The profiles and significant regions related to the chemical bindings are given in Figure 5.11. Overall, the peaks were precisely at the same wavenumber ranges for each group, and a more reliable distinction between the samples could be made by observing the spectra.

Details of the regions were previously explained in detail in sections 4.2.3 and 5.2.2. Overall, the peaks at 3800 – 3000 cm^{-1} range occurred due to OH stretching, indicating water presence, with a slight possibility of aliphatic and phenolic structures (Casiraghi et al. 2008; Xu et al. 2023). Absorbance order in the water region follows the same trend as the amount of water added for the samples, which is also similar to the moisture contents of the crumbs (Table 5.6): B30, BH30, B15, BH15, STD, H15 and H30 (in descending order).

The next peaks at 3000 – 2800 cm^{-1} are associated with the C-H stretching, representing the presence of carbonyl groups. The other minor peak around 1750 cm-1 shows C=O stretching, which is firmly attributed to the presence of the ester fatty acid group. The absorbance trends in this region are also in line with the crude fat contents of

the flours, when the mixture ratio is also considered: H30, H15, BH30, BH15, STD, B15, B30 (in decreasing order).

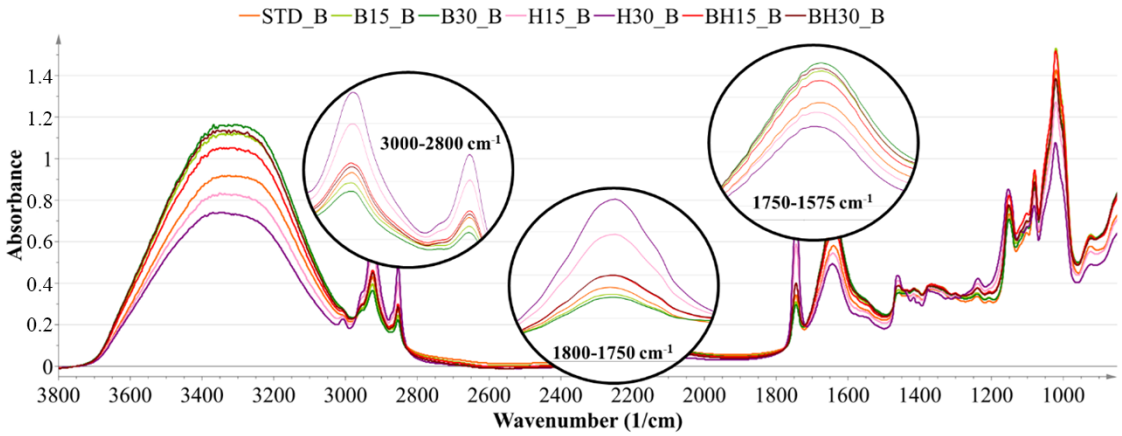

Figure 5.11. FT-IR spectra of the crumbs of fresh GF-YF bread samples

The following peaks around 1650, 1550, and 1240 cm^{-1} are associated with Amide I, II and III, respectively. These peaks also give an idea about the protein contents of the breads, which are strongly related to the protein contents of the flours. The absorbances in decreasing order (B30, BH30, B15, STD, H15, H30), with an unexpectedly lower absorption performance for H15 and H30, were probably related to amino acids of H being more susceptible to degradation than those of B.

The region between 1200 – 1000 cm^{-1} is known as the fingerprint region, due to the presence of the characteristic peaks for the polysaccharides (Sinelli, Casiraghi, and Downey 2008; Căpraru et al. 2009; Skendi,Papageorgiou and Papastergiadis 2021). At wavenumbers 1460 and 1150 cm^{-1}, absorbances of H30 and H15 were higher than others, implementing that these wavenumbers can be related to fiber. At 1080 and 1020 cm^{-1}, BH15 and B15 had stronger peaks, where H15 and H30 had the lowest, which proposes these could be related to the amount of slowly digestible starches present in these samples (section 5.8). According to Figure 5.12, the peaks evaluated in the previous paragraphs are actually lower than those of the doughs. This can be related to the moisture loss occurred during baking, as well as starch and protein degradation caused by the baking process (180 °C).

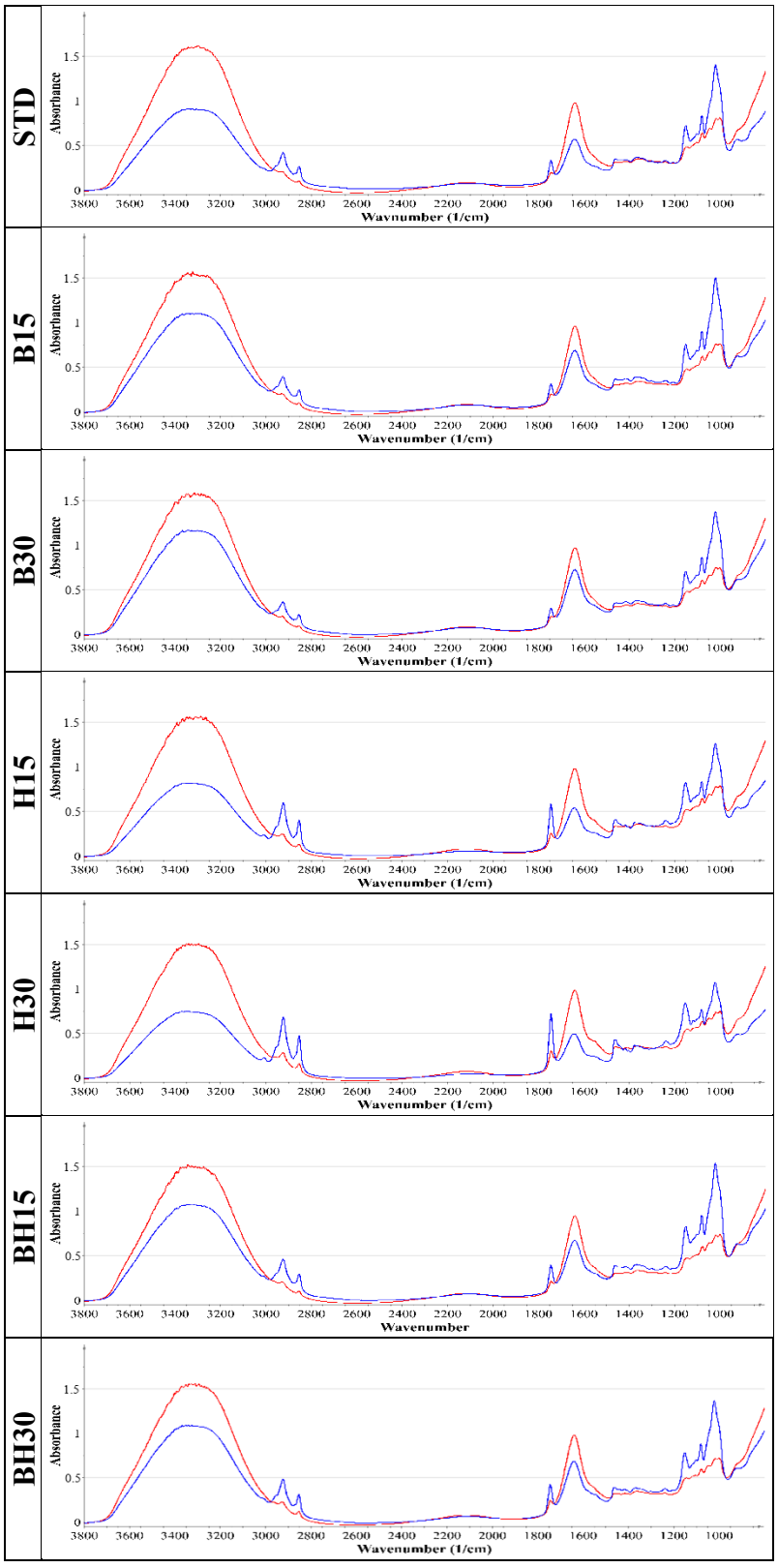

Figure 5.12. Dough and bread FT-IR spectra comparison of STD, B15, B30, H15, H30, BH15 and BH30. Blue: bread; red: dough

Decreases in the water region (3800 – 3000 cm-1) of the spectra were expected as some of the water was lost during baking. Probably, as a result of water removal, peaks related to fat can be observed clearer in the spectra of the breads than those of the doughs. On the other hand, a significant decrease in the Amide-I region (1750-1575 cm-1) was observed.

Even though water loss allowed other molecules to stretch or vibrate more freely, protein degradation by heating probably caused the absorption at this region to be lower. The peptide bonds, amide I and II, are known to be sensitive to conformational changes. Any decrease in those bands can be related to increase in the ß sheets, which leads to a decrease in the α-helix content (Rondeau et al. 2007; Ji et al. 2020). In fact, Bikaki et al. (2021) showed that peptides are subjected to amide bond cleavage during thermal food processing. Based on this information, it can be said that the absorbance values in the amide bands may be affected.

In the fingerprint region (1200 and 1000 cm^{-1}), there was a noticeable increase in the absorbance values. In a study investigating the effect of microwave treatment on potato starch (Kumar et al. 2020), a similar increase in 1200 – 1000 cm^{-1} range was observed. They suggested that the increase could be associated with the starch structure modification and intra-molecular hydrogen bond development, due to the evaporation of water during the microwave treatment. Using a similar approach, the increase in the absorbance at specific wavenumbers can be explained by using both baking loss and changes in the starch structure (for example, gelatinization) due to thermal processing (i.e., baking).

5.8. FT-IR Analysis of the Stored GF-YF Breads

A clear discrimination between the stored bread samples (STD, H30, BH30) can be made just by observing their FT-IR profiles (Figure 5.13). As expected from previous FT-IR analyses (section “Dough” and “GF-YF Bread”), compositional differences between the stored samples of STD, H30, and BH30 can be observed. Being the most moist among the stored samples, BH30 gave the highest peak in the water region. In the regions related to the fat presence, H30 stand out, even though BH30 also resulted in a

relatively higher absorbance than STD. Amide and fingerprint regions also follow a trend closer to those of fresh breads.

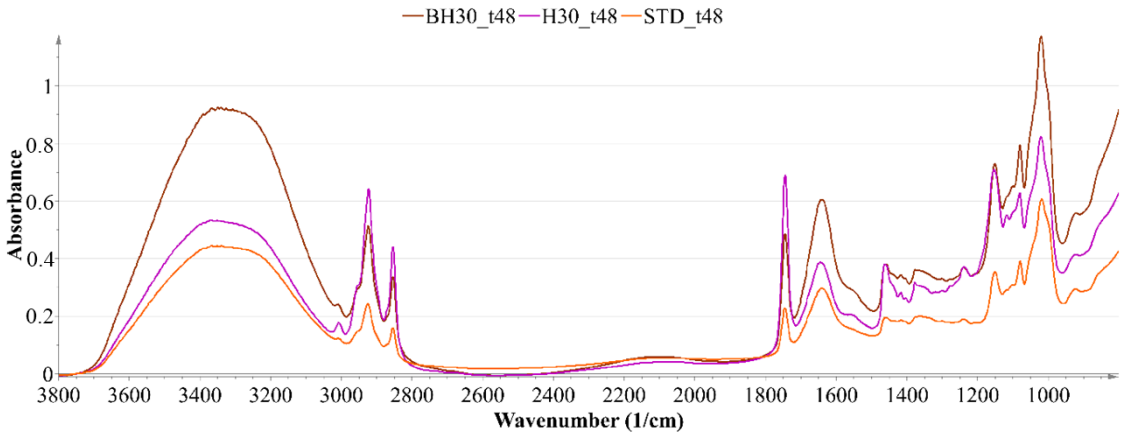

Figure 5.13. FTIR spectra of stored breads: STD (orange), H30 (purple) and BH30 (brown)

A clear discrimination between the stored bread samples (STD, H30, BH30) can be made just by observing their FT-IR profiles. As expected from previous FT-IR analyses (sections 5.2.2 and 5.7), compositional differences between the stored samples of STD, H30, and BH30 can be observed.

Being the most moist among the stored samples, BH30 gave the highest peak in the water region. In the regions related to the fat presence, H30 stood out, even though BH30 also resulted in a relatively higher absorbance than STD. Amide and fingerprint regions also follow a trend closer to those of fresh breads.

Differences between the spectra of dough, fresh bread and stored samples can also be observed in Figure 5.14, with the changes in STD being more noticeable. Even though fresh and stored breads of H30 and BH30 indicate less reduction in the fat-related wavenumbers, slight differences could still be observed.

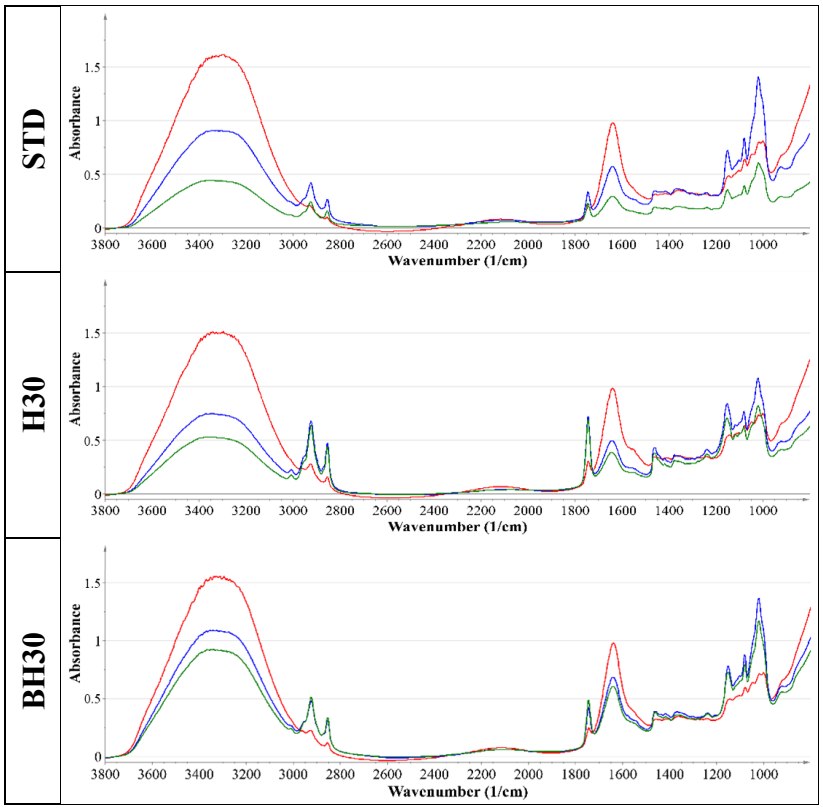

Figure 5.14. FT-IR comparison of dough (red), fresh bread (blue) and stored bread (t=48 h, green) loaves of STD, H30, and BH30

In several studies, the fingerprint region (1200-800 cm-1) was selected to monitor the staling of the breads, especially the peaks at 1047 and 1020 cm-1. (Özkoç et al. 2009; Masure, Fierens, and Delcour 2016; Fu et al. 2021; Kotsiou et al. 2021; Mouzakitis et al. 2022; Kotsiou et al. 2022). As expected, a decrease in the intensities of the absorbances in this range, especially around 1040 and 1020 cm-1, was observed for STD, H30, and BH30. Based on these values, it can also be seen that H30 and BH30 are much less susceptible to staling in terms of starch (crystalline and amorphous regions), since basically 30% of their starch structure differs from that of STD.

