DEVELOPMENT OF GLUTEN-FREE BREAD FORMULATIONS BASED ON CHICKPEA FLOUR: OPTIMIZATION OF FORMULATION, EVALUATION OF DOUGH PROPERTIES AND BREAD QUALITY

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ABSTRACT

DEVELOPMENT OF GLUTEN-FREE BREAD FORMULATIONS BASED ON CHICKPEA FLOUR: OPTIMIZATION OF FORMULATION, EVALUATION OF DOUGH PROPERTIES AND BREAD QUALITY

The main objective of this study was to develop chickpea flour-containing gluten-free bread formulations having improved physicochemical, nutritional and sensory properties. Increasing roasted chickpea flour (RCF) levels resulted in decreased cohesiveness and springiness, and darker color. Hardness and chewiness decreased and specific volume increased when water level increased. With increasing hydroxypropyl methylcellulose levels, softer crumb was obtained. Optimized formulation contained almost 25% RCF. Model validation revealed good agreement between predicted and measured responses. Based on the optimized formulation, roasted, raw or dehulled chickpea flour-containing rice flour blends, doughs and breads were prepared and analyzed. Compared to rice flour control, reduced retrogradation tendency was observed for chickpea flour-containing blends. Although gas retention capacities of chickpea flour-containing doughs were slightly lower than rice dough, 5-12% higher CO_2 was produced. Chickpea flour addition increased both storage (G') and loss (G") moduli. Bread containing RCF exhibited higher specific volume (2.89 mL/g), ~2.5 fold softer crumb and lower staling rate than raw and dehulled chickpea-containing formulations. Chickpea enrichment resulted with increased protein (24-36%), ash, fat and total phenolic contents; besides, reduced available starch levels (~14%). Breads fortified with dehulled and roasted chickpea flour were superior in terms of low rapidly digestible starch and high *in vitro* protein digestibility, respectively. Improved sensory quality was observed with an increased overall acceptability from 5.31 to 6.58-6.84. Addition of sourdough (30%) increased *in vitro* protein digestibility; however, slight decrease occurred in sensory attributes. Chickpea flour-enriched gluten-free rice bread resulted in improved quality characteristics depending on pretreatments applied to chickpea grain.

ÖZET

NOHUT UNU BAZLI GLUTENSİZ EKMEK FORMÜLASYONLARININ GELİŞTİRİLMESİ: FORMÜLASYON OPTİMİZASYONU, HAMUR ÖZELLİKLERİ VE EKMEK KALİTESİNİN DEĞERLENDİRİLMESİ

Bu çalışmanın temel amacı gelişmiş fizikokimyasal, besinsel ve duyusal kalitede, nohut unları ile zenginleştirilmiş glutensiz ekmek formülasyonları geliştirmektir. Kavrulmuş nohut ununun artan seviyeleri iç yapışkanlık ve esneklik değerlerinde azalmaya ve renkte koyulaşmaya neden olmuştur. Formülasyondaki su seviyesi arttıkça sertlik ve çiğnenebilirlik değerlerinde düşüş, hacim değerinde artış gözlenmiştir. Hidroksipropil metilselüloz miktarındaki artış daha yumuşak ekmek içi oluşumuna neden olmuştur. Optimum formülasyonda yaklaşık %25 oranında kavrulmuş nohut unu bulunmaktadır. Model validasyonu sonucuna göre tahminlenen ve ölçülen yanıtların uyumlu olduğu görülmüştür. Optimum formülasyon dikkate alınarak kavrulmuş, kuru ve kabuğu ayrılmış nohut unu içeren pirinç temelli un karışımları, hamur ve ekmek örnekleri hazırlanmış ve analizlenmiştir. Nohut unu içeren un karışımlar sadece pirinç unu içeren kontrole göre daha az retrogradasyon eğilimine sahiptir. Nohut unlu hamurların gaz tutma kapasiteleri kontrole göre daha az olmakla beraber, %5-12 daha fazla CO_2 üretim gerçekleşmiştir. Depolama (G') ve kayıp (G'') modülleri nohut unu ilavesiyle artmıştır. Kavrulmuş nohut unu içeren ekmek kuru ve kabuğu ayrılmış nohut unu içerenlere göre daha büyük özgül hacime (2.89 mL/g), yaklaşık 2.5 kat yumuşak ekmek içine ve düşük bayatlama oranına sahiptir. Nohut unu ilavesi protein (%24-36), kül, yağ ve toplam fenolik madde miktarında artış, kullanılabilir nişasta miktarlarında azalış (~%14) sağlamıştır. Kabuğu ayrılmış ve kavrulmuş nohut unu içeren ekmeklerde sırasıyla yavaş sindirilen nişasta miktarı ve *in vitro* protein sindirilebilirliği artmıştır. Duyusal kalite artmış, genel beğeni 5.31'den 6.58-6.84'e yükselmiştir. Ekşi maya ilavesi (%30) *in vitro* protein sindirilebilirliğini artırmış; bununla birlikte, duyusal kalitede azalmaya yol açmıştır. Glutensiz ekmeklerin kalitesinin nohuta uygulanan ön işlemlerden etkilendiği görülmüştür.

Dedicated to my family

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LIST OF ABBREVIATIONS

BD	Breakdown
CCD	Central composite design
CD	Celiac disease
db	Dry basis
GF	Gluten-free
GFB	Gluten-free bread
CF	Chickpea flour
DCF	Dehulled chickpea flour
ESEM	Environmental Scanning Electron Microscopy
fb	Flour basis
FC	Foaming capacity
FS	Foaming stability
FV	Final viscosity
GI	Glycemic index
GT	Gelatinization temperature
HPCM	Hydroxypropyl methylcellulose
LAB	Lactic acid bacteria
PV	Pasting viscosity
RF	Rice flour
RCF	Roasted chickpea flour
RDS	Rapidly digestible starch
RSM	Response surface methodology
SB	Setback
SD	Sourdough
SDS	Slowly digestible starch
SEM	Scanning Electron Microscopy
TPC	Total phenolic content
WBC	Water binging capacity

CHAPTER 1

INTRODUCTION

Food is necessary for humankind as the source of energy and eating is a part of the daily social life. However, our bodies give different responses to all the foods consumed, either in positive or negative way. Gluten, a storage protein group in wheat, rye and barley, may cause some health problems in many individuals. Celiac disease (CD), wheat allergy and non-celiac gluten sensitivity (NCGS) are the main gluten-related disorders affecting broad spectrum of population (Catassi et al., 2013). Among these, CD is an autoimmune metabolic disease with a prevalence of 1% of population (Reilly & Green, 2012). In CD patients, the consumption of gluten-containing foods leads to small intestine damage and absorption of nutrients is negatively affected. The only remedy for celiac and other gluten-related diseases is exclusion of gluten from the diet. The gluten-free (GF) diet is a real challenge especially for celiac patients since very low amounts of gluten can trigger the symptoms.

Although the GF product market increasing continuously, the existing products still do not fulfill the need completely. Moreover, there is a demand for GF products having available prices and also improved physicochemical, nutritional and sensorial quality. More importantly, the starchy flours used in the GF products resulted in nutrient deficiency, especially in terms of protein. In order to overcome this problem, protein-rich raw materials such as legume flours can be used in the formulations.

Legumes are plants of Leguminosae family, which are mostly planted for their grains called pulses such as dry peas, dry beans, chickpeas, lentils and cowpeas (FAO, 2016b). In order to create public awareness of the benefits of pulses, 2016 has been declared as the International Year of Pulses (FAO, 2016b). Pulses are listed as one of the most sustainable crops due to their nitrogen fixing property and low water requirement. Moreover, consumption of pulses is highly recommended due to their high nutrient composition. Besides, they are free from gluten. Chickpea is one of the most important pulses mostly produced in Turkey India, Australia and Pakistan (FAO, 2016a). Chickpea can be consumed as meal or snack; a special type of roasted chickpea snack, which is widely consumed in Turkey and countries nearby, is called leblebi.

Bread has been a staple food for centuries in human life. Therefore, its fortification would considerably affect the nutrient intake of persons. For this reason, breadwas chosen as the model food in this study. Roasted chickpea flour was used as the main ingredient to fortify rice flour-based gluten-free bread (GFB). This selection was mainly based on its high nutritional value. Besides, possible utilization of the broken kernels, which are obtained as the by-product of leblebi production, would be an economical ingredient. Moreover, chickpea proteins are known as a good protein source having several functional properties. Therefore, the utilization of chickpea-based flour was thought to enhance the quality of GFB. However, it is known that heat processing of grains causes many alterations in the structure of carbohydrates, proteins and their functional properties (Ma et al., 2011) and also macronutrient bioavailability. Those changes may also alter the dough and bread quality. Therefore, in this study, raw chickpea flour and dehulled chickpea flour were also employed in the evaluation of flour, dough and bread properties in order to investigate the effects of roasting and dehulling. To the best of our knowledge, the effect of the utilization of roasted or dehulled chickpea flour in a rice flour-based GFB formulation has not been investigated before. In addition, although there are many studies related to wheat sourdough fermentation, limited results are reported concerning gluten-free sourdough. Accordingly, sourdough fermentation was performed for the roasted chickpea-rice flour formulation using *Lactobacillus sanfranciscensis*, which is the most typical bacterium isolated from sourdough microflora.

The objectives of this dissertation is to evaluate the effects of roasted chickpea flour, hydroxypropyl methylcellulose (HPMC) and water on GFB quality and to optimize their levels; to characterize the physicochemical structure of flours used as raw materials; to evaluate the effects of raw, roasted and dehulled chickpea flour on rice flour-based gluten-free dough and bread formulations; to evaluate the effects of sourdough fermentation on roasted chickpea-rice flour-based GFB quality. Within the scope of this thesis, determination of pasting properties of flour blends; consistency, leavening properties and rheology analysis of dough; bread quality during storage and bread *in vitro* starch digestibility analysis were carried out at the Department of Food, Environmental and Nutritional Sciences (DeFENS) of the University of Milan, Italy.

CHAPTER 2

LITERATURE REVIEW

2.1. Celiac Disease

Gluten proteins are the storage proteins in wheat consisting of gliadins and glutenins (Shewry & Tatham, 1997). Gliadin and glutenin are responsible for dough viscosity and elasticity, respectively (Wieser, 2007). This behavior makes the wheat flour unique in terms of bakery technology. Upon hydration of wheat flour during dough kneading, gluten proteins absorb water and start to unfold. Hydrophobic interactions and sulfhydryl-disulfide interchange reactions cause the formation of polymer structures interacting with each other via hydrogen bonding, hydrophobic associations and disulfide cross-linking (Fennema, 1996). The obtained film network is capable of holding gas and is responsible for dough strength. The extent of intermolecular disulfide bonds has a primary role in rheological properties of the obtained dough (Fennema, 1996; Shewry & Tatham, 1997).

Even though gluten has technological importance, consumption of gluten-containing foods might have negative effects on some individuals. Celiac disease, gluten enteropathy, is an autoimmune metabolic disease affecting 1 of 100 people in the population (Fasano & Catassi, 2001; Reilly & Green, 2012). Although it has high prevalence, diagnosed cases constitute only cery small amount (2-3%) of overall patients due to the unspecific symptoms (Aydoğdu & Karakoyun, 2013). People having celiac are required to follow a completely gluten-free diet as a lifestyle to have a healthy life.

Celiac disease is seen in people having genetic predisposition. Almost all patients have human leukocyte antigen (HLA) class II genes; HLA-DQ2 and HLA-DQ8 (Kupfer & Jabri, 2013). Although genetic background is required, external factors such as consumption of gluten-containing food in early childhood, short breastfeeding period and severe depressions could trigger the disease (Di Sabatino & Corazza, 2009).

Celiac disease has a very complex mechanism as shown in Figure 2.1. After consumption of gluten containing foods, gluten is reached to small intestine through

gastrointestinal tract. The partially digested gluten leads to the formation of undigested peptides. Upon their transfer to lamina propria, negatively-charged glutamate having high affinity to HLA-DQ2 and HLA-DQ8 is formed by tissue transglutaminase (tTG) (deamidation) (Kupfer & Jabri, 2013). Also, this enzyme is capable of catalyzing covalent crosslinking between lysine and glutamine residues (Meresse et al., 2009). The activated $CD4^{+}$T-cells reveal interferon-γ (IFN-γ) affecting the proinflammatory dendritic cells responsible for mucosal damage (Kupfer & Jabri, 2013; Wieser & Koehler, 2008). Addition to this mechanism, innate immune response that is characterized by rapid increase in intraepithelial lymphocytes, particularly interleukin 15 (IL-15), also plays a part (Malalgoda & Simsek, 2016). In this mechanism, natural killer receptors like NKG2D expressed by intraepithelial lymphocytes (IELs), which are stimulated by IL-15, leads to epithelial cell damage by recognizing MICA, MICB and HLA-E on epithelium (Kupfer & Jabri, 2013; Malalgoda & Simsek, 2016). Moreover, serum IgA and IgG antibodies are released by activated B-cells (Wieser & Koehler, 2008).

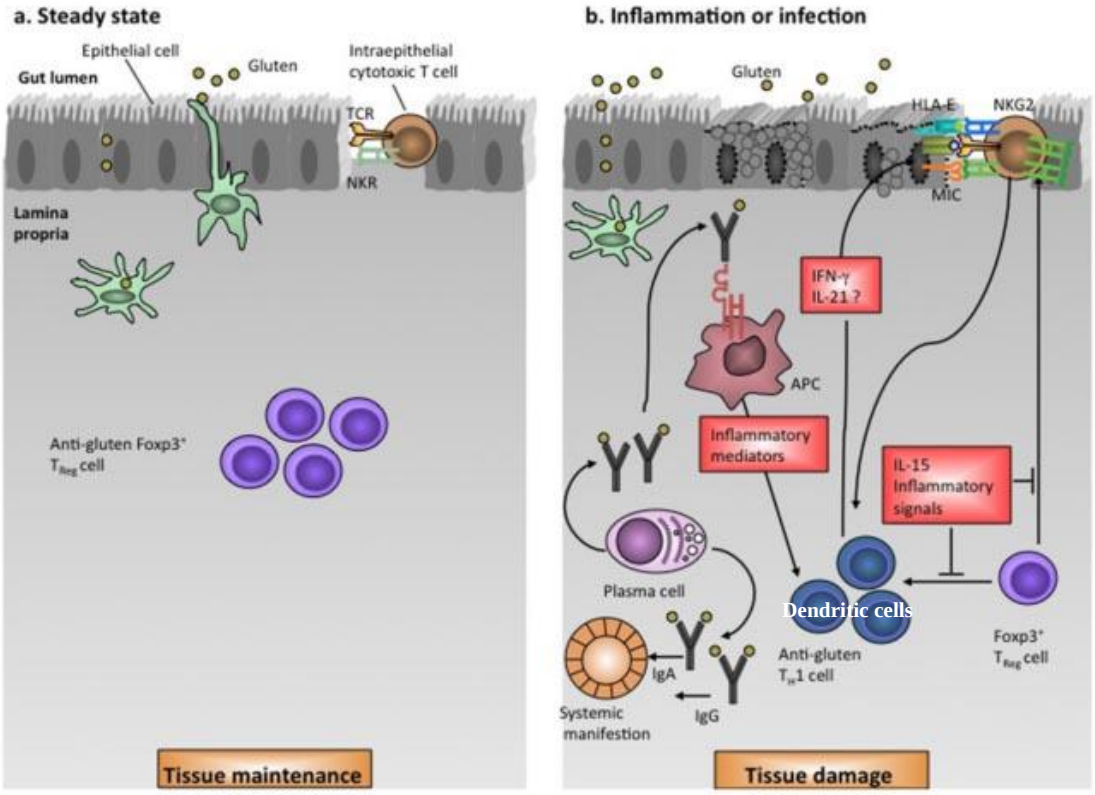

Figure 2.1 Schematic representation of adaptive and innate response by showing inflammated mucosa compared to healthy mucosa (Source: Kupfer & Jabri, 2013).

2.1.1. Symptoms & Diagnosis

Typical symptoms such as chronic diarrhea, abdominal distention, failure to thrive and some atypical symptoms like iron deficiency anemia, abdominal pain, low bone mineral density and dermatitis herpetiformis could be observed in celiac patients (Fasano & Catassi, 2001; Mearin, 2007). Due to some unspecific symptoms, the diagnosis usually takes time or the patient undergoes a treatment for another disease due to wrong diagnosis. Therefore, the diagnosed patients constitute only a small part of overall patients. This phenomenon was represented as “iceberg model” in the literature (Logan, 1991).

People having the symptoms were subjected to blood tests for diagnosis. Positive responses to anti-tissue transglutaminase (tTG) and anti-endomysium (EMA) tests are related with the presence of celiac disease. However, endoscopy and small intestine biopsy should be applied to confirm the positive blood tests. Since it is a genetic disease, first degree relatives of diagnosed individuals should also be screened for the disease.

2.1.2. Gluten-Free Diet

After diagnosis, persons have to follow a strict diet consisting of foods free from gluten. In order to classify the food products as GF, a general and detectable GF level has to be set. However, the gluten level triggering immunological response varies between individuals (Catassi et al., 2007; Greco et al., 2011; Laurin et al., 2002). The existing gluten limit for GF foods is set as 20 ppm by Codex Alimentarius Commission (2008). In the same Codex standard, GF foods are divided into two categories; foods containing ingredient(s) 1) without wheat and 2) obtained from wheat but special processes were applied to remove gluten. The wheat-containing ingredients are listed as wheat (durum, spelt, KAMUT), rye, barley and crossbreds of them (Codex Alimentarius Commission, 2008, p. 2). The situation of oats is controversial; although most of the celiac patients are thought to tolerate oats, the possible cross contamination with wheat-containing cereals during harvesting affects it negatively. At this point, the Codex standard leaves the decision of allowance the consumption of non-contaminated oats to the countries.

As regards the detection of gluten level in foods, reliable and practical techniques are required to analyze such small amounts of gluten. The method of analysis was stated to be immunologic method or other techniques having at least same sensitivity and specificity; the antibody should detect the toxic fractions of the gluten and do not exhibit cross reactions with other proteins and components of the food; the results should be qualitatively obtained and the minimum detection limit is set as ≤ 10 mg gluten/kg (Codex Alimentarius Commission, 2008). Among the methods, Enzyme-linked Immunoassay (ELISA) R5 Mendez Method was recommended as the standard analysis method.

Severe health problems and villous atrophy may occur upon going off the diet. Therefore, the availability and variety of commercial products labelled as GF and avoidance of consumption of cross-contaminated foods are of great importance. Although the products on the market is increasing in number, they are lack of primary nutritional elements, especially protein and dietary fibre, and have higher prices compared to gluten-containing counterparts (Grehn et al., 2001; Hallert et al., 2002; Mariani et al., 1998; Thompson, 2000). Utilization of starches in GF formulations is the main reason for the low nutritional value of the GF products. Another reason for low protein and nutrient content is the production of one type of bread for both celiac patients and phenylketonuria patients.

2.2. Gluten-Free Dough & Bread

2.2.1. Roles of Bread Basic Formulation Components

GF bread is a complex food having many factors that affect the quality (Figure 2.2). Ingredients (flours, starches, hydrocolloids, enzymes, dietary fibers, proteins) and processing technologies (sourdough fermentation, partial baking) could be manipulated to obtain improved bread quality. With the alteration of every individual factor, the final product can have different characteristics. In general, the essential components found in almost every recipe are GF flour, water, salt, sugar and yeast, which have several functions in terms of bread quality.

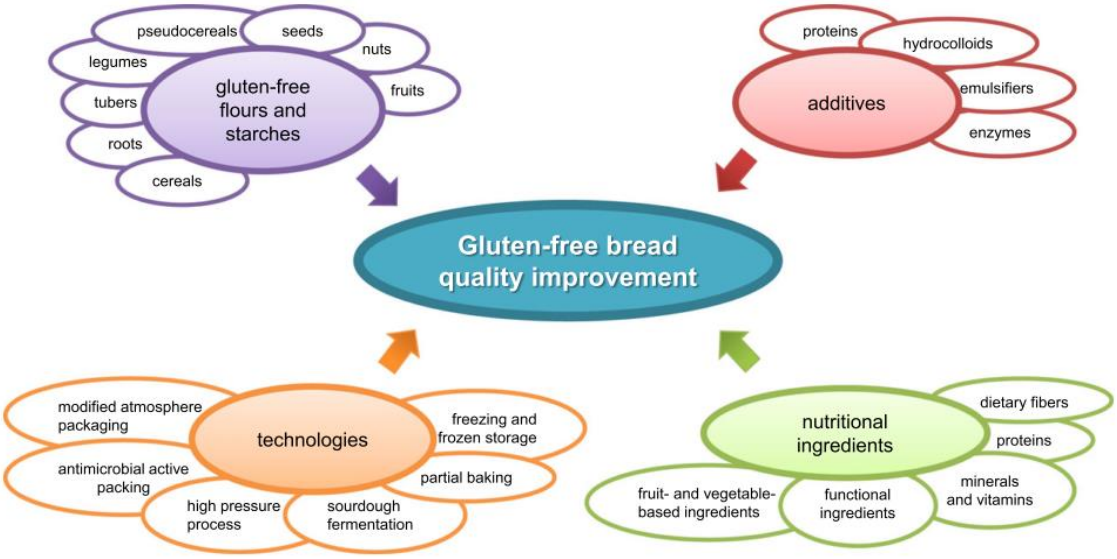

Figure 2.2. Factors affecting gluten-free bread quality
(Source: Caprilles et al., 2014)

2.2.1.1. Flour

In GF bread development studies, rice flour is commonly used due to its GF and hypo-allergenic nature and also similar color with wheat flour. Apart from rice, also corn, millet, teff, pseudocereals (buckwheat, quinoa, amaranth), legumes (chickpea, kidney bean, faba bean, pea, soybean, lentil), chestnut and carob bean can be used in GF product formulations.

Flours consist of carbohydrates (starch and non-starch polysaccharides), protein, fat, minerals and vitamins. Starch is present either as a component of flour or can be added as in the refined starch form to bread formulation. Several structural changes occur as the starch-water slurry is heated and stirred (Figure 2.3). During mixing, starch granules absorb water and behave like a filler in the matrix. Upon gelatinization, amylose leached from the disrupted granule during cooking with the help of shear occurred by mixing. It is known that amylose and amylopectin have different roles during starch retrogradation (Ottenhof & Farhat, 2004). Although individual and/or co-retrogradation of amylose and amylopectin can be occurred, amylopectin retrogradation occurs more slowly than amylose retrogradation. From technological point of view, amylose retrogradation is essential for setting up the initial loaf volume. Also, the

plasticizer effect of water enhances the movements of amylopectin chains, which speed up the crystal formation (Zeleznak & Hoseney, 1986).

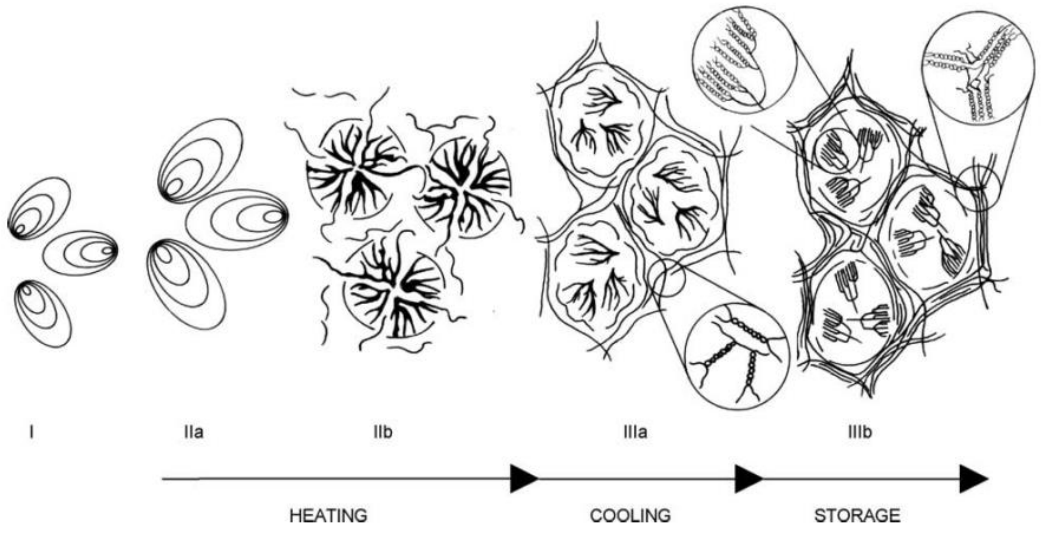

Figure 2.3. Effects of heating, cooling and storage on starch structure in a starch-water slurry (I, Native starch granules; IIa, gelatinisation and swelling; IIb, granule disruption-starch paste formation; IIIa, amylose network; IIIb, amylopectin retrogradation (Source: Goesaert et al., 2005).

Another important flour component is protein. As mentioned before, gluten is responsible for viscoelasticity of the dough and entrapment of gas inside the dough till the end of baking. However in GF dough system, the absence of gluten negatively affected the dough rheology. Moreover, the extensive usage of starches makes starch properties to be more dominant on dough rheology and bread quality than proteins. Due to both its nutritional benefits and also several technologically important functional properties such as water holding, foaming, gelling and emulsifying capacities (Boye et al., 2010; Ma et al., 2011), existence of protein in GFB formulations is of great importance. Due to the increasing awareness of the benefits of protein enrichment, several recent studies are concentrated on fortification of both wheat bread and GFB in terms of proteins (Crockett et al., 2011; Marco & Rosell, 2008; Miñarro et al., 2012). In this respect, legume flours are considered as excellent protein sources. Resulting from this fact, raw, roasted and dehulled chickpea flours were decided to be used in rice-based GF dough and bread systems in this thesis study.

2.2.1.1.1. Chickpea Flour

Chickpea (*Cicer arietinum* L.) is a widely grown pulse especially in India, Australia, Pakistan and Turkey (FAO, 2016a). According to legume production statistics of Turkey, among all other pulses grown, chickpea is placed on top with the production of 460.000 tons (43% of total pulses) in 2015 (TUIK, 2016). Chickpea flour is one of the most suitable raw materials to be used for nutritional enrichment of foods due to its high protein (23-27%), dietary fiber, vitamin B1 and B2, phosphorus and potassium content and low glycemic index (GI) (Dodok et al., 1993; Goni & Valentin-Gamazo, 2003; Ramulu & Udayasekhara Rao, 1997; USDA, 2016). Apart from this, chickpea is rich-in lysine which is a limiting amino acid for a number of cereals. This property leads to the combined usage of cereal flours (such as rice flour) and chickpea flour to yield a balanced amino acid content in GF products.

Leblebi, a type of roasted chickpea, is a very popular traditional snack food consumed in Turkey and some of the nearby countries (Kökselet al., 1998). Approximately 20% of chickpea is used for the production of this special type of roasted chickpea (Coşkuner & Karababa, 2004; Gürsul & Batu 2010). During processing several steps such as tempering, moistening, resting and roasting are applied as shown in Figure 2.4 (Coşkuner & Karababa, 2004). The water in rehydrated chickpea grains turns into steam upon roasting. Therefore, air spaces that are responsible for grain volume increase and fragile structure are formed (Köksel et al., 1998). Although some modifications in carbohydrates and proteins occurred throughout processing (Coşkuner & Karababa, 2004), no significant changes in ash, fiber and protein contents were reported (Sağlam, 2006). The starch is not completely gelatinized due to very limited hydration (Köksel et al., 1998). The hulls of the chickpea grain are lost almost completely. Besides, some chickpea kernels are split into half during processing and separated as by-product. Following the powdering step, these broken parts can be reintroduced to industry via their utilization in many food formulations as a nutritious and cheap ingredient.

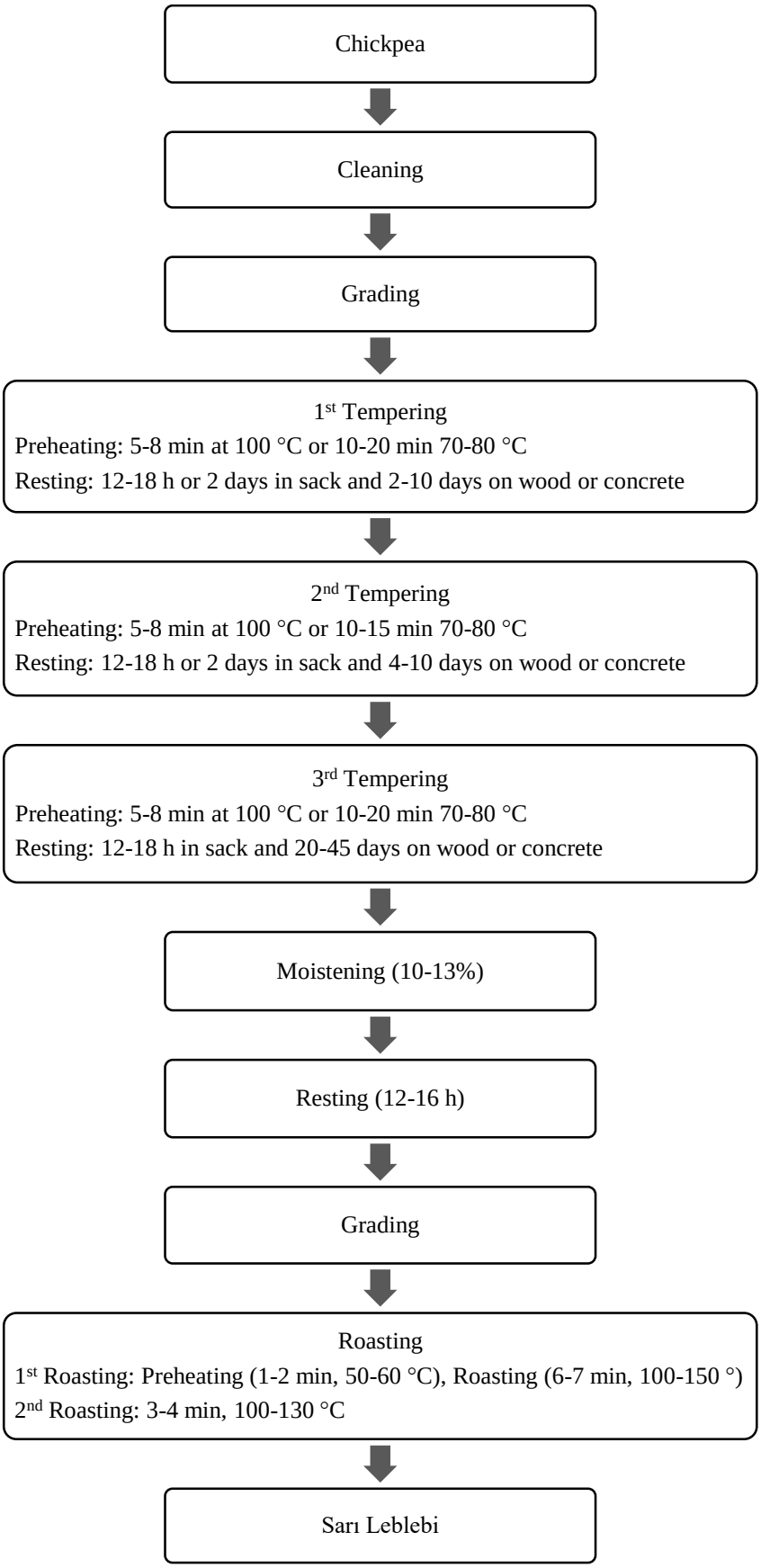

Figure 2.4. Sarı Leblebi production (Adapted from: Coşkuner & Karababa, 2004)

The chickpea grain consists of three parts: seed coat or hulls (15%), cotyledons (84%) and embryo (1%) (Aykroyd & Doughty, 1982). Dehulling process may cause several changes in chickpea. Soluble, insoluble and total dietary fiber contents are decreased (Ghavidel & Prakash, 2007). Since the antinutritional factors present mostly in the hulls, phytic acid and tannin levels are also lowered (Ghavidel & Prakash, 2007). The levels of minerals are reported to be reduced, however, their bioavailability is increased (Ghavidel & Prakash, 2007).

There are many studies in which the effects of chickpea flour on the quality of wheat bread were evaluated (Mohammed et al., 2012; Rizzello et al., 2014; Utrilla-Coello et al., 2007; Yamsaengsung et al., 2010; Zafar et al., 2015) and a study related to roasted chickpea flour fortified wheat bread (Baik & Han, 2012). However, studies related to the development of yeast-leavened GFB formulations containing chickpea flour are limited (Aguilar et al., 2015; Burešová et al., 2014; Miñarro et al., 2012; Ouazib et al., 2016).

2.2.1.2. Hydrocolloids

Hydrocolloids are polysaccharides and proteins having technologically important functions such as foam and emulsion stabilization, gelation and thickening (Phillips & Williams, 2000). They are obtained from botanical (cellulose, pectin, guar gum, locust bean gum, etc.), algal (agar, carrageenan), microbial (xanthan, cellulose, dextran) and animal (whey protein, chitosan, casein, gelatin) sources (Phillips & Williams, 2000).

Hydrocolloids are included into the GFB formulations to partially replace gluten functionality, therefore, to improve the dough handling properties and bread structure. The most commonly used hydrocolloid in GF products is hydroxypropyl methylcellulose (HPMC). HPMC, E464, is a cellulosic derived from cellulose by chemical modification (Murray, 2000). It is soluble in cold water and undergoes reversible thermal gelation (Murray, 2000; Mariotti et al., 2013). The main factors affecting the properties of HPMC are the type of substitution of the cellulose, the average chain length or degree of polymerisation (DP) of the cellulose molecules and the degree of substitution of the chain (Murray, 2000). DP is related with the chain length of the polymer and with the increase of DP viscosity increased.

HPMC acts as emulsifier, thickener and stabilizer (Phillips & Williams, 2000). Like other hydrocolloids, HPMC has water binding capacity and forms gels upon heating causing an increase in viscosity and also unlike other hydrocolloids has surface-active property (BeMiller, 2008). By locating in the gas liquid interface, it stabilizes the gas cells inside the dough and avoids their coalescence as explained based on a starch bread model in Figure 2.5 (Schober, 2009). In case of no hydrocolloid presence, starch granules and yeast tend to fall down. With the addition of a hydrocolloid, the dough components are suspended as a result of viscosity increase. The addition of HPMC to this system increases the stability of gas bubbles; moreover, avoids the coalescence of the gas bubbles due to its film forming function and surface activity caused by the presence of hydrophobic side groups (Mariotti et al., 2013).

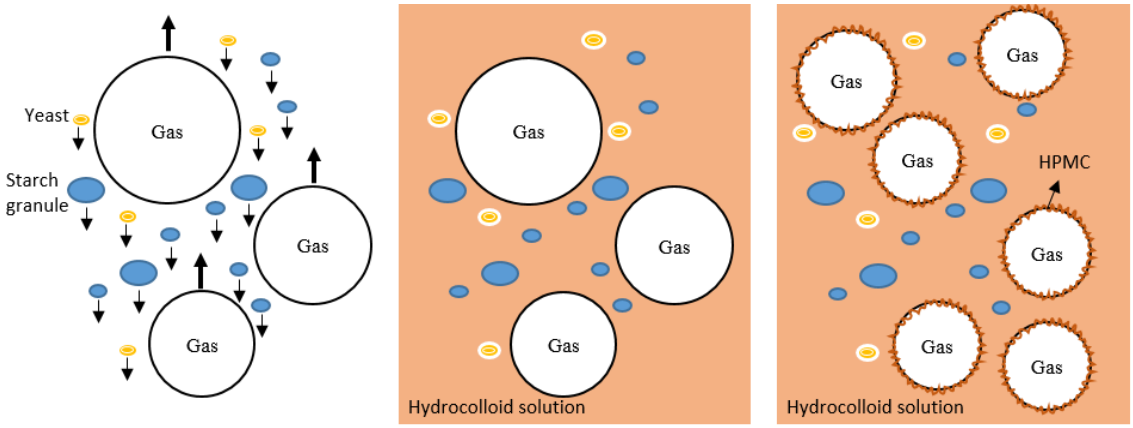

Figure 2.5. Starch bread models when (a) no hydrocolloid, (b) with hydrocolloid (e.g. xanthan gum), (c) with surface-active hydrocolloids like HPMC (Adapted from: Schober, 2009).

The presence of HPMC in GF dough leads to an improvement in visco-elastic properties of the GF dough (Demirkesen et al., 2010; Manchebo et al., 2015; Moreira et al., 2011; Ronda et al., 2015; Sivaramakrishnan et al., 2004) mainly by increasing both the viscous and elastic moduli. As regards to the GFB, HPMC caused a decrease in firmness, staling rate and bake loss and an increase in specific volume (Ahlborn et al., 2005; Bárcenas & Rosell, 2005; Mariotti et al., 2013; Mezaize et al., 2009). Besides, addition of HPMC and other hydrocolloids increases the dietary fiber content of the bread and positively affects the health (Mir et al., 2016).

2.2.1.3. Water

Water is one of the basic ingredients in bread formulation that have many important functions. Water is responsible for hydration and solubilization of the components of the bread formulation. Mixing also contributes the hydration by transfering water to the unhydrated parts of the flour mix until all particles are fully hydrated (Arendt et al., 2008). Increased levels of water are required for the dough formulations including hydrocolloids due to their enhanced water holding capacity and thickening properties. Dough consistency could be manipulated by changing the amount of water added to dough formulation. Physical changes like gas bubble expansion, chemical changes such as starch gelatinisation, textural properties and shelf-life of bread also affected by water content (Wagner at al., 2007). By virtue of plasticizing ability of water, phase transition occurs and the amorphous regions of the starch granule turn into rubbery state from glass state (Arendt et al., 2008). During storage of the bread, alterations in the water distribution inside the crumb and crust affect the staling rate.

