INFLUENCE OF DIFFERENT INGREDIENTS AND TECHNOLOGIES IN GLUTEN-FREE BREAD QUALITY

Núria Aguilar Puig | 2014

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Departament de
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Doctorat en Ciència dels Aliments

Núria Aguilar Puig

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Planta de Tecnologia dels Aliments UAB

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TECNIO Be tech. Be competitive

Dra. MARTA CAPELLAS PUIG i Dra. ELENA ALBANELL TRULLÁS, professores titulars del Departament de Ciència Animal i dels Aliments de la Universitat Autònoma de Barcelona,

FAN CONSTAR que NÚRIA AGUILAR PUIG ha realitzat, sota la seva direcció, el treball titulat "Influence of different ingredients and technologies in gluten-free bread quality" que presenta per optar al grau de Doctor en Ciència dels Aliments.

I perquè així consti, signen el present document a Bellaterra, Cerdanyola del Vallès, el dia 12 de desembre del 2014.

Dra. Marta Capellas Puig

Dra. Elena Albanell Trullás

La autora de esta tesis ha disfrutado de una beca FPU (AP2009-0859) del Ministerio de Educación, Cultura y Deporte de España

"Que el teu aliment sigui la teva medicina, i que la teva medicina sigui el teu aliment"

Hipòcrates

Aquesta tesi està dedicada a totes aquelles persones que per algun motiu o altre segueixen una dieta lliure de gluten

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Publications and presentations related to this thesis

Publications

Title: Desarrollo de masa madre para pan sin gluten
Authors: Aguilar Puig, N., Miñarro Vivas, B., Albanell Trullás, E., Capellas Puig, M.
Reference: Alimentaria 2013, 443, 50-52

Title: Effect of tiger nut-derived products in gluten-free batter and bread
Authors: Aguilar, N., Albanell, E., Miñarro, B., Guamis, B., Capellas, M.
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Title: Chickpea and tiger nut flours as alternatives to emulsifier and shortening in gluten-free bread
Authors: Aguilar, N., Albanell, E., Miñarro, B., Capellas, M.
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Title: Influence of final baking technologies in partially baked frozen gluten-free bread quality
Authors: Aguilar, N., Albanell, E., Miñarro, B., Gallardo, J., Capellas, M.
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Scientific communications

Title: New ingredients in gluten-free formulations
Authors: Aguilar, N., Miñarro, B., Albanell, E., Guamis, B., Capellas, M.
Meeting: 10th European Young Cereal Scientists and Technologists Workshop
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Title: Effect of tiger nut flour sourdough in gluten-free bread
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Meeting: 11th European Young Cereal Scientists and Technologists Workshop
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Title: Tiger nut flour and chickpea flour as an alternative to shortening in gluten-free bread
Authors: Aguilar, N., Miñarro, B., Albanell, E., Capellas, M.
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ABSTRACT

The increase of people that follow a gluten-free diet due to a gluten-related problem such as celiac disease, non-celiac gluten sensitivity, wheat allergy or because they perceive gluten-free diet as healthier, is causing an important rise of gluten-free products market. Besides, since gluten gives unique viscoelastic properties to wheat dough, gluten-free products, and specially bread, usually have poorer quality compared to their gluten-containing counterparts. For these reasons, continuous development of gluten-free bread formulations to improve their organoleptic and nutritional characteristics and shelf-life is needed.

The influence of different ingredients (tiger nut derived products, chickpea flour, shortening, emulsifier and chestnut flour) and technologies (sourdough and final baking technologies) in gluten-free bread quality has been evaluated. In the first study, the use of tiger nut milk, tiger nut milk by-product and tiger nut flour was assessed in order to replace soya flour. Tiger nut milk improved gluten-free bread characteristics (batter rheology, specific volume, texture and consumers' preference), tiger nut flour rendered breads that were similar to soya flour breads, and tiger nut milk by-product impaired gluten-free bread quality, giving a harder and darker crumb. In the second study, chickpea flour and/or tiger nut flour were added into gluten-free bread in order to partially or totally replace emulsifier and/or shortening. The combination of both flours maintained bread characteristics (bake loss, specific volume, crust and crumb color, and crumb hardness) even when shortening and/or emulsifier were reduced or eliminated. To evaluate the effect of spontaneously fermented chestnut flour sourdough in gluten-free bread, a third study was performed. Chestnut flour sourdough improved gluten-free bread specific volume and crumb texture and structure. However, it had no effect on yeasts and moulds growth and decreased consumers' preference, as sourdough breads were less sweet. Finally, in the fourth study the influence of three final baking technologies (convection oven, microwave oven and microwave oven with susceptor packaging material) in partially baked frozen gluten-free bread was evaluated. Final baking in microwave oven did not induce crust browning and increased crumb hardness. In contrast, microwave oven using susceptor packaging material changed crust color and rendered breads similar to those that were finally baked in convection oven.

RESUM

L'augment de persones que segueixen una dieta lliure de gluten perquè pateixen agluna afecció relacionada amb el gluten com la malaltia celíaca, la sensibilitat al gluten no celíaca o l'al·lèrgia al blat, o perquè perceben que la dieta sense gluten és més saludable, està causant un creixement important del mercat dels productes sense gluten. A més, com que el gluten aporta propietats viscoelàstiques úniques a la massa de blat, els productes sense gluten i, sobre tot el pa, en general tenen una qualitat inferior als seus homòlegs amb gluten. Per aquestes raons, és necessari el desenvolupament continu de formulacions de pa sense gluten per millorar-ne les propietats organolèptiques i nutricionals i la vida útil.

En aquesta tesi s'ha estudiat la influència de diferents ingredients (productes derivats de la xufla, farina de cigró, greix hidrogenat, emulsionant i farina de castanya) i tecnologies (massa mare i tecnologies de cocció final) en la qualitat del pa sense gluten. En el primer estudi es va avaluar la utilització de l'orxata, el residu d'orxata i la farina de xufla per tal de substituir la farina de soja. L'orxata va millorar les característiques del pa sense gluten (reologia de la massa, volum específic, textura i preferència dels consumidors), la farina de xufla va donar uns pans similars als pans obtinguts amb farina de soja, i el residu d'orxata va empitjorar la qualitat del pa sense gluten, resultant molles més dures i fosques. En el segon estudi es van afegir farina de cigró i/o xufla al pa sense gluten per tal de substituir total o parcialment l'emulsionant i/o el greix hidrogenat. La combinació de les dues farines va permetre mantenir les característiques del pa (pèrdues per cocció, volum específic, color de la crosta i la molla i, duresa de la molla) fins i tot quan es va reduir o eliminar el greix hidrogenat i/o l'emulsionant. Per avaluar l'efecte de la massa mare de farina de castanya fermentada espontàniament en el pa sense gluten, es va realitzar un tercer estudi. La massa mare de farina de castanya va millorar el volum específic, la textura i l'estructura de la molla del pa sense gluten. No obstant això, no va tenir efecte en el creixement de fongs filamentosos ni llevats, i va reduir la preferència dels consumidors ja que el pa amb massa mare era menys dolç. Finalment, en el quart estudi es va investigar la influència de tres tecnologies de cocció final (forn de convecció, forn de microones i forn de microones amb material d'envasament amb susceptors) en el pa sense gluten precuit i congelat. La cocció final al forn de microones no va causar enfosquiment de la crosta i va augmentar la duresa de la molla. En canvi, el forn de microones amb material d'envasament amb susceptors va

canviar el color de la crosta i va donar lloc a uns pans similars als obtinguts amb la cocció final al forn de convecció.

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Abbreviation Key

CD	Celiac disease
CSL	calcium stearoyl lactylate
DATEM	diacetil tartaric acid esters of mono- and diacylglycerols
DH	Dermatitis herpetiformis
DM	distilled monoglylcerides
FW	Flour weight
G'	Storage modulus
G"	Loss modulus
GA	Gluten ataxia
GF	Gluten-free
GFD	Gluten-free diet
GMS	glycerol monostearate
HLA	Human leukocyte antigen
HMW	High molecular weight
HP	High pressure
HPMC	Hydroxipropilmethylcelulose
LAB	Lactic acid bacteria
LMW	Low molecular weight
MAP	Modified atmosphere packaging
NCGS	Non-celiac gluten sensitivity
SPM	Susceptor packaging material
SSL	sodium stearoyl lactylate
TTA	Titratable acidity
WA	Wheat allergy
WDEIA	Wheat dependent exercise-induced anaphylaxis

CHAPTER 1:
GENERAL INTRODUCTION

1. Who follows a gluten-free diet?

1.1. Celiac disease

The definition of celiac disease (CD) has changed over the years and recently a new definition that includes the immunological and clinical research advances has been published in ESPGHAN guidelines for the diagnosis of CD (Husby et al., 2012).

"Celiac disease is an immune-mediated systemic disorder elicited by gluten and related prolamines in genetically susceptible individuals and characterized by the presence of a variable combination of gluten-dependent clinical manifestations, Celiac disease-specific antibodies, Human Leukocyte Antigen (HLA)-DQ2 or HLA-DQ8 haplotypes, and enteropathy. Celiac disease-specific antibodies comprise autoantibodies against transglutaminase 2, including endomysial antibodies, and antibodies against deamidated forms of gliadin peptides."

Two conditions are necessary to develop CD: ingestion of gluten and a genetic predisposition to celiac disease (Catassi and Fasano, 2008; Pietzak, 2012) and, nowadays, the only treatment for CD is a lifelong gluten-free diet (GFD) avoiding wheat, rye and, barley in all patients and oats in less than 5% of patients (see section 3.1.), according to World Gastroenterology Organization (2007). In addition, gluten traces could induce intestinal damage in CD patients, and thus, the diet must be very strict. To follow a GFD is difficult due to limited availability, high cost and possible cross contaminations of gluten-free (GF) products. For this reason, studies on alternative therapeutic strategies for CD are being investigated: gluten enzyme degradation to degrade intestinal gluten; polymers that bind and sequester gluten in the small intestine; larazotide acetate, which is an octopeptid that prevents intestinal permeability induced by gliadin; or a vaccine to induce gluten tolerance (Kaukinen et al., 2014; Lerner, 2010; Mäki, 2014).

As mentioned before, HLA haplotypes related to celiac disease are HLA DQ2 and/or HLA DQ8 and are present in 95% of celiac patients. Populations that have not these HLA, present a low risk to develop CD. However, up to 40% of the population present these HLA alleles but most do not develop CD (Catassi and Fasano, 2008; Green, 2009). Recently, Abadie et al. (2011) suggested a role of environmental factors and other genetic factors in CD pathogenesis as they observed different levels of CD prevalence in countries (e.g. Finland

and Russia) with similar levels of wheat consumption and predisposing HLA expression. However, in most studied countries, the authors found a significant correlation between CD prevalence and wheat consumption and between CD prevalence and HLA (DQ2 and DQ8) frequency.

Other genetic factors associated with CD pathogenesis are mainly related with autoimmune diseases like type I diabetes, autoimmune thyroid disease, rheumatoid arthritis, autoimmune liver disease and other diseases such as Down syndrome, Williams syndrome, Turner syndrome, and cystic fibrosis, which increase the risk of CD (Pietzak, 2012). Ivarsson et al. (2003a) suggested that females have an increased risk of suffering CD.

Some environmental factors that affect CD manifestation are related with birth and first months of life: it seems that children born in the summer have increased risk for CD compared with those born in winter (Ivarsson et al., 2003b) and that cesarean delivery is associated with CD (Decker et al., 2010). It has also been observed that early infections are associated with increased risk for CD (Myléus et al., 2012). Breast-feeding delays or reduces the risk of developing CD (Akobeng et al., 2006; Ivarsson et al., 2002). The introduction of large amounts of gluten during the first year of life increases CD risk (Branski et al., 2006), however, if the introduction of dietary gluten is within the period of breast-feeding the CD risk is reduced (Ivarsson et al., 2002).

Ingestion of gluten proteins produces peptides fragments that are absorbed through the mucosal layer of small intestine. The peptide fragment of 33 residues called 33-mer is resistant to hydrolysis due to its high proline content and is one of the fragments that induce toxicity. Anti-tissue transglutaminase of CD patients deaminates these peptides resulting in strong affinity of them for HLA-DQ2 or DQ8 on antigen-presenting cells. These cells stimulate T helper 1 response, which leads to intestinal damage (Pietzak, 2012; Qiao et al., 2004). Typical intestinal damage in CD patients is characterized by loss of absorptive villi and hyperplasia of the crypts and, when gluten containing cereals are removed from the diet, the intestinal damage is recovered (Fasano and Catassi, 2001).

Clinical manifestations of CD are wide and include classic symptoms (typically in toddler and young child) such as abdominal distension, anorexia, irritability, chronic or recurrent diarrhea, failure to thrive or weight loss, vomiting, muscle wasting and fatigue; or non-classic symptoms (typically in older child and adult) including arthritis, aphthous stomatitis,

constipation, dental enamel defects, dermatitis herpetiformis (DH), hepatitis, iron-deficient anemia, pubertal delay, recurrent abdominal pain and short stature. However, some celiac patients, especially adults, present the silent form of the disease and they have no symptoms or minimal complains (Catassi and Fasano, 2008; Rivera et al., 2013). Subjects that present potential form of CD (positivity of endomysial antibody and/or anti-tissue transglutaminase antibodies, predisposing HLA DQ2 or DQ8) have minimal or none intestinal damage but have an increased risk for developing CD. The untreated CD implies the increased risk of suffering associated complications like osteoporosis, impaired splenic function, neurologic disorders, infertility or recurrent abortion, ulcerative jejunoileitis and cancer (Catassi and Fasano, 2008; Fasano and Catassi, 2001).

The DH is a skin manifestation of CD with rash on the elbows, knees, buttocks and scalp, presenting cutaneous IgA deposits. Patients with DH have CD and 65-75% of them show villous atrophy in the upper small intestinal mucosa. The diagnosis is based on skin biopsy and serological evidence of CD autoimmunity. This illness is treated with GFD which ameliorates skin and intestinal abnormalities and prevents from associated disorders and complications (autoimmune diseases, iron-deficient anemia, osteoporosis and malignancy); and dapsone (diaminodiphenylsulfone), an anti-inflammatory antibiotic that suppresses the skin inflammation. It is rare in African and Asian populations and is most common in European individuals, with a prevalence of 0.01, 0.04 and 0.06% in the UK, Sweden and Finland, respectively. This dermatitis can be presented at any age although 40 years is the mean age of onset, and is more common in men than in women (Pietzak, 2012; Salmi et al., 2011; Sapone et al., 2012; Zone, 2005).

Serological tests comprising IgA or IgG anti-tissue transglutaminase antibodies, IgA anti-endomysial antibodies, IgG deamidated gliadin peptides and HLA-DQ2 or HLA-DQ8 are frequently used to diagnose CD. However, a biopsy from small intestine is still necessary to confirm the disease. Marsh classification divides the intestinal damage according to its severity (Figure 1.1). Marsh 1: normal villous with intraepithelial lymphocytosis. Marsh 2: crypt hypertrophy and intraepithelial lymphocytosis. Marsh 3a: partial villous atrophy. Marsh 3b: subtotal villous atrophy. Marsh 3c: total villous atrophy. Diagnosed CD patients usually have villous atrophy degree of Marsh 3. Moreover, when a patient is diagnosed from CD, it is recommended to do serologic tests in all first-degree family members (Fasano and Catassi, 2012; Green, 2009; Hill et al., 2005). The improvement of diagnostic tools for CD

detection has led to an increase of diagnosed CD patients. According to Fasano and Catassi (2001) the prevalence of CD can be represented by the "iceberg model" where the superficial part of the iceberg represents the diagnosed CD and the submerged part are the patients that are undiagnosed. The "water line" of the iceberg is the ratio of diagnosed to undiagnosed cases and depends on awareness, diagnostic facilities and variations in clinical intensity.

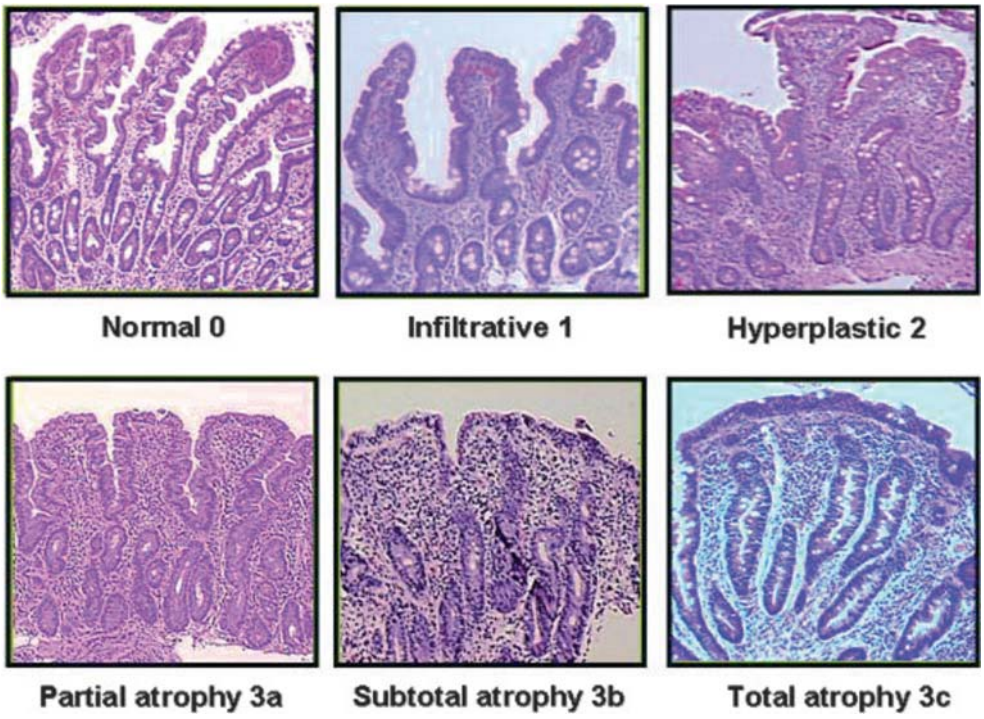

Figure 1.1. Small intestinal mucosa damage of celiac disease according to Marsh classification. Figure from: Rivera et al., 2013 (© 2013 John Wiley & Sons A/S. Published by John Wiley & Sons Ltd).

In the past, CD was classified as a rare disorder that only appeared in young European children. Nevertheless, nowadays it is known that CD is found worldwide including populations such as saharian children in Algeria (which represent the highest prevalence of CD in the world), and from India, Middle East, North Africa and South America (Catassi et al., 1999; Makharia et al., 2011; Malekzadeh et al., 2005; Mandal and Mayberry, 2000) and it can be presented at any age, even in the elderly (Vilppula et al., 2009). Furthermore, CD is considered one of the commonest lifelong disorders affecting 1% of the world population (Husby et al., 2014; Rivera et al., 2013). In Europe, the CD prevalence is also 1% but this value changes depending on the country evaluated: in adult population, CD prevalence is 2.4% in Finland, 0.3% in Germany, and 0.7% in Italy (Mustalahti et al., 2010). In Spain,

prevalence of CD ranges from 1/118 in child population to 1/389 in adult population (Ministerio de Sanidad y Consumo, 2008).

1.2. Non-celiac gluten sensitivity

Non-celiac gluten sensitivity (NCGS) is an emerging disorder related to gluten intake, but different from CD and wheat allergy (WA), which has also been described as gluten hypersensitivity or gluten intolerance. The NCGS is characterized by gastrointestinal or extra-intestinal symptoms similar to CD that improve or disappear when the patient follows a GFD and reappear when gluten is reintroduced (Molina-Infante et al., 2014; Sapone et al., 2012; Tonutti and Bizzarro, 2014; Volta and De Giorgio, 2012). Another characteristic of NCGS is that no intestinal damage is present. The symptoms of NCGS are abdominal pain, abdominal distension/bloating, diarrhea, constipation, eczema, rash, headache, foggy mind, fatigue, depression, anemia, numbness in legs and arms, joint pain (Tonutti and Bizzarro, 2014; Volta and De Giorgio, 2012).

The mechanism by which gluten induces symptoms in NCGS is still unknown (Molina-Infante et al., 2014). However, Sapone et al. (2011) postulated that NCGS is associated with gluten-induced activation of innate immune response, whereas CD is related to adaptive and innate immunity.

Since there are no specific biomarkers to identify this disorder, diagnosis criteria for NCGS is based on discarding CD (negative CD serology, negative duodenal histopathology) and WA (negative immune-allergy tests to wheat), together with possible presence of biomarkers of native gluten immune-reaction (anti-gliadin antibodies), resolution of the symptoms with gluten withdraw and reappearance when gluten is reincorporated (Sapone et al., 2012; Tonutti and Bizzarro, 2014).

The prevalence of NCGS is estimated to be 6% of the USA population and 10% of Spanish population and the treatment of this disease is a GFD, although it is not clear if gluten is the only cause of this disorder or other wheat compounds could also play a role in this disease (Molina-Infante et al., 2014).

1.3. Wheat allergy

Another disease related to gluten is WA which is an immune-mediated disease like CD but, in WA, IgE antibodies trigger the allergic reaction. It can be classified into food allergy, wheat dependent exercise-induced anaphylaxis (WDEIA), baker's asthma and contact urticaria (Sapone et al., 2012).

Codex Alimentarius includes wheat as responsible for most of food allergies. Classic WA (food allergy) is related to wheat ingestion and could affect skin, gastrointestinal track or respiratory tract. Time interval between wheat ingestion and allergic reaction could be immediate (few hours) or non-immediate (from several hours to 1-2 days). The treatment for WA consists in avoiding wheat, rye and barley proteins (Di Sabatino et al., 2013; Hischenhuber et al., 2006; Sapone et al., 2012).

Wheat ingestion and subsequent physical exercise induce an allergenic response caused by ω_5-gliadins in patients that suffer from WDEIA. Clinical symptoms range from generalized urticaria to anaphylaxis (Sapone et al., 2012; Tatham and Shewry, 2008). It is difficult to diagnose WDEIA since exercise level and wheat ingested quantity are variable (Hischenhuber et al., 2006).

Baker's asthma is caused by the inhalation of wheat, cereal flours and dusts and is one of the most prevalent occupational diseases in most countries. The α-amylase inhibitors from wheat are the most important allergens related to this disorder (Di Sabatino et al., 2013; Tatham and Shewry, 2008). Contact urticaria is characterized by erythema and eczema of skin when there is contact with wheat (Di Sabatino et al., 2013).

Diagnosis of WA is based on skin prick tests and in vitro IgE assays (Di Sabatino et al., 2013; Sapone et al., 2012). The prevalence of WA ranges between 0.2-0.9% in adults and 0.4-1.3% in children from both Europe and USA (Morita et al., 2012).

1.4. Gluten-free diet as a treatment in other diseases

Gluten Ataxia (GA) is and idiopathic sporadic ataxia associated with positive anti-gliadin antibodies with or without enteropathy. The GA is characterized by insidious onset and the

mean age of manifestation is 53 years. Its symptoms are related to gait or limb ataxia. Limb ataxia has been detected in 90% of patients with GA and affects lower limbs more frequently than upper. Generally, GA is not related with gastrointestinal symptoms (less than 10%) but one third of GA patients' present enteropathy on duodenal biopsy. The GA patients with prolonged exposure to gluten present an irreversible loss of cerebellar Purkinje cells and thus, early diagnosis and treatment with GFD improve ataxia and prevent its progression. GA patients have increased prevalence of autoimmune diseases, as it happens with CD patients. (Di Sabatino et al., 2013; Hadjivossilou et al., 2008; Pietzak, 2012; Sapone et al., 2012).

There are other diseases that, despite they are not related to gluten intake, are treated with GFD. The effect of GFD has been experimented on patients suffering from schizophrenia, autism, dementia, attention deficit hyperactivity disorder and multiple sclerosis. However, it is not clear if GFD is effective on these cases, and further research is needed (Marí-Bauset et al., 2014; Pietzak, 2012; Sapone et al., 2012). Moreover, up to 15% of the population present symptoms similar to intestinal bowel syndrome which improve with a GFD (Biesiekierski et al., 2013).

1.5. The gluten-free market

The selection of wheat varieties with higher gluten content over the past 10,000 years has led to increase the proportion of toxic gluten peptides 33-mer and seems to be the explanation for the adverse reactions of human organism to gluten. In addition, gliadin increases epithelial permeability and oxidative stress, and induces apoptosis (Biesiekierski et al., 2013; Molberg et al., 2005; Sapone et al., 2012). A recent study observed that apparently asymptomatic patients with positive endomysial antibodies showed benefits like improved gastrointestinal symptoms, reduced indigestion, anxiety and better health when followed a GFD, and most of evaluated subjects expected to continue the GFD (Kurppa et al., 2014).

Although there is lack of scientific evidences of the effect of GFD on healthy population, it has gained popularity in recent years. Some people perceive this diet as healthier and claims such as better sleep, increased energy, thinner thighs and faster weight loss are associated to GFD. For this reason, GF market increased a 28% between 2004 and 2011 in USA. It has

been estimated that GF products generated 2.6 billion dollars in US market in 2012, and it is expected that it will exceed 5 billion dollars in 2015 (Gaesser and Angadi, 2012; Marcason, 2011; Sapone et al., 2012). Over than 15% of consumers are following a GFD, nevertheless, people suffering CD represent a minority of the whole GF market (The Gluten-Free Agency, 2014).

2. Gluten-free bread

2.1. What is gluten?

The Codex Alimentarius Commission defines gluten as a protein fraction from wheat, rye, barley, oats or their crossbred varieties and derivatives that is insoluble in water and 0.5 M NaCl, to which some persons are intolerant. Most patients with CD can tolerate limited quantities of uncontaminated oats. However, the contamination of oats with wheat, rye or barley is usual and, for that reason, oats are included in the standardized definition of gluten (Codex Alimentarius Commission, 2008; Rashid and Khan, 2011).

According to Osborne classification, cereal proteins can be divided into four groups depending on their solubility: albumins, which are water soluble; globulins, which are soluble in salt solutions; prolamins (named gliadin in wheat, secalin in rye, hordein in barley, avenins in oat, oryzin in rice, zein in maize and kaifirine in sorghum and millet), which are soluble in alcohol–water mixtures; and glutelins (named glutenin in wheat, secalinin in rye, hordenin in barley), which are soluble in diluted acid or alkali (Belitz et al., 2009; Edwards, 2007). Albumins and globulins are mainly metabolic proteins (such as enzymes and enzymes inhibitors) present in the aleurone layer and embryo of the grain cereal. Prolamins and glutelins are storage proteins located in the grain endosperm and their function is to provide the embryo with nitrogen and aminoacids during germination and represent the 80-85% of wheat proteins (Arendt et al., 2008; Wieser et al., 2014).

The gliadin fraction of wheat protein is the most CD-toxic, however, if it is hydrolyzed with acid, it can be harmless. The glutenin fraction of wheat has been reported to be nontoxic, weakly toxic or toxic but it should be considered that glutenin fraction can be contaminated by gliadins. The prolamins of rye (secalins) and barley (hordeins) also induce CD toxicity

(Wieser et al., 2014). All toxic cereals (wheat, rye and barley) belong to the same tribe *Triticeae*. Oats, which toxicity is controversial, belong to the same subfamily (*Pooideae*) as toxic cereals, and the safe cereals (rice, corn, sorghum and millet) have separate evolutionary lines: corn, sorghum and millet belong to subfamily *Panicoideae* and rice belongs to subfamily *Bambusoideae*.

In bakery science, the term gluten is referred to the formation of a viscoelastic mass when wheat flour is mixed with water due to the interaction of gliadins (wheat prolamins) and glutenins (wheat glutelins). Gluten proteins give water absorption capacity, cohesivity, viscosity and elasticity to wheat dough, making it to have unique baking properties (Arendt et al., 2008; Khan and Nygard, 2006; Wieser, 2007). The viscoelastic properties of gliadins and glutenins allow to develop the gluten network in wheat dough which is able to retain the gas produced during fermentation providing a porous, spongy and elastic crumb after baking (Belitz et al., 2009; Khan and Nygard, 2006).

Gliadins (wheat prolamins) are monomeric proteins termed α-, β-, γ-, and ω-gliadins according to their electrophoretic mobility (from fastest to slowest). Their molecular weight range from 28,000 to 55,000 and their structure is formed by single-chain polypeptides with intrachain disulfide bonds. Gliadins represent 40-50% of total wheat protein and their aminoacid composition is characterized by very high levels of glutamine (35%), high levels of proline (20%) and low levels of arginine, lysine, histidine, aspartic acid and glutamic acid. Gliadins contribute to viscosity and extensibility of wheat dough as, when hydrated, have little elasticity and are less cohesive than glutenins (Cornell, 2003; Khan and Nygard, 2006; Wieser, 2007).

Glutenins (wheat glutelins) are polymeric proteins linked by interchain disulfide bonds with a molecular weight ranging from 500,000 to more than 10 million. Reducing agents brake glutenins into subunits that can be divided into high molecular weight (HMW) subunits (67,000-88,000) and low-molecular-weight (LMW) subunits (32,000-35,000). HMW subunits are lower in glycine and higher in valine, isoleucine, leucine and phenylalanine compared to LMW. Between 30 and 45% of wheat protein are glutenins and they have high quantity of glutamine (13%) and proline. Glutenins size influences dough characteristics and glutenin macropolymer amount in wheat flour is correlated with dough strength and loaf volume. Glutenins contribute to dough strength and elasticity since, when hydrated, are

cohesive and elastic (Cornell, 2003; Khan and Nygard, 2006; Wieser, 2007).

2.2. Definition of gluten-free products

The GF foods are defined by Codex Alimentarius Commission (Codex Alimentarius Comission, 2008) as dietary foods:

a) *consisting of or made only from one or more ingredients which do not contain wheat (i.e., all* Triticum *species, such as durum wheat, spelt, and kamut), rye, barley, oats or their crossbred varieties, and the gluten level does not exceed 20 mg/kg in total, based on the food as sold or distributed to the consumer, and/or*

b) consisting of one or more ingredients from wheat (i.e., all Triticum *species, such as durum wheat, spelt, and kamut), rye, barley, oats or their crossbred varieties, which have been specially processed to remove gluten, and the gluten level does not exceed 20 mg/kg in total, based on the food as sold or distributed to the consumer.*

The standard also points out that the allowance of pure, uncontaminated oats may be determined at the national level and that products processed to reduce gluten content until 20-100 mg/kg must not be labelled as GF (Codex Alimentarius Comission, 2008; Rashid and Khan, 2011).

2.3. The challenge of gluten-free bread making

As mentioned before, gluten is responsible for wheat dough characteristics and thus, lack of gluten renders GF dough which is less cohesive and elastic than wheat dough, and cannot retain gas formed during fermentation. Actually, the behavior of GF dough is more similar to a batter than to a dough. For that reason, GF products, and specially bread, are characterized by having low volume, pale light crust, crumbly texture, poor crumb structure and mouth-feel, and short shelf-life. Therefore, the production of GF products, particularly bread, with good baking and sensory characteristics is a big challenge and researchers have been studying different ingredients such as allowed flours, hydrocolloids, emulsifiers, shortenings, proteins, enzymes, and technologies in order to replace gluten. These

alternatives inevitably lead to an increase in bread production cost (Moroni et al., 2009; O'Shea et al., 2014; Rosell et al., 2014; Zannini et al., 2012).

Bread is a staple food in many countries and it is the base of food pyramid due to its nutritional profile as it provides macronutrients, micronutrients and some minerals (Rosell, 2011). Because of its formulation, nutritional quality of GF products is usually inadequate due to the deficiency of B vitamins, minerals, fiber and protein, and excess of fat (mainly saturated) and carbohydrates with high glycemic index. (Matos and Rosell, 2011; Miranda et al., 2014; Thompson, 2009; Zannini et al., 2012). Thus, the improvement of nutritional profile of GF bread is another challenge for food researchers and industry.

3. Ingredients used for gluten-free bread making

3.1. Cereal flours

Cereals are monocotyledon grasses from the family *Poaceae* or *Graminaceae* and are the most important staple food worldwide. Wheat, rye and barley (gluten containing cereals) come from the same subfamily (*Pooideae*) and the same tribe (*Triticeae*). Oats belong to the same subfamily of wheat, rye and barley but to a different tribe (*Aveaneae*). Maize, millet, rice and sorghum (cereals without gluten) have separate evolutionary lines than other cereals. Cereal grains are a source of carbohydrates (70-80%), proteins (8-13%), B-vitamines and tocopherols. In this section, the cereals that do not contain gluten and are used as GF bread ingredients are exposed.

Maize (*Zea mays* L. ssp. *mays*) or corn is an economically important crop plant and a model plant for studying genetics which was originated ~7,000-12,000 years ago in the region of Mexico and Guatemala (Wrigley et al., 2004). In 2013, total maize production in the world was 1,016.7 million tonnes and the US was the major producer (34.3% of the world production) followed by China (21.4% of the world production). In Spain, maize production was 4,853,600 tonnes in 2013 (FAOSTAT, 2014). Maize flour, maize starch (or corn starch) and maize prolamins (zein) can be used to produce GF bread. However, literature on maize flour GF bread is limited. Olatunji et al. (1992) elaborated GF bread with maize flour and raw cassava starch in a proportion of 70:30. Maize flour breads were studied by Sanni et al.

(1998), who used maize flour and maize starch (70:30) and by Edema et al. (2005) who used blends of 80-90% of maize flour and 10-20% of soya flour. Brites et al. (2010) evaluated the effect of maize varieties, milling process, formulation and processing variables on the quality of GF maize bread. De la Hera et al. (2012) assessed the influence of maize flour particle size on GF bread making (see section 4.1.1). Recently, Hager and Arendt (2013) investigated the effect of hydroxypropylmethylcellulose (HPMC), xanthan gum and their combination in different GF breads based on maize or other flours (see section 3.10).

Andersson et al. (2011) stated that zein protein, which is the prolamin of maize, improved GF bread quality when it was added together with hydrocolloids. However, zein-starch dough and bread had no acceptable quality.

Millet is a term referred to "small seed grain" and includes different cereals that can be very different one to each other although all of them are from the same grass family (*Poaceae*) and from the two tribes *Paniceae* and *Chlorideae*. The most important millet species are pearl millet, foxtail millet, proso millet, finger millet, teff, fonio, Japanese millet and kodo millet (Taylor and Emmambux, 2008; Wrigley et al., 2004). World production of millet in 2013 was 29.9 million tonnes being India (36.5% of the world production) and Nigeria (16.8% of the world production) the most important millet producers (FAOSTAT, 2014).