5.9. Starch Digestion Results

Foods high in starch, such as gluten-free breads, are known for their predominant rapidly digestible starch (RDS) since almost all starch is gelatinized during digestion. On the other hand, slowly digestible starch (SDS) is slowly broken down in the small intestine. It is responsible for the induction of the gradual increase in postprandial plasma

glucose and the insulin levels (de la Hera, Rosell and Gomez 2014; Culetu et al. 2021). Overall, the glucose release of H30, BH30 and the control sample, STD, during 120 minutes of digestion were determined (Table 5.11 and Figure 5.15). As a result of the experiments, the effect of 30% replacement of the rice flour and corn starch blend with hazelnut (H30) and white bean-hazelnut blend (BH30) on RDS and SDS was investigated (Table 5.11 and Figure 5.16). To avoid any confusion, RDS, SDS and the released glucose amounts were all expressed in terms of g glucose per 100 g sample.

According to Table 5.11 and Figure 5.15, which covers the glucose release during gastric and intestinal phases of the analysis, STD released more glucose than other samples because its main ingredients were corn starch and rice flour, which are known for their starchy structure. BH30 released the lowest amount of glucose, where BH30 and H30 showed similar trends to each other throughout the whole digestion process. That was probably because their structure being different than STD by at least 30% due to replacement of rice flour-corn starch blend. Besides, H30 and BH30 differed from each other by at most 15% since the only difference between them was the presence of bean flour.

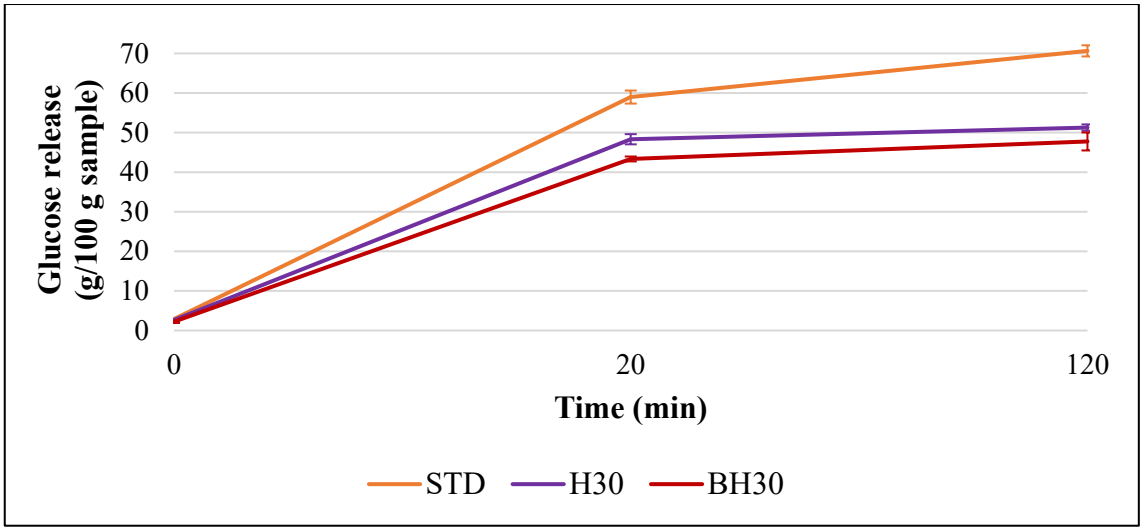

Figure 5.15. Glucose released during gastric and intestinal digestion phases (0^{th}, 20^{th} and 120^{th} minutes) of samples STD, H30, and BH30

There are actually several factors that combinedly affect the rate of starch digestion including the gelatinization properties, starch damage, size, composition, and

structure of the starch granules, physical encapsulation and protein and lipid content of the food matrix (Wolter et al. 2013). Those factors and highly viscous structure of dough, and dense bread made of high protein and high fiber flour replacements can explain the reduction in the amount of RDS and SDS, when this study is considered. In this kind of matrices, the act of amylase enzyme is limited to the starch molecules as explained in the study of Sasaki (2018). Furthermore, the amount of water in the formulations were found to be effective since it takes part in the determination of the degree of starch gelatinization in the study of de la Hera, Rosell and Gomez (2014). In the same study, it was also stated that pancreatic α-amylase affinity was dependent on that degree since it also affects the initial rate that amylase digests the native starch. In fact, the particle size decreases the digestion rate because it causes a reduction in the surface area that is exposed to the digestive enzymes. Hence, the first step in digestion is directly influenced by the enzyme binding and absorption. Overall, inhibited or slowed digestion are actually among the factors affecting the glycemic index (GI), as it was claimed that more compact structure lowers the glycemic response (Fardet et al. 2006).

Table 5.11. Starch digestion results of STD, H30, and BH30

Parameter	**STD**	**H30**	**BH30**
Gastric Phase Glucose Release (g glucose/100 g sample)	2.90 ± 0.05^{A}	2.66 ± 0.07^{A}	2.23 ± 0.33^{B}
Intestinal Phase - 20th min Glucose Release (g glucose/100 g sample)	58.98 ± 1.65^{A}	48.32 ± 1.29^{B}	43.33 ± 0.62^{C}
Intestinal Phase - 120th min Glucose Release (g glucose/100 g sample)	70.64 ± 1.41^{A}	51.27 ± 0.80^{B}	47.76 ± 2.27^{C}
FSG (g glucose/100 g sample)	4.03 ± 0.06^{B}	3.99 ± 0.30^{B}	4.35 ± 0.06^{A}
RDS (g RDS/100 g sample)	49.46 ± 1.53^{A}	39.90 ± 0.89^{B}	35.08 ± 0.51^{C}
SDS (g SDS/100 g sample)	10.50 ± 0.22^{A}	2.65 ± 0.44^{B}	3.98 ± 1.49^{B}
Decrease in Gastric Phase Glucose Release (%)	-	8.48 ± 2.57	$23.00 \pm 11.30^{*}$
Decrease in RDS (%)	-	19.30 ± 0.70	$29.03 \pm 1.18^{*}$
Decrease in SDS (%)	-	74.74 ± 4.16	62.07 ± 14.18

$^{A-C}$, mean values in the same row with different superscript letters are significantly different ($p \leq 0.05$)

* indicates significantly higher mean in two-sample comparisons ($p \leq 0.05$)

Lipids are considerably effective in inhibition of starch digestion as the starch-lipid complex alters the pasting properties, oil and water absorption capacity, solubility,

swelling capacity and viscosity of the starch. This complex occurs when the crystalline structure of the starch during heating, where double helix of the amylose is expanded to fit in the lipid, in which electrostatic bonds are formed and stability is obtained (Cervantes-Ramírez et al. 2020; Sengupta, Chakraborty and Mazumder 2022; Sun et al. 2023). Hazelnut, which was used in H30 and BH30, contains a high amount of fat (Table 4.1, Table 5.1, Turan, Çapanoğlu and Altay 2015) and its fatty acid profile is dominated by oleic (MUFA, 73.6-82.6%) and linoleic (PUFA, 9.8-16.6%) acids (Sun et al. 2022). So, those unsaturated fatty acids are actually prone to bind to the double helix of the amylose, which in turn, inhibits or slows down starch digestion.

Protein basically acts as a physical barrier between the starch and digestive enzymes and reduces starch digestibility (Xu et al. 2022). Hence, increase in the protein content of foods can increase the resistance to starch hydrolysis, and consequently decreases rate of the starch digestion (Li et al. 2021). Rate of starch digestion can also be affected by the type of protein, due to the differences in their structures and interactions (Liu et al. 2021). Basically, protein presence increases the amount of hydrogen bonds between starch and protein, consequently conserving the starch structure and inhibiting or slowing down starch digestion (Lu et al. 2021b). Amino acids such as threonine, serine and tyrosine are known for containing a large number of hydroxyl groups, which may result in binding of those amino acids to the active site of the porcine pancreatic α-amylase through the hydrogen bonds. This, in turn, can inhibit enzyme activity as a result of change in the enzyme conformation, thus preventing the substrate (i.e., starch) from binding to the active side of the enzyme (Lu et al. 2021a). Mentioned amino acids have been reported to be present in hazelnut (Köksal et al. 2006; Alasalvar et al. 2003), which replaces at least 15% of the total flour (i.e., rice flour and corn starch blend) in H30 and BH30. Therefore, it can be claimed that protein content of hazelnut flour in those formulations takes an important part in physical and enzymatic inhibition of starch digestion.

The importance of the effect of fat and protein on starch digestion in the food matrix is indisputable. However, regarding the food matrices containing fat and protein along with starch, the effect of fat and protein on starch digestion should be examined in terms of the interactions of these three compounds, as well. The reason for this is basically the ternary interaction between those three components (Wang et al. 2017a; Zheng et al. 2018), according to the results of rapid visco analyzer (Zhang and Hamaker 2003) and high pressure size exclusion chromatography (Zhang, Maladen and Hamaker 2003).

Starch, lipid (fatty acids) and protein can form the ternary complexes during a heating and cooling process. Previous studies actually showed that the aliphatic tail of the fatty acids interacted with amylose and the negatively charged carboxyl group of fatty acids joins the protein. The associations between those components in the food matrices affect the pasting properties, gel structure, starch retrogradation and consequently, starch digestion (Wang et al. 2020). Subsequently, Ye et al. (2018) reported that degree of starch hydrolysis increases significantly when lipid and proteins were removed from the milled long-grain rice samples. In the same study, they also showed that presence of lipids were considerably more effective than proteins at decreasing the starch digestibility. In another study, it was reported that proteins and fatty acids (e.g., linoleic acids) showed a synergistic effect on reducing the starch digestion, while type of protein was also effective (Lin et al. 2020). Regarding this result, it was claimed that protein addition would promote lipid interaction since protein is known to improve emulsifying properties and consequently solubility of lipids, which directly affects the starch-lipid formation in the food complexes (Chao et al. 2018; Wang et al. 2017b). Overall, H30 and BH30 both contains flours that has considerable amounts of fat (H) and protein (B and H). So, interactions between protein, fat and starch can actually takes a very important part in slowing down or inhibiting starch digestion of those formulations. Regarding the effect of lipid and protein presence in food complexes, Wang et al. (2020) suggested that subject would be understood better if more in-depth research is performed including the digestion simulation in oral cavity and the acidic environment of the stomach as well as small intestine. This would actually be useful for all sorts of enriched foods as well, since digestion of starch actually starts in the mouth with the enzymes in the saliva, when the effect of enrichment on glycemic index is considered.

Additionally, hazelnut flour has a significant amount of fiber, which is also known to alter the starch structure, as well as its digestion. In fact, Yemenicioğlu et al. (2019) explained that increased hydrocolloid related viscosity that may be caused by high fiber content, the interactions between enzyme and its substrate is decreased, so nutrient absorption, hence glucose release can be lowered.

Moreover, in another study, the effect of fiber-enriched flour was reported to be highly dependent on the amylose proportion in the starch (Sasaki and Kohyama 2012). Besides, the authors reported that the use of gums such as xanthan and guar suppresses the starch digestibility of corn and rice starches that contains high amounts of amylose. Rapidly and slowly digestible starch (RDS and SDS) were evaluated using the glucose

released during digestion data where the in vitro starch digestion was mainly carried out to test the hypothesis suggesting that inclusion of H and B decreases the RDS.

According to data presented in Figure 5.15 and Table 5.11, addition of 30% H based on 100 g flour can decrease the RDS (39.90 g/100 g sample) by 19.3%, whereas addition of 30% B and H blend per 100 g flour can decrease the RDS (35.08 g/100 g sample) by 29.03% with respect to STD (rice flour-corn starch GF bread, $p<0.01$). In particular, it can be clearly seen that the addition of hazelnut four significantly caused a decrease in RDS, whereas its SDS also reduced, hence suggesting that its presence would increase the fiber content of the final product.

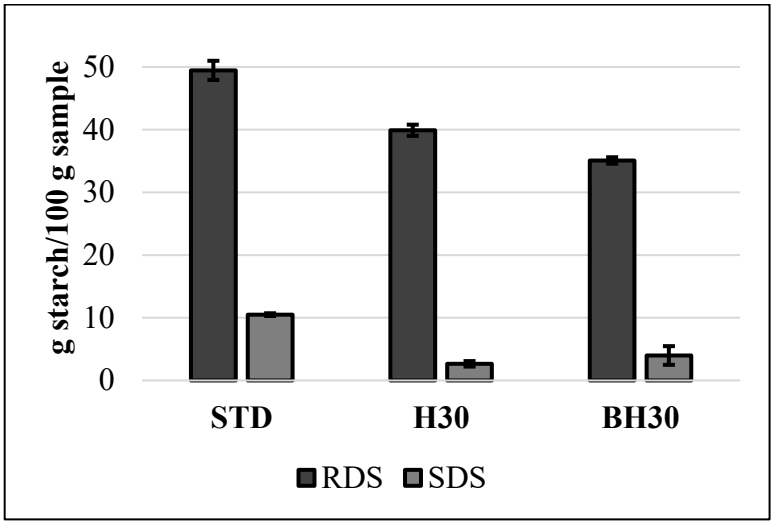

Figure 5.16. Rapidly (RDS, black) and slowly digestible starch (SDS, gray) contents of STD, H30, and BH30

As an example of SDS and RDS determination, a similar approach to this study was followed by Kahraman et al. (2022), in which they prepared gluten-free breads using chickpea (i.e., raw, roasted and dehulled) and rice flours, and reported that incorporation of chickpea flour significantly decreased the total glucose content, as well as RDS. In another study, Güler and Şensoy (2023) investigated the effect of psyllium fiber on the in vitro starch digestion of wheat-based flat dough pieces cooked with different processing methods (i.e., steaming, and roasting). They concluded that processing methods and fiber addition could alter starch gelatinization (processing and fiber) properties, dispersion behavior (processing methods), enzyme mobility (fiber addition) and thus, starch

digestion. More specifically, the addition of psyllium fiber acted as a physical barrier and thickening agent, which resulted in restricted mobility of the enzymes. On the contrary, no change in terms of RDS or SDS was reported in a gluten-free cake study in which 50% of rice flour was replaced with bean flour, where use of pea and lentil flours significantly decreased RDS and use of chickpea flour significantly reduced SDS (Gularte, Gómez and Rosell 2012). In another study, the effect of partial replacement with ripe and unripe gac fruit on the in vitro starch digestion were investigated, in addition to the physicochemical properties and sensory acceptability of functional pasta (Chusak et al. 2020). The authors reported that replacement with both unripe and ripe gac fruit flour (5-15%) significantly decreased total glucose release ($p<0.05$, except 5% replacement with unripe gac fruit flour) and RDS content ($p<0.05$), but no significant change in SDS was observed.