2.2.2. Gluten-Free Dough Properties and Analysis

Dough properties are directly affected by the ingredients and also process conditions (mixing time, speed, equipment design, etc.). The rheological properties of dough could alter the final bread quality dramatically. The target of rheological measurements is to gain insight of the mechanical properties of material quantitatively, to learn about the composition and structure, and to simulate the process conditions in order to measure the performance of the material during processing (Dobraszczyk & Morgenstern, 2003). In order to come up with a desired viscoelastic and leavening properties, effects of each factor regarding dough quality should be carefully examined by using proper measuring systems.

Descriptive and fundamental tests are used for measuring dough properties. Descriptive tests are rapid, easy to perform and widely used for a long time (Lazaridou & Biliaderis, 2009). However, due to variable and undefined geometry, and uncontrolled and non-uniform strain and stress states, the fundamental tests gained more importance (Dobraszczyk & Morgenstern, 2003). On the other hand, fundamental tests

are time consuming, need technical knowledge in interpretation of results and the instruments have high prices (Lazaridou & Biliaderis, 2009).

Brabender farinograph, mixograph, extensograph, Chopin alveograph, amylograph and rheofermentometer are the instruments used for empirical measurements (Dobraszczyk & Morgenstern, 2003). Brabender farinograph is the basic device for water absorption and consistency (Brabender units, BU) measurement, which constantly records the power required to mix the dough continually (Lazaridou & Biliaderis, 2009). Extensograph and Chopin alveograph are used to measure extention by applying the uniaxial and biaxial expansion, respectively (Lazaridou & Biliaderis, 2009). Rheofermentometer is used for the measurement of gas retention and rheological behavior during proofing. The pasting properties of flour and starch slurries are measured using Rapid Viscoanalyzer (RVA) and Brabender Micro Visco-Amylo-Graph (MVA). The principle of amylograph is based on the measurement of viscosity (BU) during heating, holding and cooling periods. The changes in the viscosity reveal the gelatinization and retrogradation behavior of starch in the sample slurry.

Fundamental rheological tests consist of small and large deformation tests (Lazaridou & Biliaderis, 2009). Dynamic oscillation tests, tube viscometers and extension measurement instruments are used in this context (Dobraszczyk, & Morgenstern, 2003). Rheometer is used for dynamic oscillatory and creep-recovery testing. With these tests, viscoelastic properties of dough such as elastic (G') and viscous (G'') moduli, damping factor ($\tan \delta = G''/G'$) and creep compliance (J) can be determined. Texture analysis devices such as Texture Analyzer (Stable Micro Systems, UK) and Universal Testing Machine (Instron, USA) can be used for large deformation to measure dough extention, stickiness, extrusion behavior (back and forward extrusion) and hardness (texture profile analysis).

The GF flours differ in rheological behaviours from wheat flour. Sivaramakrishnan et al. (2004) evaluated viscoelastic properties of rice and wheat flour and showed that rice flour has higher viscous and elastic moduli than wheat flour as shown in Figure 2.6. This is the result of more solid behavior of rice flour than wheat flour. Increasing frequencies cause increasing moduli for wheat dough, however for rice dough this increase is not dramatic. They concluded that rice flour is unable to form a definable structure in case of no hydrocolloid presence due to being lack of binding agent such as gluten. For several GF dough formulations, many research groups also

reported a higher G′ than G″, which means a solid-elastic behavior (Aguilar et al., 2015; Galle et al., 2012; Hüttner et al., 2010; Sciarini et al., 2012).

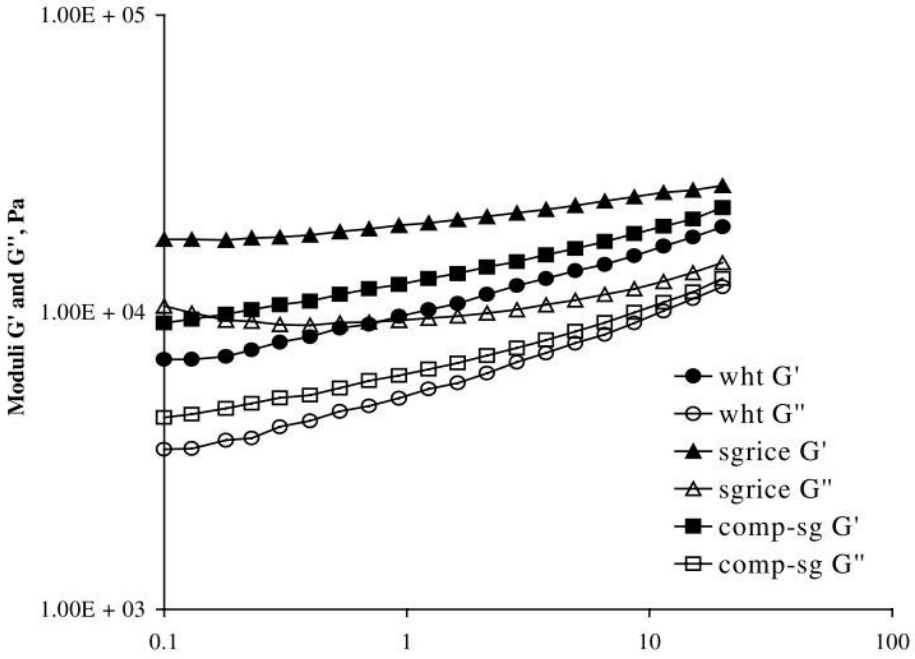

Figure 2.6. The elastic (G′) and viscous (G″) moduli of wheat and rice dough (wht, wheat; sgrice, short grain rice; comp-sg, 50% wheat and 50% short grain rice) (Source: Sivaramakrishnan et al., 2004).

2.2.3. Gluten-Free Bread Quality Parameters and Analysis

High loaf volume, homogeniously distributed gas cells in the crumb, good crust color, soft crumb, acceptable flavor, long shelf-life and slow staling can be listed as the attributes desired to be in a high quality GFB. For the evaluation of these parameters several appropriate techniques can be utilized.

Bread volume can be measured by seed displacement method by using a bread volumeter. Currently, new laser-based devices are developed for this purpose. Altough these devices give accurate results, they are expensive.

Textural parameters of bread crumb can be measured both with sensory analysis and by using texture analyzers. In order to get a comparable and universal data, Texture Profile Analysis (TPA) can be performed by applying two successive uniaxial

compression on the sample. Parameters like hardness, springiness, cohesiveness, resilience, chewiness and adhession are calculated from generated force-time plots shown in Figure 2.7. Hardness (N) is defined as the maximum peak force obtained in the first compression. Springiness is related with elasticity of food and calculated as the ratio of time for second compression (time 4-5) to first compression (time 1-2). Cohesiveness is the property regarding the disintegration of the tested food and obtained from proportion of total area in cycle 2 (area 4-6) to cycle 1 (area 1-3). Resilience is calculated by the ratio of area obtained during withdrawal of the probe (area 2-3) to area obtained during compression (area 1-2) in the first cycle. Chewiness (N) is obtained by the multiplication of hardness, cohesiveness and springiness, and gives evidence of mastication behaviour.

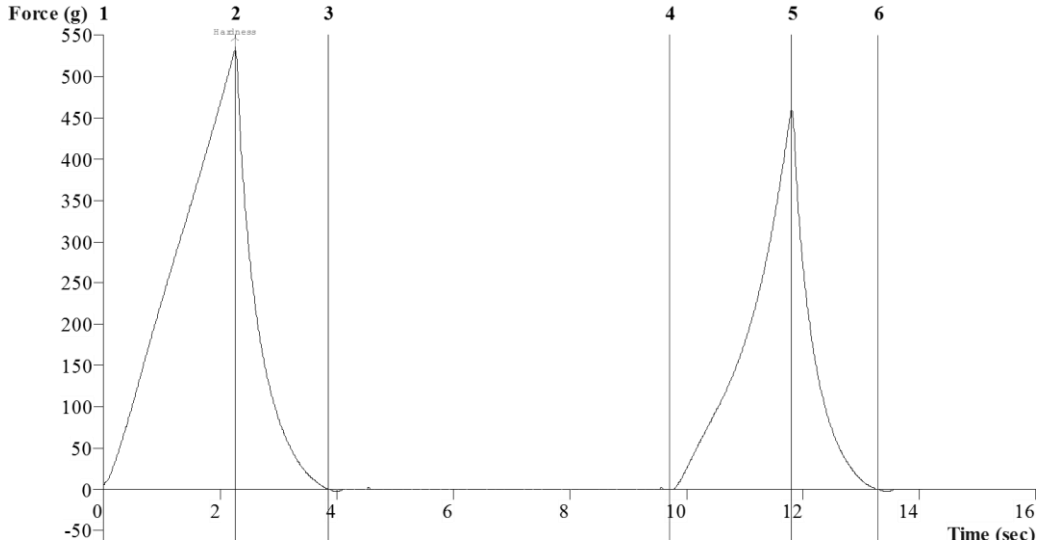

Figure 2.7. Force-Time curve of TPA

2.2.4. Staling of Gluten-Free Bread

Staling is the main problem that affects the shelf-life of bread, especially GFBs. In most of the GF product formulations, because of its plain taste, neutral color and widespread production, rice flour is preferred as the main ingredient. In contrast with these advantages, rice breads are prone to rapid staling (Kadan et al., 2001).

The underlying mechanism of staling is very complex and not completely identified yet. Main reasons for bread staling are proposed as inability of keeping water inside the bread structure, uncontrolled distribution of water inside the bread (migration of water from crumb to crust) and starch retrogradation (Gray & Bemiller, 2003). Starch components, amylose and amylopectin, contribute to starch retrogradation. Amylose retrogradation, which has a role in crumb setting, occurred immediately during cooling of the bread. However, retrogradation of amylopectin is slower than amylose (Singh et al., 2003), which makes it one of the predominant factors regarding staling. Apart from water and starch, other bread components are thought to have effects of bread staling. Even some researchers do not agree on the effect of gluten on staling, the presence of high amounts of gluten was also found to contribute to the softness of the crumb by increasing the amount water that plasticizes (Curti et al., 2014). In some of the studies effect of gluten on staling was linked with the dilution effect on starch (Kim & D'Appolonia, 1977). On the other hand, the importance of the interactions between starch-gluten and starch-starch rather than their presence or absence was suggested as the most important point (Every et al., 1998; Martin et al., 1991). Also in this study, the hydrogen bond formation between gelatinized starch granules was proposed as the reason for staling.

Staling of bread can be evaluated by using several data obtained from texture analysis, differential scanning calorimetry (DSC), thermogravimetric analysis (TGA) and Fourier transform infrared spectroscopy (FT-IR). Main bread quality parameters affecting consumer acceptability are related to the textural attributes of the crumb. Increase in hardness of the bread crumb, which can be measured by Texture profile analysis (TPA), can be used to interpret the staling ratio. As regards DSC thermograms, increasing amylopectin crystallization during storage can be evidenced with an endothermic peak formation due to entalphy increase (Demirkesen et al., 2014; Kadan et al., 2001). The occurance of retrogradation is also estimated with FT-IR spectra. The ratio of the intensities of the peaks at the wavenumbers of 1043 and 1023 cm^{-1} is considered as the indicator of crystalline structure (Sun et al., 2014). During storage, the increase in this ratio is linked with staling.

Although the staling kinetics of wheat bread (Angioloni & Collar, 2009; Le-Bail, Agrane, & Queveau, 2012) and gluten-free bread (Novotni et al., 2012; Ronda & Roos, 2011) have been investigated by few researchers, more studies are necessary to contribute to the explanation of exact mechanism.

2.2.5. Nutritional Properties of Gluten-Free Products

Although there is an increasing tendency towards fortification of GF products, most of gluten-free breads on the shelves are made from refined flours and starches which are low in protein, dietary fiber, vitamins and minerals. Although current studies mostly focus on the improvements of the physicochemical quality, nutritional enhancement of the products is recently gaining importance. As compared to wheat-based counterparts, GF products cannot support primary macro and micronutrients to consumers. Aiming to produce only a single product both for celiac and phenylketonuria (PKU) people is another reason for this low nutritional value.

Glycemic index (GI) term is used to evaluate blood glucose response of foods. After a 10-12 overnight fast, tested food containing 50 g carbohydrate is consumed by the subjects within a specific time period. Capillary blood samples obtained by finger prick are analysed and used for the determination of GI. Incremental area under the blood glucose-time curve is used to calculate GI in terms of glucose response to a standard food (FAO, 1998). Apart from *in vivo* test, GI can be also estimated by *in vitro* techniques which are more practical than *in vivo* techniques (Goni et al., 1997). For this purpose, simulated gastric digestion protocols can be used (Englyst et al., 2000; Goni et al., 1997). With the simulation of the conditions of mouth, stomach and small intestine, it is possible to evaluate the starch and protein bioavailability and the fate of the nutritional components after ingestion. The starch hydrolysis, predicted GI and nutritionally important starch fractions can be determined. The nutritionally important starch fractions can be classified as slowly digestible starch (SDS), rapidly digestible starch (RDS) and resistant starch (RS) (Englyst et al., 1992). Their difference is coming from the characteristics of glycemic response: RDS and SDS are defined with large and small glycemic responses, respectively, however RS is considered to have no glycemic response since it does not hydrolysed and absorbed in small intestine.

For measuring GI, white bread or glucose can be used as the standard food. However, it should be kept in mind that 1.4 times higher GI are obtained when white bread is used as standard food rather than glucose (FAO, 1998). According to GI values, foods are classified as low (<55), medium (55-69) or high (>70) GI foods (Venn & Green, 2007). Several factors play an important role on the GI as listed in Figure 2.8 (FAO, 1998).

Another parameter as important as GI is glycemic load (GL) which is obtained after the multiplication of GI by amount of carbohydrate present in a portion of the same food (Riccardi et al., 2008). The usage of GL term provides both qualitative and quantitatative measure of carbohydrate consumption. Fan et al. (2012) reported that although there is a link between GL and coronary heart disease and stroke, GI is only slightly linked with coronary hearth disease and no association is found with stroke.

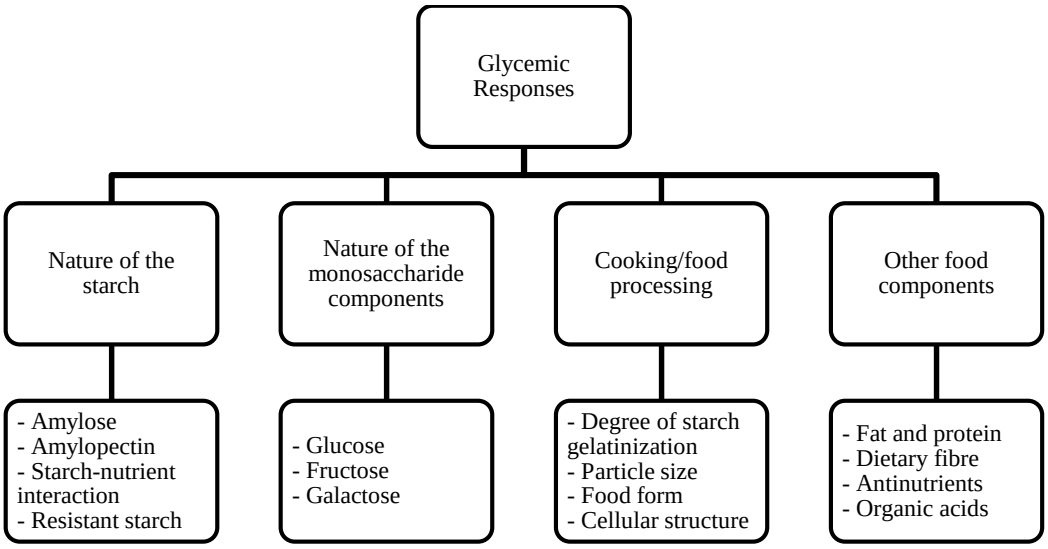

Figure 2.8. Factors affecting glycemic response (Adapted from: FAO, 1998)

It is reported that there is a link between celiac and type 1 diabetes (Holmes, 2001). Due to this fact, individuals having celiac disease should avoid consuming high GI gluten-free products. However, according to GI table (Atkinson et al., 2008), rice breads and GF rice-flour-based breads have high GI values (GI=88-103, $GI_{wheat\ bread}$=100). Since bread is a staple food, it is vital to have actions to reduce the GI of those products. In this context, low GI flours such as legume flours could be incorporated into rice flour-based GF bread formulation. The addition of hydrocolloids, organic acids produced during sourdough fermentation and processing techniques (partial baking etc.) may also considerably affect the glycemic response (Novotni et al., 2012). Although in recent years, the nutritional evaluation of GF products are gaining interest, more effort is required to reveal the effects of the ingredients and process conditions. Some articles of the existing literature are listed in Table 1.2.

Table 2.1. Literature related with nutritional fortification and analysis of gluten-free products

Product	**Tested Nutritional Property**	**Results**	**Reference**
Iron-fortified amaranth GFB	*In vitro* bioavailability of selected iron compounds	• Ferric pyrophosphate was suggested due to high bioavailability and acceptable sensorial quality	Kiskini et al., 2007
Commercial GFBs	*In vitro* starch digestibility	• Low protein and high starch content • High rapidly digestable starch content • Predicted GI (pGI) values between 83 and 96 (High GI class)	Segura & Rosell, 2011
Partially baked sourdough GFB	*In vivo* glycemic index	• Control, 7.5, 15, 22.5 and 30% of sourdough addition resulted in GI of 68±7, 59±6, 52±3, 54±6 and 61±6, respectively.	Novotni et al., 2012
Commercial GFB (buckwheat, oat, quinoa, sorghum or teff flour)	*In vitro* starch digestibility	• All breads were high GI (> 70) • pGI (wheat bread=100): Commercial<Oat≤Sorghum≤Teff<Buckwheat<Quinoa • pGL(50g portion): Commercial<Teff =Buckwheat<Oat=Quinoa<Sorghum	Wolter et al., 2013
Rice flour+potato starch based GFB with 0, 4, 8, 10 and 12% of inulin-type fructans (ITFs)	*In vitro* starch digestibility *In vivo* glycemic index	• Increasing ITFs levels reduced pGI and pGL. • The *in vivo* reductions were higher than *in vitro* analysis.	Capriles & Arêas, 2013
Commercial GFB, cakes, biscuit, pasta	*In vivo* glycemic index	• GI values ranged from 37.5 to 66.7 (Low or medium GI).	Scazzina et al., 2014
GFB with rice flour (fine and coarse) and varying water levels	*In vitro* starch digestibility	• Flour having small particle size and high water level increased the RDS and pGI.	de la Hera et al, 2014
Germinated brown rice flour bread	*In vitro* protein and starch digestibility	• Germination reduced the pGI and protein digestibility. • Phytic acid content decreased, total phenolic content increased.	Cornejo et al, 2015
Bean flour-enriched gluten free rice spaghetti	*In vitro* starch digestibility	• Resistant starch content increased. • pGI decraesed.	Giuberti et al, 2015
Buckwheat, quinoa, sorghum, teff sourdough bread	*In vitro* starch digestibility	• Only sorghum, teff and wheat breads fermented with *Lactobacillus plantarum* FST1.7 showed decreased pGI. • Starch digestibility depends on type of flour and strain.	Wolter et al., 2014b

2.2.6. Sourdough and Gluten-Free Bread

Sourdough is a traditional fermented semi-product which is the mixture of flour and water. Sourdough microflora mainly consists of lactic acid bacteria and yeasts. Many bioconversions are carried out by *Lactobacillus* strains through the utilization of proteins, carbohydrates, fats and phenolics in sourdough (De Vuyst & Neysens, 2005; Gänzle, 2014). Although obligately homofermentative and facultatively or obligately heterofermentative *Lactobacillus* strains are typical in sourdough environment, *Leuconostoc*, *Weissella*, and *Pediococcus* species are also frequently present (De Vuyst & Neysens, 2005). Among the LAB, the most commonly isolated strains are *L. sanfranciscensis*, *L. plantarum* and *L. brevis*. On the other hand, *L. pontis, L. reuteri, Pediococcus pentosaceus, Leuconostoc mesenteroides, L. delbrueckii* ssp., *L. casei, L. alimentarius, L. fermentum, L. rossiae* have also been isolated. The most common yeasts are *Saccharomyces cerevisiae, S. exiguus, Candida holmii, C. krusei, Pichia norvegensis* and *Hansenula anomala* (De Vuyst & Neysens, 2005; Gobbetti, 1998). It is known that *S. cerevisiae* in sourdough microflora comes from the bakery environment.

There are basic differences among sourdough LAB with respect to their reductive capacity. Glucose is metabolized by homofermentative and facultative heterofermentative strains through Emden-Meyerhoff pathway, whereas pentose-phosphate pathway was used by heterofermentative lactobacilli for hexose metabolism (Vermeulen et al., 2006). The most important lactic acid bacterium in the rye and wheat sourdoughs is *L. sanfranciscensis* (Gobbetti & Corsetti, 1997). Usually in sourdough, *L. sanfranciscensis* shows mutualistic associations with maltose negative yeasts such as *C. humilis*. Although *L. sanfranciscensis* cannot use fructose as carbon source, fructose is reduced to mannitol by the mannitol dehydrogenase activity. As shown in Figure 2.9, fructose and also oxygen are used as electron acceptors. Lactate, acetate, ethanol and CO_2 are formed due to the metabolism of *L. sanfranciscensis*. Mechanism of some metabolic activities of *L. sanfranciscensis* strains such as the proteolytic system and amino acid catabolism (Vermeulen et al., 2005; Gallo et al., 2005) and exopolysaccharide (EPS) synthesis (Tieking & Gänzle, 2005) have been studied previously.

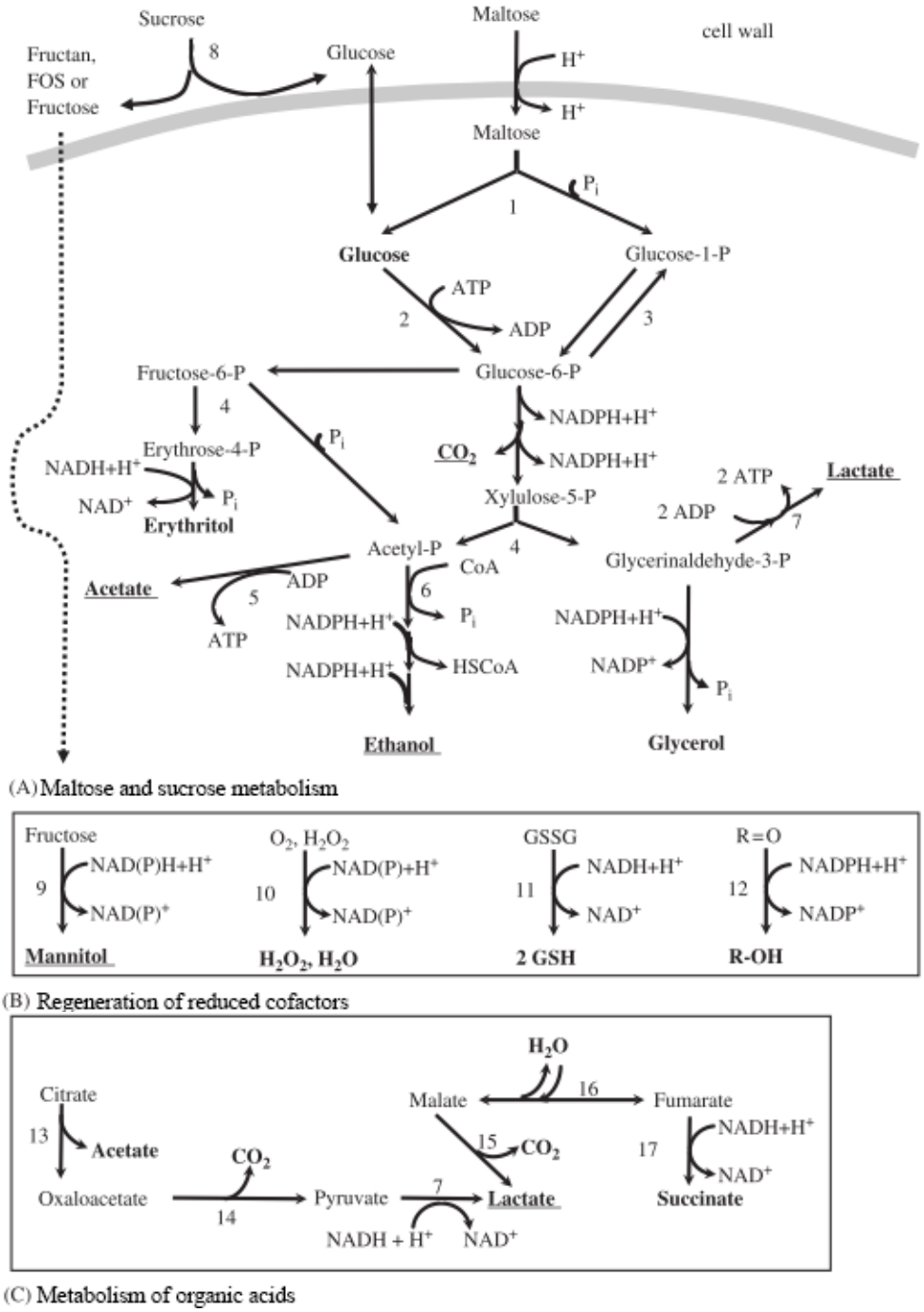

Figure 2.9. Carbohydrate metabolism of *L. sanfranciscensis* (Source: Gänzle et al., 2007). 1) maltose-phosphorylase 2) hexokinase 3) phosphoglucomutase 4) phosphoketolase 5) acetate kinase 6) phosphotransacetylase, further metabolism to ethanol via acetaldehyde- and alcohol dehydrogenases 7) lactate dehydrogenase 8) cell-wall bound fructosyltransferase. A majority of *L. sanfranciscensis* strains does not exhibit fructosyltransferase activity and is unable to metabolize sucrose. FOS or fructan are generally not metabolized by strains of *L. sanfranciscensis* 9) mannitol dehydrogenase 10) NADH oxidase 11) glutathione dehydrogenase 12) short-chain alcohol dehydrogenase 13) citrate lyase 14) oxaloacetate decarboxylase 15) malolactic enzyme 16) fumarase 17) succinate dehyrogenase (not present in *L. sanfranciscensis* but activity in *L. pontis, L. reuteri* and *L. fermentum*).

Due the fermentation products obtained after the biochemical conversions and, accordingly, the increase in organic acid levels, many improvements may occur in dough and bread quality (Gobbetti & Corsetti, 1997; Gänzle, 2014). Breads obtained by sourdough technique is characterized as shelf-stable (Dalie et al., 2010; Gänzle & Vogel, 2003; Moore et al., 2008), having high nutritional value (Hammes & Gänzle, 1998; Moroni et al., 2009; Poutanen et al., 2009) enhanced texture (Arendt et al., 2007; Lacaze et al., 2007; Moore et al., 2008) and aroma (Hansen & Schieberle, 2005). These positive effects were evidenced mostly for wheat sourdough. However, the effects on GF sourdough is still controversial. Studies related to the effects of sourdough fermentation on dough and bread properties are summarized in Table 2.2. According to this table, rice, buckwheat, sorghum, teff, quinoa, and legumes such as chickpea, lentil, bean and pea were used in sourdough fermentation. In general, sourdough addition increased the free amino acids, soluble fiber and total phenolic content. However, bread texture, glycemic index and shelf-life were differently affected as a function of starter type and dough composition. In general, type of starter strain and flour and also fermentation conditions strongly affected the dough properties and bread quality.

The beneficial effect of sourdough fermentation can be observed in terms of the legume flour based sourdough bread formulation. In order to fortify the bread, legume flours are good options. However, legume flours also contain anti-nutritional compounds such as phytic acid, condensed tannins, alkaloids, lectins, pyrimidine glycosides and protease inhibitors (Coda et al., 2015). Although some of the anti-nutritional factors can be removed by heat treatment, others like phytic acid are heat-stable and cannot be inactivated (Curiel et al., 2015). At this point, sourdough fermentation can be utilized to enhance the mineral uptake, increase the amount of bioactive compounds and decrease the level of anti-nutritional factors (Gobbetti et al., 2014).

Table 2.2. Studies related to sourdough application using gluten-free flours

Strain	Gluten-Free Flour	Results	Reference
L. amylovorus DSM19280 *L. amylovorus* DSM20531^{T}	Quinoa	• Sourdough fermented with *L. amylovorus* DSM19280 extended the shelf life of breads; concentrations of antifungal compunds such as 4-hydroxyphenyllactic acid, phloretic acid, 3-phenyllactic acid and hydroferulic acid were increased.	Axel et al., 2015
L. plantarum C48 *L. brevis* AM7	Kidney bean Chickpea Grass pea Lentil	• Free amino acids, soluble fibers, total phenols, antioxidant and phytase activities and γ-aminobutyric acid levels increased; condensed tannins decreased in sourdough samples after fermentation.	Curiel et al., 2015
Weissella cibaria MG1 *L. plantarum* FST1.7	Buckwheat Quinoa Sorghum Teff	• Decreased resistant starch in buckwheat and teff sourdough breads • GI related to the type of flour and lactic acid bacteria strain; pGI increased in buckwheat and quinoa sourdough breads, decreased in sorghum, teff and wheat bread fermented with *L. plantarum* FST1.7. • Starch hydrolysis related with factors other than presence of organic acids and resistant starch formation.	Wolter et al., 2014b
W. cibaria MG1 *L. reuteri* Y2 *L. reuteri* VIP	Sorghum	• Exopolysaccharides that were formed during sourdough fermentation significantly decreased the dough strength and elasticity; increased bread quality (softer crumb). • Dextran improved the shelf life.	Galle et al., 2012

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Table 2.2. (cont.)

Strain	Gluten-Free Flour	Results	Reference
L. fermentum (Commercial starter PL3)	Rice Extruded corn Buckwheat Corn and potato starches	• Among the breads containing 7.5, 15, 22.5 and 30% sourdough, only 15 and 22.5% of sourdough-containing ones had low glycemic index. • For the partial baked samples of the 15 and 22.5% of sourdough-containing breads, specific volume increased and crumb firmness decreased.	Novotni et al., 2012
L. reuteri TMW 1.106 *L. animalis* TMW 1.971 *L. curvatus* TMW 1.624	Rice Quinoa Buckwheat Buckwheat core	• Considerable amounts of exopolysaccharides were produced by the strains. However, exopolysaccharide levels in sourdough samples were affected by the type of flour, sucrose concentration, dough yield and inoculum amount.	Rühmkorf et al., 2012
L. plantarum FST 1.7 *L. sanfranciscensis* TMW1.52	Brown rice Buckwheat Soya Corn starch	• Increase in firmness and elasticity. • Mold growth retarded in bread having sourdough fermented with *L. plantarum* FST 1.7.	Moore et al., 2008
L. plantarum L2-1	Sorghum Potato starch	• Upon sourdough fermentation, proteins were degraded to small peptides. • Crumb defects disappeared in sourdough-containing bread. • No differences in crumb hardness observed during storage compared to control bread without sourdough.	Schober et al., 2007

2.3. Response Surface Methodology

Response surface methodology (RSM) is a statistical technique that involves the analysis, modeling and optimization of responses (Montgomery, 2005). If a response (y) is the function of the levels of factors x_1 and x_2, it is formulated as reported in the following equation with a noise or error (ϵ);

$$y = f(x_1,x_2) + \epsilon \tag{1.1}$$

In case the expected response is represented with $E(y) = f(x_1,x_2)$, the surface is represented as below and called as response surface (Montgomery, 2005).

$$\eta = f(x_1,x_2) \tag{1.2}$$

In order to explain the relationship between factors and response, three-dimensional surface plot is used as shown in Figure 2.10. Contour lines are usually plotted at the bottom part of the graph, in order to better visualize the surface plot.

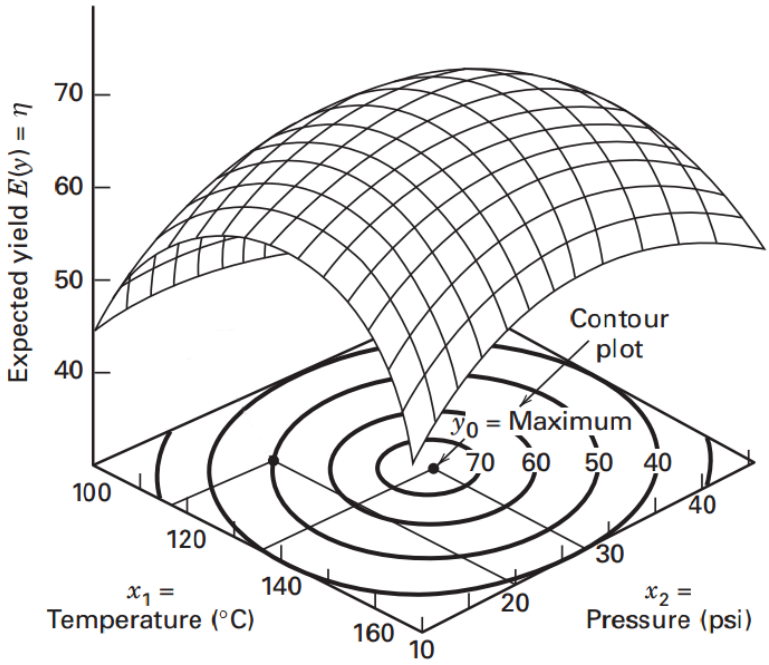


Figure 2.10. An example to three-dimensional (3-D) response surface and contour plot (Source: Montgomery, 2005).

The main advantage of using RSM compared to factorial design is the effectiveness in terms of both sources and time. With the appropriate design selection, a dramatic reduction in number of runs is achieved. There are several types of designs used for fitting the second-order models. Central composite design and Box-Behnken designs are the most commonly used types in food science and engineering.

2.3.1. Central Composite Design

The first step in response surface methodology is to decide on the best design type to fit the model. Central composite design (CCD) is one of the most commonly used designs due to i) its efficiency, ii) having full or fractional factorial design options and iii) a large design space can be covered with the usage of axial points. A CCD has 5 levels for each factor and the design is composed of 2^k factorial runs (n_F), center runs (n_C) and $2k$ axial runs (k, number of factors) (Montgomery, 2005). A three factor central composite design is shown in Figure 2.11. The center points are useful to estimate pure error and curvature. Addition of 3 to 5 center runs is recommended (Montgomery, 2005). Axial or star runs are the factor combinations where one factor has the alpha value (+/-) and all other factors are on center point. An alpha (α) value was chosen in order to determine the location of axial points and it is generally higher than 1. The CCD could be blocked if there is a considerable effect of time on the response. Therefore, the design could be divided into few parts (blocks) in order to exclude the effects of blocks on responses (Montgomery, 2005). These models are called orthogonally blocked models.

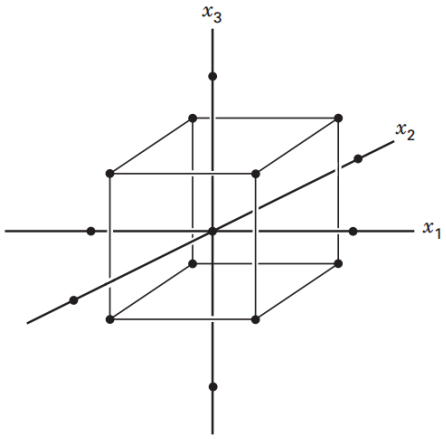


Figure 2.11. Central composite design having three factors (k = 3)
(Source: Montgomery, 2005).

After collecting the data, each response is modelled based on appropriate approximation. The response could be modelled with a first-order model, in case there is a linear relationship with factors and responses (Equation 1.3). In case of the presence of curvature, a second-order polynomial model could be utilized (Equation 1.4).

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_k x_k + \epsilon \quad (1.3)$$

$$y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum\sum_{i<j} \beta_{ij} x_i x_j + \epsilon \quad (1.4)$$

2.3.2. Optimization and Validation

After obtaining model equations for each response, the region of desirability should be determined. The desirability function can be used for this purpose. First of all, restrictions are set for each response variable (y_i) which resulted in desirability functions (d_i) (Montgomery, 2005). This function is constructed as $0 \leq d_i \leq 1$ if the response is in the range, $d_i = 1$ if the response is at the target and $d_i = 0$ if it is outside an acceptable region. The overall desirability is maximized as shown in the Equation 1.5 (m, responses) (Montgomery, 2005).

$$D = (d_1 x\ d_2\ x \ldots x\ d_m)^{1/m} \quad (1.5)$$

The goal for each response can be set as maximize, minimize, in range or target by using the software packages such as Design-Expert (Stat-Ease Inc., Minneapolis, USA). The overall desirability function was generated according to those restrictions having values between 0 and 1.

For all RSM studies, final vital step is to validate the obtained model. Validation reflects how well the response values predict the experimental measurements. The validation trails cover the analysis of the sample having factor combinations in the optimal region. It is recommended to carry out validation experiments on more than one

selected points. Then the measured responses were compared with the model predictions in order to see if there is any variation between them.

2.3.3. Using Response Surface Methodology in Gluten-Free Bread Development

GFB is a complex food having many components. Each component has its own function and there can be an interaction between each other. Because of this fact, the appropriate balance between all the ingredients, including water, in the GFB formulations is of great importance. Since one-by-one alteration of the levels of several components by applying factorial designs are time-consuming and expensive to perform, RSM can be applied with minimum numbers of runs by spending less time. Despite its usefulness and necessity in GFB formulation development purposes, there are only few studies in which RSM is used in recent years as shown in Table 2.3. According to the table, main target for almost every GFB development study is the adjustment of water level due to the different water holding capacities of each flour and hydrocolloid. Besides, consistency, which is a very important parameter in terms of dough and bread quality, is strongly affected by water level. Therefore, water was selected as a factor in common.