Teff (*Eragrostis tef*) is a kind of millet mainly grown in Ethiopia and its flour has a nutritional profile similar to wheat flour. Although teff has no gluten forming proteins, a type of bread called "injera" is produced in Ethiopia. Teff flour is mixed with water, fermented and 20% of the fermented batter is removed and cooked to obtain a viscous dough called "absit" which is mixed again with the uncooked dough to obtain "injera" bread. Therefore, viscoelastic properties of "absit" could be used for GF bread making (Zannini et al., 2012). Hager and Arendt (2013) investigated the effect of HPMC, xanthan gum and their combination on different GF breads based on teff or other flours (see section 3.10). Renzetti et al. (2008) evaluated the effect of transglutaminase on GF batters and breads based on teff or other flours. A teff sourdough destined to GF bread production was developed by Moroni et al. (2011).

Oats (*Avena sativa*) are a staple food in Germany, Ireland, Scotland and the Scandinavian countries. In 2013, 23.8 million tonnes of oats were produced in the world being Russia (20.7% of the world production) and Canada (16.3% of the world production) the major oats

producers. In Spain, oats production was 956,800 tonnes in 2013 (FAOSTAT, 2014). Oats have high nutritional value containing unsaturated fatty acids, B-vitamins and minerals. In addition, oats have health benefits that are related to their soluble fiber and β-glucan content, which contribute to blood glucose levels regulation and blood cholesterol reduction (Hüttner and Arendt, 2010; Richman, 2012; Wrigley et al., 2004). Oats have been typically avoided in GFD but there is a controversy about its toxicity for CD patients. Oats can be contaminated with wheat, rye or barley during grain harvesting, transport, storage and processing. The latest studies suggest that the risk of oats consumption for CD patients could be less noxious than first thought and that it may be related to oats strain (Richman, 2012; Rosell et al., 2014). The Codex Alimentarius Commission (2008) stated that oats can be tolerated by most, but not all, people who are gluten-intolerant and thus, the acceptance of oats that are not contaminated with wheat, rye, or barley in foods covered by the Codex standard may be determined at the national level. The introduction of uncontaminated oats into GFD would suppose that more products would be available for CD patients and they would benefit from nutritional properties of oats (Hüttner and Arendt, 2010; Richman, 2012).

Rice (*Oryza sativa*) is one of the most important staple foods in the world providing 27% of the total energy intake and 20% of dietary protein intake in developing countries, and 4% of total energy intake in developed countries. The rice grain is rich in complex carbohydrates, and represents a source of proteins, minerals, and vitamins, mainly of B group (Rosell and Marco, 2008). Rice production in 2013 was 745.7 million tonnes being China (27.3% of total production) and India (21.3% of total production) the world's most important rice producers. In 2013, Spain produced 851,500 tonnes of rice (FAOSTAT, 2014).

Rice flour is one of the most suitable for GF bread making due to its bland taste, white color, digestibility and hypoallergenic characteristics and GF based on rice flour or starch can be considered the standard GF breads (Rosell and Marco, 2008). Moreover, rice low sodium and prolamin content and the presence of easily digestible carbohydrates are valuable properties for products intended for patients that suffer allergies (Rosell et al., 2014). However, rice flour has deficient baking qualities because rice proteins do not provide the viscoelastic characteristics necessary to retain gas formed during dough fermentation (Zannini et al., 2012). For that reason, addition of hydrocolloids (typically HPMC) and/or other flours and starches are usually utilized for rice GF bread production (Schober, 2009).

Sorghum (*Sorghum bicolor* L. Moench) is an important staple food in many arid parts of the world since it can grow in drought conditions where other cereals fail. The most important sorghum producers in the world in 2013 were USA (16.1% of the world production) and Nigeria (10.9% of the world production), followed by Mexico (10.3% of the world production). Total world production was 61.4 million tonnes and Spain production was 44,300 tonnes in 2013 (FAOSTAT, 2014). Sorghum and maize are related members of the subfamily *Panicoidae* that pertain to *Poaceae* family. Between 70-90% of the total grain protein are prolamins called kafirins (Schober and Bean, 2008). Several studies about GF bread based on sorghum have been reported and it seems that 20-30% of pure starch and high water levels are required to formulate GF bread with this flour (Schober, 2009). Starches (Onyango et al., 2011), hydrocolloids (Velázquez et al., 2012), emulsifiers (Onyango et al., 2009), sourdough starters (Schober et al., 2007) and enzymes (Onyango et al., 2010a,b) can improve the quality of sorghum GF bread.

3.2. Pseudocereal flours

Pseudocereals are plants that produce starchy grains like cereals but, on the contrary, they are dicotyledonous instead of monocotyledonous plants. Due to their nutritional properties such as protein content and quality, and fiber and mineral content, the interest for pseudocereals has increased, and specially, for the production of GF products.

Amaranth (*Amaranthus* spp.) was a staple food of Aztecs but after the Spanish conquest it was forbidden due to religious causes. However, due to its nutritional properties, the interest for amaranth has spread once more and this crop has been rediscovered. In Central and South America amaranth is still cultivated and, in China, USA and some areas of Europe, this pseudocereal is also produced as a minor crop for human nutrition purposes (Schoenlechner et al., 2008; Wrigley et al., 2004).

Amaranth starch has small size granule and is high in amylopectin giving good stability among to freezing and thawing and to retrogradation (Schoenlechner et al., 2008). The protein content of amaranth ranges from 11.7% to 18.4% and is higher than most of cereal grains and other pseudocereals as quinoa and buckwheat (Mariotti et al., 2009). Amaranth protein is very low in prolamins and high in albumins and globulins and its aminoacid profile

is more balanced than that of some cereals as it is high in lysine, which is reported to be low in cereals, and its essential aminoacid content is 47.7% of protein (Drzewiecki et al., 2003). Amaranth has between 6.6% and 10.3% of fat (2-3 times higher than in cereals) which is rich in unsaturated fatty acids, specially, linoleic acid, and it also contains tocotrienols and squalene (Alvarez-Jubete et al., 2010a; Schoenlechner et al., 2008). Moreover, amaranth is a good source of dietary fiber (8-17%), some vitamins (B group and vitamin E) and some minerals (Ca, Mg and Fe) (Singh and Singh, 2011).

Some researchers have studied the effect of amaranth flour on GF bread and they have observed that it improved viscoelastic properties of GF batter (Mariotti et al., 2009), the quality of GF bread and its nutritional characteristics (Alvarez-Jubete et al., 2009; Gambus et al., 2002; Schoenlechner et al., 2010).

Buckwheat is a pseudocereal that can be classified into common buckwheat (*Fagopyrum esculentum*) or tartary buckwheat (*Fagopyrum tataricum*) and originates from China. Major producers and consumers of buckwheat are Russia, China, Kazakhstan and Ukraine. The total buckwheat production in 2013 was 2.5 million tonnes being Russia the first producer in 2013 (32.7% of world production) and China the second (28.8% of world production) (FAOSTAT, 2014). Recently, the interest for buckwheat has increased due to its healthy properties as its regular consumption may improve cholesterol and lipid metabolism and insulin resistance, and prevent obesity, hypertension and cardiovascular diseases (Takahama et al., 2011; Wrigley et al., 2004).

Buckwheat contains high amounts of resistant starch (33.5-37.8% of total starch) which is an interesting ingredient to formulate foods with low glycemic index (Schoenlechner et al., 2008). Protein content of buckwheat is 12.5% (on dry basis), with salt-soluble globulins being the main. Its aminoacid composition is well balanced and rich in lysine and arginine compared to cereals and 39% of its protein is composed by essential amino acids (Alvarez-Jubete et al., 2009; Drzewiecki et al., 2003; Schoenlechner et al., 2008). Lipid content is 2.1% on dry basis, with 80% of unsaturated fatty acids, mainly linoleic, oleic and palmitic (Alvarez-Jubete et al., 2009; Steadman et al., 2001). Buckwheat is rich in fiber (29.5% on dry basis, 20-30% of which is soluble fiber), minerals (Mg, Se, Fe, K, Ca, Cu, Mn and Zn), vitamins (thiamine or B_1, riboflavin or B_2 and pyridoxine or B_6), flavonoids and polyphenols (Alvarez-Jubete et al., 2009; Wrigley et al., 2004).

There are several studies on the effect of buckwheat flour in GF bread quality. Hager and Arendt (2013) reported higher specific volume and softer crumb on buckwheat GF bread compared to maize flour bread. The increase of buckwheat flour (10, 20, 30 and 40%) in GF bread based on corn starch improved its specific volume, texture and delayed staling (Wronkowska et al., 2013). Mariotti et al. (2013) stated that 40% of buckwheat flour improved GF bread quality due to the better leavening properties related to the rise of fiber that increased batter viscosity. Moreover, Alvarez-Jubete et al. (2009) observed that buckwheat enhanced nutritional value of GF bread. However, the increase of buckwheat flour in GF bread based on rice flour increased crumb hardness although all breads tested presented acceptable sensory properties (Torbica et al., 2010).

Chia (*Salvia hispanica* L.) is considered a pseudocereal and an oilseed due to its high oil content. It was first used as early as 3500 BC as one of the basic foods of Central American in pre-Columbian civilizations. Nowadays, it is still being cultivated in South and Central America and Australia (Ayerza and Coates, 2011; Coates, 2011). Chia provides health benefits related to cardiovascular disease, obesity, intestinal transit, type II diabetes and some types of cancer due to its nutritional profile since it is rich in proteins (19-23%), antioxidants, fiber and unsaturated fatty acids, being the plant source with the highest amount of α-linolenic acid (68%) compared with camelina, perilla and flax (Ayerza and Coates, 2011; Costantini et al., 2014; Sandoval-Oliveros and Paredes-López, 2012). Moreira et al. (2012, 2013a) evaluated the effect of chia flour in GF batters based on chestnut flour and observed that chia flour together with hydrocolloids improved chestnut flour batter properties. Costantini et al. (2014) introduced 10% of chia flour into GF buckwheat bread and found an improvement of nutritional value (increasing protein, fiber, omega-3 and omega-6 content) without impairing bread quality (specific volume, crust and crumb color).

Quinoa (*Chenopodium quinoa* Willd) is a crop originated in the Andean region that was the staple food of Incas, who named it “the mother grain” since they believed that quinoa was a sacred gift from their gods. However, after the Spanish conquest quinoa was replaced for other grains and it only remained in isolated regions (Vega-Gálvez et al., 2010; Wrigley et al., 2004). Nowadays, quinoa is still cultivated mainly in Bolivia (48.8% of total production) and Perú (50.4% of total production). World production was 103,418 tonnes in 2013 (FAOSTAT, 2014). According to Food and Agriculture Organization of the United Nations (FAO), 2013 was the international year of quinoa and it was emphasized that quinoa can

play an important role in eradicating hunger, malnutrition and poverty (FAO, 2013). However, in 2013, the price of quinoa increased more than 86% in Perú (Perú this week, 2013).

Starch content of quinoa (67.4%) is lower than that of most cereals, and is low in amylose and resistant starch (Schoenlechner et al., 2008). Its protein content is higher than cereals, as it contains between 12.5-16.7% of protein, with 38.7% of essential aminoacids, which are well balanced and rich in lysine and methionine (Vega-Gálvez et al., 2010; Drzewiecki et al., 2003). Fat content of quinoa ranges from 5.5% to 8.5% and it has a higher unsaturated/saturated ratio than amaranth and buckwheat, with linoleic acid being the most abundant fatty acid (50.2-53.1%) followed by oleic acid (23.3-26%) (Schoenlechner et al., 2008; Vega-Gálvez et al., 2010). The amount of fiber in quinoa and cereals is similar. Quinoa has higher content of calcium, magnesium, zinc and specially iron than cereals and its mineral content is twice as high as in cereals (Schoenlechner et al., 2008; Wrigley et al., 2004). Quinoa is rich in thiamine, folic acid and vitamin C and contains more riboflavin, vitamin E and carotene than rice, barley or wheat (Vega-Gálvez et al., 2010). Moreover, quinoa is a source of flavonoids and its oil contains 1.5% of sterols (Alvarez-Jubete et al., 2010b).

Elgeti et al. (2014) observed that quinoa white flour stabilized gas formed during fermentation which generated GF breads with a homogenous crumb structure, increased specific volume (33%) and soft crumbs. Similar findings were reported by Alvarez-Jubete et al. (2010c) who observed higher specific volume and softer crumb when quinoa flour was added into GF bread based on rice flour and potato starch, due to natural emulsifiers present in quinoa flour. Nutritional value of GF bread was enhanced by adding quinoa flour (Alvarez-Jubete et al., 2009).

3.3. Legume flours

Legumes are plants from the family *Fabaceae* or *Leguminosae* that produce seeds in pods. Legumes are a source of protein, complex carbohydrates, fiber, and minerals, and consumed with cereals provide a well-balanced aminoacid profile. Legumes can be added to GF bread formulations as legume flours or as protein isolates due to its high protein content and

functionality. Legume flours are interesting for GF bread making due to their nutritional properties and the functional characteristics of their proteins.

Carob is the seed produced by the carob tree (*Ceratonia siliqua* L.) which is a leguminous evergreen tree that grows in the Mediterranian region. Spain is the main producer and, in 2012, generated the 24.6% of the total world production, followed by Italy (18.9%), and Portugal (14.1%), being the total world production of 162,911 tonnes (FAOSTAT, 2014). Locust bean gum or carob gum is obtained by milling carob seed endosperm after removal of the hull and the germ. It is widely used in food industry and in bakery products and, in GF bread, it improves loaf volume and crumb structure (Barak and Mudgil, 2014). The germs that are removed to obtain locust bean are milled to obtain carob germ flour which has almost 50% of protein that is high in lysine, arginine and caroubin. Caroubin is a water-insoluble protein from carob seed germ that has rheological properties similar to gluten (Bengoechea et al., 2008). Due to the properties of caroubin, some authors have studied the effect of carob germ flour on GF bread (Miñarro et al., 2012; Smith et al., 2012). Tsatsaragkou et al. (2012, 2014a, b) studied the effect of carob flour on GF bread formulations and concluded that it enhanced rheological, nutritional and baking properties when an adequate proportion of carob flour/water was used.

Chickpea (*Cicer arietinum* L.) is an herbaceous, annual legume that was the first legume crop domesticated 10,000 years ago in Turkey (Wrigley et al., 2004). Chickpea cultivars are divided into Kabuli chickpea, characterized by larger seed size and cream color that is grown in West Asia, North Africa, North America and Europe; and Desi chickpea, with smaller seed size and darker color that is mostly grown in Asia and Africa and represents 80-85% of the total chickpea (Boye et al., 2010; Kaur and Singh, 2005). In 2013, 13.1 million tonnes of chickpea were produced in the world, and India contributed to the 67.4% of the world production. Turkey is still cultivating chickpea and, in 2013, produced 3.9% of the total world production (FAOSTAT, 2014). Chickpea has potential health benefits related to cardiovascular disease, type II diabetes, digestive diseases and some cancers (Jukanti et al., 2012). Miñarro et al. (2012) reported that chickpea reduced GF bread crumb hardness and increased bread volume due to the emulsifying properties of chickpea protein. Moreover, they stated that chickpea flour could substitute soybean flour to obtain an allergen-free bread.

Soybean (*Glycine max* L.) was probably originated in the north and central regions of China 4,000-5,000 years ago and was introduced into Europe before 1737 and into North America in 1765 (Olaoye and Ade-Omowaye, 2011; Wrigley et al., 2004). In 2013, 247.6 million tonnes of soybean were produced in the world, being USA the major producer (34%) followed by Brasil (29.6%) and Argentina (17.8%) (FAOSTAT, 2014). Soybean has different health benefits such as reduction of cholesterol levels, prevention of cardiovascular diseases, improvement of glucose tolerance, improvement of irritable bowel syndrome, and anti-inflammatory and anti-carcinogenic effects on digestive system. In 1999, the Food and Drug Administration approved the health claim that soya may reduce the risk of coronary heart disease due to the effect of soya protein together with low saturated fatty acids and cholesterol diet (Mateos-Aparicio et al., 2008).

Soybean has been used to formulate GF bread due to its nutritional properties and the functionality of its protein, which can help to substitute gluten. Different authors have successfully formulated GF bread with soybean flour. The incorporation of 0.5% of soybean flour improved GF bread texture (Sánchez et al., 2002). Sciarini et al. (2010) found that inactive soybean flour improved batter characteristics, loaf specific volume and delayed staling of GF bread based on rice or corn flours. In addition, active soybean flour improved GF bread quality (volume and crumb structure) but semiactive or inactive soybean flour had no this positive effect on GF bread based on rice flour and cassava starch (Ribotta et al., 2004). Rice GF bread had the highest loaf volume and the lowest hardness when germinated soybean flour was added compared to raw, steamed and roasted soybean flours, however, heat treatments of soybean flour (steamed and roasted) improved flavor and consumer sensory scores (Shin et al., 2013). On the contrary, Miñarro et al. (2012) observed that 54.4% of consumers preferred GF bread elaborated with soybean flour instead of GF breads containing other legume flours (chickpea, carob germ or pea). However, soya is an allergenic ingredient and, for that reason, the substitution of soya for other non-allergenic ingredients in GF bread making has also been studied (Miñarro et al., 2012).

As mentioned before, legume proteins present functional properties and thus, they can be used to improve GF bread quality. Different authors have studied the effect of legume protein isolates such as soybean, pea or lupin (Crockett et al., 2011a; Mariotti et al., 2009; Miñarro et al., 2012). Ziobro et al. (2013) supplemented GF bread with non-gluten proteins and observed that soya, pea and lupin protein isolates strengthened viscoelastic properties of GF

dough, reduced crumb hardness and chewiness, and increased bread volume, except for soya isolate, which reduced it.

3.4. Other flours

Chestnut tree is cultivated for its nut, timber, tannins and because of its contribution to forestry landscape. There are three chestnut growing areas in the world: (a) Asia, which is the most important and where the specie *Castanea mollissima* is mainly cultivated in China; (b) Europe and Turkey, the second main area, where the predominant specie is *Castanea sativa*; and (c) North America, where *Castanea dentata* is found naturally but is being substituted by hybrids. European chestnut was probably originated 90 million years ago in the eastern Mediterranean region (Pereira-Lorenzo and Ramos-Cabrer, 2004). In 2012, almost 2 million tonnes of chestnuts were produced in the world, and China generated 82.5% of the world production (FAOSTAT, 2014). Chestnut has health benefits due to its nutritional composition since it contains considerable amounts of dietary fiber, minerals (Ca, P, K, Mg, Cu, Fe, Zn) and vitamins (E, C, A, B group) (Sacchetti et al., 2004; De Vasconcelos et al., 2010).

Some articles have reported the effect of chestnut flour on rheological properties and baking characteristics of GF batters and breads. Chestnut flour particle size influenced batter characteristics, as the increase of particle size increased G' and G" (Moreira et al., 2010). In addition, 70% of commercial chestnut flour or 25% of low particle size chestnut flour rendered GF rice batters with suitable properties (Moreira et al., 2013b). The addition of chestnut flour into GF rice bread enhanced its batter rheological characteristics, specific volume, crumb texture and crust color (Demirkesen et al., 2010a; 2011a). Chestnut flour prevented the formation of large pores providing GF rice breads with a uniform crumb structure (Demirkesen et al., 2012). Demirkesen et al. (2013) observed that the use of chestnut flour together with xanthan gum, guar gum and emulsifier in GF rice bread significantly delayed its staling by reducing moisture loss and hardness. These authors stated that chestnut fiber was responsible for most of the obtained results since the entanglement of fibers improved GF batter rheological characteristics allowing to entrap more air bubbles. These batters rendered breads with higher volume and better crumb texture and structure.

However, when chestnut flour content exceeded the optimum level (30-40%), the fiber restricted the expansion of gas cells, resulting in lower bread volume and harder crumb.

Tiger nut (*Cyperus esculentus* L.) is an underutilized crop that produces rhizomes from the base and somewhat spherical edible tubers (Adejuyitan et al., 2009). It was originated during the ancient Egypt in 5,000 BC and it is thought to be the third most ancient domesticated crop after emmer wheat and barley. It was probably introduced into Europe during the Middle Age by the Arabs (Defelice, 2002; Ezeh et al., 2014; Sánchez-Zapata et al., 2012). Nowadays, tiger nut is also cultivated in Mediterranean regions, especially Spain and Egypt, and also in other African countries (Northen Nigeria, Niger, Mali, Senegal, Ghana, and Togo) as well as in American countries (Chile, Brasil, USA) and also in China and Australia. However, it is considered a weed in many countries. In Spain, it is an important crop for the production of tiger nut milk (*horchata de chufa*) which is a refreshing drink (Defelice, 2002; Sánchez-Zapata et al., 2012). Tiger nut has health benefits related to the prevention of diseases like coronary heart diseases, obesity, diabetics and gastro intestinal disorders (Adejuyitan, 2011) and, according to Khare (2007), it is a digestive tonic, promotes diuresis and menstruation. Tiger nut tuber is rich in carbohydrates, lipids (high content of oleic acid), fiber, some minerals (K, P, Ca), and vitamin E and C (Sánchez-Zapata et al., 2012).

Demirkesen et al. (2011b) evaluated the effect of different ratios of tiger nut flour/rice flour and observed that conventionally baked bread and infrared-microwave combination baked bread had the most acceptable characteristics (crumb hardness and specific volume) when using 10% or 20% of chestnut flour, respectively. The increase of tiger nut flour in GF rice bread reduced bake loss due to the increase of fiber that retained more water. Crumb hardness was reduced and bread specific volume increased because of fiber content of tiger nut enhanced gas retention and water holding capacity of batter.

3.5. Starches

Starch is the main carbohydrates reservoir in plants and is constituted by two types of glucose polymers, amylose, that is formed by α-(1,4) linkages, and the highly branched amylopectin, composed by α-(1,4) linked linear chains units that are connected between them by α-(1,6) linkages. The typical proportion of amylose and amylopectin is 20-30% and 70-80%,

respectively. Starch gelatinization takes place when starchy food is heated with enough water: starch granule absorbs water and swells, and then, starch amylose is leached out the starch granule resulting in a viscous slurry or paste. In wheat bread making, starch plays an important role in dough rise during baking since crumb structure is set due to starch gelatinization which in turn, affects loaf volume and texture. Retrogradation, the realignment of amylose and amylopectin chains, occurs during the cooling and storage of the gelatinized starch and, in wheat bread making, amylopectin retrogradation is one of the main causes of bread firming during storage. Starch functionality depends on amylose and amylopectin structure and proportions and it can be modified by different mechanisms which, in turn, affect bread characteristics (Abdel-Aal, 2009; Goesaert et al., 2008a, b). Different reports have been published about the effect of starches on GF bread making. Below, studies on the effect of native (maize, potato, rice and cassava) and modified starches are summarized.

Onyango et al. (2011) studied the effect of different concentrations (10, 20, 30, 40 and 50%) of native starches (cassava, maize, potato and rice) on GF sorghum bread. The increase of starch reduced batter consistency and increased bread volume. The positive effect of starch is explained by the reduction of sorghum flour which has large irregularly shaped endosperm and bran particles that can deform and puncture gas bubbles. Crumb hardness was reduced with increasing starch contents. However, at 50% of sorghum flour substitution, bread containing cassava starch had the softest crumb. Cassava or rice starch rendered breads with higher crumb cohesiveness and resilience than maize or potato starch. In conclusion, breads containing cassava or rice starch performed better than breads elaborated with maize or potato starch. The addition of 50% cassava starch rendered GF with the best crumb properties, which were maintained during storage (96 h).

The combination of maize starch, cassava starch and rice flour for the elaboration of GF bread has been reported. According to Sánchez et al. (2002) the optimum formulation, that rendered GF bread with high crumb grain score and bread score, contained 74.2% maize starch, 17.2% rice flour and 8.6% cassava starch. However, Ballesteros-López (2004) reported that 45% rice flour, 35% maize starch and 20% cassava starch rendered breads with good characteristics.

Wheat starch with less than 20 ppm of gluten can also be used to elaborate GF breads since wheat starch provides higher water absorption, dough density and bread volume than other

GF starches (rice, maize, potato) (Deutsch et al., 2008; Houben et al., 2012). *Aspergillus niger* peptidase degrades the gluten that remains in wheat starch (0.3-5%) obtaining GF wheat starch with similar properties than original wheat starch but with lower viscosity (Walter et al., 2014).

Native cassava starch rendered GF sorghum breads with better crumb properties than pre-gelatinized cassava starch because gelatinized starch formed a stiff and inelastic dough. The hydration of starch polymer chains thickened the dough and, the cross-linking of starch polymer had a gelling action on dough (Onyango et al., 2010a). Resistant corn and potato starch increased G' and G" of GF batter, reduced crumb hardness and increased soluble (18%) and insoluble (137%) dietary fiber (Korus et al., 2009). Resistant starch also promoted crumb elasticity and porosity in rice flour GF bread (Tsatsaragkou et al., 2014b).

The addition of 10 and 15% of hydroxylpropyl distarch phosphate or acetylated distarch adipate increased the volume of GF bread based on maize starch and improved crumb structure (lower cell size and high number of cells) and texture (increase of elasticity and reduce of hardness and chewiness) (Ziobro et al., 2012). Moreover, both modified starches also affected rheological characteristics of GF batter increasing storage and loss moduli (G' and G") (Witczak et al., 2012).

3.6. Non-vegetable proteins

In order to substitute gluten and to improve nutritional value in GF bread making, proteins from animal origin can also be added since some of non-gluten proteins play a role as structure and texture-forming agents (Deora et al., 2014).

Milk products have functional and nutritional properties as they contain proteins that can form a network and provide essential aminoacids and calcium. However, most of celiac patients suffer from lactose-intolerance, because they have low levels of lactase due to the villous atrophy, and dairy products containing lactose are not adequate for them. For that reason, high protein and low lactose dairy products like sodium caseinate, milk protein isolate and whey protein isolate or concentrate, are more appropiated and it has been shown that they can improve GF bread quality. The addition of milk proteins in GF bread increased

loaf volume and sensory characteristics. Moreover, it has been shown that caseinate acts as an emulsifier in GF breads (Gallagher et al., 2003; Houben et al., 2012; Krupa-Kozak et al., 2013; Stathopoulos, 2008). Miñarro (2013) substituted water by liquid whey from cheese industry for the elaboration of GF bread and observed a thicker batter, lower bake loss and higher bread crumb hardness.

Egg proteins can be added in GF bread formulations as foaming agents, crumb stabilizers and to obtain good shape. Egg powder improved volume and increased the number of cells in crumb of GF bread due to the protein network formed (Moore et al., 2006). Addition of egg white solids at 15% in GF bread with cassava starch and HPMC increased loaf volume and improved crumb structure (Crockett et al., 2011a). Schoenlechner et al. (2010) observed that albumen (egg white powder) improved texture and sensory scores of GF breads. Ziobro et al. (2013) also reported a positive effect of albumin on GF bread volume.

Other protein sources such as surimi and collagen have also been studied to formulate GF bread. Surimi improved crumb texture, bread volume and crust color of GF bread (Gormley et al., 2003). Ziobro et al. (2013) observed that collagen increased acceptability of smell and crumb color of bread, and strengthened viscoelastic properties of GF dough.

Miñarro et al. (2010) introduced unicellular protein from yeast to GF bread formulations and observed that it reduced bake loss, crumb lightness and bread acceptance.

3.7. By-products from vegetable industry

The valorization of by-products or wastes from food industry is becoming a trend due to the awareness of sustainable development together with the economic crisis. Food by-products can be reused directly, which could imply health risk, or processed, to obtain a new product suitable for food or other industries.

In the fruit and vegetable industry, by-products represent one third of the initial food and may have a negative impact on the environment. However, these by-products have high nutritional value containing dietary fiber, vitamins, minerals and bioactive compounds, and functional properties like gelling and water binding, and thus, they can be used as low cost ingredients (O'Shea et al., 2012).

Korus et al. (2012) used defatted blackcurrant and strawberry seeds as dietary fiber supplement in GF bread. They observed that the addition of 15% of these by-products changed the rheological properties of GF dough. These fibers reduced crumb hardness and affected bread color and only defatted strawberry seeds added at 5 and 10% improved bread volume. Both fibers enriched GF bread with protein, dietary fiber and polyphenols.

O'Shea et al. (2013, 2015) studied the effect of orange pomace, obtained from orange juice industry, in GF bread formulation. They observed that orange pomace improved GF batter characteristics and concluded that 5.5% of this by-product, 94.6% of water and 49 min of fermentation contributed to the optimum formulation.

3.8. Shortenings

Lipids play a role in wheat bread making and, besides flour lipids, they can be added as shortenings. Often, shortenings are described as added fats or oils that tenderize or shorten bread or cakes texture (Smith and Johansson, 2004). However, the term "shortening" refers particularly to a group of solid lipids formulated for baking applications. They are defined as crystalline lipids and oils from vegetable and/or animal origin with a composition of 100% lipid approximately (Pareyt et al., 2011).

An important factor that determines shortening functionality is its solid fat content (Ghotra et al., 2002). For that reason, to elaborate shortenings, the lipids are partially or fully hydrogenated to increase their solids content. Another important factor is the crystal structure of the solid fat: α, β' or β, ordered from low to high stability. In bakery products, the β' crystal form is the most desirable (Pareyt et al., 2011; Smith and Johansson, 2004).

Brooker (1996) reported that lipid crystals can adsorb onto gas-liquid interface of air bubbles during mixing and later, in the baking step, this lipid becomes a cell membrane which allows the gas cell to grow better. In consequence, solid fat contributes to volume, crumb structure and texture of bread (Autio and Laurikainen, 1997; Chin et al., 2010). Sufficient solid fat strengthens dough and improves gas retention, but too much solid lipid can inhibit rising during fermentation (Chin et al., 2010; Ghotra et al., 2002; Rogers et al., 1988). Liquid fat cannot produce this effect but it lubricates dough, provides a moister mouthfeel, and

tenderizes the crumb and the crust. Moreover, shortening can reduce the firming rate when non defatted flour is used since shortening acts through the flour lipids (Rogers et al., 1988). Smith and Johansson (2004) also observed that increasing solid fat in wheat bread increased loaf volume and reduced the rate of staling. To sum up, shortenings plasticize and lubricate the dough, and improve loaf volume, crumb structure and shelf-life of bread.

In 1970, Hart et al. studied the effect of shortening addition in sorghum GF bread and observed that shortening reduced crumb hardness. More recently, other authors are including shortenings in GF bread formulations (Demirkesen et al., 2010a, 2010b; Miñarro et al., 2010, 2012; Schober, 2009; Sciarini et al., 2012a, b).

Schoenlechner et al. (2010) added vegetable fat powder instead of shortening in GF bread and concluded that it had not significant effect on bread quality but when it was incorporated together with albumen, they improved bread acceptance. Sunflower oil and olive oil reduced water absorption, dough stability, apparent viscosity and storage modulus of GF doughs based on chestnut flour (Moreira et al., 2012).

3.9. Emulsifiers

Emulsifiers are surface-active compounds that contain both hydrophilic and lipophilic groups. They lower the tension of oil-water interface allowing the emulsion formation and stabilization due to their amphiphilic nature. Emulsifiers are classified according to its hydrophilic/lipophilic balance (HLB) value that ranges from 0 to 18. Emulsifiers with low HLB values are lipophilic, while high HLB value emulsifiers are hydrophilic (Dickinson, 1993; Whitehurst, 2004).

All emulsifiers are synthetic except lecithin which is present in animal or vegetal products. Monoglycerides and diacetil tartaric acid esters of mono- and diacylglycerols (DATEM) can occur naturally but the ones that are used as food additives are synthetic (Dickinson, 1993). There is the hypothesis that synthetic emulsifiers could increase intestinal permeability promoting allergic and autoimmune diseases (Csáki, 2011) and, thus, reduction or elimination of synthetic emulsifiers from GF bread could benefit the intestinal health of celiac patients.

In the food industry, emulsifiers are used for many applications and, in bread making they are added to strength the dough or soften the crumb (Stampfli and Nersten, 1994). DATEM, sodium stearoyl lactylate (SSL), calcium stearoyl lactylate (CSL), and polysorbate are commonly used as dough strengtheners. They can associate with gluten improving its ability to retain gas, which increases dough height during proofing (Gómez et al., 2004; Pareyt et al., 2011; Stampfli and Nersten, 1994). Monoglycerides are the typical crumb softeners and can delay bread staling because they interact with starch (Gómez et al., 2004; Pareyt et al., 2011). Amylose-monoglycerides complex is insoluble in water, does not gelatinize and, therefore, it cannot recrystallize during cooling and bread staling is delayed (Stampfli and Nersten, 1994). Lecithins are also used for their crumb softening effect but they cannot associate with starch and thus, they have no effect on bread staling. Besides, lecithins have a weakening effect on wheat dough (Pareyt et al., 2011; Stampfli and Nersten, 1994). However, Gómez et al. (2004) reported that lecithin enriched with lysophospholipids delayed crumb hardening and, at high concentrations (0.7%), it strengthened the dough.

In GF bread making, emulsifiers are also added due to their functionality. Some authors have studied the effect of emulsifiers in GF bread and the results obtained have been divergent, probably because of the different nature and concentrations of the emulsifiers used, and the variety of GF bread formulations tested (Demirkesen et al., 2010b; Nunes et al., 2009; Onyango et al., 2009; Purhagen et al., 2012; Sciarini et al., 2012a). Table 1.1 shows a summary of the effect of different emulsifiers used in GF bread.

3.10. Hydrocolloids

Hydrocolloids or gums are a group of water-soluble polysaccharides with different chemical structure which provides diverse functional properties. They can be used in food industry as emulsifiers, stabilizers, gelling, thickening or foaming agents, with the purposes of texture properties improvement or control of water mobility, and they are also utilized in bakery products (BeMiller, 2008).

In GF bread making, hydrocolloids are used to substitute gluten since they increase dough consistency and improve gas holding ability, and contribute to improve texture, appearance

Table 1.1. Effect of emulsifiers in gluten-free bread making.

