ÇUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES

MSc THESIS

Ahmed I. A. ALSAIQALI

EFFECTS OF PSEUDOCEREALS ON THE QUALITY OF GLUTEN FREE DOUGH AND BREAD

DEPARTMENT OF FOOD ENGINEERING

ADANA-2018

ABSTRACT

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Supervisor : Assoc. Prof. Dr. M. Sertaç ÖZER
Year: 2018, Pages: 105
Jury : Assoc. Prof. Dr. M. Sertaç ÖZER
: Prof. Dr. Hüseyin ERTEN
: Asst. Prof. Dr. Ali Emrah ÇETİN

The baking properties of the pseudocereals as potential healthy and good-quality ingredients using different levels of hydrocolloids in gluten-free breads were studied. The pseudocereals significantly showed higher levels of protein, oil, ash and dietary fiber than corn starch. The results of the present study suggest that the pseudocereals can represent a good-healthy alternative to ingredients used in the manufacture of gluten-free breads. The increasing of moisture content, specific volume, *L* values of crumb color, and hardness of the crumb texture of all breads was associated with the increase in the level of hydrocolloids (2%, 3%, and 4%). Increasing the ratio of pseudocereal flours (10%, 20%, and 30%) showed a dark dough, also, resulted in a reduction in specific volume, *L* values of bread color and an increase in the moisture content and hardness of the crumb texture in all bread formulations (except in quinoa breads). The bread formulations prepared with the lowest ratio of pseudocereal flours (10%) at the high hydrocolloid concentration (4%) produced bread with good-quality better than that of the control bread in the acceptability. In contrast, the bread formulations prepared using the highest ratio of pseudocereal flours (30%) at the low hydrocolloid concentration (2%) produced bread with poor-quality, compared to the control bread.

Key Words: Celiac Disease, Gluten-Free, Bread, Pseudocereals, Hydrocolloids

ÖZ

YÜKSEK LİSANS TEZİ

YALANCI TAHILLAR ILE ZENGINLEŞTIRILMIŞ GLUTENSIZ EKMEK ÜRETIMI

Ahmed I. A. ALSAIQALI

ÇUKUROVA ÜNİVERSİTESİ
FEN BİLİMLERİ ENSTİTÜSÜ
GIDA MÜHENDİSLİĞİ ANABİLİM DALI

Supervisor : Assoc. Prof. Dr. M. Sertaç ÖZER
Yıl: 2018, Sayfalar: 105
Jury : Doç. Dr. M.Sertaç ÖZER
: Prof. Dr. Hüseyin ERTEN
: Dr. Öğr. Üyesi Ali Emrah ÇETİN

Çalışmada; amarant, kinoa ve chia (yalancı tahıllar) tahıllar ile farklı oranlarda gamların kullanılmasının glutensiz ekmekler üzerindeki etkisi araştırılmıştır. Yalancı tahılların protein, yağ, kül ve diyet lif içeriği mısır nişastasından daha yüksek çıkmıştır. Çalışmanın sonucunda; yalancı tahılların glutensiz ekmeklerin üretiminde kullanılan bileşenlere iyi ve sağlıklı bir alternatif teşkil edebileceğini göstermektedir. Kullanılan hidrokolloid oranlarının artmasıyla (%2, %3, %4) glutensiz ekmeklerde nem içeriği, spesifik hacim, ekmek içi renk (L) ve sertlik değerlerinin de arttığı gözlemlenmiştir. Yalancı tahıl un oranlarının artmasıyla (%10, %20, %30) daha koyu renkte hamur elde edilmiş, glutensiz ekmeklerin ise nem içeriği ve ekmek içi sertlikleri artarken spesifik hacim ve renk değerlerinin azaldığı belirlenmiştir. Düşük yalancı tahıl unları (%10) ve yüksek hidrokolloid konsantrasyonları kullanılmasıyla kontrol ekmeğine göre daha kabul edilebilir olarak gözlemlenmiştir. Düşük oranlı hidrokolloid (%2) ve yüksek konsantrasyonlu yalancı tahıl kullanılan glutensiz ekmeklerin kontrol ekmeklerine göre daha düşük kalitede olduğu belirlenmiştir.

Anahtar Kelimeler: Çölyak Hastalığı, Glutensiz, Ekmek, Yalancı Tahıllar, Hidrokolloidler

GENIŞLETILMIŞ ÖZET

Buğday, dünyadaki en yaygın tahıllardan biridir. Buğday ununda bulunan gluten proteinlerinin fonksiyonel özelliklerinden dolayı buğday birçok gıda ürününde kullanılmaktadır.

Gluten' in ekmek teknolojisi için önemine rağmen, çölyak hastalığı gibi glutene intoleransı olan kişiler için bazı sağlık sorunlarına neden olmaktadır. Çölyak; buğdayda bulunan gluten proteininin gliadin alt fraksiyonu ve arpa ile çavdarda bulunan benzer protein gruplarını içeren gıdaların tüketilmesi sonucunda, ince bağırsak mukozasında hasara neden olmaktadır. Buna bağlı olarak, besinlerin emiliminde yetersizlik başta olmak üzere birçok belirtiler görülmektedir. Bu hastalığa sahip kişilerde görülen en yaygın semptomlar: ishal veya kabızlık, kilo kaybı, karın ağrısı, şişkinlik ve aşırı gaz, yorgunluk, osteoporoz, depresyon ve bazı nörolojik bozukluklar olarak bildirilmiştir. Ayrıca, besinlerin emilimindeki azalmaya bağlı olarak birçok mineral ve vitamin eksikliği de görülmektedir.

Genetik bir hastalık olan çölyak, günümüzde, dünya nüfusunun yaklaşık % 1-2'inde olmak üzere yaygın bir şekilde görülmektedir. Bu hastalığın tedavisinde günümüzde geçerli ve etkili tek yöntem 'Glutensiz Diyet' (GD) uygulaması olup bu diyete ömür boyu devam edilmesi gerekmektedir. Çölyak hastalarının diyetlerinde; buğday, arpa ve çavdar içeren gıdalar yer almamalıdır. Bu nedenle, buğday, arpa ve çavdar içeren gıdaların tüketiminden kaçınmaları gerekmektedir.

Son yıllarda, bir yandan çölyak hastalığının teşhisindeki artışa, öte yandan moda diyetlere bağlı olarak glutensiz ürünlere olan ilgi giderek artış göstermektedir. Glutensiz ürünlerde hammadde olarak genellikle farklı kaynaklardan sağlanan nişastalar kullanılmaktadır. Özellikle ekmekte yapıya viskoelastik özellik kazandıran gluten' in eksikliği sonucunda nihai üründe düşük hacim, zayıf renk ve tekstürel özellikler gibi kalite kayıpları olarak ortaya çıkmaktadır. Ayrıca, glutensiz ekmeklerin, buğday unundan üretilen ekmekler ile karşılaştırıldığında daha düşük protein, lipit, vitamin, mineral ve lif içeriğine sahip olduğu bilinmektedir.

Ekmek başta olmak üzere glutensiz ürünlerde karşılaşılan bu sorunları gidermek, besinsel özellikleri geliştirmek ve duyusal nitelikleri iyileştirmek amacıyla hidrokolloidler ve yüzey aktif maddeler başta olmak üzere çeşitli gıda katkı maddeleri kullanılmaktadır. Ayrıca glutensiz hammadde olarak da karabuğday, sorghum, kinoa, amarant ve chia gibi fonksiyonel özelliklere sahip yalancı tahıl kullanımına yönelik çalışmalar da hız kazanmaktadır.

Bu çalışmada, mısır nişastası bazlı glutensiz ekmek üretiminde 3 farklı konsantrasyonda (%2, %3, %4) Metil selüloz, Karboksimetil selüloz, Ksantan zamk ve Guar zamk olmak üzere 4 farklı hidrokolloid ve %10, %20, %30 oranında olmak üzere 3 farklı yalancı tahıl (kinoa, amarant ve chia) ile bunların kombinasyonları kullanılmıştır.

Yalancı tahılların; protein, yağ, kül, toplam nişasta ve diyet lifi analizleri yapılmıştır. Çalışmada kullanılan yalancı tahılların, mısır nişastasından önemli ölçüde daha yüksek düzeyde; protein, yağ, kül ve diyet lifi içerdiği belirlenmiştir.

Hidrokolloid oranındaki artışa bağlı olarak tüm örneklerin nem içeriğinde artış gözlenmiştir. Yalancı tahıl kullanım oranındaki artışa koşut olarak tüm ekmek örneklerinin nem oranında artış tespit edilmiştir. Bu durum hidrokolloidlerin yüksek su tutma kapasitesi ve yalancı tahılların diyet lif içeriği ile açıklanabilir.

Genel olarak hidrokolloid oranındaki artışa bağlı olarak spesifik hacim değerlerinde artış tespit edilirken yalancı tahıl miktarındaki artışın hacim üzerinde olumsuz etkileri olduğu belirlenmiştir. Bu durum, yapıya destek veren glutenin yer almadığı glutensiz ürünlerde çökme problemi olarak karşımıza çıkmaktadır.

Tüm örneklerde, hidrokolloid oranındaki artışa bağlı olarak ekmeğin iç rengin açılmıştır. Yalancı tahılların oranındaki artışa bağlı olarak ise, ekmeğin hem iç hem de kabuk rengin koyulaşılmasına neden olduğunu tespit edilmiştir.

Kontrol örneğinde hidrokolloid oranındaki artışa bağlı olarak sertlik değeri de artış göstermiştir. Aynı şekilde genel olarak üretilen tüm ekmeklerde hidrokolloid oranındaki artış ve yalancı tahıl konsantrasyonundaki yükselmeye bağlı olarak sertlik değeri artış göstermiştir.

Yüksek hidrokolloid konsantrasyonda (% 4) düşük oranlarda yalancı tahıl unu (% 10) ile hazırlanan ekmek formülasyonları, kabul edilebilirlik açısından kontrol ekmeğinden daha iyi özellik göstermiştir. Buna karşın, düşük hidrokolloid konsantrasyonda (% 2) ve yüksek oranda yalancı tahıl unu ile hazırlanan (% 30) ekmek formülasyonları kontrol ekmeğine kıyasla düşük kaliteye sahiptir. Genel olarak, düşük oranlarda yalancı tahıl unu (% 10) ile hazırlanan glutensiz ekmek formülasyonları daha iyi kalite ekmek özellikleri göstermiştir.

Bu çalışmanın sonuçları, yalancı tahılların gluten içermeyen ekmeklerin üretiminde kullanılan bileşenlere iyi ve sağlıklı bir alternatif teşkil edebileceğini göstermektedir.

ACKNOWLEDGMENT

Alhamdulillah, I praise and thank Allah SWT for granting me the strength, inspiration, and courage to complete this thesis.

I express my sincere appreciation and deepest gratitude to my supervisor, Assoc. Prof. Dr. M. Sertaç ÖZER, for his supervision and continuous support. His constructive comments and suggestions throughout the experimental and thesis works have contributed to the success of this study.

Besides my supervisor, I extend sincere appreciation to the rest of my thesis committee Prof. Dr. Hüseyin ERTEN and Asst. Prof. Dr. Ali Emrah ÇETİN, for discussing my thesis and their insightful comments.

I owe my special thanks to all the technicians and office staffs of Department of Food Engineering, without their cooperation and kindness, this work could not have been completed.

Finally, but first in my heart, I would like to express my thanks and the deep sense of gratitude to my parents, for the overwhelming love and care they bestow upon me, the ones who have supported me and without whose proper directions it would not have been possible for me to complete my higher education.

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LIST OF ABBREVIATIONS

CD	: Celiac Disease
GF	: Gluten-Free
GFD	: Gluten-Free Diet
WHO	: World Health Organization
FAO	: Food and Agricultural Organization
GS	: Gluten Sensitivity
MC	: Methylcellulose
CMC	: Carboxymethylcellulose
HPMC	: Hydroxypropylmethylcellulose
LBG	: Locust Bean Gum
DATEM	: Purawave and Diacetyltartaric Ester of Monoglycerides
RSM	: Response Surface Methodology
SSL	: Sodium Stearoyl Sactylate
DM	: Distilled Monoglycerides
LC	: Lecithin
TC	: Total Cholesterol
LDL	: Low Density Lipoprotein
GSH	: Glutathione
TBARS	: Thiobarbituric Acid Reactive Substances
GFC	: Gluten-Free Control
A	: Amaranth
Q	: Quinoa
C	: Chia
M	: Mixture

GO	: Glucose Oxidase
IDF	: Insoluble Dietary Fiber
SDF	: Soluble Dietary Fiber
TPA	: Texture Profile Analysis

1. INTRODUCTION

Wheat is one of the most common grain in the world. It is the most widely cultivated crop with more than 25000 different varieties worldwide (Sapone et al., 2012). Wheat is used mainly in many food products like bread, pasta, noodles, bulgur, and couscous, or as a component in food processing (Sapone et al., 2012). This is attributed to the functional properties of gluten proteins found in wheat flour (Lindsay and Skerritt, 1999; Moore et al., 2006, Sciarini et al., 2008; Mariotti et al., 2009; Sapone et al., 2012).

Bread has been one of the principal constituents of the human diet for a long time. Wheat is importantly used in breadmaking beside other cereals which could be used to a lesser extent. In general, wheat flour, water, salt and yeast with the addition of non-essential ingredients are used in wheat breadmaking (Goesaert et al., 2005).

Gluten is the most important protein in cereals and particularly in wheat, it is characterized as the main structural protein and functional component of wheat. It mainly provides viscoelastic properties of dough required to produce high-quality products (Lindsay and Skerritt, 1999; Moore et al., 2006; Sciarini et al. 2008; Mariotti et al., 2009; Sapone et al., 2012). However, gluten is the rubbery mass that remains after wheat dough washing in order to remove starch granules and water-soluble constituents. This protein determines the unique baking quality of wheat. The gluten protein comprises two roughly equal parts in accordance to solubility in alcohol-water solutions: the soluble gliadin which provides the viscosity and extensibility to the dough, and the insoluble glutenin which is responsible for dough strength and elasticity (Wieser, 2007).

Nevertheless, gluten consumption causes some health problems for people who have allergic reactions or intolerances to gluten like celiac disease (CD) (Mariotti et al., 2009). In 1888, Dr. Samuel Gee described CD as a disorder considering it a condition of childhood, he reported that CD occurs commonly during the first five years of childhood particularly in the second year, indicating that the diet is the main part of celiac patients treatment (Auricchio and Troncone, 1996). CD is (also known as Celiac Sprue) an autoimmune disease induced by the ingestion of any food containing gluten proteins in wheat, barley, and rye, causing small intestinal inflammation, villous atrophy, and crypt hyperplasia in genetically susceptible persons at any age and commonly in childhood (Ciacci et al., 2002; Shan et al., 2002; Collin et al., 2004; Green and Cellier 2007; Hall et al., 2009; Cabrera-Chávez and Calderón de la Barca, 2010). The consumption of these foods leads to damage in the mucosa of the small intestine causing malabsorption of nutrients (Thompson et al., 2005). Consequently, celiac patients should avoid the ingestion of any foods contain wheat, barley, and rye in their diets.

Today, CD is known as a common genetic disease with a prevalence of 1-2% in the world population (Rodrigo, 2006; Sapone et al., 2012), and generally, its incidence is higher in women (2-3 times) than in men (Green and Cellier 2007). In Turkey, the prevalence of celiac patients is 0.2-0.3% of total population (Sozer, 2009).