Table 2.3. Studies involving response surface methodology in gluten-free bread development

Experimental Design	Independent Variables (Factors)	Responses	References
CCD	Water, HPMC and/or xanthan	Loaf volume, crumb hardness, area of cells, wall thickness	Hager & Arendt, 2013
Box-Behnken	HPMC, yeast β-glucan, whey protein isolate	Spread ratio, specific volume, texture (Hardness, cohesiveness, springiness, chewiness), crumb color, moisture	Kittisuban et al., 2014
D-optimal	Orange pomace, water, proofing time	Specific volume, cell volume, number of cells, moisture (at 2 h and 24 h), hardness (at 2 h and 24 h)	O'Shea et al., 2015
Box-Behnken	Resistant starch, proteins, water	Moisture, firmness, elasticity, total porosity, surface porosity, cell density	Tsatsaragkou et al., 2014
CCD	Carob germ flour, water, HPMC	Specific volume, hardness	Smith et al., 2012
CCD	Cornstarch/cassava starch, Rice flour/cassava starch Categoric factors: Soy flour (0-0.5%)	Specific volume, crumb-grain score, bread score	Sanchez et al., 2002
CCD	Soy flour, dry milk	Batter softness, specific volume, crumb grain score, bread score, bread protein content	Sanchez et al., 2004
RSM	β-glucan concentrates from oat and barley, water	Dough rheology, bread specific volume, weight loss, texture (Hardness, resilience, cohesiveness, chewiness, ΔHardness-1day, ΔHardness-7day), Crust L*, Crust h, Crumb C*	Ronda et al., 2015
CCD	HPMC, water	Specific volume, loaf height, crumb firmness, number of cells	McCarthy et al., 2005
Simplex Centroid Design	Proportion of rice flour, maize starch, wheat starch	Loaf specific volume, texture (Firmness, cohesiveness, resilience), crust brightness (L*), cell density, appearance, taste, overall acceptability	Mancebo et al., 2015a
Box-Behnken	HPMC, psyllium, water	Dough rheological properties (Dynamic oscillatory test and creep-recovery test), bread specific volume, hardness	Mancebo et al., 2015b

CHAPTER 3

OPTIMIZATION OF GLUTEN-FREE BREAD

This chapter consists of the evaluation of the effects of roasted chickpea flour (RCF), HPMC and water levels on rice-based GFB quality. Specific volume, bake loss, moisture content, color of crumb and crust and textural features were evaluated. The optimum region was determined using the desirability method. The obtained model was validated by comparing model predictions with measured responses obtained from breads having optimum factor combinations.

3.1. Materials and Methods

3.1.1. Materials

The rice flour and instant dry yeast used in this study were kindly provided by Pakmaya (As Gıda, Turkey). Roasted chickpea was obtained from the local markets and milled by using a laboratory mill. The other ingredients were HPMC (Benecel F4M, Ashland, USA), instant yeast (Pakmaya, Turkey), sugar, salt, sunflower oil and water.

3.1.2. Experimental Design

Central composite design (CCD) with three independent numeric factors, which are RCF (X_1: 10-25%, flour basis (fb)), HPMC (X_2: 0.5-2%, fb) and water (X_3: 85-105%, fb), was constructed. The lower (-1) and upper (1) levels were chosen according to the results of preliminary breadmaking trials. In total, 8 factorial points, 6 center points (0, 0, 0) and 6 axial points (alpha=1.63299) were included in the design (Table 3.1). Moreover, the design was orthogonally blocked; each baking day was set as a block (3 blocks). Contour and three-dimensional (3-D) surface plots were obtained and evaluated to assess the effects of individual variables on each response.

Table 3.1. Coded and actual levels of independent variables

Independent Variables (Factors)	Coded Levels				
	-1.63299	-1	0	1	1.63299
RCF (g/100 g flour), X_1	5.25	10	17.5	25	29.75
HPMC (g/100 g flour), X_2	0.03	0.5	1.25	2	2.47
Water (mL/100 g flour), X_3	78.67	85	95	105	111.33

RCF, roasted chickpea flour; HPMC, hydroxypropyl methylcellulose.

The second-order polynomial model equation (Equation 3.1) was used to fit the data and to predict the responses (Montgomery, 2005).

$$y=\beta_0+\sum_{i=1}^{k}\beta_i x_i+\sum_{i=1}^{k}\beta_{ii}x_i^2+\sum\sum_{i<j}\beta_{ij}x_i x_j+\epsilon \quad (3.1)$$

In this equation, *y*, response variable; *xi* and x_j, factors; *k*, number of factors; β_0, intercept; β_i, β_{ii} and β_{ij}, linear, quadratic and interaction regression coefficients, respectively. For three factors (k=3) the equation is written as in Equation 3.2.

$$y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{11}X_1^2+\beta_{22}X_2^2+\beta_{33}X_3^2+\beta_{12}X_1X_2+\beta_{13}X_1X_3+\beta_{23}X_2X_3 \quad (3.2)$$

The model suitability was evaluated by considering R^2, adjusted-R^2, p-value and lack of fit (LOF) of the model. To fit the model, the insignificant terms were eliminated and the obtained reduced models were used to estimate predicted responses for the optimum formulations. Design Expert 7.0.0 (Stat-Ease, Minneapolis, USA) was used for the construction of the experimental design and statistical evaluation of the data.

3.1.3. Bread Preparation

For the dough preparation, rice flour, RCF (10-25%), HPMC (0.5-2%), sugar (2%), salt (1.5%) and instant yeast (2.5%) were blended in a stand mixer (KitchenAid, KSM150, USA) at speed 2 for 1 min. Then, water (85-105%) and sunflower oil (5.27%) were added and mixed for the first 2 min at speed 2 and the following 3 min at speed 4.

All the ingredients were added based on flour basis (fb). The obtained dough (180 g) was placed in a baking pan (bottom: 5 cm x 9 cm, top: 6 cm x 9.5 cm, height: 7 cm) and proofed at 32 °C and 85% relative humidity for 30 min. The proofed loaves were placed in a preheated deck oven (Enkomak, Turkey) and baked for 45 min at 225 °C (top) and 205 °C (bottom). The loaves were removed from the pans and cooled at ambient temperature for 2 h until analysis. Three loaves for each formulation were prepared.

3.1.4. Specific Volume and Bake Loss

The volume of bread was measured by seed displacement method using a bread volumeter (Şimşek Laborteknik, Ankara, Turkey). Ratio of loaf volume to loaf weight was used for the specific volume (cm^3/g) calculation. The ratio of the differences in dough and bread weights to dough weight was reported as bake loss (%).

3.1.5. Moisture Content of Bread Crumb

The moisture content of the bread crumbs was determined by oven drying. The crumb (3-4 g) was cut from the center of the bread slice and dried at 105 °C for 16 h. Moisture content was calculated as the percent of the decrease in weight.

3.1.6. Color of Crust and Crumb

The color of the bread crust and crumb were measured by a colorimeter (Konica Minolta, CR-400, Japan) according to LAB color space with L^* (Lightness), a^* (+a, red; -a, green) and b^* (+b, yellow; -b, blue) parameters. The color change (ΔE) occurred during baking was calculated as in Equation 3.3 by using the color values of batter (L_0^*, a_0^*, b_0^*) and bread (L^*, a^*, b^*) for each formulation. At least six measurements per crust and crumb were taken and averaged.

$$\Delta E=\sqrt{(L^*-L_0^*)^2+(a^*-a_0^*)^2+(b^*-b_0^*)^2} \quad (3.3)$$

3.1.7. Texture Profile Analysis

Texture characteristics of bread crumbs were evaluated by texture profile analysis (TPA) using TA.XT plus Texture Analyzer (Stable Micro Systems, Surrey, UK) equipped with a 5 N load cell and 25 mm aluminum cylinder probe. The bread slices having thickness of 25 mm were compressed in the center to 40% of their original height with a test speed of 1.00 mm/s. Hardness (N), cohesiveness, resilience, springiness and chewiness (N) were calculated using Exponent software 6.1.9 (Stable Micro Systems, UK). Six slices for each formulation were analyzed and averaged.

3.2. Results and Discussion

3.2.1. Evaluation of Design Model

The response data obtained from CCD were given in Table 3.2. In order to improve the model fit, inverse square root and square root data transformations were applied to hardness and chewiness data, respectively. After fitting the data to the full second order polynomial model, regression equations and coefficients were observed (Table 3.3). All the models have significant p-value ($p<0.05$) except the crust a^* and b^* responses. For this reason, these responses were not considered in model predictions. Considering R^2 and adjusted-R^2 values, the models for each response seemed adequate. For these full models, specific volume and moisture data showed significant model fit as a result of LOF tests.

Table 3.2. Experimental design, independent factors and responses

	Actual Levels					Crumb Textural Parameters						Crust Color				Crumb Color			
Sample	RCF	HPMC	Water	Bake Loss (%)	Specific Volume (cm^3/g)	Moisture (%)	Hardness (N)	Cohesiveness	Resilience	Springiness	Chewiness (N)	*L**	*a**	*b**	ΔE	*L**	*a**	*b**	ΔE
1	25	0.5	105	21.24	2.29	51.95	5.78	0.62	0.34	0.92	3.60	41.37	13.04	32.06	38.51	68.19	2.36	23.66	9.10
2	10	0.5	85	18.31	1.74	47.41	10.12	0.59	0.33	0.91	5.94	57.86	8.24	33.39	30.17	68.84	0.94	18.56	11.34
3	25	2	85	16.42	1.71	46.86	14.43	0.58	0.33	0.91	8.43	52.93	14.13	35.79	30.65	67.98	2.77	23.69	8.68
4	10	2	105	19.25	2.29	52.59	3.94	0.71	0.41	0.96	2.78	60.21	12.25	38.43	34.20	75.15	0.27	17.23	6.24
5	17.5	1.25	95	19.89	2.27	49.65	5.04	0.63	0.35	0.92	3.15	51.56	12.71	35.01	33.73	68.76	1.58	20.79	9.32
6	17.5	1.25	95	19.96	2.24	49.71	5.19	0.67	0.38	0.96	3.35	52.77	20.84	7.21	32.35	71.35	1.80	21.38	8.05
7	25	0.5	85	17.51	1.82	46.99	9.92	0.61	0.33	0.87	6.00	51.43	15.18	37.49	33.54	66.08	2.83	23.68	10.59
8	10	0.5	105	19.53	1.99	52.02	5.08	0.68	0.40	0.99	3.46	52.31	12.01	35.49	37.00	68.11	0.27	16.56	11.69
9	17.5	1.25	95	19.02	2.14	49.41	5.65	0.66	0.37	0.95	3.84	54.38	15.17	38.58	35.34	68.58	1.36	20.21	9.66
10	10	2	85	17.12	1.66	47.38	11.25	0.61	0.35	0.92	6.71	63.42	9.05	34.16	27.17	72.57	0.78	18.28	7.73
11	25	2	105	18.66	2.31	52.47	3.82	0.67	0.36	0.92	2.60	49.83	15.04	34.72	33.65	67.80	1.95	21.90	8.67
12	17.5	1.25	95	18.81	2.13	49.75	5.11	0.67	0.38	0.93	3.41	54.19	14.72	37.77	34.63	68.20	1.37	20.27	9.83
13	17.5	1.25	95	17.78	2.12	50.38	5.10	0.66	0.37	0.94	3.48	59.33	10.91	36.05	28.86	71.46	1.38	21.18	7.89
14	17.5	1.25	78.67	15.72	1.46	46.25	16.44	0.61	0.33	0.88	10.28	61.85	10.47	35.21	25.21	70.39	2.08	21.79	7.57
15	17.5	1.25	95	18.69	2.12	49.93	5.22	0.68	0.38	0.92	3.84	55.66	13.59	37.16	32.79	71.62	1.25	20.57	7.21
16	29.75	1.25	95	18.52	2.08	49.37	5.38	0.64	0.36	0.91	3.47	45.86	17.17	34.49	35.46	67.34	2.89	24.14	8.20
17	17.5	1.25	111.33	21.51	2.48	53.06	2.83	0.71	0.41	0.94	2.15	59.02	11.09	36.56	29.80	69.92	0.82	19.77	8.61
18	5.25	1.25	95	18.53	2.01	49.73	6.43	0.70	0.42	0.93	4.48	58.85	12.07	38.17	36.95	72.89	-0.36	14.54	8.70
19	17.5	2.47	95	17.47	2.05	48.06	5.11	0.65	0.37	0.91	3.30	47.86	16.42	35.51	38.10	71.05	1.30	20.75	7.41
20	17.5	0.03	95	18.91	1.75	49.52	7.40	0.64	0.37	0.95	5.18	48.73	15.21	35.65	37.15	66.06	1.64	20.66	11.80

RCF, roasted chickpea flou; HPMC, hydroxypropyl methylcellulose.

Table 3.3. Regression coefficients of the models for individual responses

	Crumb Textural Parameters								Crust Color				Crumb Color			
Regression Coefficients	Bake Loss (%)	Specific Volume (cm^3/g)	Moisture (%)	Hardness (N)[a]	Cohesiveness	Resilience	Springiness	Chewiness (N)[b]	*L**	*a**	*b**	ΔE	*L**	*a**	*b**	ΔE
β_0	19.04**	2.17***	49.79***	0.44***	0.66***	0.37***	0.94*	1.81***	54.57**	14.75	31.85	33.02*	69.96**	1.46***	20.72***	8.68**
β_1	-0.028	0.042	-0.13	0.0006	-0.015**	-0.016***	-0.013*	-0.022	-4.46***	1.81*	-0.56	0.40	-1.78***	0.97***	2.85***	-0.058
β_2	-0.56**	0.046*	-0.11	0.016*	0.0061	0.0034	-0.001	-0.045	1.65	0.30	0.33	-0.90	1.53***	-0.089*	-0.091	-1.39***
β_3	1.41***	0.27***	2.36***	0.094***	0.034***	0.023***	0.024***	-0.431***	-1.99*	0.51	0.16	2.20*	0.23	-0.34***	-0.61***	-0.071
β_{12}	-0.27	-0.036	-0.019	-0.0003	-0.0035	-0.0011	0.000	0.023	-0.44	-0.01	-0.34	-0.24	-1.16*	-0.037	-0.27*	0.84**
β_{13}	0.33	0.025	0.095	0.0015	-0.011	-0.0093*	-0.014*	-0.049	-0.55	-1.03	-1.61	-0.74	0.011	-0.015	0.16	-0.045
β_{23}	-0.071	0.065*	0.16	0.028**	0.0087	0.0017	-0.001	-0.135*	1.16	0.31	0.81	-0.22	0.13	-0.022	-0.10	-0.046
β_{11}	-0.16	-0.040	0.0058	-0.015*	-0.0015	0.0012	-0.005	0.042	-0.84	-0.30	1.35	1.04	-0.0058	-0.041	-0.47***	0.043
β_{22}	-0.28	-0.094**	-0.28	-0.018*	-0.011*	-0.0054	0.002	0.069	-2.36*	0.15	1.07	1.58	-0.59	0.036	0.041	0.48*
β_{33}	-0.12	-0.066	0.043	-0.013	-0.0073	-0.0058	-0.003	0.176**	2.19*	-1.74	1.19	-2.22*	0.0094	0.029	0.070	-0.091
p-value	0.0017	<0.0001	<0.0001	<0.0001	0.0010	0.0009	0.0180	0.0006	0.0076	0.3398	0.9975	0.0479	0.0028	<0.0001	<0.0001	0.0037
R^2	0.9189	0.9677	0.9656	0.9703	0.9290	0.9309	0.8457	0.9395	0.8790	0.6038	0.1212	0.7948	0.9078	0.9937	0.9936	0.9006
Adjusted-R^2	0.8276	0.9313	0.9269	0.9370	0.8491	0.8532	0.6722	0.8715	0.7429	0.1580	-0.8675	0.5639	0.8040	0.9865	0.9865	0.7888
LOF (p-value)	0.1659	0.0019	0.0472	0.0522	0.9600	0.9189	0.7944	0.0784	0.1028	0.7885	0.8624	0.2028	0.5413	0.5305	0.6901	0.3694

[a] 1/square root (Hardness)

[b] square root (Chewiness)

Levels of statistical significance * $p<0.05$, ** $p<0.01$ and *** $p<0.001$.

To obtain the reduced models, the insignificant factors in the models were eliminated. The resulting ANOVA table was given in Table 3.4 and the regression equations for responses having significant models were given in Table 3.5. It can be seen that the reduced models have improved adjusted-R^2 values when compared with the full models. Also, the LOF test for moisture content response changed to insignificant. With this improvement, only specific volume response remained with significant LOF. Although having significant LOF, the p-value, R^2 and adjusted-R^2 for this response are found satisfactory. In this situation, comperative evaluation of the predicted and measured data obtained from validation experiments should be carefully done in order to confirm model adequacy. Similarly, in some of the previous RSM studies (Kittisuban et al., 2014; Schober et al., 2005), the models having significant LOF were also considered in the optimization because of high R^2.

Table 3.4. Analysis of variance of the fitted second-order polynomial models (Reduced models)

	Source of Variation	Sum of Squares	DOF	Mean Square	F-value	p-value	R^2	Adj R^2
Bake Loss	Block	2.47	2	1.23				
	Model	30.61	2	15.30	39.02	< 0.0001	0.8388	0.8173
	Residual	5.88	15	0.39				
	LOF	5.45	12	0.45	3.14	0.1880		
	Pure Error	0.43	3	0.14				
	Total	38.96	19					
Specific Volume	Block	0.03	2	0.014				
	Model	1.19	5	0.240	28.01	< 0.0001	0.9211	0.8882
	Residual	0.10	12	$8.530*10^{-3}$				
	LOF	0.10	9	0.011	120.31	0.0011		
	Pure Error	$2.828*10^{-4}$	3	$9.428*10^{-5}$				
	Total	1.32	19					
Moisture	Block	0.10	2	0.052				
	Model	74.44	1	74.44	266.89	< 0.0001	0.9434	0.9399
	Residual	4.46	16	0.28				
	LOF	4.30	13	0.33	6.09	0.0814		
	Pure Error	0.16	3	0.054				
	Total	79.01	19					
Hardness	Block	$1.832*10^{-3}$	2	$9.159*10^{-4}$				
	Model	0.130	6	0.022	39.04	< 0.0001	0.9551	0.9307
	Residual	$6.252*10^{-3}$	11	$5.684*10^{-4}$				
	LOF	$5.986*10^{-3}$	8	$7.483*10^{-4}$	8.44	0.0533		
	Pure Error	$2.659*10^{-4}$	3	$8.863*10^{-5}$				
	Total	0.14	19					
Cohesiveness	Block	$2.706*10^{3}$	2	$1.353*10-^{3}$				
	Model	0.020	4	$5.124*10^{-3}$	16.10	< 0.0001	0.8321	0.7804
	Residual	$4.137*10^{-3}$	13	$3.182*10^{-4}$				
	LOF	$2.763*10^{-3}$	10	$2.763*10^{-4}$	0.60	0.7616		
	Pure Error	$1.374*10^{-3}$	3	$4.579*10^{-4}$				
	Total	0.027	19					
Resilience	Block	$1.384*10^{-3}$	2	$6.922*10^{-4}$				
	Model	0.011	3	$3.743*10^{-3}$	27.21	< 0.0001	0.8536	0.8222
	Residual	$1.925*10^{-3}$	14	$1.375*10^{-4}$				
	LOF	$1.279*10^{-3}$	11	$1.163*10^{-4}$	0.54	0.8039		
	Pure Error	$6.463*10^{-4}$	3	$2.154*10^{4}$				
	Total	0.015	19					
Springiness	Block	$2.349*10^{-4}$	2	$1.174*10^{-4}$				
	Model	0.011	3	$3.773*10^{-3}$	20.27	< 0.0001	0.8129	0.7728
	Residual	$2.606*10^{-3}$	14	$1.861*10^{-4}$				
	LOF	$1.382*10^{-3}$	11	$1.257*10^{-4}$	0.31	0.9360		
	Pure Error	$1.223*10^{-3}$	3	$4.078*10^{-4}$				
	Total	0.01	19					
Chewiness	Block	$3.152*10^{-3}$	2	$1.576*10^{-3}$				
	Model	3.03	4	0.76	31.34	< 0.0001	0.9060	0.8771
	Residual	0.31	13	0.024				
	LOF	0.30	10	0.030	5.16	0.1019		
	Pure Error	0.017	3	$5.764*10^{-3}$				
	Total	3.35	19					

(cont. on next page)

Table 3.4. (cont.)

	Source of Variation	Sum of Squares	DOF	Mean Square	F-value	p-value	R^2	Adj R^2
*Crust L**	Block	12.60	2	6.30				
	Model	501.54	5	100.31	12.48	0.0002	0.8388	0.7716
	Residual	96.41	12	8.03				
	LOF	88.92	9	9.88	3.96	0.1426		
	Pure Error	7.49	3	2.50				
	Total	610.55	19					
Crust ΔE	Block	0.91	2	0.46				
	Model	140.95	2	70.48	9.20	0.0025	0.5509	0.4911
	Residual	114.89	15	7.66				
	LOF	105.96	12	8.83	2.97	0.2011		
	Pure Error	8.93	3	2.98				
	Total	256.75	19					
*Crumb L**	Block	9.63	2	4.81				
	Model	84.10	3	28.03	26.96	< 0.0001	0.8524	0.8208
	Residual	14.56	14	1.04				
	LOF	11.11	11	1.01	0.88	0.6238		
	Pure Error	3.45	3	1.15				
	Total	108.29	19					
*Crumb a**	Block	0.22	2	0.11				
	Model	14.23	3	4.74	409.83	< 0.0001	0.9887	0.9863
	Residual	0.16	14	0.01				
	LOF	0.13	11	0.01	1.03	0.5584		
	Pure Error	0.03	3	0.01				
	Total	14.61	19					
*Crumb b**	Block	1.66	2	0.83				
	Model	116.92	5	23.38	252.44	< 0.0001	0.9906	0.9867
	Residual	1.11	12	0.09				
	LOF	0.75	9	0.08	0.69	0.7093		
	Pure Error	0.36	3	0.12				
	Total	119.70	19					
Crumb ΔE	Block	5.67	2	2.83				
	Model	34.70	4	8.68	27.52	< 0.0001	0.8944	0.8619
	Residual	4.10	13	0.32				
	LOF	3.05	10	0.31	0.87	0.6223		
	Pure Error	1.05	3	0.35				
	Total	44.47	19					

Table 3.5. Regression equations of fitted models

Response	Final equation (Coded)
Bake Loss	$Y=18,67 - 0.56\ X_2 + 1.41\ X_3$
Specific Volume	$Y=2.14 + 0.046\ X_2 +0.27\ X_3 + 0.065\ X_2\ X_3 -0.091\ X_2^2 – 0.064\ X_3^2$
Moisture	$Y= 49.63 + 2.36\ X_3$
Hardness	$Y^a =0.43+6.35*10^{-4}\ X_1 + 0.016\ X_2 + 0.094\ X_3 +0.028\ X_2\ X_3 – 0.015\ X_1^2 -0.017\ X_2^2$
Cohesiveness	$Y= 0.65 – 0.015\ X_1 +6.07*10^{-3}\ X_2 + 0.034\ X_3 – 0.011\ X_2^2$
Resilience	$Y= 0.37 – 0.016\ X_1 + 0.023\ X_3 - 9.32*10^{-3}\ X_1\ X_3$
Springiness	$Y= 0.93 – 0.013\ X_1 + 0.024\ X_3 – 0.014\ X_1\ X_3$
Chewiness	$Y^b = 1.89 – 0.045\ X_2 – 0.43\ X_3 – 0.13\ X_2\ X_3 + 0.17\ X_3^2$
Crust *L**	$Y= 53.93 – 4.46\ X_1 + 1.65\ X_2 – 1.99\ X_3 – 2.30\ X_2^2 + 2.25\ X_3^2$
Crust ΔE	$Y = 34.88 + 2.20\ X_3 – 2.40\ X_3^2$
Crumb *L**	$Y = 69.56 – 1.78\ X_1 + 1.53\ X_2 – 1.16\ X_1\ X_2$
Crumb *a**	$Y = 1.47 + 0.97\ X_1 – 0.089\ X_2 – 0.34\ X_3$
Crumb *b**	$Y = 20.80 + 2.85\ X_1 – 0.091\ X_2 – 0.61\ X_3 – 0.27\ X_1\ X_2 – 0.48\ X_1^2$
Crumb ΔE	$Y= 8.65 – 0.058\ X_1 – 1.39\ X_2 + 0.84\ X_1\ X_2 + 0.48\ X_2^2$

X_1, roasted chickpea flour; X_2, HPMC; X_3, water.
[a] 1/square root (Hardness)
[b] square root (Chewiness)

3.2.2. Effect of RCF, HPMC and Water on Bake Loss and Moisture Content

The bake loss values of the samples were ranged between 15.72 to 21.51% (Table 3.2) and affected by HPMC and, more dominantly, water level (Table 3.3). No significant effect of RCF addition was found. The highest bake loss was observed at the point where water addition level was at maximum and HPMC level was at minimum (Figure 3.1a). Dough having excess water in case of low levels of HPMC seemed unable to keep water inside the dough during baking. Apart from dough properties, crumb morphology may affect the bake loss; according to the visual observations on the crust surface, the bread samples with more cracks on the crust had higher bake loss since more water was vaporized from the inner parts of the loaf.

Increasing bake loss values with increasing water addition levels were reported in a study that evaluated the rice flour-HPMC bread (de la Hera et al., 2014). The reports related with the effect of chickpea flour addition on bake loss of GF bread are scarce. In a corn starch-based GFB formulation, a slight increase in bake loss was reported upon the presence of 7.8% chickpea flour (Aguilar et al., 2015).

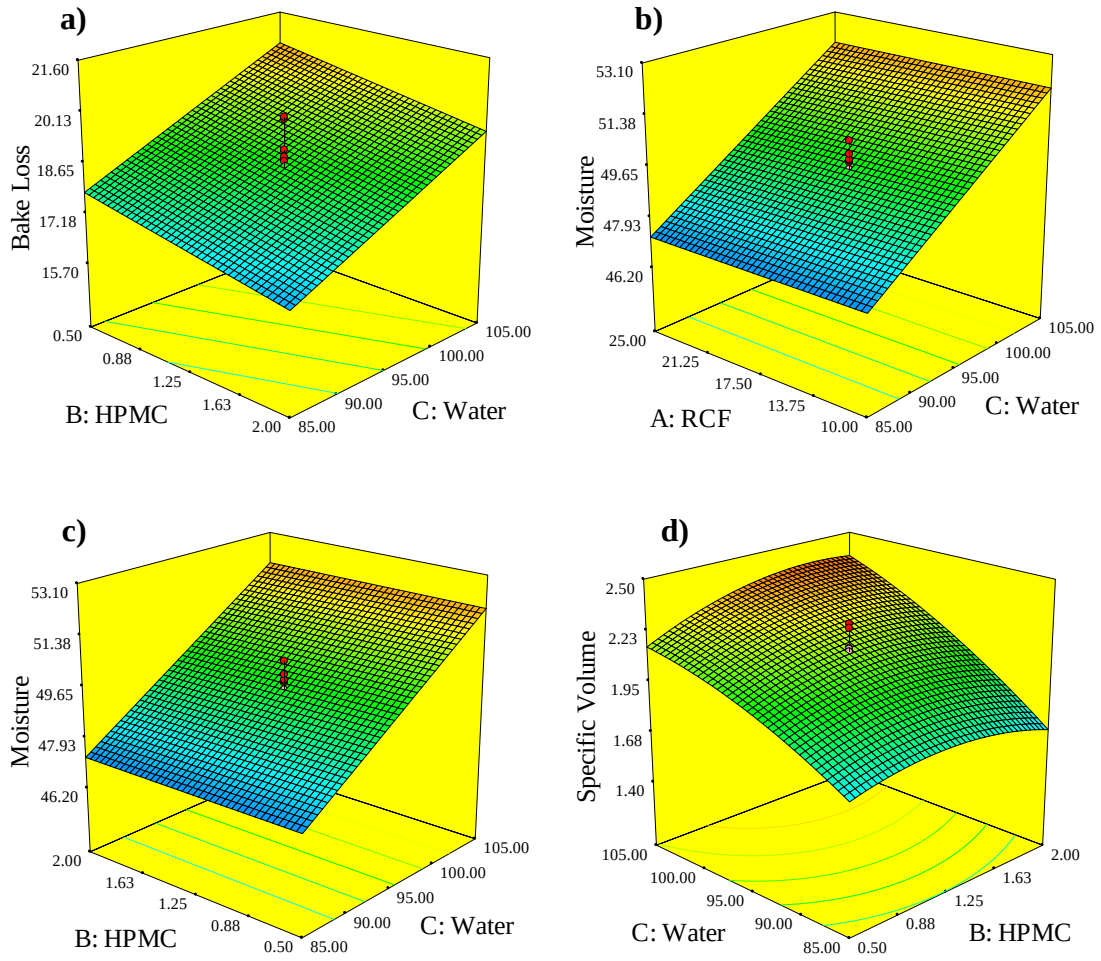


Figure 3.1. 3-D response surface plots for a) bake loss, b-c) moisture content and d) specific volume as influenced by (A) Roasted chickpea flour (RCF) (%), (B) HPMC (%) and (C) water (%).

The moisture content results were shown in Table 3.2. According to results, the only significant and positively related factor was water addition level (Table 3.3) (Figure 3.1b-c). Regarding the RCF and HPMC levels, no significant effects on moisture content were observed ($p>0.05$). Similarly, O'Shea et al. (2015) evaluated the effects of water, orange pomace and proofing time on moisture content of rice-flour-potato starch based bread and found water level as the only effective factor. In general, high amounts of water were required in GF dough formulations to obtain well-leavened dough and soft crumb texture (Gallagher et al., 2003). In that sense, GF dough can be called as "batter" due to relatively low consistency values compared to wheat dough. Apart from the effect of water on dough consistency, bread firmness is also low when bread had relatively high levels of moisture (Rogers et al., 1988).

3.2.3. Effect of RCF, HPMC and Water on Specific Volume

One of the most important attributes concerning bread quality is specific volume. It is well-described that, most of the GFB have dense structure resulting in lower specific volume compared to wheat bread.

The specific volumes of bread samples (1.46 and 2.48 cm^3/g) were given in Table 3.2. Our results suggested that HPMC and water addition levels influenced the specific volume to a great extent together with their interaction (β_{23}) and quadratic (β_{22}) effect of HPMC (Table 3.3). The highest specific volume was achieved at high levels of water (Figure 3.1d). When water level increased, the presence of high amounts of HPMC resulted in increased specific volume. However, specific volume was decreased slightly when the HPMC levels were moved towards high and low limits. Since HPMC influences the dough consistency, it can be concluded that extremely low and high dough consistencies affected the specific volume in a negative way. This could be ascribed to the influence of consistency on gas retention and expansion. The positive effects of HPMC on specific volume were evidenced in previous studies and attributed to its ability to retain water, form gel network (Marco & Rosell, 2008) and stabilize gas cells (Schober, 2009). Previous research findings are also in agreement with the adverse effect of high levels of HPMC on volume (McCarthy et al., 2005; Sabanis & Tzia, 2011).

RCF addition level, another factor in this study, had little and insignificant effect on specific volume (p-value=0.066). Similarly, a slight increase in loaf specific volume was reported in a recent study in which toasted chickpea flour bread was evaluated (Ouazib et al., 2016).

3.2.4. Effect of RCF, HPMC and Water on Crumb Texture

The hardness values of the bread samples were in 2.83-16.44 N range as shown in Table 3.2. With the data transformation, p-value of LOF was altered to insignificant (0.0522) from significant (0.0087; data not shown). The hardness of the breads was affected by HPMC, water, their interaction (β_{23}) and also quadratic terms coming from RCF (β_{11}) and HPMC (β_{22}) (Table 3.3). The bread became softer when HPMC and water levels increased (Figure 3.2a). Although up to 25% RCF presented in our

formulations, only a slight and statistically insignificant increase in hardness was observed (Figure 3.2b).

Similar findings related to the effects of water and HPMC on hardness were reported in the previous studies (de La Hera, Rosell & Gomez, 2014; Peressini et al., 2011). HPMC, via film forming and gas cell stabilizing effects (BeMiller, 2008; Schober, 2009), may contribute positively to crumb softness. Furthermore, the hardness results were in agreement with specific volume results. As volume increases, the crumb becomes less dense and soft. As far as we know, there is no literature finding on hardness of roasted chickpea-enriched rice flour-based GF bread. However, chickpea flour (7.5%) addition to corn starch-based bread was reported to have no significant effect on hardness (Aguilar et al., 2015). The negative effect of protein isolate addition on crumb was reported previously (Matos & Rosell, 2014). In that sense, no hardening effect of roasted chickpea flour, which is rich-in protein, seemed advantageous.

Cohesiveness reflects the internal resistance of food structure and it is inversely related with crumbling (Onyango et al., 2011). The bread samples in this study had cohesiveness values in the range of 0.58-0.71 (Table 3.2). According to the results, both the levels of RCF and water and also the quadratic term of HPMC (β_{22}) significantly influenced the cohesiveness of bread (Table 3.3). Maximum cohesiveness was achieved at the low levels of RCF and high levels of water (Figure 3.2c). This finding was in good agreement with a previous study in which the positive effect of increasing water levels on rice bread quality was reported (de La Hera et al., 2014).

As it is shown in Table 3.2, resilience values of GFB crumb (0.33-0.42) were significantly ($p<0.05$) affected by the levels of RCF, water and their interaction (β_{13}) (Table 3.3). At the lowest RCF and the highest water levels, resilience was at its maximum (Figure 3.2d). Similarly to resilience, springiness is also related to the elasticity and showed similar trend with resilience (Figure 3.2e). The springiness values of the bread samples were found between 0.87 and 0.99.

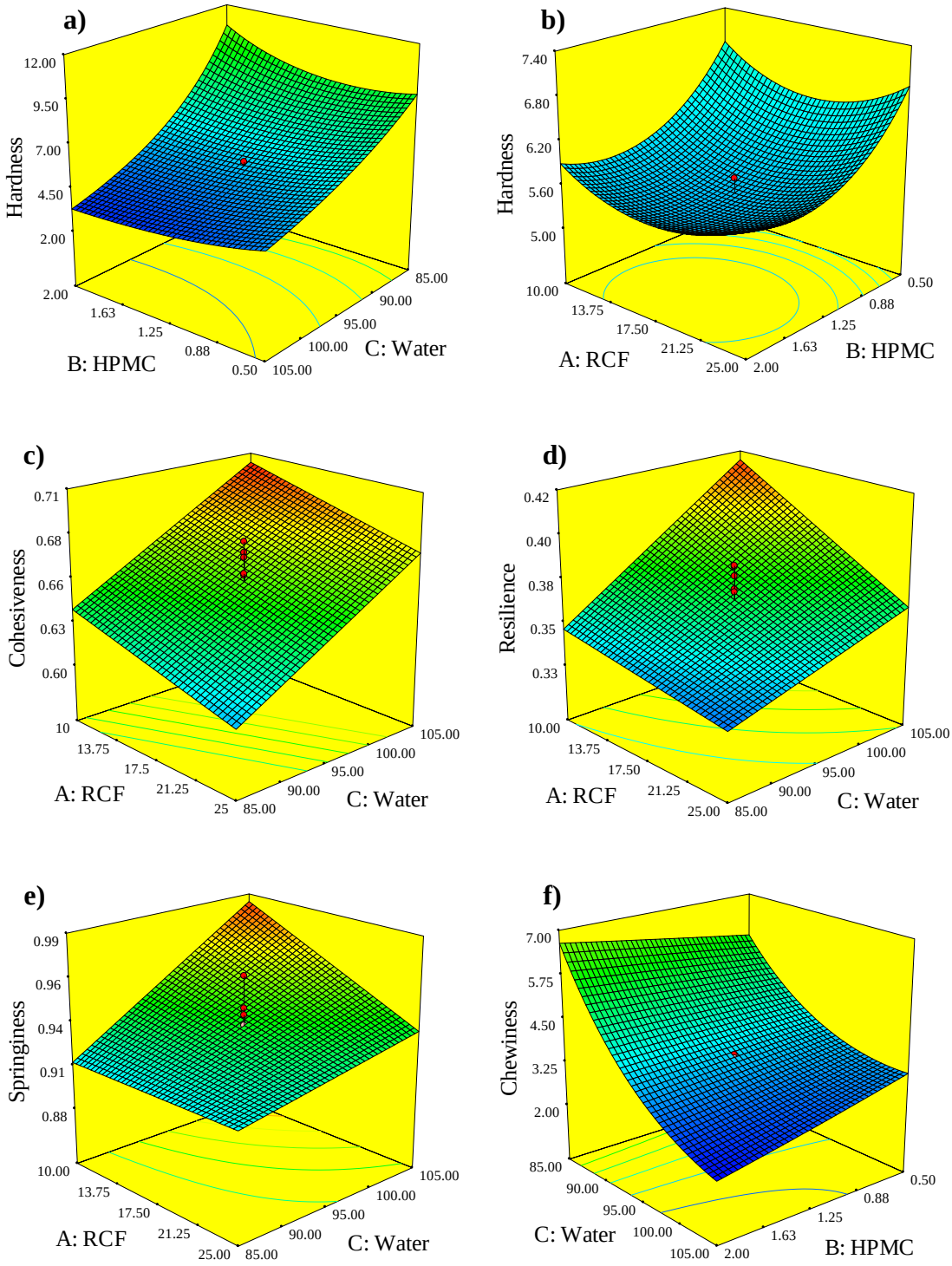


Figure 3.2. 3-D response surface plots for a-b) hardness, c) cohesiveness, d) resilience, e) springiness and f) chewiness as influenced by (A) Roasted chickpea flour (RCF) (%), (B) HPMC (%) and (C) water (%).