	DATEM							DM			GMS						SSL							CSL		Lecithin		
Flour base/reference	A	B			C	D	E	B			F				C		B			C		E		C		B		
Emulsifier %	0.5	0.3	0.45	0.6	2.4	3	1	0.3	0.65	1.0	1	2	3	4	0.4	2.4	0.3	0.4	0.5	0.4	2.4	1	0.4	2.4	0.1	0.3	0.5
Effect of emulsifier																											
· Batter strengthening	X	X	X	X			X								X	X				X	X	X	X	X	X	X	X
· Gas retention increase	X																										
· Batter volume increase													X														
· Water retention increase	X					X																					
· Bread volume increase	X			X				X	X	X	X	X	X	X			X	X	X						X	X	X
· Bread volume decrease						X																X					
· Crumb softening	X				X								X			X					X			X			
· Crumb hardening																						X					
· Staling delay					X					X						X			X		X		X	X			
· Staling increase																						X			X	X	X
· Number of cells increase							X															X					
· Mean cell area decrease																											
· Bake loss increase																									X	X	X

Emulsifiers: diacetil tartaric acid esters of mono- and diacylglycerols (DATEM), distilled monoglylcerides (DM), glycerol monostearate (GMS), sodium stearoyl lactylate (SSL), calcium stearoyl lactylate (CSL) and lecithin.
Flour base/reference: (A) rice flour (Demirkesen et al., 2010b); (B) rice flour and potato starch (Nunes et al., 2009); (C) cassava starch and sorghum flour (Onyango et al., 2009); (D) deglutenized wheat starch (Purhagen et al., 2012); (E) rice four, cassava starch and full-fat active soya flour (Sciarini et al., 2012a); (F) cassava flour and defatted soya flour (Defloor et al., 1991).

and shelf-life. However, they should be added in small proportions and it is important to evaluate their effect on each GF formulation since an inadequate use can reduce GF bread quality and they are expensive. Xanthan gum and HPMC are the most used hydrocolloids in GF bread making. Xanthan gum is an extracellular heteropolysaccharide formed through a fermentation process by *Xanthomonas campestris*. HPMC is a cellulose ether derived from alkali-treated cellulose by linking hydroxypropyl and methyl groups to the β-1,4-D-glucan cellulosic backbone (Anton and Artfield, 2008; Hager and Arendt, 2013; Mandala and Kapsokefalou, 2011). Crockett et al. (2011b) evaluated the effect of xanthan gum and low and high methoxy HPMC on GF rice cassava dough and concluded that high methoxy HPMC performed better than others in this kind of dough. Hager and Arendt (2013) reported that, depending on the GF formulation were hydrocolloids are added, they functionality can be different. In their study, HPMC improved volume in teff and maize breads but reduced it in rice bread, had no effect in buckwheat bread and softened the crumb of all breads studied. Xanthan gum reduced bread volume of all breads, reduced crumb hardness of maize bread, increased crumb hardness of teff and buckwheat bread and had no effect on rice bread texture.

Some authors have studied the effect of other hydrocolloids such as guar gum, carrageenan, pectin, carboxymethylcellusose, agarose, β-glucan, tragacanth gum, alginate, locust bean gum on GF bread and batter (Demirkesen et al., 2010b; Lazaridou et al., 2007; Moreira et al., 2013a; Sabanis and Tzia, 2010; Sciarini et al., 2012a).

3.11. Other ingredients

Water is essential in bakery products since it solubilizes ingredients, hydrates proteins and carbohydrates, and helps to develop gluten network. In final product, moisture content contributes to bread quality and shelf-life. In GF bread making, the level of water addition has an important influence on GF bread quality since high water content reduces crumb firmness, increases specific volume and bread circumference and gives large pore size and a low number of cells. The GF batters are characterized by having a high amount of water which is not retained by any protein network and are difficult to handle (Arendt et al., 2008; Cauvain and Young, 2006; Schoenlechner et al., 2010).

Yeast or baker's yeast (*Saccharomyces cerevisiae*) is a biological leavening agent form the fungi kingdom that is responsible for producing CO_2, ethanol and aroma compounds due to sugars fermentation. The CO_2 released is retained in the dough, allowing it to rise, and ethanol is evaporated during baking. In GF bread making, the lack of gluten reduces the gas retention during proofing and, thus, shorter proofing times are applied. The optimum temperature for yeast fermentation is 28-32ºC (Belitz et al., 2009; Cauvain and Young, 2006).

Baking powder is a chemical leavening agent used in bakery products. It consists of a CO_2-generating agent that is usually sodium carbonate and an acid (disodium dihydrogen phosphate or monocalcium phosphate). The interaction of water, acid, chemical CO_2-generating agent and heat (~90 ºC) produces a chemical reaction that releases CO_2. The amount of acid needed to release the maximum CO_2 from the chemical CO_2-generating agent is called "neutralization value" and depends on the acid chemical composition. Baking powder is used in bakery products such as cakes, biscuits, cookies, pastries and some yeast-raised products (Belitz et al., 2009; Cauvain and Young, 2006). Since GF batter consistency is weak and gas retention during proofing is poor, baking powder provides gas during baking, which contributes to obtain breads with higher volume and softer crumb.

Salt (sodium chloride) plays an important role on the flavor of baked products and it also has effect on water activity and shelf-life. Salt limits yeast growth and for that reason it is important to balance the addition of salt on yeast leavened products. It has been shown that salt consumption is related to high pressure and cardiovascular disease. Thus, as bread is one of the most important dietary salt sources (20-25%) it is necessary to control salt addition in bread making (Cauvain, 2003; Cauvain and Young, 2006; Quílez and Salas-Salvado, 2012).

Sugar (sucrose) is used in bread making as flavor agent, starter for yeast fermentation and it also influences crust color due to its role on Maillard reaction. Moreover, sugar has an important function in structure formation, as it delays the gelatinization temperature of the starch. However, high levels of sugar inhibit yeast activity (Cauvain, 2003; Cauvain and Young, 2006). Morais et al. (2013) compared different sugar substitutes for GF bread making and observed that bread elaborated with raw sugar was the sweetest and provided breads with the highest specific volume. Bread elaborated with fructooligosaccharides received the

highest acceptance, while stevia bread showed the highest yeast flavor intensity and the lowest specific volume.

4. Technologies applied for gluten-free bread making and preservation

4.1. Bread making technologies

4.1.1. Milling

The particle size of the flour that results from the milling process can influence dough characteristics and consequently final bread quality, particularly, loaf volume. In wheat bread, fine wheat flour increases dough cohesiveness. Moreover, the milling process to obtain reduced particle sizes increases starch damage which, in turn, also affects bread quality (Hoseney, 1994; Li et al., 2014).

De la Hera et al. (2012) reported that low particle size of maize flour reduced dough development during fermentation and, in contrast, high particle size rendered breads with improved volume and reduced crumb firmness. They concluded that a coarse particle size is more appropriate for maize flour GF bread. The same research group also evaluated the effect of particle size and water content on rice GF bread. They stated that coarse rice flour together with high hydration (90-110%) rendered GF breads with better volume and texture and, in contrast, fine flours reduced gas retention during fermentation (De la Hera et al., 2013, 2014).

Trappey et al. (2014) reported that sorghum flour GF breads elaborated with 60% extraction flour with low particle size rendered better quality (high volume, soft crumb, crumb structure) than 100% or 80% extraction flour with higher particle size. They observed that the starch damage induced during particle size reduction had a positive correlation with water absorption and was a significant predictor for loaf volume and crumb firmness.

4.1.2. Mixing

Mixing is the first step of bread making and is one of the most important processes. The objectives of mixing are: to uniformly incorporate all ingredients, to hydrate the flour and other dry ingredients and to develop gluten. In wheat bread, mixing allows gluten network formation and thus, mixing characteristics are important to achieve the desired dough properties and bread characteristics. Moreover, the effect of mixing in dough and bread characteristics is related to the amount of air incorporated during this step. Air bubbles formed during mixing can expand due to the incorporation of gas released from yeast during fermentation and cause dough rising. A good distribution of these air bubbles will promote uniform expansion during baking and provide breads with fine crumb structure. (Haegens, 2006; Lai and Lin, 2006).

Although gluten development does not occur during mixing of GF batters, this step also affects GF bread characteristics. Gómez et al. (2012) stated that mixing parameters (mixing arm, speed and time) influenced GF dough characteristics and bread quality and the effect was higher with high hydrated dough. Higher volumes and softer breads were obtained with wire whip mixing arm, lower mixing speeds and longer mixing time. However, in wheat bread, overmixing reduced bread volume because dough falls, probably due to gluten network damage (Lai and Lin, 2006).

4.1.3. Enzymes

Enzymes are widely used in food industry, especially in baking industry, as technological aids. They are proteins that are denaturalized during baking and, thus, they are not active in the final baked product. They are considered as clean label compounds and they can substitute different additives due to their capacity to catalyze some chemical reactions (Rosell, 2009).

The activity of enzymes that induce protein cross-links such as transglutaminase allows protein network formation in the absence of gluten which, in turn, improves GF dough viscoelastic properties and GF bread quality (Gujral and Rosell, 2004a; Moore et al., 2006; Renzetti et al., 2008; Storck et al., 2013).

Gujral and Rosell (2004b) stated that glucose oxidase increased elastic and viscous modulus of rice flour dough rendering GF breads with improved volume and texture, which permitted the reduction of HPMC in the formulation. Renzetti and Arendt (2009a) observed that the use of glucose oxidase on corn and sorghum GF bread increased volume and reduced top collapsing, but it had no effect on buckwheat and teff GF breads. Protease treatment on brown and normal rice GF bread resulted in an improvement of volume and texture (Renzetti and Arendt, 2009b). However, other authors reported that protease had no effect on volume and texture of corn and teff breads and impaired buckwheat and sorghum bread texture. (Hamada et al., 2013; Kawamura-Konishi et al., 2013).

The effect of lipase, protease and two amylases on GF bread was studied by Martínez et al. (2013), who observed that the addition of lipase improved bread volume and texture but protease reduced volume, while amylases had no significant effect. Lipase and amylases reduced initial crumb hardness and only lipase had positive effect on crumb hardening during storage.

Gujral et al. (2003) evaluated starchy hydrolyzing enzymes α-amylase of intermediate thermostability and cyclodextrin glycoxyl transferase on GF rice bread and reported that both enzymes improved bread volume and reduced crumb hardness as well as limited amylopectin retrogradation during storage. However, breads treated with α-amylase had sticky texture and did not show any delay in staling.

4.1.4. High pressure processing

High pressure (HP) is a technology used in different food products to inactivate vegetative microorganisms and enzymes. HP technology also produces changes in food biopolymers, such as proteins and starch that can change the final texture of the food product (Gallagher, 2009). For example, HP treatment of lupin (Chapleau and De Lamballerie-Anton 2003) and soybean (Denda and Hayashi, 1992) proteins enhanced emulsifier properties of these proteins. In gluten, HP promoted disulfide bonds, strengthening the gluten network (Apichartsrangkoon et al., 1998).

Vallons et al. (2010) treated sorghum batters with 200-600 MPa at 20 ºC and stated that pressures lower than 300 MPa reduced batter viscoelastic properties. However, this effect was not observed when a blocker of free thiol groups was added, indicating that thiol/disulphide interchain reactions produced sorghum protein depolymerization which was the cause of the reduction of batter strength. In contrast, sorghum batters treated with pressures higher than 300 MPa, showed an increase of consistency probably due to pressure-induced starch gelatinization. This research group also studied the effect of HP treatment on rice, teff and buckwheat flours and observed that rice and teff protein suffered polymerization by thiol/disulphide-interchain reactions but this effect was not observed on buckwheat protein, probably due to the absence of free thiol groups. Batter rheological properties of rice and buckwheat flours improved with the increase of pressure but teff batter was only strengthened when it was treated with pressures higher than 200 MPa (Vallons et al., 2011).

4.1.5. Sourdough

Sourdough is an old technology used since the ancient Egypt for the elaboration of leavened baked goods. Egyptians discovered that mixing water and flour and letting time to ferment, a dough with increased volume was obtained. When this fermented dough was added to fresh dough, the resulting baked product was soft and light (Cappelle et al., 2013). Nowadays, sourdough is still used for the elaboration of artisanal bread and also in the bakery industry. This technology is based on the fermentation of flour and water by native lactic acid bacteria (LAB) and yeasts (as it was done during the ancient times) or by the addition of starters (Aponte et al., 2013; Moroni et al., 2009). The research of this old technology has gained importance and scientific literature reports that sourdough plays an important role in bread making. During sourdough fermentation, LAB and yeasts develop enzymatic activities (e.g. proteases, amylases, lipases, phytases) that generate substances (e.g. exopolysacharides, gluco-/fructo-oligosacharides, antimicrobial compounds, aroma compounds, organic acids) that help in the improvement of bread quality (Gänzle et al., 2008; Gobbetti, 1998; Gobbetti et al., 2008, 2014; Katina et al., 2005; Tieking and Gänzle, 2005). The benefits that sourdough provides to bread are listed below:

- Increase of bread volume because of the increase of gas retention.

- Improvement of bread texture due to crumb softening.
- Partial or total replacement of hydrocolloids.
- Elongation of bread shelf-life by reducing its staling and by generating antimicrobial substances that prevent microbial growth.
- Nutritional quality improvement due to the increase of mineral biodisponibility by phytase activity, the reduction of glycemic index, and the generation of prebiotic compounds.
- Improvement of sensory profile by generating aroma compounds.

Different authors have reported that sourdough also imparts these benefits to GF bread quality (Moore et al., 2007, 2008; Novotni et al., 2012; Schober et al., 2007) and GF sourdoughs based on different flours (amaranth, buckwheat, chestnut, chickpea, millet, quinoa, rice, sorghum, soya or teff), spontaneously fermented or with starters addition, have been studied (Aponte et al., 2013; Coda et al., 2010; Moroni et al., 2010, 2011; Schober et al., 2007; Sterr et al., 2009).

Moreover, gluten toxicity can be diminished or eliminated by using lactobacilli and fungal proteases and, thus, sourdough can be a promising technology for the elaboration of GF bread with gluten containing flours (Gobbetti et al., 2008; Rizzello et al., 2007). Actually, production of wheat and rye bread that is tolerated by celiac patients has been reported (Di Cagno et al., 2004, 2008). In addition, Calasso et al. (2012) have stated that the use of sourdough in GF baked goods could be useful for the intestinal damage recovery of celiac patients during the early stage of the GFD.

4.1.6. Baking

Baking process is the last step in bread making. Heat can be transferred by radiation, convection or conduction and steam injection can be used during the initial baking stages to promote a proper crust formation. During baking, oven heating allows dough to be converted into bread which is a light, readily digestible and flavorful product. In the course of this process, different mechanisms take place: gas expansion which allows oven spring, starch swelling and gelatinization, protein coagulation, moisture lost, crust formation, Maillard reactions and caramelization (Cauvain and Young, 2006; Chang, 2006).

In GF bread making, Demirkesen et al. (2011a, b, 2012) applied infrared-microwave combination oven as an alternative to conventional oven. They optimized a GF formulation based on rice and chestnut flour and the infrared-microwave baking conditions and used 46.5% chestnut flour, 0.62% emulsifier, 40% infrared and 30% microwave power for 9 min to obtain a bread that had a comparable quality (color, specific volume and firmness) than conventionally baked bread. In addition, infrared-microwave baking notably reduced baking time (Demirkesen et al., 2011a). Besides, they also evaluated the effect of infrared-microwave combination oven on GF bread based on rice flour and tiger nut flour and concluded that 10/90 tiger nut/rice flour GF bread conventionally baked had similar characteristics (color, specific volume and firmness) than 20/80 tiger nut/rice flour GF bread baked in infrared-microwave combination ovens (Demirkesen et al., 2011b). Later, they reported higher values of pore area fraction, total number of cells and number of small pores in chestnut-rice GF breads baked in infrared-microwave combination oven compared to chestnut-rice GF breads conventionally baked (Demirkesen et al., 2012).

4.2. Preservation technologies

4.2.1. Freezing and partial baking

The relatively short shelf-life of bread, characterized by an increase of crumb hardness and a reduction in flavor and aroma that lead to a decrease in consumer acceptance, is an issue for baking industry. Freezing temperatures can be applied on dough or on partial baked bread. However, partial baked bread can also be stored at refrigeration or room temperatures. These processes increase bread shelf-life, making it available according to consumers' demand and decreasing bread wastes.

The application of freezing temperatures is one of the technologies that are widely applied to extend bread shelf-life. Dough can be frozen before proofing to interrupt bread making process and continue it according to bread demand. However, freezing-thawing of dough promotes changes on dough structure (e.g., reduction of gluten cross-linking, starch damage) as well as decrease of yeast cells activity that lead to a reduction of gas production and retention which, in turn, results in a reduction of bread volume and deterioration of texture. To solve these problems, strong wheat flour, freeze-tolerant yeasts and additives such as

hydrocolloids and emulsifiers can be used (Bárcenas et al., 2003; Dodić et al., 2007; Selomulyo and Zhoy, 2007).

Partial baking consists in the interruption of baking process together with the storage of partially baked bread at freezing, refrigeration or room temperature, and the bread receives the final baking just before its selling or consumption. This technology is an alternative to full baking process that allows to elongate bread shelf-life, to have fresh bread at any time and to reduce bread wastes. The quality of the final product depends on the storage conditions. Partially baked frozen bread can be stored for 12 months without microbial spoilage, but after 6 weeks, a texture impairment is observed. After final baking, partially baked frozen bread has lower volume, denser structure and harder crumbs than directly baked breads. To overcome these drawbacks, hydrocolloids can be added (Bárcenas et al., 2003; Bosmans et al., 2014; Mandala et al., 2008; Škara, et al., 2013).

These technologies can also be applied in GF bread making and different authors have studied them. GF frozen dough enriched with protein (amaranth flour) was less affected by the freezing process than non-enriched dough, but it was more sensitive to storage conditions (Leray et al., 2010). GF bread obtained from GF frozen dough had lower specific volume, harder crumb and more homogeneous cell size (Mezaize et al., 2010). Sciarini et al. (2012b) reported that partially baked GF bread stored at 4 ºC during 7 days had lower specific volume, harder crumbs and smaller cell area than full-baked breads but these negative effects were diminished by the addition of hydrocolloid. Novotni et al. (2012) also studied partially baked frozen technology on GF breads and concluded that it enhanced shelf-life and availability of GF bread. The effect of freezing storage temperatures of GF full-baked bread have also been studied: GF breads stored at -28 ºC during 7 days had similar properties than fresh breads, however, the quality diminished when breads were stored at -14 ºC, due to the higher amount of unfrozen water that could accelerate the reactions responsible for bread staling (Ronda and Roos, 2011).

4.2.2. Packaging

Modified atmosphere packaging (MAP) is a technology that extends shelf-life of different food products by altering proportions of surrounding atmospheric gases. The packaging

materials used for this purpose have to prevent gases to escape from the package. In bakery products, 20-50% of CO_2 is used to inhibit yeasts and moulds growth and 80-50% of N_2 is used as inert filling gas to displace oxygen (Hempel et al., 2013; Kotsianis et al., 2002). The effect of MAP on bakery products is triple: chemical, slowing oxidation; microbiological, inhibiting yeasts, moulds and bacteria growth; and physical, reducing moisture loss (Smith, 1993). Sabanis et al. (2009) stated that GF bread stored under MAP had lower crumb firmness and moisture content and remained softer during 6 days of storage.

Active packaging is a technology used for food preservation that is based on changing packaging conditions to maintain and/or improve safety and/or organoleptic properties, in which a chemical substance (the active compound) is incorporated into the packaging material (Soares et al., 2002). Gutiérrez et al. (2011) observed that active packaging containing essential cinnamon oil inhibited microbial growth and maintained sensorial quality of GF bread better than MAP technology.

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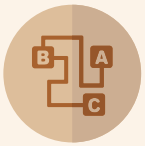
CHAPTER 2: STATEMENT OF THE PROBLEM, OBJECTIVES AND WORKING PLAN



1. Statement of the problem

Nowadays, gluten-free (GF) products present in the market have poor quality compared with their gluten-containing counterparts and, therefore, many efforts on GF products development are made to overcome this problem. Research on GF bread has increased over the last 10 years and different ingredients and technologies have been studied. Flours and starches from different sources have been proposed to be incorporated into GF bread. Proteins from animal and vegetable origin and additives such as hydrocolloids have also been used to mimic gluten viscoelastic properties. Emulsifiers and fats have been incorporated to improve GF bread quality. In addition, technologies such as sourdough, enzymes, high pressure, partial baking and freezing, or infrared-microwave combination oven baking have been studied. Nevertheless, continuous research on ingredients and/or technologies is required to further improve GF bread sensory and nutritional quality and extend its shelf-life.

2. Objectives

2.1. General objective

The general objective of this thesis was to study the influence of different ingredients and technologies in GF batter and bread characteristics in order to improve GF bread quality and shelf-life.

2.2. Specific objectives

- To study the effect of tiger nut flour, tiger nut milk and tiger nut milk by-product in GF batter and bread characteristics in order to substitute soya flour from GF formulations.
- To evaluate the influence of chickpea and tiger nut flours in GF batters and breads in order to partially or totally replace emulsifier and/or shortening from GF formulations.

- To study spontaneously fermented chestnut flour sourdough and to evaluate its effect in GF bread quality.
- To investigate the effect of different final baking technologies in partially baked frozen GF bread characteristics.

3. Working plan

Four experiments were carried out to address the specific objectives:

- **Experiment 1**: Effect of tiger nut derived products in GF batter and bread.
- **Experiment 2**: Chickpea and tiger nut flours as alternatives to emulsifier and shortening in GF bread.
- **Experiment 3**: Chestnut flour sourdough for GF bread making.
- **Experiment 4**: Influence of final baking technologies in partially baked frozen GF bread quality.

Previously to each experiment, preliminary studies were performed to develop and optimize the formulations (Table 2.1). In experiments 2 and 4, salt and sugar contents were reduced to improve nutritional characteristics. Formulations from experiment 3 had an increased content of fiber due to the amount of chestnut flour. For that reason, a rheological study was conducted to optimize water content of each formulation.

Table 2.1. Formulations used in each experiment. Ingredients are expressed as percentage of flour weight (% FW).

	Experiment 1				Experiment 2				Experiment 3						Experiment 4
Ingredient (% FW)	**TM**	**TMB**	**TF**	**SF**	**S**	**C**	**T**	**CT**	**C15**	**C20**	**C25**	**S15**	**S20**	**S25**	
Water	-	103	103	103	103	103	103	103	107.6	111.2	111.8	92.6	91.2	86.8	103
Corn starch	90.2	95	95	95	100	92.2	91.4	83.6	85	80	75	85	80	75	92.2
Tiger nut milk	103	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tiger nut milk by-product	-	5	-	-	-	-	-	-	-	-	-	-	-	-	-
Tiger nut flour	-	-	5	-	-	-	8.6	8.6	-	-	-	-	-	-	-
Soya flour	-	-	-	5	-	-	-	-	-	-	-	-	-	-	-
Chickpea flour	-	-	-	-	-	7.8	-	7.8	-	-	-	-	-	-	7.8
Chestnut flour	-	-	-	-	-	-	-	-	15	20	25	-	-	-	-
Sourdough	-	-	-	-	-	-	-	-	-	-	-	30	40	50	-
Sugar	5.8	5.8	5.8	5.8	4.2	4.2	4.2	4.2	5.8	5.8	5.8	5.8	5.8	5.8	4.2
Shortening	5	5	5	5	5·2.5·0	5·2.5·0	5·2.5·0	5·2.5·0	5	5	5	5	5	5	5
Salt	2.5	2.5	2.5	2.5	1.7	1.7	1.7	1.7	2.5	2.5	2.5	2.5	2.5	2.5	1.7
Baking powder	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Dry yeast	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Xanthan gum	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Emulsifier	2	2	2	2	2·1·0	2·1·0	2·1·0	2·1·0	2	2	2	2	2	2	2

- **Experiment 1**: TM (tiger nut milk), TMB (tiger nut milk by-product), TF (tiger nut flour), SF (soya flour).
- **Experiment 2**: S (starch), C (chickpea), T (tiger nut), CT (chickpea-tiger nut). Three levels of shortening (5, 2.5 and 0%) and three levels of emulsifier (2, 1 and 0%) were used.
- **Experiment 3**: C15, C20 and C25 were control formulations with 15, 20 or 25% FW of chestnut flour. In S15, S20 and S25, chestnut flour was added as sourdough.
- **Experiment 4**: Only one formulation was used to compare different final baking treatments.

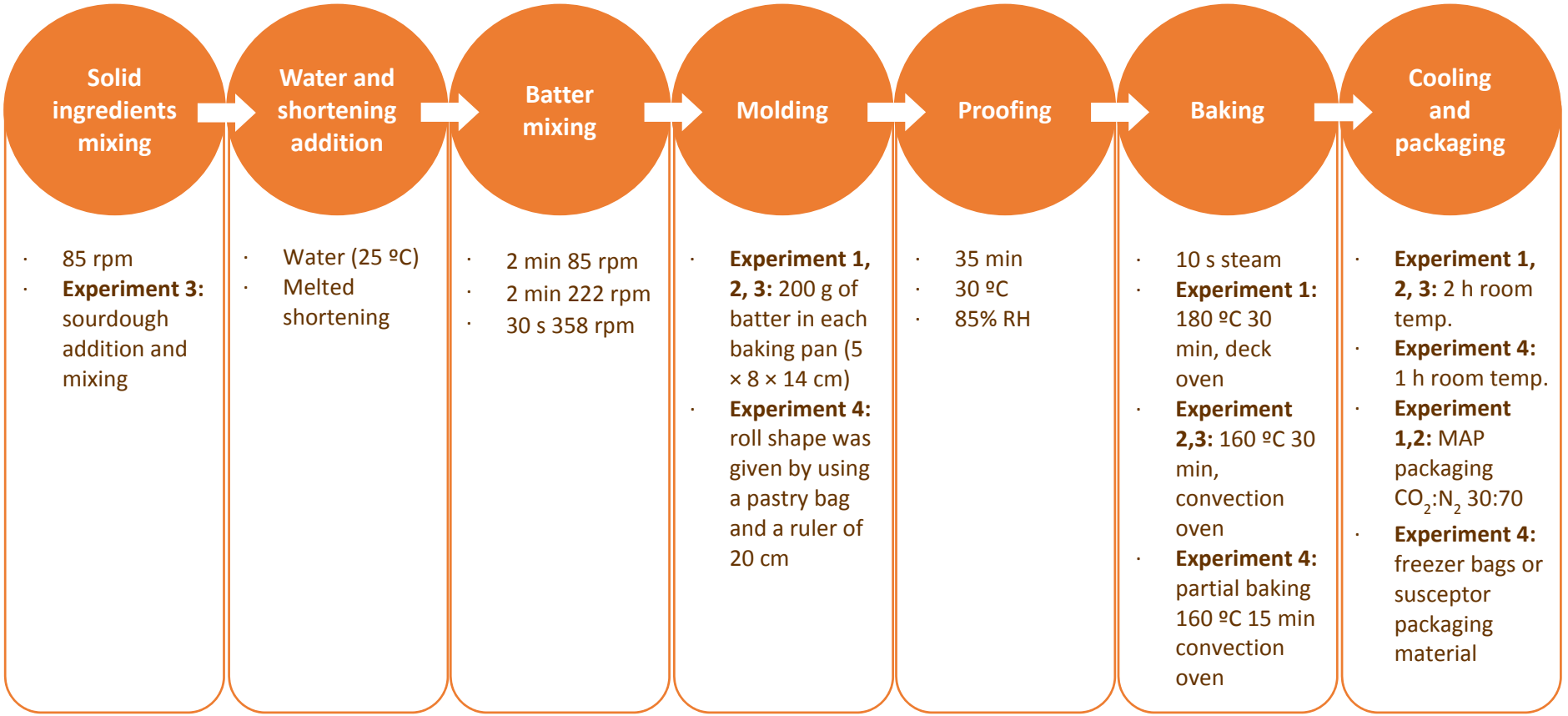


Figure 2.1. Bread making procedure.

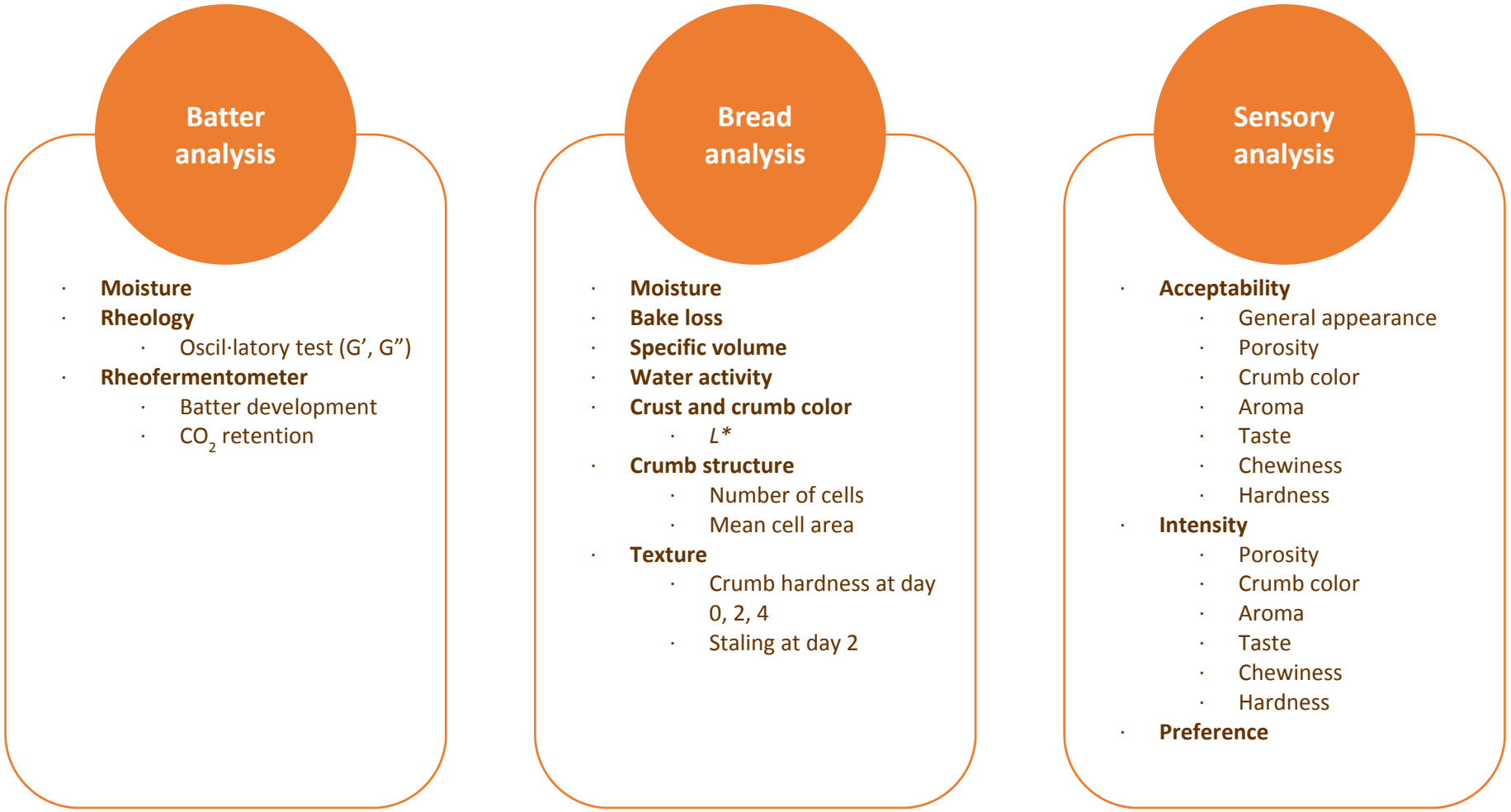


Figure 2.2. Experiment 1: Effect of tiger nut derived products in gluten-free batter and bread.

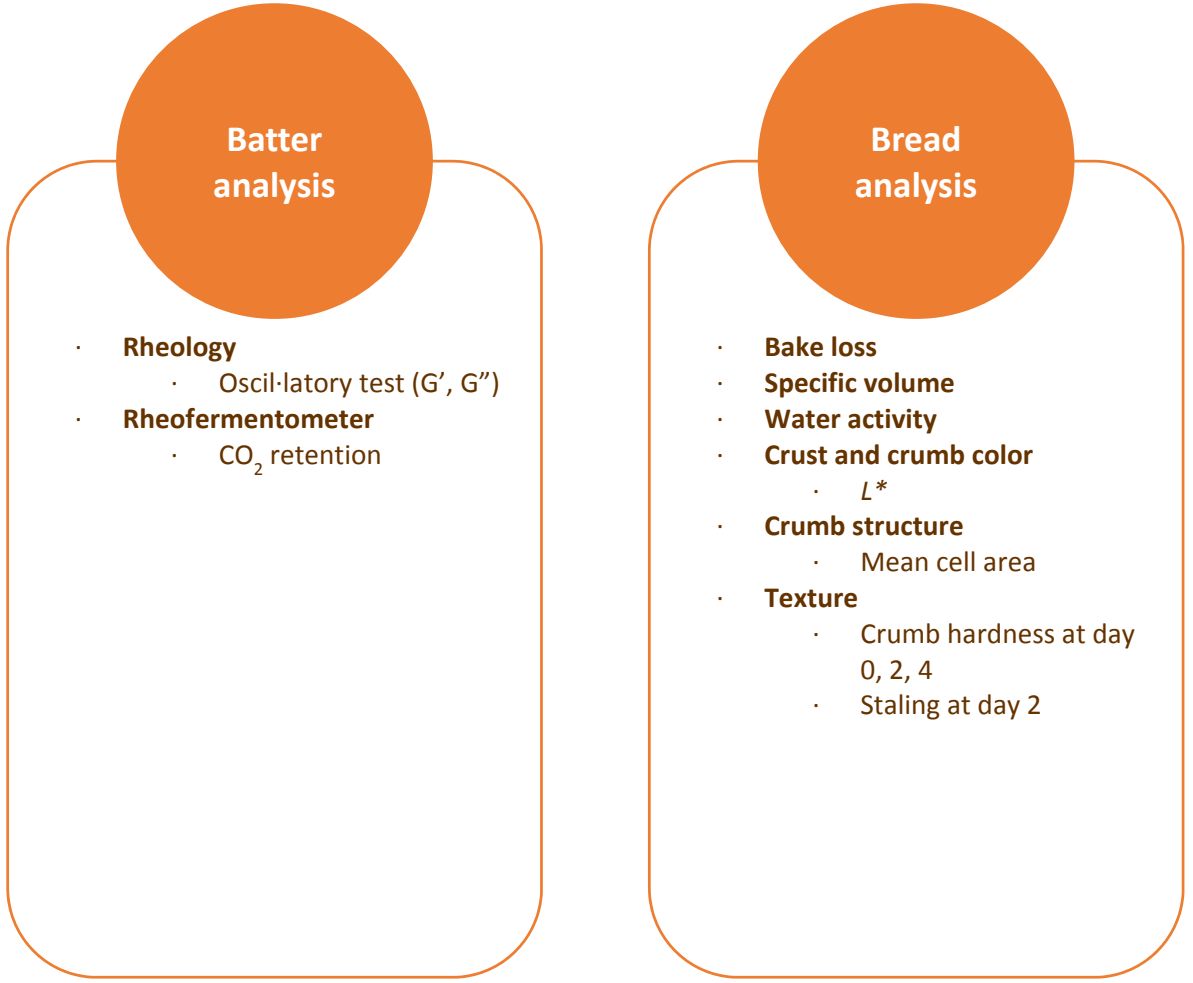


Figure 2.3. Experiment 2: Chickpea and tiger nut flours as alternatives to emulsifier and shortening in gluten-free bread.