There are also other methods applied to the samples prior to the in vitro starch digestion. For instance, Zhuang, Wang and Yang (2023) evaluated the starch fractions of potato starch with various salts by freeze-drying the samples prior to the analyses. In fact, there were some other studies that also dried their samples to preserve their samples and avoid gelatinization possibility. These studies worked on freeze-drying (Larder et al. 2018; Rewthong et al. 2011; Wang, Liu and Wang 2016; Gularte, Gómez and Rosell 2012), ethanol-drying (Wang, Liu and Wang 2016) and hot air/oven drying methods (Wang, Liu and Wang 2016; Rewthong et al. 2011), in order to increase the efficiency of the digestibility method.

CHAPTER 6

MULTIVARIATE STATISTICAL ANALYSIS

In this chapter, multivariate statistical analyses (MVA) for selected physical properties of fresh and stored GF breads with and without yeast and spectroscopic profiles of flours (R, C, I-R, I-C, B, H), GF-YF dough, bread, and stored bread are discussed. All data were scaled on the center and no filters (e.g., MSC or derivative) were applied unless specified.

6.1. PCA of FT-IR Spectra

In this section, MVA results of the FT-IR spectra of flours and GF-YF bread samples are discussed. The number of readings performed for FT-IR spectra of GF yeast bread was insufficient for multivariate analysis.

6.1.1. PCA of FT-IR Spectra of Flours

PCA score and loading plots of flour samples (R, I-R, C, I-C, B, and H) are presented in Figures 6.1. As explained in Section 3.2.1.15, five pellets were prepared from each sample, where each pellet was read five times. The PCA model was prepared and analyzed after the mean spectra of each pellet was calculated.

Noticeable discriminations were observed among samples, after applying standard normal variate (SNV) and 2nd derivative filters, due to their differences in some of their chemical and physical properties. Corn starches (I-C and C) and rice flours (R and I-R) were separated from bean (B) and hazelnut (H) flours according to PC1. Besides,

no difference between B and H regarding PC2 was observed while starches and rice flours were separated.

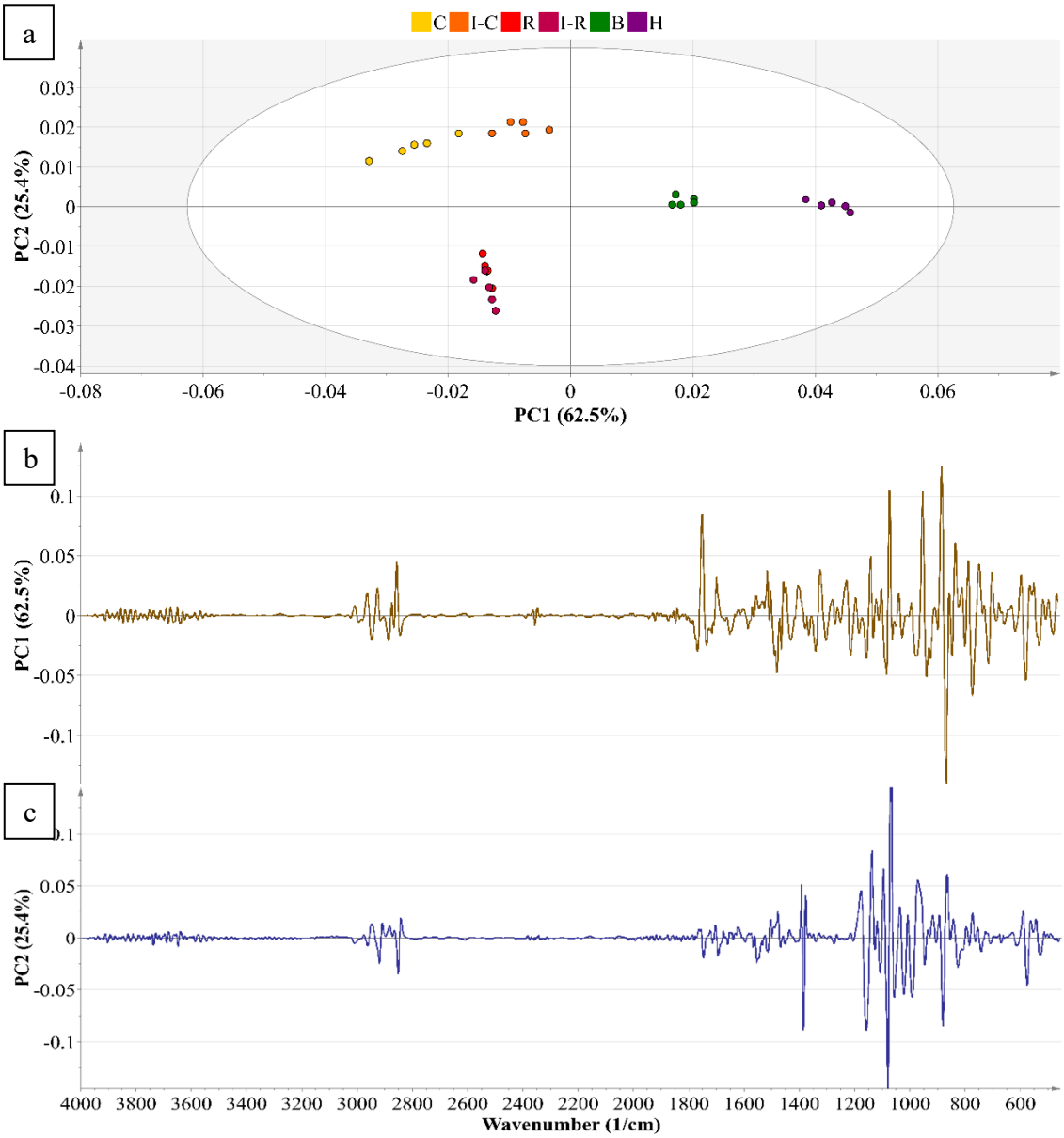

Figure 6.1. PCA score (a) and loading plots according to PC1 (b) and PC2 (c) of FT-IR profiles of the flours used in this study (R^2= 99.2% and PC=8)

The contribution of transmittance values in certain wavelength ranges to the principle components could be observed more precisely and reliably when loading plots of PC1 and PC2 were examined separately. It was determined that there was a strong relationship between both components and transmittance, especially between the wavenumbers 1800 and 450 cm^{-1}.

Relatively minor contributions belonged to 3800 – 3600 (O-H stretching, Casiraghi et al. 2008; Xu et al. 2023) and 3000 – 2800 cm-1 (C-H stretching). Another fat-related peak (1800-1700, C=O stretching) was associated with PC1 (strongly positive) and PC2 (weakly negative).

The peaks around 1650 (Amide I), 1550 (Amide II), and 1240 (Amide III) were related to the protein contents of the samples and positively affiliated with PC1. Furthermore, H had a higher score than B in terms of PC1 since the proportions of its chemical composition elements other than fat increased. In addition, the peaks between both 1200 and 1000 cm^{-1} showed positive and negative associations with PC1 and PC2, respectively. In other words, the peaks within the fingerprint region caused the samples to have positive and negative scores in terms of PC1 and PC2, affecting their position on the scatter plot. One of the most important transmittance values which mainly separated corn starch and rice flour samples were at 1040 (crystalline starch structure) and 1020 (amorphous region of starch) cm-1. The difference between their transmittances around 1100 cm^{-1} was also effective.

Overall, rice flour and corn starch samples diverged on the scatter plot based on their starch characteristics (PC2), whereas they were discriminated from bean and hazelnut flours according to the difference in their protein contents (PC1).

6.1.2. PCA of FT-IR Spectra of GF-YF Bread Doughs

PCA score and loading plots of the GF-YF bread doughs are shown in Figures 6.2. No filters were applied to the data since the formulations were separable on their own when they were center-scaled. Absorbances on the specific wavenumbers mostly contributed both PC1 and PC2. Hence, examination of their loading plots in form of line plots were preferable as there were more than 3000 data points. H-containing samples diverged from STD according to PC1 (72.7%), whereas B-containing samples separated from H15 and H30 by PC2 (15.0%).

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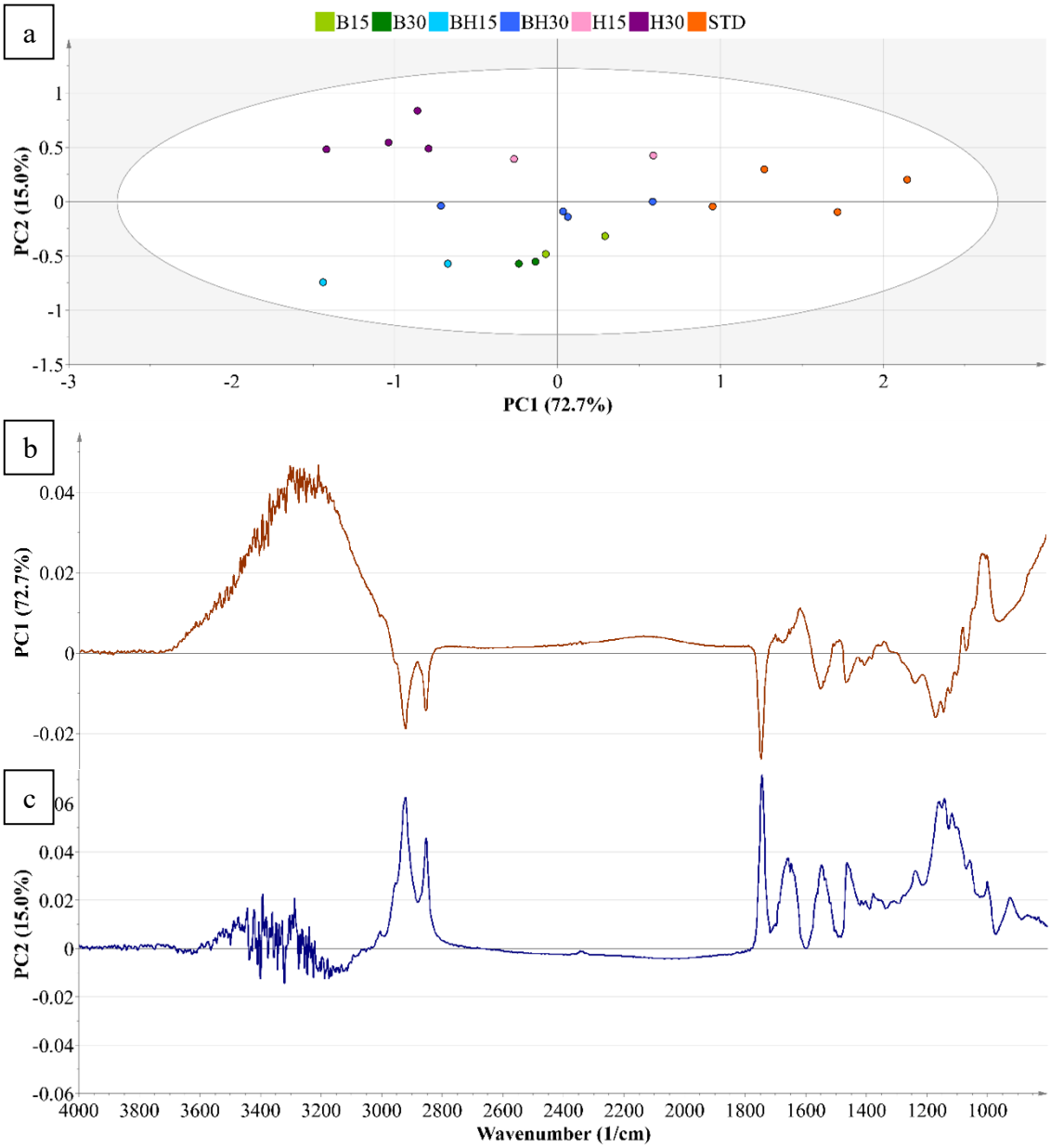

Figure 6.2. PCA score (a) and loading plots according to PC1 (b) and PC2 (c) of FT-IR profiles of the GF-YF bread doughs (R^2= 97.2% and PC=8)

There was a strong and positive relationship between PC1 and the absorbance values in the O-H stretching region (3800-3600 cm^{-1}), whereas fat-related interactions were on the negative side. In addition, it was observed that protein content (i.e., Amide I bond, at 1650 cm^{-1}) had almost no effect on the PC1 (contribution score around 0.01). Still, probably the relatively stronger negative contribution of Amide II and III (1550 and 1240 cm^{-1}) was able to separate STD from other samples on the PC1 axis since its dough had relatively lower amount of protein due to the chemical compositions of its flours, I-

R and I-C. Namely, presence of amide I dragged B15 and B30 slightly to the positive side (PC), whereas Amide II and III was more effective on keeping them on the negative axis. On the other hand, fat-contents affected H15 and H30 more than protein. Fluctuations were observed in the starch (fingerprint, 1200-100 cm-1) region; however, the positive contributions of crystalline structure and the amorphous region of starch (1040 and 1020 cm^{-1}, respectively) were more effective for STD, as it had the highest scores on PC1. In addition, hazelnut-containing bread formulations gave higher absorbance values on the regions that were believed to be fiber-related (i.e., 1460 and 1150 cm^{-1}).

In summary, higher absorbance values on the fat, starch, and protein content of H15 and H30 kept those samples on the top-left (i.e., negative PC1 and positive PC2) while lower fat-content pushed other formulations to the negative PC2 side. On the other hand, composition B was more effective in placing BH30, since it was closer to B15 and B30. And hazelnut level (7.5%, BH15) kept BH15 on the negative PC1 side, while STD with its relatively lower protein, fiber and fat content was closer to the origin in terms of PC2 and had higher scores on PC1.

6.1.3. PCA of FT-IR Spectra of GF-YF Breads

PCA score and loading plots of the fresh GF-YF breads are presented in Figure 6.3. Standard normal variate (SNV) was applied to the data and the absorbances were center-scaled. Absorbance values on the specific wavenumbers mostly contributed both PC1 and PC2. Hence, examination of their loading plots in form of line plots were more preferable as there were more than 3000 data points. samples diverged from each other according to PC1 (88.3%), and PC2 (10.0%) with a total explanation of the data variation of 98.3%.

First of all, it should be noted that the strong relationship between absorbance values in the water region (3800-3000 cm^{-1}) and the principal components weakened as the doughs lost considerable amounts of water. Besides, a slight reduction in the relative divergent strength of absorbance peaks in the wavenumber range 1750 – 1200 cm^{-1} was also observed for PC2.

In brief, a clear distinction on PC1 between the H15 and H30 breads and STD, B15, B30, BH15 and BH30 was observed. This was probably caused by their relatively higher fat and fiber contents. Additionally, B30, B15 and BH30, which were claimed to have higher protein than other formulations, were separated from other samples by the contribution of their absorbance to PC2.