Chewiness is the product of hardness, springiness and cohesiveness. Low chewiness value indicates the easiness of breaking the bread in the mouth (Matos & Rosell, 2012). As a consequence of the transformation applied to cohesiveness data, LOF was altered from significant (p-value=0.0377; data not shown) to insignificant (p-value=0.0784) (Table 3.3). Among the factors tested, only water level significantly influenced the chewiness of GFB (Table 3.3). At the highest level of water addition, chewiness was minimized when HPMC level was at the highest level (Figure 3.2f). Since hardness is a component in the calculation of chewiness, the 3-D surface plots for hardness and chewiness exhibited similar trends.

3.2.5. Effect of RCF, HPMC and Water on Color

In Table 3.3, the significant factors on crumb and crust color can be seen. Lightness value of crumb was significantly influenced by RCF and HPMC levels ($p<0.05$) (Table 3.3). The highest RCF and the lowest HPMC levels in the formulations caused the darkest crumb (Figure 3.3a). Similar to lightness, crumb a^* was significantly affected by RCF and HPMC. As regards the crumb b^*, it was significantly influenced by RCF and water levels ($p<0.05$). It seemed that the main factor significantly ($p<0.001$) affecting all the crumb color parameters was RCF and its increasing levels resulted in increased darkness, yellowness and redness (Figure 3.3a-c). The alteration of crumb color was due to the incorporation of yellow-brown colored RCF (L^*=78.96±0.08, a^*=2.92±0.03, b^*=29.75±0.08) into the whitish rice flour (L^*=93.55±0.01, a^*=-0.11±0.01, b^*=6.63±0.03). The color improvement caused by RCF addition can be considered advantageous since the darker crumb color is desirable by consumers compared to pale color (Campo et al., 2016). As regards the HPMC, in a rice starch-based GFB development study (Kittisuban et al., 2014), increased crumb lightness was reported in case of the presence of increased levels of HPMC, which is in agreement to our findings. When considering crumb ΔE value, among the factors used, HPMC addition, it's quadratic effect (β_{22}) and RCF-HPMC interaction (β_{12}) influenced this parameter (Table 3.3). The highest color change was observed at low RCF and HPMC levels, however the lowest color change was found at low RCF and high HPMC levels (Figure 3.3d).

The RCF and water levels and also quadratic terms belonging to HPMC (β_{22}) and water (β_{33}) significantly influenced the crust L^* ($p<0.05$) (Figure 3.3e). With high p-value (<0.001), the most dominant factor was found as RCF (Table 3.3). Compared to chickpea flour, roasted chickpea has darker color due to the heat application. Therefore, the presence of RCF in bread formulations resulted in darker crust. In a previous study by Aguilar et al. (2015), darker color was obtained in chickpea and tiger nut flour added corn starch-based GFB formulation. On the other hand, the high protein content of roasted chickpea could enhance the formation of dark-colored Maillard reaction products on the crust surface. Maillard reaction occurs between reducing sugars and amino acid present in the food as a result of heat application. It should be noted that, acrylamide is a chemical could be formed after Maillard reaction and it is considered as a probable carcinogenic substance (Klauning, 2008; Rice, 2005). According to a survey study, roasted chickpea (leblebi) contains 12 µg/kg acrylamide and wheat bread has 38 µg/kg acrylamide (Ölmez et al., 2008). Those acrylamide levels are low compared to roasted corn (194 µg/kg) and some bakery products such as biscuits (198 µg/kg). In that sense, the addition of roasted chickpea flour within the levels in this study seems not to increase the acrylamide content considerably. The crust color change during baking was only affected significantly by water level (Table 3.3) and there was a positive relationship between them until a certain water addition level (up to 95 mL/100 g flour) (Figure 3.3f). As mentioned in Section 3.2.3, the increase in water level resulted in volume increases. As the volume increases, crust surface reachs to the top heating surface of the oven, which could be the reason of the increased crust color change.

Nowadays, the consumer preferences were shifted towards dark colored bread (Campo et al., 2016). The increase in whole-seed flour-containing bread consumption due to its positive health effects may lead to the formation of a perception that dark crumb and crust color indicate healthful bread. However, rice flour, which has light color, is the basic flour type used in GFB formulations because of its non-allergenic nature and good breadmaking properties. Therefore, the roasted chickpea flour can be added to the formulations to improve the color of crumb and crust.

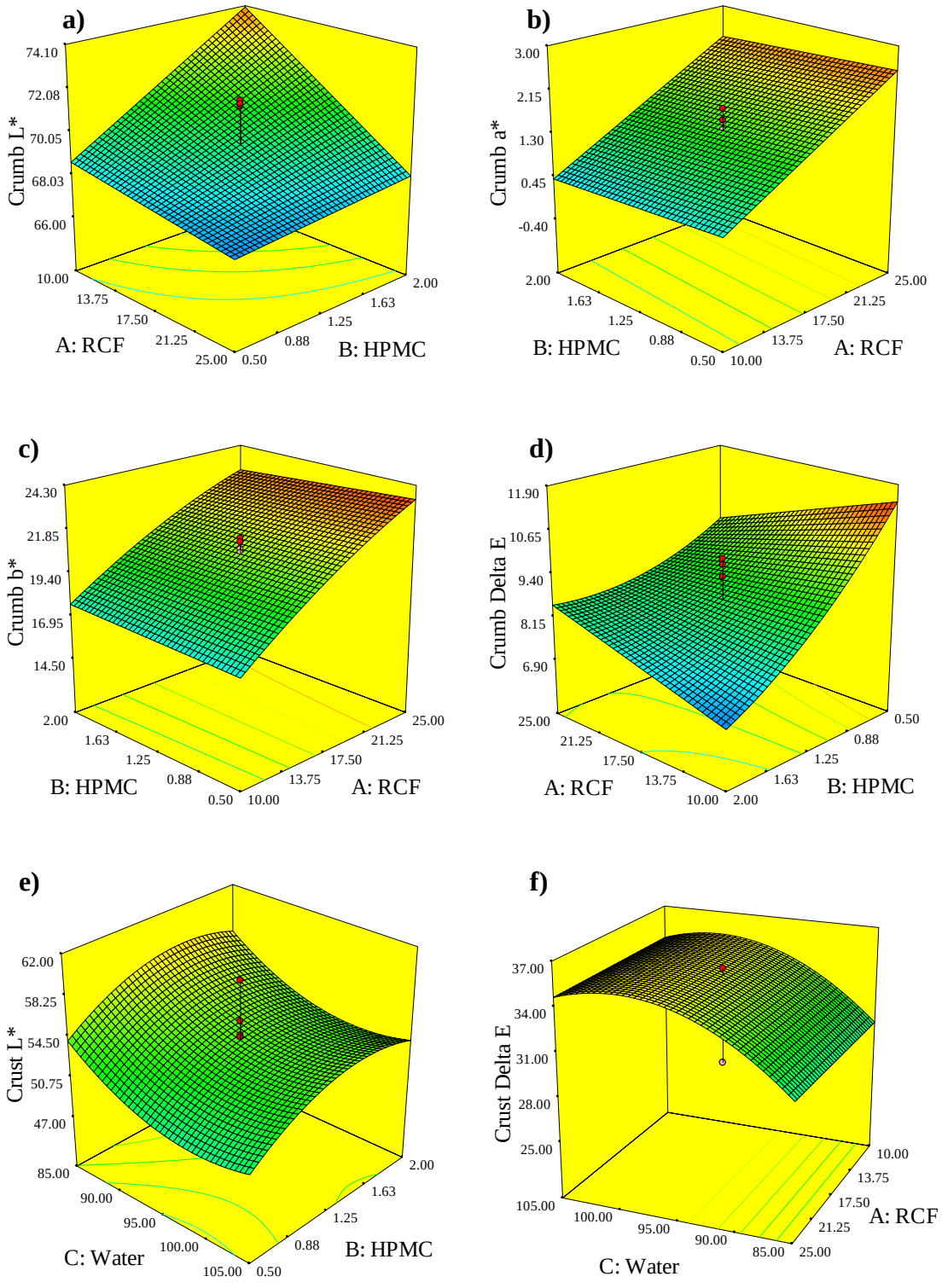


Figure 3.3. 3-D response surface plots for a-d) crumb and e-f) crust color as influenced by (A) Roasted chickpea flour (RCF) (%), (B) HPMC (%) and (C) water (%).

3.2.6. Optimization and Validation

Numerical optimization was utilized to determine optimum factor levels by using responses having significant models. For this purpose, desirability method was applied with the following settings: RCF level was set at maximum; specific volume and cohesiveness maximized; hardness and chewiness were minimized; other responses were kept in their ranges. In addition, the lower limit for specific volume and the upper limit for hardness were set as 2.2 cm^3/g and 5 N, respectively. Based on these restrictions, the software suggested the maximum desirability of 0.745 which could be considered as high (Figure 3.4).

For the model validation, three points in the desirable region were selected and bread samples having these factor levels were baked (Table 3.6). When the measured results were compared with the predicted response values, most of the responses were found to be in 95% prediction interval (PI). That means predicted values of this study, corresponded well with the measured values (Table 3.6).

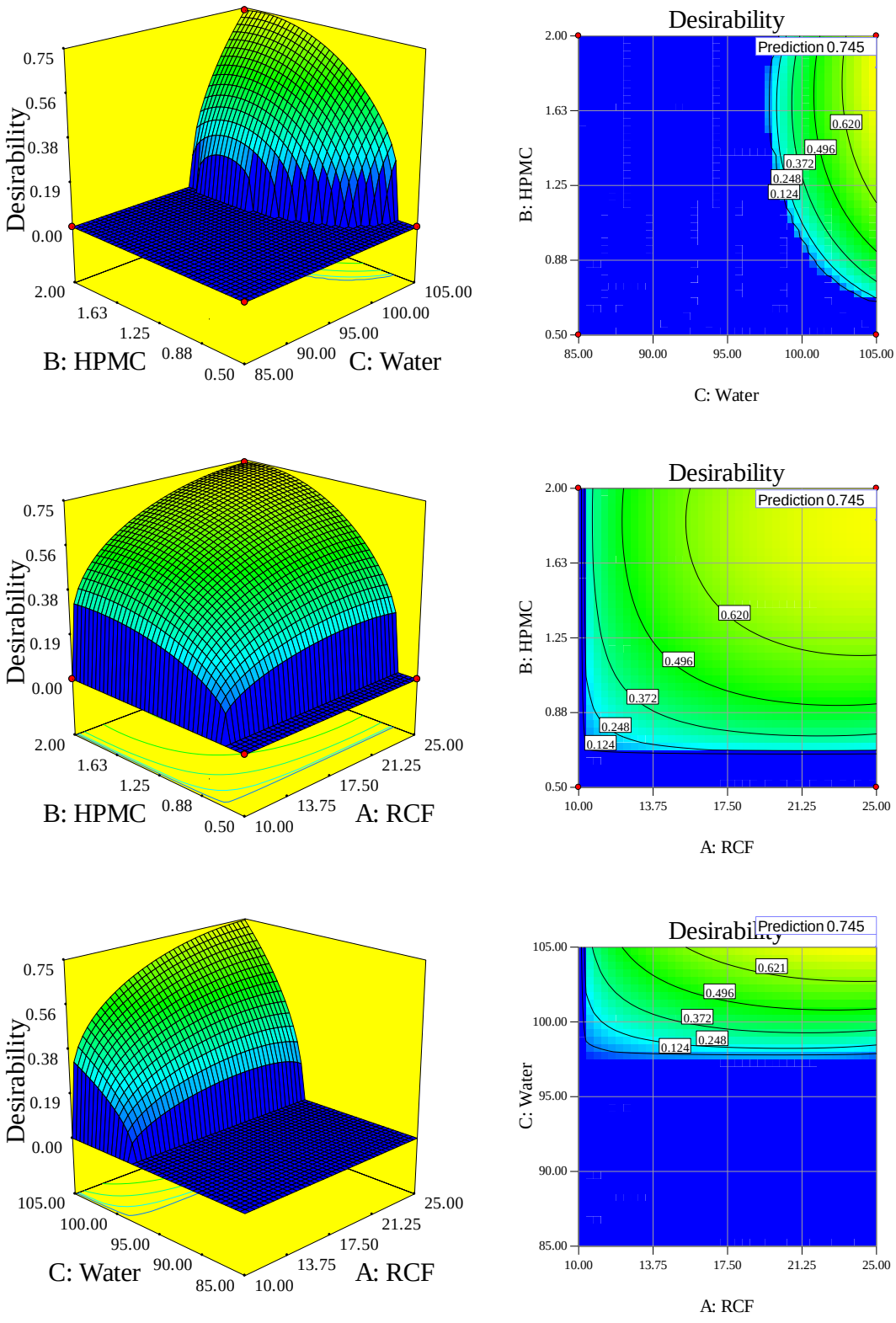


Figure 3.4. 3-D response surface and contour plots for desirability function for optimized model. (A) Roasted chickpea flour (RCF) (%), (B) HPMC (%) and (C) water (%).

Table 3.6. Predicted vs. measured response values for GFB sample at optimum factor combinations

	Levels					
Factors	**Sample 1**		**Sample 2**		**Sample 3**	
RCF (%)	1.00		0.98		0.52	
HPMC (%)	0.79		0.63		0.15	
Water (%)	1.00		0.97		1.00	
Desirability	0.745		0.726		0.670	
Response	**Predicted**	**Measured**	**Predicted**	**Measured**	**Predicted**	**Measured**
Bake Loss (%)	19.64	19.78 ± 0.26	19.68	19.13 ± 0.39	19.99	19.72 ± 0.43
Specific Volume (cm^3/g)	2.38	2.17 ± 0.06	2.37	2.28 ± 0.01	2.36	2.24 ± 0.01
Moisture (%)	52.00	51.24 ± 0.11	51.93	51.73 ± 0.24	52.00	51.37 ± 0.01
Hardness (N)	3.53	3.76 ± 0.40	3.61	4.07 ± 0.43	3.63	3.96 ± 0.73
Cohesiveness	0.67	0.69 ± 0.02	0.67	0.67 ± 0.04	0.68	0.67 ± 0.04
Resilience	0.36	0.39 ± 0.01	0.36	0.38 ± 0.04	0.38	0.38 ± 0.04
Springiness	0.93	0.94 ± 0.01	0.93	0.93 ± 0.01	0.94	0.92 ± 0.01
Chewiness (N)	2.20	2.50 ± 0.25	2.30	2.90 ± 0.08	2.56	2.63 ± 0.27
Crust *L**	49.61	47.85 ± 0.70	49.88	47.56 ± 1.26	52.06	52.33 ± 1.75
Crust Δ*E*	34.69	35.70 ± 0.32	34.76	34.93 ± 1.04	34.69	31.70 ± 1.28
Crumb *L**	68.08	65.61 ± 0.74	68.07	67.64 ± 1.58	68.78	68.03 ± 0.77
Crumb *a**	2.04	2.21 ± 0.12	2.04	1.60 ± 0.04	1.63	1.54 ± 0.05
Crumb *b**	22.28	23.96 ± 0.55	22.32	23.04 ± 0.12	21.51	22.17 ± 0.45
Crumb Δ*E*	8.45	9.46 ± 0.22	8.42	8.88 ± 1.38	8.49	8.73 ± 1.40

RCF, roasted chickpea flour; HPMC, hydroxypropyl methylcellulose.
Values are mean ± SD.

3.3. Conclusions

In this part of the study, it was observed that quality parameters of bread were affected by the levels of RCF, HPMC and water. Due to its significant effects on almost all parameters, special attention should be given to the adjustment of water level in GFB development studies. The beneficial effects of HPMC on bake loss, specific volume and softness of the bread were evidenced. Although increasing RCF amount resulted in decreased cohesiveness and springiness, crust and crumb color was improved and more appealing brownish color was observed. RCF, up to 25%, was successfully used to fortify rice flour based GFB formulations containing HPMC. From economical point of view, in case of the utilization of broken roasted chickpea, by-product obtained during RCF production, relatively low-cost GFB could be produced. Since the obtained bread has a simple formulation consisting of two types of flour and HPMC, it could be used as a basis for further bread development studies.

CHAPTER 4

DOUGH CHARACTERIZATION

This part of the thesis was carried out to compare dough properties of the optimized rice-based formulation enriched with roasted chickpea flour with raw chickpea flour and dehulled chickpea flour containing counterparts. Dough formulation having only rice flour was also evaluated as the reference sample. Within the scope of this section flour properties (proximate composition, particle size distribution, microstructure and color), flour blend properties (water binding and foaming capacities, and pasting properties) and dough properties (consistency, leavening during proofing and rheology) were evaluated.

4.1. Materials & Methods

4.1.1. Materials

The flours used in this study were: rice flour, roasted, dehulled and dried raw chickpea flours. Rice flour (RF) (Pakmaya, Turkey) and dehulled chickpea flour (DCF) (Ingredion, Germany) were obtained as in the flour form. Roasted chickpea and dried chickpea were milled by using a laboratory mill to obtain roasted chickpea flour (RCF) and chickpea flour (CF) having particle size ≤ 1 mm. The other ingredients were HPMC (Benecel F4M, Ashland, USA), instant yeast (Pakmaya, Turkey), sugar, salt and sunflower oil.

4.1.2. Flour Properties

4.1.2.1. Proximate Composition

The moisture content of the flour samples was determined via oven drying at 105 °C until reaching a constant weight. The total nitrogen content of samples was determined according to the Official Standard Method AOAC 920.87 (1999) by using and a block digestion system (Kjeldatherm, C. Gerhardt GmbH & Co. KG, Germany) and a distillation system (Vapodest 50s, C. Gerhardt GmbH & Co. KG, Germany). The protein content was then calculated using 5.95 and 6.25 as conversion factors for rice and chickpea flours, respectively. For the fat content determination, automatic extraction system (Soxtherm, Gerhardt, Germany) was used with hexane as the extraction solvent. The ash content was analyzed according to AACC (1999) by using a muffle furnace (Protherm, Turkey). Total carbohydrates (TC) were calculated by taking the difference [100-(proteins + lipids + ash)]. The results were expressed as percentage on dry basis (db).

4.1.2.2. Particle Size Distribution

The flour samples were analyzed to assess their particle size distributions. Samples (50 g) were placed in an analytical sieve shaker (Octagon Digital, Endecotts Ltd., England) equipped with 6 sieves with 40, 90, 125, 250, 500 and 1000 μm openings. Plastic balls having diameters of 3 cm were placed on two sieves (500 and 1000 μm). Each fraction was collected after sieving at amplitude 8 for 10 min. The analysis was replicated twice and the results were given as percentage of each fraction on 100 g flour.

4.1.2.3. Water Binding Capacity of Flour Blends

The reference sample, RF, and chickpea-rice flour blends having ratio of 1:3 for RCF:RF, CF:RF or DCF:RF were prepared and each were investigated for their water binding capacities (WBC). Each sample (2 g) was mixed with 24 mL of deionized

water, shaken for 60 min on a shaker (KS 130 Basic, IKA, Germany) and centrifuged at 3460xg at 25 °C for 10 min (Universal 320R, Hettich, Germany). The supernatant was carefully removed and the weights of the tubes were recorded. The results were given as percentage of water held by the dry sample. The results are the average of at least two measurements.

4.1.2.4. Foaming Properties of Flour Blends

Foaming properties, foam capacity and foam stability, of flour blends, which were prepared as in Section 4.1.2.3, were determined according to Shevkani et al. (2015b) with some modifications. Flour blend (2 g) was mixed with 50 mL deionized water and homogenized for 2 min (Ultra-Turrax T 25, 18G Dispenser, IKA, Germany). Foaming capacity (FC, %) was calculated as the volume ratio of foam to initial slurry, and foam stability (FS, %) was given as the ratio of foam volume measured after 60 min to initial foam volume. At least three replicates were performed.

4.1.2.5. Flour Color

The color of the flour samples was measured by a colorimeter (Konica Minolta, CR-400, Japan) according to LAB color space with L^* (Lightness), a^* (+a, red; -a, green) and b^* (+b, yellow; -b, blue) parameters by using glass sample cup. At least ten measurements were recorded for each flour sample and they were averaged.

4.1.2.6. Pasting Properties of Flour Blends

The rice flour (RF) and the flour blends (RF+RCF, RF+CF, RF+DCF) were prepared as in Section 4.1.2.3 and analyzed for their pasting properties by using Brabender® Micro-Visco-Amylograph (MVA) (Brabender OHG, Duisburg, Germany) according to Cappa et al. (2013a). Sample slurry was prepared by dispersing sample (12 g) in distilled water (100 mL). Flour and water weights were scaled on 14% sample moisture basis. The slurry was stirred at 250 min^{-1}, heated from 30°C to 95°C with a ramp of 3°C, held at 95°C for 30 minutes, cooled to 50°C and held at that temperature

for 30 min and finally cooled to 30°C. The measured indices were gelatinization temperature (GT (°C), temperature when the initial viscosity increase occurred); peak viscosity (PV (Brabender units, BU), maximum paste viscosity during heating), breakdown (BD (BU), viscosity decrease index during holding at 95°C, calculated by the difference between the peak viscosity and the viscosity obtained after the holding period); final viscosity (FV (BU), paste viscosity at the end of the cooling), and setback (SB (BU), index of the viscosity increase during cooling, difference between FV and the minimum viscosity obtained after the holding period at 95 °C). The analysis was performed in triplicate.

4.1.2.7. Scanning Electron Microscopy (SEM) Analysis of Flours

The microstructure of flours was analyzed with SEM. Double-sided carbon tape having flour samples on one side was attached to an aluminum stub and coated with gold under vacuum (0.09 mbar). The images were captured by scanning electron microscope (XL 30S FEG, Philips) under a voltage of 2.0 kV.

4.1.3. Dough Properties

4.1.3.1. Dough Preparation and Consistency Measurement

According to the blend investigated, four doughs were prepared: RF, RF+RCF, RF+CF and RF+DCF. The RF+RCF was the optimized formulation obtained in Chapter 3. The recipe consisted of 100% RF or flour blends having 1:3 ratio for RCF:RF, CF:RF or DCF:RF, according to the optimized recipe and HPMC, sugar (2%), salt (1.5%), instant yeasts (2.5%), sunflower oil (5.27%) and water, based on flour weight. The water amount added to each formulation was determined according to the desired dough consistency assessed on the optimized dough (RF+RCF) by using the Brabender Farinograph (Brabender OHG, Germany). For the consistency measurement purpose, the dry components (flours, HPMC, instant yeast, sugar and salt) were added to the farinograph bowl (300 g capacity) and mixed for 1 min. Following water addition, vegetable oil was added at the end of subsequent 2 min. The dough was eventually

mixed for 8 min at 25°C and consistency was recorded (125±5 BU). The other doughs (RF, RF+CF and RF+DCF) were prepared by using the same procedure, and the water was added accordingly to reach the same final consistency of the optimized dough.

4.1.3.2. Dough Development during Proofing

4.1.3.2.1. Rheofermentometer Measurements

The leavening behavior of dough samples was evaluated with Chopin Rheofermentometer F3 (Chopin, Villeneuve-La-Garenne, Cedex, France) according to a method developed for GF dough samples (Cappa et al., 2013a; Mariotti et al., 2006). Briefly, the dough (300 g) was placed in the instrument bowl and the weight support (254 g) of the instrument was placed on the sample. The proofing was carried out at 30 °C for 60 min. Maximum and final height of dough (Hm and Hf, mm), time necessary to reach maximum height (T1, min), time of dough porosity appearance (Tx, min), total CO_2 production ($CO_{2\text{-TOT}}$, mL), CO_2 retention ($CO_{2\text{-RET}}$, mL), released CO_2 ($CO_{2\text{-REL}}$, mL) and coefficient of retention (Rc, %) were measured.

4.1.3.2.2. Image Analysis

As a parallel test to rheofermentometric test, leavening properties of dough samples were measured by using image analysis with the method developed by Cappa et al. (2013a). The dough samples (10 g), prepared using Brabender Farinograph, were weighed in Petri dishes and leavened for 60 min. Every 10 minutes, samples were scanned by using a flatbed scanner (HP ScanJet 8300, Hewlett-Packard, CA, USA) at 600 dpi. The images were processed using Image Pro-Plus (4.5.1.29, Media Cybernetics Inc, MD, USA) software. The area (mm^2) of the dough was measured and the area increase (%) during proofing was calculated. Six petri dishes per each sampling time were analyzed.

4.1.3.3. Rheological Properties of Dough

The dynamic oscillatory measurements were carried out on Physica MCR300 Rheometer (Anton Paar, Graz, Austria) equipped with a corrugated parallel plate system (PP25/P2, diameter: 25 mm) having a gap of 2 mm. The dough samples were prepared and rested for 60 min at 25 °C before each measurement. The instant yeast was not included in the formulation in order to avoid perturbation of the system. The dough was loaded between the plates and the excess amount was trimmed off. In order to avoid moisture loss during analysis, a humidity cover (H-PTD 150) having water trap and wet pads was used, and mineral oil was carefully applied to the dough borders. After five minutes of resting to relax stresses, the measurements were carried out and data was recorded by using Universal Software US200 (version 2.5) (Anton Paar, Ostfildern, Germany). For the determination of linear viscoelastic region, strain sweep tests were performed at a constant frequency of 1 Hz and in the range of 0.01-100% strain. Frequency sweep tests were carried out in the range of 10 to 0.1 Hz at a constant strain of 0.04%. For both tests, storage modulus (G′, Pa), loss modulus (G″, Pa) and damping factor (tan δ, G″/G′) were calculated. For each formulation, the analysis was performed on two separate doughs, each having at least two replications.

4.1.4. Statistical Analysis

Statistical evaluation of the data was performed by using MINITAB 16 (Minitab Inc., U.S.). The results were given as “mean ± SD”. The significance of the data was tested by analysis of variance (ANOVA) at $p<0.05$ and, in the significant models, means were compared by Tukey’s test at 95% confidence interval.

4.2. Results and Discussion

4.2.1. Flour Properties

4.2.1.1. Proximate Composition

The proximate compositions of the rice flour and the three types of chickpea flour are given in Table 4.1. Since heat was applied during the roasting process, RCF showed almost three times lower moisture content than CF. Also the dehulling process resulted in a moisture reduction, but at a lower extend than RCF. In general, the chickpea flours were rich in protein, fat and ash in comparison to rice flour, consequently, the total carbohydrates were present in lower concentration. According to the results, the usage of all the chickpea flours as raw materials in bread formulations is quite promising in terms of nutritional quality improvement.

Roasting caused no statistical change in the amount of protein whereas the dehulled chickpea contained 10% less protein. Also, 25% of ash reduction observed for DCF can be linked with the removal of mineral rich hulls. According to Ghavidel & Prakash (2007), dehulling decreased moisture and ash contents andon the contrary, increased protein content. In literature, some modifications in carbohydrates and proteins and roasted aroma formation were reported as a consequence of the roasting process (Coşkuner & Karababa, 2004). Besides, no significant changes in ash, fiber and protein contents during processing were observed (Sağlam, 2006).

Table 4.1. Proximate compositions of flour samples

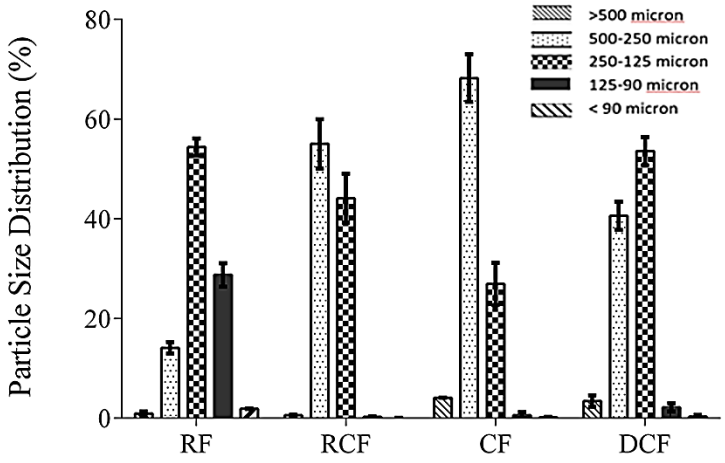
Flour	Moisture (%)	Protein (% db)	Fat (% db)	Ash (% db)	TC (% db)
RF	11.85 ± 0.09^{a}	8.29 ± 0.18^{c}	1.28 ± 0.19^{c}	0.68 ± 0.00^{b}	89.75
RCF	3.25 ± 0.06^{d}	23.10 ± 0.79^{a}	7.57 ± 0.08^{a}	2.68 ± 0.03^{a}	66.65
CF	9.15 ± 0.06^{b}	23.52 ± 0.30^{a}	5.71 ± 0.24^{b}	3.09 ± 0.01^{a}	67.68
DCF	7.12 ± 0.00^{c}	21.15 ± 0.41^{b}	7.55 ± 0.05^{a}	2.32 ± 0.77^{a}	68.98

RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour; TC, total carbohydrates.
Values are mean ± SD.
Means having different letters at the same column are significantly different ($p<0.05$).

4.2.1.2. Particle Size Distribution

As reported in Figure 4.1, the particle size distribution of the flours covered a wide range; however, the most important fractions were the intermediate classes (90-125 µm, 125-250 µm and 250-500 µm) for rice flour and 125-250 µm and 250-500 µm classes for the 3 types of chickpea flour. In general, if the sample particle size is summarized in two classes (i.e. lower or higher than 250 µm), the differences become more evident. In fact, according to the percentage of particles having diameters higher than 250 µm, the flours could be ranked as CF>RCF>DCF>RF.

The particle size distribution may be affected by the structure of the kernel and also by the milling process which was constantly conducted in this study (Schober, 2009). As regards the RCF in comparison with CF, upon the expansion of air in the chickpea kernel during production, volume increases and gas cells are formed. The presence of air gaps makes the kernel very brittle and after milling the obtained flour has relatively small particle size. The particle size distribution may affect many properties such as the hydration rate, the pasting behavior and, certainly, the final product quality.



Particle Size	RF (%)	RCF (%)	CF (%)	DCF (%)
>250 µm	15.54	52.04	75.68	45.17
<250 µm	84.46	47.96	24.32	54.83

Figure 4.1. Particle size distributions of flour samples (RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour).

4.2.1.3. Water Binding Capacity of Flour Blends

The WBC was measured for the rice flour and the blends prepared with the same flour ratio used in the dough formulations. The addition of RCF, CF and DCF to RF caused a decrease in WBC (Figure 4.2). This effect was predominant especially for DCF flour. In literature it is reported that flour having low particle size has high water binding capacity (Kim & Shin, 2014). However, DCF, which is the chickpea flour having the smallest particle size, had the lowest water binding capacity. This finding can be attributed to the losses of husks during dehulling process. Since husks are sources of non-starch polysaccharides and proteins (Kumar et al., 2012), their absence may modify flour water binding capacity and thus affect dough rheology (Witczak et al., 2015).

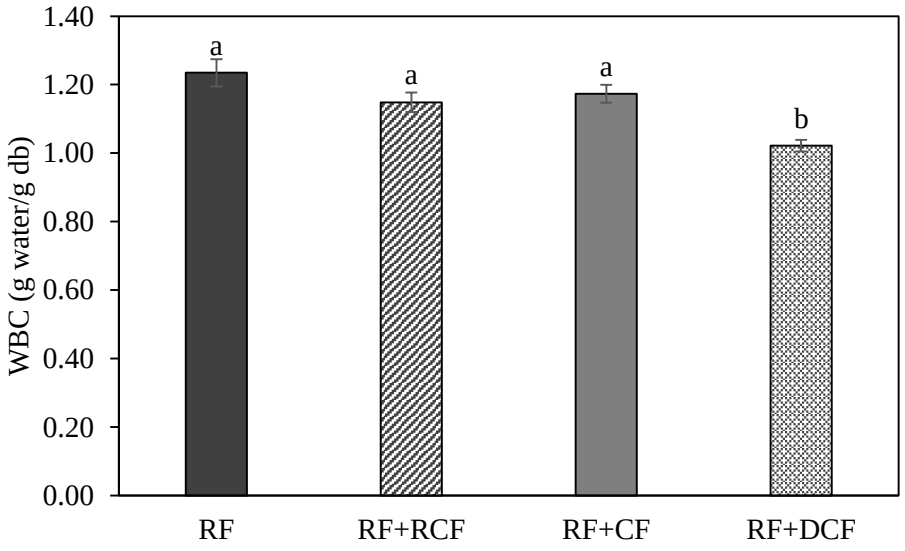


Figure 4.2. Water binding capacities of flour blends used in gluten-free dough and bread formulations (RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour). Means having different letters are significantly different ($p<0.05$).

4.2.1.4. Foaming Properties of Flour Blends

The foaming capacity and stability results were given in Figure 4.3. Increased foam formation was observed in chickpea-containing blends ($p<0.05$). However, RF+RCF showed significantly lower FC compared to CF and DCF containig blends. In comparison to raw chickpea flour, reduced foaming capacity of roasted chickpea flour was also previously reported by Ma et al. (2011). The negative effect of roasting on FC was attributed to the decreased protein solubility caused by the protein denaturation.

As regards the foam stability given in Figure 4.3b, rice flour exhibited no detectable foam during analysis. In agreement with foaming capacity results, with the chickpea flour addition, rice flour gained foam stability. However, the foam stability of RF+RCF was lower than RF+CF and RF+DCF. In contrary to our results, Ma et al. (2011) found no differences between raw and roasted chickpea flour in terms of foam stability. This is probably due to the variations in roasting process; in our study, roasting was applied to chickpea grain rather then the flour and sequential heating and roasting aprocesses were applied instead of one stage roasting (80 °C for 1 min) as carried out in Ma et al. (2011).

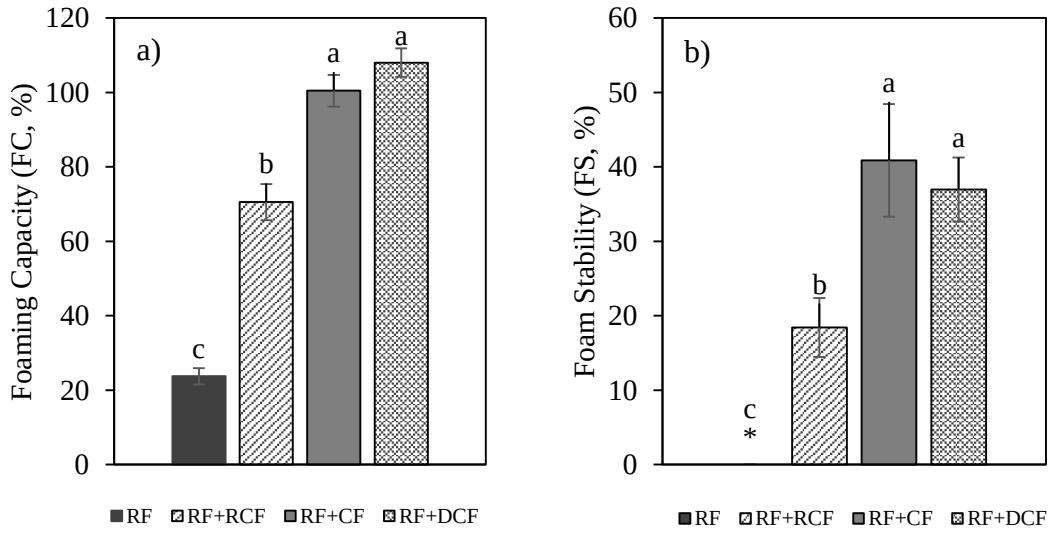


Figure 4.3. a) Foaming capacity and b) foam stability of flour blends used in gluten-free dough and bread formulations (RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour; * no detectable foam). Means having different letters are significantly different ($p<0.05$).

4.2.1.5. Flour Color

The *L**, *a** and *b** values of the flours were given in Table 4.2. All flour samples were significantly different from each other in terms of all the color parameters ($p<0.05$). RF was the lightest flour sample having the lowest *L**. Roasting process resulted in darker flour and also resulted in increased *a** and *b**. DCF showed increased lightness and decreased *a** and *b** compared to CF and RCF.

Table 4.2. Color of the flours

Samples	***L****	***a****	***b****
RF	93.12 ± 0.04^{a}	-0.09 ± 0.01^{c}	5.88 ± 0.06^{d}
RCF	80.99 ± 0.04^{d}	2.79 ± 0.04^{a}	26.43 ± 0.02^{a}
CF	86.58 ± 0.23^{c}	0.92 ± 0.00^{b}	22.46 ± 0.30^{b}
DCF	91.74 ± 0.04^{b}	-0.54 ± 0.01^{d}	16.31 ± 0.02^{c}

RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour.
Values are mean ± SD.
Means having different letters at the same column are significantly different ($p<0.05$).