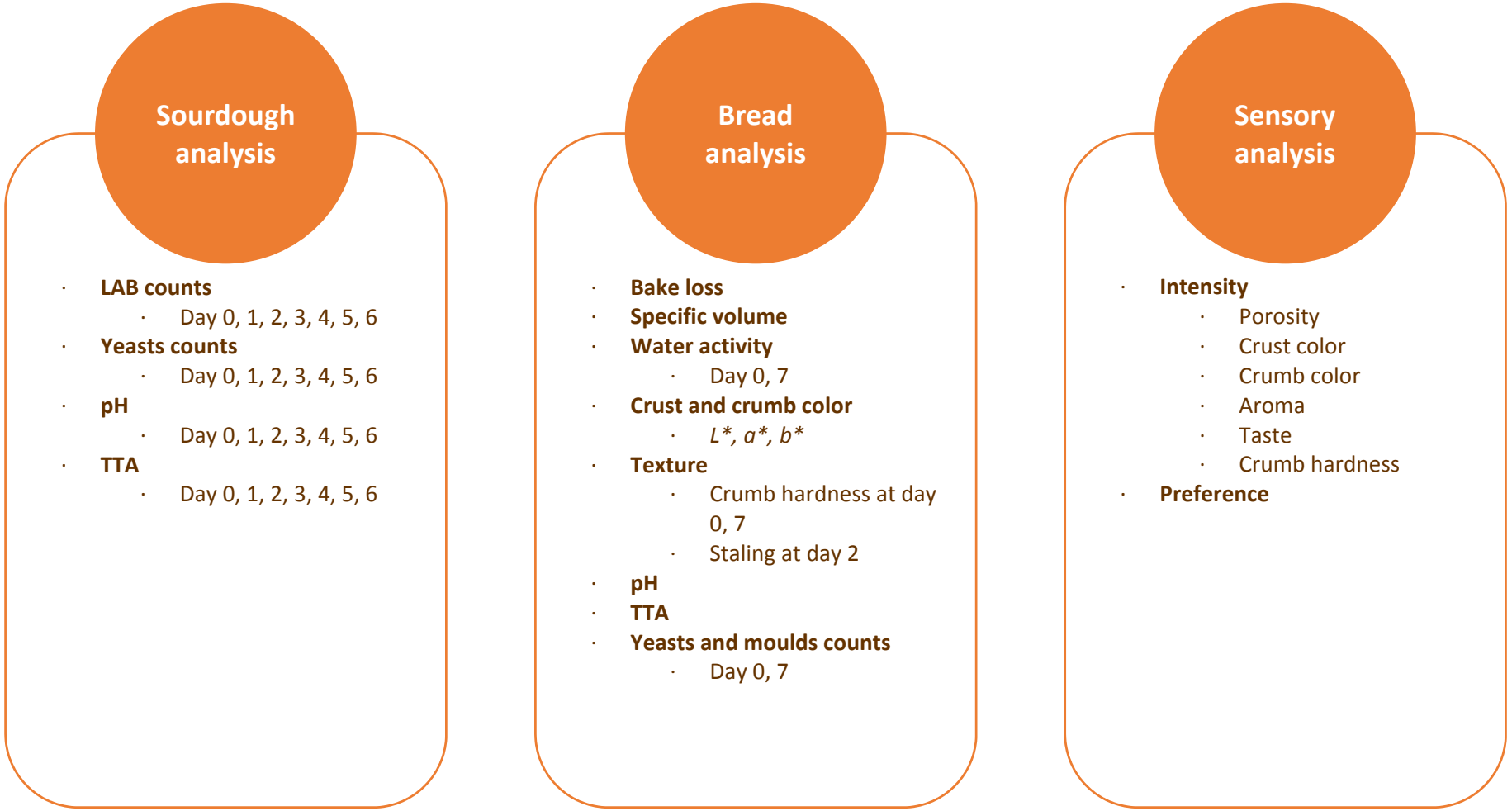


Figure 2.4. Experiment 3: Chestnut flour sourdough for gluten-free bread making.

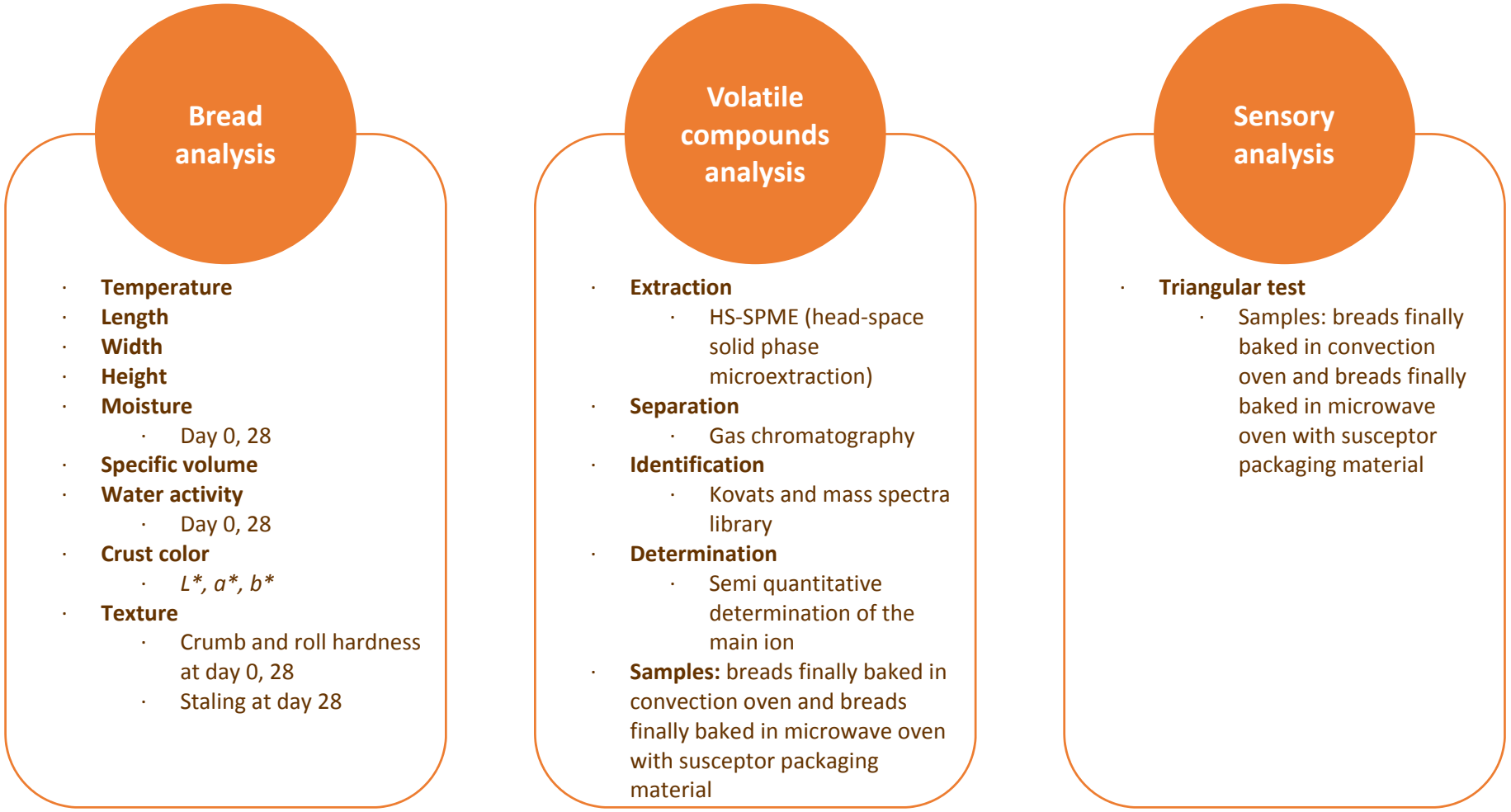


Figure 2.5. Experiment 4: Influence of final baking technologies in partially baked frozen gluten-free bread quality.

CHAPTER 3: EFFECT OF TIGER NUT DERIVED PRODUCTS IN GLUTEN-FREE BATTER AND BREAD

(EXPERIMENT 1)

Abstract

Tiger nut is a tuber used to produce tiger nut milk that yields a high quantity of solid waste, which can be dried and used as fiber source. The objective of this paper was to evaluate the quality of gluten-free bread formulated with different tiger nut derived products in order to substitute soya flour (which is an allergen ingredient) and, at the same time, increase the use of tiger nut derived products. Four gluten-free formulations based on corn starch and containing tiger nut milk, tiger nut milk by-product, tiger nut flour or soya flour (as reference formulation) were studied. Tiger nut milk increased G' of gluten-free batter and rendered breads with the softest crumb (502.46 g ± 102.05), the highest loaf specific volume (3.35 cm^3/g ± 0.25), and it was the most preferred by consumers (61.02%). Breads elaborated with tiger nut flour had similar characteristics than soya flour breads (except in color and crumb structure). The addition of tiger nut milk by-product resulted in a hard (1047.64 g ± 145.74) and dark (L^* = 70.02 ± 3.38) crumb bread, which was the least preferred by consumers. Results showed that tiger nut is a promising ingredient to formulate gluten-free baked products.

Keywords

Celiac disease, gluten-free bread, tiger nut flour, tiger nut milk, tiger nut milk by-product

1. Introduction

Celiac disease is one of the most common lifelong disorders in many areas of the world (Catassi and Fasano, 2008) and its detection has increased with the availability of improved and more accessible diagnostic tools. Therefore, demand of gluten-free (GF) products is also increasing and there is a need for GF quality products, especially bread. Production of high quality GF bread is a big challenge because gluten network confers unique viscoelastic properties to bread dough that render breads with good baking characteristics. For this reason, most of GF bakery products have an inferior quality than wheat counterparts (Gallagher et al., 2003a; Thompson, 2009).

Many GF formulations contain soya (*Glycine max* L.) flour as a protein source. Our research group has developed a formulation that contains soya flour and offers good baking (high volume and soft crumb) and sensory characteristics (Miñarro et al., 2012). However, soya associated digestive and allergy problems are leading to more research into alternative ingredients in order to obtain not only GF bread, but also allergen-free bread that has a wider consumer market.

Tiger nut (*Cyperus esculentus* L.) is a tuber rich in carbohydrates, lipids, fiber, some minerals (K, P, Ca), and vitamin E and C. It has moderate protein content with more essential amino acids content (mg/g protein) than the FAO/WHO recommended protein standard (Bosch et al., 2005). Nutritional properties of tiger nut and, by extension, its derived products are mainly related with its lipidic profile and fiber content. Tiger nut lipidic profile is similar to olive and hazelnut oils (Coşkuner et al., 2002). Ingredients with high fiber content are particularly interesting in GF diets as it has been reported that fiber intake of celiac patients is low (Thompson et al., 2005).

In Spain, tiger nut is mostly cultivated for the production of "horchata de chufa" or tiger nut milk that is a non-alcoholic and refreshing beverage mainly consumed during summer period, but it is underutilized in many countries in the world (Sánchez-Zapata et al., 2012). For the elaboration of tiger nut milk, tiger nut tubers are washed, soaked, grinded and pressed to separate tiger nut milk from tiger nut solid waste (tiger nut milk by-product). This solid waste, with a high content of insoluble fiber, is an economic and environmental problem for food industry and is used for animal feed, combustion or composting (Sánchez-Zapata et al.,

2009). Tiger nut milk is also used as a flavoring agent in ice cream, and tiger nut flour is added to biscuits and other bakery products (Coşkuner et al., 2002; Sánchez-Zapata et al., 2012). Recently, some researchers have used tiger nut to improve the nutritional profile or the technological characteristics of some products. Chinma et al. (2010) obtained acceptable wheat-based cakes with 30% of tiger nut flour, with better nutritional characteristics than 100% wheat cake. Demirkesen et al. (2011) observed that 10 or 20% of tiger nut flour improved GF rice bread conventionally baked or infrared-microwave combination baked, respectively.

Considering the nutritional profile of tiger nut derived products and the fact that they are not labelled as allergens, these ingredients could be adequate to formulate healthy and allergen-free GF breads. At the same time, this application would contribute to increase their use and exploitation. Therefore, the aim of this research was to evaluate the use of tiger nut flour, tiger nut milk and tiger nut milk by-product to formulate GF breads.

2. Materials and methods

2.1. Raw materials

Ingredients used for batter and bread formulation were: tap water, corn starch (Syral Iberia S.A.U., Zaragoza, Spain), white sugar (Azucarera Ebro S.L., Madrid, Spain), tiger nut milk and tiger nut milk by-product (Artgelato S.L., Barcelona, Spain), tiger nut flour (Tigernuts Traders S.L., Valencia, Spain), soya flour (Trades, Barcelona), shortening (Puratos, Sils, Spain), iodized refined salt (Sal Costa S.A., Barcelona), baking powder (Panreac Química S.L.U., Castellar del Vallès, Spain), dry yeast (Lallemand Iberica S.A., Cachofarra, Portugal), xanthan gum, and emulsifier: citric acid esters of mono and diglycerides and sucrose fatty acid esters (Degussa Texturant Systems, Paris, France).

Figure 3.1 shows tiger nut ingredients processing flowchart and Table 3.1 shows tiger nut ingredients and soya flour composition. Moisture, ash, lipids, protein, sugars, and soluble and insoluble fiber content were determined according to official methods (AOAC, 2005). Carbohydrate and starch content were calculated per difference.

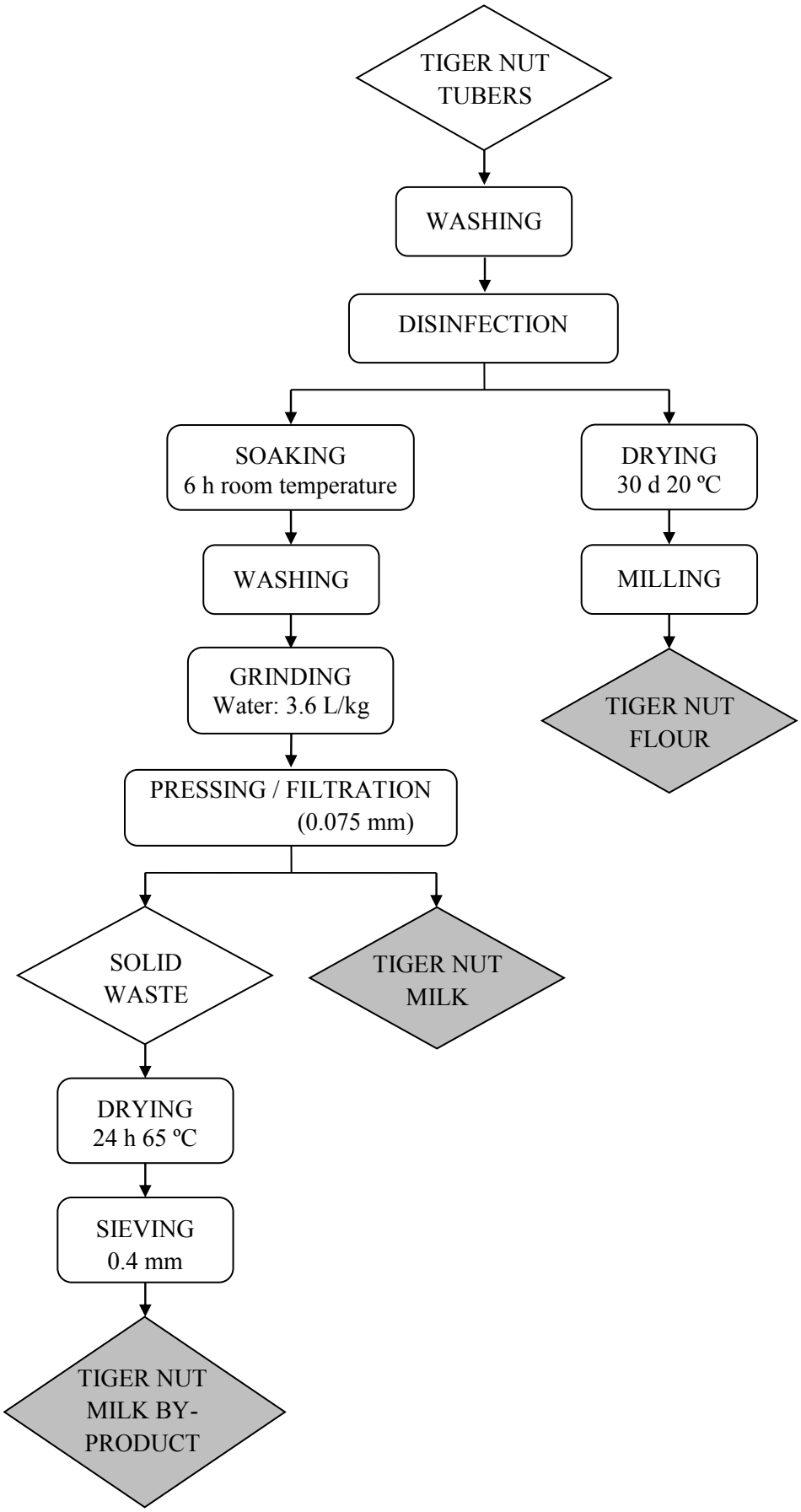


Figure 3.1. Flowchart for the production of tiger nut derived products.

Table 3.1. Nutritional composition of tiger nut derived products and soya flour (%) and nutrients of these ingredients provided to final batter formulation expressed in percentage of flour weight (% FW).

	Tiger nut milk		Tiger nut milk by-product		Tiger nut flour		Soya flour	
	%	% FW	%	% FW	%	% FW	%	% FW
Moisture	90.50	92.99	6.70	0.34	6.80	0.34	6.90	0.35
Ash	0.32	0.33	1.65	0.08	2.45	0.12	5.30	0.27
Fat	3.90	4.01	16.00	0.80	28.40	1.42	24.3	1.22
Protein	1.10	1.13	5.50	0.28	5.10	0.26	36.60	1.83
Carbohydrates	1.83	1.88	39.60	1.98	38.00	1.90	22.10	1.11
Starch	0.40	0.41	28.20	1.41	19.80	0.99	-	-
Sugars	1.43	1.47	11.40	0.57	18.20	0.91	-	-
Total fiber	2.35	2.41	30.55	1.53	19.25	0.96	4.80	0.24

Four formulations were obtained (Table 3.2): tiger nut milk (TM), tiger nut milk by-product (TMB), tiger nut flour (TF) and soya flour (SF). Soya flour was used as a reference, as it is a formulation developed in our group with good baking and sensory characteristics (Miñarro et al., 2012). In TMB and TF formulations, the tiger nut derived ingredient substituted the same amount of soya flour from the SF formulation. In TM formulation, tiger nut milk substituted water and soya flour. Previous studies indicated that adjusting all formulations at the same liquid ingredient volume was enough to obtain breads with good baking characteristics. However, as tiger nut milk had a significant solid content (9.5%), the corresponding weight of corn starch was removed to counterbalance the solid content of this ingredient.

2.2. Bread making procedure

Bread was elaborated at CERPTA (Centre Especial de Recerca Planta de Tecnologia dels Aliments) food processing plant. Powder ingredients were weighed and kneaded in a mixer (Sammic S.L., Gipuzkoa, Spain) at low speed (85 rpm). Water (25 ºC ± 1) or tiger nut milk (25 ºC ± 1) and melted shortening were added to powder ingredients. Batter was mixed for 2 min at low speed (85 rpm), 2 min at medium speed (222 rpm) and 30 s at high speed (385 rpm). Batter was weighed in 200 g portions in each baking pan (5 × 8 × 14 cm) and proofed

in a chamber (Salva, Lezo, Spain) for 35 min at 85% RH and 30 ºC. Finally, the baking process was carried out in a classic deck oven (Sveba-Dahlen AB, Fristad, Sweeden) at 180 °C for 30 min with steam injection during 10 s at the start of baking. After baking, loaves were depanned and cooled for 2 h at room temperature. Bread samples were packed in a modified atmosphere of 70% N_2: 30% CO_2 and stored for 4 days at 20 ºC ± 1. Three independent batches of each formulation were elaborated.

Table 3.2. Gluten-free formulations (TM: tiger nut milk; TMB: tiger nut milk by-product; TF: tiger nut flour; SF: soya flour) expressed in % of flour weight (% FW).

Ingredient (% FW)	TM	TMB	TF	SF
Water	-	102.75	102.75	102.75
Corn starch	90.24	95	95	95
Tiger nut milk	102.75	-	-	-
Tiger nut milk by-product	-	5	-	-
Tiger nut flour	-	-	5	-
Soya flour	-	-	-	5
Sugar	5.8	5.8	5.8	5.8
Shortening	5	5	5	5
Salt	2.5	2.5	2.5	2.5
Baking powder	2.5	2.5	2.5	2.5
Dry yeast	2	2	2	2
Xanthan gum	2	2	2	2
Emulsifier	2	2	2	2

2.3. Batter analysis

Moisture of batters was analyzed using a gravimetric method. To measure batter moisture, 3 g of sample were weighed and mixed with sand to increase drying area. Batters were dried with a forced air oven at 98-100 ºC to constant weight and moisture percent was calculated per difference.

For rheology studies, viscoelastic behavior was analyzed by oscillatory frequency sweep in the linear viscoelastic range. Batters were prepared as explained before but excluding yeast to avoid gas bubbles formation during the analysis (Lazaridou et al., 2007; Miñarro et al., 2012), and were placed between corrugated plates (35 mm diameter, with a 2 mm gap) of a controlled stress and strain rheometer, Haake Rheo Stress 1 (Thermo Scientific, Karlsruhe, Germany). Temperature was regulated at 30 ºC ± 0.1 with a Haake SC100 water bath (Thermo Scientific). Excess batter was removed and the exposed edges were covered with water to avoid desiccation. Batter rested for 5 min before being tested to allow relaxation. During test, the whole system was covered to prevent changes in temperature. Preliminary strain sweep tests at 1 Hz of frequency were performed, which showed that a strain of 0.05% was within the linear viscoelastic region. Storage modulus (G') and loss modulus (G") were obtained from frequency sweep tests performed at frequencies between 0.1 and 10 Hz with a target strain of 5×10^{-4} (0.05%). Three independent batches and at least two repetitions were analyzed.

A Rheofermentometer F3 (Chopin, Villeneuve-la-Garenne, France) was utilized to measure CO_2 production, loss and retention and batter height. The analysis was performed with 315 g of batter at 28.5 ºC for 3 h. A cylindrical weight of 500 g was used to run the test. Three independent batches were evaluated.

2.4. Bread characteristics

Moisture (%) of breads were measured according to AOAC standard method (AOAC, 2005) and three replicates were analyzed.

Initial batter weight and weight of bread after cooling were measured. Bake loss was calculated using the formula: bake loss (%) = (initial weight of batter - weight of bread after cooling) ×100 / initial weight of batter. Loaf volume was measured by the method of displacement of millet seeds (Griswold, 1962) with a bread volumeter (Chopin, Villeneuve-la-Garenne Cedex, France). Specific volume was calculated using the formula: specific volume (cm^3/g) = volume (cm^3) / weight (g). Water activity was measured by the dew-point method using an Aqualab hygrometer (Aqualab Series 3TE, Decagon Devices Inc., Pullman, USA).

A Hunter Lab colorimeter miniScan XTE (Hunter Associates Laboratory INC, Reston, Virginia, USA) was used and CIE *L** values (lightness) were measured with a D65 illuminant and a standard observer of 10º. Crust color was measured on six zones of the top of each loaf and crumb color was measured at four equidistant points from the center of each slice.

A flatbed scanner (HP ScanJet N6310c, Hewlett Packard) and its supporting software were used to capture bread images with a resolution of 300 dots per inch (dpi). From each batch, 9 slices of each sample were processed. A 4 × 4 cm square field of each slice was evaluated, which captured the majority of the crumb area of individual slice of bread. Sigma Scan Pro 5 software (Systat software Inc, Chicago, USA) and a threshold method were used to analyze mean cell area and total number of cells.

Texture analysis was carried out using AACC (74-09) standard method at 0, 2 and 4 days of storage at 20 ºC ± 1, with a TA-TX2 texture analyzer (Stable Micro Systems, Surrey, UK) using a cylindrical probe of 35 mm diameter. Uniform slices of 12.5 mm thickness were obtained by cutting the bread transversely using an automatic cutter. Two slices were stacked together to obtain uniform slices of 25 mm. Probe speed was set to 2 mm/s to compress the center of bread crumb to 40% of its original height. Hardness values, corresponding to the maximum peak force of the first compression, were recorded. Staling percent was calculated using the formula: staling (%) = (hardness day 2 / hardness day 0) × 100.

Three independent batches of each formulation were elaborated to determine bake loss, loaf specific volume, water activity, color, texture, and cell size. In each experiment, three breads from each formulation were analyzed.

2.5. Sensory analysis

Sensory analysis of bread was performed by 59 volunteers recruited among university staff and students. Testers were both female and male, aged between 18 and 58, and regular bread consumers. To study sensory characteristics of different breads, consumers evaluated acceptability and intensity of each attribute (porosity, crumb color, aroma, taste, chewiness and hardness) with a nine-point hedonic scale. At the end of the test, consumers were asked to select the most and the least preferred bread (results of preference test are expressed in %

of preference). One slice of each sample, randomly codified using three digits, was analyzed simultaneously, 24 h after bread making.

2.6. Statistical analysis

Results were analyzed by analysis of variance (ANOVA) using the general linear models procedure of Statistical Analysis System (SAS, 9.1 version). Student-Newman-Keuls test was applied to compare sample data. Evaluations were based on a significance level of $p < 0.05$.

3. Results and discussion

3.1. Batter characteristics

Batter rheology results are shown in Figure 3.2. All batters presented higher storage modulus values (G') than loss modulus values (G") in all range of frequencies studied, indicating a solid elastic-like behavior of all samples. TMB and TM showed the highest G' values compared with TF and SF, which remained very close to each other. Different water and fiber content of batters, as well as fiber size, may account for batter consistency differences. Demirkesen et al. (2010) found an increase in G' when fiber content of GF batter increased due to the presence of chestnut flour, probably caused by the association of fibers. Therefore, TM and TMB batters had higher G' than TF and SF because of its higher fiber content expressed in % of flour weight (% FW) (Table 3.1). However, TMB formulation that had lower fiber content than TM (Table 3.1) presented higher G'. This behavior could be attributed to the different size of fiber present in tiger nut milk by-product compared to tiger nut milk. The fiber particle size plays an important role in batters rheological behavior, as reported by Gómez et al. (2010) who stated an increase of G' and G" with increasing fiber size in wheat layer cake batters. In this research, tiger nut milk was filtered (0.075 mm) during its obtaining process (Figure 3.1) and, in consequence, the size of fiber particles from tiger nut milk by-product was higher than fiber size from tiger nut milk, and thus, TMB batter presented higher G' compared to TM.

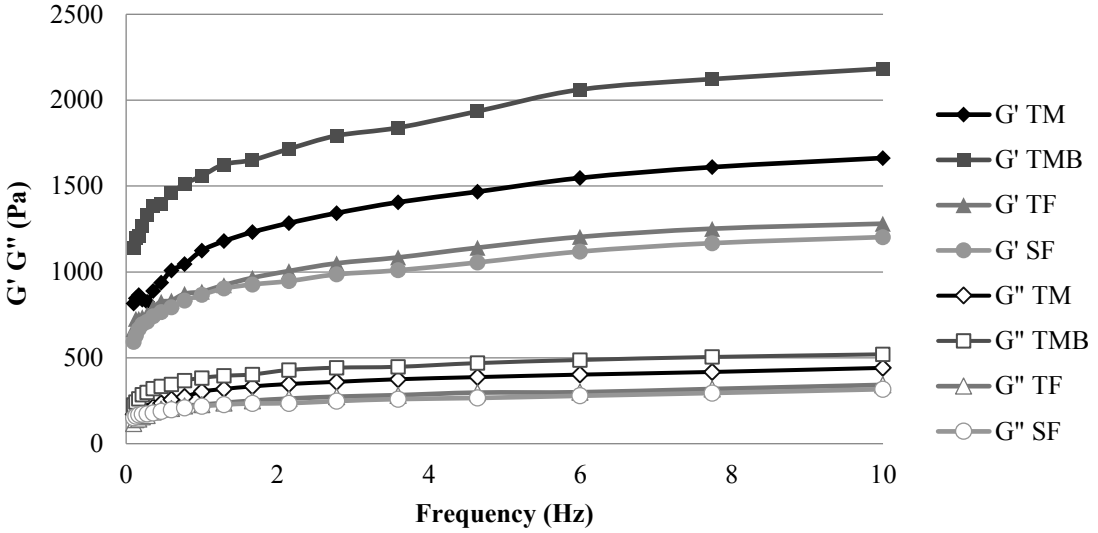


Figure 3.2. Batter parameters from oscillatory test (G' and G"). TM: tiger nut milk; TMB: tiger nut milk by-product; TF: tiger nut flour; SF: soya flour.

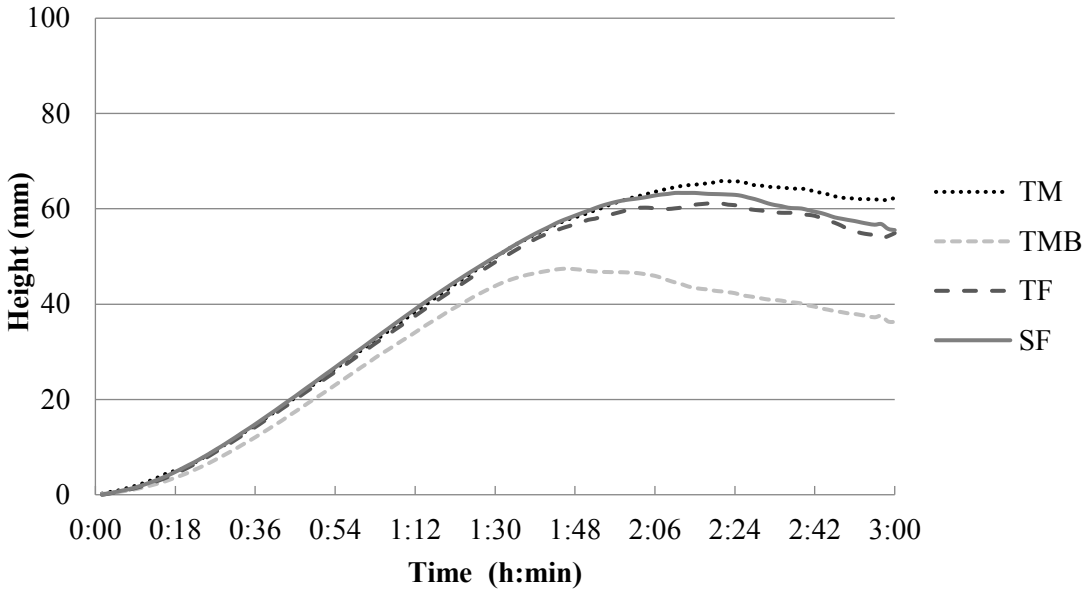


Figure 3.3. Batter parameters from rheofermentometer analysis (height of batter development). TM: tiger nut milk; TMB: tiger nut milk by-product; TF: tiger nut flour; SF: soya flour.

Higher G' values of TM batter than TF or SF batters may be related to the low moisture content of TM batter (47.31% ± 0.07) compared to TF and SF (51.13% ± 0.12 and 51.07%

± 0.28, respectively). Moisture of TM batter decreased because water was substituted by tiger nut milk, which contained 9.5% of solids (Table 3.1). Lazaridou et al. (2007) and Ronda et al. (2013) also observed higher values of G' when water content decreased in GF formulations. Moreover, higher G' values of TM batter compared to TF and SF batters, might also be related to fiber content, as tiger nut milk provides higher fiber content (expressed in % FW) than tiger nut flour or soya flour (Table 3.1). Demirkesen et al. (2010) also found an increase in G' when incrementing chestnut flour content, which increased fiber concentration of GF formulations. Figure 3.3 presents batter development results from the rheofermentometer test during 3 h of fermentation. The TMB batter had the lowest development curve, as well as the lowest gas released during 3 h of fermentation (1417.00 ml ± 34.70; $p < 0.05$). The low batter development of this batter could be related to its rheological characteristics, as TMB batter had the highest G' and thus, was too consistent to allow optimum development during fermentation. Furthermore, the low CO_2 released by TMB batter could be related to the low proportion of directly fermentable sugars that tiger nut milk by-product provides to the formulation, expressed in % FW (Table 3.1).

3.2. Baking characteristics

Table 3.3 shows baking characteristics of breads. The TM and TMB breads had lower bake loss values compared with TF and SF breads, while there were no differences between TM and TMB. On the one hand, initial water contents of the batters can partially explain bake loss differences, as TM batter was the one with the lowest water content (TM: 47.31% ± 0.07; TMB: 50.94% ± 0.16; TF: 51.13% ± 0.12; SF: 51.07% ± 0.28). On the other hand, tiger nut milk and tiger nut milk by-product were the ingredients that supplied the highest quantity of fiber to TM and TMB formulations respectively, expressed in % FW (Table 3.1), which would increase its water retention ability contributing to a lower bake loss. Moreover, Sánchez-Zapata et al. (2009) pointed out that tiger nut milk solid waste had a high water holding capacity, greater than other fibrous residues (soybean, sugar cane, pear and coconut) that also would help to reduce water loses during baking and cooling in TMB bread. The different type of fiber from tiger nut milk by-product in comparison with tiger nut milk would explain that TMB and TM breads had the same bake loss values although different initial water and fiber contents.