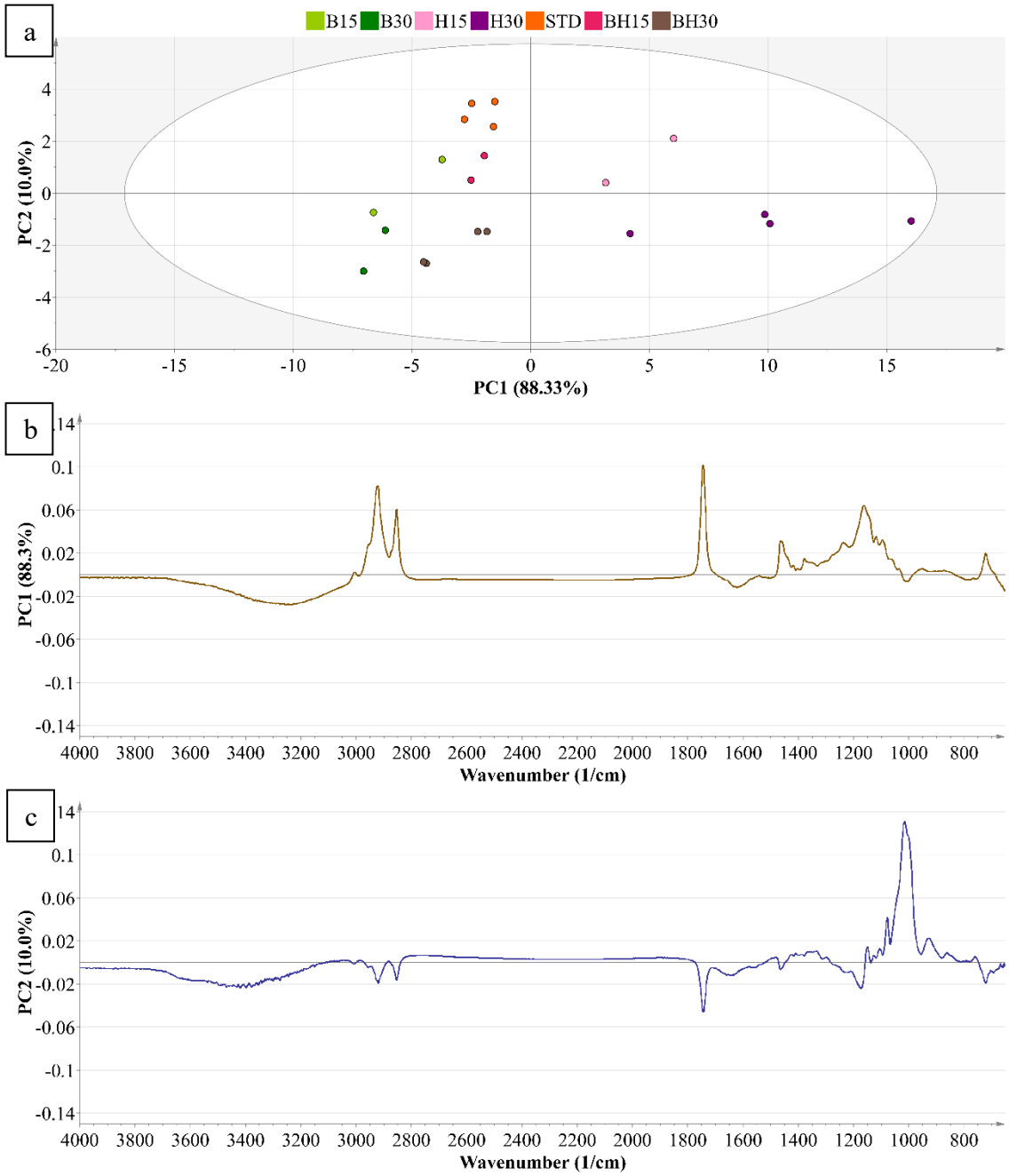

Figure 6.3. PCA score (a) and loading plots according to PC1 (b) and PC2 (c) of FT-IR profiles of the fresh GF-YF breads (R^2= 99.9% and PC=7)

As explained in the first paragraph, although the effect of absorbance in the water region on the components has decreased, it has not completely disappeared. In fact, especially in PC2, water seem to make a considerable contribution to the separation of samples relative to PC2, following starch and the esters at 1750 cm^{-1}. Because it is clear that B30, which has more water in the crumb than other samples, in addition to not having a considerably absorbance at 1040 and 1020 cm^{-1}, has a higher score than H30 in the negative region of PC2. On the other hand, STD had absorbances very close to B15 and BH15, which were expected to be closest in terms of their chemical compositions according to this multivariate analysis. BH15, STD, and one average spectra of B15 were located on the negative and positive axes of PC1 of PC2, respectively; even though there was a significant variation in B15.

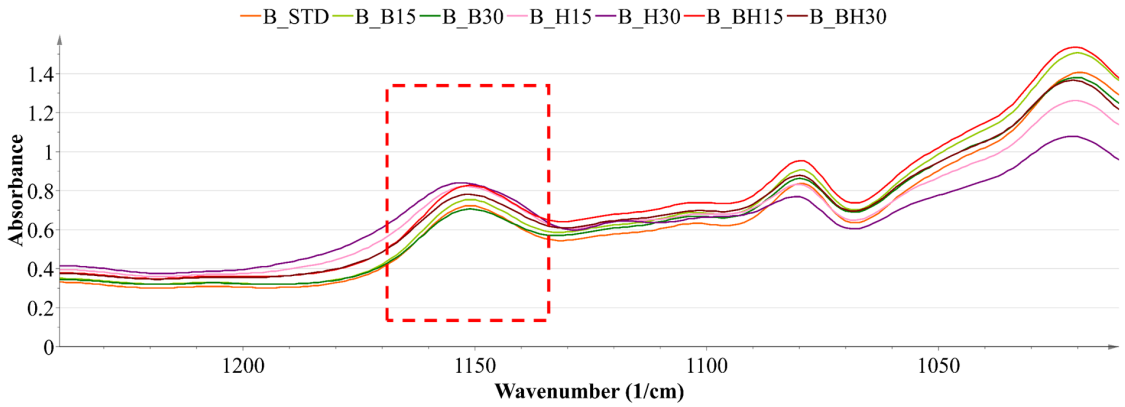

Figure 6.4. Fresh bread spectra between the wavenumbers 1250 and 1000 cm^{-1}

In summary, bread samples were separated based on their fat (2950, 2850 and 1750 cm^{-1}) contents and difference in their absorbance value around 1150 cm^{-1} on PC1, whereas fat (1750 cm^{-1}), protein (1650 cm^{-1}, amide I), and starch contents (especially in the crystalline and amorphous regions, 1040 and 1020 cm^{-1}) as well as the absorbance values around 1150 cm^{-1} were more effective on PC2.

There were not any clear statement regarding the wavenumber 1150 cm^{-1} or related compounds. However, a clear difference was observed between fresh bread spectra. Since the highest two peaks belong to the formulations containing hazelnut (H30

and H15, respectively), the wavenumber can be associated with the fiber or phenolics, which were found in hazelnut more than other samples (Figure 6.4).

6.1.4. PCA of FT-IR Spectra of Stored and Fresh GF-YF Breads

PCA score and loading plots of the fresh and stored GF-YF breads are presented in Figures 6.5. No filters were applied to the data since the formulations were separable on their own when they were center-scaled. Absorbances on the specific wavenumbers mostly contributed both PC1 and PC2. Hence, examination of their loading plots in form of line plots were more preferable as there were more than 3000 data points. Stored samples diverged from their respective fresh breads mainly according to PC1 (91.8%), whereas fresh samples separated from each other by both PC1 and PC2 (6.73%) with a total explanation of the data variation of 98.53%. This model was established to compare fresh and stored breads and it was slightly more successful than the fresh bread model, as only B-containing (i.e., B15 and B30) and other "intermediate" formulations (i.e., BH15 and H15) were excluded from the comparison.

According to the scatter and loading plots, PC1 separated the samples mainly based on their moisture content (3800-3000 cm-1) and absorbances between 1740 and 800 cm-1. On the other hand, sample scores on the PC2 were mainly affected by their absorbances in fat (3000-2800, and 1800-1670 cm-1), and around 1450 cm-1. Overall, fresh and stored H30 breads had significantly higher amounts of fat an expected to contain relatively higher fiber; hence they were located on the top-left of the scatter plot. The special peak at approximately 1150 cm-1 was observed in H30, BH30, H15, BH15, B30, B15, and STD breads. Higher absorption might be due to the contents of hazelnut and bean flours which have higher fat, fiber and phenolics.

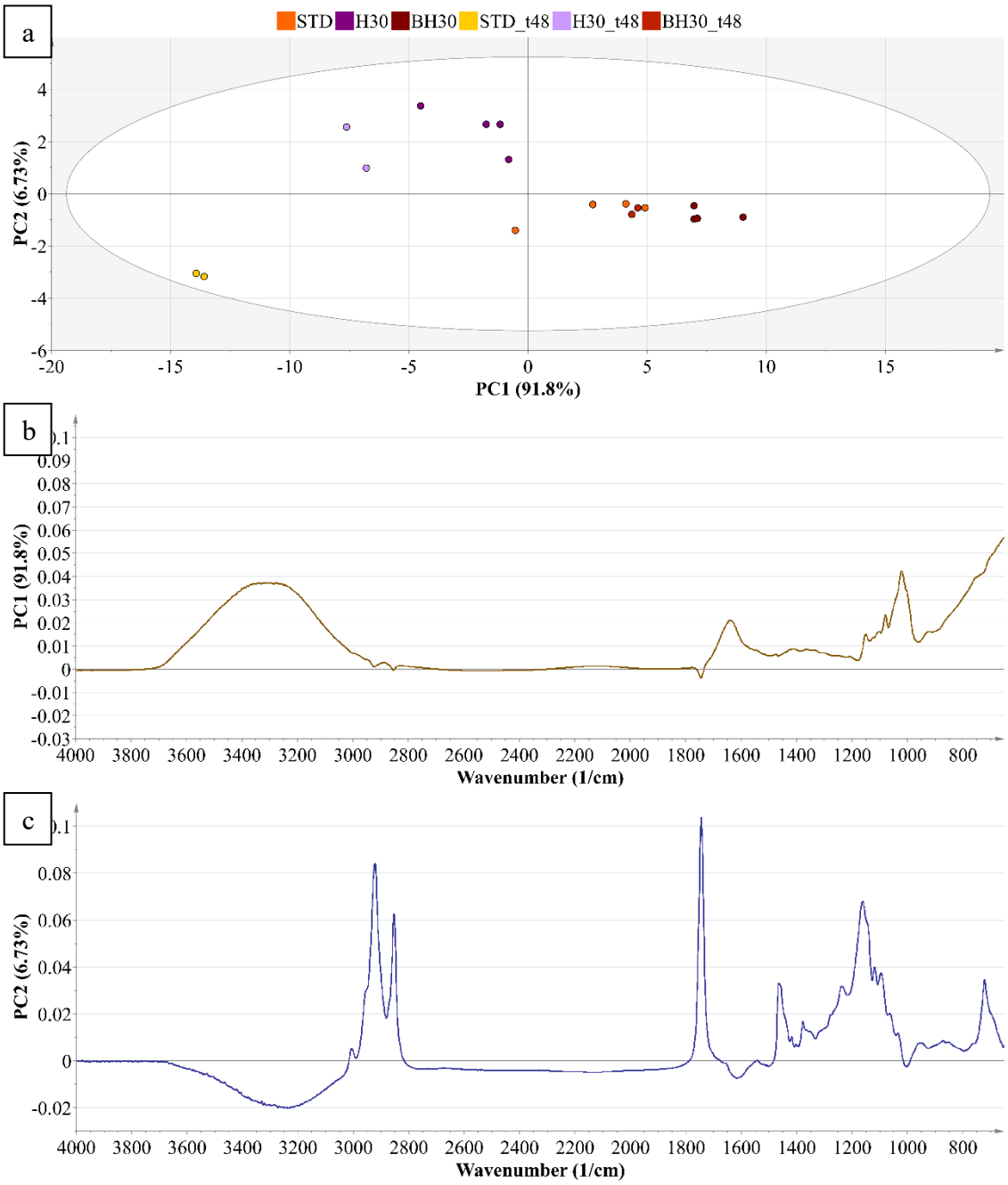

Figure 6.5. PCA score (a) and loading plots according to PC1 (b) and PC2 (c) of FT-IR profiles of the fresh and stored GF-YF breads (R^2= 99.9% and PC=7)

To sum up, fresh and stored hazelnut breads (H30) were distinctively separable from STD and BH30 (both fresh and stored) breads due to the contributions of their absorbances to both PC1 and PC2. STD and stored BH30 showed an interesting similarity in their absorbances, as they were nearly inseparable according to the model. Bread that underwent the most changes during storage was STD since starch retrogradation was

expected to occur more in STD since it was expected to contain the most starch among the samples.

6.2. PCA of GF Dough and Dough Physical Properties

In this section, the MVA results of GF yeast and GF-YF breads and doughs (if necessary) were discussed. Overall, PCA was determined to be a valuable tool for discriminating samples based on their FT-IR profiles and most of their physical properties at once instead of analyzing them individually using ANOVA and correlation coefficients.

6.2.1. PCA of Fresh GF Yeast Bread Physical Properties

Principal Component Analysis was applied for the physical properties (e.g., color, hardness, specific volume, baking loss, water activity, and moisture) of the fresh GF yeast bread samples. As shown in Figure 6.6a, all breads except BH15 and BH30 can be separated from each other. The similarity between BH15 and BH30 were also reported in chapter 5. The model was able to explain 85% of the data, using Principal Components 1 and 2 (PC1 and PC2) with contribution levels of 56.3% and 28.7%, respectively.

According to the loading plot of the data, breads that have the hardest and the most yellow crumbs (H30) are clustered on the right side of the scatter plot (Figure 6.6b). It can also be seen that the lightest crust and crumb colors and the highest baking loss belong to STD, as it was located on the bottom right of the scatter plot. H15 was reported to have some properties (specific volume, crumb lightness, baking loss, and so on) similar to STD in Chapter 4, and its position on the scatter plot validated it. Furthermore, crumbs and slices with the highest moisture belonged to B30, as the properties and the samples had the highest score on PC2. B15, on the other hand, was located between samples BH15-BH30 and B30. When the flour properties were also considered, it can be claimed that PC1 (56.3%) was successful at separating the samples that contained hazelnut flour from others, whereas PC2 (28.7%) split those prepared using white bean flour.