4.2.1.6. Microstructure of Flour Samples

The scanning electron micrographs of rice and chickpea flours can be seen in Figure 4.4. In rice flour, very small (<5 μm) polyhedral starch granules have been found (Hager et al., 2012). As seen in Figure 4.4, although there were large granules (≤18 μm), rice starch granules were smaller compared to chickpea starch granules and they were aggregated. Regarding chickpea flours (RCF, CF, DCF), starch granules exhibited spherical shape covered with protein fragments in agreement with Aguilera et al. (2009). For all samples, intact starch granules were detected, although partial gelatinization of starch occurred in RCF. Similarly, Köksel, Sivri, Scanlon, & Bushuk (1998) stated that the starch was not completely gelatinized during roasted chickpea processing due to limited rehydration of the kernels. For RCF, residues of the air cells formed upon processing can be seen partly in the images. On the other hand, in

agreement with Ma et al. (2011), roasting and dehulling decreased the starch granule size; CF≤27 µm, RCF≤18 µm, DCF≤23µm.

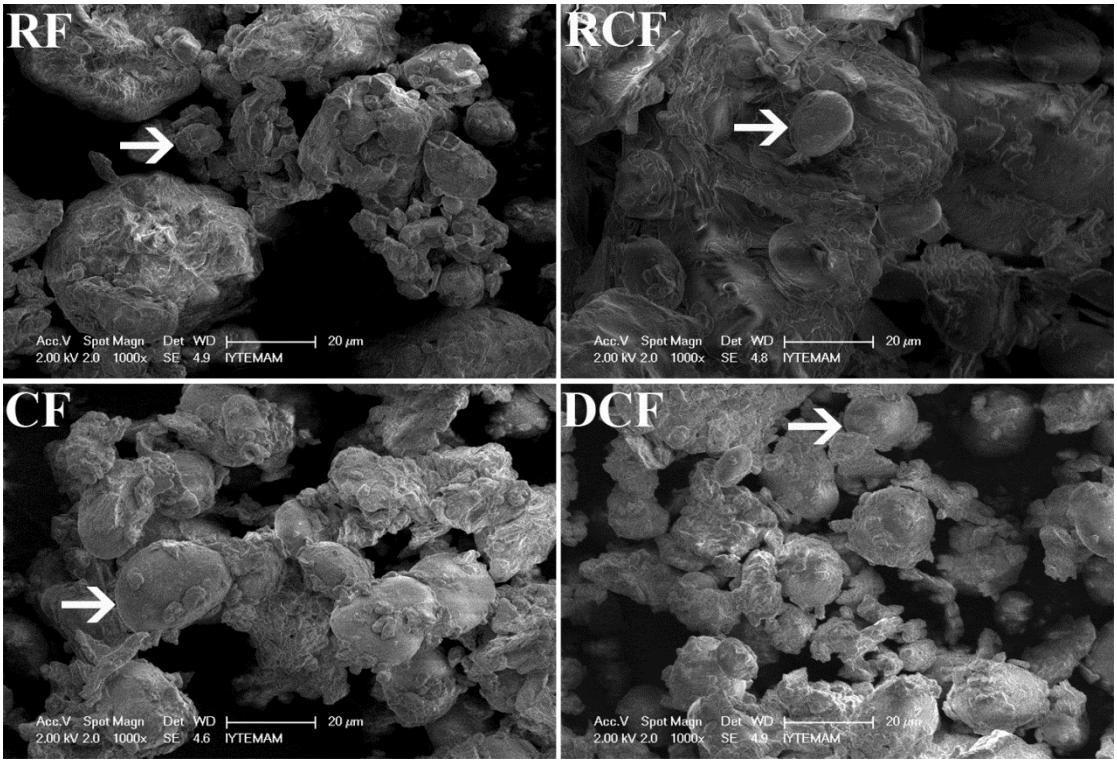


Figure 4.4. SEM images of flour samples (x1000). The starch granules were shown with the arrow (RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour).

4.2.1.7. Pasting Properties of Flour Blends

The pasting curves of flour blends and the corresponding indices are reported in Figure 4.5 and Table 4.3, respectively. During heating, starch granules start to uptake water and swell, then a paste is obtained. During the holding period at 95°C the starch gel viscosity decreases due to the shearing force applied (Fennema, 1996). On cooling, a new viscosity increase takes place due to the reordering of starch molecules. The starch gelatinization behavior has a strong effect on baked foods.

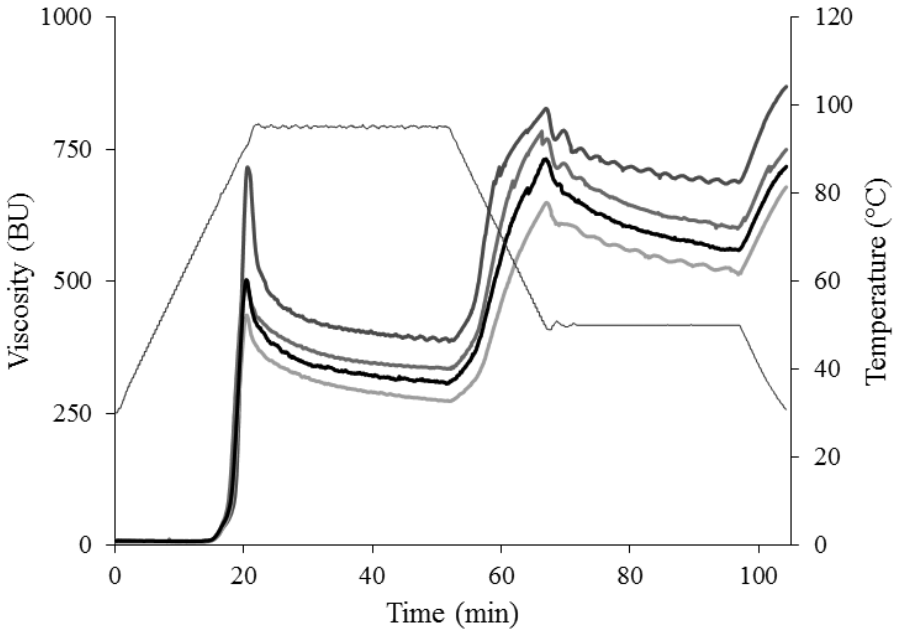


Figure 4.5. Viscoamylograph curve of flour blends (RF (▬), RF+RCF (▬), RF+CF (▬), RF+DCF (▬), Temperature (▬)) (RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour).

It is well known that the pasting properties are mainly affected by the starch quantity and quality and to a minor extent by the flour particle size. All blends, due to the lower starch content in comparison to the rice flour, showed lower peak viscosity, breakdown, final viscosity and setback in comparison to the rice flour. This fact underlines that chickpea flour addition, besides giving rise to a lower strength gel, slowed down paste retrogradation. Among the pasting parameters of the three blends, PV, the maximum viscosity reached during the heating period, was lower for RF+RCF to indicate that roasting had some effects, although minor, on starch characteristics. In fact, roasting was performed with the addition of a small amount of water and thus the heat treatment did not exhibit a relevant effect on pasting behavior. Similarly, FV was lower for the same blend indicating a lower tendency to increase viscosity upon cooling.

It was also reported that both FV and SB usually increase as particle size decreases (Kim & Shin, 2014). Since the particle size of the chickpea flour samples was CF>RCF>DCF and FV was DCF≥>CF>RCF, our results seemed to be partially in agreement with this statement; this can be explained by the complexity of the phenomena which occurs during the gelatinization and retrogradation of starch.

Table 4.3. Pasting properties of flour blends used in gluten-free dough and bread formulations

	GT (°C)	PV (BU)	BD (BU)	SB (BU)	FV (BU)
RF	77.15 ± 0.07^{bc}	716.50 ± 7.78^{a}	326.50 ± 12.02^{a}	478.50 ± 9.19^{a}	868.50 ± 13.44^{a}
RF+RCF	77.60 ± 0.00^{a}	435.50 ± 12.02^{c}	161.50 ± 9.19^{c}	404.00 ± 7.07^{b}	678.00 ± 9.90^{c}
RF+CF	76.80 ± 0.14^{ab}	503.50 ± 2.12^{b}	196.50 ± 6.36^{b}	410.00 ± 4.24^{b}	717.00 ± 0.00^{b}
RF+DCF	77.40 ± 0.14^{c}	498.00 ± 5.66^{b}	164.00 ± 4.24^{bc}	415.00 ± 5.66^{b}	749.00 ± 4.24^{b}

RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour; WBC, water binding capacity; GT, gelatinization temperature; PV, pasting viscosity; BD, breakdown; SB, setback; FV, final viscosity.
Values are mean ± SD.
Means having different letters at the same column are significantly different ($p<0.05$).

4.2.2. Dough Properties

4.2.2.1. Dough Consistency

In Chapter 3, the optimization of a rice+roasted chickpea flour-based GFB formulation was carried out. The current chapter aimed to investigate the dough properties of the optimized formulation (RF+RCF), versus the one including CF. However, during the production of roasted chickpea, hulls were removed almost completely. For this reason, the RF+DCF formulation was also included into the experimental plan. The 100% rice dough was produced and characterized as a reference sample. In order to exclude the effects of different dough consistencies, all the dough samples were fixed to 125±5 BU which was the consistency of the optimized RF+RCF dough. The water amounts added to RF, RF+RCF, RF+CF, RF+DCF were 101.14%, 104.70%, 99.47% and 90.07% (fb), respectively. The dramatically lower water addition level of RF+DCF is in aggrement with the significantly lower water binding capacity of the same blend (see Section 4.2.1.3). The dough consistencies are mainly affected by the different moisture content of the flours and by the ability of the flours to interact with water.

4.2.2.2. Dough Development during Proofing

According to the results obtained from the Rheofermentometer test, maximum and final dough heights were the highest for RF dough (Table 4.4). The CO_2 retention capacity of RF+RCF sample (99.0%) was higher than RF+CF (97.9%) and RF+DCF (98.1%), and slightly lower than RF (99.4%) (Table 4.4). This suggests a slight weakening of the dough containing chickpea flours which were in any case able to retain the majority of the CO_2 produced by the yeast. The advantages of using the chickpea flours can be noticed in terms of leavening time before the dough porosity appearance (Tx) and total CO_2 produced. In fact, in the three formulations containing chickpea flours, the production of CO_2 was 5-12% higher than in RF. This can be due to the faster activities of the yeast in these samples. Accordingly, RF+CF and RF+DCF were also characterized by an earlier appearance of Tx.

Table 4.4. Dough leavening properties

Dough	Hm (mm)	Hf (mm)	Tx (min)	$CO_{2\text{-TOT}}$ (mL)	$CO_{2\text{-REL}}$ (mL)	$CO_{2\text{-RET}}$ (mL)	Rc (%)
RF	49.2	49.2	-	783	5	779	99.4
RF+RCF	41.7	35.8	-	821	8	812	99.0
RF+CF	41.3	36.9	40.5	875	18	857	97.9
RF+DCF	43.2	41.0	45.0	879	17	862	98.1

RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour; Hm, dough maximum height; Hf, dough final height; Tx, time of dough porosity appearance; $CO_{2\text{-TOT}}$, total gas production; $CO_{2\text{-REL}}$, CO_2 released by the dough; $CO_{2\text{-RET}}$, CO_2 retained by the dough; Rc, gas retention coefficient.

The dough leavening behavior was also followed by image acquisition of the doughs placed in Petri dishes during 60 min period. This technique was proposed as an alternative tool to rheofermentometric test by allowing to interpret dough development by means of dough area increase (Cappa et al., 2013a). The obtained Petri images are visible in Figure 4.6a. There were no significant differences ($p > 0.05$) in the dough area increases among the samples (Figure 4.6b). It could be attributed to the same consistency values of doughs. Furthermore, for each formulation, the area increase

results were highly correlated with the dough height (mm) values obtained from the rheofermentometer test (R^2=0.989) (Figure A.1).

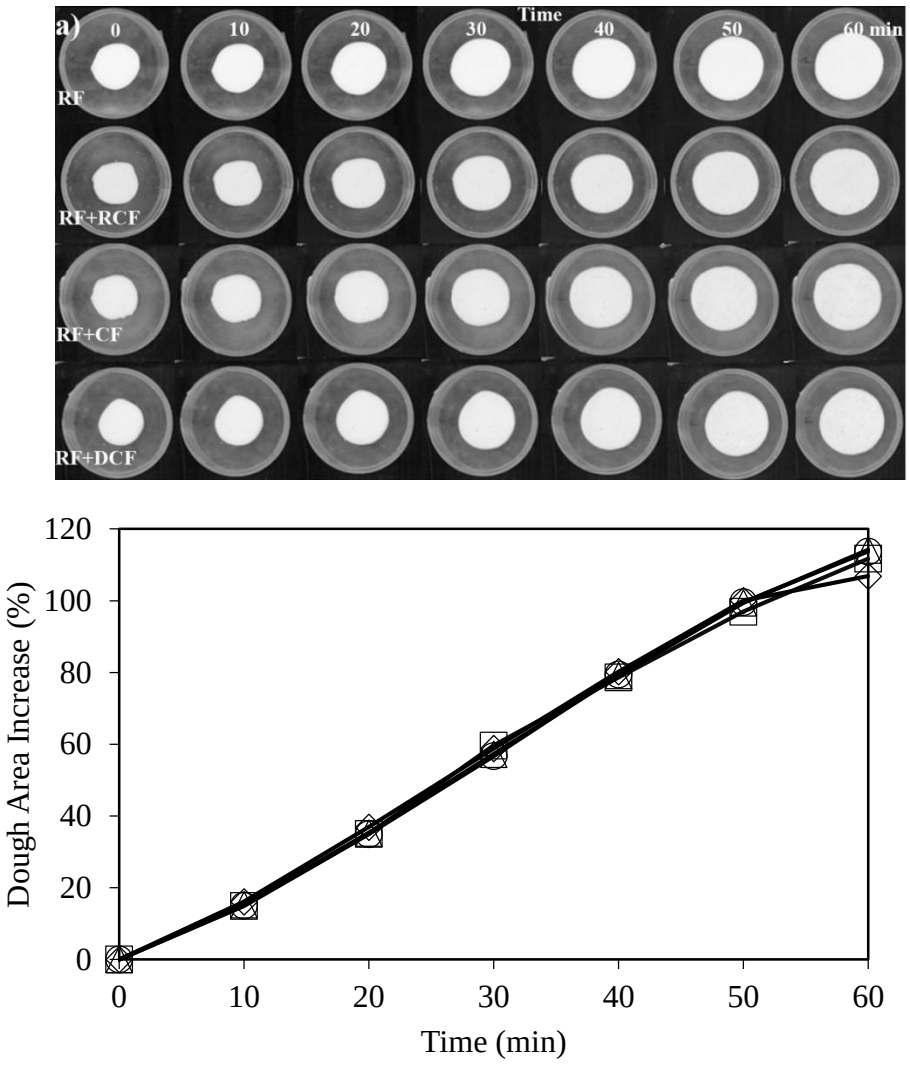


Figure 4.6. a) Images of petri dishes with gluten-free dough samples during proofing b) Dough area increase (%) during proofing (60 min) (RF (■), RF+RCF (▲), RF+CF (♦) and RF+DCF (●) (RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour).

4.2.2.3. Dough Rheology

Rheological measurements performed at small deformation are frequently used to characterize the dough behavior. These measurements preserve the dough structure and allow to obtain information on the viscoelastic characteristic of the samples.

The frequency sweep curves are reported in Figure 4.7 and Figure 4.8. For all dough formulations, G' values were higher than G" to indicate a solid-like behavior (Figure 4.7). This behavior is agreed with literature regarding the rheology of GF batters (Galle et al., 2012; Hüttner et al., 2010; Sciarini et al., 2012) and gels (Cappa et al., 2016; 2013b). The addition of chickpea flours into rice dough formulation caused the increase of both G' and G". In particular, DCF and CF showed the highest and similar values at all the frequencies investigated. RCF had an intermediate behavior. However, tan δ was not affected by the chickpea addition and remained constant for all the formulations (0.41±0.02, 0.41±0.02, 0.42±0.02, 0.37±0.01 for RF, RCF+RF, CF+RCF and RF+DCF at 1 Hz, respectively) and was lower than 1 at all frequencies (Figure 4.8). Similar increase in G' and G" was previously observed after addition of chickpea (Aguilar et al., 2015) and soy protein in the starch-based GF dough (Ziobro et al., 2013).

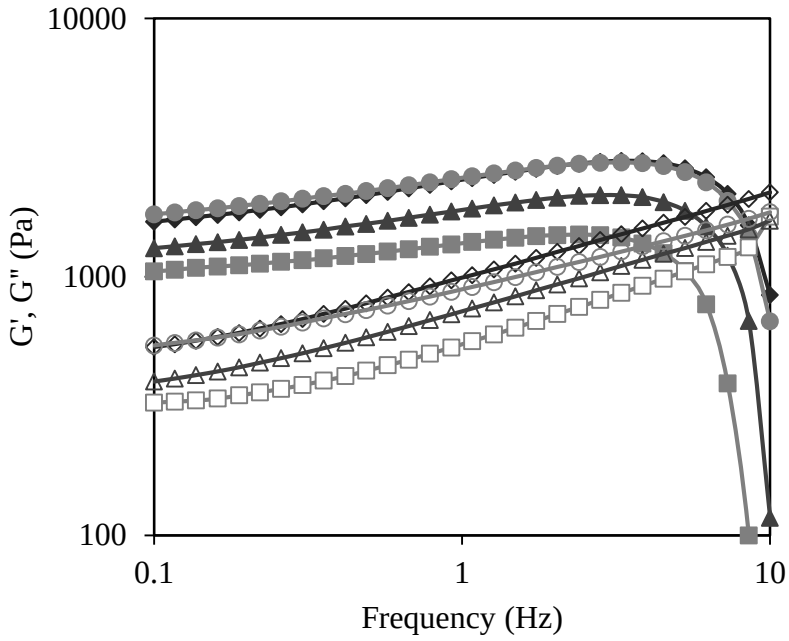


Figure 4.7. Storage (G', dark) and loss modulus (G", white) of gluten free dough samples (RF (■), RF+RCF (▲), RF+CF (♦) and RF+DCF (●)). (RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour).

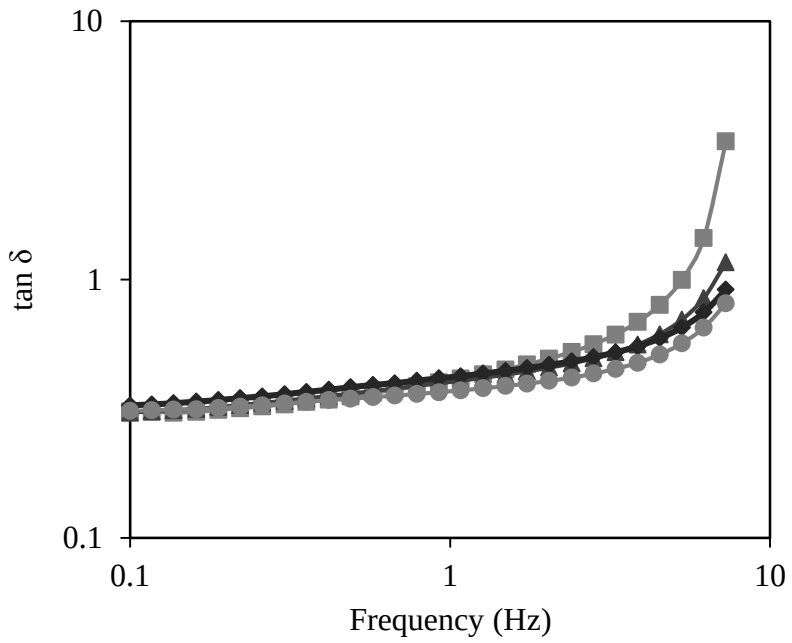


Figure 4.8. Damping factor (tan δ) of gluten free dough samples (RF (■), RF+RCF (▲), RF+CF (♦) and RF+DCF (●)). (RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour).

4.3. Conclusions

Using pulse flours in food formulations are of great importance for nutrient fortification. In this part of the study, effects of roasted, dehulled and raw chickpea flour addition up to 25% in rice dough formulations were evaluated. Beyond the increase of protein and fat content, the addition of chickpea flour created positive effects on the technological attitude of the dough with decreased starch content. The dough development of the formulations containing chickpea flours were slightly lower than the reference samples, however high CO_2 retention were evidenced. Lower leavening times were suggested to obtain the maximum dough development. Therefore, the predetermined proofing time of 30 min was reasonable in that sense. Also the viscoelastic properties of the dough were positively affected by the chickpea flour addition, in fact, higher storage modulus were obtained. The viscoamylographic test underlined also a slower retrogradation tendency (lower SB) of the slurry containing chickpea flours, this could be promising for baking food application. The results of this part open new possibilities to the usage of chickpea flours.

CHAPTER 5

BREAD CHARACTERIZATION & SHELF-LIFE

In this chapter, quality of fresh and stored rice-based breads enriched with roasted, raw or dehulled chickpea flour were evaluated based on physical, chemical, nutritional and sensorial quality. Bread formulation having only rice flour was also evaluated as the reference sample.

5.1. Materials &Methods

5.1.1. Materials

The flours used in this study were: rice flour, roasted, dehulled and dried raw chickpea flours. Rice flour (RF) (Pakmaya, Turkey) and dehulled chickpea flour (DCF) (Ingredion, Germany) were obtained as in the flour form. Roasted chickpea and dried chickpea were milled by using a laboratory mill to obtain roasted chickpea flour (RCF) and chickpea flour (CF) having particle size ≤ 1 mm. The other ingredients were HPMC (Benecel F4M, Ashland, USA), instant yeast (Pakmaya, Turkey), sugar, salt and sunflower oil.

The chemicals used are acetone (24201, Riedel-de Haën), amyloglucosidase (A7095, Sigma), Folin-Ciocalteu's reagent (109001, Merck), gallic acid (G7384, Sigma), guar gum (G-4129, Sigma), hexane (34859, Sigma-Aldrich), invertase (I4504, Sigma), N_{α}-*p*-Tosyl-L-arginine methyl ester hydrochloride (TAME) (T4626, Sigma-Aldrich), pancreatin from porcine pancreas (P7545, Sigma), pepsin (P7000, Sigma) and sodium carbonate (13418, Sigma-Aldrich).

5.1.2. Methods

5.1.2.1. Bread Preparation

Bread samples were prepared according to the optimized recipe consisted of 100% RF or flour blends having 1:3 ratio for RCF:RF, CF:RF or DCF:RF, HPMC, sugar (2%), salt (1.5%), instant yeasts (2.5%), sunflower oil (5.27%) and water (amounts required to reach 125±5 BU), based on flour weight. For the dough preparation, dry ingredients were blended in a stand mixer (Hobart Corporation, Troy, Ohio, USA) at speed 2 for 1 min. Then water and oil were added and mixed at speed 2 for 2 min and at speed 4 for 3 min. The obtained dough (150 g) was placed in baking pans and proofed at 32°C and 85% relative humidity for 30 min in a proofing cabinet (HC0020, Haereus Vötsch, Frommern, Germany). The proofed loaves were placed in a preheated deck oven (Lotus P4, Treviso, Italy) and baked for 45 min at 225 °C (top) and 205 °C (bottom). The loaves were removed from the pans and cooled at ambient temperature for 2 h until analysis.

5.1.2.2. Bake Loss, Specific Volume & Height

Just after baking, loaves were immediately weighed. Bake loss (%) was calculated as [Weight (Batter-Bread)/Weight (Batter)]x100.

The volume of bread was measured by seed displacement method. Measurements were repeated twice for each bread loaf. Volume to weight ratio gives the specific volume (cm^3/g) of bread samples. The heights (mm) of the loaves were recorded using a callipers.

5.1.2.3. Moisture Content and Water Activity of Bread

The moisture content of the slice and crumb were determined by oven drying. Briefly, a half slice from the center of the bread and a crumb piece cut from the center of a slice (3-4 g) were weighed and dried at 105 °C 16 h. Moisture content was calculated as percent of decrease in weight.

Water activity of bread crumb was evaluated using water activity measuring device (CX-2, AquaLab, USA).

5.1.2.4. Color of Crust and Crumb

The color of the bread crust and crumb were measured by a colorimeter (Chroma Meter II Reflectance, Minolta, Japan) according to Lab color space as *L** (Lightness), *a** (+a, red; -a, green) and *b** (+b, yellow; -b, blue) parameters. At least six measurements for crust and crumb were taken and averaged. At least ten loaves were evaluated and the measurements were averaged.

5.1.2.5. Texture Analysis

Texture characteristics of bread crumbs were evaluated by texture profile analysis (TPA) using TA-HD*plus* Texture Analyzer (Stable Micro Systems, Surrey, UK) equipped with a 50 kg load cell and a 25 mm cylinder probe. The bread slices having thickness of 25 mm were compressed in the center to 40 % of its original height with a test speed of 1.00 mm/sec. Hardness, cohesiveness, springiness and chewiness were calculated using a software (Exponent software 6.1.9, Stable Micro Systems, UK). At least six slices for each formulation were analyzed and the average values were given. The rate of staling ([Hardness $_{95h}$-Hardness $_{2h}$]/Hardness $_{2h}$) was also calculated for each sample.

5.1.2.6. Image Analysis of Bread Crumb

The crumb samples were evaluated for porosity according to Cappa et al. (2013a). Samples were scanned at 600 dpi by using flatbed scanner (HP SCANJET 8300, Hewlett-Packard, CA, USA) and images were converted to grey scale. The images were processed using Image Pro-Plus (4.5.1.29, Media Cybernetics Inc, MD, USA) software. Based on their sizes ($0.1 \leq x < 0.2$ mm^2, $0.2 \leq x < 0.5$ mm^2, $0.5 \leq x < 1$ mm^2, $1 \leq x < 5$ mm^2, $5 \leq x < 10$ mm^2 and $x \geq 10$ mm^2), the holes classified into six

groups. For each group, number and area of the holes were given as the percentage of the total number and image area.

5.1.2.7. Environmental Scanning Electron Microscopy (ESEM) Analysis of Bread Crumb

The microstructure of bread samples were analyzed with environmental scanning electron microscopy (Quanta 250 FEG, FEI). The bread samples were carefully cut and attached to double-sided carbon tapes. The images were captured without any pretreatment.

5.1.2.8. Nutritional Evaluation of Bread

5.1.2.8.1. Protein Content

The total nitrogen content of samples was determined according to the Official Standard Method AOAC 920.87 (1999) by using and a block digestion system (Kjeldatherm, C. Gerhardt GmbH & Co. KG, Germany) and a distillation system (Vapodest 50s, C. Gerhardt GmbH & Co. KG, Germany). The protein content was then calculated using 5.95 and 6.25 as conversion factors for rice and chickpea breads, respectively.

5.1.2.8.2. Fat Content

For the fat content determination, automatic extraction system (Soxtherm, Gerhardt, Germany) was used with hexane as the extraction solvent. Sample (4 g) was weighed in the special white cups and placed in extraction beakers of the device and hexane was added until it covers the sample. Extraction was performed at 150 °C and the extracted fat weight was recorded and percentage of fat in dry matter was calculated. The analysis was performed in at least duplicate.

5.1.2.8.3. Ash Content

The ash content was analyzed according to AACC (1999). Briefly, 3 g of bread was weighed into a crucible and incinerated in a muffle furnace (Protherm, Turkey) at 550 °C until the sample residue becomes light gray-white. Ash content was calculated as shown in Equation 5.1.

$$\text{Ash (\%)} = [\text{Residue weight (g)}/ \text{Bread weight (g)}] \times 100 \quad (5.1)$$

5.1.2.8.4. Total Phenolic Content

5.1.2.8.4.1. Extraction of Phenolic Compounds

The phenolic compounds were extracted from flour and bread according to Alves et al. (2016) with some modifications. Briefly, 1 g of sample (flour or bread) was mixed with 10 mL of acetone (70%, v/v) and placed in a shaker (Reax 2, Heidolph, Germany). After 1 h shaking (400 rpm) in the dark, tubes were centrifuged (Universal 320R, Hettich, Germany) at 4000 rpm, 20 °C for 5 min and supernatant was transferred to another tube. This procedure was repeated 3 times and all the supernatants were combined and stored at -20 °C until the analysis. Extractions were performed in duplicate.

5.1.2.8.4.2. Determination of Total Phenolic Content

The total phenolic content of the acetone/water extracts were determined according to Singleton et al. (1999) and Sakač et al. (2011) with some modifications. The sample extract (0.5 mL) was mixed with distilled water (7.5 mL) and Folin-Ciocalteu's reagent (0.5 mL), respectively. After 5 minutes, sodium carbonate (20%, w/v) solution (1.5 mL) was added. The tubes were vortexed and incubated for 120 minutes on a shaker (KS 130 Basic, IKA, Germany) in a dark place at room temperature. The absorbances were recorded at 760 nm with a spectrophotometer (UV-1601, Shimadzu, Japan) at room temperature. Standard curve (Figure B.1) was prepared

by using gallic acid and the results were expressed as gallic acid equivalent (GAE) (mg GAE/ 100 mg sample, db). Analysis was performed in duplicate with two repeated measurements.

5.1.2.8.5. *In vitro* Starch Digestibility

The nutritionally important starch fractions in breads were determined according to Englyst et al. (2000). Briefly, gluten-free breads were minced by using manual meat mincer. These "as eaten" samples (containing 500-600 mg starch) were then treated with pepsin-guar gum mixture (10 mL) for 30 min at 37 °C. Then, 0.5 M sodium acetate buffer (5 mL) and 5 glass balls were added. After the addition of 5 mL enzyme mixture containing pancreatin, amyloglucosidase and invertase, the tubes were incubated at 37 °C in a water bath equipped with a shaker. In order to calculate rapidly digestible starch (RDS) and slowly digestible starch (SDS), samples (0.2 mL) were taken after 20 and 120 min, respectively, placed in ethanol (4 mL), centrifuged and the released glucose was measured. Available starch content was calculated as the sum of SDS and RDS. For total glucose (TG) content, tubes taken after 120 min were kept in boiling water bath for 30 min, further treated with amyloglucosidase (40 μL) at 70 °C for 30 min and 100 °C for 10 min, sequentially. Finally, ethanol (12 mL) was added to the tubes, tubes were centrifuged and glucose contents were measured. Samples treated only with water, without any enzymatic process, were used to determine free sugars.

The glucose contents were measured by High Performance Liquid Chromatography (HPLC) (Series 200, Perkin Elmer, USA) equipped with 4 x 250 mm CarboPac™ PA1 column (Dionex, USA) and PAD detector (ED50, Dionex, USA). Sample (20 μL) was injected to column at room temperature using NaOH (160 mM) as the mobile phase at a flow rate of 1 mL/min. Arabinose was used as internal standard.

5.1.2.8.6. *In Vitro* Protein Digestibility

5.1.2.8.6.1. Measurement of Enzyme Activity of Pancreatin

The enzyme used in *in vitro* protein digestibility analysis was pancreatin from porcine pancreas. The trypsin activity of the enzyme was determined according to Minekus et al. (2014). As the substrate, N_{α}-*p*-Tosyl-L-arginine methyl ester hydrochloride (TAME) was used. At least four concentrations of pancreatin were prepared and increase in absorbance was recorded at 247 nm and 25°C (2450, Shimadzu, Japan) for 10 min. The slopes of the initial linear portion of the curves were calculated. The result was given as TAME units/mg.

5.1.2.8.6.2. Determination of *In Vitro* Protein Digestibility

The *in vitro* protein digestibility (IVPD) of bread samples was determined according to Hsu et al. (1977) with some modifications. Instead of trypsin, pancreatin was used as digestive enzyme. In order to obtain same particle size, dried bread samples were sieved (≤ 1 mm). Bread was mixed with ultrapure water to obtain 6.25 mg crude protein/mL and rehydrated at 4 °C for 1h. The pH of the sample mix was adjusted to 8.0 and the temperature was set as 37 ± 0.3 °C on a hot plate equipped with a thermocouple. The pancreatin having 7.53 TAME units/mg was prepared and pH was adjusted to 8.0. The enzyme solution was added into the sample solution and pH change was recorded for 10 min. The pH reached at 10 min (x) was used to calculate the *in vitro* protein digestibility (Y) by using equation below;

$$Y = 210.46 - 18.10x \quad (5.1)$$

5.1.2.9. Sensory Evaluation of Bread Samples

Among the staff and students of the Department of Food Engineering at Izmir Institute of Technology, 21 panelists (17 women, 4 men) aged between 24 and 53 attended the sensory panel of gluten-free bread. All participants were familiar with sensory evaluation technique. However, the participants were not celiac patients and only few of them had been tasted gluten-free bread before. Therefore, a training session aiming to introduce gluten-free bread was carried out before sensory analysis.

5.1.2.9.1. Panel Training

The training session consisted of two parts. First part includes a presentation in which general properties of gluten-free bread and the main differences between gluten-free bread and wheat bread were discussed; pictures of several gluten-free breads from literature were shown. In the second part, two types of commercial gluten-free bread samples were provided to the participants in order to get familiar with the sensorial quality of gluten-free bread.

5.1.2.9.2. Sensory Analysis

The sensory evaluation was carried out one day after baking in sensory evaluation laboratory having individual panel booths equipped with white light. Whole loaves of the bread samples and a quarter of a slice (crumb with top and bottom crusts) were given. The slices were served in white plastic plates and each sample was coded with different three-digit numbers. A glass of water was also given to panelists to clean their palate. The panelists were asked to evaluate appearance, crust and crumb color, odor, texture, flavor and overall acceptability according to their personal-liking via using 9-point hedonic scale (9, like extremely; 1, dislike extremely). The sensory evaluation form and the hedonic scale were given in Table C.1 and Figure C.1. Samples having scores higher than 5 were considered as acceptable.

5.1.2.10. Statistical Analysis

Statistical evaluation of the data was performed by using MINITAB 16 (Minitab Inc., U.S.). The results were given as "mean ± SD". The significance of the data was tested by analysis of variance (ANOVA) at $p<0.05$ and, in the significant models, means were compared by Tukey's test at 95% confidence interval.

5.2. Results & Discussions

5.2.1. Evaluation of Fresh Bread

5.2.1.1. Bake Loss, Height and Specific Volume

The bake loss, height and specific volume results are shown in Table 5.1. As regards the bake loss parameter, RCF, CF and DCF addition was not able to significantly change this parameter ($p>0.05$).

According to specific volume results, RF+CF exhibited significantly low specific volume compared to other samples ($p<0.05$). Although RF+RCF and RF+DCF caused increasing specific volumes, this change was not statistically significant compared to RF. Previously in optimization part (Chapter 3), the positive effect of RCF addition was evidenced. The negative effect of CF could be attributed to its husk content. Since DCF is the dehulled counterpart of CF, the slightly higher specific volume of RF+DCF compared to RF+CF strengthen this hypothesis. During leavening, husks in CF may have disrupted the gas cells, forced the gas inside the cells to move out of the dough and caused a decrease in final volume (Onyango et al., 2011).

Table 5.1. Quality parameters of gluten-free bread after 2 hours from baking

	RF	RF+RCF	RF+CF	RF+DCF	p-value	R^2
Height Max (mm)	4.69 ± 0.23^{ab}	5.00 ± 0.07^{a}	4.34 ± 0.03^{b}	4.66 ± 0.04^{ab}	0.026	87.92
Specific Volume (mL/g)	2.63 ± 0.11^{ab}	2.89 ± 0.03^{a}	2.51 ± 0.11^{b}	2.75 ± 0.04^{ab}	0.036	85.75
Bake Loss (%)	19.62 ± 0.47^{a}	20.43 ± 0.74^{a}	20.44 ± 0.18^{a}	20.77 ± 0.57^{a}	0.365	51.24
Crumb color						
*L**	75.07 ± 0.05^{a}	70.56 ± 1.16^{b}	75.47 ± 0.39^{a}	75.09 ± 0.02^{a}	0.004	95.62
*a**	-2.21 ± 0.19^{a}	-2.02 ± 0.04^{a}	-3.13 ± 0.21^{b}	-2.71 ± 0.22^{ab}	0.011	92.07
*b**	7.75 ± 0.07^{d}	20.59 ± 1.00^{a}	16.47 ± 0.22^{b}	13.42 ± 0.09^{c}	0.000	99.40
Crust color						
*L**	72.48 ± 1.08^{a}	56.02 ± 0.53^{b}	52.90 ± 0.39^{b}	48.96 ± 1.14^{c}	0.000	99.55
*a**	-0.50 ± 0.17^{d}	7.14 ± 0.20^{c}	8.58 ± 0.08^{b}	9.80 ± 0.03^{a}	0.000	99.94
*b**	24.55 ± 0.35^{b}	33.49 ± 0.17^{a}	32.86 ± 0.53^{a}	31.30 ± 1.08^{a}	0.000	98.44

RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour.
Values are mean ± SD.
Mean values having different letters in the same row are significantly different ($p<0.05$).

In respect to our findings, among chickpea flours, the utilization of RCF is advantageous over CF addition due to the significantly higher (15%) specific volume ($p<0.05$). Until now, there is no available information regarding the behavior of roasted chickpea flour in rice-based GFB. Only in a recent study, the specific volume of toasted chickpea bread is found to be 4% higher than raw chickpea bread (Ouazib et al., 2016). The results of a limited number of studies related with the chickpea utilization in GFB showed variations due to the level of chickpea flour in the formulation and the type of coexisting flours in the blend. Burešová et al. (2014) found no statistical difference between the specific volumes of rice (100%, fb) and chickpea (100%, fb) bread. In a starch-based GFB development study (Aguilar et al., 2015), only a slight increase in specific volume was observed with 7.8% chickpea flour addition. Miñarro et al. (2012) showed that chickpea flour added corn-starch based GFB showed enhanced specific volume values by virtue of the higher foam stabilizing properties of chickpea protein.