The bread with the highest loaf specific volume was TM, while no differences were observed among the other formulations (Table 3.3). This high volume could be explained by the feasible role played by tiger nut amylases during soaking and grinding processes in tiger nut milk production (Figure 3.1), including first hours after elaboration until refrigeration temperature is reached. These steps are performed with water, which extracts soluble components and activates tiger nut amylases, so starch would be partially converted to dextrins and sugars during and after tiger nut milk production process. Ejoh et al. (2006) observed a higher free reducing sugar content in tiger nut milk compared with tiger nut tubers, which could be explained by a higher amylase activity in tiger nut milk. Goesaert et al. (2009) suggested that amylase functionality may be related to the reduction of dough viscosity during starch gelatinization, thus prolonging oven rise and resulting in an increased loaf volume.

Table 3.3. Baking characteristics of gluten-free breads (TM: tiger nut milk; TMB: tiger nut milk by-product; TF: tiger nut flour; SF: soya flour).

	TM	TMB	TF	SF
Bake loss (%)	$10.38^{b} \pm 0.75$	$10.21^{b} \pm 0.95$	$11.30^{a} \pm 0.58$	$11.35^{a} \pm 1.11$
Loaf specific volume (cm^3/g)	$3.35^{a} \pm 0.25$	$2.68^{b} \pm 0.29$	$2.88^{b} \pm 0.18$	$2.89^{b} \pm 0.23$
Water activity	$0.970^{b} \pm 0.003$	$0.975^{a} \pm 0.002$	$0.974^{a} \pm 0.001$	$0.973^{a} \pm 0.001$
Number of cells/cm^2	$38.46^{b} \pm 9.21$	$46.76^{a} \pm 5.55$	$36.20^{b} \pm 6.61$	$45.41^{a} \pm 7.07$
Mean cell area (mm^2)	$4.83^{a} \pm 1.39$	$3.31^{b} \pm 0.43$	$5.15^{a} \pm 1.29$	$3.17^{b} \pm 0.42$
Crust color (L^* values)	$52.18^{d} \pm 2.05$	$60.19^{b} \pm 4.81$	$63.22^{a} \pm 4.19$	$58.59^{c} \pm 3.28$
Crumb color (L^* values)	$72.30^{b} \pm 2.51$	$70.02^{c} \pm 3.38$	$73.15^{b} \pm 2.87$	$77.50^{a} \pm 3.61$
Moisture (g/100g product)	$42.54^{c} \pm 2.09$	$46.85^{a} \pm 1.35$	$47.43^{a} \pm 0.89$	$45.82^{b} \pm 1.59$

[a-d] Values labelled with a different letter in the same row are significantly different ($p < 0.05$).

Gularte et al. (2012) observed a relationship between bread volume and batter consistency. They stated that when batter consistency increased, specific volume of breads also increased, but when batter was too much consistent, its development was limited and bread specific volume decreased. This observation agrees with our results as TM batter, with higher consistency than TF and SF (Figure 3.2), rendered the highest loaf specific volume (Table 3.3), while TMB batter, with the highest consistency, (Figure 3.2) rendered the breads with

the lowest specific volume (Table 3.3). Therefore, TMB batter structure was too consistent to allow batter rising during fermentation and baking.

As observed in the digital images (Figure 3.4), a lower number of cells, but of a larger size, were found in TM and TF crumbs. On the contrary, TMB and SF crumbs had more cells, which were smaller (Table 3.3). Large cells of TF bread did not involve an increase in bread volume; in contrast, the open structure of TM, due to larger alveolus size, gave the breads with the highest volume. Large cells in breads with high volume have found in other studies (Blanco et al., 2011; Lynch et al., 2009). Schober et al. (2005) observed that the presence of a high amount of sugars, due to increased susceptibility to amylase activity in damaged starch, gave breads with large mean cell area, small number of cells, and soft crumb, according to TM bread results obtained in our study.

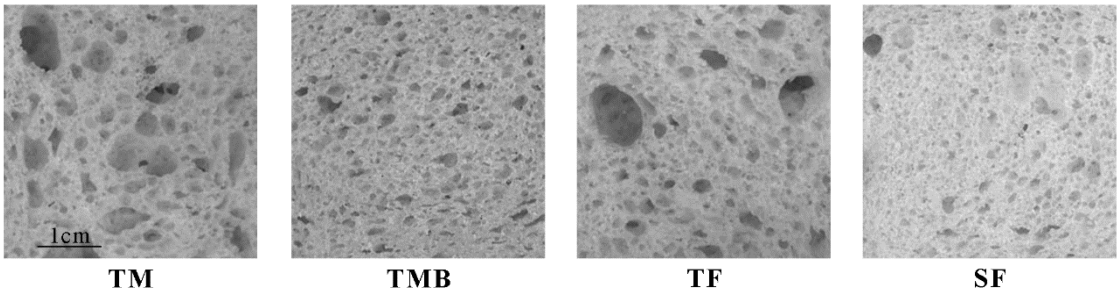


Figure 3.4. Digital images of gluten-free breads crumb (TM: tiger nut milk; TMB: tiger nut milk by-product; TF: tiger nut flour; SF: soya flour).

Lightness values of crusts and crumbs are reported in Table 3.3. The TM bread had the darkest crust while TF had the lightest. The dark crust color of TM bread agrees with its higher content of free sugars and protein, as crust color is a result of sugar caramelisation and Maillard browning, which is influenced by the distribution of water and the reaction of reducing sugars and amino acids. Goesaert et al. (2009) reported that amylase activity increases the level of reducing sugars, promoting the formation of Maillard reaction products, which, in their turn intensify bread crust color. Regarding to crumb color, all breads containing tiger nut derived products presented lower L^* values than SF breads. Demirkesen

et al. (2011) and Chinma et al. (2010) also observed decrease in crumb lightness due to the addition of tiger nut flour in GF and wheat bread, respectively.

3.3. Texture

Hardness and staling rate results are listed in Table 3.4. Crumb of TMB bread was the hardest during all days of analysis. On day 0, TM bread presented the softest crumb but on day 2, there were no significant differences between TM, TF and SF formulations.

Table 3.4. Texture results of gluten-free breads (TM: tiger nut milk; TMB: tiger nut milk by-product; TF: tiger nut flour; SF: soya flour).

	TM	TMB	TF	SF
Hardness (g)				
Day 0	$502.46^{d,z} \pm 102.05$	$1047.64^{a,z} \pm 145.74$	$661.49^{c,y} \pm 155.10$	$741.96^{b,z} \pm 65.90$
Day 2	$1429.35^{b,y} \pm 370.39$	$2402.55^{a,y} \pm 303.30$	$1593.67^{b,x} \pm 349.18$	$1613.26^{b,y} \pm 202.32$
Day 4	$1644.67^{c,x} \pm 408.62$	$2726.94^{a,x} \pm 184.96$	$1760.77^{c,x} \pm 373.35$	$2035.95^{b,x} \pm 401.19$
Staling (%)				
Day 2	$284.45^{a} \pm 50.98$	$236.13^{b} \pm 39.56$	$245.42^{b} \pm 57.47$	$218.86^{b} \pm 32.08$

[a-d] Values labelled with a different letter in the same row are significantly different ($p < 0.05$).
[x-z] Hardness values labelled with a different letter in the same column are significantly different ($p < 0.05$).

Gallagher et al. (2003b) studied the effect of moisture content in GF bread texture and concluded that breads with higher water content had softer crumb. In contrast, in our study, TM bread was the softest although it had the lowest water content (Table 3.3). Gujral et al. (2003) found a decrease in crumb hardness when α-amylase was added into rice flour GF bread. This observation agrees with our hypothesis of amylase activity in tiger nut milk, as TM crumb was the softest on day 0. Moreover, tiger nut milk provided to TM formulation the highest lipid content expressed in % FW (Table 3.1). This could contribute to explain the softest crumb of TM bread on day 0, as lipids can interact with amylose during baking (Pareyt et al., 2011), which is the responsible of initial crumb firmness (Goesaert et al., 2005). Furthermore, some authors have found softer breads when loaf specific volume was

higher (Gallagher et al., 2003b; Miñarro et al., 2010; Sabanis et al., 2009). In this study, this relationship was clearly observed in TM bread.

Although TM bread had the softest crumb on day 0, it showed the highest staling rate, probably due to its low water content (Table 3.3). The second softest bread was TF, and suffered less staling than TM. Demirkesen et al. (2011) also observed a decrease in crumb hardness when 5 and 10% of rice flour was substituted by tiger nut flour in GF bread. The TMB bread consistently showed the hardest crumb throughout the analysis, according to its fiber size and content, as previously discussed. Demirkesen et al. (2012) and Leray et al. (2010) observed an increase in crumb hardness when GF bread and wheat bread were enriched with fiber.

3.4. Sensory evaluation

Results from consumer test are shown in Table 3.5. Acceptability results show that TM bread obtained the highest scores in texture and taste attributes. Consumer comments pointed out that it had a sweet taste. The TM bread was selected as the most preferred by 61.02% of the consumers. Therefore, TM bread was the most preferred due to its texture and taste. In contrast, TMB bread was only preferred by 1.69% of consumers. In addition, TMB obtained the lowest scores in general appearance, aroma and hardness acceptability that explain the low preference for this bread.

Regarding to intensity results, TM and TF breads obtained the highest score in porosity, in agreement with cell size results. Consumers classified TMB bread as the hardest and did not detect any differences among other formulations. This indicates that staling, calculated from instrumental analysis results at day 2, had already developed one day after baking, when consumer test was performed. Consumers identified SF crumb color as the whitest, in accordance to instrumental measurements. No differences in taste intensity were found, but TMB and TF breads received a higher aroma intensity score.

Table 3.5. Intensity and acceptability values of sensory parameters of gluten-free breads (TM: tiger nut milk; TMB: tiger nut milk by-product; TF: tiger nut flour; SF: soya flour).

	Acceptability score[d]			
	TM	**TMB**	**TF**	**SF**
Porosity	$6.12^{a} \pm 1.71$	$5.46^{a} \pm 1.92$	$6.20^{a} \pm 1.67$	$5.90^{a} \pm 1.86$
Crumb color	$5.73^{bc} \pm 1.69$	$5.20^{c} \pm 1.91$	$6.20^{ab} \pm 1.49$	$6.56^{a} \pm 1.78$
Aroma	$5.98^{a} \pm 1.50$	$4.64^{b} \pm 1.92$	$5.98^{a} \pm 1.92$	$5.85^{a} \pm 1.77$
Taste	$6.46^{a} \pm 1.56$	$4.32^{b} \pm 1.89$	$4.97^{b} \pm 2.08$	$5.17^{b} \pm 2.29$
Chewiness	$6.47^{a} \pm 1.55$	$4.46^{c} \pm 1.96$	$4.85^{c} \pm 1.90$	$5.61^{b} \pm 1.95$
Hardness	$6.56^{a} \pm 1.58$	$4.34^{c} \pm 2.00$	$5.54^{b} \pm 1.97$	$5.97^{ab} \pm 1.92$
	Intensity score			
	TM	**TMB**	**TF**	**SF**
Porosity	$6.61^{a} \pm 1.47$	$5.08^{b} \pm 2.21$	$6.27^{a} \pm 1.40$	$4.92^{b} \pm 1.81$
Crumb color	$6.56^{a} \pm 1.28$	$6.95^{a} \pm 1.86$	$5.25^{b} \pm 1.46$	$2.80^{c} \pm 1.87$
Aroma	$5.00^{b} \pm 1.74$	$6.02^{a} \pm 1.81$	$6.20^{a} \pm 1.47$	$5.27^{b} \pm 1.68$
Taste	$5.12^{a} \pm 1.41$	$5.05^{a} \pm 1.21$	$5.14^{a} \pm 1.41$	$4.59^{a} \pm 1.40$
Chewiness	$3.46^{b} \pm 1.70$	$4.47^{a} \pm 1.90$	$3.75^{b} \pm 1.89$	$3.36^{b} \pm 1.85$
Hardness	$3.56^{b} \pm 1.67$	$5.10^{a} \pm 2.27$	$3.47^{b} \pm 1.77$	$3.95^{b} \pm 1.85$
General Appearance	$6.12^{a} \pm 1.81$	$5.24^{b} \pm 2.01$	$6.53^{a} \pm 1.36$	$6.12^{a} \pm 1.99$
Most preferred (%)	61.02	1.69	16.95	20.34

Mean values ± standard deviations of 59 consumers.
[a-c] Values labelled with a different letter in the same row are significantly different ($p < 0.05$).
[d] Acceptability descriptors: "dislike extremely" (left of the nine-point scale) and "like extremely" (right of the nine-point scale).

4. Conclusions

Gluten-free breads containing tiger nut milk showed better baking (higher specific volume, lower bake loss and softer crumb) and sensory characteristics (texture and taste) than soya flour breads, due to the sugar, fat and fiber content that tiger nut milk provided to tiger nut milk formulation. Tiger nut and soya flours gave similar characteristics to breads, except in color and crumb structure. Tiger nut milk by-product impaired bread quality (darkest color and hardest crumb) due to its fiber size and content. In conclusion, tiger nut milk and tiger nut flour are good alternatives to soya flour for the production of gluten-free breads with increased fiber content and free of allergens.

Acknowledgments

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CHAPTER 4: CHICKPEA AND TIGER NUT FLOURS AS ALTERNATIVES TO EMULSIFIER AND SHORTENING IN GLUTEN-FREE BREAD

(EXPERIMENT 2)

Abstract

Chickpea protein has good emulsifying properties, which improve gluten-free bread volume. Tiger nut is a tuber with high lipid content of healthy fatty acid profile. Taking into account these characteristics, the effect of chickpea and tiger nut flours addition to gluten-free batters and breads in order to partially or totally replace emulsifier and/or shortening in gluten-free formulations has been studied. Four formulations were compared: corn starch; 7.8% chickpea flour and corn starch; 8.6% tiger nut flour and corn starch; 7.8% chickpea flour + 8.6% tiger nut flour and corn starch. The combination of three levels of shortening (5, 2.5 and 0%) and three levels of emulsifier (2, 1 and 0%) was evaluated in each basic formulation. Chickpea flour increased bread specific volume but tiger nut flour reduced it. When chickpea protein and emulsifier were added in the formulation, shortening increased G'and specific volume, and reduced initial crumb firmness. Bread elaborated with both chickpea and tiger nut flour maintained its baking characteristics (bake loss, specific volume, crust and crumb color and, crumb hardness) even when shortening and/or emulsifier were reduced or eliminated.

Keywords

Gluten-free bread, shortening, emulsifier, chickpea flour, tiger nut flour

1. Introduction

Gluten-free (GF) products demand, especially bread, is increasing as a result of the increase of celiac disease diagnosis (Cureton and Fasano, 2009). Nevertheless, production of high quality GF bread is a big challenge due to the absence of gluten, which confers unique viscoelastic properties to dough. To overcome this challenge, GF bread formulations incorporate a range of flours from cereals (rice, sorghum, millet), pseudocereals (quinoa, amaranth, buckwheat) or legume flours (soya, chickpea, carob, pea); starches from corn, potato or cassava; and ingredients such as proteins, hydrocolloids, emulsifiers and shortenings that improve their sensory properties and shelf-life, but also lead to an increase of final price (Miñarro et al., 2012; Moroni et al., 2009; Zannini et al., 2012).

Chickpea (*Cicer arietinum* L.) is a legume rich in protein, dietary fiber, carbohydrates, folate and trace minerals (Fe, Mo, Mn) (Meng et al., 2010). Some authors have studied functional properties of chickpea proteins, reporting good emulsifying and foaming characteristics (Boye et al., 2010; Karaca et al., 2011) as well as high oil absorption capacity (Aydemir and Yemenicioglu, 2013). The functional properties of chickpea protein provide good baking characteristics in GF and wheat breads elaborated with chickpea flour (Miñarro et al., 2012; Mohammed et al., 2012).

Tiger nut (*Cyperus esculentus* L.) is an important crop in Spain that is used to produce a milky beverage called "horchata de chufa", although it is underutilized in many countries in the world. It is a tuber rich in carbohydrates, lipids, fiber, some minerals (K, P, Ca), and vitamin E and C (Sánchez-Zapata et al., 2012). This tuber has 23-31% of lipid content with a fatty acid profile similar to olive and hazelnut oil, which confers healthy properties to tiger nut tuber, together with its high fiber content (8-15%) (Alegría-Torán and Farré-Rovira, 2003; Sánchez-Zapata et al., 2012). Tiger nut flour could be used in bakery products (Chinma et al., 2010) as well as to formulate GF bread with good baking and nutritional characteristics (Aguilar et al., 2014; Demirkesen et al., 2011).

Emulsifiers in wheat bread can be classified, according to its functionality, as dough strengtheners or crumb softeners (Stampfli and Nersten, 1994). Some authors have incorporated emulsifiers in GF formulations in order to strengthen the dough and/or soften the crumb (Demirkesen et al., 2010; Nunes et al., 2009; Onyango et al., 2009; Purhagen et al., 2012; Sciarini et al., 2012). In 2011, Csáki reported that synthetic emulsifiers, commonly

used in bakery industry, could increase intestinal permeability, favoring the incidence of allergic and autoimmune diseases. As inhibition of intestinal barrier dysfunction is recommended for celiac disease treatment (Paterson and Turner, 2008), reduction or elimination of synthetic emulsifiers from GF bread could benefit the intestinal health of celiac patients.

Shortening is defined as crystalline lipids and oils from vegetable and/or animal origin with a composition of 100% lipid approximately. Shortening plasticizes and lubricates dough, increases dough rise, oven spring and loaf volume, as well as improves crumb structure and shelf-life of wheat bread (Autio and Laurikainen, 1997; Chin et al., 2010; Ghotra et al., 2002; Pareyt et al., 2011). Shortening is usually incorporated in GF bread to improve its quality (Aguilar et al., 2014; Demirkesen et al., 2010; Miñarro et al., 2012; Schober, 2009; Sciarini et al., 2012). In addition, shortening showed a crumb softening effect in sorghum GF bread (Schober, 2009). Nevertheless, shortening increases the caloric intake and may imbalance the nutritional profile of GF bread due to the increase of fat content with a high proportion of saturated fatty acids.

Considering the properties of chickpea proteins and tiger nut lipid content, their flours could be used to improve GF formulations, providing a cleaner label and a better nutritional quality. The aim of this research was to study the effect of chickpea and tiger nut flours, separately and combined, in GF batters and breads in order to partially or totally replace emulsifier and/or shortening from GF formulations.

2. Materials and methods

2.1. Raw materials

Ingredients used for the elaboration of GF bread and batter were: tap water, corn starch (Syral Iberia S.A.U., Zaragoza, Spain), chickpea flour (El Granero Integral S.L., Madrid), tiger nut flour (Tigernuts Traders S.L., Valencia, Spain), shortening (Puratos, Sils, Spain), white sugar (Azucarera Ebro S.L., Madrid, Spain), baking powder (Panreac Química S.L.U., Castellar del Vallès, Spain), xanthan gum (Degussa Texturant Systems, Paris, France), emulsifier (Degussa Texturant Systems), dry yeast (Lallemand Iberica S.A., Cachofarra, Portugal), and iodized refined salt (Sal Costa S.A., Barcelona).

Commercial chickpea flour nutritional composition expressed in % was: 8.2 moisture, 3.0 ash, 6.1 fat, 19.6 protein, 54.2 carbohydrates, 50.2 starch, 4.0 sugars, < 0.1 soluble fiber, 8.8 insoluble fiber. Commercial tiger nut flour was sieved through a 0.5-mm sieve to avoid the incorporation of particles leading to sandy texture in bread. Nutritional composition (expressed in %) of the sieved tiger nut flour was: 6.7 moisture, 3.0 ash, 28.6 fat, 5.4 protein, 44.3 carbohydrates, 26.2 starch, 18.1 sugars, < 0.1 soluble fiber, 11.9 insoluble fiber. Shortening used contained refined vegetable fats and oils, tocopherol-rich extract (E-306), ascorbyl palmitate (E-304) and beta-carotene (E-160a). Emulsifier was composed of citric acid esters of mono- and diglycerides and sucrose fatty acid esters.

Four basic formulations were compared (Table 4.1): starch (S), chickpea (C), tiger nut (T) and chickpea-tiger nut (CT). Decreasing concentrations of shortening (5, 2.5 and 0%) and emulsifier (2, 1 and 0%) and the combination of all them were evaluated in the four basic formulations (S, C, T, CT). Therefore, a total of 36 formulations (9 from each basic formulation) were compared in this study (Table 4.1). Maximum concentrations of shortening and emulsifier (5 and 2%, respectively) and chickpea flour concentration (7.8%) were those used in previous studies from our research group (Miñarro et al., 2012; Aguilar, et al., 2014). The amount of tiger nut flour was determined in preliminary studies.

Table 4.1. Gluten-free formulations (S: starch; C: chickpea flour; T: tiger nut flour; CT: chickpea and tiger nut flours) expressed in % of flour (starch + flour) weight (FW).

Ingredient (% FW)	S	C	T	CT
Water	103	103	103	103
Corn starch	100	92.2	91.4	83.6
Tiger nut flour	-		8.6	8.6
Chickpea flour	-	7.8	-	7.8
Sugar	4.2	4.2	4.2	4.2
Shortening	5-2.5-0	5-2.5-0	5-2.5-0	5-2.5-0
Salt	1.7	1.7	1.7	1.7
Baking powder	2.5	2.5	2.5	2.5
Dry yeast	2	2	2	2
Xanthan gum	2	2	2	2
Emulsifier	2-1-0	2-1-0	2-1-0	2-1-0

2.2. Batter analysis

For rheology measurements, batters were prepared as described by Aguilar et al. (2014) but without dry yeast. Oscillatory test was performed as described by Miñarro et al. (2012). A target strain of $5x10^{-4}$ (0.05%) was used in the frequency sweep test as preliminary studies demonstrated that it was within the linear visoelastic range. At least two repetitions of three independent batches were analyzed.

To study GF batter behavior during fermentation a Rheofermentometer F3 (Chopin, Villeneuve-la-Garenne, France) was utilized, which measured CO_2 retention during 45 min of fermentation at 30 ºC. The analysis was performed with 315 g of batter prepared as explained before and a cylindrical weight of 500 g was used to run the test. Three independent batches were analyzed.

2.3. Bread making

Bread was made as described by Aguilar et al. (2014) method except that, in the present study, breads were baked in a convection oven (Sveba-Dahlen AB, Fristad, Sweeden) at 160 °C for 30 min, with steam injection for 10 s at the start of baking.

2.4. Bread Analysis

Loaf volume, bake loss, crumb hardness and crust and crumb color were evaluated by the methods described by Miñarro et al. (2012).

Image analysis (mean cell area) and staling were measured as described by Aguilar et al. (2014).

To evaluate bread characteristics, three independent productions of each formulation were developed. In each experiment, three breads from each formulation were analyzed.

2.5. Statistical analysis

Results were analyzed by analysis of variance (ANOVA) using the general linear models procedure of Statistica 7 software. Student-Newman-Keuls method was applied for comparison of sample data. Evaluations were based on a significance level of $p < 0.05$. Statistical analysis of batter rheology was performed with G' values at 10 Hz.

3. Results and discussion

3.1. Batter characteristics

Figure 4.1 shows rheology results from frequency sweep. All 36 formulations presented higher storage modulus (G') than viscous modulus (G") values, which indicates a solid elastic-like structure in the frequencies range studied. Rheology results show that the incorporation of chickpea flour in GF batter increased G' as C and/or CT formulations tended to present higher G' ($p < 0.05$) than formulations without chickpea flour (S and T).

Shortening reduction and/or elimination resulted in a decrease of G' ($p < 0.05$) in the presence of emulsifier. The reduction of G' due to lower shortening content may be related with the plasticizer and lubricant effect of shortening (Ghotra et al., 2002; Pareyt et al., 2011). However, in the absence of emulsifier, reduction or elimination of shortening had no significant effect ($p > 0.05$) in S and T formulations and resulted in a higher G' ($p < 0.05$) in C batters, while in CT, shortening elimination caused a decrease of G' ($p < 0.05$). It seems that in CT formulations without emulsifier, tiger nut flour allowed the increase of G' when shortening was present, probably due to its lipid content (28.6%) that permit the emulsification of chickpea protein.

The elimination of emulsifier resulted in a higher G' ($p < 0.05$) in all formulations with 2.5% and 0% of shortening. In contrast, when 5% shortening was present, reduction or elimination of emulsifier caused a significant decrease of batter consistency (G') in C and S formulations, but it did not affect G' values of batters containing tiger nut flour (T and CT). The effect of emulsifiers in GF batter rheology depends on the type of emulsifier, specifically on the HLB value. HLB (hydrophilic/lipophilic balance) is a system to classify emulsifiers by their hydrophilic/lipophilic nature and range from 0 to 18.

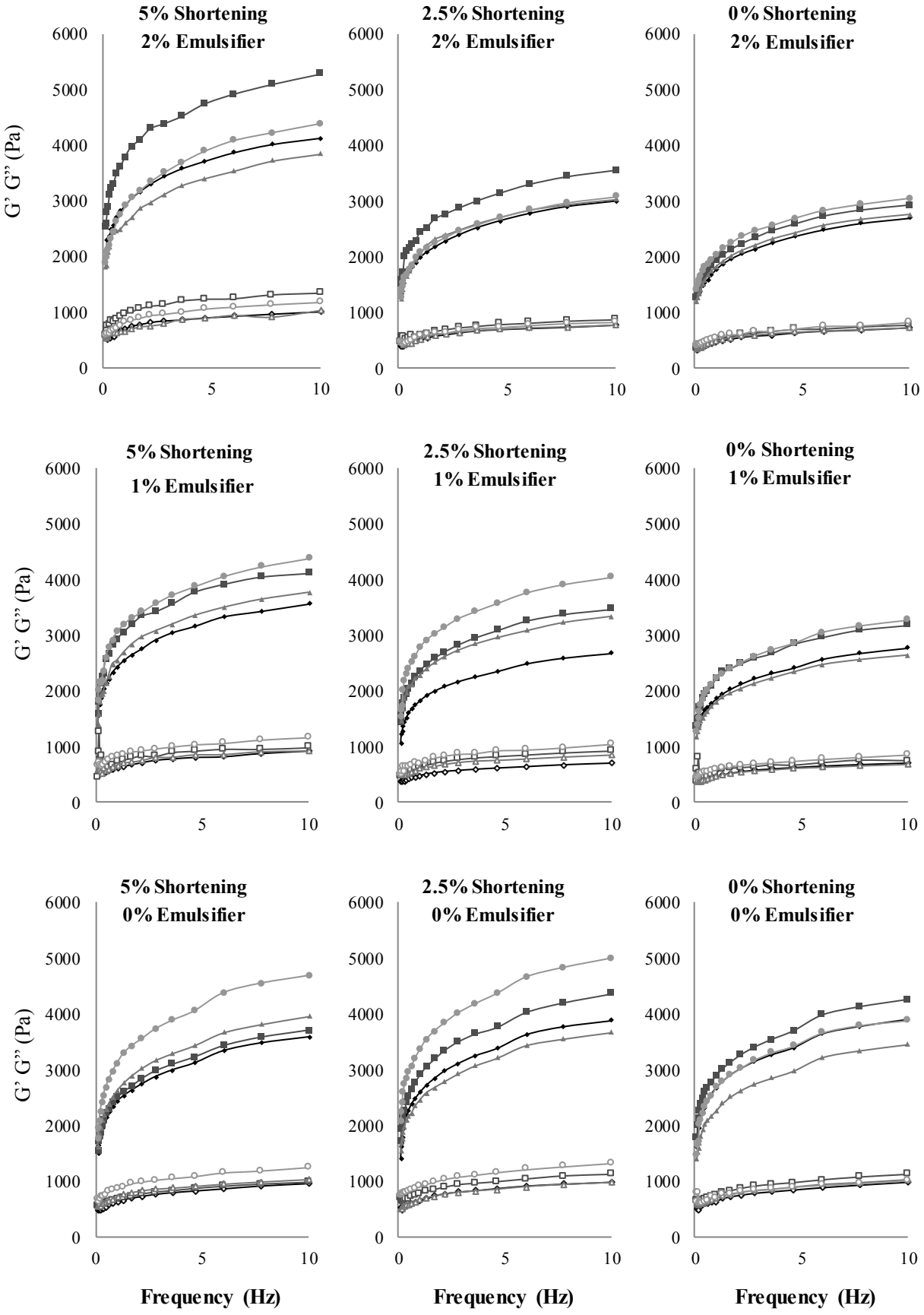

Figure 4.1. Storage [G' (gray)] and loss [G'' (white)] modulus of gluten-free batters [starch (◇), chickpea (□), tiger nut (△), chickpea-tiger nut (○)] with different concentrations of shortening and emulsifier.

High HLB values refer to high hydrophilic nature and, in contrast, low HLB values indicate high lipophilic nature (Whitehurst, 2004). Nunes et al. (2009) reported that addition of soy lecithin as emulsifier in GF batter reduced G' and G" due to its high hydrophobicity since more water was available in the system. Although the emulsifier used in the present study (citric acid esters of mono- and diglycerides and sucrose fatty acid esters) had medium hydrophobicity (HLB = 8-9) it also reduced G' values of GF batters. However, Sciarini et al. (2012) observed an increase of G' and G" when different emulsifiers, diacetil tartaric acid esters of mono- and diacylglycerols (DATEM) or sodium stearoyl lactylate (SSL) were added into GF batters.

Shortening could not be eliminated without negatively affecting batter consistency except when no emulsifier was added. Thus, when no shortening was added, G' values increased in absence of emulsifier. Emulsifier could be eliminated without causing a decrease in G' values, except for S and C formulations with 5% of shortening.

There were no significant ($p > 0.05$) differences in CO_2 retention (results not shown) when comparing different shortening and emulsifier concentrations, indicating that these ingredients did not influence the retention of gas released during fermentation.

3.2. Baking characteristics

Figure 4.2A shows specific volume results of all breads studied. In most of cases C and S breads presented higher volume ($p < 0.05$) than T and CT. Therefore, the presence of tiger nut flour significantly ($p < 0.05$) impaired bread specific volume. Demirkesen et al. (2011) reported an increase of bread specific volume of rice flour based GF bread when tiger nut flour was added, due to the role of tiger nut fat (that plasticized dough), and tiger nut fiber (that improved gas retention and water holding capacity). In the present study, this effect was not observed, due to the differences in the GF formulations compared i.e. rice flour base instead of corn starch, higher water content, higher shortening content, absence of emulsifier. Miñarro et al. (2012) also observed an increase of specific volume in GF bread elaborated with chickpea flour due to the good emulsifying stability index of chickpea protein (Paredes-López et al., 1991). In our study, C formulation is also the one that renders the breads with the highest specific volume.

Although shortening increases bread specific volume (Pareyt et al., 2011), in the present study shortening increased volume only in C, T and CT formulations that contained 2% of emulsifier. Shortening had no effect on S formulations, which may be related to their lack of protein as shortening interacts with protein enabling an increase of bread volume (Pareyt et al., 2011).

Elimination of emulsifier rendered breads with significant ($p < 0.05$) higher volume in all S and C breads as well as in T breads without shortening. Different authors have observed that emulsifiers increase wheat bread volume (Gómez et al., 2004; Nunes et al., 2009; Stampfli and Nersten, 1994). However, addition of emulsifiers in GF bread has different effects on bread specific volume. Sciarini et al. (2012) did not observe an increase in GF bread volume adding DATEM or SSL. Purhagen et al. (2012) found a decrease in bread specific volume when added DATEM as emulsifier in GF bread. On the contrary, Nunes et al., (2009) reported an increase of GF bread volume when different emulsifiers were added: lecithin, DATEM, distilled monoglycerides (DM) or SSL. Probably, the effect of emulsifiers on GF bread depends on the formulation where they are acting e.g. flour fat content or presence of shortening in the formulation. Moreover, Gómez et al. (2004) reported that emulsifiers delayed fermentation and did not have any positive effect on bread volume in breads with short fermentation time. This observation could be also applied to the present study, as breads were proofed for 45 min. Furthermore, bread specific volume is highly related with batter characteristics. According to Gularte et al. (2012), GF batters with high G' render breads with higher specific volume. In our study, this relationship was only observed when emulsifier was eliminated from S and C formulations with 2.5% or 0% of shortening, in which G' and specific volume increased.

Shortening had no significant ($p > 0.05$) effect on bake loss (Figure 4.2B) except in one case (T bread with 0% of emulsifier and 2.5% of shortening). Reduction of emulsifier from 2% to 1% did not affect bake loss ($p > 0.05$) but its elimination resulted in a higher bake loss ($p < 0.05$). Purhagen et al. (2012) stated that emulsifiers [glycerol monostarate (GMS), SSL or DATEM] improved water holding capacity when added into GF bread. This could explain the higher bake loss observed when emulsifier was eliminated. C breads showed significantly higher bake loss than CT when containing 5% shortening and/or 0% emulsifier.