The common symptoms of CD are wide and divided into classical intestinal (diarrhea or constipation, weight loss, abdominal pain, bloating and excessive gas), and non-classical intestinal (anemia, fatigue, osteoporosis, depression and neurological disturbances) (Woodward, 2007; Barker and Liu, 2008; Sapone et al., 2012; Skendi et al., 2018). Some mineral and vitamin deficiencies can also occur; vitamin B12 deficiency and iron deficiency may cause anemia, vitamin D deficiency results in rickets in children and osteomalacia in adults, and vitamin K deficiency leads to coagulopathy (Woodward, 2007; Barker and Liu, 2008; Sapone et al., 2012; Skendi et al., 2018).

The clinical manifestations of CD are not completely confined to the gastrointestinal tract, in some cases, CD is associated with other autoimmune conditions like thyroid disease and type 1 diabetes (Barker and Liu, 2008). Intestinal biopsy is the only way to evaluate mucosal damage associated with CD and to diagnose CD definitely (Altuntaş et al., 1998, Ciacci et al., 2002).

The only real and effective treatment for this disease is, at present, a lifelong adherence to a Gluten-Free Diet (GFD) (Ciacci et al., 2002; Collin et al., 2004; Gallagher et al., 2004; Rashid et al., 2005; Green and Cellier, 2007; Barker and Liu, 2008; Mariotti et al., 2009; Choung et al., 2016; Guandalini and Discepolo, 2016).

In the past few years, GF cereals like corn, sorghum, millet or pseudocereals like buckwheat, quinoa and amaranth were used for the production of GF products, and this is a significant challenge for food research to reach high-quality GF products (Schoenlechner et al., 2010). In general, although gluten-containing grains should be avoided in GFDs of celiac patients, different types of starches, grains, legumes, tubers can be used as flour substitutes in baking as shown in Table 1.1. (Green and Cellier 2007). However, the use of pseudocereals are significantly useful to produce GF products due to their high nutritional value comparing to wheat flours and they have no negative effects on the final products.

Table 1.1. Fundamentals of the gluten-free diet

Gluten-containing grains	Wheat, rye, barley.
Gluten-free grains	Rice, amaranth, buckwheat, corn, millet, quinoa, sorghum, teff and oat.
Sources of GF starches	Cereal grains: Amaranth, buckwheat, corn, millet, quinoa, sorghum, teff and rice. Tubers: Arrowroot, jicama, taro, potato and tapioca. Legumes: Chickpeas, lentils and beans.

From: (Green and Cellier 2007).

The aim of this study was to use some pseudocereal flours (amaranth, quinoa, and chia) and corn starch with different ratios as a alternative to wheat flour using several hydrocolloids (MC, CMC, Xanthan gum and Guar gum) beside emulsifiers (SSL) in order to (1) improve the technological properties of dough and bread, (2) to enhance the nutritive value of GF breads, and (3) to avoid the negative effects caused by ingestion of gluten, particularly for celiac patients. The final product quality will be based on obtained results during practical experiments, also the best ratio for used pseudocereal flours will be determined according to these results.

2. LITERATURE REVIEW

2.1. Celiac Disease (CD)

To date, CD is considered as a common disease with an increasing prevalence of global population, it can be seen in all ages and especially in children. In general, Table 2.1. presents symptoms and signs which could be classified into intestinal features (Feighery, 1999).

Table 2.1. Symptoms (and related signs) of celiac disease

Age	Symptoms	Related signs
Infancy (less than 2 years)	Diarrhoea	Miserable, pale
	Abdominal distension	Enlarged abdomen
	Failure to thrive	Low weight, lack of fat, hair thinning
	Anorexia, vomiting	
	Psychomotor impairment	Muscle wasting
Childhood	Diarrhoea or constipation	
	Anaemia	
	Loss of appetite	Short stature, osteoporosis
Adulthood	Diarrhoea or constipation	
	Anaemia	
	Aphthous ulcers, sore tongue and mouth	Mouth ulcers, glossitis, stomatitis
	Dyspepsia, abdominal pain, bloating	Weight loss
	Fatigue, infertility, neuropsychiatric symptoms	Anxiety, depression
	Bone pain	Osteoporosis
	Weakness	Myopathy, neuropathy

From (Feighery, 1999).

The only accepted therapy is a lifelong avoidance of all gluten-containing cereals from diets. Thereby, celiac patients are advised to consume natural Gluten-Free (GF) foods like vegetables, fruits, unprocessed meat, fish, and poultry as a part of GFD, as well as GF products like bread, pasta, cakes, and cookies (Lee et al., 2007), which are produced from GF flours like corn, rice, pseudocereals (amaranth, buckwheat, chia, and quinoa). In contrast, the foods which must be avoided in GFD (foods not allowed) are; (1) breads or any food made from these grains (wheat, rye, barley, oat and any grains contain gluten); (2) processed foods which use gluten-derivatives as thickeners and fillers (e.g. salad dressings, canned soups and processed cheese); (3) and drugs which contain gluten as pill or tablet binders (Gallagher et al., 2004) as shown in Table 2.2.

Table 2.2. Types of foods allowed and not allowed in a GFD

Type	**Food type**	**Example**	**References**
Foods allowed in a GFD	Naturally GF foods	Vegetables, fruits, and unprocessed meat, poultry and fish	(Lee et al., 2007)
	GF substitute foods	GF pasta, bread, and crackers	
Foods not allowed in a GFD	Any foods contain gluten	Breads and other products	(Gallagher et al., 2004)
	Processed foods contain gluten-derivatives	Thickeners and fillers	
	Medications contain gluten	Pill or tablet binders	

Celiac patients should receive the suitable kinds of food in their diets making sure these foods are devoid of gluten and considering the possibility of the presence of other diseases in celiac patients which may force them to consume other kinds of food.

In 1979, Codex Alimentarius which was created by World Health Organization (WHO) and Food and Agricultural Organization (FAO) adopted the standard for GF foods for who have an intolerance to ingestion of gluten such as individuals with CD or Gluten Sensitivity (GS). This standard was amended two times in 1983 and 2015. According to this standard, the GF foods be defined as dietary foods are:

A. Consisting of/or made only from ingredients which are free of wheat (all *Triticum* species), rye, barley, oats or their crossbred varieties, with total gluten level less than 20 mg/kg.
B. Consisting of ingredients from wheat (all *Triticum* species), rye, barley, oats or their crossbred varieties, which have been particularly prepared to be gluten-free, with total gluten level less than 20 mg/kg.
C. A mixture of (A) and (B) with total gluten level of less than 20 mg/kg (Codex Alimentarius, 2015).

In Turkey, the composition and labeling of foods produced for individuals with gluten intolerance comply with the following rules:

A. The amount of gluten in the food which will be presented to the final consumer consisting of one or more components derived from wheat, barley, oats, rye or their hybrid varieties especially which is processed to reduce the gluten level produced for individuals with gluten intolerance cannot exceed 100 mg/kg.
B. The labeling, advertising, and promotion of the products specified in the (A) label are used with the phrase "very low gluten". The phrase "gluten-free" could be used provided that the gluten level in the foods which will be presented to the final consumer does not exceed 20 mg/kg.

C. Oats found in the content of foods produced for individuals with gluten intolerance must be produced in order to prevent the contamination of wheat, barley, rye, or hybrid varieties of them. The gluten content of this oat cannot exceed 20 mg/kg.
D. The amount of gluten in food containing one or more components that are derived from wheat, barley, oats, or rye, including hybrid varieties, which is to be produced for individuals with gluten intolerance, also, to be presented to the final consumer cannot exceed 20 mg/kg. The phrase "gluten-free" is used in the labeling, advertisement, promotion of these products.
E. Replacing components for wheat, rye, barley, or oat, also their hybrid variety; and together with components made from wheat, rye, barley, oats or rye varieties including their hybrid variety are subjected to special treatment to reduce the level of gluten. These procedures must be applied for provisions (A), (B), and (C), but not for provision (D) for the foods which were produced to the individuals with gluten intolerance.
F. The phrase "very low gluten" and "gluten-free" in (b) and (d) should be found on the label of the food, close to the name of the food (Resmî Gazete, 2012).

However, a lifelong adherence to GFD is very difficult particularly for children wellbeing (Collin et al., 2004; Rashid et al., 2005; Guandalini and Discepolo, 2016). This may affect their psychosocial wellbeing (Collin et al., 2004; Rashid et al., 2005). On the other hand, the majority of products and processed foods contains gluten because wheat is widely used in their preparation. This limit the chance of finding GF products easily relative to their counterparts of gluten-containing products.

A study demonstrated that packaged GF foods are not easily available and limited. It does not exist in all markets and grocery stores making the task of getting these foods is difficult for celiac patients, and force them to buy from specialty stores or via the internet. Also, the cost of GF products is 2-3 times more expensive than that of their gluten-containing counterparts (Lee et al., 2007). In fact, this problem is increasing in developing countries. This can be attributed to the lack of sufficient knowledge about CD.

In Turkey, because of the manufacture of GF products is quite low, most of these products are imported from outside at a high cost. Thus, they are sold at exorbitant prices in the Turkish markets. There is, therefore, an urgent need for the production of these products locally in Turkey and make its final cost acceptable for all (Sozer, 2009).

In the past recent years, the demand for GF products has been significantly increased especially GF breads because of (1) the increasing prevalence of CD among people, or other allergic reactions to gluten (Moore et al., 2006; Lazaridou et al., 2007; Schoenlechner et al., 2010; Marti and Pagani, 2013), (2) being the fact that breads are normally basic ingredient of the diet for people (Moore et al., 2006), while (3) some people prefer to exclude gluten-containing products for health reasons (Marti and Pagani, 2013). For these reasons, the interest on the GF grains has been increased to find suitable substitutes for gluten-containing grains in order to produce GF products especially high-quality bread.

Several studies (Pink and Creamer, 1967; Altuntaş et al., 1998; Ertekin et al., 2005; Rashid et al., 2005; Abu-Zekry et al., 2008) have been conducted on individuals worldwide to investigate CD.

A study was conducted in order to assess the response of the 54 celiac patients after following a GFD, the results showed 70 % of patients (38 cases) recovered quickly to normal situation within 6 months, in contrast, 30 % of patients (16 cases) did not respond well or the response was partial to the diet (Pink and Creamer, 1967).

In Egypt, a study demonstrated that CD is a common disease among Egyptian children, and many celiac patients remain without a diagnosis, which would lead to late complications of CD (Abu-Zekry et al., 2008).

In Turkish studies, Ertekin et al. (2005) found that CD prevalence is significantly high in healthy schoolchildren. Also, they confirmed that children with iron deficiency and malnutrition need to be evaluated well due to the increased CD prevalence in Turkey. Other authors have illustrated that CD may be a cause of short stature in Turkish children, indicating the early diagnosis of CD and starting immediately in treatment are important to avoid untreated CD complications and to obtain optimal growth (Altuntaş et al., 1998).

A delay in the diagnosis of celiac disease predisposes to incremental use of medicines and health care services and reduced well-being. Improved awareness and at-risk group screening could be an effective means to diminish the delay at the population level (Fuchs et al., 2018).

A Canadian study was conducted on children with CD across Canada: confirmed that compliance with a GFD is very necessary to prevent the late complications of CD in future. The compliance also includes providing support and education to these children and their families, because strict adherence to a GFD for lifelong can be harder especially for children and may causes some problems for their psychosocial wellbeing (Rashid et al., 2005).

A Swedish study showed that a high intake of gluten during the first two years of age increases the risk of CD at least two times in children with genetic risk factors for CD (Andren Aronsson et al., 2016).

However, for several thousand years, gluten-containing cereals especially wheat have been used widely in bakery products. Since these grains and their flours contain gluten proteins, they must be avoided in GF bread production. In contrast, GF cereals like corn, rice, millet, and pseudocereals like amaranth, buckwheat, and quinoa are only able to be used in the manufacture of gluten-free bakery products (Houben et al., 2012). In addition, starches, gums, and hydrocolloids are used in order to simulate the viscoelastic properties of gluten in the production of GF products. This can be attributed to their structure forming and water binding properties, and their ability to improve gas retaining capacity (Gallagher et al., 2004; Mariotti et al., 2009; Marti and Pagani, 2013).

On the other hand, many GF breads have no good technological and nutritional quality (like dry crumb, poor flavor, and mouthfeel) in comparison to their wheat counterparts (Mariotti et al., 2009). The presence of gluten provides unique functional properties like extensible dough with good gas holding properties and gives breads with good crumb structure after post-baking as well. Gluten properties become apparent when water is added to wheat flour during dough mixing. In contrast, the absence of gluten leads to a liquid batter instead of dough. This can result in the emergence of quality defects like crumbling texture, poor color and other defects in baked breads (Gallagher et al., 2004).

In general, the formulations of GF breads are mainly to incorporate different kinds of starches, dairy proteins, non-gluten proteins, and hydrocolloids into a GF flour (like rice and corn flours) as a flour base (Toufeili et al., 1994; Gallagher et al., 2004; Mariotti et al., 2009).

2.2. Gluten Free Bread Ingredients

In general, breadmaking requires main ingredients which are indispensable in the manufacture of breads such as flour, water, yeast, sugar, and salt besides some secondary ingredients which can be used according to the needs (colorings, preservatives, acidity regulators, thickeners, emulsifiers, flavor enhancers glazing agents sweeteners, and hydrocolloids).

2.2.1. Corn Starch

Corn starch has been used rather than wheat flour as a base flour with some substances to produce GF breads since it does not contain gluten. In a study, it has been found that a mixture of 8.6% cassava starch, 17.2% rice flour, and 74.2% corn starch resulted in GF breads with satisfactory results in terms of taste and appearance, and it was suitable for celiac patients. Also, bread texture was improved when 0.5% soy flour was added (Sanchez et al., 2002). In a subsequent study (Sanchez et al., 2004), an increase from 1 to 7.3% in the protein content of GF bread was observed when 7.5% soy flour and 7.8% dry milk were added to a previous formulation (Sanchez et al., 2002), but small changes occurred in the sensory properties and the specific volume of GF bread.

Skendi et al. (2018) developed GF breads from rice flour and corn starch a fixed ratio 1:1 with acorn meal addition (5%, 15%, and 25%). Acorn supplemented GF breads were nutritionally improved in terms of total phenolics. The specific volume of GF breads significantly decreased with increasing the addition of acorn while crumb hardness was increased.

López et al. (2004) also reported that a mixture of 20% cassava starch, 45% rice flour and 35% corn starch presented GF breads with pleasant flavor, good external appearance, well-distributed cells, and uniform crumb.

Sciarini et al. (2008) confirmed that the addition of soy flour to corn and rice flours contributed well to improve batter and bread properties. Soybean proteins could give a structure which can retain more air (CO_2) during dough mixing. Also, they reported that soy proteins have a high water-holding capacity which helps reduce the bread staling rate. Overall, a mixture of corn, rice, soy flours produced high-quality breads: low staling rate, high specific volume, soft texture, and good crumb.

2.2.2. Hydrocolloids

Hydrocolloids (food gums) are high-molecular-weight hydrophilic biopolymers which are used as functional ingredients in the food industry. The term "hydrocolloid" includes all polysaccharides extracted from microbial sources, seaweed and plant, also, gums derived from plant exudates, and modified biopolymers made by enzymatic or chemical treatment of cellulose or starch (Dickinson, 2003).

A growing interest for hydrocolloids in food industry has been observed in recent years, due to the significantly increasing demand of GF products, particularly GF breads, and this can be attributed to the increasing prevalence of CD among people recently.

Several studies confirmed the potential utilization of hydrocolloids in the bakery industry. Gums include a number of water-soluble polysaccharides with various chemical structures giving a range of unique functional properties which enable them to be extensively used as additives in the food industry like thickening agents, foaming agents, emulsifiers, stabilizers, inhibitors, and improvers of water retention and texture properties (Rosell et al., 2007).