5.2.1.2. Crust and Crumb Color

The results of color measurements are presented in Table 5.1. Regarding the crumb lightness, the only formulation that significantly differed from RF was RF+RCF ($p<0.05$). The addition of CF and DCF did not change this parameter. However, in case

of crumb redness, only CF addition showed a significant effect ($p<0.05$). All the formulations had significantly different yellowness value ($p<0.05$); RF+RCF had the highest and DCF had the lowest yellowness among chickpea-containing breads. The crumb color is affected directly by the color of flours used in formulation. Since RCF had the darkest color among flours used in this study, the resulting crumb of RF+RCF bread was expected to be the darkest one. Similarly by Ouazib et al. (2016), a dark crumb color was reported for toasted chickpea bread.

The crust lightness also differed significantly for all samples ($p<0.05$). Such in the crumb color, flour color is the most important factor affecting the crust color. Due to the white color of rice flour, RF had the lightest crust. All chickpea-containing breads showed increased crust darkness. Although RF+DCF had a light crumb, this sample showed the darkest crust color among other chickpea flour-containing samples. The crust redness was significantly different for all samples ($p<0.05$); lowest for RF and highest for RF+DCF. The addition of chickpea flours increases the yellowness value regardless of the type ($p<0.05$). Upon baking, the crust becomes darker due to the Maillard reaction that takes place on the crust within amino acids and reducing sugars. The incorporation of chickpea flour would contribute to this reaction by leading an increase in protein and sugar content of the bread.

5.2.1.3. Crumb Porosity

The images of gluten-free bread slices are given in Figure 5.1. Altough all samples had high porosity values (above 40%), RF+RCF had the highest porosity among them (Table 5.2). Since this bread had the softest crumb according to texture profile analysis, the highly porous crumb structure seemed to be responsible for the soft crumb. Also for the other samples, crumb porosity and hardness showed a similar relationship. According to holes number, for RF, most of the pores belonged to 1-5 mm^2 class, while chickpea flour addition led to the formation of a higher amount of small size holes. In fact, in these samples about 45% of holes had a dimension lower than 0.5 mm^2. This result has to be correlated with the high foaming capacity of chickpea flours as previously reported (See Section 4.2.1.4).

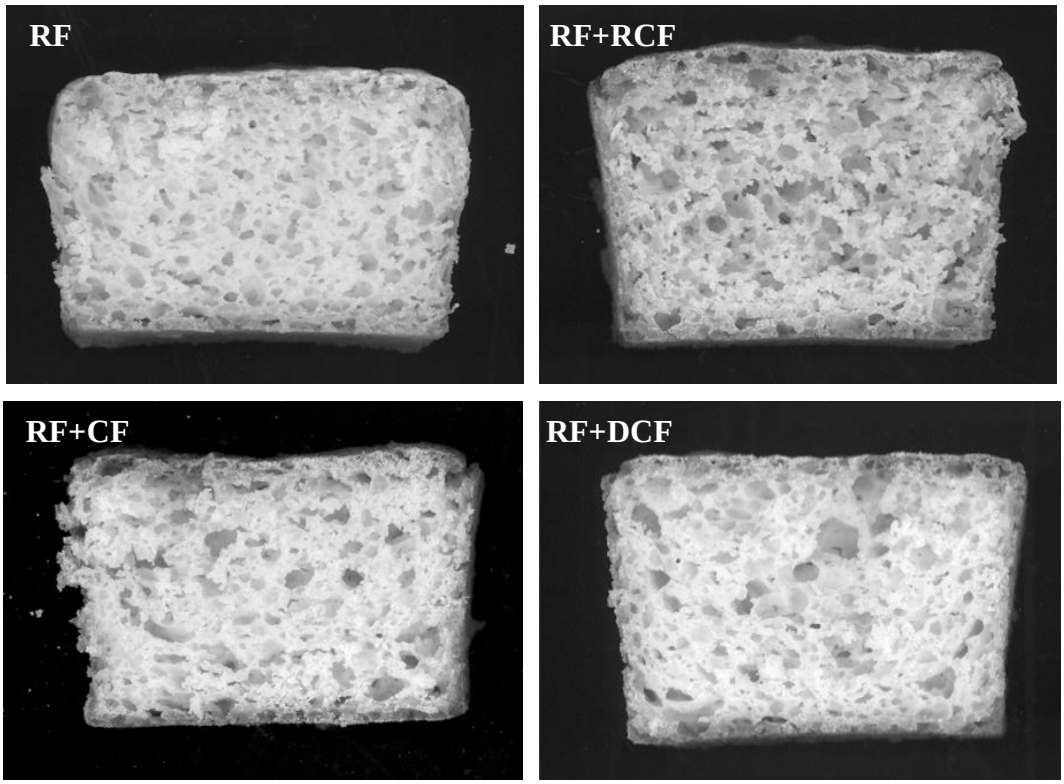


Figure 5.1. Photographs of gluten-free bread slices **(**RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour).

Table 5.2. Crumb porosity and cell distribution of gluten-free bread crumbs

	RF	RF+RCF	RF+CF	RF+DCF	p-value	R^2
	Crumb Porosity (%)					
	45.36 ± 2.87^{ab}	51.41 ± 2.30^{a}	41.49 ± 0.11^{b}	41.84 ± 1.25^{b}	0.02	89.38
mm^2	Distribution of cells (%)					
0.1-0.2	18.03 ± 0.44^{b}	23.16 ± 0.07^{a}	23.02 ± 0.21^{a}	21.99 ± 0.41^{a}	0.000	98.81
0.2-0.5	18.84 ± 3.90^{a}	22.23 ± 1.42^{a}	21.69 ± 0.91^{a}	23.04 ± 1.04^{a}	0.367	51.09
0.5-1	15.37 ± 1.52^{a}	16.70 ± 0.61^{a}	17.17 ± 1.17^{a}	17.21 ± 0.42^{a}	0.365	51.24
1-5	33.80 ± 2.63^{a}	25.81 ± 0.11^{b}	27.92 ± 0.67^{b}	29.07 ± 0.15^{ab}	0.017	90.25
5-10	8.21 ± 1.34^{a}	5.33 ± 0.20^{b}	4.76 ± 0.05^{b}	5.29 ± 0.01^{b}	0.022	88.87
>10	5.44 ± 1.46^{a}	6.77 ± 1.66^{a}	5.43 ± 1.15^{a}	3.40 ± 0.34^{a}	0.204	64.74

RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour.
Values are mean ± SD.
Mean values having different letters in the same row are significantly different ($p<0.05$).

5.2.1.4. Microstructure of Bread Crumb

The ESEM micrographs of breads are seen in Figure 5.2. All crumbs showed continuous starch and protein structures. Both intact and gelatinized starch granules could be seen in all micrographs. Intact starch granules were evidenced in SEM images, even if the formulations contained very high amount of water. This finding was also previously highlighted in buckwheat, quinoa, sorghum, teff and wheat breads by Wolter et al. (2014c). In our study, RF+CF and RF+DCF exhibited higher amounts of intact starch granules with larger sizes. The size of the granules in the crumb was certainly affected by the size of the starch granules of the flours (Figure 4.4). As previously mentioned (see Section 4.2.1.6), RF had relatively small starch granules compared to all types of chickpea flours. Besides, RCF exhibited smaller granules than raw chickpea flour due to the effect of roasting. Therefore relatively small intact starch granules in the crumbs of these two breads were reasonable. Bread containing RCF shows a more "fuse" and continuous structure where starch granules are less defined in the final bread. RCF underwent a double heat treatment; at first, during leblebi production with limited amount of water, and second, during baking in presence of a higher water level.

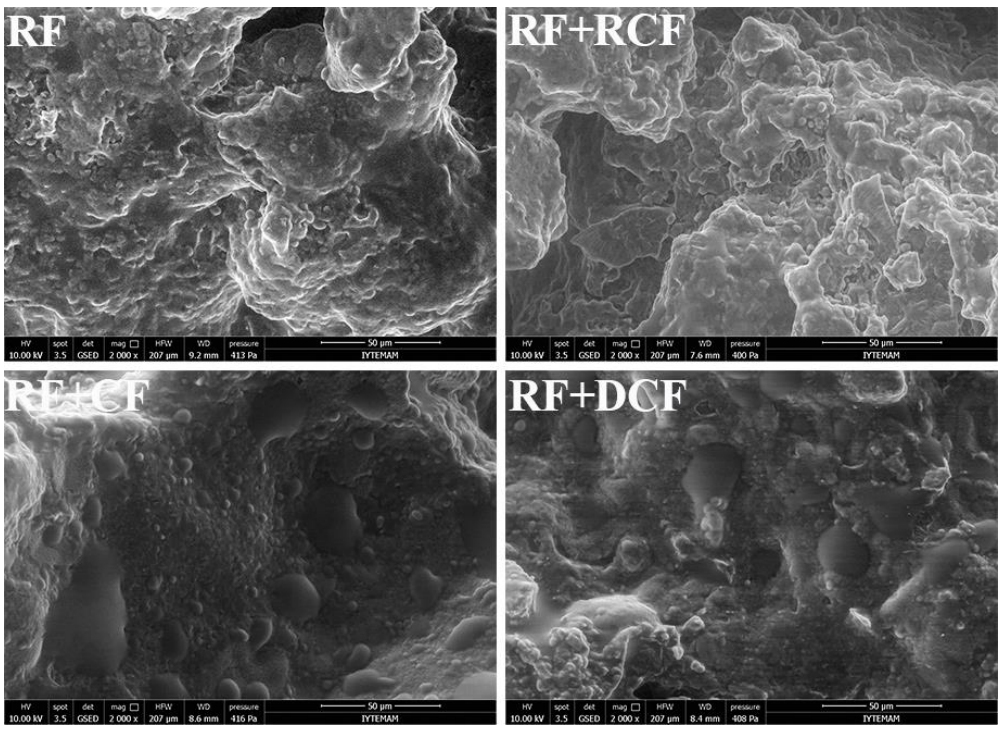


Figure 5.2. ESEM micrographs of gluten-free breads (x2000) (RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour).

5.2.2. Bread Quality during Storage

In this part, the results of moisture content, water activity and textural parameters for both fresh (after 2 h from baking) and stored (after 23, 47, 71 and 95 h from baking) breads are discussed.

5.2.2.1. Moisture Content & Water Activity of Bread

Since the gluten-free dough formulations in this study contained high amounts of water, the moisture content of bread samples were also high; 40.15-43.01% and 49.92-52.68% for slice and crumb, respectively (Table 5.3). Although crumb moisture loss during 95 h storage was significant ($p<0.05$) for all crumbs, moisture decrease was higher for RF+CF (5.37%) and RF+DCF (5.53%). RF+RCF exhibited the lowest moisture loss value (3.26%) at the end of 95 hours. Similarly, only 2-5% crumb moisture loss was reported by Cappa et al. (2013a) for GFB fortified with *Psyllium* and sugar beet fibre.

When considering the slice moisture loss, significant decreases were observed for only RF+CF (3.22%) and RF+DCF (3.94%) ($p<0.05$). At all storage times, RF+DCF showed significantly lower slice moisture content compared to the other breads. Similarly, moisture loss of slice after 95 hours storage was the highest for RF+DCF and the lowest for RF+RCF (2.00%). Relatively higher reduction in slice moisture (9-16%) was previously reported by Cappa et al. (2013a). Lower slice moisture loss in our study could be ascribed to the use of plastic bags instead of paper bags for bread storage.

The water activity of fresh crumbs was 0.994±0.006, 0.989±0.006, 0.992±0.007 and 0.993+0.007 for RF, RF+RCF, RF+CF and RF+DCF, respectively. No significant effect of flour type and storage time on crumb a_w was observed.

Table 5.3. Changes in moisture content of breads during 95 hours of storage.

	Time (h)	RF	RF+RCF	RF+CF	RF+DCF	p-value	R^2
Crumb Moisture (%)	2	52.68 ± 0.10^{Aa}	52.50 ± 0.04^{Aab}	52.26 ± 0.01^{Ab}	49.92 ± 0.11^{Ac}	0.000	99.76
	23	52.49 ± 0.07^{Aa}	52.23 ± 0.20^{ABa}	52.10 ± 0.18^{Aa}	49.90 ± 0.78^{Ab}	0.017	90.36
	47	51.75 ± 0.02^{Ba}	51.65 ± 0.20^{BCab}	51.13 ± 0.15^{Bb}	48.89 ± 0.05^{ABc}	0.000	99.40
	71	51.21 ± 0.32^{BCa}	51.18 ± 0.17^{CDa}	50.37 ± 0.12^{Ca}	47.56 ± 0.68^{Bb}	0.002	96.66
	95	50.61 ± 0.04^{Ca}	50.79 ± 0.01^{Da}	49.46 ± 0.14^{Db}	47.15 ± 0.19^{Bc}	0.000	99.66
Crumb Moisture loss during storage (%)		3.92	3.26	5.37	5.53		
P		0.000	0.000	0.000	0.004		
R^2		98.03	97.31	99.20	93.03		
Slice Moisture (%)	2	42.92 ± 0.96^{Aa}	42.97 ± 0.09^{Aa}	43.01 ± 0.48^{Aa}	40.15 ± 0.05^{Ab}	0.014	91.13
	23	42.74 ± 0.93^{Aa}	42.80 ± 0.17^{Aa}	42.75 ± 0.30^{ABa}	39.94 ± 0.16^{Ab}	0.011	92.22
	47	42.11 ± 0.73^{Aa}	42.38 ± 0.19^{Aa}	41.95 ± 0.05^{ABCa}	39.41 ± 0.34^{Ab}	0.006	94.33
	71	41.30 ± 0.40^{Aa}	41.63 ± 0.01^{Aa}	41.21 ± 0.12^{Ca}	38.65 ± 0.71^{Ab}	0.006	94.36
	95	41.90 ± 0.06^{Aa}	42.11 ± 0.76^{Aa}	41.62 ± 0.44^{BCa}	38.57 ± 0.43^{Ab}	0.005	94.58
Slice Moisture loss during storage (%)		2.39	2.00	3.22	3.94		
P		0.281	0.066	0.011	0.034		
R^2		57.96	78.13	89.74	83.57		

RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour.
Values are mean ± SD.
[a-c] Means having different letters in the same row are significantly different (P<0.05).
[A-D] Means having different letters in the same column are significantly different (P<0.05).

5.2.2.2. Crumb Texture

Textural parameters of GFB play an important role in terms of consumer preferences. In case of GFB, in particular, quick staling is one of the main problems. In order to evaluate staling, TPA is commonly used since it gives a more complete description of bread texture; besides hardness, other parameters such as cohesiveness, springiness and chewiness can be evaluated by the double compression curves.

The changes in crumb hardness during storage are shown in Figure 5.3. For fresh breads, RF+CF (13.37±0.87 N) and RF+DCF (14.04±0.57 N) presented significantly ($p<0.05$) higher hardness compared to RF (8.42±1.38 N) and RF+RCF (5.49±0.58 N) breads. In particular, bread containing RCF presented a softer crumb even if the moisture content of this bread is not significantly different from that of RF and RF+CF breads. As discussed in crumb porosity part, the hardness values were in accordance with crumb porosity; samples having higher porosity had the lower crumb hardness.

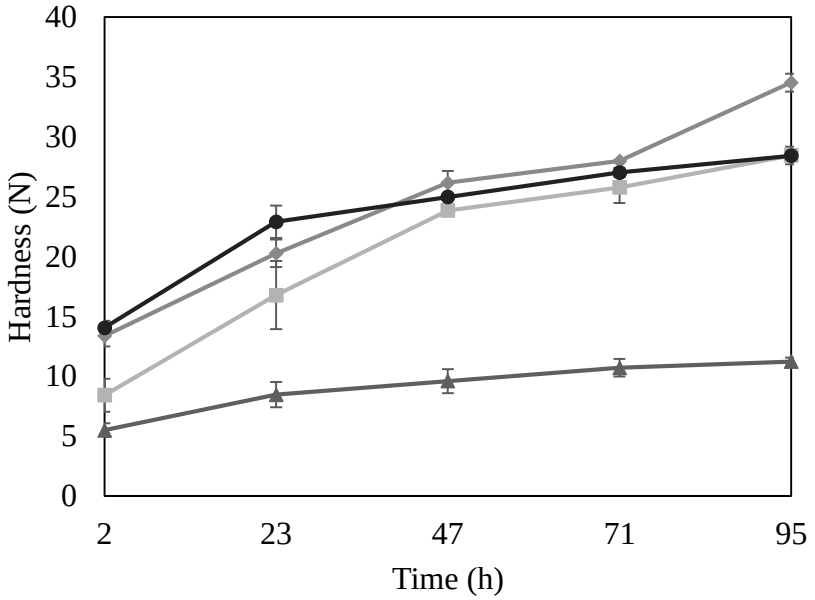


Figure 5.3. Crumb hardness values of gluten free breads (RF (■), RF+RCF (▲), RF+CF (♦) and RF+DCF (●)). (RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour).

Within 47 h, in particular in the first 23 h, the rate of increase in firmness was rapid for all samples. However, the hardening of RF+RCF was significantly lower than other breads during storage. At the end of 95 h, hardness values of the breads were: RF, 28.45±0.73 N; RF+RCF, 11.22±0.34 N; RF+CF, 34.53±0.74 N; RF+DCF, 28.42±0.08 N. This means that RF+RCF was 2.54 times softer than RF at the end of storage period. The rate of staling was the highest for RF (2.38) and lowest for RF+DCF (1.02) and RF+RCF (1.04). RF+CF had moderate staling rate (1.58) compared to the other samples. By having low initial crumb hardness and low staling rate, RF+RCF bread exhibited superior quality.

In a recent study, relatively softer crumb was obtained for toasted (180 °C, 20 min) chickpea flour bread (167 N) compared to raw chickpea bread (251 N) (Ouazib et al., 2016). Compared to the hardness value obtained for our sample roasted chickpea flour containing sample, RF+RCF, the toasted chickpea bread prepared in Ouazib et al. (2016) exhibited 15 times higher hardness. There are two probable reasons for this difference; they used one type of flour in each formulation and the heat was applied to chickpea grain in different conditions. According to Ouazib et al. (2016), for toasting, heat was applied to chickpea grain only once (180 °C, 20 min). In our dissertation study, the roasted chickpea grains were obtained after at least three tempering steps and at least one roasting step as explained in the flow diagram of leblebi production in the literature review section (Figure 2.4). Moreover, tempering steps were followed by resting periods and also a moistening process was applied during roasted chickpea production. Additionally, the roasted chickpea grains lost the hulls during processing.

Cohesiveness is an indicator of the internal resistance of bread. High levels of cohesiveness are desirable to obtain less crumbling bread structure. During mastication, less cohesive bread starts to fractionate in the mouth (Onyango et al., 2011). Compared to rice bread (0.66±0.01), significant decrease in crumb cohesiveness of chickpea-containing breads (0.54-0.56) was observed as seen in Figure 5.4 ($p<0.05$). Sharp decrease in cohesiveness values of all crumbs was observed during storage, particularly in the first 23 h ($p<0.05$). After 95 h, cohesiveness values decreased to RF, 0.21±0.01; RF+RCF, 0.14±0.0.01; RF+CF, 0.15±0.00; RF+DCF, 0.14±0.01. When compared to the cohesiveness of toasted chickpea-containing breads (0.43) evaluated in Ouazib et al. (2016), our samples showed higher values. Burešová et al. (2014) reported that rice bread exhibited higher cohesiveness value (0.76) compared to chickpea bread (0.58) which highlighted the negative effect of chickpea flour on cohesiveness. This effect

could be attributed to high protein content of chickpea flour. In agreement with this discussion, in previous studies, protein-enriched breads were reported to exhibit low cohesiveness values. In such a study (Shevkani et al., 2015a), the addition of protein isolates from white cowpea was found to decrease cohesiveness of GF rice cakes.

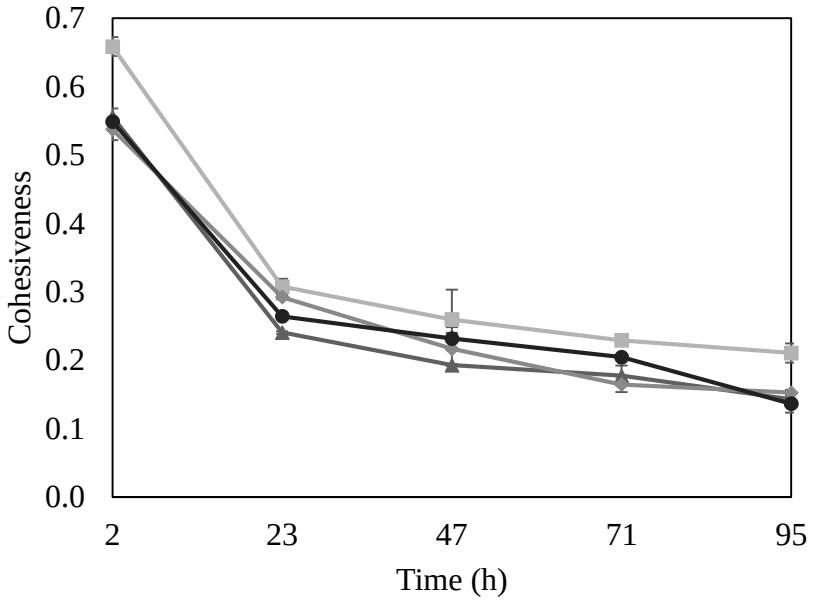


Figure 5.4. Crumb cohesiveness values of gluten free breads (RF (■), RF+RCF (▲), RF+CF (♦) and RF+DCF (●)). (RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour).

As an indicator of elasticity of bread crumb, springiness is considered. When compared to RF, presence of chickpea flours (RCF, CF and DCF) in bread formulations caused a slight but statistically significant decrease in springiness from 0.93+0.01 (RF) to 0.89+0.00 (RF+RCF), 0.87+0.00 (RF+CF) and 0.88+0.02 (RF+DCF) (Figure 5.5). After 95 h of storage, springiness values decreased to RF, 0.74±0.02; RF+RCF, 0.56±0.0.01; RF+CF, 0.62±0.01; RF+DCF, 0.63±0.03. Ouazib et al. (2016) reported springiness values of fresh raw (0.85) and toasted (0.70) chickpea bread which are relatively low compared to our findings. Although increased springiness was observed in protein isolate-containing GF rice muffins (Shevkani et al., 2015a), in our study, addition of high protein containing chickpea flour to GFB did not cause such an effect.

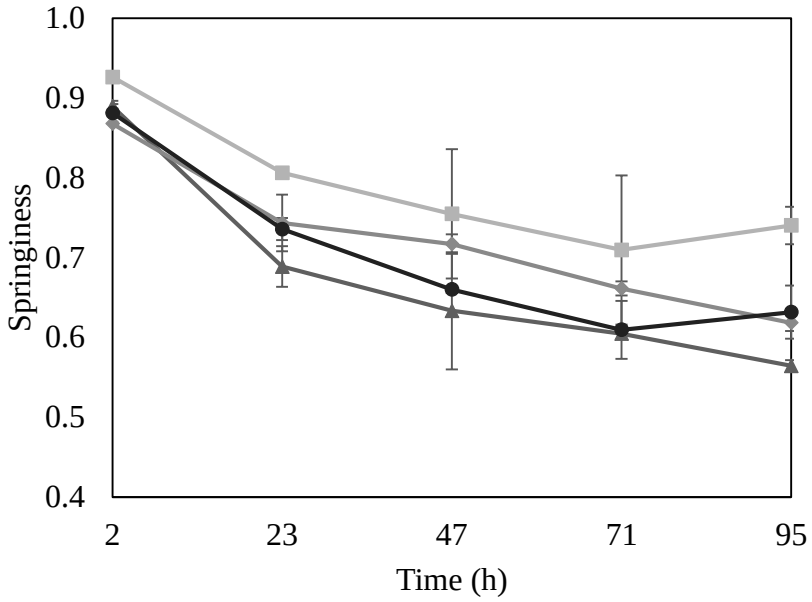


Figure 5.5. Crumb springiness values of gluten free breads (RF (■), RF+RCF (▲), RF+CF (♦) and RF+DCF (●)). (RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour).

Chewiness is defined as the energy required for chewing a solid food until it becomes ready for swallowing (Abdelghafor et al., 2011). Low chewiness is an indication of how easy to break the bread in the mouth (Matos & Rosell, 2012). It is the product of hardness, springiness and cohesiveness. From the chewiness results (Figure 5.6), it was seen that for all storage times sample RF+RCF had the lowest chewiness value. Similar behavior for heat treated chickpea flour containing breads was observed in a previous study (Ouazib et al., 2016), however the chewiness values were dramatically higher compared to our samples due to formulation differences. Considering hardness results, there is a relationship with chewiness; as the crumb become softer, chewiness decreased. The breads with low chewiness is advantageous for the consumption of infant and elderly people.

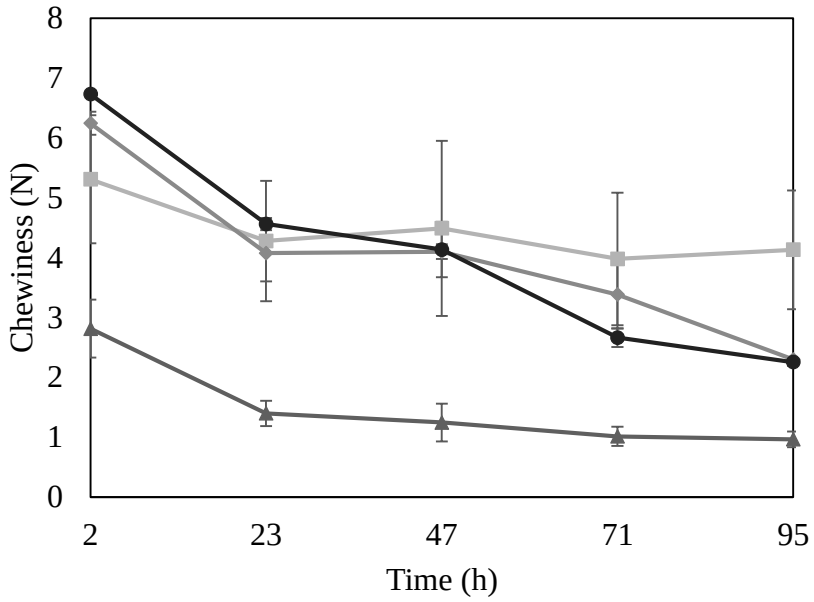


Figure 5.6. Crumb chewiness values of gluten free breads (RF (■), RF+RCF (▲), RF+CF (♦) and RF+DCF (●)). (RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour).

5.2.3. Nutritional Quality

5.2.3.1. Proximate Composition

The protein, fat and ash and total starch contents of fresh bread samples were shown in Table 5.4. The substitution of rice flour with RCF, CF and DCF increased the protein content 35.91, 33.95 and 23.77%, respectively. Moreover, ash and fat levels were significantly increased and available starch content decreased in chickpea-fortified breads ($p<0.05$). Therefore, chickpea flour enrichment of GF rice bread considerably increased the nutritional value of bread.

Table 5.4. Proximate composition of gluten-free breads

Bread	Protein (% dm)	Fat (% dm)	Ash (% dm)	Available Starch (% dm)
RF	9.72 ± 0.08^{b}	1.61 ± 0.38^{c}	2.01 ± 0.03^{b}	86.94 ± 0.60^{a}
RF+RCF	13.21 ± 0.03^{a}	4.58 ± 0.07^{b}	2.45 ± 0.01^{ab}	75.20 ± 0.59^{c}
RF+CF	13.02 ± 0.14^{a}	4.78 ± 0.10^{b}	2.78 ± 0.09^{a}	75.79 ± 0.90^{c}
RF+DCF	12.03 ± 0.71^{a}	5.86 ± 0.16^{a}	2.63 ± 0.21^{a}	78.43 ± 1.29^{b}

RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour.
Values are mean ± SD.
Means having different letters at the same column are significantly different ($p<0.05$).

5.2.3.2. Total Phenolic Content

Many polyphenolic compounds such as flavonols, flavone glycosides and proanthocyanidins (oligomeric and polymeric) are found in chickpea (Shahidi & Ambigaipalan, 2015). The results of phenolic content analysis of flours and bread were given in Figure 5.7 and Figure 5.8, respectively. In comparison to RF (44.55 ± 4.94 mg GAE/100 g dm), chickpea flour had significantly higher ($p<0.05$) total phenolic content regardless of the type of processing applied (RCF, 119.63 ± 0.85; CF, 118.32 ± 3.56; DCF, 115.98 ± 4.52 mg GAE/100 g dm). In a previous study, the TPC content of raw chickpea extracted with 80% acetone was found as 1.41±0.08 mg GAE/g (Xu & Chang, 2007). In a study comparing raw and toasted chickpea flour, a significant increase in TPC was observed after toasting (Fares & Menga, 2012). This increase was attributed to the compounds like Amadori and Heyns products formed during toasting. Toasting also increased the TPC of buckwheat grains (Szawara-Nowak et al., 2014).

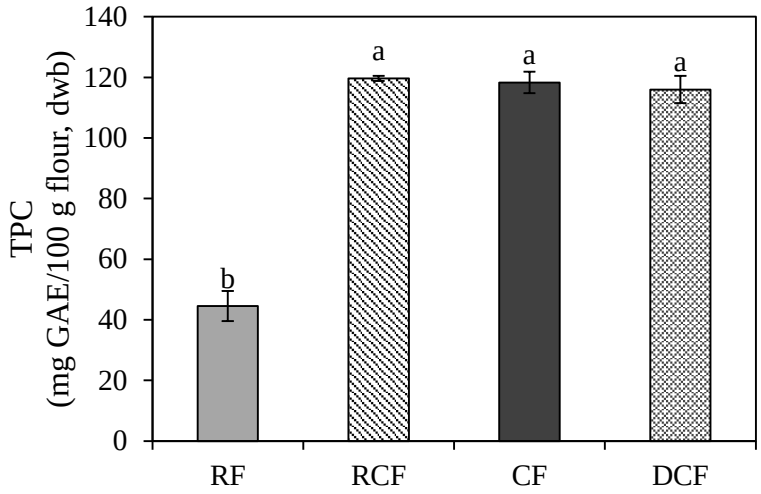


Figure 5.7. Total phenolic content of flours (RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour). Means having different letters are significantly different ($p<0.05$).

As seen in Figure 5.8, bread samples possessed significantly different TPC ($p<0.05$). RF (49.36 ± 2.47 mg GAE/100 g dm) had the lowest TPC. RF+RCF (80.52 ± 5.13 mg GAE/100 g dm) had higher TPC compared to RF+CF (65.29 ± 2.25) and RF+DCF (71.87 ± 2.05).

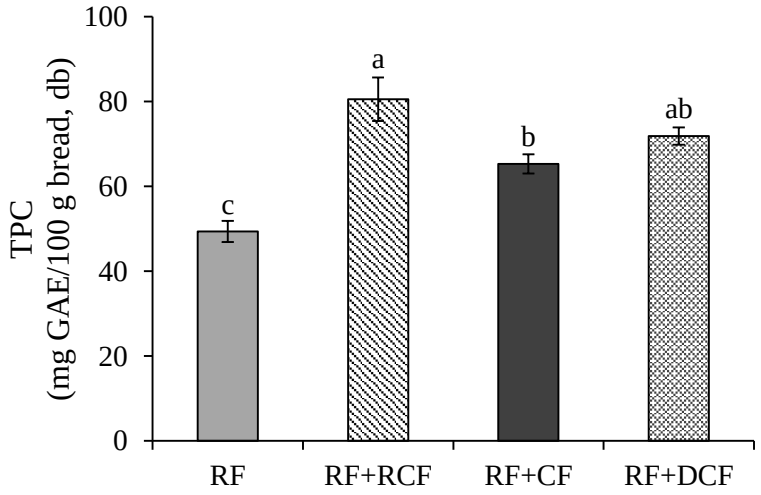


Figure 5.8. Total phenolic content of breads (RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour). Means having different letters are significantly different ($p<0.05$).

5.2.3.3. *In Vitro* Starch Digestibility

The free sugar composition of gluten-free bread samples were given in Figure 5.9. Monosaccharide contents of breads containing chickpea flours were higher than RF. RF+CF has the highest maltose, fructose and glucose content. The total glucose contents of RF, RCF, CF and DCF were 48.7±1.5%, 44.4±1.6%, 45.60.8% and 46.8±1.3%, respectively.

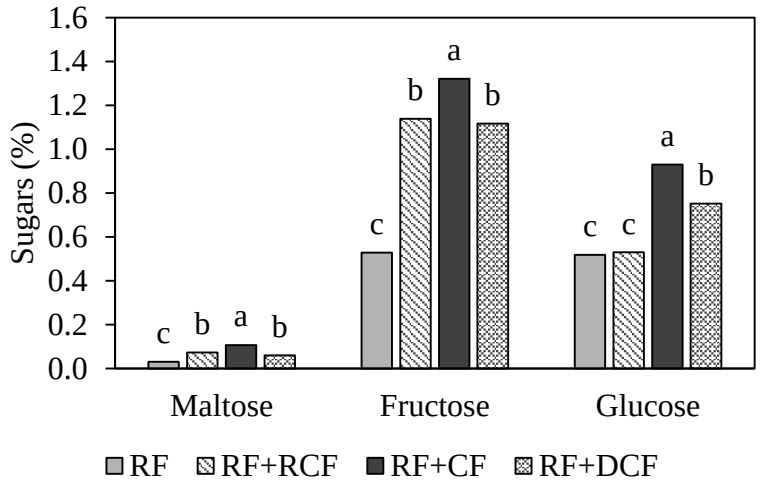


Figure 5.9. Free sugar composition of gluten-free breads (RF (■), RF+RCF (■), RF+CF (■) and RF+DCF (■)). (RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour). Means having different letters for the same type of sugar are significantly different ($p<0.05$).

The nutritionally important starch fractions, RDS and SDS, based on total starch amount were given in Figure 5.10. These two fractions were associated to glycemic index as low RDS and high SDS content were considered as low glycemic index foods. For GF products, RDS fraction is more dominant than SDS (de La Hera, Rosell, & Gomez, 2014; Gularte, Gómez, & Rosell, 2011). Our results are consistent with these findings; for all the bread formulations RDS was higher than SDS. No statistically significant changes were observed between RDS values of RF (93.2 ± 0.7) and RF+RCF (92.9 ± 0.8) breads. On the other hand, slight decrease was observed in RDS fraction for RF+CF (90.8 ± 0.7) and the decrease was the highest for RF+DCF (85.8 ±

0.7). In Wolter et al. (2013), the factors affecting the starch digestibility were listed as size of starch granules, existence of gelatinization, starch composition, protein and lipid content of bread. Therefore, the relatively low RDS content of CF and DCF containing breads might be attributed to the presence of intact starch granules in breads as seen in ESEM micrographs. Also, the partial gelatinization of chickpea starch during roasting could make it much susceptible to digestion enzymes. Another effective factor is the particle size of the flour. Since the surface area increases in flours having smaller particle size, the digestibility is increased (de La Hera et al., 2014). Our results were partial agreement with this hypothesis as the CF had the biggest and RF had the smallest flour particle sizes. However DCF possessed the lowest RDS, its particle size is lower than both CF and RCF. Additionally, in a previous study, the effect of dehulling on *in vitro* starch digestibility of chickpea flour was evaluated and it was reported that dehulling increases the starch digestibility due to the reduced levels of antinutrients with dehulling process (Ghavidel & Prakash, 2007). However, the bread is a complex system having multi components and several molecular interactions and structural changes occur during baking as well. On the other hand, it should be noted that cultivar differences might affected the starch digestibility. The dietary fiber (soluble and insoluble) and resistant starch was found to be changed with variety of lentils (Wang et al., 2009). The dehulled chickpea flour used in this study was obtained from a commercial company in Europe, however, chickpea and roasted chickpea were Turkish cultivars.

In literature, chickpea was defined as low glycemic index (28 ± 9) grain (Atkinson et al., 2008). However in this study, even the breads with chickpea flour showed high levels of RDS. In wholegrain, the starch was packed in the grain and although gelatinized, it is surrounded with other macromolecules (proteins, fats). However, in case of flour utilization, milling process could have disrupted the starch granules and make them accessible for digestive enzymes. In a GF cake study in which 50% of rice flour was replaced with chickpea, pea, lentil and bean flours, it was also observed that chickpea flour-containing formulation had the highest RDS (86.6±3.8) and lowest SDS (7.3±5.4) content than other breads (Gularte et al., 2011). Chung et al. (2008) reported a relatively higher RDS and hydrolysis extent for chickpea flour compared to lentil flour and ascribed it to relatively lower protein and amylose content of chickpea flour compared to lentil flour. In Zafar et al. (2015), the incorporation of 25% chickpea flour into whole wheat bread formulation did not affect the glycemic

response significantly compared to wheat bread without chickpea flour even if chickpea flour had high amylose, dietary fiber and resistant starch content.

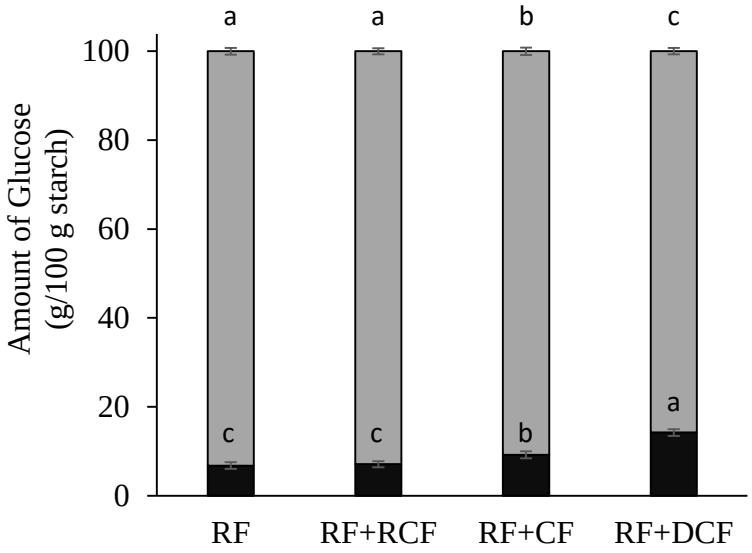


Figure 5.10. RDS and SDS contents of gluten-free breads (RDS (■), SDS (■)). (RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour). Means having different letters for the same type of nutritionally important starch fractions are significantly different ($p<0.05$).