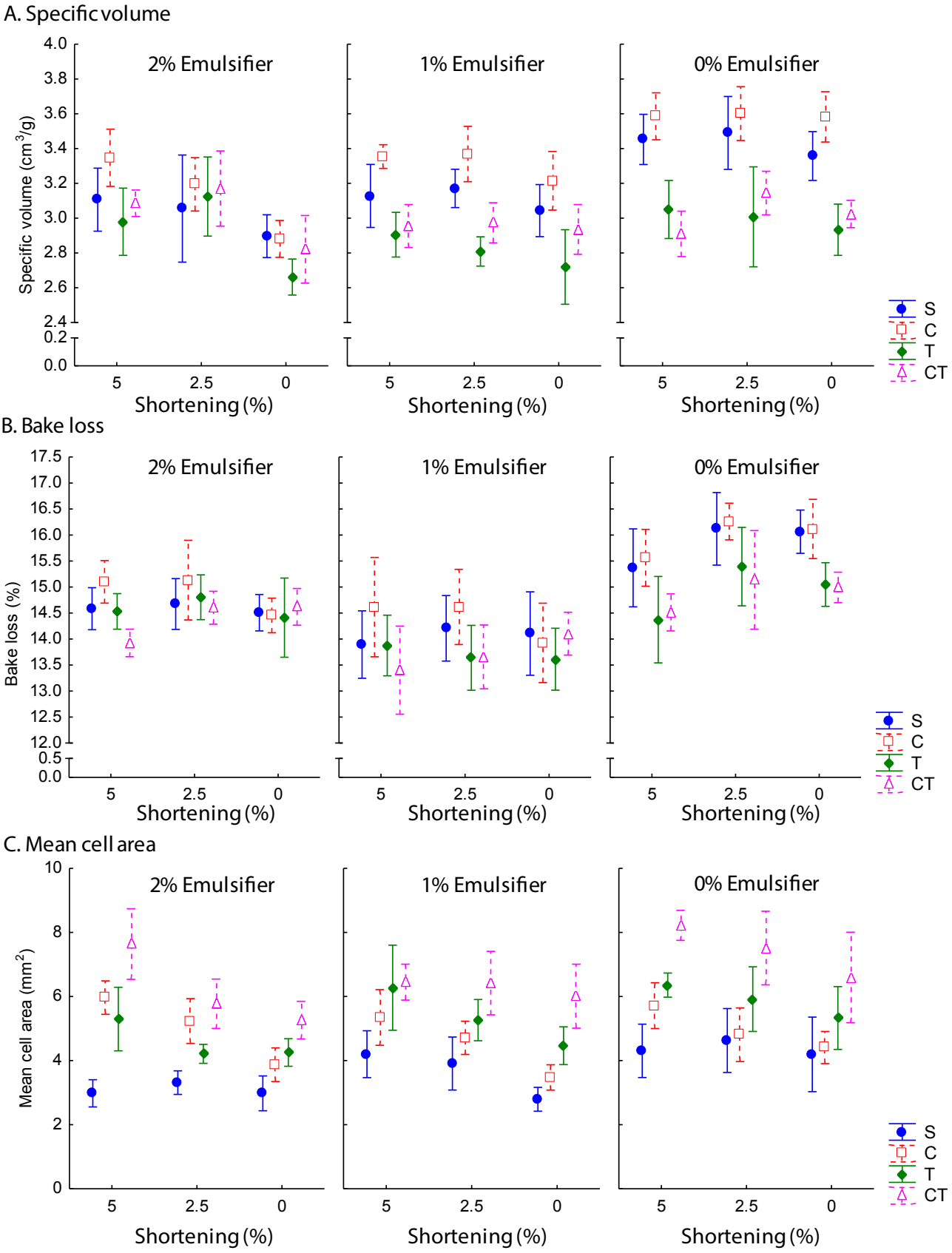

Figure 4.2. Baking characteristics of gluten-free breads (S: starch, C: chickpea, T: tiger nut, CT: chickpea-tiger nut) with different concentrations of shortening and emulsifier. Bread specific volume (A), bake loss (B) and mean cell area (C).

Shortening reduction and/or elimination resulted in a decrease of mean cell area ($p < 0.05$) (Figure 4.2C). In contrast, reduction and/or elimination of emulsifier, increased significantly ($p < 0.05$) mean cell area in S, T and CT formulations and had no effect in C breads. The highest and lowest cell areas were observed in CT and S formulations respectively. A negative correlation between cell area and number of cells was found ($r = -0.65$; $p < 0.05$). Thus, breads with higher mean cell area had lower number of cells. The observed effect of emulsifier on crumb structure (generating lower mean cell area) was expected, as emulsifiers stabilize air bubbles and prevent coalescence (Gant et al., 1995; Nunes et al., 2009).

Crust and crumb color results (L^* values) are shown in Figure 4.3. Addition of chickpea and/or tiger nut flours rendered breads with darker crust ($p < 0.05$) due to the amino acids and sugars provided by chickpea and tiger nut flours respectively, which contributed to Maillard reaction. Actually, the darkest crusts were obtained with combination of both flours. In general, emulsifier and shortening levels had no significant ($p > 0.05$) effect on crust lightness.

Breads containing tiger nut flour (T and CT) presented darker crumbs ($p < 0.05$) as well as higher a^* values (results not shown) compared with S and C breads due to the tiger nut flour color ($L^* = 68.59 \pm 0.03$, $a^* = 3.63 \pm 0.05$, $b^* = 18.13 \pm 0.04$), which is darker than chickpea flour ($L^* = 86.94 \pm 0.04$, $a^* = 2.47 \pm 0.03$, $b^* = 22.11 \pm 0.07$) and, of course, corn starch ($L^* = 97.10 \pm 0.02$, $a^* = -0.39 \pm 0.29$, $b^* = 5.74 \pm 0.02$). Shortening level had no significant ($p > 0.05$) effect on crumb lightness. Darker crumbs ($p < 0.05$) were observed in S, T and CT breads without emulsifier compared to 1 and 2% emulsifier, which is probably related with the higher cell area observed in these breads that affected crumb color measurement. In fact, a negative correlation between crumb color (L^* values) and cell area was observed ($r = -0.60$; $p < 0.05$). In contrast, emulsifier did not affect crumb color nor cell area in C breads.

3.3. Texture

Figure 4.4 shows crumb hardness results on day 0, 2 and 4. Crumb hardness of all breads increased ($p < 0.05$) during storage.

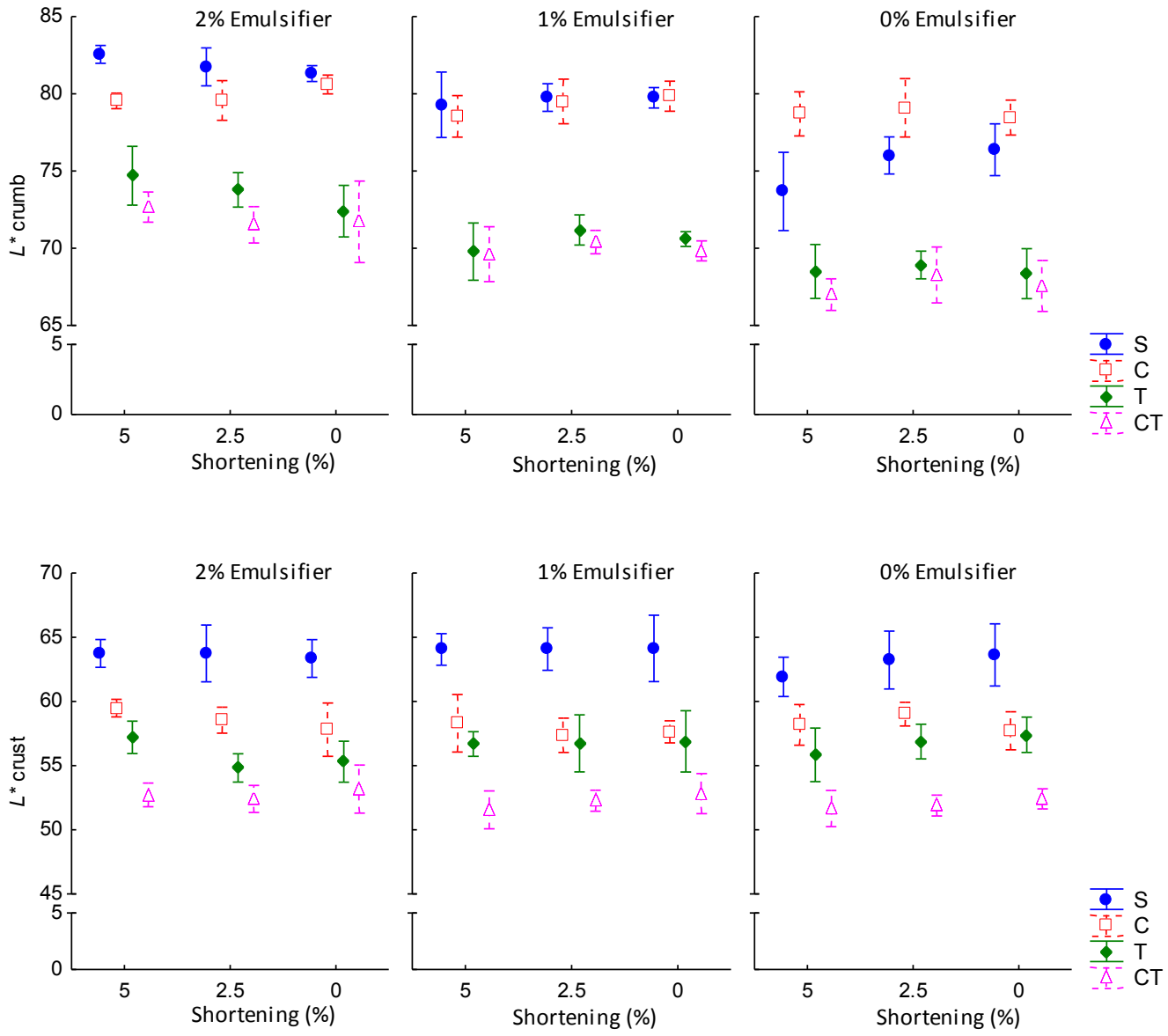

Figure 4.3. Crust and crumb color (lightness) of gluten-free breads (S: starch, C: chickpea, T: tiger nut, CT: chickpea-tiger nut) with different concentrations of shortening and emulsifier.

On day 0, shortening had different effects in crumb hardness. In general, crumb hardness of S and C breads with 1 or 2% of emulsifier increased when shortening was eliminated. This effect was also observed in T breads with 2% of emulsifier. In contrast, shortening level had no significant effect on CT breads crumb hardness. Reduction of emulsifier from 2% to 1% caused an increase ($p < 0.05$) of initial crumb hardness except in CT bread without shortening. However, hardness of breads without emulsifier was not different ($p > 0.05$) from

hardness of breads with 2% of emulsifier except in T and CT breads containing 5% of shortening.

On day 2, elimination of shortening rendered significantly harder crumbs only in three formulations: S and C breads that contained 1% of emulsifier and in T breads containing 2% of emulsifier. Elimination of emulsifier did not increase crumb hardness ($p > 0.05$) in any formulation. In general, crumb hardness on day 2 of CT breads was not affected by the concentration of shortening and emulsifier used ($p > 0.05$).

On day 4, shortening concentration did not affect crumb hardness ($p > 0.05$). Elimination and reduction of emulsifier did not increase crumb hardness ($p > 0.05$) of any bread, except S with 0% of shortening and 1% of emulsifier. Breads containing chickpea flour (C and CT) had significantly softer crumbs compared to S breads, although there were no differences in absence of emulsifier.

The use of shortening and emulsifiers delays bread staling of wheat bread (Autio and Laurikainen, 1997; Ghotra et al., 2002; Gómez et al., 2004; Pareyt et al., 2011; Stampfli and Nersten 1994) and GF bread (Nunes et al., 2009; Onyango et al., 2009; Schober, 2009). Nevertheless, in most cases of this study, there were no significant ($p > 0.05$) differences on bread staling when different concentrations of shortening and emulsifier were used (results not shown). In general, staling of CT breads was lower than S breads. This was probably due to the higher protein content of CT formulations, which would compete with starch for water absorption, and thus, delays its retrogradation. In this sense, Nilufer-Erdil et al. (2012), suggested that protein/starch complexes hinder starch/starch complexes and reduce starch retrogradation. Moreover, as the fat content of bread formulations plays an important role on crumb tenderness, the tiger nut flour oil (28.6%) could contribute to explain the lower crumb hardness on CT formulations.

The highest crumb hardness at day 0 observed in different formulations without shortening was expected, as shortening lubricates bread, tenderizes crumb and improves aeration (Chin et al., 2010). Furthermore, shortening acts as a barrier to moisture migration and prevents crumb hardening (Chin et al., 2010; Pareyt et al., 2011).

Emulsifiers, especially monoglycerides, can associate with amylose preventing it to participate in gel formation and thus reducing the extent of cristallization (Pareyt et al., 2011;

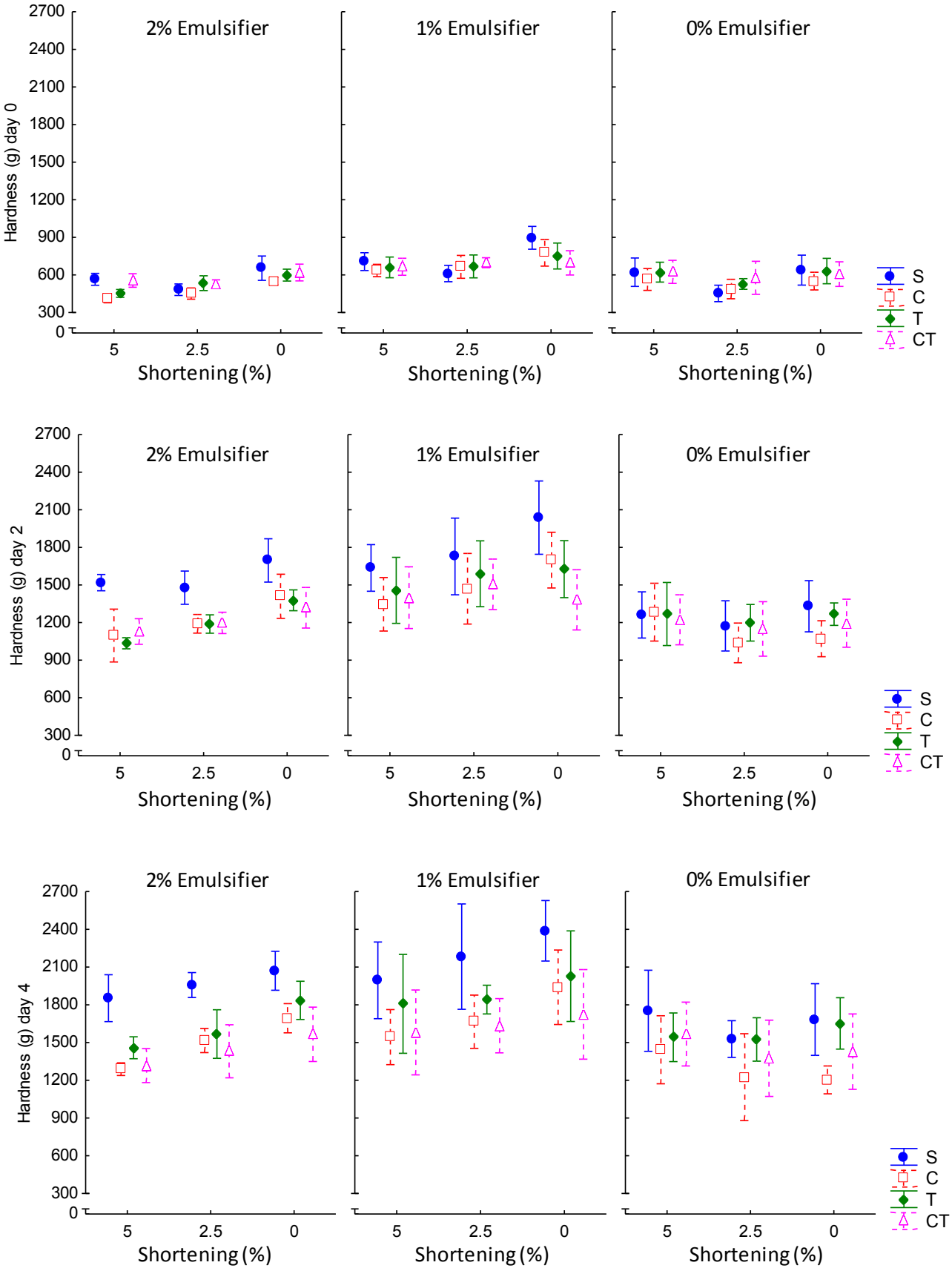

Figure 4.4. Crumb hardness on day 0, 2 and 4 of gluten-free breads (S: starch, C: chickpea, T: tiger nut, CT: chickpea-tiger nut) with different concentrations of shortening and emulsifier.

Purhagen et al., 2012). Some authors have studied the effect of emulsifiers in GF bread and have obtained different results. Nunes et al. (2009) did not find significant ($p > 0.05$) differences on crumb hardness at day 0 using different emulsifiers (lecithin, DATEM, DM or SSL) in GF bread. Sciarini et al. (2012) observed that emulsifiers (DATEM or SSL) increased initial crumb hardness but, on the contrary, Onyango et al. (2009) and Purhagen et al. (2012) noticed that emulsifiers (GMS, SSL or DATEM) gave softer crumbs.

In this research, the effect of the emulsifier on initial crumb hardness depended on its concentration, as 1% of emulsifier gave harder crumbs than 2%, except in one case (CT without shortening). There were no significant ($p > 0.05$) differences between 2 and 0% of emulsifier except in two cases (T and CT containing 5% of shortening). Therefore, it is important to select the correct type and proportion of emulsifier to have the proper effect on GF bread (Houben et al., 2012).

In our study, the effect of the reduction of shortening and/or emulsifier in crumb hardness was lower in CT breads than in S, C and T breads, probably due to interactions between chickpea protein and tiger nut lipids from their respective flours, which would compensate the effect of shortening and emulsifier on crumb texture. These results indicate that the use of tiger nut flour together with chickpea flour could substitute shortening and emulsifier without negatively affecting crumb softness.

4. Conclusions

Chickpea flour increased storage modulus as well as bread specific volume while tiger nut flour reduced bread specific volume and gave darker crumbs. The presence of both flours gave breads with darker crusts. Bread with both chickpea and tiger nut flour maintained baking characteristics when shortening and/or emulsifier were reduced or eliminated. Apparently, interactions between chickpea protein and tiger nut lipids compensated the effect of the elimination of shortening and emulsifier on crumb texture.

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CHAPTER 5: CHESTNUT FLOUR SOURDOUGH FOR GLUTEN-FREE BREAD MAKING

(EXPERIMENT 3)

ABSTRACT

The objective of this research was to study spontaneously fermented chestnut flour sourdough and to evaluate its effect in gluten-free bread quality. Lactic acid bacteria (LAB) and yeasts counts, pH and titratable acidity (TTA) of chestnut flour sourdough were measured during 6 d of sourdough propagation. Chestnut flour sourdough fermented during 5 d with back-slopping every 24 h with 33% of the ripe sourdough was selected to elaborate gluten-free bread. Control breads contained 15, 20 or 25% of chestnut flour and sourdough breads contained the same amount of chestnut flour added as chestnut flour sourdough. Chestnut flour sourdough improved bread specific volume, rendered breads with lighter crusts, reduced crumb hardness at day 0 and day 7, reduced pH and rendered breads with stronger aroma as evaluated in consumers test. However, chestnut flour sourdough had no effect on yeasts and moulds growth during 7 d of bread storage and it did not influence bread taste.

Keywords

Gluten-free bread, sourdough, chestnut flour, celiac disease

1. Introduction

Population that follows a gluten-free (GF) diet because suffering wheat allergy, celiac disease or gluten sensitivity is growing and, therefore, demand of GF products and specially bread, is also increasing (Rosell et al., 2014). Since lack of gluten impairs GF bread baking and sensory characteristics as well as its shelf-life, a wide range of additives including hydrocolloids, proteins, emulsifiers, shortenings and enzymes are being used to improve GF bread quality (Hager and Arendt, 2013; Martínez et al., 2013; Miñarro et al., 2010; 2012; Nunes et al., 2009; Schober et al., 2009).

Sourdough is a traditional technology used in bread making based on the fermentation of a mixture of flour and water either by native lactic acid bacteria (LAB) and yeasts or by added starters (Aponte et al., 2013; Moroni et al., 2009). Sourdough plays an important role in bread making: increases bread volume due to the improvement of CO_2 retention; improves bread texture as softer crumbs are obtained; elongates bread shelf-life by reducing staling and by generating antimicrobial substances that prevent microbial spoilage; enriches nutritional quality by increasing mineral biodisponibility because of phytase activity and mineral solubility and by reducing glycemic index; and enhances sensory profile. It has been stated that sourdough also improves texture, aroma, nutritional aspects and shelf-life of GF bread and sourdough biotechnology is emerging for industrial GF bread making (Gobbetti et al., 1998; 2008; 2014; Katina et al., 2005; 2006; Moroni et al., 2009). Furthermore, Rizzello et al. (2007) reported that selected sourdough lactobacilli and fungal proteases can eliminate gluten toxicity during long fermentations.

The interest in using chestnut (*Castanea sativa* Mill.) flour in bakery products is increasing due to its nutritional and health benefits as it contains 4-7% of high quality proteins with essential aminoacids, 20-30% of sugar, 50-60% of starch, 4-10% of fiber, 2-4% of fat, and vitamins and minerals such as vitamin E, B group, K, P, and Mg (Chenlo et al., 2007; Sacchetti et al., 2004). As chestnut flour is a GF flour it could also contribute to improve nutritional profile of GF breads since their content in fiber and vitamin B is usually low (Moroni et al., 2009). Demirkesen et al. (2010) evaluated the effect of chestnut flour to formulate GF rice bread and concluded that 30% of chestnut flour addition was the optimum. Moreira et al. (2013) studied the rheological behavior of different blends using rice flour and chestnut flour with different particle size and indicated that the blend with 30% of chestnut

flour with a particle size of 169 μm or the blend with 25% of chestnut flour with low particle size (77 μm) performed better than other formulations evaluated.

Aponte et al. (2013) developed a spontaneously fermented sourdough with chestnut flour and assessed the impact of fermentation on volatile organic compounds formation during sourdough maturation using gas chromatography coupled to mass spectrometry and identified 59 volatile compounds. However, the effect of spontaneously fermented chestnut flour sourdough on GF bread has not been evaluated yet.

The objective of this research was to study spontaneously fermented chestnut flour sourdough and to evaluate its effect in GF bread quality.

2. Materials and methods

2.1. Raw materials

Ingredients used for the elaboration of GF bread were: tap water, corn starch (Syral Iberia S.A.U., Zaragoza, Spain), chestnut flour (Castaña del Bierzo, Mesa del Castaño del Bierzo, León, Spain), white sugar (Azucarera Ebro S.L., Madrid, Spain), shortening (Puratos, Sils, Spain), iodized refined salt (Sal Costa S.A., Barcelona, Spain), baking powder (Panreac Química S.L.U., Castellar del Vallès, Spain), dry yeast (Lallemand Iberica S.A., Cachofarra, Portugal), xanthan gum (Degussa Texturant Systems, Paris, France) and emulsifier: citric acid esters of mono and diglycerides and sucrose fatty acid esters (Degussa Texturant Systems).

2.2. Sourdough production and analysis

Chestnut flour and sterilized tap water (1:1) were used to start fermentation. The mixture was incubated at 25 ºC and propagated by back-slopping every 24 h, adding 33% or 10% of the previous sourdough to a new fresh mixture of chestnut flour and water, during 6 d. Aseptic conditions were guaranteed during all process working under sterile conditions.

The LAB and yeasts counts were evaluated at the beginning of the process (day 0) and at day 1, 2, 3, 4, 5 and 6 of sourdough propagation. To count LAB, 10 g of sourdough were

diluted with 90 ml of peptone water (Oxoid Ltd, Basingstocke, England) and homogenized for 30 s at 300 rpm in a Stomacher® 400 Circulator (Worthing, UK). Then, 1 ml of decimal dilutions were plated on Man Rogosa Sharpe (MRS) agar (Oxoid Ltd) and incubated for 48 h at 30 ºC. Yeasts were counted on Saboraud dextrose agar (Oxoid Ltd) using supplemented with 0.1 g/l of chloramphenicol (Oxoid Ltd) and incubated at 25 ºC for 5 d.

Total titratable acidity (TTA) and pH were measured at the beginning of the process (day 0) and at day 1, 2, 3, 4, 5 and 6 of the sourdough propagation. The pH was directly determined with a pH meter (Crison Instruments S.A., Alella, Spain). TTA was evaluated on 10 g of sourdough homogenized with 90 ml of distilled water and expressed as the amount (ml) of 0.1 M NaOH to get a pH of 8.3, according to the method described by Coda et al. (2011).

Three independent batches were elaborated to evaluate sourdough LAB and yeasts counts, pH and TTA.

2.3. Bread making

Chestnut flour sourdough fermented during 5 d with back-slopping every 24 h with 33% of the ripe sourdough was selected to elaborate GF bread.

Bread was elaborated according to Aguilar et al. (2014) method except that, in the present study, breads were baked in a convection oven (Sveba-Dahlen AB, Fristad, Sweeden) at 160 °C for 30 min, with steam injection during 10 s at the start of baking. After 2 h of cooling, breads were stored in plastic bags at room temperature during 7 d.

Six formulations were obtained (Table 5.1). Control formulations C15, C20 and C25, contained 15, 20 and 25% of chestnut flour respectively, expressed as % of flour weight (% FW) (corn starch + chestnut flour). Previous studies were performed to adjust water level of control formulations to obtain the same rheological characteristics (G*) in them. Sourdough formulations S15, S20 and S25, contained the same amount of chestnut flour and water than their respective controls but, in this case chestnut flour and part of the water were added as sourdough. The content of chestnut flour sourdough in batter formulations expressed as % of total ingredients was: 13.1% in S15, 17.2% in S20 and 21.4% in S25.

Table 5.1. Gluten-free bread formulations expressed in % of flour weight (% FW). C15, C20 and C25 are control formulations with 15, 20 or 25% FW of chestnut flour.

Ingredient (% FW)	C15	C20	C25	S15	S20	S25
Water	107.6	111.2	111.8	92.6	91.2	86.8
Corn starch	85	80	75	85	80	75
Chestnut flour	15	20	25	-	-	-
Sourdough[a]	-	-	-	30	40	50
Sugar	5.8	5.8	5.8	5.8	5.8	5.8
Shortening	5	5	5	5	5	5
Salt	2.5	2.5	2.5	2.5	2.5	2.5
Baking powder	2.5	2.5	2.5	2.5	2.5	2.5
Dry yest	2	2	2	2	2	2
Xanthan gum	2	2	2	2	2	2
Emulsifier	2	2	2	2	2	2

[a] Chestnut flour and part of the water were added as sourdough.

2.4. Bread analysis

Loaf volume, bake loss, water activity, crust and crumb color and crumb texture were evaluated by the methods described by Miñarro et al. (2012).

To count yeasts and moulds, 10 g of bread were diluted with 90 ml of peptone water (Oxoid Ltd) and homogenized for 30 s at 300 rpm in a Stomacher® 400 Circulator (Worthing, UK). Then, 0.1 ml of decimal dilutions of bread samples in peptone water (Oxoid Ltd) were plated on Saboraud dextrose agar supplemented with 0.1 g/l of chloramphenicol and incubated at 25 ºC for 5 d.

For pH and TTA measurements, 10 g of bread were homogenized with 90 ml of distilled water in a Stomacher® 400 Circulator (Worthing, UK) for 30 s. From this solution, pH was directly determined with a pH meter (Crison Instruments S.A.) and TTA was evaluated as previously explained (see section 2.2).

To evaluate bread characteristics, three independent productions of each formulation were developed. In each experiment, three breads from each formulation were analyzed.

2.5. Sensory analysis

Sensory analysis of bread was performed 24 h after bread making by 54 volunteers recruited among university staff and students. Testers were both female and male, aged between 18 and 64, and regular bread consumers which evaluated each sensory attribute (crust color, crumb color, porosity, crumb hardness, aroma and taste) with a seven-point intensity scale. Control and sourdough bread samples with the same chestnut flour content were randomly codified using three digits and compared in pairs.

2.6. Statistical analysis

Results were analyzed by analysis of variance (ANOVA) using the general linear models procedure of Statistica 7 software. Student-Newman-Keuls method was applied for comparison of sample data. Evaluations were based on a significance level of $p < 0.05$.

3. Results and discussion

3.1. Sourdough characteristics

Figure 5.1 shows the evolution of chestnut flour sourdough LAB and yeasts populations. Initial LAB counts were 3.0 ± 0.1 log CFU/g and after 2 d of propagation increased to *ca.* 9 log CFU/g. Counts remained constant between days 2 and 5 and increased ($p < 0.05$) to *ca.* 9.5 log CFU/g at day 6. There were no differences ($p > 0.05$) between 10% and 33% of sourdough used for back-slopping.

No yeasts were detected in the initial sourdough mixture at day 0 and, 1 d of incubation, counts were 2.8 ± 0.5 log CFU/g. From day 1 to day 2, yeasts growth stopped in sourdough renewed at 33% and decreased ($p < 0.05$) in sourdough renewed at 10%. The pause in yeasts growth was probably caused by the pH drop observed in the same time interval (Figure 5.2).

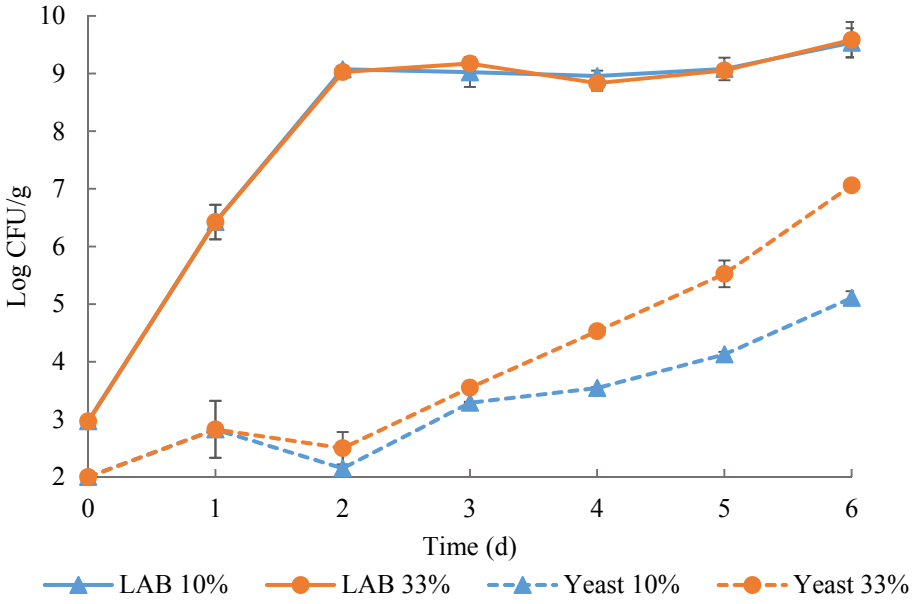

Figure 5.1. Evolution of LAB (lactic acid bacteria) and yeasts at each refreshment step during 6 days of propagation at 25 ºC of sourdough renewed with 33% or 10% of the ripe sourdough. The detection limit for LAB and yeasts counts is 1 log CFU/g and 2 log CFU/g, respectively.

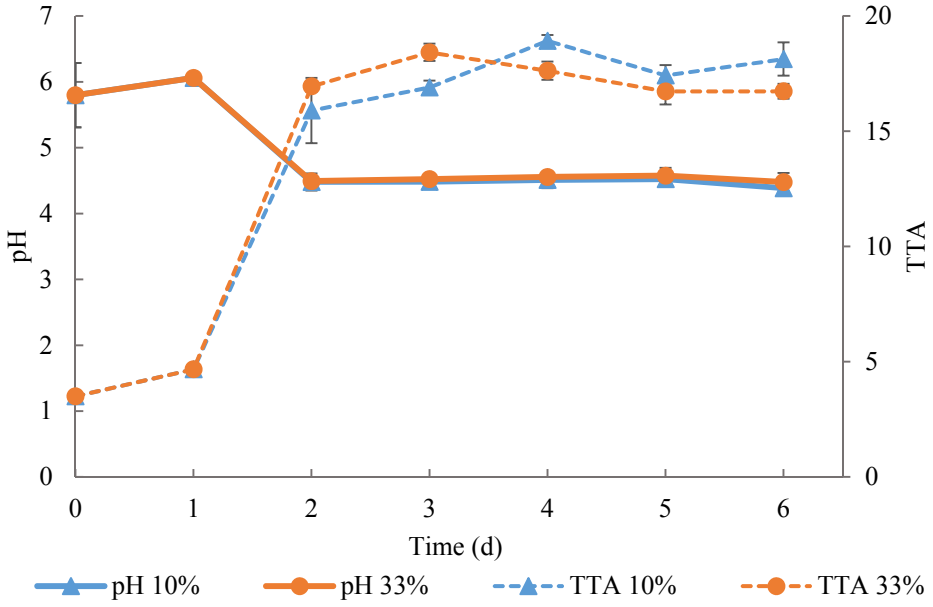

Figure 5.2. Evolution of pH and TTA (titratable acidity) at each refreshment step during 6 days of propagation at 25 ºC of sourdough renewed with 33% or 10% of the ripe sourdough.

Nevertheless, yeasts rapidly adapted to new media conditions and retook growth from day 2. Moroni et al. (2010) also observed a reduction of yeasts counts at day 2 in teff and buckwheat sourdoughs. At day 6, sourdough renewed at 33% presented higher ($p < 0.05$) yeasts counts than sourdough renewed at 10%, LAB:yeasts ratio being 100:1 for the first, and 10000:1 for the second. Sourdoughs evaluated in this study had cell counts similar to those of typically mature sourdoughs: $>10^8$ CFU/g LAB and yeasts counts orders of magnitude lower (Ehrmann and Vobel, 2005; Gobbetti et al., 2008).

Figure 5.2 shows pH and TTA results from sourdough renewed at 10% and 33%. Initial pH of sourdough was 5.8 ± 0.5, it slightly increased to 6.1 ± 0.1 at day 1 and, at second day of propagation, it had already decreased until *ca.* 4.5. This pH remained constant until day 6. There were no significant differences between pH results from both sourdoughs.

The initial TTA value was 3.5 ± 0.2 and increased to *ca.* 16 at day 2. The maximum increase of TTA was recorded at day 2, accordingly to pH drop. Aponte et al. (2013) studied chestnut flour sourdough characteristics inoculated with bakers' yeast and renewed every 24 h with 10% of the ripe sourdough and reported higher pH and lower TTA values compared with the present study, probably due to higher LAB counts observed in our research.

3.2. Bread characteristics

Table 5.2 shows results of bread characteristics. The increase of chestnut flour concentration in control breads caused a decrease in specific volume. Interestingly, this effect was not observed in sourdough breads as S20 and S25 had higher volumes than S15. In these breads, sourdough effect compensated the negative effect of chestnut flour on bread volume. The positive effect of sourdough on bread specific volume could also be observed comparing control and sourdough breads. Previous studies have also reported an increase of GF bread specific volume when sourdough was added (Coda et al., 2010; Novotni et al., 2012; Schober et al., 2007).

Demirkesen et al. (2010) reported the effect of chestnut flour mixed with rice flour at different ratios (0/100, 10/90, 20/80, 30/70, 40/60, 50/50 and 100/0) on bread specific volume and observed that bread volume increased as the chestnut/rice flour ratio increased,

up to 30/70. The high amount of fiber in chestnut (8.7-11.7%) (Pereira-Lorenzo et al., 2006) could explain the results obtained in this study since certain amount of fiber could improve GF bread volume due to its gas retention capacity and the viscoelastic characteristics that fiber provides to dough, but too much fiber reduces bread volume (Demirkesen et al., 2010; 2011; Sabanis et al., 2009). In our study, on the contrary, specific volume of control breads decreased with increasing ratio of chestnut flour/corn starch, up to 25/75. These divergent results could be attributed to differences in bread formulations, not only related to the flour components but also to the gums and emulsifiers used.