Hydrocolloids are extensively applied as additives in the food industry to improve the product texture, to slow down the retrogradation of the starches, to increase moisture retention, to extend the product quality during time, and finally to be used in the formulations of GF breads (Rojas et al., 1999).

In GF baking, hydrocolloids can be applied as a gluten replacer because of their fibrillar character. Bonding involved in hydrocolloid networks and gluten networks is different. Cationic cross-linking, hydrogen bonding, and hydrophobic interactions are common in hydrocolloid fibril formation. Gluten networks involve the formation of hydrogen bonding, hydrophobic associations, electrostatic interactions, dipole-dipole interactions, and van der Waals interactions. The effects of reducing and oxidizing agents are substantial in gluten development, but no effect of redox reagents is found on hydrocolloids, which do not contain sulfhydryl groups (BeMiller, 2008; Houben et al., 2012). However, different gels with diverse properties can be formed using different hydrocolloids and combinations of hydrocolloids (BeMiller, 2008).

It is worth highlighting that the effects of hydrocolloids on the rheological properties of dough and the bread quality depend on the specific options of the specific hydrocolloid used (e.g. chemical modification, chain length, molecular mass, and molecular structure), the addition level of hydrocolloid, and the type of flour, starch, and other ingredients used, as well as the process parameters used (like temperature, pH value, and shearing) (Hüttner and Arendt, 2010; Houben et al., 2012). Also, the selection of the proper combination of hydrocolloids are very important to obtain high-quality GF breads (Hüttner and Arendt, 2010).

Hydrocolloids act as structuring agents in GF matrixes (Morreale et al., 2018). They are essential ingredients to improve the texture and the appearance of final GF breads. They have the ability to improve gas retaining capacity, to provide higher dough consistency, and also to improve longer shelf-life. These improvements can be attributed to the structure-forming properties of hydrocolloids (Mariotti et al., 2009). Therefore, the addition of hydrocolloids can be successfully incorporated in GF bread formulations. Gums are important in the improvement of GF bread quality, resulting in high bread volume, good crumb structure, high cell-average-size and staling rate over storage (Hüttner and Arendt, 2010; Sciarini et al., 2010; Houben et al., 2012).

2.2.2.1. Cellulose derivatives

Cellulose is the most abundant organic substance in nature (Zecher and Gerrish, 1997, Kohajdová and Karovičová, 2009). It constitutes around one-third of all vegetable matter in the world (Zecher and Gerrish, 1997). The structure of a cellulose molecule is a polymer composed of two repeating anhydroglucose units (*β*-glucopyranose residues) linked by 1,4-glucosidic linkages (Kohajdová and Karovičová, 2009). Cellulose is insoluble in water and is indigestible by the human body (Chinachoti, 1995, Zecher and Gerrish, 1997).

Cellulose derivatives are among the common hydrocolloids used to replace gluten (Morreale et al., 2018). Cellulose derivatives such as methylcellulose (MC), carboxymethylcellulose (CMC), and hydroxypropylmethylcellulose (HPMC) are obtained by chemical modification of cellulose (Kohajdová and Karovičová, 2009).

Methylcelluloses (MCs) comprise a family of cellulose ethers. The importance of these nonionic cellulose ethers is based on four key attributes; (1) the ability to form thermal gels that melt upon cooling, (2) surface activity, (3) efficient thickening, and (4) film-forming ability (Coffey et al., 2006). MC and MHPC have the unique property of being insoluble in hot water but soluble in cold water (Zecher and Gerrish, 1997).

CMC is a cellulose derivative with carboxymethyl groups (–CH2–COOH) bound to some of the hydroxyl groups. It dissolves quickly in cold water and is used for controlling viscosity without gelling. Its solutions tend to be both highly viscous and stable, but the viscosity decreases during heating (Selomulyo and Zhou, 2007).

In general, cellulose derivatives (CMC, MC, and MHPC) are used industrially in bakery products, frozen desserts, dairy products, soft drinks, structured and coated products, and other applications (Zecher and Gerrish, 1997). However, cellulose derivatives have been utilized in the preparation of bakery and pastry products for many years. Cellulosics provide functionality as stabilizers, thickeners, flour strengtheners, shelf-life extenders, volume improvers and processing aids. For example, CMC and MC tend to lose moisture during baking and make a gel. This gelation possesses some advantages in bakery products such as GF breads (Zecher and Gerrish, 1997).

2.2.2.2. Guar gum

Guar gum is a galactomannan derived from the seed of a leguminous plant *Cyamopsis tetragonolobus*, it has been widely utilized as a food additive because of the very high viscosity of its aqueous solutions (Miyazawa and Funazukuri, 2006). Guar gum is used industrially as a thickening and stabilizing agent in a wide assortment of foods, usually in amounts less than 1% of food weight (Slavin and Greenberg, 2003). The incorporation of guar gum into certain types of foods like bread, biscuits and breakfast cereals improves their palatability (Kohajdová and Karovičová, 2009).

In baked goods, it is used to improve the mouthfeel and to change their rheological properties, to enhance the shelf life through moisture retention, and to delay bread staling (Kohajdová and Karovičová, 2009). In GF baking, this gum was also used as a food additive (Ćurić et al., 2007; Sozer, 2009; Demirkesen et al., 2010).

2.2.2.3. Xanthan gum

Xanthan gum is a main commercial biopolymer; its production exceeds 20000 tons yearly from several commercial sources (Khan et al., 2007). It is a complex exopolysaccharide obtained from the plant-pathogenic bacterium *Xanthomonas campestris* (Becker et al., 1998). Xanthan gum is a microbial gum permitted for use in foods as a thickener, stabilizer, emulsifier, foam enhancer and suspending agent (Chinachoti, 1995).

Xanthan gum is a white to cream colored free-flowing powder soluble in water (whether hot or cold), but insoluble in most organic solvents (Khan et al., 2007). This gum is unique to other polysaccharides because it provides high viscosity solutions at low concentrations, the viscosity of xanthan gum is little affected by temperature variation and stable over a wide pH range (Khan et al., 2007).

Xanthan gum improves the cohesion of starch granules, providing a structure to bakery products. It is used to augment water binding during baking and storage and it, therefore, increases the shelf life of baked products and refrigerated dough (Khan et al., 2007). Also, it contributes to the product smoothness, air incorporation and air retention in the product, improves the texture, volume, and moisture retention in refrigerated dough and GF breads (Kohajdová and Karovičová, 2009).

Table 2.3. presents several studies successfully used hydrocolloids in GF bread making (Hüttner and Arendt, 2010).

Table 2.3. Hydrocolloids used in GF bread making

Gums	Gelling properties	Effect on GF bread
Agarose	Form gels upon heating	Increased loaf volume Decreased uniformity of crumb Retarded bread staling
Guar gum	Highly viscous solutions, no gelling properties	Even cell size distribution in crumb Retarded bread staling
LBG	Slightly soluble in cold water, dissolves in water at 85 C Form gels with kappa-type carrageenans and xanthan	Increased height of the bread loaves Retarded bread staling
HPMC	Form gels upon heating	Increased specific volume
	Some interfacial activity and ability to form films	Improved gas retention and water absorption
Pectin	Lowmethoxyl pectins form gels in presence of calcium ions	Increased crumb porosity Retarded bread staling
Xanthan gum	High viscosity, pseudoplastic solutions (unaffected by temperature, pH, salt)	Good crumb structure
		Decreased crumb porosity
	Forms gels with agarose, kappa-type carrageenans, locust bean gum, konjac gum	Decreased loaf volume and increased crumb firmness

Source: (Hüttner and Arendt, 2010)

However, generally, the main role of the addition of hydrocolloids is to mimic gluten properties as gluten substitutes, especially in the absence of gluten in the manufacture of GF products. In this respect, among the hydrocolloids which have been used for this purpose are xanthan gum (Christianson et al., 1974; Ahlborn et al., 2005; Ćurić et al., 2007; Demirkesen et al., 2010; Mohammadi et al., 2014), arabic gum (Toufeili et al., 1994), guar gum (Ćurić et al., 2007; Sozer, 2009; Demirkesen et al., 2010), hydroxypropylmethylcellulose (HPMC) (Nishita et al., 1976; Sivaramakrishnan et al., 2004; Ahlborn et al., 2005; McCarthy et al., 2005; Demirkesen et al., 2010), carboxymethylcellulose (CMC) (Mohammadi et al., 2014), methylcellulose (MC) (Toufeili et al., 1994), etc.

Toufeili et al. (1994) reported that the GF breads could be formulated from a mix based on rice flour and corn starch using methylcellulose, arabic gum, and egg albumen as gluten substitutes. They pointed out that arabic gum had a minor impact on the sensory properties of breads, while its presence conferred strength to the dough. All final breads had a light-yellow crumb, corn flavor, and a faster staling rate compared to wheat bread.

The rheological properties of GF bread formulations based on rice flour using different hydrocolloids (xanthan gum, guar gum, HPMC, pectin, locust bean gum (LBG), xanthan–guar, and xanthan–LBG) with or without emulsifiers (Purawave and diacetyltartaric ester of monoglycerides (DATEM)) to find the best GF bread formulation were studied by Demirkesen et al. (2010). They reported that the presence of emulsifiers and hydrocolloids were necessary for getting the desired physical properties in dough formulations. Xanthan gum, xanthan–LBG, and xanthan–guar blend were the most effective hydrocolloids to improve dough structure.

Extruded blends of cornmeal and defatted soybean flour were combined in a 1:1 mixture with rice flour to produce GF bread formulation. Rheological properties of dough and baking characteristics were studied with or without using different hydrocolloids (guar gum, xanthan gum, pectin and cellulose). The optimal formulation, which gave the best outcomes in terms of specific volume, crumb elasticity, softness, and porosity, was 75 cornmeal/ 25 defatted soybean flour using 3% guar gum (w/w rice flour). All samples prepared from extruded flours characterized by high protein content and acceptable sensory properties (Ćurić et al., 2007).

The effects of xanthan gum and CMC on the quality of GF bread based on corn starch, soy flour, and rice flour were studied by Mohammadi et al. (2014). Outcomes showed that formulations of (15 g/kg xanthan) and (10 g/kg xanthan + 10 g/kg CMC) had the highest acceptability score, the highest dough and bread yield, and the lowest weight loss in comparison with all the others. Xanthan gum had a major effect on increasing moisture, while CMC resulted in bigger gas cells. However, they concluded that xanthan gum is the best hydrocolloid, also, to be used in improving the quality of GF breads.

Another study was conducted by Christianson et al. (1974) to determine the influence of xanthan gum in GF bread based on cereal starches (corn and wheat starches) using xanthan gum. They reported that the production of bread based on cereal starches without xanthan gum addition resulted in breads remained flat and coarse in texture. In contrast, adding xanthan gum improved bread volume and crumb structure. Also, the xanthan-containing breads made from wheat starch were better than that of corn starches in terms of crust and texture. Corn starch produced breads with a wrinkled crust and a coarser texture.

Ahlborn et al. (2005) observed that the GF formulations made from rice, egg, tapioca, and milk proteins using xanthan gum and HPMC were able to create a matrix with starch fragments comparable to that of gluten. This matrix may limit the structural damage, and provide freshness for the GF rice bread formulation.

Optimization of water and HPMC levels in a GF bread formulation using Response Surface Methodology (RSM) was studied by McCarthy et al. (2005). This formulation was prepared primarily from rice flour, skim milk powder, and potato starch using different levels of HPMC and water. Analyses were made a day after baking. Increasing water addition significantly resulted in an increase in loaf volume and height and a decrease in crumb firmness. The best quality bread could be formulated at a level of 2.2% HPMC and 79% water.

RSM was also applied by Kittisuban et al. (2014) to investigate the effects of HPMC, yeast beta-glucan, and whey protein isolate (WPI) on GF bread formulation based on rice starch. The optimal formulation contained 4.35% HPMC, 1% yeast beta-glucan, and 0.37% WPI based on rice starch. Moreover, the bread formulation was found to be acceptable in terms of sensory analysis.

Schober et al. (2008) could present a formulation for GF bread made from zein (corn protein), corn starch, water (20g, 80g, and 75g, respectively), NaCl, saccharose, and dry yeast using HPMC (2g). Resultant bread was similar to wheat bread having a good crumb and aeration, demonstrating the role of HPMC in improving gas cell stabilization.

In general, both xanthan gum and HPMC considered as the most suitable hydrocolloids to be used in the manufacture of GF products as gluten substitutes to mimic gluten properties, to provide gas-holding properties and also to produce GF breads with high quality in terms of the sensory properties, acceptability, mouthfeel, and good crumb structure and crust.

2.2.3. Emulsifiers

Not only hydrocolloids are commonly used in the manufacture of wheat based bakery products, but also emulsifiers are employed in order to contribute emulsification and blending of ingredients, to interact with the components of the flour in the mixture for a softer crumb, and to support the properties of the shortening. Based on the chemical structure of emulsifiers, they have the ability to interact and form complexes with protein, shortening, starch, and water. Interaction of an emulsifier with the protein can improve the strength and allow better retention of CO_2 (Demirkesen et al., 2010).

The basic role of the emulsifiers is to adsorb at the surface of the freshly formed droplets, and thereby prohibit them from coalescing and being united with their neighbors to shape larger droplets again (Dickinson, 2009).

Emulsifiers have already been employed into GF formulations as optional additives. Nunes et al. (2008) investigated the effect different types of emulsifiers (diacetyl tartaric ester of monoglycerides (DATEM), sodium stearoyl lactylate (SSL), distilled monoglycerides (DM) and lecithin (LC)) on the quality and the rheological properties of GF breads and batters. The addition of the different emulsifiers led to a major increase in the specific volume. DM-containing breads produced breads with higher volumes, whereas DATEM-containing breads presented breads with lower specific volumes. Cell size and distribution (Crumb structure) were greatly affected by the addition of LC and DATEM, contrary to the addition of SSL and DM which did not demonstrate significant differences. However, emulsifiers slightly affected the melting of the starch components. In general, they reported that emulsifiers had a positive influence on the quality of GF breads. Also, the correct emulsifier and its optimal addition level can enhance GF breads.

Demirkesen et al. (2010) found that the addition of emulsifiers, particularly DATEM, improved the specific volumes and firmness of GF loaves. Also, they concluded that DATEM is the best emulsifier to be incorporated with different hydrocolloids into dough formulations to produce GF rice breads with acceptable quality.

Generally, the effect of different additives is strongly dependent on the nature and quantity of additive used, the raw material used, and water availability. Thereby, it is very difficult to predict the real effect of the additives on different formulations (Sciarini et al., 2012). In this study, the additives employed were hydrocolloids (MC, CMC, Xanthan, and Guar) besides emulsifiers (SSL).

2.2.4. Pseudocereals

In many studies (Alvarez-Jubete et al., 2009a; Alvarez-Jubete et al., 2009b; Schoenlechner et al., 2010), pseudocereals have been investigated to be introduced into the application of the GF products as GF ingredients have the ability to be a replacement for gluten-containing flours and other starches and to boost the nutritional value of GF products as well. Also, pseudocereals could be used as natural seeds, oils, or other forms to be a part of GFDs. So, at present, the importance of pseudocereals increasingly emerged for many uses not only in GF foods but also in food processing generally.