During evaluation of starch digestibility, the proportion of the food consumed should also be considered in the context of glycemic load. As seen in Table 5.4, available starch content of chickpea-containing breads were significantly lower ($p<0.05$) than rice bread. Therefore, the consumption of equal weight slices propably results in low glycemic response for chickpea containing breads due to their low available starch content. Wolter et al. (2013) came up with lower glycemic load due to lower carbohydrate content for quinoa bread even though it exhibited high glycemic index.

5.2.3.4. *In Vitro* Protein Digestibility

Among the *in vitro* protein digestibility methods, the method used in this study was selected based on its simple and less time consuming nature. Additionally, this method was suggested as a reliable technique to evaluate both raw and heat-treated chickpea samples (Tavano et al., 2016). The method is based on the measurement of the pH drop caused by the increase in H+ ions due to the breakdown of peptide bonds and formation of free carboxyl groups of amino acids (Tavano et al., 2016).

The *in vitro* protein digestibility curve of the breads was given in Figure 5.11. The highest protein digestibility was observed for RF+RCF (85.57±0.52%) and lowest for RF (83.07±0.64%). RF+CF and RF+DCF had *in vitro* protein digestibility of 84.25±0.04% and 83.58±0.29%, respectively.

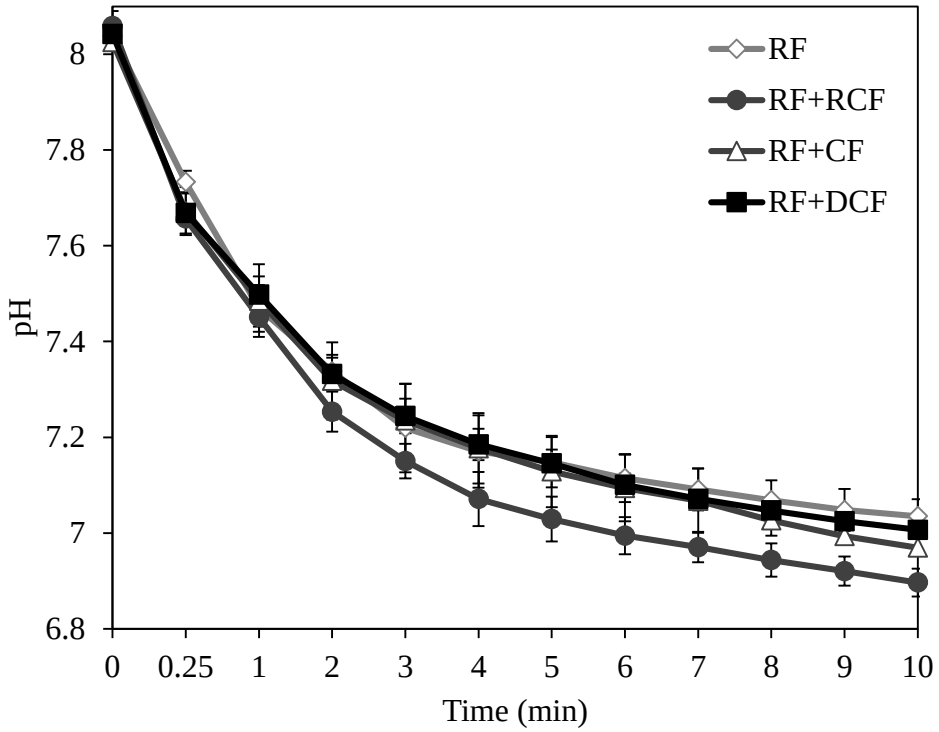


Figure 5.11. Protein digestibility curves of breads (RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour).

The increase in protein digestibility is the result of increase in protein quality and availability. The low protein digestibility has been linked with the anti-nutritional factors, such as protease inhibitors, particularly present in legumes (Coda et al., 2015). The effect of heat treatment on the reduction of Kunitz-type protease inhibitors and lectins is a very well-defined fact (Fennema, 1996). Therefore, higher protein digestibility of roasted chickpea-containing bread formulation could be explained by the so-called effect of heat-treatment. In a review related with the protein digestibility of sorghum, processes such as dry heating, grain refinement and fermentation were suggested to increase the protein digestibility of sorghum (Duodu et al., 2003).

5.2.4. Sensory Properties of Bread Samples

The gluten-free bread samples were presented in Figure 5.12 and sensory evaluation results were given in Table 5.5 and Figure 5.13. According to appearance results, sample RF had the lowest and RF+CF had the highest scores. The presence of all types of chickpea flours enhanced the appearance of the bread and resulted in increased acceptability. The appearance of the samples was a general parameter and could be affected by crust smoothness, volume and color of the loaves. As seen from Figure 5.12, RF had a cracked crust and light color. In contrast, sample RF+CF had less cracks and it has intermediate crust darkness. The crust color scores were also parallel with the appearance results. Accordingly, the consumer preferences were towards the smooth surface and brown-colored bread.

RF+RCF RF+CF RF+DCF RF

Figure 5.12. Gluten-free bread samples used in sensory evaluation (RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour).

The crumb color results showed that CF and DCF containing bread crumbs were more desirable than RF and RF+RCF (Figure 5.13). According to the results of color measurement, RF+CF and RF+DCF had relatively lighter crumb than RF+RCF and exhibited more yellowness than RF. From this aspect, panel preferences resulted towards the intermediately dark crumb.

Table 5.5. Sensory evaluation results of gluten-free breads

Sample	Appearance	Crust Color	Crumb Color	Odor	Texture	Flavor	Overall Acceptability
RF	4.68 ± 0.15^{c}	4.96 ± 0.19^{c}	5.15 ± 0.01^{c}	5.20 ± 0.13^{b}	4.51 ± 0.02^{c}	6.08 ± 0.03^{b}	5.31 ± 0.10^{b}
RF+RCF	6.77 ± 0.17^{a}	6.57 ± 0.07^{ab}	6.36 ± 0.01^{b}	5.64 ± 0.20^{b}	7.10 ± 0.00^{a}	6.39 ± 0.08^{ab}	6.73 ± 0.04^{a}
RF+CF	6.83 ± 0.25^{a}	7.07 ± 0.04^{a}	7.07 ± 0.03^{a}	5.47 ± 0.12^{b}	6.68 ± 0.15^{ab}	6.14 ± 0.13^{b}	6.58 ± 0.04^{a}
RF+DCF	6.00 ± 0.00^{b}	6.38 ± 0.27^{b}	7.12 ± 0.03^{a}	6.52 ± 0.07^{a}	6.39 ± 0.15^{b}	6.69 ± 0.13^{a}	6.84 ± 0.22^{a}

RF, rice flour; RCF, leblebi flour; CF, chickpea flour; DCF, dehulled chickpea flour.
Values are mean ± SD.
Means having different letters at the same column are significantly different ($p<0.05$).

As regards the odor, RF+DCF had significantly the highest score ($p<0.05$). Since RF+CF had significantly lower score compared to RF+DCF, dehulling seemed to enhance the odor of the bread. On the other hand, possible varietal differences in chickpea grains might also affected the sensorial properties. Concerning texture, addition of all types of chickpea flour into rice-bread formulation caused significant increase. However, RF+RCF had the highest score. This bread also showed the softest crumb according to TPA results in Chapter 3. The flavor results showed that RF+DCF had significantly desirable flavor compared to RF and RF+CF. Overall acceptability scores for all breads were higher than 5, which means that all samples were acceptable. On the other hand, all chickpea containing samples had significantly higher scores than RF ($p<0.05$). Therefore, addition of chickpea flour (raw, roasted or dehulled) could be suggested to improve acceptability of rice-based GFB.

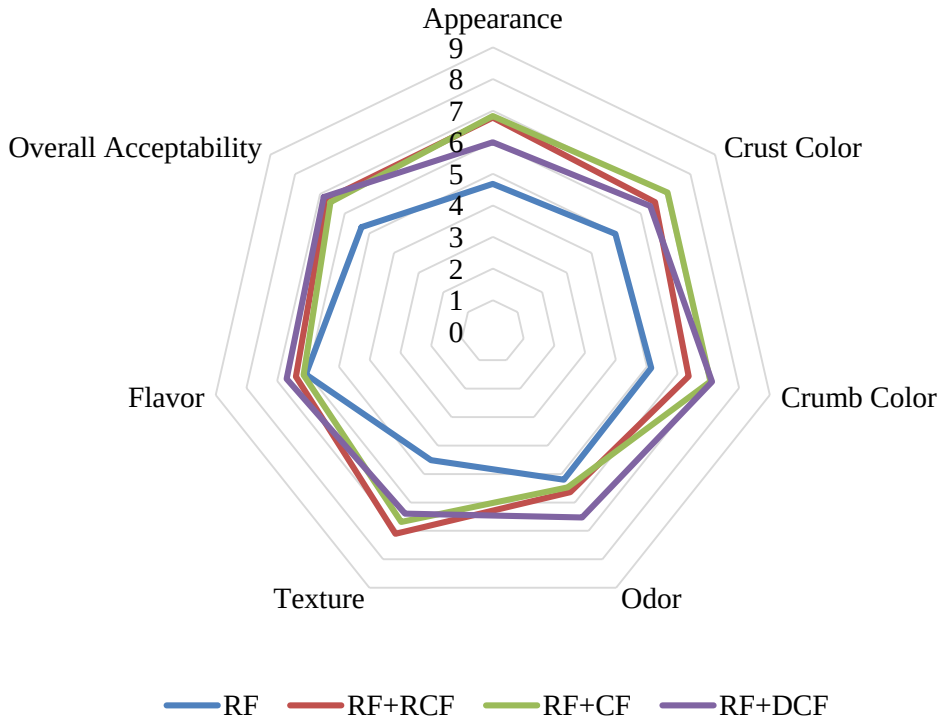


Figure 5.13. Sensory evaluation results of gluten-free bread samples (RF, rice flour; RCF, roasted chickpea flour; CF, chickpea flour; DCF, dehulled chickpea flour).

5.3. Conclusions

In this part of the study, effects of roasted, dehulled and raw chickpea flour fortification on rice based gluten-free bread were evaluated. In general, all types of chickpea flour fortification contributed the rice bread quality by affecting in different ways. Roasted chickpea flour addition found to be advantageous over other chickpea flours, since its use resulted in increased specific volume, crumb and crust darkness. Among all the chickpea breads, the one containing roasted chickpea flour became prominent due to its dramatic effect on crumb softening during storage. Significant increase in protein, ash and fat contents of the breads and reduced starch levels were achieved with the utilization of chickpea flours in the gluten-free bread formulations. Additionally, total phenolic content of the breads significantly increased after chickpea flour addition, in particular in the bread containing roasted chickpea flour. In raw and dehulled chickpea flour containing breads, RDS levels were significantly ($p<0.05$) decreased and consequently, SDS levels were increased. The protein digestibility was enhanced in chickpea flour containing breads. In addition to above-mentioned positive outcomes, partial replacement of rice flour with chickpea flours significantly ($p<0.05$) increased the sensory scores and resulted in acceptable breads by consumer. In conclusion, chickpea flour can be successfully used to fortify rice-based gluten-free bread in order to obtain high quality, nutritious and acceptable product.

CHAPTER 6

APPLICATION OF SOURDOUGH FERMENTATION

This chapter covers the evaluation of sourdough fermentation parameters of rice-based dough enriched with roasted chickpea flour by using *Lactobacillus sanfranciscensis* ED-5C as starter culture. The quality of sourdough added breads (15 and 30%) were evaluated in comparison with unfermented bread.

6.1. Materials & Methods

6.1.1. Materials

Strain used for sourdough fermentation, *Lactobacillus sanfranciscensis* ED-5C, was a Turkish sourdough isolate (Dertli et al., 2016) that was kindly provided by Assist. Prof. Dr. Enes Dertli (Department of Food Engineering, Bayburt University, Turkey). Stock solutions in 40% glycerol (104093, Merck) were prepared and stored at -80 °C.

The flours used in this study were: rice flour (Pakmaya, As Gıda, Turkey) and roasted chickpea flour (milled by using a laboratory mill and sieved (≤ 1 mm)). The other ingredients were HPMC (Benecel F4M, Ashland, USA), instant yeast (Pakmaya, Turkey), sugar, salt, sunflower oil and water.

The chemicals used were acetic acid (100063, Merck), agar (A0949, Applichem), glycerol (104093, Merck), lactic acid (L52, Aldrich), Man, Rogosa and Sharpe Medium (MRS) broth (110661, Merck), perchloric acid (311421, Aldrich), potassium chloride (KCl, 12636, Sigma-Aldrich), potassium phosphate monobasic (KH_2PO_4, 60218, Fluka), sodium chloride (NaCl, 106404, Merck), sodium hydroxide (NaOH, 06203, Riedel-de-Haën), sodium phosphate dibasic (Na_2HPO_4, 04276, Riedel-de Haën) and sucrose (A2211, Applichem). For phosphate buffered saline (PBS), NaCl (8.00 g/L), KCl (0.20 g/L), Na_2HPO_4 (1.44 g/L) and KH_2PO_4 (0.24 g/L) were dissolved in deionized water, pH was adjusted to 7.4 and sterilized at 121°C for 15 min.

6.1.2. Methods

6.1.2.1. Preparation of Growth Media & Subcultures

For subculture and cell count media, MRS agar (52.2 g/L MRS Broth, 15 g/L agar) containing 2% (w/v) sucrose was prepared. Filter-sterilized sucrose solution (45 μm syringe filter) was added to autoclaved MRS broth/agar.

L. sanfranciscensis culture (100 μL) was taken from glycerol stocks, inoculated to MRS broth (5 mL) and incubated at 30 °C for 7 h. At the end of incubation, subcultures were prepared, incubated for 16 h and subsequently used in sourdough fermentation.

6.1.2.2. Morphology & Growth Curve of *L. sanfranciscensis*

For morphological analysis and growth curve, 16 h-incubated cultures of the strain were streaked on MRS agar plates (containing 2% (w/v) sucrose) and incubated overnight at 30 °C in an anaerobic jar (AnaeroJar, Oxoid, Thermo Scientific) containing an anaerobic gas generating sachet (AnaeroGen, Oxoid, Thermo Scientific). The strain was analyzed under microscope (CX31, Olympus, Japan) after Gram staining.

The growth curve of the strain was constructed by using a microplate reader (Varioskan Flash, Thermo Scientific, USA) set at 30 °C. The absorbance was read at 620 nm for 24 h against a blank containing only MRS broth.

6.1.2.3. Sourdough Preparation

The subcultured *L. sanfranciscensis* cells were harvested via centrifugation at 5000 rpm for 15 min at 4 °C. Cells were washed with PBS (pH 7.4) and suspended in sterile water to obtain cell density of 0.5 McFarland unit using a densitometer (DEN-1, HVD Life Sciences, Austria). The obtained cell suspensions were used to prepare sourdough. *L. sanfranciscensis* cell concentration in this suspension was determined previously in at least two replicates and found as almost $6.6x10^7$ colony forming unit

(CFU)/mL. The inoculation volume used in sourdough fermentation was calculated to have a final inoculum of 10^7 CFU/g dough.

The sourdough sample was prepared according to the optimized RF+RCF bread recipe by using only rice flour, roasted chickpea flour, sterile water and culture suspension. The obtained dough was put in a glass beaker, covered with aluminum foil and incubated at 30 °C until the dough reached a pH value of 3.9 (almost 22 h). In order to compare the microbial growth and metabolite formation, control dough containing no bacterial inoculation (C) and chemically acidified dough (CAD) (lactic and acetic acid mixture (4:1, v/v)) without inoculum were also prepared.

6.1.2.4. Determination of Fermentation Parameters

In order to follow the progress of fermentation, pH and total titratable acidity (TTA) were measured and numbers of LAB were counted for each dough sample.

6.1.2.4.1. Determination of pH and Total Titratable Acidity

The pH and TTA of sourdough samples at the beginning (0 h), after 5.5 h and 22 h were determined. The pH of the sourdough was directly measured with a pH-meter (pH1100L, VWR, Germany). For the TTA measurement, sourdough (10 g) was diluted with 90 mL of deionized water and titrated with standardized 0.1 N NaOH to pH 8.5. The volume (mL) of 0.1 N NaOH consumed was recorded as TTA.

6.1.2.4.2. Organic Acid Composition

Sourdough samples taken at the end offermentation (22h) were prepared for organic acid analysis according to Thiele et al. (2002) with some modifications in centrifugation conditions as explained in Wolter et al. (2014a). Sourdough samples (500 mg) were treated with 7% perchloric acid (500 µL) for 16 h at 4 °C. The supernatant was obtained after centrifugation at 2000xg for 20 min. The filtrated supernatant (0.45 µm) was used for organic acid analysis.

Lactic and acetic acid contents of the doughs were measured by using High Performance Liquid Chromatography (HPLC) (Series 200, Perkin Elmer, USA) equipped with Aminex HPX-87H column (9 μm particle size, 300 x 7.8 mm) (Bio-Rad Laboratories, USA) and refractive index detector (Series 200, Perkin Elmer, USA). Filtered (0.45μm) dough extracts (20 μL) were injected to preheated column (65 °C) using H_2SO_4 (5 mM) as the mobile phase at a flow rate of 0.6 mL/min and analyzed for 20 min. Calibration curves of lactic and acetic acid standards were given in Figure B.2.

6.1.2.4.3. Lactic Acid Bacteria Cell Count

The LAB concentrations of sourdoughs at the beginning (0 h), after 5.5 h and 22 h were determined. The sourdough (10 g) was suspended in peptone water (90 mL) and serial dilutions were prepared. Samples (1 mL) from proper dilutions were pipetted into petri dishes and MRS agar was poured. Petri dishes were incubated for 48 h at 30 °C in an anaerobic jar (AnaeroJar, Oxoid, Thermo Scientific) containing an anaerobic gas generating sachet (AnaeroGen, Oxoid, Thermo Scientific). Colonies on plates containing 30-300 colonies were counted.

6.1.2.5. Dough & Bread Preparation

The sourdough breads were prepared as explained in Section 3.1.3. Differently, sourdough (15 and 30%) was added to formulations by replacing the same amount of flour and water to keep the dough composition and consistency constant. Besides, the ratio of RF to RCF was also kept constant as in the optimized formulation, for both control and sourdough breads. In total three bread samples were prepared: C (control, without sourdough), SD 15 (15% sourdough), SD 30 (30% sourdough).

6.1.2.5.1. Determination of pH and Total Titratable Acidity of Bread Dough

TTA and pH of the doughs used for bread preparation (C, control; SD 15, 15% sourdough; SD 30, 30% sourdough) were measured with the methods explained in Section 6.1.2.4.1.

6.1.2.6. Sourdough Bread Analysis

6.1.2.6.1. Measurement of pH & TTA

Deionized water (90 mL) was added to bread (10 g) and homogenized by using a bar blender (32BL80, Waring, USA). The pH of the homogenate was measured with a pH-meter (pH1100L, VWR, Germany). For the TTA measurement, the homogenate was titrated with standardized 0.1 N NaOH to pH 8.5. The volume (mL) of 0.1 N NaOH consumed was recorded as TTA.

6.1.2.6.2. Bake Loss and Specific Volume Measurements

Bake loss and specific volume measurements were carried out as explained in Section 3.1.4.

6.1.2.6.3. Moisture Content

Moisture content of the bread crumb was determined as explained in Section 5.1.2.3.

6.1.2.6.4. Color of Crust and Crumb

Crust and crumb color were measured as explained in Section 3.1.6.

6.1.2.6.5. Texture Analysis

Texture profile analysis (TPA) was carried out by using the method in Section 3.1.7.

6.1.2.6.6. *In Vitro* Protein Digestibility

In vitro protein digestibility of breads was carried out by using the method in Section 5.1.2.8.6.

6.1.2.6.7. Sensory Evaluation of Bread

The sensory evaluation of bread samples (C, control without sourdough; SD 15, 15% sourdough; SD 30, 30% sourdough) were carried out as explained in Section 5.1.2.9 with some differences in the gender and age of the panelists. Among the staff and students of Food Engineering Department at Izmir Institute of Technology, 21 panelists (15 women, 6 men) aged between 20 and 53 attended the sensory panel of gluten-free breads.

6.1.2.6.8. Statistical Analysis

Statistical evaluation of the data was performed by using MINITAB 16 (Minitab Inc., U.S.). The results were given as "mean ± SD". The significance of the data was tested by analysis of variance (ANOVA) at $p<0.05$ and, in the significant models, means were compared by Tukey's test at 95% confidence interval.

6.2. Results & Discussions

6.2.1. Morphology & Growth Curve of *L. sanfranciscensis* ED-5C

L. sanfranciscensis was used in this study due to its common usage in sourdough fermentation. Also this strain is a previously identified strain isolated from typical traditional wheat sourdough originated from Turkey (Dertli et al., 2016). The microscopical view of *L. sanfranciscensis* ED-5C was shown in Figure 6.1. Since *L. sanfranciscensis* is a Gram-positive lactic acid bacteria, the bacilli shaped cells have purple color. The morphology of this culture showed pure lactobacilli form as it was supplied and evidenced by the inventers.

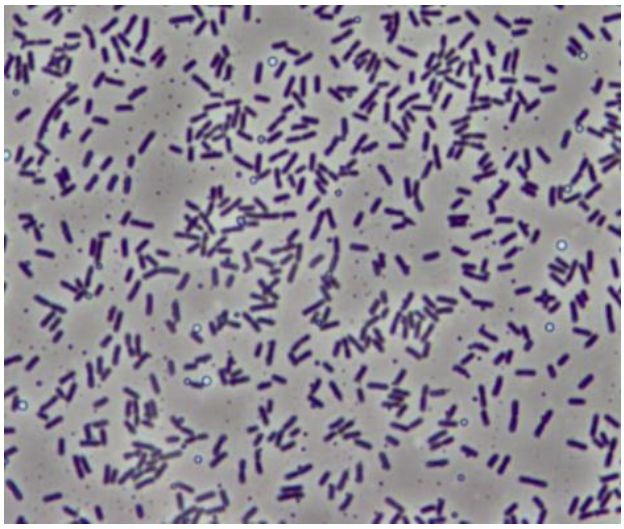


Figure 6.1 Morphology and Gram reaction of *L. sanfranciscensis* ED-5C under light microscope

The growth curve of *L. sanfranciscensis* ED-5 C was shown in Figure 6.2. This strain reached the stationary phase at the end of 20 h. In the sourdough preparation, the culture from glycerol stock was incubated for 7 h and subcultured. Besides, sourdough was inoculated with this subculture after 16 h of incubation. Therefore, subculture and inoculum were taken when the strain was in exponential growth phase.

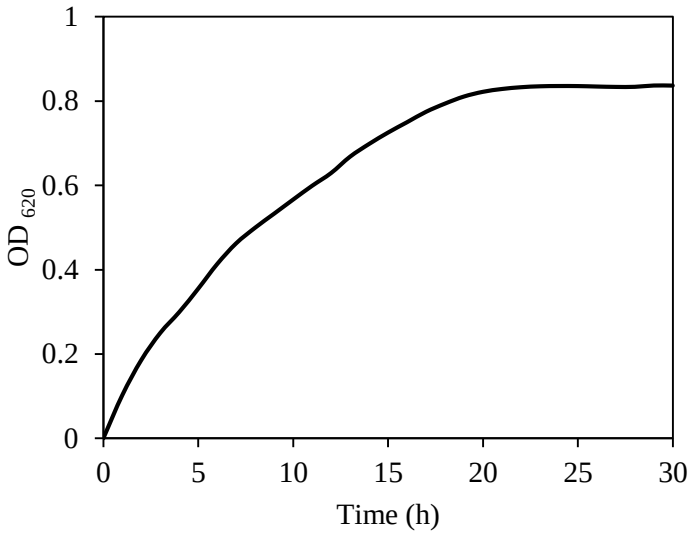


Figure 6.2. Growth curve of *L. sanfranciscensis* ED-5C

6.2.2. Fermentation Parameters & Organic Acid Content of Sourdough

The pH, TTA and LAB counts were shown in Figure 6.3. During fermentation, significant changes in pH, TTA and LAB count of C and SD were observed. For CAD, no considerable change in pH and TTA was obtained. At the end of fermentation, C, CAD and SD reached to pH 5.25±0.01, 3.94±0.03 and 3.98±0.04; TTA of 5.03±0.04, 11.50±0.00 and 10.39±0.23, respectively. Studies related with the sourdough fermentation evidenced the importance of strain/flour type and fermentation conditions on pH and TTA. In a study by Axel et al. (2015), it was observed that quinoa sourdough prepared with *L. amylovorus* DSM19280 as the starter culture reached a pH of 3.9 and TTA of 35.7 at the end of 48 h fermentation. Compared to wheat sourdough (16.3 mL), considerably high TTA of the quinoa sourdough was attributed to high buffering capacity of mineral-rich quinoa. In another study, chickpea sourdough inoculated with *L. plantarum* C48 and *L. brevis* AM7 reached pH of 4.0-4.1 and TTA of 22.4-22.6 (Curiel et al., 2015).

LAB numbers in the doughs were significantly different throughout the whole fermentation period ($p<0.05$). In comparison to C and SD, very limited LAB growth occurred in CAD due to the growth limiting effect caused by highly acidic environment. LAB count for C was found closer to SD at the end of fermentation as seen in Figure

6.3. However, metabolite formation occurred to a higher extent in SD compared to C, due to the activity of *L. sanfranciscensis* starting from the beginning of the fermentation. The use of starter cultures in sourdough production is also advantageous in terms of industrial production, where short and efficient fermentation times are required.

The organic acid contents at the end of fermentation (at 22h) were given in Table 6.1. For all doughs, dramatically high amounts of lactic acid were produced compared to acetic acid. In particular, sample SD had lactic acid:acetic acid ratio of 34:1. Similarly, in a study related to amaranth sourdough fermentation, lactic and acetic acid contents were found to be as 136.6-178.1 mM and 1.9-7.6 mM, respectively (Sterr et al., 2009). On the other hand, sample CAD was prepared by adding lactic and acetic acid in a ratio of 4:1. This is a general ratio used to prepare a chemically acidified dough in many sourdough studies. However, according to our results, it seems that a higher ratio is required for the preparation of the acidification solution in order to obtain a more realistic dough acidification.

Table 6.1. Lactic and acetic acid contents of doughs at the end of fermentation

Sample	Lactic Acid (mmol/kg dough)	Acetic Acid (mmol/kg dough)
C	73.18 ± 1.14 ^{b}	1.87 ± 0.13 ^{b}
CAD	131.11 ± 28.83 ^{ab}	29.53 ± 6.03 ^{a}
SD	182.60 ± 5.79 ^{a}	5.41 ± 0.20 ^{b}

C, control; CAD, chemically acidified dough; SD, sourdough.
Values are mean ± SD.
Means having different letters in the same column are statistically significant ($P<0.05$).

Although the final LAB counts were very similar for C and SD, the organic acid levels were significantly different ($p<0.05$). This difference was the result of the metabolism of *L. sanfranciscensis* even at the early stages of fermentation. The advantage of sourdough fermentation using a starter in terms of time efficiency and higher rates of metabolite formation was clearly demonstrated with this result.

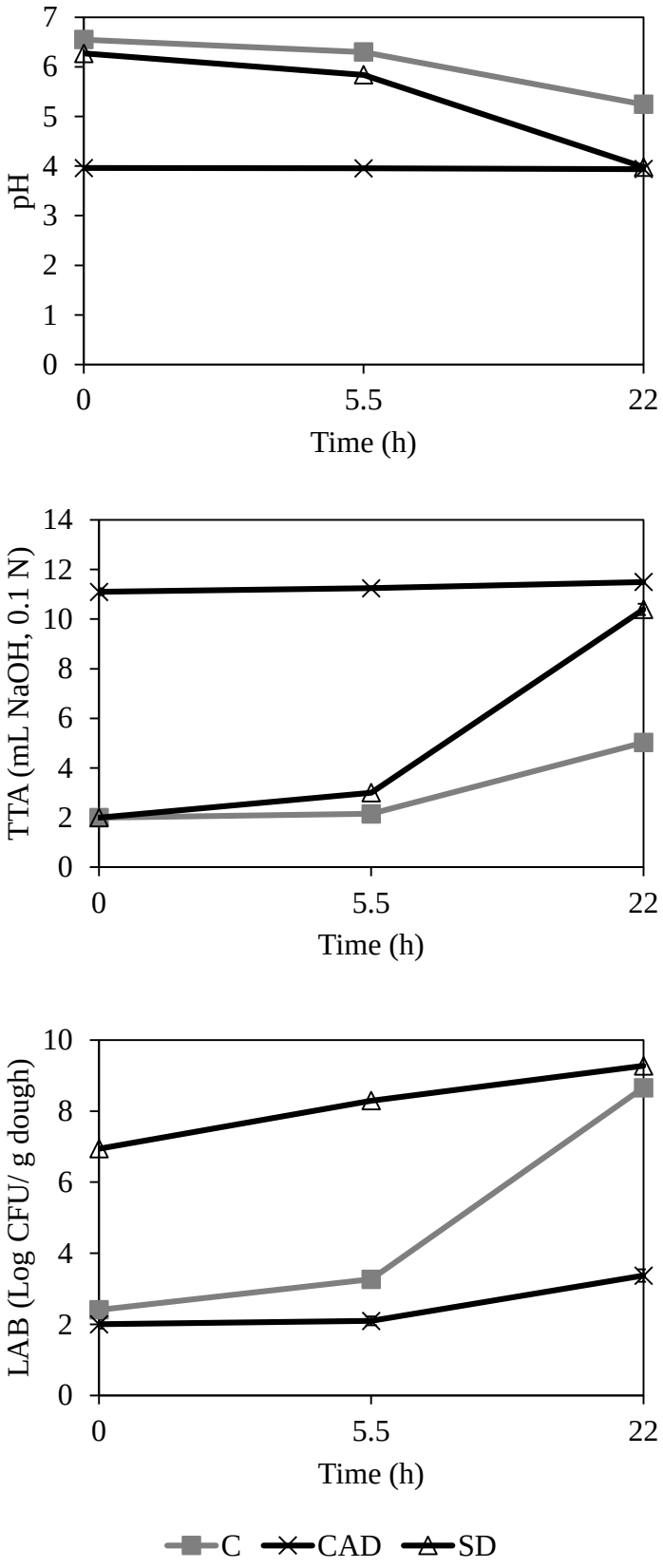


Figure 6.3. Fermentation parameters at 0, 5.5 and 22 h a) pH b) TTA c) LAB (C, control; CAD, chemically acidified dough; SD, sourdough)

6.2.3. Acidity and pH of Bread Dough

The pH and TTA of the dough samples prepared for breadmaking were seen in Figure 6.4. The increasing sourdough levels caused significant decrease in pH and increase in TTA proportional to the sourdough addition level ($p<0.05$). The dough containing 30% sourdough had 13.7% lower pH and 69.5% higher TTA than control sample without sourdough.

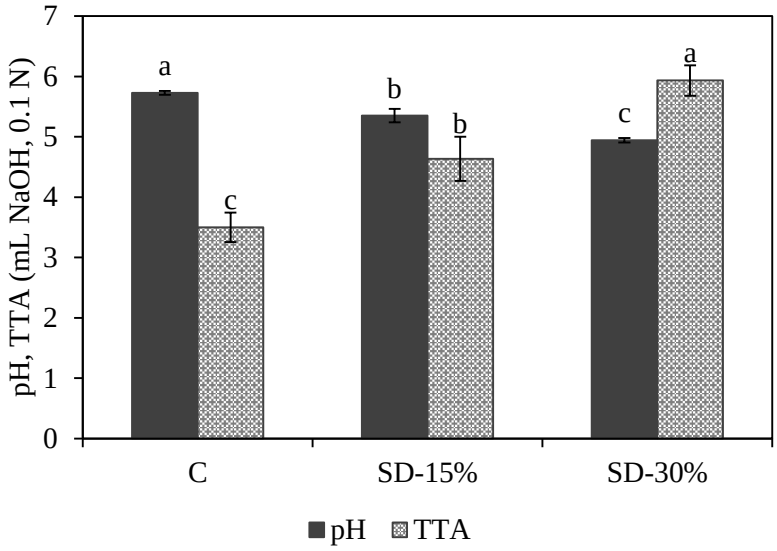


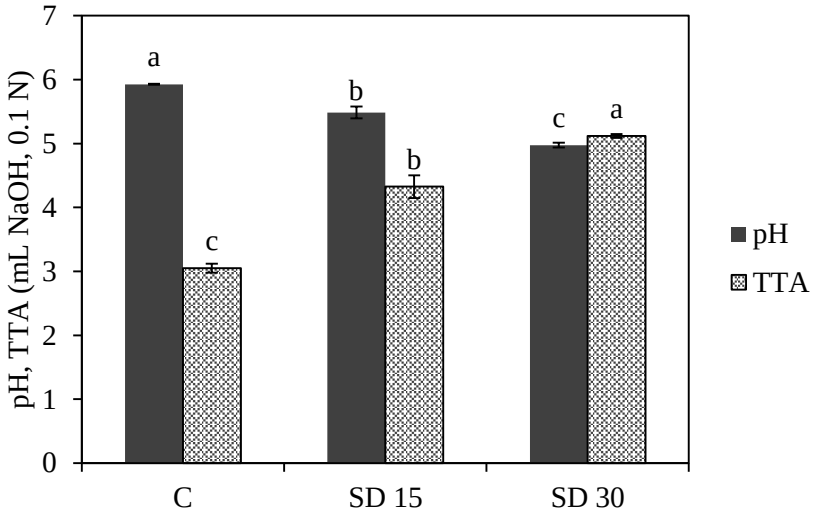
Figure 6.4. TTA and pH of doughs used for breadmaking (C, control; SD 15, 15% sourdough; SD 30, 30% sourdough). Means having different letters within each parameter are significantly different ($p<0.05$).

6.2.4. Sourdough Bread Properties

6.2.4.1. Acidity and pH of Bread

In Figure 6.5, the pH and TTA of control and breads containing 15 and 30% of sourdough were seen. Increasing sourdough levels in gluten-free bread formulation caused a gradual and significant ($p<0.05$) decrease in pH and increase in TTA. SD 30 had 16.1% lower pH and 40.4% higher TTA than C. Likewise, in a quinoa sourdough

bread contained 20% of 48 h-fermented sourdough, the pH decreased 16% and TTA increased 69.7%, respectively (Axel et al., 2015). Wolter et al. (2014b) reported the pH and TTA of buckwheat, quinoa, sorghum and teff sourdough breads as 4.9-5.4 and 4.5-11.3, respectively. The changes in pH and the presence of organic acids might affect particularly the physicochemical properties, sensorial quality and shelf-life of bread.



	C	SD 15	SD 30
pH	5.93 ± 0.01^{a}	5.49 ± 0.09^{b}	4.97 ± 0.04^{c}
TTA (mL NaOH, 0.1 N)	3.05 ± 0.07^{c}	4.33 ± 0.18^{b}	5.12 ± 0.03^{a}

Figure 6.5. TTA and pH of breads (C, control; SD 15, 15% sourdough; SD 30, 30% sourdough). Means having different letters within each parameter are significantly different ($p<0.05$).

6.2.4.2. Bake Loss and Specific Volume

The bake loss values of the breads showed no differences ($p>0.05$): C, 19.60±0.14%; SD 15, 20.00±0.14% and SD 30, 19.45±0.49%. Similarly, no significant effect of sourdough addition was reported in most of the previous studies (Galle et al., 2012; Schober et al., 2007).

The specific volume and height of the bread samples were given in Figure 6.6. Although the specific volume decreased with the increasing levels of sourdough, only 30% of sourdough addition caused a significant change ($p<0.05$). As expected, the height of the loaves decreased in the same manner. The specific volume of sourdough breads could be affected by the strain and flour types used in the formulation. In literature, although some researchers reported increased volumes of sourdough breads (Axel et al., 2015; Novotni et al., 2012), considerable numbers of studies showed no significant differences (Galle et al., 2012; Schober et al., 2007; Wolter et al., 2014c).

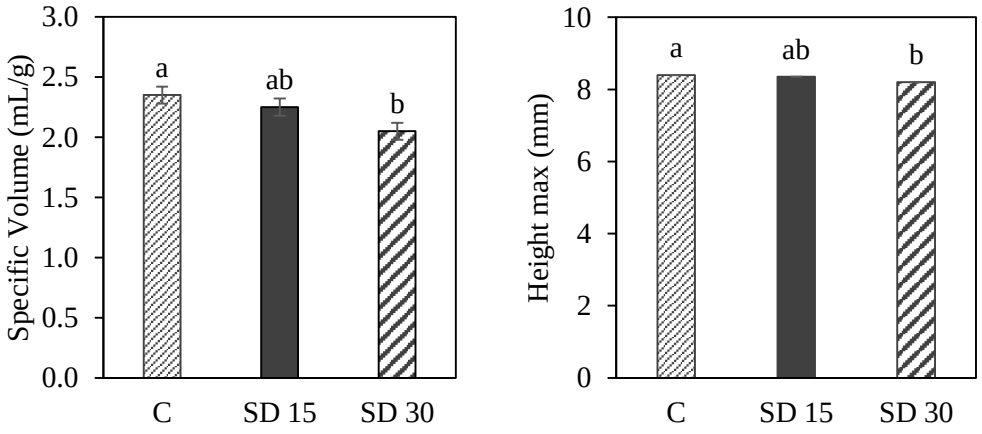


Figure 6.6. Specific volume and height of breads (C, control; SD 15, 15% sourdough; SD 30, 30% sourdough). Means having different letters are significantly different ($p<0.05$).