Chestnut flour concentration did not affect bake loss values but sourdough did, as S15, S20 and S25 had higher bake loss than C15, C21 and C25. Water activity values did not reflect bake loss results, as no differences between water activity of breads were observed at day 0. All breads suffered a significant reduction of water activity during 7 d of storage. Wolter et al. (2014) reported that sourdough (buckwheat, quinoa, sorghum, teff or wheat) fermented with *Weissella cibaria* MG1 did not influence water activity of bread. They also observed that quinoa and sorghum sourdoughs reduced bake loss and wheat sourdough increased it, while buckwheat and teff sourdoughs had no significant effect on bake loss. Galle et al. (2012) and Moroni et al. (2011) neither found differences between bake loss of GF sourdough breads and their controls.

Crust and crumb color were significantly influenced by chestnut flour % and sourdough addition (Tables 5.2 and 5.3). In crumb, the increase in chestnut flour % resulted in a decrease in L^* values and an increase in a^* and b^* values while, in crust, the increase in chestnut flour % resulted in a decrease in L^* and b* values and an increase in a^* values. In general, sourdough addition rendered crumbs with lower L^*, a^* and b^* values than their controls. All sourdough bread crusts were lighter (higher L^*) and less reddish (lower a^* values) than their counterparts. The darkening effect of chestnut flour on bread crumb and crust was expected due to the dark color of this ingredient ($L^* = 85.57 \pm 0.20$; $a^* = 1.23 \pm 0.01$; $b^* = 14.93 \pm 0.09$). Moreover, crust browning is explained by the high sugar content of chestnut flour, which contributes to Maillard reaction and caramelization during baking (Demirkesen et al., 2010). According to this, sourdough breads presented paler crusts than their controls because sugars were consumed during fermentation and could not contribute to crust browning to the same extent. Breads with 25% of chestnut flour were harder than breads with 15% and 20% at day 0. In contrast, after 7 d of storage, no differences due to %

of chestnut flour were observed. Demirkesen et al. (2010) studied the effect of 0-100% of chestnut flour blended with rice flour and observed that 30% of chestnut flour rendered the softest breads and, when chestnut flour content exceeded this optimum level, a firmer texture was obtained due to the increase in fibre content.

Sourdough breads had softer crumbs than their controls either at day 0 and 7, although all breads became harder after 7 d of storage. Moore et al. (2007; 2008), Novotni et al. (2012), Schwab et al. (2008) and Wolter et al. (2014) also found that sourdough softened the crumb of GF bread. However, Moroni et al. (2011) observed the opposite effect of sourdough on GF crumb hardness and Schober et al. (2007) did not find differences in crumb hardness of GF bread elaborated with sourdough.

Breads containing 25% of chestnut flour showed lower pH than C20 and C15 although no significant differences in TTA were observed. As expected, sourdough reduced bread pH and increased TTA. Moreover, an increasing % of sourdough caused a related decrease in pH and a related increase in TTA. Chestnut flour contains organic acids such as malic, oxalic, citric and ascorbic (Ribeiro et al., 2007) that would explain the slight decrease in pH in control breads with 25% of chestnut flour. GF bread pH values described in the literature range between 5.9 and 6.3, which are lower than the results of our study (7.51-7.71) probably due to formulations used. For that reason, the pH of resulting sourdough breads were higher (6.82-7.11) than those reported by other authors (4.6-5.9) (Moore et al., 2007; 2008; Moroni et al., 2011; Novotni et al., 2012; Wolter et al., 2014).

No yeasts and moulds could be detected at day 0. After 7 d of storage, yeasts and moulds counts were slightly higher than 5 log CFU/g in all breads and no effect that could be attributed to chestnut flour % or sourdough addition was observed. It has been shown that sourdough LAB produce antifungal compounds that prevent yeasts and moulds growth and improve bread shelf-life (Cizeikiene et al., 2013; Rizzello et al., 2011). In GF bread, Moore et al. (2008) has reported the antifungal activity of sourdough fermented with *Lactobacillus plantarum* FST 1.7. However, sourdough fermented with *Weissella cibaria* MG1 did not improve the microbial shelf-life of GF bread (Wolter et al., 2014). LAB present in the sourdough evaluated in our study probably did not produce enough antifungal compounds to reduce yeasts and moulds growth.

Table 5.2. Bread characteristics. C15, C20 and C25 are control formulations with 15, 20 or 25% of flour weight of chestnut flour. In S15, S20 and S25, chestnut flour was added as sourdough.

	C15	C20	C25	S15	S20	S25
Specific volume (cm^3/g)	1.94^{c} ± 0.07	1.83^{d} ± 0.09	1.76^{e} ± 0.09	2.07^{b} ± 0.13	2.20^{a} ± 0.13	2.27^{a} ± 0.08
Bake loss (%)	10.41^{b} ± 0.42	10.73^{b} ± 0.48	10.67^{b} ± 0.45	11.04^{a} ± 0.49	11.31^{a} ± 0.55	11.31^{a} ± 0.49
Water activity						
Day 0	$0.975^{a,x}$ ± 0.002	$0.975^{a,x}$ ± 0.002	$0.973^{a,x}$ ± 0.003	$0.976^{a,x}$ ± 0.001	$0.975^{a,x}$ ± 0.001	$0.975^{a,x}$ ± 0.001
Day 7	$0.972^{a,y}$ ± 0.001	$0.970^{b,y}$ ± 0.001	$0.970^{b,y}$ ± 0.002	$0.972^{ab,y}$ ± 0.001	$0.971^{ab,y}$ ± 0.001	$0.970^{b,y}$ ± 0.002
Crust color						
*L**	51.75^{b} ± 1.67	50.45^{c} ± 1.43	47.98^{d} ± 1.36	56.70^{a} ± 1.52	52.23^{b} ± 1.30	50.33^{c} ± 1.12
*a**	17.90^{bc} ± 0.30	18.02^{b} ± 0.36	18.59^{a} ± 0.26	16.72^{d} ± 0.59	17.82^{c} ± 0.31	17.87^{bc} ± 0.29
*b**	35.20^{b} ± 1.37	34.11^{c} ± 0.87	32.58^{d} ± 1.31	37.35^{a} ± 0.54	35.61^{b} ± 0.96	33.90^{c} ± 0.99
Crumb color						
*L**	71.41^{a} ± 0.93	68.96^{b} ± 1.02	66.48^{d} ± 0.87	71.68^{a} ± 0.94	67.70^{c}± 0.91	64.58^{e} ± 0.77
*a**	4.87^{d} ± 0.21	5.82^{b} ± 0.24	6.58^{a} ± 0.19	4.06^{e} ± 0.18	5.11^{c} ± 0.24	5.80^{b} ± 0.21
*b**	17.76^{c} ± 0.40	18.47^{b} ± 0.44	19.14^{a} ± 0.36	15.46^{f} ± 0.40	16.61^{e} ± 0.44	17.11^{d} ± 0.36
Hardness (g)						
Day 0	$1094.42^{b,y}$ ± 107.72	$1060.75^{b,y}$ ± 83.00	$1138.80^{a,y}$ ± 85.97	$824.78^{d,y}$ ± 77.07	$810.90^{d,y}$ ± 82.23	$884.19^{c,y}$ ± 113.25
Day 7	$2653.38^{a,x}$ ± 240.76	$2696.80^{a,x}$ ± 226.68	$2556.75^{a,x}$ ± 282.49	$2191.28^{b,x}$ ± 188.43	$2184.33^{b,x}$ ± 325.77	$2171.12^{b,x}$ ± 208.82
pH	7.69^{a} ± 0.03	7.71^{a} ± 0.04	7.51^{b} ± 0.06	7.11^{c} ± 0.10	6.93^{d} ± 0.07	6.82^{e} ± 0.10
Ttritatable acidity (TTA)	0.63^{c} ± 0.16	0.61^{c} ± 0.11	0.75^{c} ± 0.17	2.44^{b} ± 0.43	2.69^{b} ± 0.33	3.16^{a} ± 0.43
Yeast and moulds counts (Log CFU/g)						
Day 0	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Day 7	5.36^{a} ± 0.09	5.35^{a} ± 0.42	5.59^{a} ± 0.11	5.30^{a} ± 0.29	5.26^{a} ± 0.75	5.34^{a} ± 0.21

$^{a-f}$ Values labelled with a different letter in the same row are significantly different ($p < 0.05$). $^{x-y}$ Values labelled with a different letter in the same column from the same parameter are significantly different ($p < 0.05$). n.d.: not detected. Detection limit for yeasts and moulds counts: 2 log CFU/g.

3.3. Sensory evaluation

Results from sensory evaluation are shown in Table 5.3. Each sourdough bread was compared with its control. Although instrumental measurement of color indicated that sourdough influenced crust and crumb color, consumers could not perceived these differences. In the case of the pair C25-S25, consumers punctuated S25 crust color significantly darker (higher score) than C25, while instrumental measurement results showed that sourdough breads had lighter crusts than their controls. Adding ≥ 17.2% of sourdough increased pore size as S20 and S25 breads received higher ($p < 0.05$) scores in porosity than C20 and C25 respectively, as can also be observed in digital images from Figure 5.2. High porosity in S20 and S25 breads is probably related to their higher volumes as sourdough helps to retain gas generated during bread fermentation, giving larger pores and resulting in a higher loaf volume (Gobbetti et al., 2008; Moore et al., 2007). According to sensory analysis, no differences in crumb hardness between breads elaborated with sourdough and controls were observed. Therefore, the softening effect of sourdough that was shown by texture analysis was not detected by consumers.

Addition of ≥ 17.2% of sourdough gave breads with stronger aroma. However, sourdough had no effect on taste intensity. Aponte et al. (2013) identified 59 volatile compounds from chestnut flour sourdough coming from microbial metabolism, lipid oxidation, caramelisation, Maillard reaction and genetic and environmental factors, and concluded that the wide variety of volatile metabolites could contribute to enrich bread flavor, taste and aroma. Nevertheless, when consumers were asked to select the preferred bread comparing each sourdough bread with its control only 32%, 21% and 23% of them preferred S15, S20 or S25, respectively, than their control counterparts. Consumers that preferred sourdough bread, selected it because of its taste, aroma and texture. Control breads were mostly preferred due to the sweet taste provided by chestnut flour but sourdough fermentation reduced sweet taste in bread, probably influencing consumers' preference. Coda et al. (2010) reported a decrease in sweetness in sourdough breads compared with their controls.

Table 5.3. Sensory results from consumers test. C15, C20 and C25 are control formulations with 15, 20 or 25% of flour weight of chestnut flour. In S15, S20 and S25, chestnut flour was added as sourdough.

	Crust color[c]	Crumb color[c]	Porosity[c]	Crumb hardness[c]	Aroma[c]	Taste[c]
C15	3.99[a] ± 0.94	3.48[a] ± 1.08	3.56[a] ± 0.99	3.71[a] ± 1.27	3.40[a] ± 1.34	3.69[a] ± 1.11
S15	4.00[a] ± 0.87	3.21[a] ± 1.04	3.86[a] ± 1.02	3.71[a] ± 1.08	3.76[a] ± 1.27	3.70[a] ± 1.18
C20	4.30[a] ± 0.79	3.90[a] ± 0.72	3.34[b] ± 0.91	3.89[a] ± 1.01	3.31[b] ± 1.09	3.71[a] ± 1.03
S20	4.17[a] ± 0.72	4.09[a] ± 0.86	4.53[a] ± 0.97	4.03[a] ± 1.10	4.21[a] ± 1.29	3.98[a] ± 1.15
C25	4.47[b] ± 0.86	4.85[a] ± 0.89	3.79[b] ± 1.17	4.37[a] ± 1.10	3.68[b] ± 0.98	4.06[a] ± 1.01
S25	4.88[a] ± 0.90	4.91[a] ± 0.90	5.62[a] ± 0.90	4.20[a] ± 1.13	4.88[a] ± 1.17	4.24[a] ± 1.27

Mean values ± standard deviations of 54 consumers.
[a-b] Values labelled with a different letter in the same column are significantly different ($p < 0.05$).
[c] Intensity descriptors: crust/crumb color (very light to very dark), porosity (very small cells to very large cells), crumb hardness (very soft to very hard), aroma (very low intensity to very high intensity), taste (very low intensity to very high intensity).

Figure 5.3. Digital images of gluten-free breads studied. C15, C20 and C25 are control formulations with 15, 20 or 25% of flour weight of chestnut flour. In S15, S20 and S25, chestnut flour was added as sourdough.

4. Conclusions

Results show that spontaneously fermented chestnut flour sourdough can contribute to improve chestnut flour bread characteristics. Sourdough increased bread specific volume, porosity and aroma intensity, reduced crumb hardness and rendered breads with paler crusts. Chestnut flour sourdough improved bread shelf-life as it provided softer crumbs than chestnut flour after 7 d of storage. However, this sourdough had no effect on yeasts and moulds growth after 7 d of bread storage and it negatively affected consumers' preference, probably due to the reduction of the characteristic sweet taste of chestnut flour caused by sourdough fermentation.

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CHAPTER 6: INFLUENCE OF FINAL BAKING TECHNOLOGIES IN PARTIALLY BAKED FROZEN GLUTEN-FREE BREAD QUALITY

(EXPERIMENT 4)

Abstract

The effect of final baking in convection oven (FBC), microwave oven (FBM) and microwave oven with susceptor packaging material (FBMS) in partially baked (PB) frozen gluten-free bread characteristics was investigated. Specific volume and crust color of loaves were measured at day 0. Bread moisture, water activity and crumb and crust texture (at 15, 45 and 90 min after baking) were analyzed at day 0 and after 28 d of frozen storage (-18 ºC). Volatile compounds from breads baked in convection oven or microwave oven with susceptor packaging material were also evaluated. Bread finally baked in convection oven or in microwave oven with susceptor packaging increased crust browning. Crumb and roll hardness increased with time after final baking (measured at 15, 45, 90 min) and after 28 d of frozen storage. Bread finally baked in microwave oven was the hardest, due to high water losses. At day 0, bread finally baked in convection oven had softer crumb than bread finally baked in microwave oven with susceptor packaging but, after 28 d of frozen storage, there were no differences between them. Moreover, final baking in convection oven and in microwave oven with susceptor packaging rendered gluten-free breads that could not be distinguished in a triangular test and had the same volatile compounds profile. In conclusion, final baking in microwave oven with susceptor packaging material could be an alternative to final baking in convection oven.

Keywords

Gluten-free, partially baked bread, convection oven, microwave oven, susceptor packaging material

1. Introduction

In recent years, the interest in gluten-free (GF) products is increasing due to the growing number of diagnosed patients with gluten related diseases such as wheat allergy, celiac disease and gluten sensitivity (Rosell et al., 2014). Nevertheless, GF bread has inferior sensory and nutritional quality compared to wheat bread, as it usually presents crumbly texture, low volume, poor crust color, taste and aroma, short shelf-life, high glycemic index, and low protein and high fat content (Arendt et al., 2009; Gallagher et al., 2003; Matos and Rosell, 2011; Miñarro et al., 2012). To increase shelf-life, some of commercial GF roll breads are partially baked and consumers have to bake them in the oven during 5-15 min at high temperatures (170-200 ºC), which represents a considerable amount of time and energy. Final baking in microwave oven could be an alternative to convection oven, which would reduce time and energy consumption.

In a convection oven, heat is transferred by convection from the hot air that is circulating inside the oven to the food surface (Stigter et al., 2001). Microwave heating occurs when electromagnetic energy is converted to heat because of the agitation of water molecules and charged ions exposed to microwaves. In microwave oven, heat is transferred by conduction and convection. It is faster than conventional heating as microwaves directly penetrate into food materials (Tang and Resurreccion, 2009). However, microwave heating cannot induce browning or crispness in food because the air around the product is cold, and thus, surface temperature is not high enough for Maillard reactions neither for high evaporation rate that allows crispness development. The packaging material known as susceptor can solve this limitation as it has a metallized plastic film that absorbs microwaves and increases the temperature on the food surface, making it brown, and crispy (Chandrasekaran et al., 2013; Sahin et al., 2002).

Another problem with bread and, particularly, GF bread, is its short shelf-life as it stales rapidly and, hence, increases waste product and economic losses (Bárcenas et al., 2003; Moore et al., 2008). Partial baking of bread and storage at freezing conditions is a combination of technologies that reduces wastes and allows to adapt bread offer to consumers' demand, improving GF bread shelf-life and availability (Novotni et al., 2012; Poinot et al., 2008; Ronda and Roos, 2011; Sciarini et al., 2012).

Sensory properties as well as physical characteristics of different GF breads have been widely investigated. However, only Poinot et al. (2009) has studied volatile compounds of GF bread. Bread formulation, as well as the modification of bread making process can influence volatile compounds released from bread which, in turn, affect to overall odorant perception (Poinot et al., 2008).

The objective of this research was to investigate the effect of final baking in convection oven, microwave oven and microwave oven with susceptor packaging material (SPM) in partially baked frozen GF bread characteristics.

2. Materials and methods

2.1. Materials

Ingredients and amounts (expressed in percentage of corn starch + chickpea flour weight) used for the elaboration of GF bread were: 103% tap water, 92.2% corn starch (Syral Iberia S.A.U., Zaragoza, Spain), 7.8% chickpea flour (El Granero Integral S.L., Madrid, Spain), 5% shortening (Puratos, Sils, Spain), 4.2% white sugar (Azucarera Ebro S.L., Madrid), 2.5% baking powder (Panreac Química S.L.U., Castellar del Vallès, Spain), 2% xanthan gum (Degussa Texturant Systems, Paris, France), 2% emulsifier: citric acid esters of mono and diglycerides and sucrose fatty acid esters (Degussa Texturant Systems), 2% dry yeast (Lallemand Iberica S.A., Cachofarra, Portugal), and 1.7% iodized refined salt (Sal Costa S.A., Barcelona, Spain).

The SPM used was Quiltwave® (Graphic Packaging Intl, Igualada, Spain), composed of light paper laminated on both sides with polyethylene terephthalate film, one of them susceptor metallized. The laminated cells, or 'quilts' are designed to expand when exposed to microwave energy to increase heat flux to food surface.

Freezer bags (Albal®, Cofresco Ibérica S.A., Alcobendas, Spain) composed of 3 layers of low density polyethylene were used to store bread at -18 ºC.

2.2. Bread making procedure

Bread batter was elaborated as reported by Aguilar et al. (2014). As it was too liquid and sticky to form rolls, the shape of each roll was given with a pastry bag. The pastry bag was filled with batter and pressed to extrude the batter through a circular nozzle of 2.4 cm of diameter. To standardize roll length, a ruler of 20 cm was used and rolls were placed over a multiple baguette pan. Rolls were proofed in a chamber (Salva, Lezo, Spain) at 85% RH and 30 ºC for 30 min. Partial baking was performed in a convection oven (Sveba-Dahlen AB, Fristad, Sweeden) at 160 °C for 15 min with steam injection during the first 10 s. These breads were directly analyzed as PB (partially baked) or cooled for 1 h (Figure 6.1). After cooling, breads received one of the following treatments:

a) final baking in

i) convection oven at 160 ºC for 5 min (FBC, day 0) or

ii) microwave oven at 800 W for 2 min (FBM, day 0)

b) packaging in freezer bag, freezing (-18ºC) and storage (-18 ºC) for 28 d, thawing for 1 h at room temperature and

i) directly analyzed as PB breads (day 28) or

ii) finally baked in convection oven at 160 ºC for 5 min (FBC, day 28) or

iii) finally baked in microwave oven at 800 W for 2 min (FBM, day 28)

c) packaging with SPM and

i) finally baked in microwave oven at 800 W for 2 min (FBMS, day 0) or

ii) frozen and stored at -18 ºC for 28 d, thawed for 1 h at room temperature and finally baked in microwave oven at 800 W for 2 min (FBMS, day 28).

Three independent batches of each treatment (PB, FBC, FBM, FBMS) were prepared.

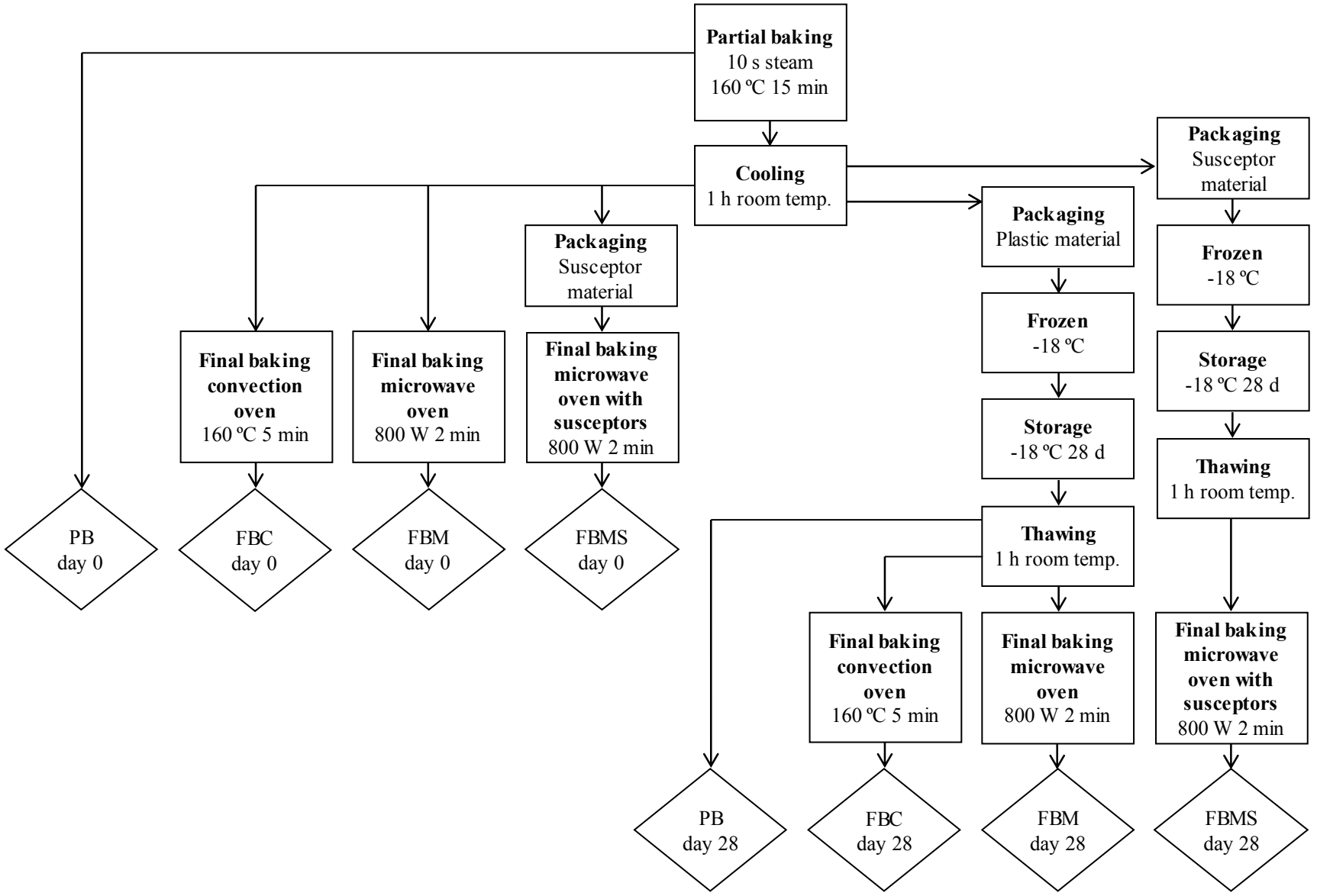

Figure 6.1. Flowchart of baking technologies applied and gluten-free breads obtained

2.3. Bread analysis

Temperature was measured after baking in the center of the loaf with a Testo 720 probe (DCL Diseño y Metrología S.L., Vitoria, Spain). Length, width and height were measured at day 0 with a ruler. Loaf volume was measured at day 0 by the method of seed displacement. Specific volume was calculated using the formula: specific volume (cm^3/g) = volume (cm^3) / weight (g). Bake loss results could not be analyzed due to the difficulty of measuring the initial batter weight, as rolls were prepared in a multiple pan.

Moisture (%) and water activity were measured according to AOAC (2005) and Aguilar et al. (2014) respectively, at days 0 and 28.

Crust color at day 0 was measured recording CIE L^*, a^* and b^* values as in Aguilar et al. (2014).

Crumb hardness and cohesiveness were measured as in Aguilar et al. (2014) on days 0 and 28 at 15, 45 and 90 min after final baking (FBC, FBM, FBMS) or after thawing (PB). A cylindrical probe of 25 mm diameter was used. Roll hardness was analyzed at 3 different points of bread surface: in the middle of the roll and at 4 cm from the middle on both sides. Hardness values, corresponding to the maximum peak force of the first compression, were recorded and crumb cohesiveness values were calculated (area of work during second compression divided by the area of work during first compression). The % of increase in crumb and roll hardness after 28 d of frozen storage was calculated for hardness measurements at 15, 45 and 90 min using the formula: (hardness day 28 / hardness day 0) × 100.

A triangular test was performed at day 0 by 24 bread consumers aged from 21 to 54 to compare FBMS and FBC breads. Three samples of bread, two identical and one different, were presented in the same plastic dish and were named using three-digit numbers randomly. Consumers received different sequences of the randomly named samples and were asked to identify the different sample.

2.4. Volatile compound analysis

The extraction of volatile compounds of FBC and FBMS breads was carried out by head-space solid phase microextraction (HS-SPME). A DVB/CAR/PDMS fiber (divinylbenzene/carboxen/polydimethylsiloxane, 50/30 μm × 20 mm, Supelco, Bellefonte, PA, USA) was used for microextraction. Bread was prepared as previously explained and, after final baking (FBC or FBMS), it was frozen at -30 ºC. After 24 h of freezing it was crushed (including crust and crumb) using a kitchen food processor (A320R1 Moulinex, Lyon, France) and 0.6 g of crushed bread and a magnetic stirring bar were placed in a 20 mL flask which was then sealed with a silicone septum and immersed in a water bath at 35 ºC for 10 min to reach equilibrium. Then, the fiber was exposed to headspace for 60 min with continuous stirring. Finally, the fiber was inserted into a gas chromatograph injector port for thermal desorption of the extracted volatiles and maintained during 15 min.

Separation and identification of the volatile compounds was performed using an Agilent 6890 gas chromatograph coupled to a 5975 MSD mass spectrometer (Agilent Technologies, Palo Alto, CA). A JandW HP-INNOWAX capillary column (60 m × 0.25 mm × 0.25 μm) with a bonded polyethylene glycol (PEG) high polarity stationary phase was used. Volatile compounds were desorbed in the injection port in splitless mode at 250 °C for 3 min, but the fiber was maintained in the injection port for a total of 15 min for cleaning. Helium was used as the carrier gas at a flow rate of 1 ml/min. Initial column temperature was kept at 50 °C for 5 min. Then, temperature was increased to 170 °C at 5 °C/min, afterwards to 230 °C at 18 °C/min and, finally, held for 8 min at 230 ºC.

Volatile compounds were identified by relative retention time of every chromatographic peak and by comparing their mass spectra with those of mass spectra from Wiley 6.0 library. In addition, C8-C20 aliphatic hydrocarbon standards dissolved in methanol (Sigma-Aldrich, St. Louis, MO, USA) were used to calculate Kovats retention indexes (RI) using the formula proposed by Van Den Dool and Kratz (1963) (RI_{calc}), which were compared with those reported in the literature (RI_{lit}). A semi quantitative determination of the main ion (m/z) for each volatile compound was performed and the results were expressed as total area $\times 10^3$.

2.5. Statistical analysis

Results were analyzed by analysis of variance (ANOVA) using the general linear models procedure of Statistica 7 software. Student-Newman-Keuls method was applied for comparison of sample data. A correlation matrix of different parameters evaluated was also performed. Evaluations were based on a significance level of $p < 0.05$.

3. Results and discussion

3.1. Bread characteristics

Table 6.1 shows some bread physicochemical characteristics of PB, FBC, FBM and FBMS breads. Although there were no differences between the shape and volume of breads (height, width, length or volume), the weight of FBM bread was lower than the rest. This difference on bread weight affected FBM specific volume results, which was the highest because of its lower weight, not because of its volume.

As expected, all final baking technologies reduced bread moisture as FBM, FBMS and FBC had lower moisture % than PB at day 0 and at day 28 ($p < 0.05$). At day 0, FBC had higher moisture content than FBM and FBMS, probably due to the lower temperatures achieved inside the bread after final baking in convection oven (69.37 ± 5.95 ºC) compared to microwave oven (80.68 ± 3.01 ºC) or microwave oven with SPM (83.87 ± 2.4 ºC), since microwave heating occurs inside food due to interaction of microwaves with charged particles and water (Sumnu and Sahin, 2005). The higher moisture content of FBMS compared to FBM can be attributed to the presence of plastic layers of SPM which probably avoided water loss during microwave baking.

Frozen storage during 28 d did not influence moisture % of breads since no differences ($p > 0.05$) in this parameter were observed when comparing data from day 0 and day 28, although a tendency to decrease was observed. Ronda and Roos (2011) did not find differences in crumb or crust moisture of GF bread after storing at -28 ºC during 7 days but when storage temperature was -14 ºC, they observed a significant increase of crust moisture due to the migration of water from crumb to crust, although crumb moisture remained constant.

Freezing storage did not influence water activity of bread crumbs as no differences ($p > 0.05$) were found between this parameter at day 0 and at day 28. As expected, there was a positive correlation between moisture and water activity at day 0 ($r = 0.73$; $p < 0.05$) and at day 28 ($r = 0.52$; $p < 0.05$).

Table 6.1. Physicochemical characteristics of gluten-free breads (PB: partially baked; FBC: final baking in convection oven; FBM: final baking in microwave oven; FBMS: final baking in microwave oven with susceptor packaging material).

	PB	FBC	FBM	FBMS
Specific volume (cm^3/g)	$2.72^{b} \pm 0.16$	$2.76^{b} \pm 0.26$	$2.92^{a} \pm 0.12$	$2.72^{b} \pm 0.19$
Volume (cm^3)	$272.22^{a} \pm 30.01$	$277.05^{a} \pm 28.48$	$268.89^{a} \pm 44.04$	$300.83^{a} \pm 36.05$
Weight (g)	$100.48^{ab} \pm 11.59$	$100.52^{ab} \pm 7.72$	$92.02^{b} \pm 14.32$	$110.73^{a} \pm 12.89$
Height (cm)	$4.26^{a} \pm 0.30$	$4.19^{a} \pm 0.40$	$3.84^{a} \pm 0.13$	$4.22^{a} \pm 0.37$
Width (cm)	$5.51^{a} \pm 0.28$	$5.54^{a} \pm 0.30$	$5.31^{a} \pm 0.19$	$5.62^{a} \pm 0.26$
Length (cm)	$21.08^{a} \pm 0.29$	$21.68^{a} \pm 0.50$	$21.13^{a} \pm 0.89$	$21.63^{a} \pm 0.48$
Moisture (%)				
Day 0	$44.40^{a} \pm 0.77$	$43.83^{b} \pm 0.95$	$39.44^{d} \pm 1.26$	$42.27^{c} \pm 1.20$
Day 28	$43.22^{a} \pm 1.02$	$41.90^{b} \pm 1.27$	$36.14^{c} \pm 1.89$	$41.62^{b} \pm 0.85$
Aw				
Day 0	$0.983^{a} \pm 0.001$	$0.979^{b} \pm 0.003$	$0.975^{c} \pm 0.002$	$0.976^{c} \pm 0.003$
Day 28	$0.984^{a} \pm 0.001$	$0.980^{a} \pm 0.005$	$0.975^{b} \pm 0.003$	$0.976^{b} \pm 0.005$
Color				
*L**	$73.58^{a} \pm 3.69$	$65.41^{b} \pm 1.87$	$73.29^{a} \pm 1.86$	$65.48^{b} \pm 1.73$
*a**	$9.81^{b} \pm 2.38$	$14.51^{a} \pm 0.82$	$9.52^{b} \pm 1.29$	$14.45^{a} \pm 0.73$
*b**	$36.09^{b} \pm 2.11$	$39.57^{a} \pm 0.53$	$36.43^{b} \pm 2.66$	$39.12^{a} \pm 0.88$

[a-d] Values labelled with a different letter in the same row are significantly different ($p < 0.05$).

Final baking in microwave oven did not induced changes in crust color, as FBM breads showed no differences compared to PB ($p > 0.05$). However, final baking in microwave oven with SPM and convection oven increased crust browning since FBC and FBMS breads showed lower lightness (L^*) and higher a^* and b^* values than PB and FBM breads ($p < 0.05$). During conventional baking, crust browning is a result of Maillard reactions produced by free reducing sugars and proteins when temperature is high enough. During microwave

heating, heat is absorbed by the food and the surface of the product cannot reach as high temperatures because the air surrounding the food is cold. Thus, microwave heating cannot induce browning (Sahin et al., 2002; Sumnu and Sahin, 2005). However, SPM incorporates metallized plastic film that absorbs microwaves converting them into heat which is transferred by conduction and radiation, allowing the increase of the temperature on the surface of the food product, making it brown (Chandrasekaran et al., 2013; Sahin et al., 2002). Icoz et al. (2004) observed browning in wheat bread only if SPM was used in microwave baking.

Bread texture was measured at 15, 45 and 90 min after baking at day 0. After 28 d of frozen storage, it was measured at 15, 45 and 90 min after thawing in case of PB and at 15, 45 and 90 min after final baking in case of FBC, FBM and FBMS. Figure 6.2 shows crumb and roll hardness results at day 0 and day 28. At day 0, crumb of finally baked breads after 15 min of baking was significantly harder than PB breads. However, no significant differences were found between crumb hardness of PB and FBC breads measured at 45 and 90 min. At all measuring times (15, 45 and 90 min), FBM showed the hardest crumb ($p < 0.05$) compared to FBMS and FBC, and FBMS crumb was significantly harder than FBC. These results could be related with bread humidity results at day 0, since a negative correlation between crumb hardness and moisture at day 0 was found ($r = -0.84$; $p < 0.05$). De la Hera et al. (2014) and Gallagher et al. (2003) also reported this relation between crumb texture and bread moisture in GF breads.