Generally, pseudocereals are safe seeds for celiac patients as they contain very little or no prolamins (storage proteins). Also, they are important energy sources and have the high nutritional value of protein, fat, dietary fiber and minerals like calcium, iron, and zinc. Thereby, the use of pseudocereals to produce GF breads boosts the nutritional value of breads, and this is also important and required for celiac patients in their diets (Alvarez-Jubete et al., 2009a). In addition, the natural emulsifiers found in the pseudocereal flours confer crumb cohesiveness and softness without negative effects in the acceptability or the sensory properties of resultant breads (Alvarez-Jubete et al., 2009b).

However, Table 2.4. presents the nutrients of amaranth, quinoa, and wheat seeds. In the comparison, the protein, the oil, and the ash contents of amaranth and quinoa are higher than those of wheat. The dietary fiber content of wheat is lower than that of amaranth and higher than that of quinoa (Alvarez-Jubete et al., 2009a).

Table 2.4. Chemical composition % of amaranth, quinoa and wheat seeds

Seed	Protein	Oil	Total starch	Dietary fiber	Ash
Amaranth	16.5±0.3	5.7±0.3	61.4±0.8	20.6±1.1	2.8±0.0
Quinoa	14.5±0.3	5.2±0.1	64.2±1.3	14.2±0.6	2.7±0.0
Wheat	12.0±0.1	2.5±0.1	63.0±1.4	17.4±1.2	1.5±0.0

From: (Alvarez-Jubete et al., 2009a).

In addition, minerals levels are higher in all pseudocereals seeds than those in wheat. Amaranth seeds contain the highest levels of calcium, magnesium, and iron, while quinoa seeds exhibit the highest content of zinc as shown in Table 2.5. (Alvarez-Jubete et al., 2009a).

Table 2.5. Content of certain minerals in amaranth, quinoa and wheat seeds

Seed	Calcium	Magnesium	Zinc	Iron
Amaranth	180.1±6.1	279.2±1.1	1.6±0.0	9.2±0.2
Quinoa	32.9±3.3	206.8±6.4	1.8±0.0	5.5±0.5
Wheat	34.8±0.0	96.4±3.7	1.2±0.1	3.3±0.1

From: (Alvarez-Jubete et al., 2009a).

These features and the others make these grains valuable for the food processing and, particularly, for the manufacture of GF products. They are used as high-quality GF flours and strong fairly substitutes of gluten-containing cereals.

2.2.4.1. Amaranth

Amaranth is a pseudocereal crop. It is also a dicotyledonous grain cultivated in many countries in South and Central America, Africa, and Asia, and mainly cultivated in the Andean countries (like Peru and Bolivia) for *Amaranthus caudatus* species (Tapia-Blacido et al., 2007). Amaranth is an important pre-Hispanic crop and was considered as a part of the diet of many people like Incas, Aztecs, Mayas and other pre-Colombian civilizations (Sanz-Penella et al., 2013).

Amaranth can be consumed as a part of diets as whole grains or its flour can be used to prepare some kind of foods (Sanz-Penella et al., 2013). It is generally incorporated in bakery products due to its high nutritional value (Sindhuja et al., 2005; Bodroža-Solarov et al., 2008; Oszvald et al., 2009; Sanz-Penella et al., 2013), or in GF products because it is gluten-free grains besides its high nutritional value (Alvarez-Jubete et al., 2009a; Alvarez-Jubete et al., 2009b).

Sindhuja et al. (2005) observed that the amaranth flour affects the dough properties and the pasting characteristics. Replacement of wheat flour with 25–30% amaranth flour improved the cookie quality. Higher levels of the amaranth flour resulted in cookies with lower hardness. The addition of emulsifiers (like lecithin and glycerol monostearate) contributed to the improvement of the cookie quality. Other researchers reported that the addition of amaranth grains to wheat flour have nutritional benefits and contribute to increasing the content of zinc, manganese, calcium, and magnesium (Bodroža-Solarov et al., 2008). Increases in the content of crude fibers and squalene in supplemented breads were observed. They also reported that the sensory characteristics of resultant breads produced by the addition of 10-15% amaranth grain were acceptable. While the supplementation of 20% amaranth resulted in loaves with lower specific volumes compared to the control (Bodroža-Solarov et al., 2008).

However, a study on the investigated the effects of incorporation of amaranth albumin proteins on the functional properties of wheat dough, reported that amaranth albumins are able to interact with gluten proteins found in wheat flour through disulfide bonds due to free SH groups, giving similar effects to wheat glutenin subunits which would improve strength and stability of dough (Oszvald et al., 2009).

Sanz-Penella et al. (2013) reported that amaranth flour could be incorporated successfully as a nutritious ingredient into the bread formulations, increasing the levels of dietary fiber, ash, minerals and proteins of the resultant breads. They indicated that the level of amaranth flour addition in bakery products should not be exceeded 20 g/100 g in order to maintain product quality.

2.2.4.2. Quinoa

Quinoa (*Chenopodium quinoa*, Willd.) is an important pseudocereal and is a dicotyledonous annual crop as well (Hirose et al., 2010; Zurita-Silva et al., 2014). Quinoa is cultivated in the Andean countries of South America (particularly Peru and Bolivia) for several thousand years (Ruales and Nair, 1992; Hirose et al., 2010; Stikic et al., 2012). Also, it can be cultivated in highlands and temperate regions due to its resistance to frost and drought (Ruales and Nair, 1992).

On the other hand, FAO declared the year 2013 as The International Year of Quinoa (IYQ) in recognition of the indigenous peoples of the Andes, Quinoa has been protected, preserved and maintained by them as a food for present and future generations. They used their simple knowledge and cultivation practices without negative effects on nature (FAO, 2013).

Quinoa has higher nutritional value (like vitamins, minerals, fatty acids, and antioxidants) compared to common cereals. Its unique functional properties made it good grains able to contribute to human nutrition especially for cell processes, which require antioxidant protection of membranes (Vega-Galvez et al., 2010). A large group of individuals such as women prone to osteoporosis, people with lactose intolerance, anemia, diabetes, obesity, and others can benefit from the consumption of quinoa. This is attributed to its therapeutic features and high nutritional value, in addition to the absence of gluten (Navruz-Varli and Sanlier, 2016). Farinazzi-Machado et al. (2012)

concluded that inclusion of quinoa in diets can prevent and treat the risk factors of cardiovascular diseases which are considered as one of the main causes of death at present.

A study was carried out by Zevallos et al. (2014) to evaluate the *in-vivo* effects of consumption of 50 g of quinoa daily for 6 weeks as part of GFD of 19 adult celiac patients. They reported that, in general, the measurements of blood samples were within the normal range. The levels of total cholesterol (TC) and low density lipoprotein (LDL) were higher than those of recommended levels. Ten participants did not suffer from any CD symptoms, yet 9 participants had several symptoms (from mild to moderate) during the first 14 days. This study suggested that, the consumption of 50 g of quinoa daily for 42 days as a part of GFD of celiac patients could be safely tolerated, pointing out the importance of having further studies in order to determine the long-term effects of quinoa consumption.

Another study was conducted on 35 overweight women who consumed daily 25 g of quinoa or corn flakes for 4 weeks consecutively. There was a significant decrease in levels of serum triglyceride, thiobarbituric acid reactive substances (TBARS) and vitamin E. Unlikely urinary excretion of enterolignans was increased in both groups. There was a decrease in TC and LDL and an increase in glutathione (GSH) only in the quinoa-flakes group. This indicates a beneficial effect of quinoa consumption to improve the lipid profile, and this may be attributed to the fact that quinoa contains higher quantities of dietary fibers compared to corn (De Carvalho et al., 2014).

2.2.4.3. Chia

Chia (*Salvia hispanica* Labiatae) is an annual summer crop (Ayerza and Coates, 2009), and a pseudocereal like amaranth and quinoa. It is also called oilseed due to its high oil content (Costantini et al., 2014). Chia is especially cultivated in Mexico (Weber et al., 1991; Ayerza, 1995).

Chia contains 4.00±0.12% moisture, 19.00±0.42% protein, 34.05±0.52% oil, 33.05±0.33% fiber, and 5.15±0.23% ash. Total carbohydrate content of chia is 41.80% (Weber et al., 1991). The protein content of chia seeds is more than one and one-half to twice that of other grains, while its oil content is more than 3-10 times that of other grains. This makes chia competitive candidate to the other common grains like wheat, barley, oats, corn, rice, and amaranth (Weber et al., 1991). The importance of chia consumption has increased for human health due to its high content of alpha-linolenic fatty acid, in addition to its the beneficial health effects (Ayerza and Coates, 2011).

The influence of chia addition on the quality of GF bread based on rice flour was carried out by Steffolani et al. (2014). The results showed that the incorporation of 15% of chia improved the nutritional characteristics of GF breads without adversely affecting the sensory acceptability of the final breads.

Another study demonstrated that a mixture of chia flour and tartary buckwheat flour enhanced functional properties of breads without negative effects in comparison with the control. Chia flour-containing breads had significantly higher amounts of proteins, fibers, ash, and omega-3 fatty acids. Tartary buckwheat flour provided high amounts of flavonoids and played a role in improving the total antioxidant capacity of breads (75 %). Also, both chia and tartary buckwheat are suitable for celiac patients because they are devoid of gluten (Costantini et al., 2014).

To conclude, in general, many studies have been confirmed that the incorporation of pseudocereals in the food industry is an interesting way to increase nutritional value of many products, in particular, GF products. Pseudocereals characterized by high-nutritional value results in the final products with higher nutritional values compared to their counterparts of wheat, as well as their flours do not contain gluten.

Several studies confirm that GF products could be produced by using wheat substitutes such as GF cereal (like corn and rice flours) as GF flour base as well as various types of starches, dairy proteins, gums, and hydrocolloids, in order to simulate viscoelastic properties of gluten. Pseudocereals such as amaranth, quinoa, buckwheat, and chia have been used for the same purpose.

A study was conducted by Alvarez-Jubete et al. (2009b) to investigate the baking properties of pseudocereal flours (amaranth, quinoa, and buckwheat) as GF ingredients in GF bread formulations. The results obtained showed that bread volumes of buckwheat and quinoa breads were significantly higher than that of GF control. There was no difference in volumes between amaranth breads and GF control breads. The pseudocereal-containing GF breads, however, gave a softer crumb texture which was attributed to natural emulsifiers found in their flours. The pseudocereal-containing GF breads had no significant differences in the acceptability or the sensory properties as compared to control. This indicates the possibility of pseudocereal flours to be introduced into GF bread formulations.

The protein, fat, ash and fiber contents were significantly increased in breads, when 50% of potato starch was replaced by pseudocereals as ingredients (amaranth, quinoa, and buckwheat, respectively). The protein content of the resultant pseudocereal-containing breads were 11.6%, 10.6%, and 8.4% respectively, at least more than twice the value of the GF control bread (4.2%). The fiber content of buckwheat bread was 23.3%, followed by amaranth (17.2%), and quinoa (16.1%) compared to GF control bread. The total starch content was significantly decreased for all pseudocereal-containing breads (Alvarez-Jubete et al., 2009a).

Steffolani et al. (2014) investigated the effects of chia addition (15 g of chia flour or seed per 100 g of rice flour) on the quality of GF bread. The specific volume of resultant breads was reduced while the hardness was increased. This effect was more profound when the chia flour rather than its seeds was employed. The inclusion of chia flour gave a darker crust and crumb. In contrast, the inclusion of seeds produced breads with a better texture than control. Chia addition generally reduced the weight loss during baking.

On the other hand, Renzetti and Arendt (2009) investigated the impact of Glucose Oxidase (GO) and protease on the breadmaking performance of some GF flours (corn, sorghum, buckwheat, and teff) without using hydrocolloids in the bread formulations. The results were: (1) GO increased specific volume of corn and sorghum breads and reduced collapsing at the top. These improvements could be attributed to protein polymerization which enhances the elastic-like behavior of corn and sorghum batters. While there were no significant effects on buckwheat and teff breads. (2) Protease adversely affected the textural quality of buckwheat and sorghum breads and this was attributed to protein degradation giving liquid-like behavior of buckwheat and sorghum batters. They also indicated the protein polymerization have improvements on the breadmaking performance of GF flours. Protein structures are mainly important for textural quality of these GF breads in the absence of hydrocolloids

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3. MATERIALS and METHODS

3.1. Materials

In this study, materials were obtained from commercial sources or from local markets as shown in the following Table 3.1. keeping the same specifications and storage condition at all stages of the work and in all experiments.

Table 3.1. The list of sources of materials used

Idioms		Company	Country
Corn starch		Sunar Misir Inc.	Adana-Turkey
Seed	**Amaranth**	Yayla Agro Food Industry Inc.	Mersin-Turkey
	Quinoa	Yayla Agro Food Industry Inc.	Mersin-Turkey
	Chia	Yayla Agro Food Industry Inc.	Mersin-Turkey
Other materials	**MC**	Ashland Industries, Inc	Beveren-Belgium
	CMC	Ashland Industries, Inc	Alizay-France
	Guar Gum	Tunçkaya Chemical Substances	Istanbul-Turkey
	Xathan Gum	Tunçkaya Chemical Substances	Istanbul-Turkey
	SSL	Beyab Food Ingredients Co.	Bursa-Turkey
	Sugar	Local markets	Adana-Turkey
	Salt	Local markets	Adana-Turkey
	Compressed Yeast	Local markets	Adana-Turkey
	Sunflower Oil	Local markets	Adana-Turkey

3.2. Methods

3.2.1. Grinding and Preparation of Seeds

All types of seeds (amaranth, quinoa, and chia) were received and placed in the refrigerator at 4°C until they were milled using grain mill. Grinding process was separately done for each type of seeds using double-bladed milling machines (Spice & Herb Grinder IC-25B-China, Power 3500W, Rotation speed: 28.000 rpm). A kilogram of seeds was placed inside the machine and milled for 15 sec. This procedure was applied three times to the same amount of seeds at different times to ensure complete milling.

After grinding, the products were sieved from a 212 micron mesh and stored in airtight plastic jars. After filling these jars, they were placed in the storage room until to be used in bread making.

3.2.2. Analysis of Corn Starch

3.2.2.1. Moisture Content:

Determined according to (AACC 44-19.01, 1999).

3.2.2.2. Protein Content:

Modified Kjeldahl method was used to determine total nitrogen and the amount of the protein was calculated by multiplying with a conversion factor (5.7 is used for cereals) (AACC 46-10.01, 1999).

3.2.2.3. Oil Content:

Soxhlet method was performed using ether as a solvent. Crude fat was residue after removing of the solvent (ether) by evaporation and drying to constant weight at 100° (AACC 30-25.01, 1999).

3.2.2.4. Ash Content:

Analyzed according to (AACC 08-01.01, 1999).

3.2.2.5. Total Dietary Fiber Analysis:

Total Dietary Fiber (TDF) was determined according to (AACC 32-07.01, 1999).

3.2.3. Analysis of Amaranth, Quinoa and Chia Seeds

3.2.3.1. Moisture Content:

Determined according to (AACC 44-19.01, 1999).

3.2.3.2. Protein Content:

Kjeldahl method was used to determine total nitrogen and the amount of the protein was calculated by multiplying with a conversion factor (5.7 is used for cereals) (AACC 46-10.01, 1999).

3.2.3.3. Oil Content:

Soxhlet method was performed using ether as a solvent. Crude fat was residue after removing of the solvent (ether) by evaporation and drying to constant weight at 100° (AACC 30-25.01, 1999).

3.2.3.4. Ash Content:

Determined according to (AACC 08-01.01, 1999).

3.2.3.5. Total Dietary Fiber Analysis:

Total Dietary Fiber (TDF) was performed according to (AACC 32-07.01, 1999).