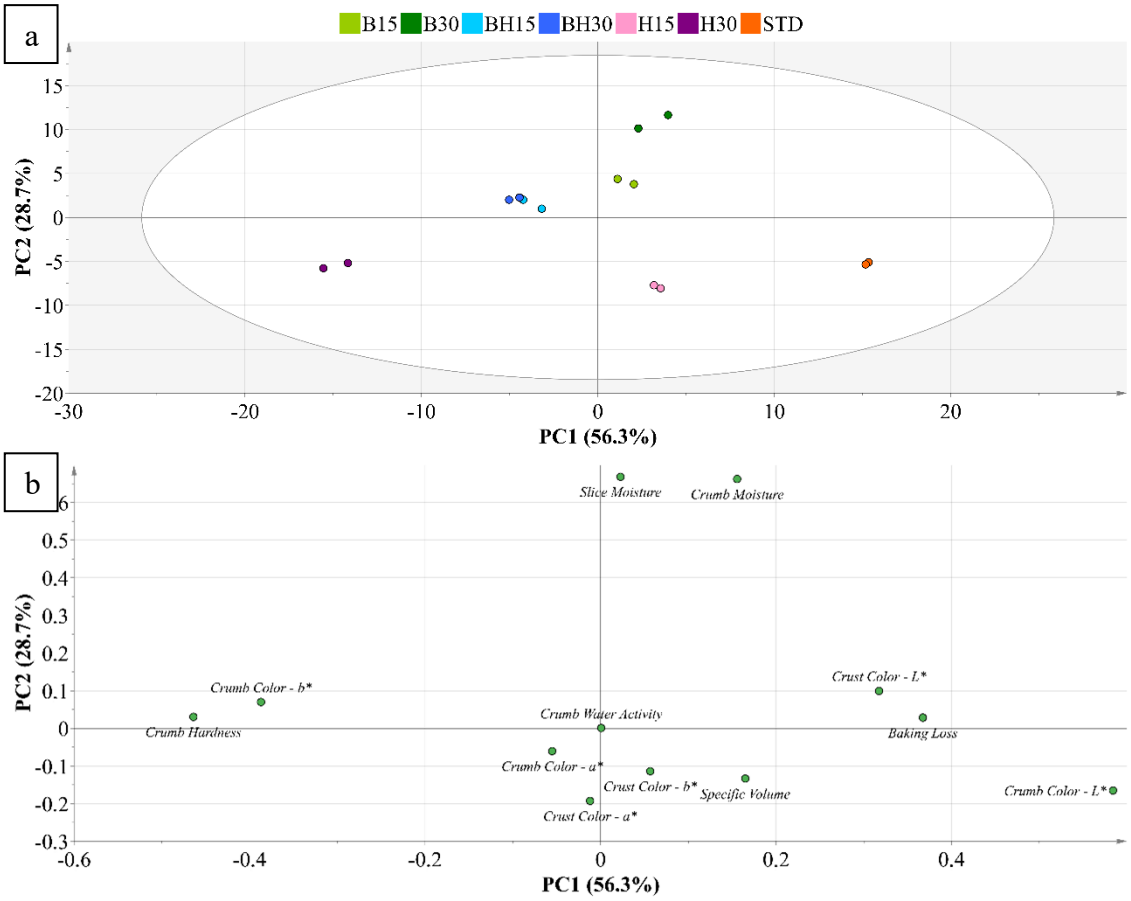

Figure 6.6. PCA score (a) and loading (b) plots of the physical properties of GF yeast breads (R^2= 98.6% and PC=4)

Overall, crumb color, baking loss, and crust and crumb lightness (L*) have strong positive relationships with PC1, whereas crumb hardness and crumb yellowness (b*) are also associated strongly but negatively. PC2 was affiliated with moisture contents of the slices and the crumbs (strongly positive) where redness (a*, crust and crumb), crust yellowness (b*), and specific volume have relatively weak but negative interactions. The specific volume also has a slightly positive effect on PC1.

6.2.2. PCA of Fresh and Stored GF Yeast Bread Properties

PCA for comparison of fresh and stored GF yeast breads was also applied for the properties mentioned in Section 6.2.1. The properties not measured for fresh and stored

samples (e.g., storage loss, baking loss, and specific volume) were not included in the multivariate analysis. PC1 (67.5%) and PC2 (22.2%) explain 89.7% of the data (Figure 6.7a), and the model had more successful PC1 than the model created for fresh samples, even with fewer properties. When the properties of both fresh and 48-h-stored samples were considered, it became slightly harder to see the effects of the flours used, yet the samples prepared by using B were on the positive side of the PC2. In addition, most of the fresh samples (all except H30) gathered on the negative side of the PC1.

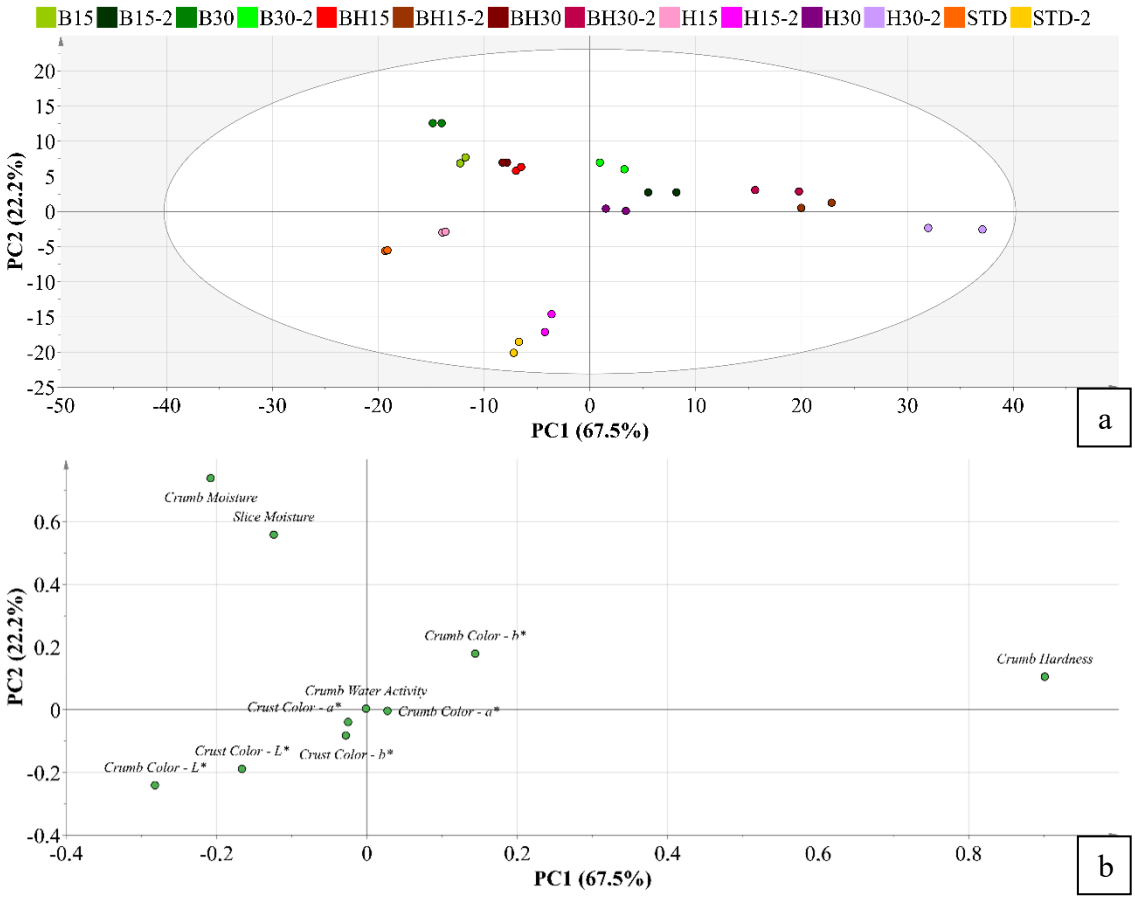

Figure 6.7. PCA score (a) and loading (b) plots of the physical properties of fresh and stored GF yeast breads (R^2= 98.9% and PC=4, 48 hour storage as indicated by "2")

According to the loading plot, samples were gathered together by both PC1 and PC2, depending on the strengths of the effects of the included properties (Figure 6.7b). Crumb hardness and moisture were critical in separating the fresh and stored samples.

Similarly, to the loading plot of fresh breads, moisture contents were again strongly associated with PC2; however, they were located on the slightly negative side of PC1, probably due to the decrease in the moisture contents of the stored breads.

On the other hand, it was also validated that STD and H15 were similar in terms of physical properties for fresh and stored breads, and they were separated from other samples since their properties had negative associations with PC1 and PC2.

Crumb hardness was negatively correlated with PC1 when only the fresh samples were considered. However, when stored samples were introduced, their affiliation became more robust and positive (than fresh bread hardness), which caused the breads with harder crumbs to be gathered on the positive side of PC1. Lightness of the crusts and crumbs (L*) were the properties that had negative associations with PC1 and PC2, whereas crumb yellowness (b*) had positive but weak relationships with them.

6.2.3. PCA of Fresh GF-YF Bread Properties

The first two components of the PCA model created to analyze the GF-YF fresh bread samples could explain 72.4% of the variations in the data, where this value is 38.5% and 33.9% for PC1 and PC2, respectively. PCA was applied on bread and dough texture (TPA and back extrusion results), color (crust and crumb, L*, a*, b*), moisture (slice and crumb), specific volume, and height change. As shown in Figure 6.8a, the breads containing only B had positive scores on PC1 and negative scores on PC2 (except for one sample of B15). On the other hand, the presence of hazelnut flour caused the properties to have negative scores on PCA1 and PCA2. It was also observed that the amount of H was more effective than B in BH formulations. It was noticed that as the amount of H increased (i.e., BH30), it showed more similar properties to H15 and H30. In other words, BH15 was closer to the STD, B15 and B30 in terms of physical properties, whereas BH30 was drawn away from them.

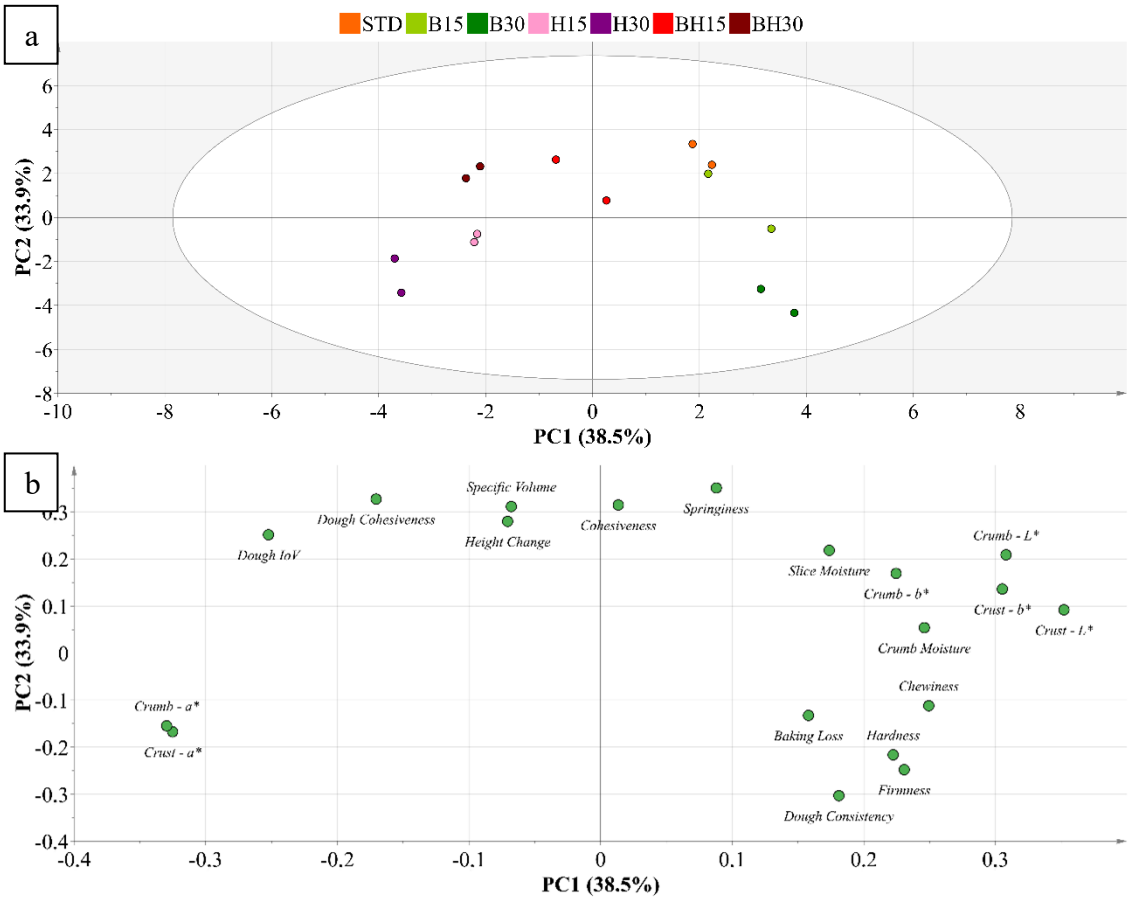

Figure 6.8. PCA score (a) and loading (b) plots of the physical properties of GF-YF breads (R^2= 89.1% and PC=4)

The strength of GF-YF bread properties was determined by comparing scores and the loading plot (Figure 6.8b). In brief, properties having closer scores to zero (-0.1 < x < 0.1) were decided to be weakly related to the component. Almost all properties had similar strengths in their associations with PC1 and PC2. More specifically, strong positive and negative affiliations of PC1 were almost all properties except cohesiveness (crumb), springiness, specific volume, and height change. Especially crust and crumb redness (a*) caused H15 and H30 to gather around the negative side of PC1 on the scatter plot. PC2 also strongly depends on most properties except crumb moisture, chewiness, and crust lightness (L*). STD, B15, and B30 were positioned closer to each other on the positive side of PC1, as this location was associated with higher moisture (slice and crumb), yellowness (crust and crumb, b*), lightness (crust and crumb, L*), baking loss and texture properties that were related to the hardness of dough and crumbs (e.g., hardness, firmness, consistency, and chewiness).

6.2.4. PCA of Fresh and Stored GF-YF Bread Properties

PCA of stored (coded by the number "-2") and fresh GF-YF bread samples is presented in Figure 6.9a. The first two components (PC1, 57.6%; PC2, 35.1%) could explain 92.7% of the data variations. First, it is safe to claim that the stored breads diverged (in the positive direction) from their fresh versions on the PC1 axis. H30, BH30, and STD were separated as described in Section 6.2.3. In addition, stored BH30 had properties similar to fresh H30. Fresh and stored STD were distinguishable from other formulations by both PC1 and PC2.