In general, at day 28, crumb hardness of finally baked breads was significantly lower than crumb hardness of PB breads measured at 15, 45 or 90 min. In fact, this difference is due to the effect of final baking since PB bread at day 28 is the same as the rest of samples after thawing and before final baking. Comparing final baking treatments, FBM bread showed the hardest crumb ($p < 0.05$) compared to FBMS and FBC. Although, at day 0, FBMS crumb was significantly harder than FBC, no significant differences were found between them at day 28, which agrees with bread moisture results at day 28. However, the correlation between crumb hardness and moisture at day 28 was not significant. This was probably due to the effect of freezing since, after the storage period of 28 d at -18 ºC, crumb and roll hardness of most breads increased ($p < 0.05$). These results agree with Ronda and Roos (2011) who detected an increase in crumb hardness during 7 d of freezing storage at -14 ºC or -28 ºC. The % of hardness increase from day 0 to day 28 was calculated for crumb and roll and for

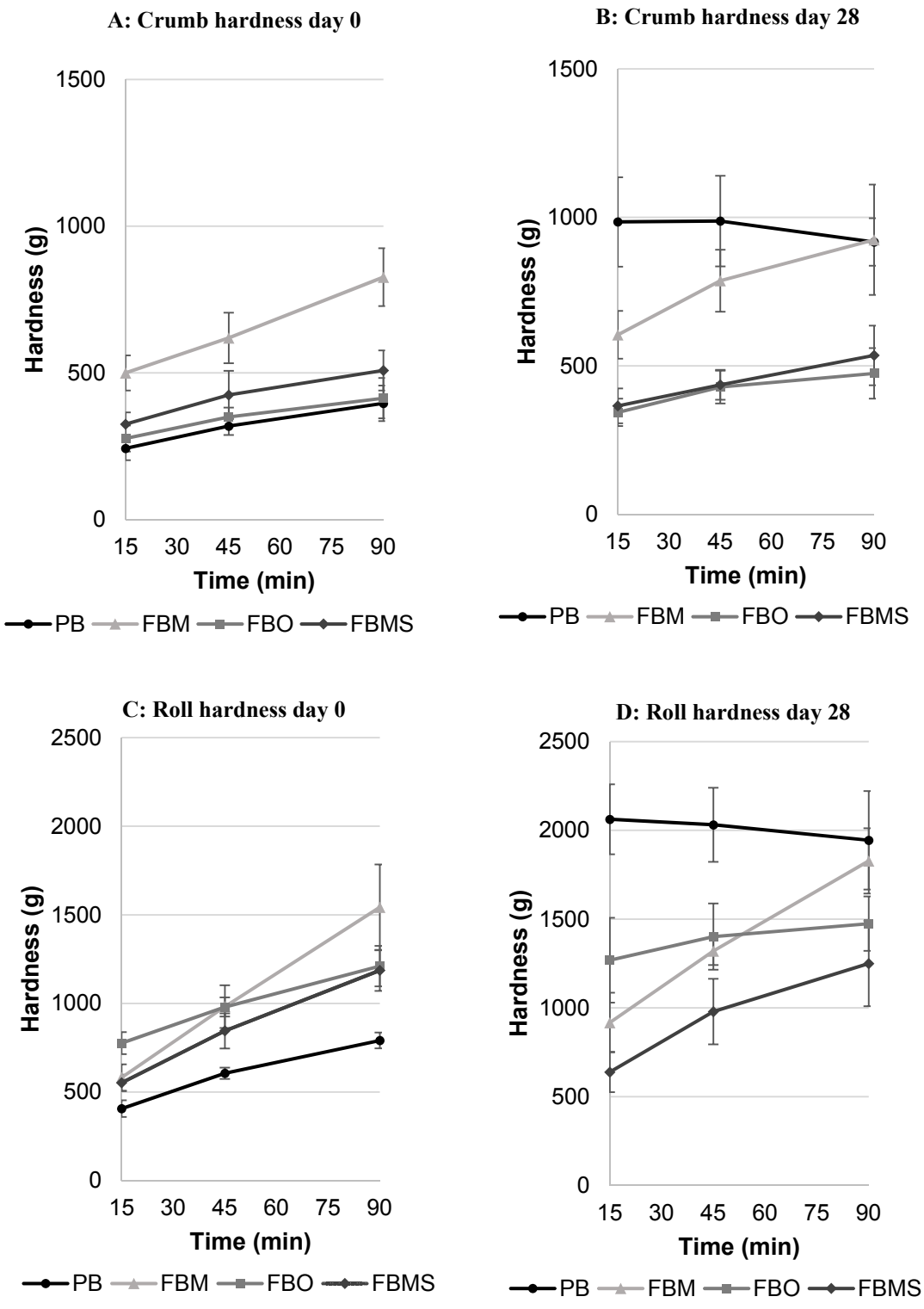

Figure 6.2. Crumb hardness at day 0 (A) and day 28 (B) and roll hardness at day 0 (C) and day 28 (D) of gluten-free breads (PB: partially baked, FBM: final baking in microwave oven, FBC: final baking in convection oven, FBMS: final baking in microwave oven with susceptor packaging material).

each time of measurement (data not shown). Differences between finally baked breads were not significant ($p > 0.05$) and only PB bread suffered an increase of crumb hardness significantly higher than finally baked breads as it did not receive the final baking treatment.

Water evaporating from food during microwave heating condenses when it contacts with cold air around the product, and thus, crust crispness development is not possible. This is one of the purposes of the use of SPM as it increases surface temperature allowing crust crispness (Chandrasekaran et al., 2013; Sahin et al., 2002). However, GF breads formulations usually do not allow to obtain crispy breads, even when they are baked in oven, due to their high water content and shortening presence. For this reason, crispness was not evaluated in our study.

Crumb cohesiveness (Table 6.2) decreased with the storage at freezing temperatures and time of measurement (15, 45 and 90 min) and it was negatively correlated with crumb hardness at day 0 ($r = -0.89$; $p < 0.05$) and at day 28 ($r = -0.85$; $p < 0.05$). Both parameters are related to freshness of bread and low cohesiveness indicates a crumbly texture.

Table 6.2. Crumb cohesiveness values of gluten-free breads (PB: partially baked; FBC: final baking in convection oven; FBM: final baking in microwave oven; FBMS: final baking in microwave oven with susceptor packaging material).

	PB	FBC	FBM	FBMS
Day 0				
15 min	$0.811^{a,l,x} \pm 0.018$	$0.775^{c,l,x} \pm 0.033$	$0.755^{d,l,x} \pm 0.027$	$0.791^{b,l,x} \pm 0.025$
45 min	$0.797^{a,m,x} \pm 0.020$	$0.760^{b,l,x} \pm 0.023$	$0.725^{c,m,x} \pm 0.038$	$0.773^{b,m,x} \pm 0.044$
90 min	$0.735^{a,m,x} \pm 0.050$	$0.712^{a,m,x} \pm 0.050$	$0.600^{b,n,x} \pm 0.044$	$0.707^{a,n,x} \pm 0.029$
Day 28				
15 min	$0.233^{c,l,y} \pm 0.059$	$0.746^{ab,l,y} \pm 0.040$	$0.724^{b,l,y} \pm 0.031$	$0.762^{a,l,y} \pm 0.063$
45 min	$0.228^{d,l,y} \pm 0.057$	$0.717^{b,m,y} \pm 0.034$	$0.659^{c,m,y} \pm 0.043$	$0.758^{a,l,x} \pm 0.034$
90 min	$0.227^{c,l,y} \pm 0.058$	$0.656^{a,n,y} \pm 0.046$	$0.547^{b,n,y} \pm 0.034$	$0.654^{a,m,y} \pm 0.077$

[a-d] Values labelled with a different letter in the same row are significantly different ($p < 0.05$). [l-n] Values labelled with a different letter in the same column within the same day are significantly different ($p < 0.05$). [x-y] Values labelled with a different letter in the same column within the same time of measurement (15, 45, 90 min) are significantly different ($p < 0.05$).

3.2. Volatile compounds

Volatile compounds from the finally baked breads with better baking characteristics (FBMS and FBC) were analyzed to assess if both methods of baking would induce the same changes in aroma profile.

Sixty-five volatile compounds from both samples were identified using the library and retention index comparison (Table 6.3). Alcohols were the components found in higher number (12). The origin of the alcohols released from bread may be due to fermentation (ethanol, 3-methyl-1-butanol), lipid oxidation (1-pentanol, 2-penten-1-ol, 1-hexanol, 1-octen-3-ol) or both form fermentation and lipid oxidation (1-propanol, 1-octanol) (Galey et al. 1994; Genot et al. 2003; Grosch 1987; Hansen and Schieberle 2005; Marie et al. 2013). Alkanes represent the second in number of volatile compounds identified (10). They included heptane, octane, dodecane, tetradecane and hexadecane, which probably came from lipid oxidation (Genot et al. 2003; Marie et al. 2013). Aldehydes (9) like pentanal, hexanal, heptanal, octanal were detected, and their origin is also attributed to lipid oxidation (Genot et al., 2003; Marie et al., 2013). Aromatic hydrocarbons (8) were identified and the origin of these compounds is more heterogenic: toluene is derived from lipid oxidation (Marie et al., 2013); benzaldehyde comes from Maillard reaction, fermentation and lipid oxidation (Galey et al., 1994; Hurrell, 1982); benzyl alcohol origin is attributed to Maillard reaction and lipid oxidation (Frasse et al., 1992; Hurrell, 1982); and phenylethyl alcohol comes from fermentation (Frasse et al., 1992; Galey et al., 1994). Ketones (7), derived from Maillard reaction (2-butanone) (Hurrell, 1982) and from both Maillard reaction and fermentation (2,3-butanedione, 2-butanone 3-hydroxy) (Jousse et al., 2002; Marie et al., 2013), were also identified. Moreover, terpenoids and furans originated by Maillard reaction and lipid oxidation, like furan 2-pentyl and 2-furanmethanol (Marie et al., 2013), pirazynes originated by Maillard reaction (Hurrell, 1982; Jousse et al., 2002), esters, acids, et al., were also identified.

Results showed that there were no significant differences between most of the volatile compounds released (Table 6.3) which agrees with the triangular test performed to assess whether consumers could perceive differences between them (results not shown). It has been shown that bread making procedure influences volatile compounds released from bread (Poinot et al., 2008), and that microwave cooked foods have lower flavor volatile compounds

as they distill of, bind to proteins or other molecules or do not develop (Brewer, 2005). As mentioned, the most abundant volatile compounds released from FBMS and FBC breads were alcohols that could be originated from fermentation and, except for two cases, no differences were detected between samples. Compounds from fermentation are influenced by ingredients used in bread formulations such as the amount of yeast (Frasse et al., 1992; Poinot et al., 2008). As both breads compared in this study shared the same formulation, the similarity of alcoholic compounds profile is justified. Similarly, a high number of volatile compounds identified were originated by lipid oxidation, which was related to the presence of shortening in the formulation. Poinot et al. (2009) identified higher quantity of compounds from lipid oxidation in GF bread than in wheat bread due to the oil content in the formulation. Marie et al. (2013) observed that lipid oxidation occurs principally during dough preparation and depends on fatty acids type and content and on the presence of antioxidants on raw matter.

Table 6.3. Volatile compounds identified and quantified (expressed in mean of area $\times 10^3$ ± SD) in FBC (final baking in convection oven) and FBMS (final baking in microwave oven with susceptor packaging material) breads.

Compound	m/z	RT	RI_{calc}	RI_{lit}	Ref	FBC	FBMS
Alcohols							
Ethanol	45	5.8	945	945	F	23141 ± 3225	20822 ± 5591
1-propanol	59	7.4	1046	1050	E	91 ± 19	52 ± 68
2-propanol, 1-methoxy	47	9.58	1151	-	-	**493 ± 97 (*)**	**312 ± 33 (*)**
3-methy-1-butanol	55	11.2	1217	1206	D	4543 ± 1386	4106 ± 2506
1-pentanol	42	12.25	1259	1267	A	205 ± 61	155 ± 63
2-penten-1-ol	57	14.08	1329	1326	D	73 ± 3	68 ± 31
1-hexanol	56	14.89	1360	1360	D	810 ± 176	634 ± 327
Ethanol, 2-butoxy	57	16.34	1415	1404	-	**105 ± 24 (*)**	**34 ± 16 (*)**
1-octen-3-ol	57	17.38	1457	1461	A	56 ± 20	52 ± 24
1,2-ethanediol diacetate	43	17.96	1478	1477	D	48 ± 18	55 ± 11
1-hexanol, 2-ethyl-	57	18.4	1495	1499	A	421 ± 134	218 ± 98
1-octanol	56	20.1	1564	1565	D	16 ± 5	14 ± 3
Alkanes							
Hexane	41	3.86	599	600	D	17 ± 8	30 ± 6
Heptane	43	4.09	685	690	D	24 ± 31	7 ± 3
Cyclohexane	56	4.23	732	705	D	29 ± 16	60 ± 14

Octane	43	4.53	814	800	C	77 ± 68	59 ± 19
Decane	57	6.66	1005	1000	C	96 ± 49	47 ± 29
Undecane	57	8.26	1090	1099	C	227 ± 110	126 ± 63
Dodecane	57	10.54	1188	1200	C	684 ± 236	365 ± 190
Tridecane	57	13.2	1294	1293	C	181 ± 38	122 ± 84
Tetradecane	57	15.87	1396	1396	C	73 ± 63	7 ± 4
Hexadecane	99	21.02	1600	1596	C	30 ± 5	37 ± 12
Aldehydes							
Pentanal	44	6.56	998	985	A	139 ± 76	123 ± 24
Hexanal	56	8.37	1095	1093	D	850 ± 867	1092 ± 356
Heptanal	70	10.76	1198	1197	D	82 ± 40	79 ± 17
Octanal	43	13.43	1303	1302	D	26 ± 16	26 ± 5
Nonanal	57	16.17	1408	1408	D	146 ± 86	146 ± 22
(t) 2-octenal	55	17.21	1450	1442	D	25 ± 13	33 ± 9
2-undecenal	70	24.92	1775	1761	D	7 ± 7	9 ± 3
(t,c) 2,4-decadienal	81	25.17	1787	1778	D	10 ± 2	15 ± 3
(t,t) 2,4-decadienal	81	25.98	1837	-	-	63 ± 14	96 ± 34
Aromatic hydrocarbons							
Toluene	91	7.66	1060	1040	A	150 ± 63	79 ± 7
Benzaldehyde	106	19.81	1552	1540	C	291 ± 23	303 ± 88
4-ethylbenzaldehyde	133	24.24	1742	1732	D	6 ± 1	7 ± 1
Benzyl alcohol	108	26.86	1896	1912	F	**33 ± 3 (*)**	**20 ± 6 (*)**
Phenylethyl alcohol	91	27.33	1935	1955	F	1243 ± 49	1453 ± 433
Phenol	94	28.4	2022	2004	C	34 ± 4	36 ± 7
Phenol, 4-ethyl-	122	30.38	2177	2195	D	36 ± 4	31 ± 30
4-vinyl-2-methoxy-phenol	135	30.79	2208	-	-	17 ± 1	22 ± 7
ketones							
2-butanone	43	5.49	922	916	E	45 ± 9	55 ± 6
2,3-butanedione	43	6.47	992	985	F	922 ± 164	1052 ± 349
3-hexanone	43	7.87	1070	1053	D	85 ± 18	83 ± 3
2-heptanone	43	10.68	1195	1160	D	39 ± 25	70 ± 45
2-butanone, 3-hydroxy-	45	13.55	1308	1295	D	3449 ± 932	3589 ± 504
2,3-octanedione	43	14.26	1336	1376	D	65 ± 34	52 ± 25
2-nonanone	58	16.03	1402	1395	B	39 ± 9	28 ± 12
Terpenoids							
Alpha-pinene	93	7.28	1039	1034	D	70 ± 31	32 ± 17
Delta-3-carene	91	9.73	1157	1127	D	63 ± 18	46 ± 13
Limonene	93	11.02	1209	1212	D	49 ± 18	40 ± 10
Junipene	109	20.94	1596	1574	D	6 ± 1	7 ± 2
Menthol	71	22.27	1654	1626	D	17 ± 4	13 ± 2
Pyrazines							
Pyrazine	80	11.62	1234	1262	E	83 ± 7	78 ± 6

Methylpyrazine	94	13	1287	1282	C	373 ± 43	333 ± 35
Pyrazine,2,5-dimethyl	42	14.44	1343	1320	D	125 ± 16	113 ± 34
Pyrazine, 2,6,-dimethyl	42	14.59	1349	1337	A	46 ± 4	38 ± 9
Furans							
Furan,2-pentyl	81	11.82	1242	1242	A	977 ± 416	782 ± 244
2-furancarboxaldehyde	96	18.14	1485	1474	C	225 ± 15	224 ± 61
2-furanmethanol	98	22.72	1672	1669	D	161 ± 23	164 ± 17
Acids							
Hexanoic acid	60	26.27	1857	1863	D	37 ± 14	23 ± 20
Acetic acid	43	17.57	1463	1466	F	190 ± 44	147 ± 27
Esters							
Ethyl acetate	43	5.3	907	885	D	198 ± 78	365 ± 250
1,3-hexadiene, 3-ethyl-2-methyl-	67	17	1441	-	-	131 ± 84	130 ± 10
Others							
Chloroform	83	7.18	1034	1022	C	213 ± 70	695 ± 974
Maltol	126	28.04	1993	2000	F	53 ± 6	60 ± 19
2-acetylpyrrole	94	28.08	1996	1952	C	18 ± 3	16 ± 3

m/z: main ion
RT: retention time
RI_{calc}: retention index calculated according to Kovats formulation
RI_{lit}: retention index found in the literature
Ref: references. A. Bianchi et al. (2008); B. Cirlini et al. (2012); C. www.odour.org.uk ; D. www.pherobase.com; E. Poinot et al. (2008); F. Poinot et al. (2010).
(*) Area of volatile compound is significantly different ($p < 0.05$).

4. Conclusions

Final baking in microwave oven was not suitable for gluten-free bread as it did not change crust color and it impaired bread texture due to the high water losses triggered by this technology. In contrast, final baking in microwave oven with susceptor packaging material induced crust browning and reduced water losses during final baking. There were no differences in triangular test nor in volatile compounds between breads that were finally baked in convection oven or in microwave oven with susceptor packaging material. The most abundant volatile compounds identified were originated from fermentation, followed by compounds from lipid oxidation. In conclusion, final baking in microwave oven with susceptor packaging material could be an alternative to final baking in convection oven, leading to a reduction of time and energy used to obtain the final product.

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CHAPTER 7: GENERAL DISCUSSION

The rise of gluten-free (GF) market due to the increase of patients diagnosed with gluten-related disorders like celiac disease, non-celiac gluten sensitivity and wheat allergy and the interest of some healthy population segments in gluten-free diet, motivates the research in GF products. As GF bread usually has low volume, pale light crust, crumbly texture, poor crumb structure and mouth-feel, short shelf-life and low nutritional profile (Gallagher et al., 2003; Thompson, 2009) studies on different ingredients and technologies are being carried out to improve its quality. The effect of different ingredients has been evaluated: tiger nut derived products to substitute soya flour (experiment 1), chickpea and tiger nut flours as alternatives to shortening and emulsifier (experiment 2) and chestnut flour (experiment 3). Moreover, the effect of different technologies has been studied: chestnut flour sourdough (experiment 3) and final baking in convection oven, microwave oven and microwave oven using susceptor packaging material (SPM) in partially baked frozen GF bread (experiment 4).

Our research group previously developed a GF formulation based on corn starch and soya flour with good baking and sensory characteristics (Miñarro et al., 2012). Due to the allergenicity of soya flour, we have focused our studies in the search of non-allergenic flours to substitute soya. For this reason, the effect of tiger nut derived products (tiger nut milk, tiger nut milk by-product and tiger nut flour) in GF bread was evaluated (experiment 1). Results showed that tiger nut milk and tiger nut flour are suitable to substitute soya flour in order to obtain a GF bread free of allergens. However, tiger nut milk by-product impaired GF bread quality, rendering breads with harder crumb texture than tiger nut flour, tiger nut milk or soya flour. This was probably due to the amount of fiber provided by tiger nut milk by-product and the high particle size of this fiber, which excessively increased batter consistency preventing enough rising during fermentation. In contrast, tiger nut milk rendered breads that were the most preferred by consumers (61%) due to its special sweet taste. The composition of tiger nut milk (sugars, fiber, lipids, starch) together with the low particle size of its fiber, due to the filtration step applied after tiger nut grinding, and the amylase activity during the entire tiger nut milk production process, could explain this results. The rheology characteristics of GF batters containing tiger nut milk were the optimum to render the maximum bread volume. Previous studies also reported the relationship between GF batter consistency and final volume observed in this research:

enough batter consistency increased specific volume but too much batter consistency decreased it (Gularte et al., 2012).

The behavior of tiger nut flour was similar to soya flour regarding to batter rheology and bread characteristics like bake loss, water activity, specific volume, crumb texture on day 2, and staling. However, tiger nut flour bread had softer initial crumb, lighter crust and darker crumb color than soya flour bread and it was less preferred by consumers because they noticed a sandy texture due to tiger nut fiber. Previous studies reported that tiger nut flour/rice flour ratios of 10/90 or 20/80 were the optimum blends to formulate GF bread baked in infrared-microwave combination oven or conventional oven, respectively (Demirkesen et al., 2011). In this thesis, it was observed that 5% of tiger nut flour could be added into a GF bread formulation based on corn starch, rendering breads similar to the ones formulated with 5% of soya flour, but free of allergens and with higher fiber content.

This study revealed that tiger nut flour could improve the results obtained with soya flour, except for its sandy texture. To overcome this drawback, tiger nut flour was sieved to eliminate the large bran particles that caused the sandy texture. This process reduced the fiber content of tiger nut flour from 19% to 12% but improved the sensory characteristics of GF breads. With this sieved tiger nut flour and taking into account the results previously obtained by our research group with chickpea flour (Miñarro et al., 2012), we decided to study the ability of these flours to partially or totally replace the shortening and/or the emulsifier that we were using in our standard formulation. Considering the lipid profile and content (23-31%) of tiger nut flour (Alegría-Torán and Farré-Rovira, 2003; Sánchez-Zapata et al., 2012) and the emulsifying properties of chickpea protein (Boye et al., 2010), the aim of the experiment 2 was to evaluate if these flours could help to maintain organoleptic characteristics while improving nutritional profile and providing a cleaner label. Moreover, reduction or elimination of emulsifiers in GF bread could contribute to improve intestinal health of celiac patients as Csáki (2011) reported that synthetic emulsifiers could increase intestinal permeability.

Four formulations were compared: corn starch; 7.8% chickpea flour and corn starch; 8.6% tiger nut flour and corn starch; 7.8% chickpea flour + 8.6% tiger nut flour and corn starch. The combination of three levels of shortening (5, 2.5 and 0%) and three levels of emulsifier (2, 1 and 0%) was evaluated in each basic formulation. All 36 breads had acceptable

characteristics in terms of volume, texture, color and crumb structure although differences between them were observed due to the effect of chickpea flour, tiger nut flour, shortening and/or emulsifier.

The addition of 7.8% of chickpea flour improved GF bread specific volume as already reported by Miñarro et al. (2012), who stated that the good emulsifying stability index of chickpea protein could explain the increase of GF bread volume. In contrast, the addition of 8.6% of tiger nut flour impaired GF bread specific volume. The use of tiger nut flour also diminished bread specific volume in formulations that contained both chickpea and tiger nut flours. Therefore, chickpea protein could not compensate the negative effect of tiger nut flour in bread volume. A reduction of staling was observed in bread containing both flours, probably due to the higher protein content, that competed with starch for water absorption and, thus, delayed its retrogradation. This experiment revealed that bread with both chickpea and tiger nut flour maintained its baking characteristics even if shortening and/or emulsifier were reduced or eliminated. Therefore, the combined used of these flours could substitute shortening and emulsifier, rendering healthier breads and with a cleaner label.

Shortening addition improved rheological properties of GF batter thanks to its lubricant and plasticizer properties only if emulsifier was present. However, the presence of tiger nut flour together with chickpea flour could substitute emulsifier because the lipid content of tiger nut flour (28.6%) permitted the emulsification of chickpea protein. For that reason, when both flours were present (chickpea and tiger nut), the positive effect of shortening on rheology properties was also observed although the absence of emulsifier. Shortening improved bread specific volume in formulations containing a source of protein (from tiger nut and/or chickpea flours) and 2% of emulsifier. Moreover, shortening reduced initial crumb hardness, but breads containing both flours (tiger nut and chickpea) did not suffer an impairment of crumb hardness due to shortening reduction or elimination, indicating that these two flours could substitute shortening without impairing crumb texture.

Some authors have studied the role of emulsifiers on GF bread and it has been reported that they could have different effect depending on the type of emulsifier used and the GF formulation where they are acting. Emulsifier improved GF batter rheology when 5% of shortening was present, but when lower amount of shortening was added, the effect of emulsifier was the opposite. Nevertheless, when GF formulation also contained tiger nut

flour, the emulsifier had no positive effect on GF batter even with 5% of shortening. Regarding to bread, the elimination of emulsifier resulted in an increase of specific volume. Crumb hardness at day 0 increased with 1% emulsifier but no changes were observed with 2% or 0%. It has been stated that emulsifiers reduce initial crumb hardness in wheat bread as they can associate with amylose (Pareyt et al., 2011). Although, in GF bread, diverse results have been reported, depending on the type of emulsifier and the formulation in which it is used (Demirkesen et al., 2010a; Nunes et al., 2009; Onyango et al., 2009; Purhagen et al., 2012; Sciarini et al., 2012). According to our results, the concentration of emulsifier is also an important factor to determine its functionality.

The research continued looking for alternative flours that could improve GF bread quality from organoleptic and nutritional points of view, like chestnut flour (experiment 3). Moreover, it integrated sourdough technology, as previous studies about chestnut flour sourdough existed in the literature but none about the effect of this sourdough in bread or GF bread quality. Thus, chestnut flour sourdough fermented spontaneously and renewed daily with 33% of the ripened sourdough was prepared, and the effect of this sourdough on GF bread quality was evaluated. Control breads with 15, 20 or 25% of chestnut flour where compared to sourdough breads with the same amount of chestnut flour added as chestnut flour sourdough.

A spontaneously fermented chestnut flour sourdough with lactic acid bacteria (LAB) and yeasts counts similar to typically mature sourdoughs was obtained (>10^8 CFU/g LAB and yeasts counts orders of magnitude lower). Aponte et al. (2013) reported higher pH and lower tritratable acidity (TTA) values on chestnut sourdough, probably due to the lower LAB counts that they achieved.

The increase of chestnut flour content reduced bread specific volume probably because of the increasing amount of fiber. The dark chestnut flour color affected bread crumb color, as lower L^* and higher a^* and b^* values were recorded when chestnut flour concentration was increased. Crust color was also affected by the increasing concentrations of chestnut flour which induced a decrease of L^* and b^* values and an increase of a^* values. Crumb hardness at day 0 was higher in breads containing 25% of chestnut flour probably due to its fiber content. However, there were no differences on crumb hardness after 7 d of storage. Bread pH was lower in breads with 25% of chestnut flour, but TTA results were not affected by the

amount of chestnut flour added. The effect of chestnut flour observed in this experiment was in accordance to the effect of tiger nut flour reported in previous results (experiment 1 and 2). Both flours contain important amounts of fiber, 4-10% in chestnut flour and 12-19% in tiger nut flour, which explain the lower volume, darker color and harder crumb texture. The addition of certain amount of fiber into GF bread can improve its volume and texture, however, when fiber content is too much high, it impairs GF bread quality (Demirkesen et al., 2010b).

Nevertheless, chestnut flour sourdough addition improved GF bread volume and compensated the negative effect of increasing concentrations of chestnut flour on this parameter. Sourdough increased bake loss but it did not affect water activity. Contradictory results about the effect of sourdough on bake loss and water activity in GF bread have been previously observed, depending on the sourdough flour type used (Galle et al., 2012; Moroni et al., 2011; Wolter et al., 2014). Breads elaborated with sourdough had lighter crusts than their counterparts. This was probably due to the reduced sugar content of sourdough breads as a result of sourdough fermentation, since sugar contributes to Maillard reaction and caramelization during baking. Sourdough had a positive effect on crumb hardness, rendering GF breads with softer crumbs at day 0 and after 7 d of storage. Sourdough reduced pH and increased TTA of breads but it had no effect on yeasts and moulds growth on bread after 7 d of storage. Sensory analysis showed that the addition of $\geq$ 17.2% of sourdough increased cell size and aroma intensity. However, most consumers preferred non-sourdough breads, probably because sourdough fermentation reduced the sugar content that chestnut flour provided to bread. Overall, spontaneously fermented chestnut flour sourdough contributed to improve chestnut flour GF bread quality.

Sensory analysis revealed consumers' preference for breads with a sweet taste. In experiment 1, they preferred bread elaborated with tiger nut milk, which provided higher sugar content than tiger nut flour or by-product, due to the amylase activity during tiger nut milk production. In experiment 3, consumers preferred breads elaborated with chestnut flour to breads elaborated with chestnut flour sourdough, which had a lower sugar content because of sourdough fermentation. These results show that these ingredients (tiger nut milk and chestnut flour) could be recommended to formulate sweet GF products.

Finally, the effect of final baking in convection oven, microwave oven and microwave oven with SPM in partially baked frozen GF bread was investigated (experiment 4). Preliminary studies were performed to develop a roll bread that could be finally baked using SPM. The GF bread formulation that contained chickpea flour used in the second experiment was selected to develop rolls because this formulation rendered batters and breads with optimal rheology and texture characteristics. However, as this batter was still too liquid and sticky to form rolls, the shape was given using a pastry bag and a ruler of 20 cm.

Results showed that although all treatments reduced bread moisture, breads that were finally baked in convection oven had higher moisture than breads finally baked in microwave independently of the use of SPM. This difference could be due to the lower inner bread temperature reached in convection oven (69.37 ± 5.95 ºC) compared to microwave oven (80.68 ± 3.01 ºC) and microwave oven with SPM (83.87 ± 2.4 ºC). However, moisture loss was lower when bread was finally baked in microwave using SPM than in microwave without SPM, probably because SPM prevented water loss during final baking.

Final baking in microwave oven without SPM could not induce crust browning as microwave heating occurs from inside the food and the surface cannot reach enough temperature for Maillard because the air surrounding is cold (Sahin et al., 2002). However, the utilization of SPM solved this problem because this material absorbs microwaves and causes a rise of bread surface temperature. In wheat bread, the SPM browning effect on crust bread was observed by Icoz et al. (2004), however, there was not any study about the effect of SPM in GF bread.

Crumb of bread finally baked in microwave at day 0 was harder than crumb of bread finally baked in microwave using SPM, which, in turn, was harder than crumb of bread finally baked in convection oven. However, after 28 d of frozen storage no differences were observed between crumb hardness of breads finally baked in microwave with SPM and convection oven. These results were related to moisture content since breads with higher moisture had softer crumbs. Indeed, a negative correlation between moisture and crumb hardness at day 0 was found ($r = -0.84$; $p < 0.05$). This relationship was also reported by De la Hera et al. (2014). Frozen storage at -18 ºC during 28 d did not significantly influence water activity neither bread moisture, although moisture values tended to decrease. Besides, it affected crumb and roll texture since harder crumbs and rolls were found at day 28 compared to day

0 as well as lower cohesiveness crumbs. Crumb hardness and crumb cohesiveness presented a negative correlation at day 0 ($r = -0.89$; $p < 0.05$) and at day 28 ($r = -0.85$; $p < 0.05$).

Volatile compounds of GF bread finally baked in convection oven or microwave oven with SPM were evaluated. Sixty-five volatile compounds were identified: 12 alcohols originated from fermentation and/or lipid oxidation; 10 alkanes and 9 aldehydes probably originated from lipid oxidation; 8 aromatic hydrocarbons from different origins (lipid oxidation, Maillard reaction or fermentation); 7 ketones derived from Maillard reaction and/or fermentation; terpenoids and furans originated by Maillard reaction and lipid oxidation, pirazynes originated by Maillard reaction, esters, acids, and other compounds. No significant differences between most of volatile compounds released were observed. Moreover, differences between both breads could not be detected in a triangular test. Volatile compounds of GF bread had been evaluated only by Poinot et al. (2009) who reported higher volatiles from lipid oxidation compared to wheat bread, due to the oil content in the formulation. The fourth research of this thesis contributes to broaden the information about volatile compounds released from GF bread.

To sum up, the positive findings in this thesis are: tiger nut milk and tiger nut flour can substitute soya flour in GF bread, the combination of chickpea and tiger nut flour can replace shortening and emulsifier, chestnut flour sourdough improves GF bread characteristics, and final baking in microwave oven using SPM is a good alternative to final baking in convection oven. The application of these findings can be advantageous for both GF consumers and industry.

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CHAPTER 8: CONCLUSIONS

The conclusions obtained in this thesis are listed below:

1. Tiger nut milk and tiger nut flour showed to be good alternatives to soya flour for the production of gluten-free breads with increased fiber content and free of allergens. Gluten-free breads containing tiger nut milk showed better baking (higher specific volume, lower bake loss, and softer crumb) and sensory characteristics (texture and taste) than soya flour breads, due to the sugar, fat, and fiber content that tiger nut milk provided to tiger nut milk formulation. Tiger nut and soya flours gave similar characteristics to breads, except in color and crumb structure. Tiger nut milk by-product impaired bread quality (darkest color and hardest crumb) due to its fiber size and content.
2. Gluten-free bread with both chickpea and tiger nut flour maintained its baking characteristics even if shortening and/or emulsifier were reduced or eliminated. Chickpea flour increased storage modulus as well as bread specific volume of gluten-free bread, while tiger nut flour reduced bread specific volume and gave darker crumbs. The effect of shortening and emulsifier depended on the concentration used and on the presence of chickpea and/or tiger nut flours.
3. Spontaneously fermented chestnut flour sourdough contributed to improve chestnut flour bread characteristics. Sourdough increased bread specific volume, porosity and aroma intensity, reduced crumb hardness and rendered breads with paler crusts. It improved bread shelf-life as it provided softer crumbs than chestnut flour after 7 d of storage. However, this sourdough had no effect on yeasts and moulds growth after 7 d of bread storage and it negatively affected consumers' preference, probably due to the reduction of the characteristic sweet taste of chestnut flour caused by sourdough fermentation.
4. Final baking in microwave oven was not suitable for gluten-free bread as it did not change crust color and it impaired bread texture due to the high water losses triggered by this technology. In contrast, final baking in microwave oven with susceptor packaging material induced crust browning and reduced water losses during final baking. There were no differences in triangular test nor in volatile compounds between breads that were finally baked in convection oven or in microwave oven with susceptor packaging material. Final baking in microwave oven with susceptor

packaging material could be an alternative to final baking in convection oven, leading to a reduction of time and energy used to obtain the final product.