3.2.4. Preparation of Gluten-Free Bread

Table 3.2. presents the different ratios of the main components (corn starch, amaranth, quinoa, and chia flour) used in bread making. Corn starch were mainly used as a base flour in all cases in different ratios. Pseudocereal flours were used in different ratios separately or as a mixture. In some formulations, they were not used at all. A

combination consisting of four types of hydrocolloids (MC, CMC, Xanthan gum, and Guar gum) with different concentrations were employed as presented in Table 3.3. The amount of water used in each bread formulation was kept constant (120 %), also emulsifier; SSL (1.5 %), sugar (2.5 %), salt (1.5 %), compressed yeast (4 %) and sunflower oil (2.5 %) were used in fixed ratios. The differences in bread formulations were in (1) the quantity of corn starch, and (2) the type and the quantity of flour and additive used.

Table 3.2. Bread formulations (%)

No.	Formulation	Level of gums	Corn Starch	Amaranth	Quinoa	Chia
1	GFC1	2	100	0	0	0
2	GFC2	3	100	0	0	0
3	GFC3	4	100	0	0	0
4	A1	2	90	10	0	0
5	A2	3	90	10	0	0
6	A3	4	90	10	0	0
7	A4	2	80	20	0	0
8	A5	3	80	20	0	0
9	A6	4	80	20	0	0
10	A7	2	70	30	0	0
11	A8	3	70	30	0	0
12	A9	4	70	30	0	0
13	Q1	2	90	0	10	0
14	Q2	3	90	0	10	0
15	Q3	4	90	0	10	0
16	Q4	2	80	0	20	0
17	Q5	3	80	0	20	0
18	Q6	4	80	0	20	0
19	Q7	2	70	0	30	0
20	Q8	3	70	0	30	0
21	Q9	4	70	0	30	0
22	C1	2	90	0	0	10
23	C2	3	90	0	0	10
24	C3	4	90	0	0	10
25	C4	2	80	0	0	20
26	C5	3	80	0	0	20
27	C6	4	80	0	0	20
28	C7	2	70	0	0	30
29	C8	3	70	0	0	30
30	C9	4	70	0	0	30
31	M1	2	90	3.33	3.33	3.33
32	M2	3	90	3.33	3.33	3.33
33	M3	4	90	3.33	3.33	3.33
34	M4	2	80	6.66	6.66	6.66
35	M5	3	80	6.66	6.66	6.66
36	M6	4	80	6.66	6.66	6.66
37	M7	2	70	10	10	10
38	M8	3	70	10	10	10
39	M9	4	70	10	10	10

GFC = Gluten-Free Control (Corn Starch 100%), A = Amaranth, Q = Quinoa, C = Chia and M = Mixture (A, Q, and C).

Table 3.3. Concentrations of gums used in bread making

Concentrations	**2 %**	**3 %**	**4 %**
MC	0.5	0.75	1.00
CMC	0.5	0.75	1.00
Guar gum	0.5	0.75	1.00
Xhnthan gum	0.5	0.75	1.00

The experiments were carried out according to the formulations presented in Table 3.2. The steps of bread making are shown in Figure 3.1.

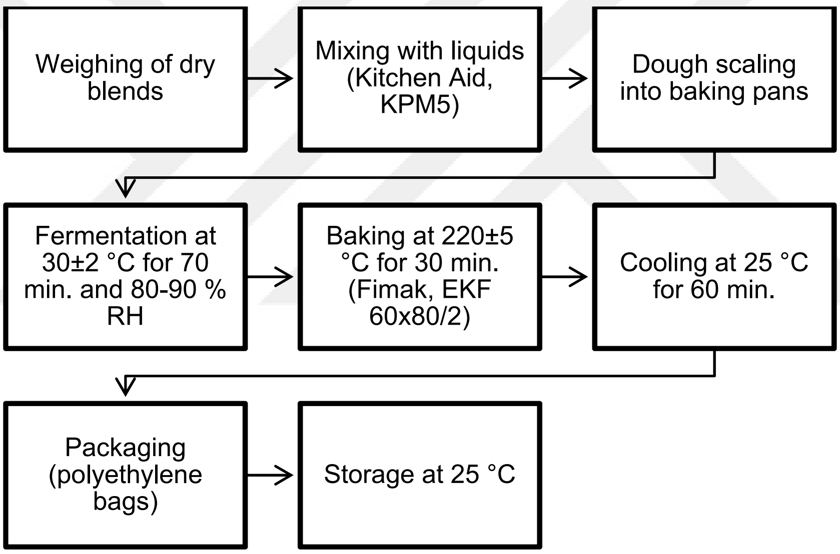

Figure 3.1. Flow chart of GF bread preparation

Firstly, all ingredients were weighed according to the formulations in Table 3.2. The following step was to place 70% of the percentage of the water into the mixer (Kitchen Aid, KPM5, St. Joseph, MI) with the addition of sugar followed by salt, oil, and yeast. They were then mixed for 60 sec. to ensure that the sugar and the salt were dissolved in water and the oil and the yeast were thoroughly mixed with water. After that, the dry components were weighed (the ratios of ingredients are different according to product code) and mixed together well, and then the rest 30% water was slowly added to the mixture in order to get a homogeneous batter. Mixing was carried out for 5 min (speed 5). The batter was scaled into baking pans after mixing: 160 g into each pan. Pans were incubated into a proofing chamber for 70 min at 30±2 °C and 80-90 % RH. The loaves were then baked in an electric oven (Fimak, EKF 60x80/2 Model) at 220 °C for 30 min. After baking, loaves were cooled for an hour at 25°C in ambient conditions and stored into sealed polyethylene bags. Breads were analyzed 6 h after baking. Six loaves were produced each time. Experiments were performed in triplicate for each type of formulation.

3.2.5. Dough Color Analysis:

ColorQuest XE was used to measure dough samples. Color reading was expressed by using the L, a, and *b* color scale (Alvarez-Jubete et al., 2009b). The *L* scale ranges from (0 black) to (100 white); the *a* scale extends from (- green hue) to (+ red hue), whereas the *b* scale ranges from (- blue) to (+ yellow) (Lazaridou et al., 2007). Dough color was measured at 3 different points for each sample. Also, the cylindrical coordinates: Chroma *C* and hue angle h were defined by the following equations (Matos and Rosell, 2012):

$$\mathrm{C} = \sqrt{a^2 + b^2}$$

$$\mathrm{h} = \tan^{-1}\left(\frac{b}{a}\right)$$

3.2.6. Bread Analyses

3.2.6.1. Moisture Content:

Measured based on (AACC 44-19.01, 1999).

3.2.6.2. Specific Volume:

The method of rapeseed displacement was used to measure the bread volume after 1 hour post-baking period (Havet et al., 2000). Specific volume was calculated by dividing bread volume by bread weight. Three measurements for each replicate were made (Sciarini et al., 2012).

3.2.6.3. Color Analysis:

Konica Minolta CM-5 was used to measure the crust and the crumb color of the fresh bread samples. Color reading was expressed by using the *L*, *a*, and *b* color scale (Alvarez-Jubete et al., 2009b). The *L* scale ranges from (0 black) to (100 white); the *a* scale extends from (- green hue) to (+ red hue), whereas the *b* scale ranges from (- blue) to (+ yellow) (Lazaridou et al., 2007). Crust and crumb color were measured at 9 different points for each loaf. Also, the cylindrical coordinates: Chroma *C* and hue angle *h* were defined by the following equations (Matos and Rosell, 2012):

$$\mathrm{C} = \sqrt{a^2 + b^2}$$

$$\mathrm{h} = \tan^{-1}\left(\frac{b}{a}\right)$$

3.2.6.4. Texture Analysis:

Crumb texture of breads was measured by conducting a texture profile analysis (TPA) using TA:XT plus Texture Analyzer (Stable Micro Systems, UK) equipped with a 5 kg load cell and a 25 mm aluminum cylindrical probe. A slice (25 mm thick) was taken from the middle of the bread and placed under the probe to conduct the test. Test speed was 1 mm/s, and compression was set at 40%. TPA analysis was conducted 24 h after baking and carried out on 3 replicates for each formulation.

3.2.6.5. Sensory Analysis:

Hedonic sensory evaluation of the fresh breads was conducted by a panel consisting of 15 non-celiac consumers. The panelists were selected from the staff and students of Çukurova University. Panelists were asked to assess the fresh GF breads according to their appearance, color, texture, taste and overall acceptability on a 5-point hedonic scale. The scale of values ranged from 1 (dislike extremely) to 5 (like extremely) as presented in Table 3.4. Water (at room temperature) was provided to the panelists to clean their palate after eating of each sample.

Table 3.4. Scales used in the consumer testing of bread

Scores
1 = Dislike extremely
2 = Dislike
3 = Neither like nor dislike
4 = Like
5 = Like extremely

3.2.7. Statistical Analysis

Results were analysed using IBM SPSS 22 statistics, Data were analysed using analysis of variance (One-Way ANOVA). Significant differences ($P < 0.05$) were determined by Duncan multiple comparison test.

4. RESULTS and DISCUSSION

4.1. Chemical Compositions of Materials

Results of the chemical analysis of corn starch, and amaranth, quinoa, and chia seeds are presented in Table 4.1.

Table 4.1. Chemical compositions of corn starch, and amaranth, quinoa, and chia seeds

Starch/Seed	Protein%	Oil%	Total Starch%	Dietary Fiber%	Ash%
Corn Starch	0.51	0.61	94.40	0.52	0.41
Amaranth	17.10	5.12	60.84	20.68	3.12
Quinoa	13.98	4.93	62.81	14.61	3.12
Chia	18.62	33.24	39.80	33.12	4.98

The protein content was significantly higher for chia (18.62%) followed by amaranth (17.1%) and quinoa (13.98%) seeds compared with corn starch (0.51%) ($P < 0.05$). The oil content of chia seed (33.24 %) was more than six times that of amaranth and quinoa seeds (5.12% and 4.93% respectively), while it was found as 0.61% for corn starch ($P < 0.05$). Chia had the lowest total starch content with a measured value of 39.80% ($P < 0.05$). Total starch of quinoa (62.81%) was higher than that of amaranth (60.81%) ($P < 0.05$). The dietary fiber for chia seed (33.12%) was higher than that of amaranth (20.68%), and more than twice that of quinoa (14.61%), while it was 0.52% for corn starch ($P < 0.05$). The ash content was significantly higher for all seeds in comparison with corn starch (0.41%), while the highest values obtained were for chia (4.98%), amaranth (3.12%) and quinoa (3.12%) ($P < 0.05$).

A compilation of some published data on the chemical composition of similar seeds is shown in the following Table 4.2. The values obtained in our study are similar to the published data (Alvarez-Jubete et al., 2009a; Weber et al., 1991).

Table 4.2. Chemical composition of amaranth, quinoa, and chia seeds

Seed	**Protein %**	**Oil%**	**Total starch%**	**Dietary fiber%**	**Ash%**	References
Amaranth	16.5±0.3	5.7±0.3	61.4±0.8	20.6±1.1	2.8±0.0	(Alvarez-Jubete et al., 2009a)
Quinoa	14.5±0.3	5.2±0.1	64.2±1.3	14.2±0.6	2.7±0.0	
Chia	19±0.42	34.05 ±0.5	41.80	33.05 ±0.3	5.15±0.2	(Weber et al., 1991)

In the study of Alvarez-Jubete et al. (2009a), the protein (16.5%), oil (5.7%), total starch (61.4%), dietary fiber (20.6%), and ash (2.8%) contents of amaranth were slightly lower (or equal), and the protein (14.5%), oil (5.2%) and total starch (64.2%) contents of quinoa were slightly higher, and dietary fiber (14.2%) and ash (2.7%) contents of quinoa were slightly lower in comparison with the composition of the present study. For chia, in the study of Weber et al. (1991), the protein (19%), oil (34.05%), total starch (41.80%) and ash (5.15%) contents were slightly higher, and dietary fiber (33.05%) was slightly lower in comparison with the composition of the present study.

4.2. Dough Color

Dough color of GF formulations was evaluated. As shown in Table 4.3. no significant differences in *L* values of GFC doughs were observed upon the increasing levels of hydrocolloids (2%, 3%, and 4%); GFC1 (an *L* value of 94.5), GFC2 (94.5), and GFC3 (94.35) ($P > 0.05$). Also, the increasing levels of hydrocolloids did not significantly affect the values of *a* and *b* except in a few cases, for example; no significant differences in *b* values between GFC1 (7.97) and GFC2 (7.96), but *b* value of GFC3 (8.2) was higher than those of GFC1 and GFC2.

The effect of increasing hydrocolloid concentration on the color of amaranth doughs tended to give a dark color (lower *L* values) except in a few cases in which the differences were not significant. This effect was observed in the formulations of 10% amaranth flour-containing doughs; A1 (91.21), A2 (90.87), and A3 (90.77) (no significant differences were observed between A2 and A3), 20% amaranth flour-containing doughs; A4 (89.09), A5 (89.05), and A6 (88.69) (no significant differences were observed between A4 and A5), and 30% amaranth flour-containing doughs; A7 (87.68), A8 (87.48), and A9 (87.27). The increasing levels of hydrocolloids, however, did not affect *a* and *b* values of amaranth doughs generally. Increasing the ratio of amaranth flour (%10, 20%, and 30%) significantly showed lower *L* values (darker dough) and higher *a* and *b* values, compared to the control ($P < 0.05$). However, among amaranth doughs, 10% amaranth flour-containing doughs (A1, A2, and A3) had higher *L* values and lower *a* and *b* values in contrast to (30%) amaranth flour-containing doughs (A7, A8, and A9) which had lower *L* values and higher *a* and *b* values. A1 (91.21) had the highest and A9 (87.27) had the lowest *L* values of amaranth doughs. The latter is due to its high hydrocolloid concentrations (4%) and the high ratio of amaranth flour (30%) in contrast to A1 (2% and 10% respectively).

Table 4.3. Color of gluten-free doughs (*L*, *a*, and *b* color scales)

Ratios of gums %	2			3			4		
Color scale	***L***	***a***	***b***	***L***	***a***	***b***	***L***	***a***	***b***
Starch 100%									
GFC	94.50^{a}	-0.47^{p}	7.97^{op}	94.50^{a}	-0.44^{p}	7.96^{op}	94.35^{a}	-0.47^{p}	8.20^{n}
Ratios of flour 10%									
Amaranth	91.21^{b}	0.79^{o}	9.62^{ef}	90.87^{c}	0.82^{o}	9.61^{ef}	90.77^{c}	0.82^{o}	9.75^{e}
Quinoa	83.95^{j}	3.10^{f}	7.82^{pq}	83.84^{jk}	3.38^{e}	8.10^{no}	83.66^{k}	3.34^{e}	8.29^{mn}
Chia	82.06^{l}	1.54^{m}	6.64^{u}	81.98^{l}	1.57^{m}	6.86st	81.84^{l}	1.57^{m}	6.70^{tu}
Mixture	84.79^{h}	2.03^{k}	7.78^{pq}	84.67^{h}	2.03^{k}	7.65^{q}	84.24^{i}	2.08^{k}	7.78^{pq}
Ratios of flour 20%									
Amaranth	89.09^{d}	1.38^{n}	10.76^{c}	89.05^{d}	1.31^{n}	10.43^{d}	88.69^{e}	1.34^{n}	10.66^{c}
Quinoa	79.96^{no}	4.18^{d}	8.71^{jk}	79.81^{op}	4.22^{d}	8.91^{i}	79.28^{q}	4.35^{c}	9.19^{h}
Chia	76.81^{s}	2.12^{k}	6.99^{s}	76.82^{s}	2.11^{k}	6.92^{s}	76.74^{s}	2.10^{k}	6.97^{s}
Mixture	80.6^{m}	2.68^{h}	8.58^{kl}	80.17^{n}	2.82^{g}	8.55^{kl}	79.69^{p}	2.87^{g}	8.47^{lm}
Ratios of flour 30%									
Amaranth	87.68^{f}	1.74^{l}	11.38^{b}	87.48^{fg}	1.76^{l}	11.56^{b}	87.27^{g}	1.82^{l}	11.82^{a}
Quinoa	76.79^{s}	4.84^{b}	9.42^{fg}	76.56st	4.97^{a}	9.81^{e}	76.23^{u}	4.75^{b}	9.48^{f}
Chia	73.01^{v}	2.47^{i}	7.37^{r}	72.98^{v}	2.40^{ij}	6.98^{s}	72.93^{v}	2.36^{j}	6.91^{s}
Mixture	77.20^{r}	3.28^{ef}	8.97^{i}	77.36^{r}	3.28^{ef}	8.81^{ij}	76.36^{tu}	3.31^{e}	9.28^{gh}

Means followed by different letters within the same columns (*L*, *a*, and *b*) are significantly different ($P < 0.05$).