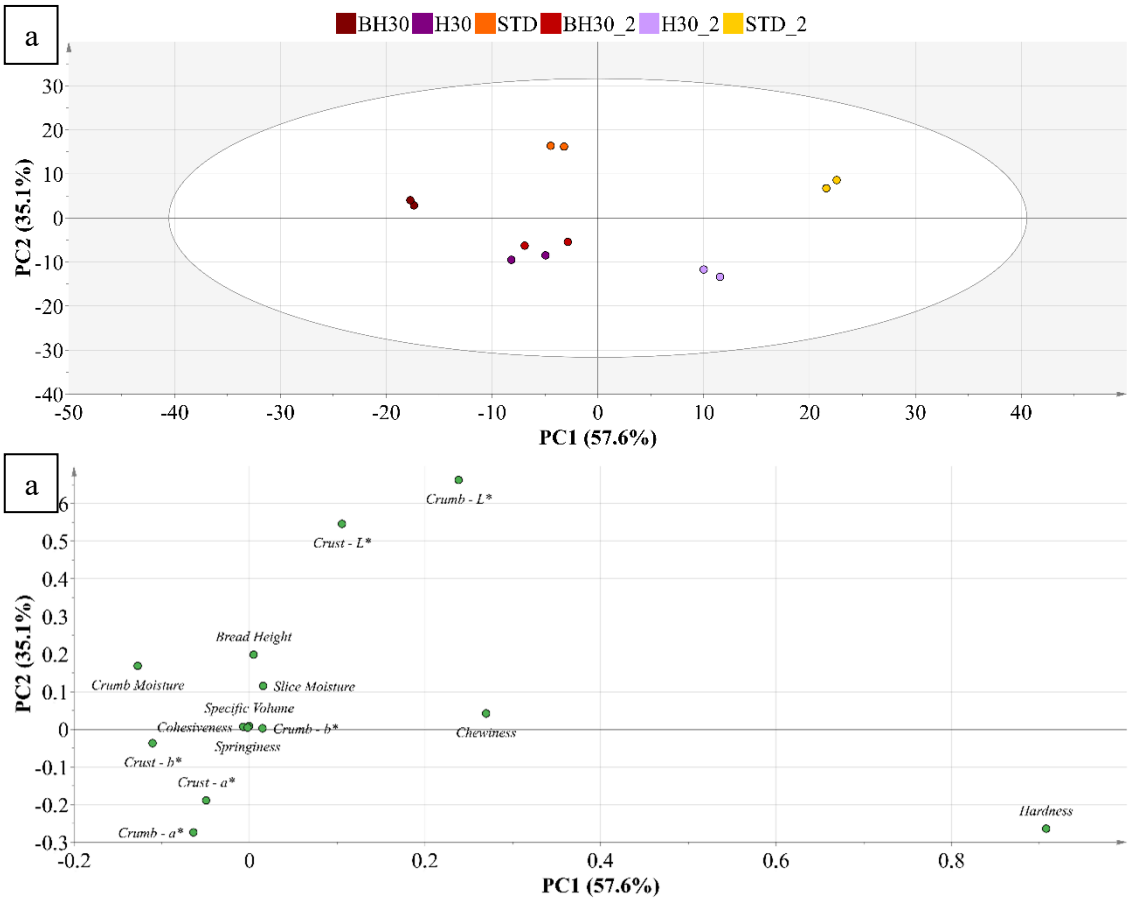

Figure 6.9. PCA score (a) and loading (b) plots of the physical properties of fresh and stored GF-YF breads (R2= 97.9% and PC=3, 48 hour storage was indicated by "2")

The loading plot shows the associations between the physical properties and the principal components (Figure 6.9b). Strong relations between PC1 and some texture-related properties (e.g., chewiness and hardness) and between lightness (crust and crumb, L*) and PC2 were observed. Crumb yellowness, springiness, specific volume, and cohesiveness were clustered in the center, suggesting that there were not any differences between them. Moisture and bread height were weakly associated with PC2, where crumb moisture also had a relatively weak (when compared to the importance of hardness) but a negative relationship with PC1. On the other hand, crust yellowness (b*), crumb, and crust redness (a*) were negatively affiliated with PC1 and PC2.

CHAPTER 7

CONCLUSIONS AND FUTURE PERSPECTIVES

White bean and hazelnut flours were used in gluten-free (GF) bread formulations, and some physical and chemical properties and mid-infrared spectroscopic profiles of flours, dough, and bread samples were determined. White bean flour was characterized as high protein and carbohydrate, whereas hazelnut flour was characterized as high fat and fiber ingredients, which were evaluated to improve nutritional and sensorial properties of rice flour-corn starch-based standard gluten-free breads.

Bread formulation experiments (with 0 to 30% bean and hazelnut flours) and optimizations were generated according to a mixture design. Regarding GF-yeast bread data, the standard bread formulation produced a high-volume (7.0 g/mL), low hardness (0.43 N) bread compared to legume and nut-containing breads. In this part of the study, bread formulated with the 15% hazelnut flour replacement of rice flour-corn starch base produced the closest product to the standard gluten-free yeast bread (3.8 g/mL and 1.59 N). In the second part of the study, gluten-free yeast-free formulations were generated based on the flour mixture in the previous part. In the case of no yeast, breads containing 30% hazelnut flour and 30% bean and hazelnut flour as a replacement for rice flour-corn starch base gave the best sensorial properties compared to standard gluten-free bread samples.

Spectroscopic profiles of dough and bread samples between 4000-800 cm-1 wave number revealed the differences among samples. A multivariate model (PCA) of mid-infrared FTIR data distinguished samples of 30% hazelnut flour (dough and bread), whereas all the breads with white bean flour with or without hazelnut flour produced similar results and formed a cluster. The stored breads separated themselves from fresh breads for 24 h or 48 h.

In starch digestion, gluten-free yeast-free hazelnut (30%), and white bean-hazelnut (30%) breads were compared to the reference bread based on 100% rice flour-corn starch in digestible starch amounts. It was determined that the standard rice flour-corn starch gluten-free bread could increase glucose levels, in terms of rapidly digestible

ones. If gluten-free bread is enriched with legume and nut flours such as white bean and hazelnut flours at 30%, the rapidly digestible starch levels can decrease by 19.30 (H30) and 29.03% (BH30).

In conclusion, legume and nut flours can be alternative, partial replacement ingredients in order to enrich bakery products nutritionally. White bean flour can increase the protein contents of bakery products sustainably, whereas hazelnut flour can improve the taste and flavor and increase the healthy fat and fiber contents. It was determined that use of bean and hazelnut flours together in the formulation improves starch digestion properties by decreasing the rapidly digestible starch.

In the future, digestion analyses can be expanded to cover different formulations of bean and hazelnut flours and their stored products to gather more information. Gluten-free and yeast-free bread can be considered as an alternative choice since it promises a considerable decrease in bread-making time, as well as producing preferable breads.