6.2.4.3. Color of Crust and Crumb

The color measurement results were given in Figure 6.7. Since same types and amounts of flours were used in all breads, no significant change was observed in crumb color parameters ($p>0.05$). On the other hand, the crust color parameters were considerably affected by sourdough addition ($p<0.05$); lightness increased and redness increased, yellowness decreased with increasing levels of sourdough in the bread. The sugars released during sourdough fermentation might trigger the formation of Maillard reaction products.

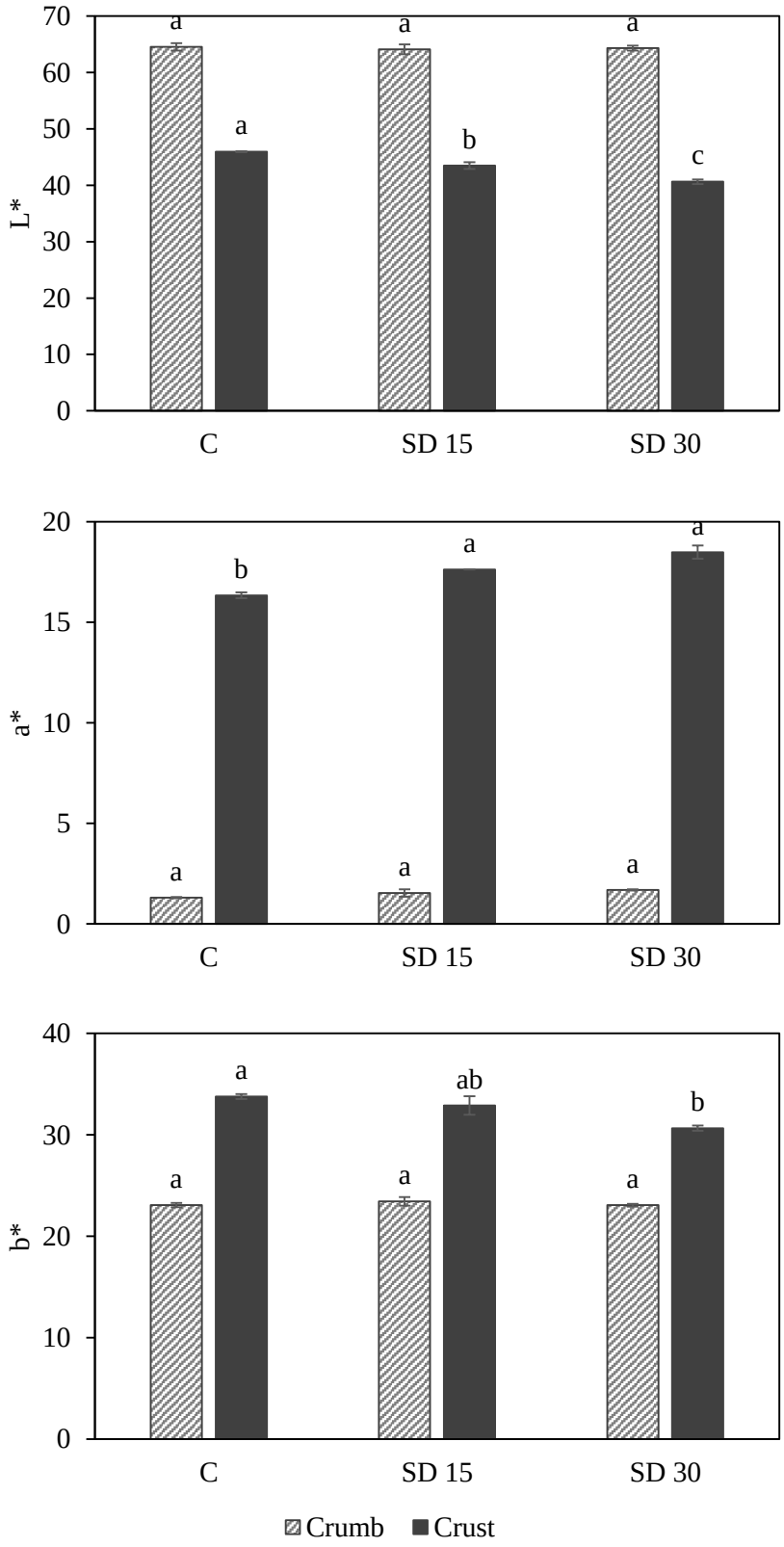


Figure 6.7. Color of crumb and crust of breads (C, control; SD 15, 15% sourdough; SD 30, 30% sourdough). Means having different letters within the same bread part are significantly different ($p<0.05$).

6.2.4.4. Moisture Content

The crumb and slice moisture contents of control and sourdough breads were seen in Table 6.2. No statistically significant differences in crumb and slice moisture were observed between samples at all storage times ($p>0.05$). Moreover, for each bread, no statistically important differences was observed in slice moisture during storage ($p>0.05$). However, the crumb moisture of individual bread samples decreased with time ($p<0.05$). The redistribution of moisture from crumb to crust is one of the most important reasons of bread staling. During storage, moisture is transferred from crumb to crust resulting in hardening of crumb and softening of crust while remaining the total bread moisture constant. Moisture loss from crust might also happen in prolonged storage times. Additionally, the breads were stored in plastic bags which limit the excess moisture loss. In one of a study that gluten-free breads were stored in paper bags, although the crumb moisture loss was very low, the slice moisture decrease was found more predominant (Cappa et al., 2013a).

Table 6.2. Crumb and slice moisture of breads during storage

		Bread		
	Time (h)	C	SD 15	SD 30
Crumb moisture (%)	2	52.00 ± 0.22^{a}	51.58 ± 0.13^{a}	51.51 ± 0.02^{a}
	23	51.20 ± 0.11^{ab}	50.84 ± 0.31^{b}	51.01 ± 0.09^{ab}
	47	50.41 ± 0.17^{bc}	50.02 ± 0.44^{c}	50.29 ± 0.09^{bc}
	71	49.71 ± 0.42^{c}	49.34 ± 0.69^{d}	49.67 ± 0.33^{c}
Slice moisture (%)*	2	41.39 ± 0.49	40.29 ± 0.23	40.38 ± 0.25
	23	40.62 ± 0.16	40.16 ± 0.69	40.37 ± 0.30
	47	40.66 ± 0.28	40.26 ± 0.77	40.17 ± 0.76
	71	40.77 ± 0.28	39.92 ± 0.56	39.87 ± 0.57

C, control; SD 15, 15% sourdough; SD 30, 30% sourdough.
Values are mean ± SD.
Means having different letters in the same column are significantly different ($p<0.05$).
* No significant changes observed between samples and during storage.

6.2.4.5. Texture

The crumb structures of the breads were seen in Figure 6.8. As the sourdough level increased, the crumb structure became coarser.

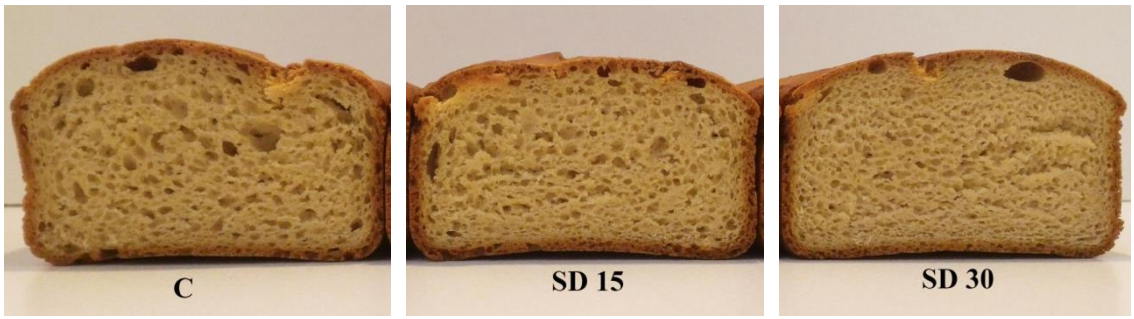


Figure 6.8. Crumb appearance of gluten-free bread samples (C, control; SD 15, 15% sourdough; SD 30, 30% sourdough).

The texture results were given in Figure 6.9. No significant differences were observed for control and sourdough containing fresh and stored breads ($p>0.05$). During storage, hardness and chewiness were increased and, cohesiveness and resilience decreased. No change was observed for springiness value ($p>0.05$). In agreement to our results, Schober et al. (2007) reported no significant differences in the hardness of the sorghum-containing sourdough and control breads at any storage time during 7 days. A reduction in crumb hardness was reported by Axel et al. (2015) for quinoa sourdough bread fermented with *L. amylovorus* DSM19280. On the other hand, Galle et al. (2012) observed increased crumb hardness for sorghum bread containing 10% and 20% of sourdough without sucrose. Differently, Moore et al. (2008) found no differences in hardness at day 0 and 2, however at the 5^{th} day of storage, bread containing sourdough had softer crumb compared to control breads.

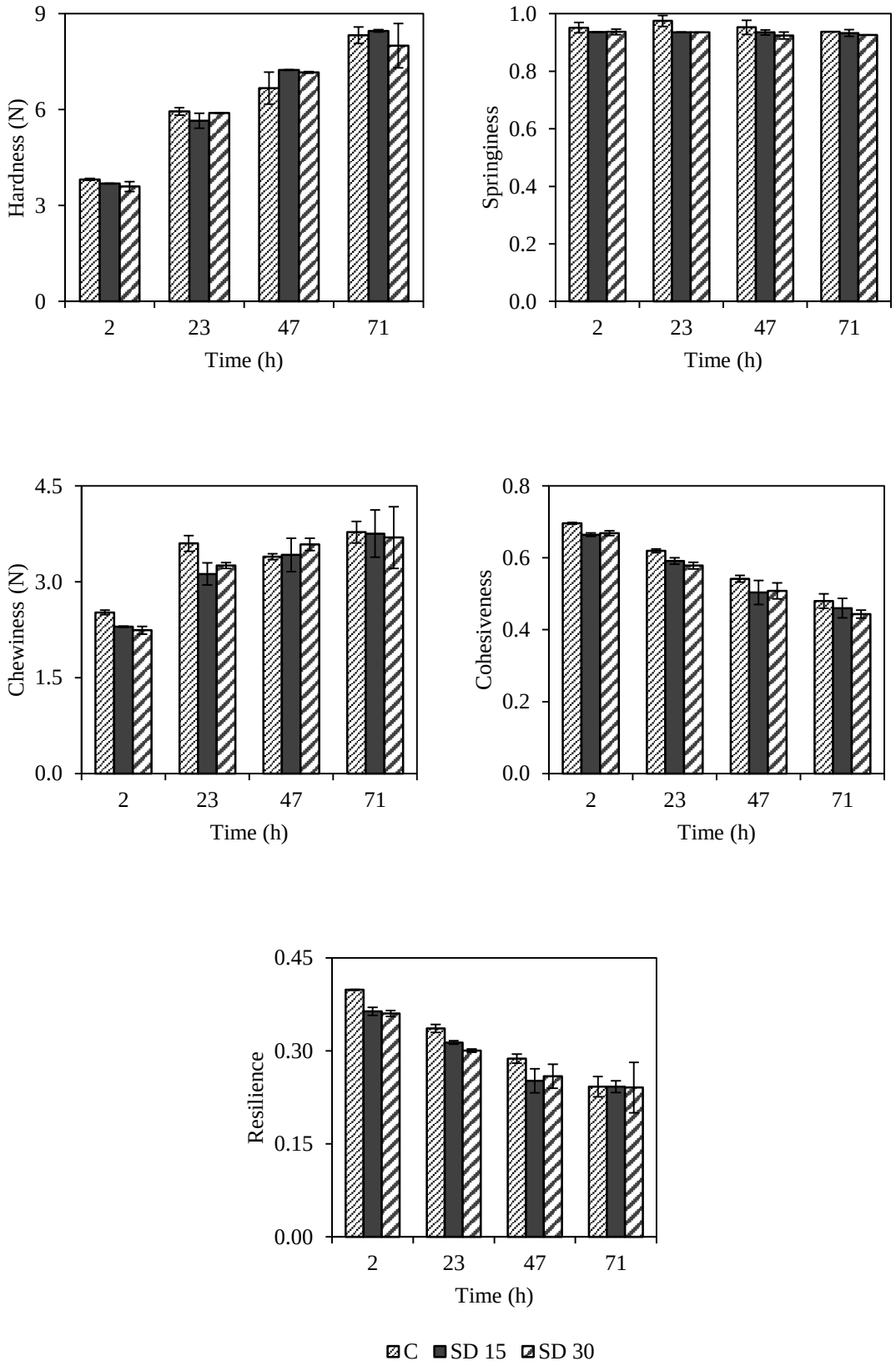


Figure 6.9. Texture parameters of breads (C, control; SD 15, 15% sourdough; SD 30, 30% sourdough).

6.2.4.6. *In Vitro* Protein Digestibility

The *in vitro* protein digestibility curve of control and sourdough breads was given in Figure 6.10. It was observed from this figure that protein digestibility was influenced by the level of inoculum. At the lower sourdough concentration such as 15%, *in vitro* protein digestibility was not affected (SD15=85.58±0.28%). At the high sourdough addition level (30%) slight, but significant increase in protein digestibility was observed ($p<0.05$); protein digestibility values were 86.32±0.27% for SD30 and 85.30±0.14% for C. In agreement with our results, the effect of sourdough fermentation on increasing protein digestibility was reported by Chinma et al. (2016).

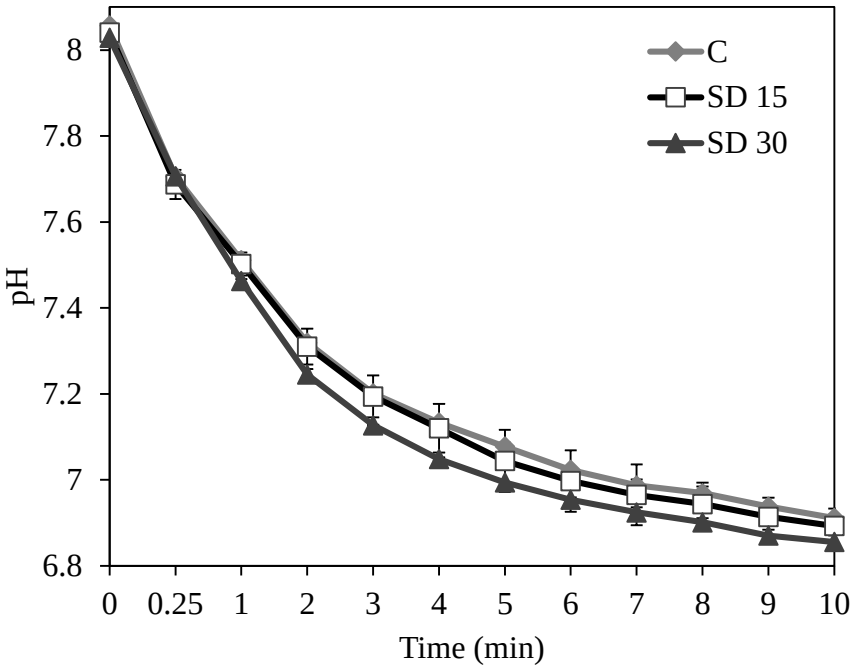


Figure 6.10. Protein digestibility curves of sourdough breads (C, control; SD 15, 15% sourdough; SD 30, 30% sourdough).

6.2.4.7. Sensory Evaluation

The sensory evaluation results were given in Table 6.3 and Figure 6.11. Increasing amounts of sourdough in bread caused a decrease in the liking of all sensory attributes. Decrease in volume and porosity might affect the appearance negatively. It is thought that, biochemical reactions, which occurred during sourdough fermentation such as production of organic acids and amino acids, were sensible at the effective sourdough addition level. It is likely to cause a profound effect especially on sensory scores related to the taste and texture of bread. Slight increase in crust color due to increasing sourdough content might be responsible for low crust color score.

Table 6.3. Sensory evaluation results of sourdough gluten-free breads (C, control; SD 15, 15% sourdough; SD 30, 30% sourdough)

Sample	Appearance	Crust Color	Crumb Color	Odor	Texture	Flavor	Overall Acceptability
C	7.33 ± 0.13^{a}	7.43 ± 0.11^{a}	7.44 ± 0.15^{a}	7.17 ± 0.01^{a}	7.18 ± 0.09^{a}	7.40 ± 0.10^{a}	7.42 ± 0.05^{a}
SD15	7.18 ± 0.10^{a}	7.11 ± 0.01^{ab}	7.28 ± 0.08^{ab}	6.85 ± 0.02^{a}	7.09 ± 0.12^{a}	7.11 ± 0.09^{a}	7.19 ± 0.13^{a}
SD 30	6.56 ± 0.31^{a}	6.82 ± 0.17^{b}	6.95 ± 0.01^{b}	6.55 ± 0.30^{a}	6.44 ± 0.08^{b}	6.29 ± 0.00^{b}	6.43 ± 0.10^{b}

C, control; SD 15, 15% sourdough; SD 30, 30% sourdough.
Values are mean ± SD.
Means having different letters in the same column are significantly different ($P<0.05$).

In Figure 6.11, the personal liking scores of each bread were given as graphical representation. Although all breads possessed acceptable sensory scores and overall acceptability higher than 5, it could be concluded that addition of 30% sourdough caused a decrease in the scores of crust and crumb color, texture and flavor.

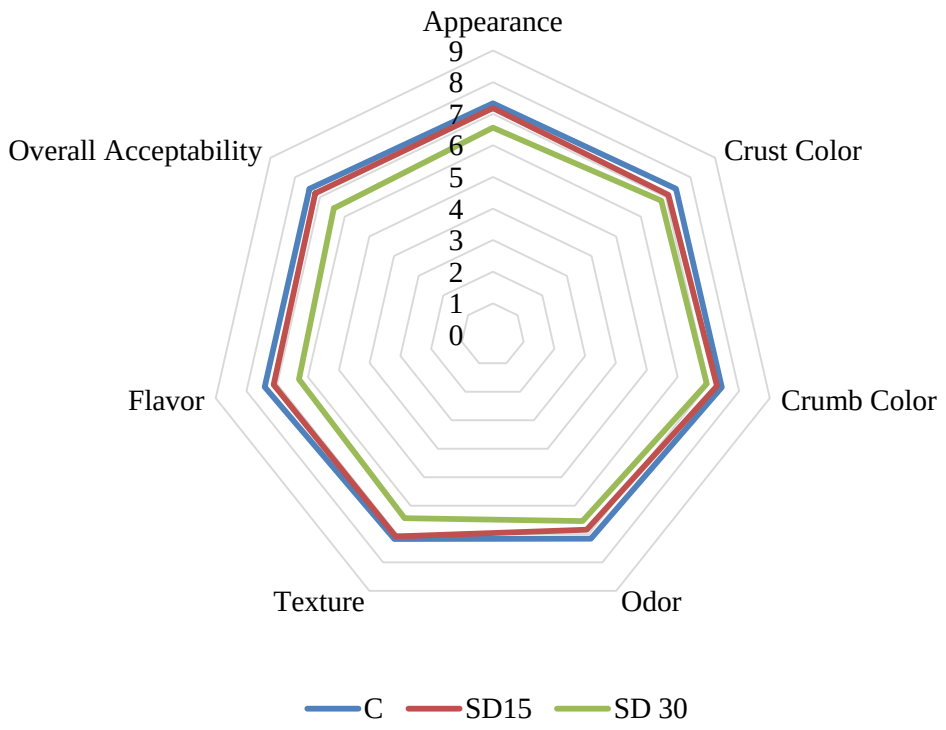


Figure 6.11. Sensory evaluation results of sourdough gluten-free breads (C, control; SD 15, 15% sourdough; SD 30, 30% sourdough)

6.3. Conclusions

L. sanfranciscensis ED-5C was used to ferment the roasted chickpea-rice flour blend. As an indicator of the adaptability of this strain to blend flour environment, in line with the lactic acid bacteria growth, organic acid production and pH decrease were observed. Inevitably, these changes leaded a gradual increase in the acidity and decrease in pH of breads containing 15 and 30% sourdough. Although the addition of 30% sourdough to the bread formulation significantly decreased ($p<0.05$) the specific volume, no significant effect was observed for textural properties of crumb even for fresh or stored bread. Control and sourdough breads showed high sensory scores, for all attributes higher than 6, however the presence of 30% sourdough in gluten-free bread significantly caused a reduction in the overall acceptability ($p<0.05$).

CHAPTER 6

CONCLUSIONS AND FUTURE PERSPECTIVES

Nowadays, numbers of people having special dietary requirements due to some health conditions. Gluten-related disorders, celiac disease in particular, have also high prevalence and can be treated by only a gluten-free diet. In order to follow a strict gluten-free diet, availability of the products is of great importance.

Recently, legumes are gaining interest due to their health benefits. Apart from their consumption as a whole grain, they can find place in many food formulations in flour form as a nutritious ingredient.

In gluten-free product development practices, obtaining a compromise between the levels of ingredients is the key to obtain high quality products. The key component in gluten-free bread formulations is water. The flours, starchs and/or hydrocolloids showed different water absoption; therefore, different levels of water are required for their hydration. Moreover, dough consistency plays a crucial role on bread characteristics and consequently water amount would be properly adjusted in order to obtain optimal quality. For this reason, most of the gluten-free bread optimization studies in literature consider water as a factor. In this PhD study, roasted chickpea flour, HPMC and water levels in rice-based gluten-free bread were optimized by using response surface methodology. The results suggested that HPMC and water amounts should be adjusted carefully due to their dramatic effects on bread quality parameters. It was evidenced that rice flour could be replaced with roasted chickpea flour up to 25%. Validation step is the essential and complementary part of an optimization process. However, it is observed that in most published articles this vital step hasn't been carried out. In this study, obtained model was validated by selecting few points in the optimum region and the breads having these factor combinations were prepared. It was observed that the measured values corresponded well with the predicted responses.

Comparative analysis of flour blends, dough and bread formulations revealed that roasting and dehulling changed the chickpea characteristics. Slower retrogradation tendency observed for chickpea flours is advantageous when they are used in baked foods. Roasted chickpea flour added bread formulation showed superior quality such as

high specific volume, soft texture and low staling rate. Raw and dehulled chickpea enriched breads exhibited low RDS levels compared to roasted chickpea flour added formulation and rice bread. On the other hand, roasted chickpea flour enriched bread exhibited high protein digestibility among all the breads. Chickpea flours enhanced the nutrient composition, mainly resulted in increased protein, ash and fat content, and decreased total starch content. All chickpea flour fortified breads showed higher sensory scores and acceptability compared to rice bread.

The effects of sourdough fermentation on wheat and rye bread have been discussed by many researchers. As regards to gluten-free sourdough bread, contradictory results were reported in literature. Differences in flour compositions and strain types were suggested as the reason of the variances in the results. In this PhD study, sourdough bread of roasted chickpea-rice flour blend has been also evaluated. Sourdough addition level of 15% was not found to be enough to affect the bread structure and characteristics. However, in 30% sourdough added bread, crumb pore size and sensory scores decreased. On the other hand no considerable change was observed in texture parameters. Despite of all, *in vitro* protein digestibility enhanced. This study is expected to contribute the literature with the first-time usage of roasted chickpea-rice flour blend in a sourdough bread formulation inoculated with *L. sanfranciscensis*. The results can form the basis for future studies. It should be noted that detailed studies are needed to further investigate the effects of fermentation conditions and starter culture combinations, and to optimize the parameters.

This study showed the successful application of roasted chickpea flour as a nutritious ingredient in gluten-free bread. The obtained product is believed to meet the consumers' needs in terms of quality and availability. The positive outcomes of this usage might be extrapolated to the usage of broken chickpea grains which are considered as a by-product and readily processed into flour. As a result, the production of relatively low-cost gluten-free bread could be achieved. It is possible that roasted chickpea flours could also be used in different types of products such as cakes, biscuits and soup formulations. Due to the above-mentioned positive outcomes, its usage in also wheat-containing products is thought to be beneficial. Moreover, due to the comprehensive scientific analysis carried out within the scope of this dissertation study, it is expected to contribute the scientific knowledge of the gluten-free field. This study is also believed to have a function to increase the awareness of celiac disease and gluten-free diet.

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APPENDIX A

CORRELATION CURVES

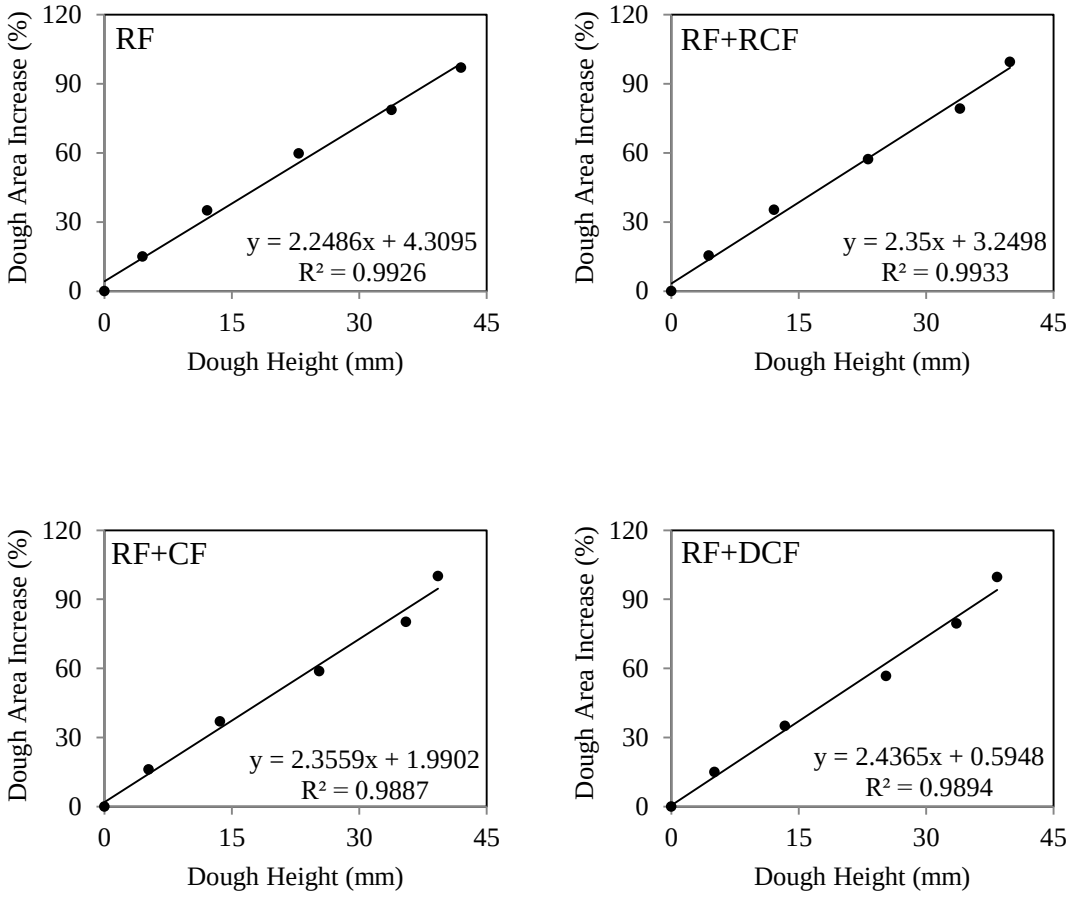


Figure A.1. Correlation between dough area increase and height obtained from image analysis and rheofermentometer, respectively.

APPENDIX B

CALIBRATION CURVES

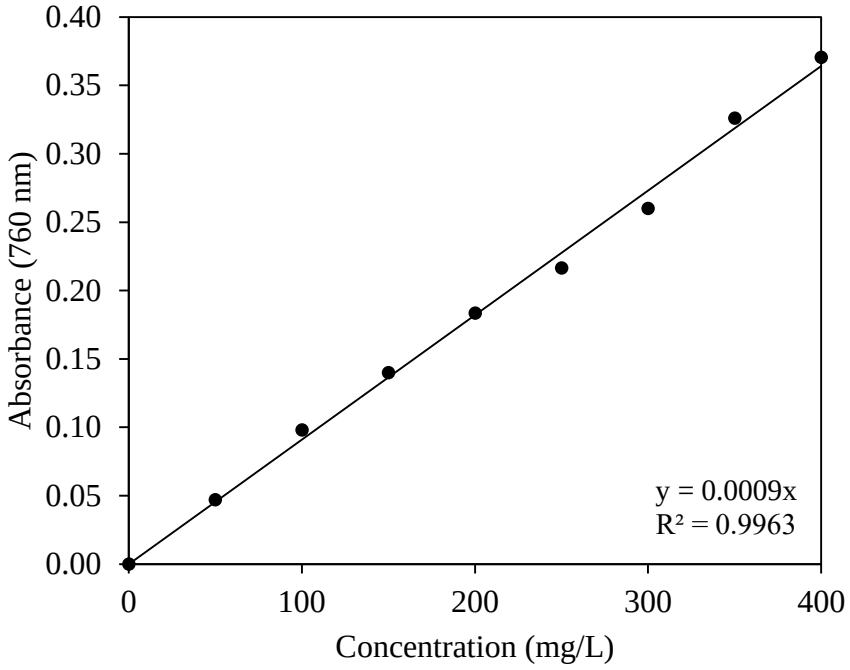
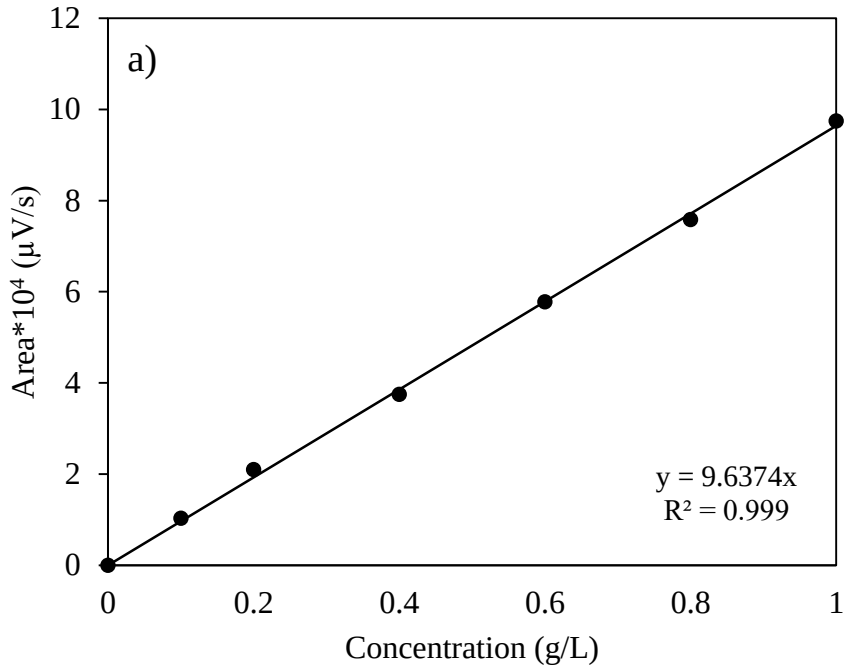


Figure B.1. Standard calibration curve of gallic acid for TPC analysis.



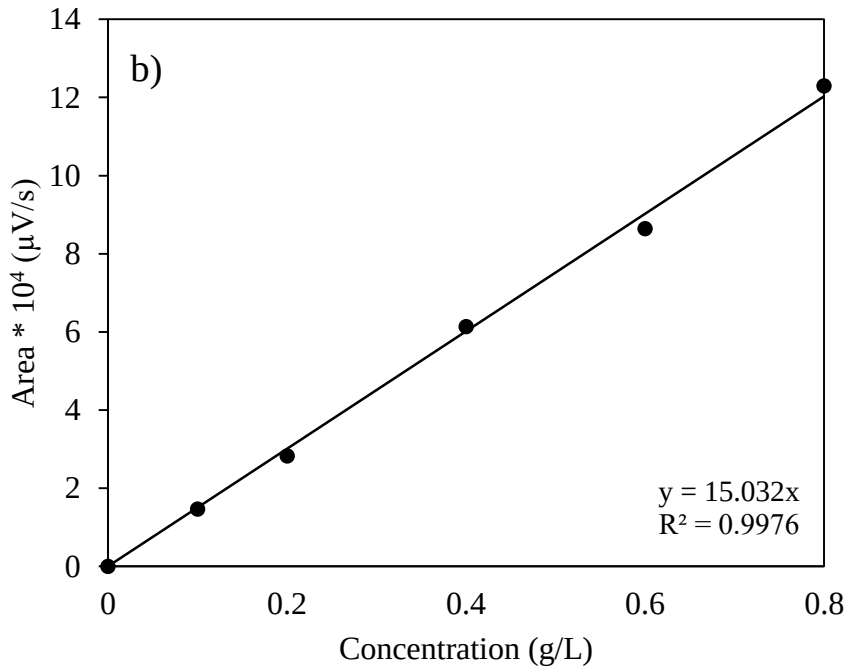


Figure B.2. Standard calibration curves of a) lactic acid and b) acetic acid for organic acid analysis in dough.

APPENDIX C

SENSORY EVALUATION FORM

Table C.1. Example of a sensory evaluation form

Gluten-Free Bread Evaluation Sheet

Name: **Date: …../…../ 2016**

Age: **Gender:**

Please score each gluten-free bread sample individually according to personal liking. Thank you.

(9 = like extremely; 1 = dislike extremely)

Sample Code	Appearance	Crust Color	Crumb Color	Odor	Texture	Flavor	Overall Acceptability
546							
328							
814							
637							

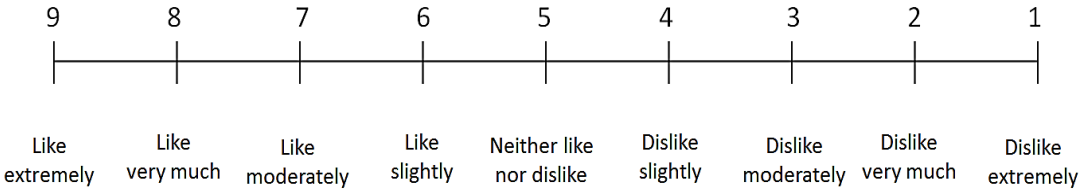


Figure C.1. 9-point hedonic scale used in sensory evaluation of bread

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EDUCATION

Ph.D.	Izmir Institute of Technology, ***Department of Food Engineering*** Thesis: *Development of Gluten-Free Bread Formulations Based on Chickpea Flour: Optimization of Formulation, Evaluation of Dough Properties and Bread Quality*	*2011-2017*
M.Sc.	Izmir Institute of Technology, ***Department of Food Engineering*** Thesis: *Structural Changes of Gliadins during Sourdough Fermentation as a Promising Approach to Gluten-free Diet*	*2008-2011*
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ACADEMIC WORK EXPERIENCE

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JOURNAL PUBLICATIONS & SELECTED CONFERENCE PROCEEDINGS

Komen, G. Baysal, A.H. & Harsa, S. (2013). Gliadin degradation ability of artisanal lactic acid bacteria, the potential probiotics from dairy products. *Journal of Nutritional Therapeutics*, 2, 163-172.

Kahraman, G., Cappa, C., Casiraghi, M.C., Harsa, S., & Lucisano, M. (2016). Use of response surface methodology for optimization of gluten-free bread formulation containing leblebi flour and evaluation of quality and digestibility parameters. *30th EFFoST International Conference*, 28-30 November, Vienna, Austria (*Oral Presentation*).

Kahraman, G., Cappa, C., Lucisano, M., & Harsa Ş. (2016). Optimization of gluten-free bread formulation containing leblebi flour and evaluation of dough and bread properties. *15th International Cereal and Bread Congress (15th ICBC)*, 18-21 April, İstanbul, Turkey, Abstract Book, 54 (*Oral Presentation*).

Komen, G. & Harsa, S. (2012). Artisanal lactic acid bacteria utilization for the development of gluten-free sourdough. *Vth Symposium on Sourdough, Cereal Fermentation for Future Foods*, Helsinki, Finland, Abstract Book, 124 (*Poster Presentation*).

Komen, G., Baysal, A. H., & Harsa, S. (2011). Structural changes of gliadins during sourdough fermentation. *11th International Congress on Engineering and Food (ICEF)*, Athens, Greece, Congress Proceedings, 219-220 (Fulltext) (*Oral Presentation*).

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HONORS & AWARDS

- Visiting Researcher at **University of Milan** (Italy), Department of Food, Environmental and Nutritional Sciences (DeFENS), **Erasmus Grant** (04/10/2015- 12/02/2016).
- Graduated among the 180 Food Engineering students with **6th highest GPA** (2008).
- **1st graduate** of **Bioengineering Minor** Degree Programme among the Food Engineering students of Ege University (2009).
- Vth International Bioengineering Congress, 2010, Izmir, **2nd place in poster presentations**
- **TÜBİTAK**, **International Scientific Meetings Fellowship**-2224 (May 2011).
- The International Life Sciences Institute (**ILSI**), **Travel Grant** to attend International Symposium on Health Benefits of Foods (October 2011).