Table 4.4. Color of gluten-free doughs (chroma *C* and hue-angle *h*)

Ratios of gums %	2		3		4	
Color scale	*C*	*h*	*C*	*h*	*C*	*h*
Starch 100%						
GFC	7.98^{op}	-1.51^{p}	7.97^{op}	-1.52^{p}	8.22^{n}	-1.51^{p}
Ratios of flour 10%						
Amaranth	9.65^{hi}	1.49^{a}	9.65^{hi}	1.49^{a}	9.78^{h}	1.49^{a}
Quinoa	8.45^{m}	1.18^{lm}	8.78^{l}	1.18^{m}	8.94^{kl}	1.19^{l}
Chia	6.81^{t}	1.34^{d}	7.04^{rs}	1.35^{d}	6.88^{st}	1.34^{d}
Mixture	8.04^{no}	1.32^{e}	7.91^{op}	1.31^{e}	8.06^{no}	1.31^{e}
Ratios of flour 20%						
Amaranth	10.85^{cd}	1.44^{b}	10.51^{f}	1.45^{b}	10.75^{de}	1.45^{b}
Quinoa	9.66^{hi}	1.12^{n}	9.85^{h}	1.13^{n}	10.17^{g}	1.13^{n}
Chia	7.31^{q}	1.28^{fg}	7.23^{qr}	1.27^{fg}	7.28^{q}	1.28^{f}
Mixture	8.99^{k}	1.27^{g}	9.00^{k}	1.25^{h}	8.95^{kl}	1.24^{hi}
Ratios of flour 30%						
Amaranth	11.52^{b}	1.42^{c}	11.69^{b}	1.42^{c}	11.96^{a}	1.42^{c}
Quinoa	10.59^{ef}	1.10^{o}	11.00^{c}	1.10^{o}	10.60^{ef}	1.11^{o}
Chia	7.78^{p}	1.25^{hi}	7.38^{q}	1.24^{i}	7.30^{q}	1.24^{i}
Mixture	9.55^{ij}	1.22^{jk}	9.40^{j}	1.21^{k}	9.85^{h}	1.23^{j}

Means followed by different letters within the same columns (C and h) are significantly different ($P < 0.05$).

The effect of increasing hydrocolloid concentration on the color of quinoa doughs was such that the doughs with higher hydrocolloid concentrations tended to have a dark color (lower *L* values) ($P < 0.05$). This effect was clear in the formulations of 10% quinoa flour-containing doughs; Q1 (83.95), Q2 (83.84), and Q3 (83.66), 20% quinoa flour-containing doughs; Q4 (79.96), Q5 (79.81), and Q6 (79.28), and 30% quinoa flour-containing doughs; Q7 (76.79), Q8 (76.56), and Q9 (76.23). The increasing levels of hydrocolloids did not significantly affect *a* values in most formulations of quinoa doughs, but ,generally, resulted in an increase in *b* values. Increasing the ratio of quinoa flour (%10, 20%, and 30%) significantly resulted in lower *L* values (darker dough) and higher *a* and *b* values, compared to the control with the exception of the *b* value of GFC1 (7.97) which was higher than that of Q1 (7.82) ($P < 0.05$). However, among quinoa doughs, 10% quinoa flour-containing doughs (Q1, Q2, and Q3) had higher *L* values and lower *a* and *b* values in contrast to (30%) quinoa flour-containing doughs (Q7, Q8, and Q9) which had lower *L* values and higher *a* and *b* values. Q1 (83.95) had the highest and Q9 (76.23) had the lowest *L* values of quinoa doughs. The latter is due to its high hydrocolloid concentrations (4%) and high ratio of the amaranth flour (30%) in contrast to Q1 (2% and 10% respectively).

No significant differences were observed in *L* values of color in all chia doughs ($P > 0.05$). This was observed in the formulations of 10% chia flour-containing doughs; C1 (82.06), C2 (81.98), and C3 (81.84), 20% chia flour-containing doughs; C4 (76.81), C5 (76.82), and C6 (76.74), and 30% chia flour-containing doughs; C7 (73.01), C8 (72.98), and C9 (72.93). The increasing levels of hydrocolloids did not significantly affect *a* values in the formulations of 10% chia flour-containing doughs (C1, C2, and C3) and 20% chia flour-containing doughs (C4, C5, and C6), but led to a decrease in 30% chia flour-containing doughs; C7 (2.47), C8 (2.4), and C9 (2.36). The increasing levels of hydrocolloids did not affect *b* values in most formulations of chia doughs. Increasing the ratio of chia flour (%10, 20%, and 30%) significantly resulted in lower *L* values (darker dough) ($P < 0.05$) and higher *a* and *b* values. In a few cases, however, no significant differences ($P > 0.05$) in *b* values were observed between C5 (6.92) and C8 (6.98) (20% and 30% chia flour respectively) and between C6 (6.97) and C9 (6.91) (20% and 30% chia flour respectively). However, among chia doughs, 10% chia flour-containing doughs (C1, C2, and C3) had higher *L* values and lower *a* and *b* values, compared to 20% and 30% chia flour-containing doughs.

Increasing hydrocolloid concentration tended to give a dark color (lower *L* values) for doughs except in few cases in which the differences were not significant. The effect was prominent in the formulations of 10% mixture flour-containing doughs; M1 (84.79), M2 (84.67), and M3 (84.24) (no significant differences were observed between M1 and M2), 20% mixture flour-containing doughs; M4 (80.6), M5 (80.17), and M6 (79.69), and 30% mixture flour-containing doughs; M7 (77.2), M8 (77.36), and M9 (76.36) (no significant differences were observed between M7 and M8). Also, the increasing levels of hydrocolloids did not affect *a* values of mixture doughs generally, but affected *b* values especially that of 30% mixture flour-containing doughs. Increasing the ratio of mixture flour (10%, 20%, and 30%) significantly resulted in lower *L* values (darker dough) and higher *a* and *b* values ($P < 0.05$). However, among mixture doughs, 10% mixture flour-containing doughs (M1, M2, and M3) had higher *L* values and lower *a* and *b* values in contrast to (30%) mixture flour-containing doughs (M7, M8, and M9) which had lower *L* values and higher *a* and *b* values. Among the mixture doughs, M1 (84.79) and M2 (84.67) had the highest and M9 (76.36) had the lowest *L* values. The latter is due to its high hydrocolloid concentrations (4%) and the high ratio of amaranth flour (30%) in contrast to M1 (2% and 10% respectively).

In general, Increasing the level of hydrocolloid often tended to result in a dark color. Increasing the ratio of pseudocereal flours produced a dark dough. This effect may be attributed to the natural color of the pseudocereals which is more darker than the color of the corn starch.

As shown in Table 4.4. in many cases, the values of chroma *C* and hue-angle *h* of the dough color were not generally affected by increasing the level of gums. Increasing the ratio of pseudocereal flours increased the *C* values and decreased the *h* values of the dough color in comparison with the control samples.

4.3. Bread Evaluation

4.3.1. Bread Moisture

Table 4.5. presents the moisture content of GF bread formulations. The addition of hydrocolloids increased bread moisture in all formulations as shown in Figure 4.1. An increase in the moisture content was observed when the concentration of hydrocolloids was increased (2%, 3%, and 4%) (GFC1 (40.50%), GFC2 (40.70%), and GFC3 (40.85%)) ($P < 0.05$).

Table 4.5. Moisture content (%) of gluten-free breads

Ratios of pseudocereal flour%	Control (100% starch)			10			20			30		
Levels of gums %	**2**	**3**	**4**	**2**	**3**	**4**	**2**	**3**	**4**	**2**	**3**	**4**
Gluten-free control	40.50^{n}	40.70^{lmn}	40.85^{j-n}	-	-	-	-	-	-	-	-	-
Amaranth	-	-	-	40.68^{lmn}	41.26^{g-l}	41.52^{e-l}	40.77^{k-n}	41.58^{e-i}	41.71^{d-h}	41.37^{f-k}	41.70^{d-h}	42.07^{cde}
Quinoa	-	-	-	40.50^{n}	40.80^{k-n}	41.00^{i-n}	40.61^{lmn}	40.93^{i-n}	41.14_{h-n}	40.61^{lmn}	40.95^{i-n}	41.42^{f-k}
Chia	-	-	-	40.54^{mn}	40.76^{k-n}	41.11^{h-n}	40.98^{i-n}	41.37^{f-k}	41.48^{e-j}	42.10^{b-e}	42.68^{ab}	42.79^{a}
Mixture	-	-	-	40.56^{mn}	40.82^{k-n}	40.85^{j-n}	41.17^{g-m}	41.79^{d-g}	41.95^{c-f}	41.81^{d-g}	42.25^{a-d}	42.54^{abc}

Means followed by different letters are significantly different ($P < 0.05$).

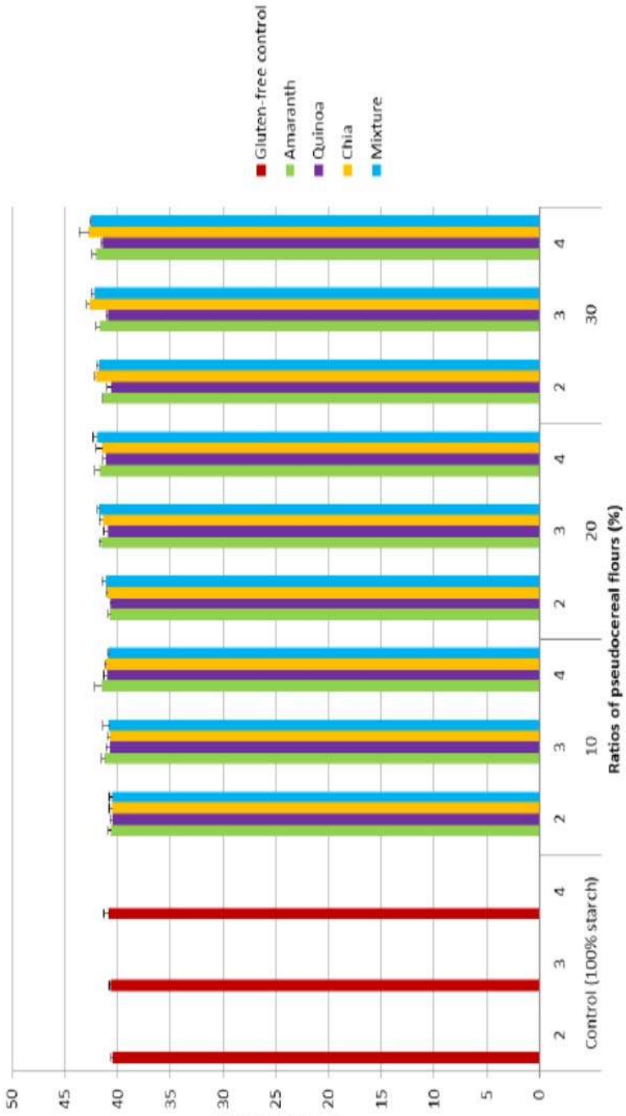

Figure 4.1. Moisture content (%) of gluten-free breads

In all ratios of amaranth flour (10%, 20%, and 30%), the moisture content of amaranth breads increased by increasing hydrocolloid concentration (2%, 3%, and 4%), and the differences were observed in the formulations of 10% amaranth flour-containing breads; A1 (40.68%), A2 (41.26%), and A3 (41.52%), 20% amaranth flour-containing breads; A4 (40.77%), A5 (41.58%), and A6 (41.71%), and 30% amaranth flour-containing breads; A7 (40.37%), A8 (41.70%), and A9 (42.07%) ($P < 0.05$). Increasing the ratio of amaranth flour (10%, 20%, and 30%) led to an increase in the moisture content of amaranth breads. All formulations of amaranth breads had moisture contents higher than those of control breads. However, among amaranth breads, the highest moisture content was found in the 30% amaranth flour-containing breads (A7, A8, and A9) in comparison to their counterparts of control breads (GFC1, GFC2, and GFC3, respectively) followed by those containing medium ratios (20%) (A4, A5, and A6) and low ratios (10%) (A1, A2, and A3) of amaranth flour. This means, A9 (42.07%) had the highest and A1 (40.68%) had the lowest moisture content among amaranth breads in comparison to the control.

In all ratios of quinoa flour (10%, 20%, and 30%), the moisture content of quinoa breads also increased by increasing hydrocolloid concentration (2%, 3%, and 4%). This effect was evident in the formulations of 10% quinoa flour-containing breads; Q1 (40.50%), Q2 (40.80%), and Q3 (41.00%), 20% quinoa flour-containing breads; Q4 (40.61%), Q5 (40.93%), and Q6 (41.14%), and 30% quinoa flour-containing breads; Q7 (40.61%), Q8 (40.95%), and Q9 (41.42%) ($P < 0.05$). Increasing the ratio of quinoa flour (10%, 20%, and 30%) resulted in an increase in the moisture content of quinoa breads generally. All formulations of quinoa breads had moisture contents higher than those of control breads except in a case of Q1(40.50%) which had the same moisture content with GFC1 (40.50%). Among quinoa breads, the highest moisture content was found in the 30% quinoa flour-containing breads (Q7, Q8, and

Q9) in comparison to their counterparts of control breads (GFC1, GFC2, and GFC3, respectively) followed by those containing medium ratios (20%) (Q4, Q5, and Q6) and low ratios (10%) (Q1, Q2, and Q3) of quinoa flour. In a few cases, however, no significant differences were found between Q4 (40.61%) and Q7 (40.61%) (20% and 30% quinoa flour respectively), and between Q5 (40.93%) and Q8 (40.93%) (20% and 30% quinoa flour respectively) ($P > 0.05$). Q9 (41.42%) had the highest and Q1 (40.50%) had the lowest moisture content among quinoa breads when compared to the control.

In all ratios of chia flour (10%, 20%, and 30%), the moisture content of chia breads also increased by increasing hydrocolloid concentration (2%, 3%, and 4%). This effect was evident in the formulations of 10% chia flour-containing breads; C1 (40.54%), C2 (40.76%), and C3 (41.11%), 20% chia flour-containing breads; C4 (40.98%), C5 (41.37%), and C6 (41.48%), and 30% chia flour-containing breads; C7 (42.10%), C8 (42.68%), and C9 (42.79%) ($P < 0.05$). Increasing the ratio of chia flour (10%, 20%, and 30%) increased the moisture content of chia breads. All formulations of chia breads had moisture contents higher than those of control breads. However, among chia formulations, the highest moisture content was found in the 30% chia flour-containing formulations (C7, C8, and C9) in comparison to their counterparts of control formulations (GFC1, GFC2, and GFC3 respectively) followed by those containing medium ratios (20%) (C4, C5, and C6) and low ratios (10%) (C1, C2, and C3) of chia flour. This means, C9 (42.79%) had the highest and C1 (40.54%) had the lowest moisture content among chia breads in comparison with the control.