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APPENDIX A

SEM FIGURES OF THE FLOURS AT DIFFERENT MAGNIFICATIONS: 250, 1000, 2500, 5000, AND 1000×

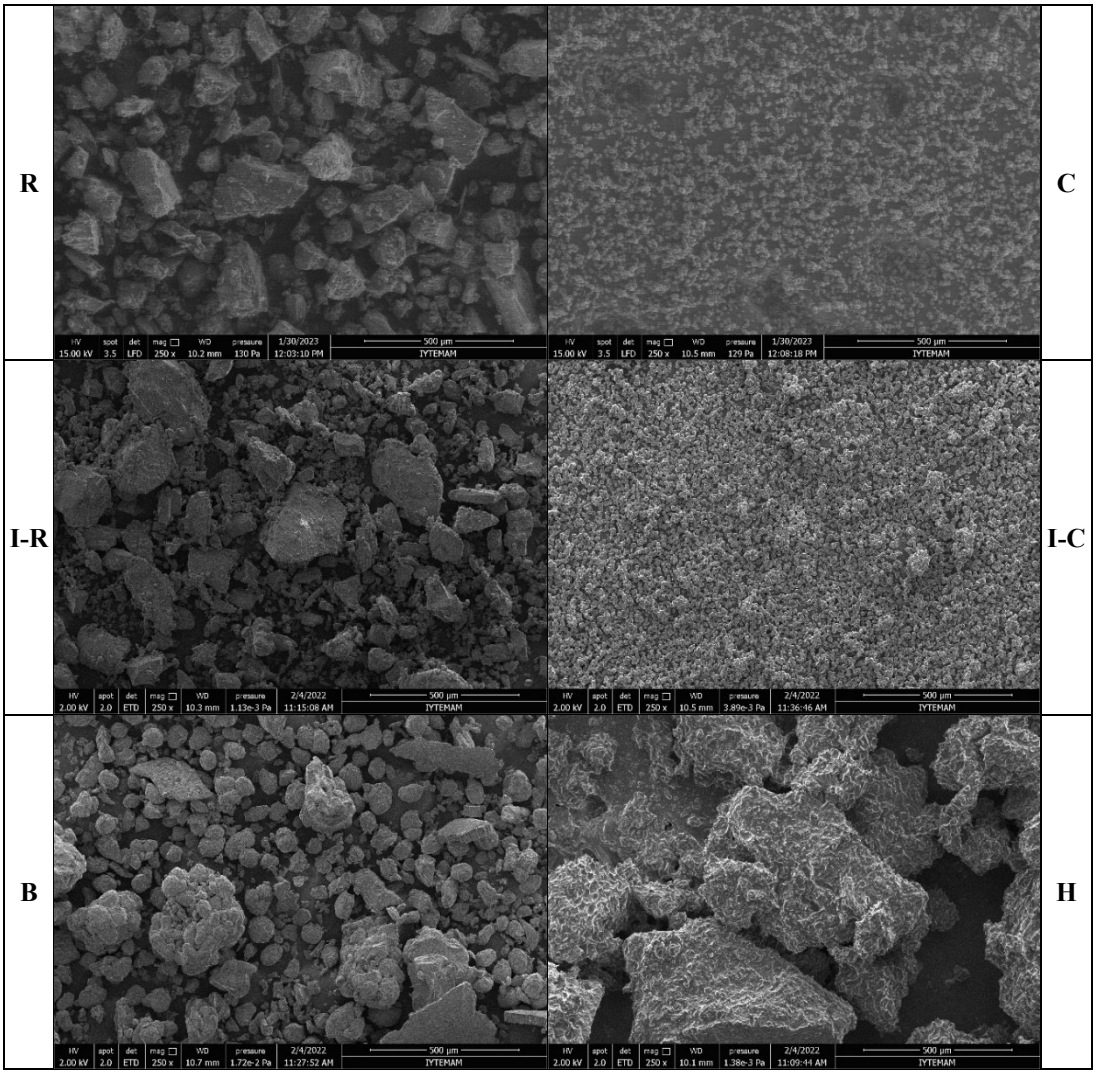

Figure A.1. SEM images of R, C, I-R, I-C, B, and H at 250× magnification

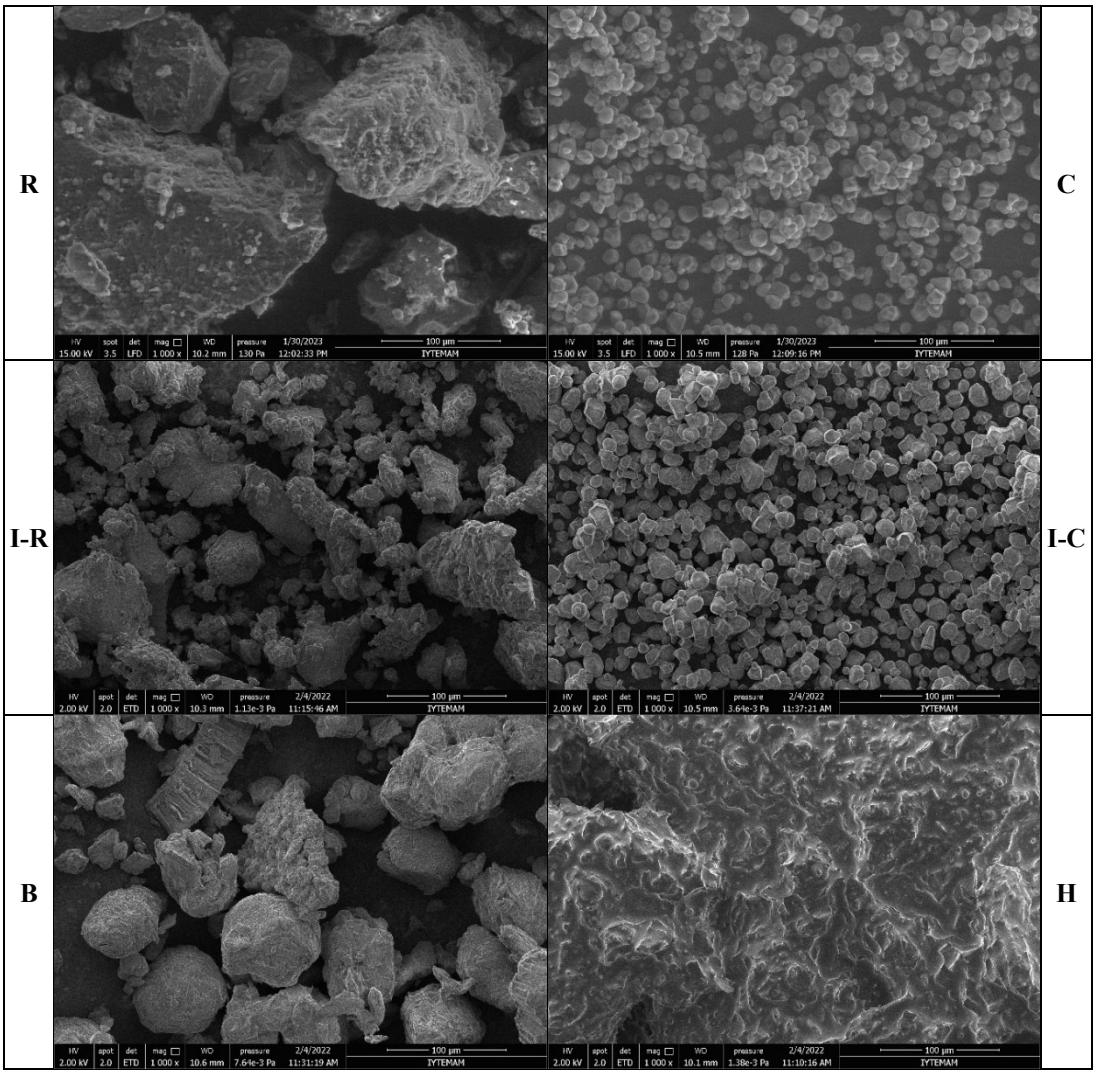

Figure A.2. SEM images of R, C, I-R, I-C, B, and H at 1000× magnification

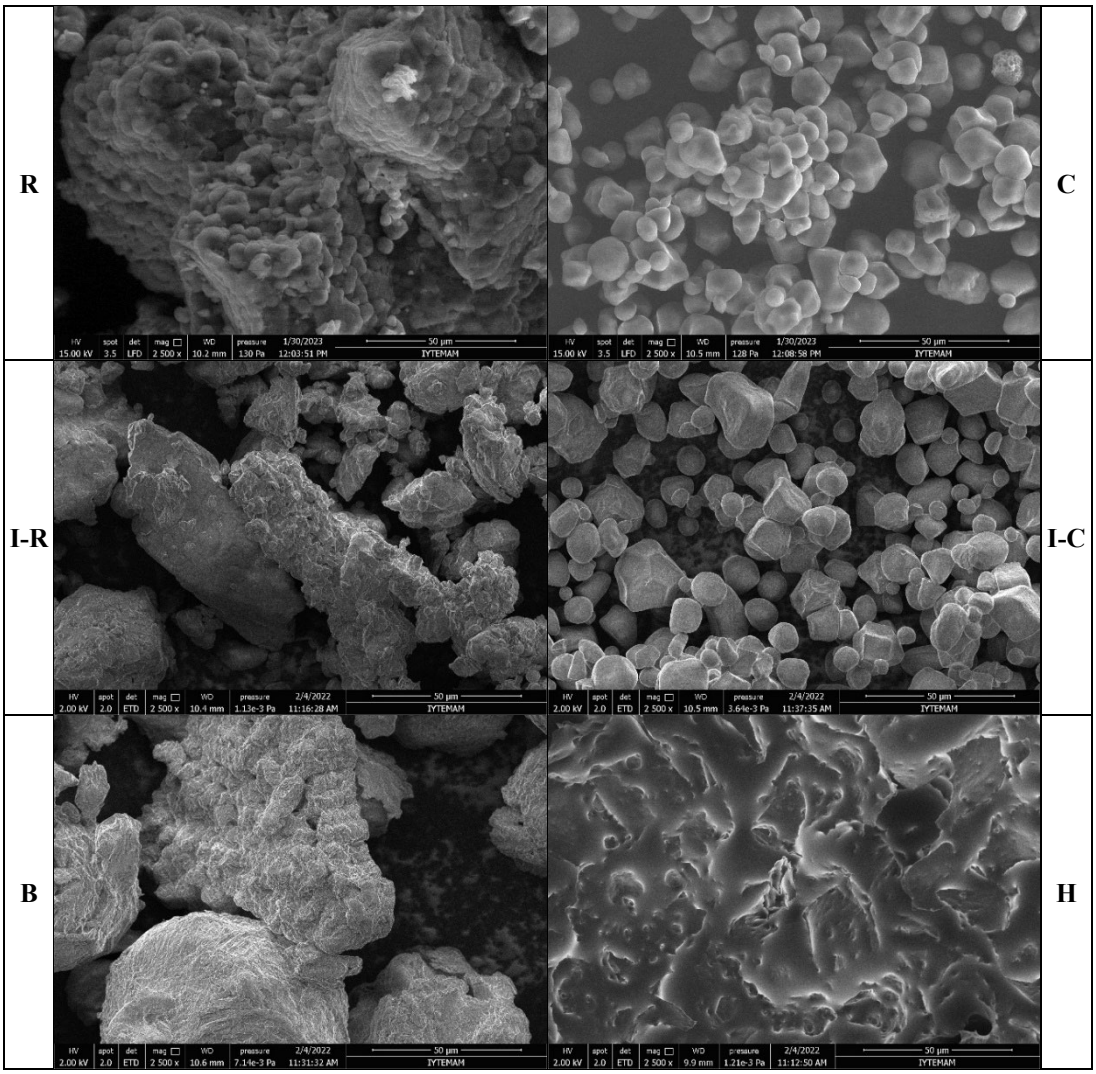

Figure A.3. SEM images of R, C, I-R, I-C, B, and H at 2500× magnification

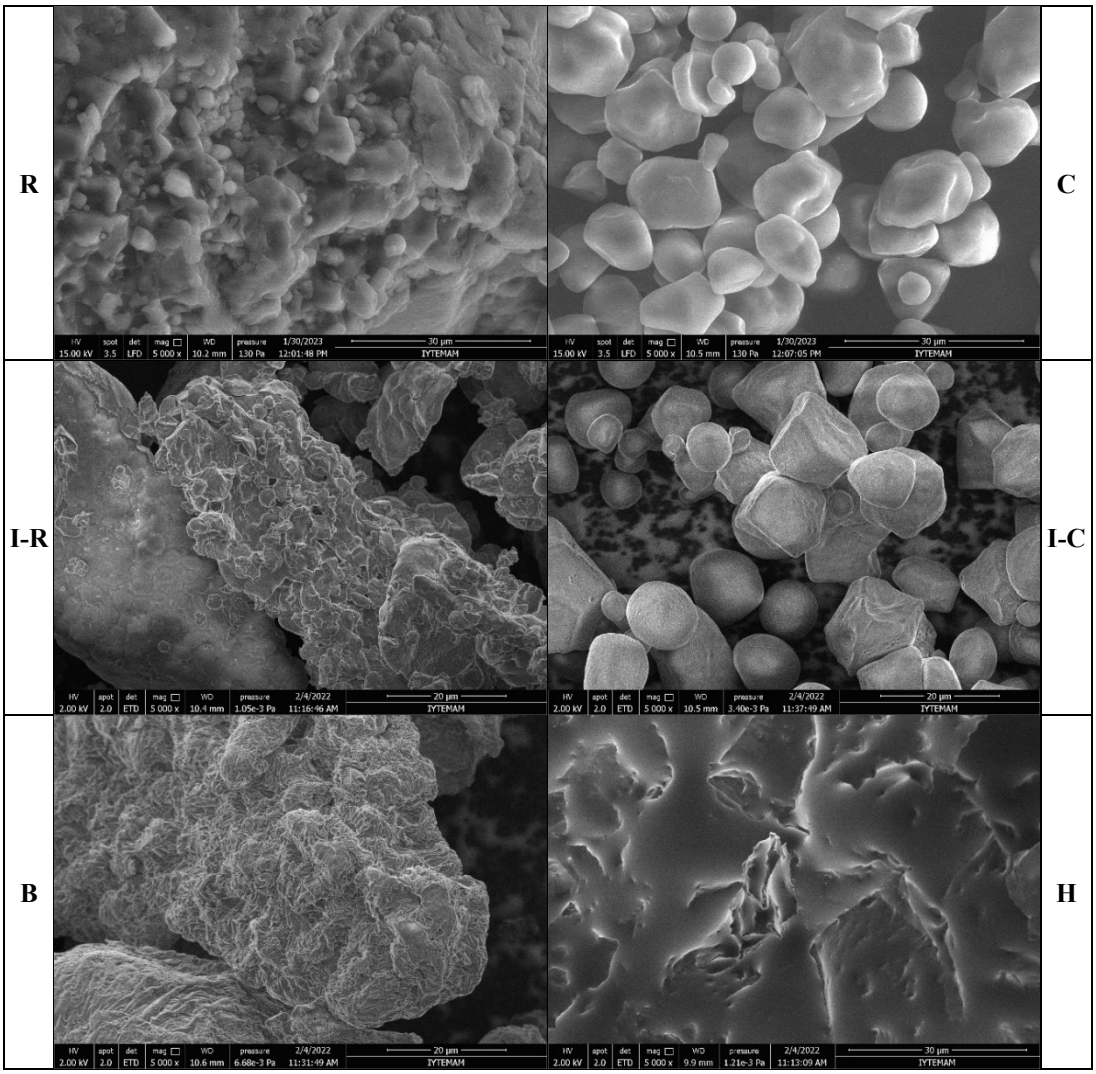

Figure A.4. SEM images of R, C, I-R, I-C, B, and H at 5000× magnification

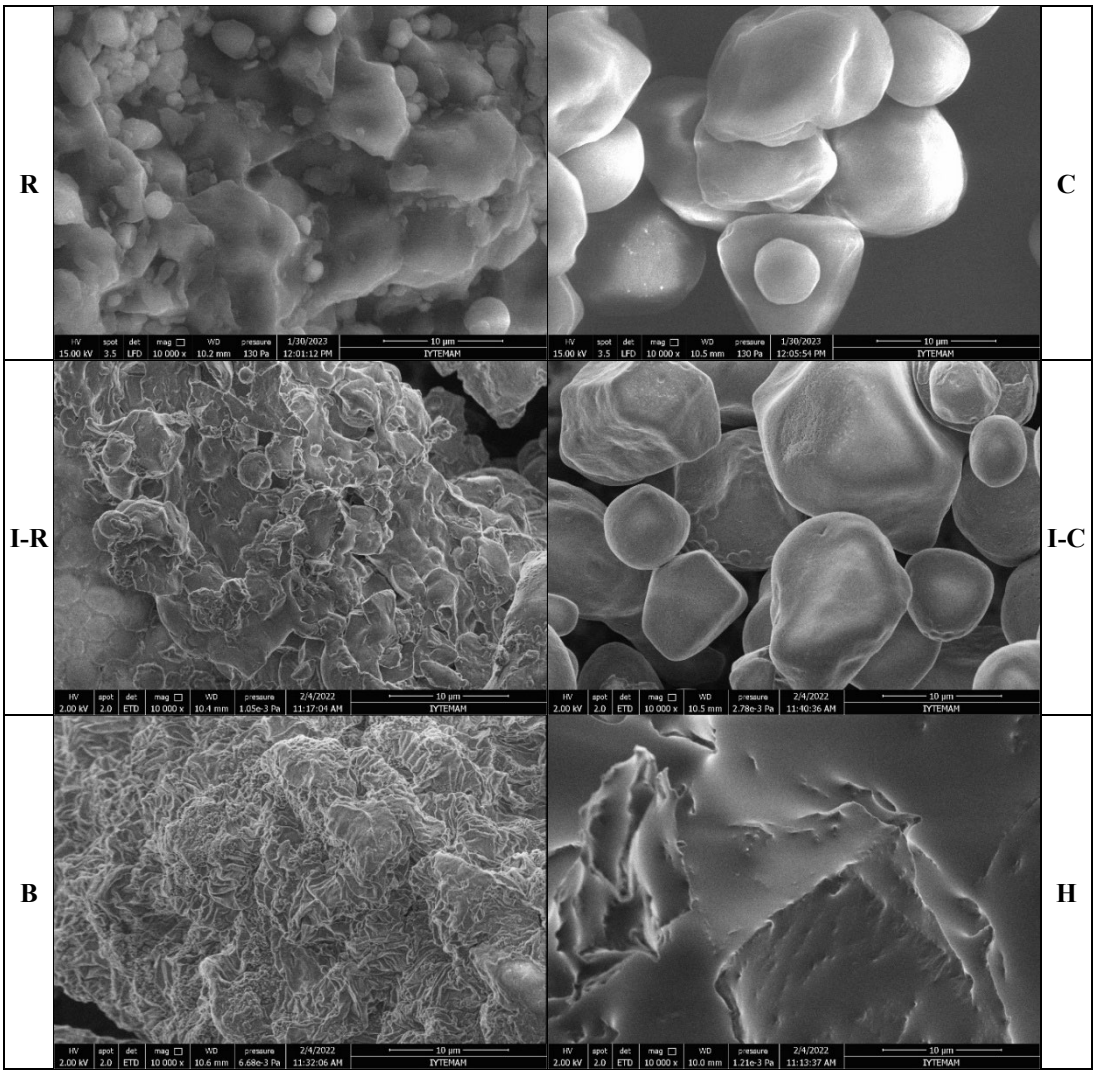

Figure A.5. SEM images of R, C, I-R, I-C, B, and H at 10000× magnification

APPENDIX B

MVA DATA AND PROFILES OF THE FLOURS

Table B.1. Pasting Properties of Flours Used In GF Yeast Breads

Sample	Gelatinization Temperature (°C)	Peak Viscosity (BU)	Breakdown Viscosity (BU)	Setback Viscosity (BU)	Final Viscosity (BU)
B	95.6 ± 0.1^{A}	44 ± 1^{C}	1 ± 1^{C}	65 ± 0^{C}	108 ± 0^{C}
C	70.0 ± 0.3^{B}	1223 ± 0^{A}	631 ± 631^{A}	973 ± 3^{A}	1548 ± 40^{A}
H*	30.3	187	97	39	127
R	70.6 ± 0.3^{B}	1065 ± 11^{B}	558 ± 16^{B}	478 ± 1^{B}	987 ± 4^{B}

*Pasting profile analysis for H could not be repeated due to the particles disturbing the MVA device.

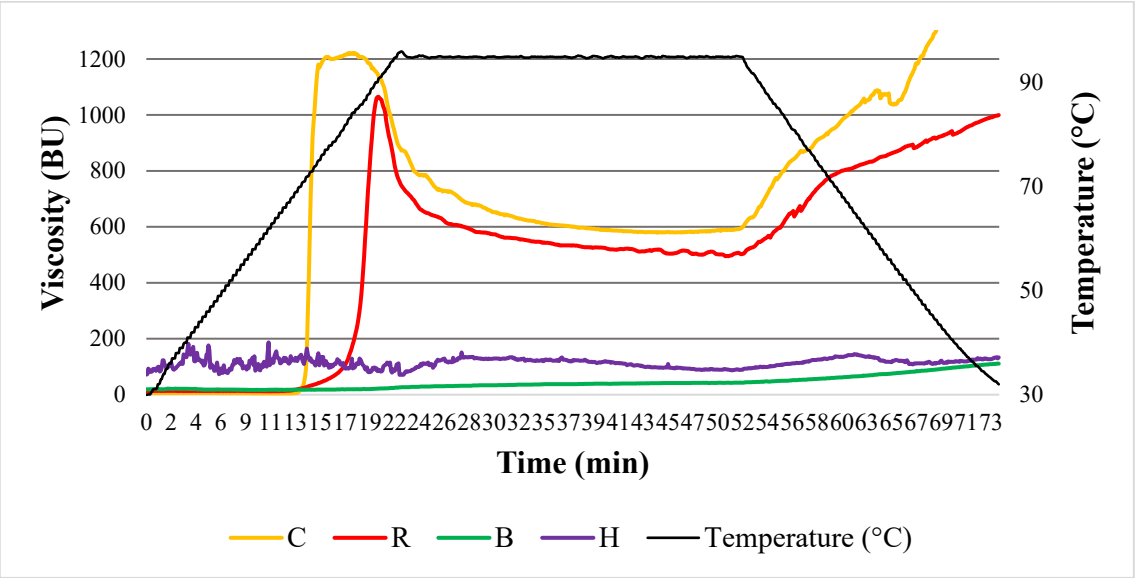

Figure B.1. Pasting profiles of the GF yeast bread flours

APPENDIX C

GF YEAST BREAD DOUGH LEAVENING DATA

Table C.1. GF yeast bread dough area, average of three measurements

Sample	Dough Area (mm^2)				
	t=0	***t=15 min***	***t=30 min***	***t=45 min***	***t=60 min***
STD	1289 ± 159	1751 ± 248	2185 ± 308	2624 ± 416	2871 ± 407
B15	1260 ± 59	1646 ± 81	2214 ± 144	2392 ± 139	2404 ± 128
B30	1087 ± 89	1293 ± 74	1647 ± 94	1772 ± 104	1778 ± 104
H15	1133 ± 106	1583 ± 163	1875 ± 203	1902 ± 195	1969 ± 202
H30	929 ± 165	1076 ± 209	1100 ± 205	1130 ± 216	1163 ± 217
BH15	1225 ± 52	1680 ± 23	1737 ± 25	1743 ± 30	1758 ± 22
BH30	1242 ± 27	1568 ± 47	1677 ± 43	1690 ± 38	1699 ± 35

Table C.2. GF yeast bread dough are increase per 15 minutes w.r.t. area at t=0

Sample	Area Increase Rate (%)			
	t=15 min	***t=30 min***	***t=45 min***	***t=60 min***
STD	35.62 ± 2.67	69.25 ± 5.16	103.04 ± 10.16	122.53 ± 13.11
B15	30.64 ± 0.87	75.88 ± 10.51	90.02 ± 10.95	91.05 ± 11.03
B30	19.12 ± 3.22	51.88 ± 7.67	63.26 ± 4.43	63.81 ± 4.51
H15	39.70 ± 1.62	65.42 ± 4.70	67.86 ± 3.62	73.73 ± 2.79
H30	15.61 ± 2.03	18.35 ± 1.08	21.53 ± 2.02	25.17 ± 1.89
BH15	37.25 ± 4.83	41.87 ± 6.01	42.36 ± 6.18	43.59 ± 6.19
BH30	26.20 ± 1.14	35.02 ± 2.25	36.06 ± 1.68	36.81 ± 1.16

APPENDIX D

PAIRWISE PEARSON CORRELATIONS OF GF YEAST FRESH BREAD PROPERTIES

Table D.1. Pairwise Pearson correlation coefficients of GF yeast fresh bread properties

Properties	Water Added (g)	Dough Area Increase (%)	Baking Loss (%)	Specific Volume (mL/g)	Slice Moisture (g/100 g)	Crumb Moisture (g/100 g)	Crumb Water Activity
Dough Area Increase (%)	0.32						
Baking Loss (%)	0.468	0.911					
Specific Volume (mL/g)	-0.102	0.794	0.795				
Slice Moisture (g/100 g)	0.92	-0.049	0.1	-0.466			
Crumb Moisture (g/100 g)	0.986	0.194	0.362	-0.226	0.955		
Crumb Water Activity	0.763	0.682	0.763	0.418	0.513	0.704	
Crumb Hardness (N)	-0.369	-0.83	-0.931	-0.716	-0.036	-0.299	-0.613

APPENDIX E

TECHNOLOGICAL PROPERTIES OF THE STORED GF YEAST BREADS

Table E.1. Properties of the GF yeast bread samples stored for 24 h

Properties	STD_{t24}	$B15_{t24}$	$B30_{t24}$	$H15_{t24}$	$H30_{t24}$	$BH15_{t24}$	$BH30_{t24}$
Storage Loss	8.4 ± 0.2	6.9 ± 0.3	7.2 ± 1.1	5.2 ± 0.3	3.5 ± 0.1	6.1 ± 0.1	6.1 ± 0.5
Crumb Moisture Content (%)	36.09 ± 0.36	42.99 ± 0.75	46.73 ± 0.70	35.84 ± 0.64	33.37 ± 0.75	37.39 ± 1.20	39.67 ± 0.92
Bread Moisture Content (%)	21.24 ± 1.15	32.26 ± 0.23	35.49 ± 1.41	24.60 ± 0.32	23.97 ± 0.57	28.12 ± 0.38	28.71 ± 0.18
Crumb Water Activity	0.955 ± 0.003	0.968 ± 0.003	0.968 ± 0.000	0.955 ± 0.004	0.939 ± 0.002	0.957 ± 0.001	0.952 ± 0.001
Crust - L*	77.8 ± 1.4	66.5 ± 0.5	71.1 ± 0.8	66.8 ± 1.7	65.3 ± 0.9	64.4 ± 0.7	64.7 ± 1.0
Crust - a*	-1.2 ± 0.7	0.7 ± 0.8	-0.8 ± 0.6	4.1 ± 1.0	1.7 ± 0.7	-0.7 ± 0.5	-0.3 ± 1.0
Crust - b*	27.3 ± 3.0	30.5 ± 0.8	28.7 ± 2.8	35.1 ± 0.8	29.3 ± 1.1	29.3 ± 1.2	29.3 ± 1.2
Crumb - L*	81.5 ± 1.5	72.8 ± 0.9	76.6 ± 0.9	77.8 ± 0.6	67.8 ± 0.4	69.5 ± 0.3	69.5 ± 0.3
Crumb - a*	-2.5 ± 0.2	-3.2 ± 0.2	-3.4 ± 0.1	-1.9 ± 0.3	-0.6 ± 0.1	-2.1 ± 0.2	-1.9 ± 0.4
Crumb - b*	5.0 ± 0.3	10.3 ± 0.6	13.2 ± 0.6	10.0 ± 0.3	17.2 ± 0.3	15.1 ± 0.8	15.4 ± 1.1
Crumb Hardness (N)	1.44 ± 0.32	13.42 ± 1.76	11.66 ± 1.08	3.62 ± 0.33	29.43 ± 4.48	20.94 ± 1.60	19.52 ± 1.02

Table E.2. Properties of the GF yeast bread samples stored for 48 h

Properties	STD_{t48}	$B15_{t48}$	$B30_{t48}$	$H15_{t48}$	$H30_{t48}$	$BH15_{t48}$	$BH30_{t48}$
Storage Loss	12.5 ± 0.1	11.2 ± 0.3	12.2 ± 0.7	9.6 ± 0.0	5.7 ± 0.4	9.3 ± 0.1	9.9 ± 0.6
Crumb Moisture Content (%)	26.17 ± 1.02	39.08 ± 0.50	43.50 ± 1.00	25.01 ± 1.57	30.57 ± 0.73	34.52 ± 0.59	36.86 ± 0.65
Bread Moisture Content (%)	19.64 ± 0.31	30.56 ± 0.38	32.68 ± 1.57	21.11 ± 0.46	22.69 ± 1.25	27.09 ± 0.61	28.42 ± 0.55
Crumb Water Activity	0.887 ± 0.016	0.960 ± 0.004	0.957 ± 0.002	0.883 ± 0.004	0.924 ± 0.001	0.939 ± 0.001	0.935 ± 0.002
Crust - L*	78.0 ± 1.1	67.3 ± 1.0	67.4 ± 1.0	66.5 ± 1.3	64.9 ± 1.1	64.2 ± 0.7	64.9 ± 1.1
Crust - a*	-1.7 ± 0.2	-0.2 ± 0.9	1.1 ± 0.7	3.8 ± 0.7	2.0 ± 0.4	-0.4 ± 0.3	2.0 ± 0.4
Crust - b*	28.9 ± 2.2	30.2 ± 1.6	31.2 ± 3.7	34.7 ± 1.8	30.9 ± 0.8	28.9 ± 1.0	30.9 ± 0.8
Crumb - L*	81.2 ± 1.5	72.0 ± 0.9	74.1 ± 0.8	76.6 ± 0.9	66.2 ± 1.5	68.6 ± 0.2	68.6 ± 0.2
Crumb - a*	-2.9 ± 0.2	-3.0 ± 0.1	-2.7 ± 0.1	-2.0 ± 0.1	-0.7 ± 0.1	-1.8 ± 0.1	-1.8 ± 0.1
Crumb - b*	6.1 ± 0.6	11.3 ± 0.2	12.9 ± 0.5	11.9 ± 1.0	16.5 ± 0.8	14.8 ± 0.4	14.8 ± 0.4
Crumb Hardness (N)	8.30 ± 1.36	22.47 ± 1.65	19.04 ± 1.53	7.42 ± 1.46	46.92 ± 9.06	34.87 ± 1.98	31.49 ± 2.84

APPENDIX F

BACK EXTRUSION PROPERTIES OF GF-YF BREADS AT DIFFERENT WATER LEVELS

Table F.1. Back extrusion properties of GF-YF breads at different water levels

Sample	Water Added (g/100 g fm)	Firmness (F1, N)	Cohesiveness (F2, N)	Consistency (A1-2, N.sec)	Index of Visc. (A2-3, N.sec)
STD_72	72	10.48 ± 0.27	-5.51 ± 0.04	35.08 ± 3.32	-7.40 ± 0.60
STD_80	80	5.44 ± 0.21	-2.43 ± 0.23	17.73 ± 2.35	-3.90 ± 1.24
STD_95	95	3.37 ± 0.07	-1.79 ± 0.11	12.81 ± 0.20	-2.70 ± 0.20
B15_110	110	4.13 ± 0.15	-2.18 ± 0.13	15.38 ± 0.49	-3.37 ± 0.19
B30_95	95	19.18 ± 0.77	-8.43 ± 0.39	68.66 ± 0.04	-7.36 ± 0.33
B30_110	110	8.39 ± 0.40	-4.48 ± 0.19	31.51 ± 2.61	-5.85 ± 0.24
B30_120	120	4.36 ± 0.39	-2.42 ± 0.08	14.95 ± 3.25	-3.56 ± 0.53
H15_95	95	3.73 ± 0.03	-1.99 ± 0.04	14.50 ± 0.18	-2.86 ± 0.05
H30_90	90	4.81 ± 0.09	-2.40 ± 0.05	19.29 ± 0.19	-3.31 ± 0.07
H30_95	95	3.65 ± 0.02	-2.01 ± 0.01	14.64 ± 0.10	-2.86 ± 0.01
BH30_90	90	8.31 ± 0.45	-4.31 ± 0.18	31.98 ± 1.20	-5.37 ± 0.23
BH30_95	95	7.32 ± 0.20	-3.65 ± 0.10	28.05 ± 0.72	-4.78 ± 0.07

APPENDIX G

PAIRWISE PEARSON CORRELATIONS OF GF-YF FRESH BREAD PROPERTIES

Table G.1. Pairwise Pearson correlation coefficients of GF-YF dough and fresh bread properties

Properties	Water%	Dough Firmness (N)	Dough Cohesiveness (N)	Dough Consistency (N.sec)	Dough Index of Viscosity (N.sec)
Firmness (N)	0.462				
D. Cohesiveness (N)	-0.332	-0.901			
Consistency (N.sec)	0.464	0.964	-0.946		
Index of Viscosity (N.sec)	-0.46	-0.934	0.931	-0.905	
Baking Loss (g/100 g)	0.703	0.386	-0.453	0.417	-0.448
Specific Volume (cm3/g)	0.056	-0.559	0.822	-0.667	0.652
Height Change (%)	-0.161	-0.429	0.517	-0.489	0.486
Slice Moisture (g/100 g)	0.388	0.019	0.236	-0.07	0.043
Crumb Moisture (g/100 g)	0.932	0.311	-0.131	0.284	-0.302
Hardness (N)	0.144	0.576	-0.602	0.545	-0.654
Cohesiveness (N)	-0.38	-0.378	0.555	-0.57	0.369
Gumminess (N)	0.042	0.516	-0.507	0.437	-0.601
Springiness	0.045	-0.338	0.589	-0.527	0.304
Chewiness (N.mm)	0.035	0.477	-0.435	0.368	-0.569
Resilience	-0.254	-0.255	0.469	-0.466	0.24

Table G.2. Pairwise Pearson correlation coefficients of GF-YF fresh bread physical and texture properties

Properties	Baking Loss (g/100 g)	Specific Volume (cm3/g)	Height Change (%)	Slice Moisture (g/100 g)	Crumb Moisture (g/100 g)
Specific Volume (cm3/g)	-0.195				
Height Change (%)	-0.333	0.37			
Slice Moisture (g/100 g)	0.05	0.333	0.439		
Crumb Moisture (g/100 g)	0.528	0.173	-0.052	0.56	
Hardness (N)	0.241	-0.549	-0.732	-0.136	0.143
Cohesiveness (N)	-0.471	0.576	0.571	0.251	-0.228
Gumminess (N)	0.148	-0.452	-0.646	-0.097	0.073
Springiness	-0.233	0.681	0.599	0.556	0.234
Chewiness (N.mm)	0.113	-0.366	-0.576	-0.035	0.089
Resilience	-0.454	0.488	0.547	0.362	-0.059

Table G.3. Pairwise Pearson correlation coefficients of GF-YF texture properties

Properties	Hardness (N)	Cohesiveness (N)	Gumminess (N)	Springiness	Chewiness (N.mm)
Hardness (N)					
Cohesiveness (N)	-0.381				
Gumminess (N)	0.974	-0.166			
Springiness	-0.37	0.815	-0.208		
Chewiness (N.mm)	0.939	-0.044	0.991	-0.076	
Resilience	-0.177	0.937	0.03	0.855	0.154

APPENDIX H

TECHNOLOGICAL PROPERTIES OF THE STORED AND FRESH GF-YF BREADS

Table H.1. Physical properties of fresh and stored GF-YF samples

Sample	Baking Loss (%)	Storage Loss (%)	Height (mm)	Height Change (%)	Specific Volume (ml/g)	Crumb Moisture (%)	Slice Moisture (%)
STD_{t0}	17.09 ± 1.10	0.00 ± 0.00	44.93 ± 1.34	86.95 ± 9.66	1.57 ± 0.02	49.74 ± 0.17	37.03 ± 1.48
$H30_{t0}$	15.91 ± 0.05	0.00 ± 0.00	39.52 ± 0.52	61.33 ± 3.57	1.35 ± 0.02	46.50 ± 0.12	33.84 ± 1.60
$BH30_{t0}$	19.74 ± 0.61	0.00 ± 0.00	36.17 ± 0.90	53.37 ± 3.19	1.48 ± 0.01	51.60 ± 0.71	34.61 ± 4.22
STD_{t48}	0.00 ± 0.00	0.09 ± 0.09	38.00 ± 6.51	1.24 ± 0.58	1.46 ± 0.07	47.05 ± 0.83	35.93 ± 2.17
$H30_{t48}$	0.00 ± 0.00	0.15 ± 0.15	37.40 ± 0.71	1.32 ± 0.13	1.32 ± 0.04	43.68 ± 0.11	33.72 ± 1.36
$BH30_{t48}$	0.00 ± 0.00	0.16 ± 0.16	35.15 ± 0.21	0.59 ± 0.08	1.35 ± 0.03	47.50 ± 1.49	34.16 ± 1.92

Table H.2. Texture properties of fresh and stored GF-YF samples

Sample	Hardness (N)	Cohesiveness	Gumminess (N)	Springiness	Chewiness	Resilience	Rate of Staling
STD_{t0}	16.38 ± 0.75	0.80 ± 0.01	13.08 ± 0.51	0.97 ± 0.01	12.70 ± 0.57	0.53 ± 0.01	0.00 ± 0.00
$H30_{t0}$	20.44 ± 1.86	0.66 ± 0.00	13.44 ± 1.19	0.88 ± 0.02	11.80 ± 0.78	0.35 ± 0.01	0.00 ± 0.00
$BH30_{t0}$	8.41 ± 0.28	0.70 ± 0.02	5.88 ± 0.04	0.93 ± 0.01	5.50 ± 0.01	0.41 ± 0.00	0.00 ± 0.00
STD_{t48}	42.97 ± 0.13	0.46 ± 0.06	19.76 ± 2.11	0.89 ± 0.01	17.53 ± 2.07	0.24 ± 0.04	1.63 ± 1.63
$H30_{t48}$	37.72 ± 1.30	0.46 ± 0.02	17.43 ± 1.24	0.79 ± 0.01	13.72 ± 1.14	0.22 ± 0.01	0.86 ± 0.86
$BH30_{t48}$	21.97 ± 1.83	0.50 ± 0.03	11.11 ± 1.59	0.87 ± 0.04	9.72 ± 1.79	0.25 ± 0.02	1.62 ± 1.62

Table H.3. Color properties of fresh and stored GF-YF samples

Sample	Crust - L*	Crust - a*	Crust - b*	Crumb - L*	Crumb - a*	Crumb - b*
STD_{t0}	72.95 ± 0.06	-2.41 ± 0.02	26.88 ± 0.14	72.03 ± 0.82	-4.14 ± 0.06	23.46 ± 0.11
$H30_{t0}$	59.07 ± 1.30	2.23 ± 0.67	28.20 ± 0.76	54.20 ± 0.25	-2.98 ± 0.00	23.49 ± 0.01
$BH30_{t0}$	64.97 ± 0.05	1.48 ± 0.08	32.04 ± 0.01	61.87 ± 0.05	2.40 ± 0.04	22.89 ± 0.08
STD_{t48}	71.49 ± 0.82	-1.67 ± 0.10	26.99 ± 1.55	73.39 ± 0.41	1.96 ± 0.04	23.83 ± 0.00
$H30_{t48}$	58.78 ± 0.23	2.81 ± 0.02	27.46 ± 0.18	57.35 ± 1.79	-3.48 ± 0.05	24.40 ± 0.12
$BH30_{t48}$	61.32 ± 0.34	2.18 ± 0.20	30.46 ± 0.59	57.91 ± 1.78	-1.84 ± 0.01	24.26 ± 0.03