In all ratios of mixture flour (10%, 20%, and 30%), the moisture content of mixture breads also increased by increasing hydrocolloid concentration (2%, 3%, and 4%). This effect was evident in the formulations of 10% mixture flour-containing breads; M1 (40.56%), M2 (40.82%), and M3 (40.85%), 20% mixture flour-containing breads; M4 (41.17%), M5 (41.79%), and M6 (41.95%), and 30% mixture flour-containing breads; M7 (41.81%), M8 (42.25%), and M9 (42.54%) ($P < 0.05$). Increasing the ratio of mixture flour (10%, 20%, and 30%) showed an increase in the moisture content of mixture breads. All formulations of mixture breads had the moisture contents higher than those of control breads. Among mixture formulations, the highest moisture content was found in the 30% mixture flour-containing formulations (M7, M8, and M9) in comparison to their counterparts of control formulations (GFC1, GFC2, and GFC3, respectively) followed by those containing medium ratios (20%) (M4, M5, and M6) and low ratios (10%) (M1, M2, and M3) of mixture flour. M9 (42.54%) had the highest and M1 (40.56%) had the lowest moisture content among mixture breads in comparison with the control.

In general, in all formulations, the moisture content is associated with the increasing of ratios of pseudocereal flours as well as hydrocolloids concentration, the effect was more evident with high ratios of pseudocereal flours (30%) at the high hydrocolloid concentration (4%) as shown in Figure 4.1.

Hydrocolloids decrease the loss of moisture content during bread storage, and thus reduce the dehydration rate of crumb. The increasing of the crumb moisture content was attributed to constant dough consistency, and also to the water binding capacity of the hydrocolloids (Guarda et al., 2004; Houben et al., 2012).

Hydrocolloids are applied in bakery products to control water absorption and, consequently, improve the shelf life of products by keeping the moisture content constant and, retarding the staling as well (Kohajdová and Karovičová, 2009).

These findings are in agreement with Guarda et al. (2004) who found that the addition of hydrocolloids increased the moisture content of the fresh bread, and that increase was more clear at high levels of hydrocolloids ($P < 0.05$). Mohammadi et al. (2014) obtained the same results and found that Xanthan-CMC significantly augmented the bread moisture (P < 0.05). Ozturk and Mert (2018) also, reported that the addition of xanthan gum to the formulation of bread prepared from corn starch had higher moisture content than that of the control breads.

On the other hand, the inclusion of the pseudocereal flours slightly increased the moisture content of the pseudocereal-containing GF breads. Alvarez-Jubete et al. (2009b) found that no significant differences were observed in the moisture content of the pseudocereal-containing breads in comparison to the GFC breads. Additionally, Steffolani et al. (2014) reported that the addition of chia (flour or seed) at a ratio of 15 g per 100 g of rice flour reduced weight loss during baking.

When comparisons were made between amaranth, quinoa, chia and mixture breads, significant differences were found. the highest moisture content was found for the 30% chia flour-containing breads. C9 (42.79%) had the highest moisture content among all the breads in comparison with the GFC breads. This is attributed to its high level of hydrocolloids and its high ratio of chia flour as well.

4.3.2. Specific Volume

The results for the specific volume of the baked breads are presented in Table 4.6. The specific volume index of all breads significantly improved by the addition of hydrocolloids as shown in Figure 4.2. - 4.7. An increase was observed in GFC1 (3.36 cm^3/g), GFC2 (3.81 cm^3/g), and GFC3 (4.02 cm^3/g) breads which contain 2%, 3%, and 4% of hydrocolloids, respectively (P < 0.05) as presented in Figure 4.3.

Table 4.6. Specific volume (cm^3/g) of gluten-free breads

Ratios of pseudocereal flour%	Control (100% starch)			10			20			30		
Levels of gums %	2	3	4	2	3	4	2	3	4	2	3	4
Gluten-free control	3.36^{k}	3.81^{d}	4.02^{a}	-	-	-	-	-	-	-	-	-
Amaranth	-	-	-	3.23^{l}	3.57^{h}	3.76^{e}	2.92^{o}	3.01^{n}	3.06^{n}	2.82^{p}	2.84^{p}	2.85^{p}
Quinoa	-	-	-	3.63^{g}	3.86^{cd}	3.92^{bc}	3.43^{j}	3.67^{fg}	3.88^{bc}	3.22^{l}	3.36^{k}	3.36^{k}
Chia	-	-	-	3.63^{g}	3.76^{e}	3.92^{b}	3.32^{k}	3.61^{gh}	3.69^{f}	3.11^{m}	3.14^{m}	3.32^{k}
Mixture	-	-	-	3.49^{i}	3.63^{g}	3.90^{bc}	3.04^{n}	3.34^{k}	3.42^{j}	2.71^{q}	2.82^{p}	3.02^{n}

Means followed by different letters are significantly different ($P < 0.05$).

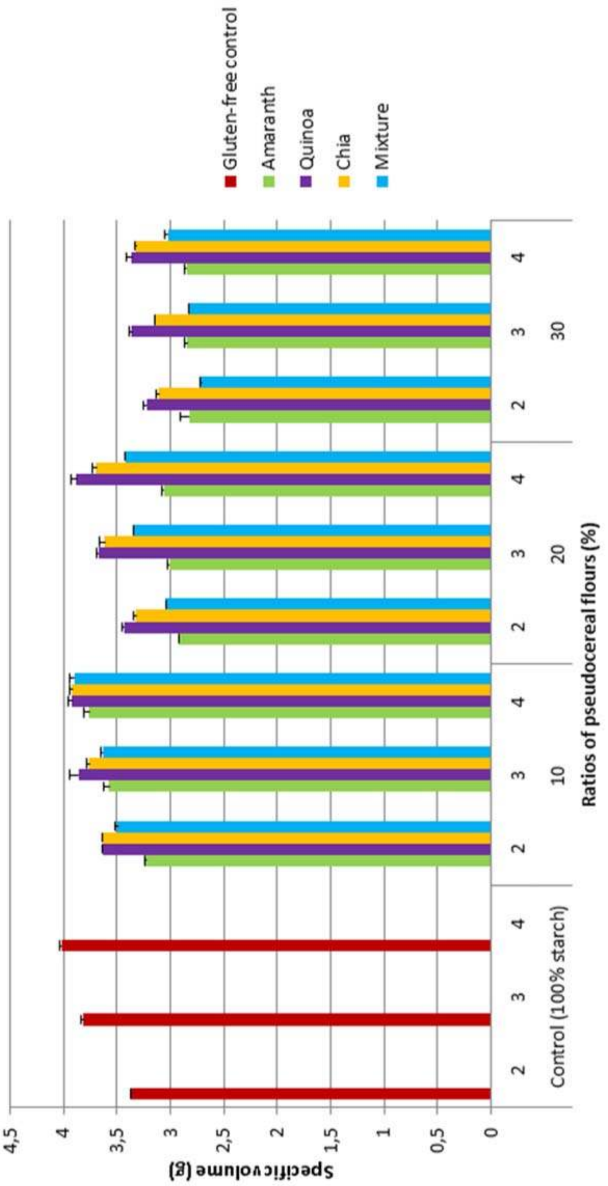

Figure 4.2. Specific volume (cm^3/g) of gluten-free breads

In the most cases, the specific volume of amaranth breads increased by increasing hydrocolloid concentration (2%, 3%, and 4%) as presented in Figure 4.2. and 4.4. Significant differences were found in the formulations of 10% amaranth flour-containing breads; A1 (3.23 cm^3/g), A2 (3.57 cm^3/g) and A3 (3.76 cm^3/g). In the formulations of 20% amaranth flour-containing breads, a significant difference was found between A4 (2.92 cm^3/g) and A5 (3.01cm^3/g) ($P < 0.05$), while no significant difference was observed between A5 (3.01 cm^3/g) and A6 (3.06 cm3/g) ($P > 0.05$). No significant differences were also found in the formulations of 30% amaranth flour-containing breads; A7 (2.82 cm^3/g), A8 (2.84 cm^3/g), and A9 (2.85 cm^3/g) ($P > 0.05$). The increase in the ratio of amaranth flour (10%, 20%, and 30%) led to a reduction in the specific volume of amaranth breads ($P < 0.05$) as shown in Figure 4.2. and 4.4. All formulations of amaranth breads had specific volumes lower than those of control breads. However, among amaranth breads, the highest specific volume was for the 10% amaranth flour-containing breads (A1, A2, and A3) in comparison with their counterparts of control breads (GFC1, GFC2, and GFC3, respectively) followed by those containing medium ratios (20%) (A4, A5, and A6) and high ratios (30%) (A7, A8, and A9). A3 (3.76 cm^3/g) had the highest and A7 (2.82 cm^3/g) had the lowest specific volume among amaranth breads in comparison with the control.

The specific volume of quinoa breads increased ($P < 0.05$) by increasing hydrocolloid concentration (2%, 3%, and 4%) except in a case as presented in Figure 4.2. and 4.5. Significant differences were found in the formulations of 10% quinoa flour-containing breads; Q1 (3.63 cm^3/g), Q2 (3.86 cm^3/g), and Q3 (3.92 cm^3/g) and 20% quinoa flour-containing breads; Q4 (3.43cm^3/g), Q5 (3.67 cm^3/g), and Q6 (3.88 cm^3/g). In the formulations of 30% quinoa flour-containing breads, a significant difference was found between Q7 (3.22 cm^3/g) and Q8 (3.36 cm^3/g), while no significant difference was obtained between Q8 (3.36 cm^3/g) and Q9 (3.36 cm^3/g) ($P >$

0.05). Increasing the ratio of quinoa flour (10%, 20%, and 30%) resulted in a reduction in the specific volume of quinoa breads ($P < 0.05$) as presented in Figure 4.2. and 4.5. Among quinoa breads, the highest specific volume was found for the 10% quinoa flour-containing breads (Q1, Q2, and Q3) in comparison with their counterparts of control breads (GFC1, GFC2, and GFC3, respectively) followed by those containing medium ratios (20%) (Q4, Q5, and Q6) and high ratios (30%) (Q7, Q8, and Q9) of quinoa flour. Q3 (3.92 cm^3/g) had the highest and Q7 (3.22 cm^3/g) had the lowest specific volume among quinoa breads in comparison with the control.

The specific volume of chia breads also augmented ($P < 0.05$) by increasing hydrocolloid concentration (2%, 3%, and 4%) except in a case as presented in Figure 4.2. and 4.6. Significant differences were found in the formulations of 10% chia flour-containing breads; C1 (3.63 cm^3/g), C2 (3.76 cm^3/g), and C3 (3.92 cm^3/g) and 20% chia flour-containing breads; C4 (3.32 cm^3/g), C5 (3.61 cm^3/g), and C6 (3.69 cm^3/g). In the formulations of 30% chia flour-containing breads, no significant difference was obtained between C7 (3.11 cm^3/g) and C8 (3.14 cm^3/g) ($P > 0.05$). while a significant difference was found between C8 (3.14 cm^3/g) and C9 (3.32 cm^3/g) ($P < 0.05$). The increase in the ratio of chia flour (10%, 20%, and 30%) reduced the specific volume of chia breads ($P < 0.05$) as shown in Figure 4.2. and 4.6. Among chia breads, the highest specific volume was measured for the 10% chia flour-containing breads (C1, C2, and C3) in comparison with their counterparts of control breads (GFC1, GFC2, and GFC3, respectively) followed by those containing medium ratios (20%) (C4, C5, and C6) and high ratios (30%) (C7, C8, and C9) of chia flour. C3 (3.92 cm^3/g) had the highest and C7 (3.11 cm^3/g) had the lowest specific volume among quinoa breads in comparison with the control.

The specific volume of mixture breads also increased ($P < 0.05$) by increasing hydrocolloid concentration (2%, 3%, and 4%) as shown in Figure 4.2. and 4.7.

Significant differences were found in the formulations of 10% mixture flour-containing breads; M1 (3.49 cm^3/g), M2 (3.63 cm^3/g), and M3 (3.90 cm^3/g), 20% mixture flour-containing breads; M4 (3.04 cm^3/g), M5 (3.34 cm^3/g), and M6 (4.42 cm^3/g), and 30% mixture flour-containing breads; M7 (2.71 cm^3/g), M8 (2.82 cm^3/g), and M9 (3.02 cm^3/g). Increasing the ratio of mixture flour (10%, 20%, and 30%) showed a reduction in the specific volume of mixture breads ($P < 0.05$) as shown in Figure 4.2. and 4.7. Among mixture breads, the highest specific volume was found in the case of 10% mixture flour-containing breads (M1, M2, and M3) in comparison to their counterparts of control breads (GFC1, GFC2, and GFC3, respectively) followed by those containing medium ratios (20%) (M4, M5, and M6) and high ratios (30%) (M7, M8 and M9) of mixture flour. This means, M3 (3.90 cm^3/g) had the highest and M7 (2.71 cm^3/g) had the lowest specific volume among mixture breads in comparison with the control.

Among the pseudocereal-containing breads, quinoa breads had better specific volume than that of chia, mixture, and amaranth breads. It is worthy to note that Q1 (3.63 cm^3/g), Q2 (3.86 cm^3/g), C1 (3.63 cm^3/g), and M1 (3.49 cm^3/g) breads had better specific volume than that of their counterparts of control breads (GFC1 3.36 cm^3/g and GFC2 3.81 cm^3/g). The specific volume of Q3 (2.92 cm^3/g), C3 (2.92 cm^3/g), and M3 (2.90 cm^3/g) breads were close to GFC3 (4.02 cm^3/g) bread. While all the amaranth breads had the lowest specific volume among the pseudocereal-containing breads ($P < 0.05$).

In general, the specific volume augmented by increasing the level of hydrocolloids with the exception of some GF breads, especially the GF breads which were prepared using high ratios of pseudocereal flours (30%). The addition of hydrocolloids significantly increased the specific volume of the GFC breads and the pseudocereal-containing GF breads with low and medium ratios (10 and 20%) with the exception of A5 and A6 (no significant difference between them), and that raise was significantly evident at high levels of hydrocolloids (4%). No significant further improvement was observed by increasing hydrocolloid concentration in the specific volume of some the GF breads with high ratios of pseudocereal flours (30%).

According to Rosell et al. (2001), hydrocolloids have the ability to improve the dough development and gas retention. An improvement in the specific volume was obtained when adding 0.1-0.5% HPMC and 0.1-0.5% xanthan gum to wheat bread formulations (Guarda et al., 2004). In a similar way, Sciarini et al. (2010) found a positive effect of hydrocolloids especially xanthan, followed by CMC on the specific volume of GF breads made of 40% rice flour, 40% corn flour and 20% soy flour ($P < 0.05$). Ozturk and Mert (2018) reported that the specific volume analysis showed that the addition of xanthan gum resulted in bigger breads than control breads.

The replacement of corn starch by each of the pseudocereal flours (amaranth, quinoa, chia and mixture flours) had a variable effect on the specific volume. Generally, the addition of the pseudocereal flours adversely affected the specific volume of the pseudocereal-containing GF breads in comparison to the GFC breads, with the exception of some formulations which were better than control.

The specific volumes of pseudocereal-containing GF breads which were prepared using low ratios of flours (10%) were the closest ones to those of GFC breads. While the lowest values of specific volumes were recorded in those prepared using high ratios of flours (30%) followed by medium ratios (20%).

Alvarez-Jubete et al. (2009b) reported that the replacement of potato starch by quinoa flour resulted in breads with higher volume ($P < 0.05$) in comparison with the control which made from 50 % rice flour and 50 % potato starch. While no difference in volume was found when potato starch replaced by amaranth flour. Steffolani et al. (2014) found that the inclusion of chia flour at a ratio of 15 g per 100 g of rice flour reduced the specific volume of breads. They reported that these differences could be attributed to the lower level of water in the formulation, because larger levels of water produce breads with larger specific volumes (McCarthy et al., 2005).

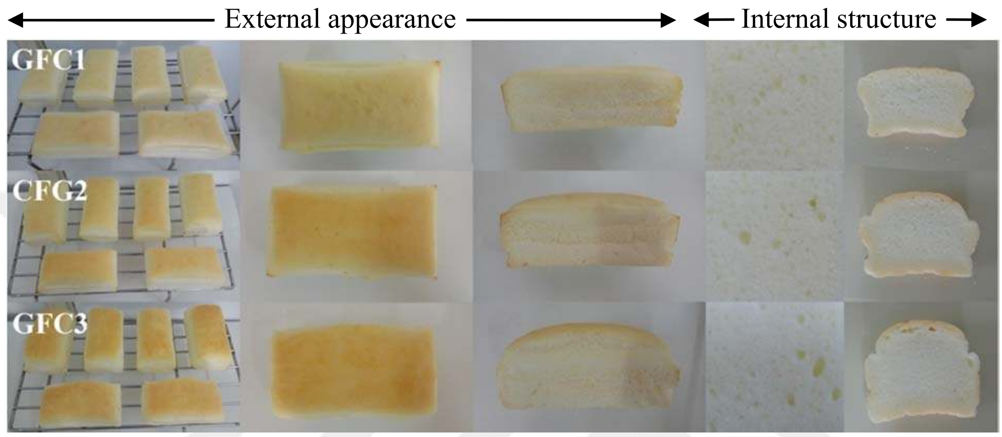

Figure 4.3. The effects of different levels of gums on (1) external appearance and (2) internal structure of gluten-free control breads.

Figure 4.4. The effects of the addition of amaranth flour with different levels of gums on (1) external appearance and (2) internal structure of GF breads.

Figure 4.5. The effects of the addition of quinoa flour with different levels of gums on (1) external appearance and (2) internal structure of GF breads.

Figure 4.6. The effects of the addition of Chia flour with different levels of gums on (1) external appearance and (2) internal structure of GF breads.

Figure 4.7. The effects of the addition of mixture flour with different levels of gums on (1) external appearance and (2) internal structure of GF breads.

4.3.3. Crust and Crumb Color of The Breads

Crust and crumb color of the GF breads were also evaluated. As shown in Table 4.7. increasing hydrocolloids concentrations (2%, 3%, and 4%) significantly resulted in a lighter color (higher *L* values) for the crumb in all bread formulations ($P < 0.05$), but did not significantly affect the values of *a* and *b* except in few cases. This effect was clearly observed in the control breads; GFC1 (an *L* value of 78.56), GFC2 (80.79), and GFC3 (83.45) as shown in Figure 4.3.

In addition, the increase in the hydrocolloid concentration significantly showed a lighter color (higher *L* values) for the crumb of amaranth breads ($P < 0.05$) as presented in Table 4.7. and Figure 4.4. The effect was evident in the formulations of 10% amaranth flour-containing breads; A1(70.30), A2 (71.86), and A3 (76.00), 20% amaranth flour-containing breads; A4 (65.17), A5 (69.07), and A6 (74.22), and 30% amaranth flour-containing breads; A7 (64.24), A8 (67.89), and A9 (71.16). Increasing the ratio of amaranth flour (%10, 20%, and 30%) significantly resulted in lower *L* values (darker bread crumb) and higher *a* and *b* values, compared to the control ($P < 0.05$) as presented in Table 4.7. and Figure 4.4. However, among amaranth breads, 10% amaranth flour-containing breads (A1, A2, and A3) had higher *L* values and lower *a* and *b* values of crumb color, in contrast to (30%) amaranth flour-containing breads (A7, A8, and A9) which had lower *L* values and higher *a* and *b* values. A3 (76) had the highest and A7 (64.24) had the lowest *L* values of the crumb of amaranth breads. The latter is due to its low hydrocolloid concentration (2%) and high ratio of the amaranth flour (30%), in contrast to A3 (4% and 10%, respectively).

Table 4.7. Crumb color of gluten-free breads (*L*, *a*, and *b* color scales)

Ratios of gums %	2			3			4		
Color scales	***L***	***a***	***b***	***L***	***a***	***b***	***L***	***a***	***b***
Starch 100%									
GFC	78.56^{bc}	-2.11^{o}	3.57^{p}	80.79^{ab}	-2.10^{o}	3.92^{p}	83.45^{a}	-2.06^{no}	4.29^{p}
Ratios of flour 10%									
Amaranth	70.30^{f-j}	-1.99^{mon}	7.21^{o}	71.86^{efg}	-1.93^{mon}	7.29^{no}	76.00^{cd}	-1.93^{mon}	7.49^{mno}
Quinoa	70.26^{f-j}	1.35^{gh}	8.98^{l}	70.84^{f-i}	1.37^{gh}	9.04^{l}	71.71^{efg}	1.19^{hi}	8.50^{lmn}
Chia	63.81^{lmn}	-0.19^{k}	8.17^{l-o}	68.46^{hij}	-0.14^{k}	8.63^{lm}	70.46^{f-i}	-0.14^{k}	8.49^{lmn}
Mixture	67.27^{jk}	-0.10^{k}	7.45^{mno}	71.47^{e-h}	-0.13^{k}	7.99^{l-o}	72.73^{ef}	-0.12^{k}	8.07^{l-o}
Ratios of flour 20%									
Amaranth	65.17^{kl}	-1.87^{mno}	11.60^{e-j}	69.07^{g-j}	-1.76^{m}	12.11^{d-g}	74.22^{de}	-1.78^{mn}	12.80^{cde}
Quinoa	61.37^{mno}	3.07^{c}	10.97^{g-k}	62.87^{l-o}	2.81^{d}	10.60^{ijk}	63.80^{lmn}	3.09^{c}	11.41^{f-k}
Chia	57.65^{qrs}	0.98^{ij}	11.27^{f-k}	60.79^{nop}	0.86^{j}	11.36^{f-k}	62.72^{l-o}	0.99^{ij}	11.6^{e-j}
Mixture	57.30^{q-t}	1.19^{hi}	10.22^{k}	59.98^{opq}	1.25$_{hi}$	10.45^{jk}	62.07^{l-o}	1.23^{hi}	10.65^{h-k}
Ratios of flour 30%									
Amaranth	64.24^{lm}	-1.14^{l}	14.59^{a}	67.89^{ijk}	-1.40^{l}	14.41^{ab}	71.16^{e-h}	-1.35^{l}	15.42^{a}
Quinoa	52.40^{v}	4.66^{b}	12.33^{c-f}	53.75^{uv}	5.12^{a}	12.98^{cd}	55.27^{r-v}	5.15^{a}	13.39^{bc}
Chia	54.33^{tuv}	1.66^{f}	12.69^{cde}	56.59^{r-u}	1.68^{f}	12.68^{cde}	58.3^{pqr}	1.58^{fg}	12.51^{c-f}
Mixture	54.32^{tuv}	2.37^{e}	11.87^{d-h}	55.03^{s-v}	2.17^{e}	11.72^{e-i}	58.18^{pqr}	2.15^{e}	12.09^{d-g}

Means followed by different letters within the same columns (*L*, *a* and *b*) are significantly different ($P < 0.05$).

Table 4.8. Crumb color of gluten-free breads (chroma *C* and hue-angle *h*)

Ratios of gums %	2		3		4	
Color scale	**C**	**h**	**C**	**h**	**C**	**h**
Starch 100%						
GFC	4.15^{o}	-1.03^{i}	4.45^{o}	-1.08^{j}	4.76^{o}	-1.12^{k}
Ratios of flour 10%						
Amaranth	7.48^{mn}	-1.30^{l}	7.55^{mn}	-1.31^{l}	7.74^{mn}	-1.32^{l}
Quinoa	9.08^{m}	1.42d^{e}	9.14^{m}	1.42^{de}	8.58^{mn}	1.43^{cd}
Chia	8.17^{mn}	-1.55^{o}	8.63^{mn}	-1.56^{o}	8.49^{mn}	-1.55^{o}
Mixture	7.45^{mn}	-1.56^{o}	7.99^{mn}	-1.55^{o}	8.07^{mn}	-1.56^{o}
Ratios of flour 20%						
Amaranth	11.75^{e-k}	-1.41^{m}	12.23^{d-h}	-1.43^{m}	12.93^{cde}	-1.43^{m}
Quinoa	11.39^{g-l}	1.30^{g}	10.96^{i-l}	1.31^{g}	11.82^{e-j}	1.31^{g}
Chia	11.32^{h-l}	1.48^{ab}	11.39^{g-l}	1.50^{a}	11.65^{f-k}	1.48^{ab}
Mixture	10.29^{l}	1.46^{bc}	10.52^{kl}	1.45^{cd}	10.72^{jkl}	1.46^{bc}
Ratios of flour 30%						
Amaranth	14.63^{ab}	-1.49^{n}	14.48^{ab}	-1.47^{n}	15.48^{a}	-1.48^{n}
Quinoa	13.18^{cd}	1.21^{h}	13.95^{bc}	1.19^{h}	14.34^{ab}	1.20^{h}
Chia	12.80^{c-f}	1.44^{cd}	12.79^{c-f}	1.44^{cd}	12.61^{d-g}	1.45^{cd}
Mixture	12.10^{d-i}	1.37^{f}	11.92^{e-j}	1.39^{f}	12.28^{d-h}	1.40^{ef}

Means followed by different letters within the same columns (C and h) are significantly different ($P < 0.05$).

Table 4.9. Crust color of gluten-free breads (*L*, *a*, and *b* color scales)

Ratios of gums %	2			3			4		
Color scale	*L*	*a*	*b*	*L*	*a*	*b*	*L*	*a*	*b*
Starch 100%									
GFC	81.06^{a}	-1.78^{s}	14.33^{k}	81.78^{a}	-1.33^{rs}	17.31^{j}	80.00^{a}	-0.67^{r}	20.22$^{e\text{-}h}$
Ratios of flour 10%									
Amaranth	74.97^{b}	0.64^{q}	18.60^{hij}	74.46^{b}	1.33^{pq}	20.5$^{d\text{-}h}$	75.09^{b}	2.05^{op}	22.26$^{b\text{-}e}$
Quinoa	71.71^{bcd}	2.86^{mno}	17.72^{ij}	72.18^{bc}	3.30^{lmn}	19.01^{hij}	69.91^{cde}	3.85$^{h\text{-}m}$	20.39$^{d\text{-}h}$
Chia	69.87^{cde}	1.86^{op}	19.00^{hij}	68.58^{cde}	2.42^{no}	20.71$^{d\text{-}h}$	68.62^{cde}	2.22^{nop}	20.51$^{d\text{-}h}$
Mixture	70.36^{cde}	2.27^{nop}	18.50^{hij}	71.58^{bcd}	2.55^{no}	19.64$^{f\text{-}j}$	70.24^{cde}	3.62^{klm}	22.70^{bcd}
Ratios of flour 20%									
Amaranth	66.85^{ef}	2.00^{op}	20.82$^{d\text{-}h}$	66.82^{ef}	3.18^{lmn}	22.66^{bcd}	68.10^{de}	3.17^{lmn}	22.72^{bcd}
Quinoa	64.50^{fg}	4.91$^{c\text{-}h}$	19.07^{hij}	64.17^{fg}	5.32$^{c\text{-}g}$	20.81$^{d\text{-}h}$	62.93^{g}	5.80$^{b\text{-}e}$	21.54$^{d\text{-}g}$
Chia	58.99^{hij}	3.73$^{j\text{-}m}$	19.59$^{f\text{-}j}$	61.08^{ghi}	3.79$^{i\text{-}m}$	20.08$^{e\text{-}i}$	60.90^{ghi}	3.89$^{h\text{-}m}$	20.61$^{d\text{-}h}$
Mixture	62.88^{g}	3.85$^{h\text{-}m}$	18.97^{hij}	61.41^{gh}	3.87$^{h\text{-}m}$	19.13$^{g\text{-}j}$	61.43^{gh}	4.13$^{h\text{-}l}$	19.99$^{e\text{-}i}$
Ratios of flour 30%									
Amaranth	63.64^{fg}	4.69$^{f\text{-}k}$	23.87^{abc}	63.23^{g}	5.29$^{c\text{-}g}$	24.14^{ab}	64.35^{fg}	5.94^{bc}	25.92^{a}
Quinoa	58.29^{hij}	6.68^{ab}	18.99^{hij}	56.65^{jk}	6.79^{ab}	19.78$^{f\text{-}i}$	56.28^{jkl}	7.20^{a}	20.66$^{d\text{-}h}$
Chia	52.93^{l}	4.85$^{d\text{-}i}$	19.39$^{f\text{-}j}$	54.63^{kl}	4.74$^{e\text{-}j}$	19.43$^{f\text{-}j}$	54.68^{kl}	4.44$^{g\text{-}k}$	19.09$^{g\text{-}j}$
Mixture	57.31^{jk}	5.86^{bcd}	20.35$^{d\text{-}h}$	57.65^{ijk}	5.53$^{c\text{-}f}$	20.43$^{d\text{-}h}$	56.03^{jkl}	6.00^{bc}	21.74$^{c\text{-}f}$

Means followed by different letters within the same columns (*L*, *a* and *b*) are significantly different ($P < 0.05$).

Table 4.10. Crust color of gluten-free breads (chroma *C* and hue-angle *h*)

Ratios of gums %	**2**		**3**		**4**	
Color scale	**C**	**h**	**C**	**h**	**C**	**h**
Starch % 100						
GFC	14.46^{n}	-1.44^{c}	17.38^{m}	-1.49^{c}	$20.24^{f\text{-}l}$	-0.49^{b}
Ratios of flour 10%						
Amaranth	18.62^{klm}	1.54^{a}	$20.55^{d\text{-}k}$	1.51^{a}	$22.36^{c\text{-}g}$	1.48^{a}
Quinoa	17.95^{lm}	1.41^{a}	$19.30^{j\text{-}m}$	1.40^{a}	$20.75^{d\text{-}k}$	1.38^{a}
Chia	$19.09^{j\text{-}m}$	1.47^{a}	$20.85^{d\text{-}k}$	1.45^{a}	$20.63^{d\text{-}k}$	1.46^{a}
Mixture	18.64^{klm}	1.45^{a}	$19.81^{h\text{-}l}$	1.44^{a}	22.98^{bcd}	1.41^{a}
Ratios of flour 20%						
Amaranth	$20.93^{d\text{-}k}$	1.48^{a}	$22.90^{b\text{-}e}$	1.43^{a}	22.94^{bcd}	1.43^{a}
Quinoa	$19.69^{i\text{-}m}$	1.32^{a}	$21.48^{d\text{-}j}$	1.32^{a}	$22.31^{c\text{-}h}$	1.31^{a}
Chia	$19.94^{g\text{-}l}$	1.38^{a}	$20.44^{e\text{-}k}$	1.38^{a}	$20.98^{d\text{-}k}$	1.38^{a}
Mixture	$19.36^{i\text{-}m}$	1.37^{a}	$19.52^{i\text{-}m}$	1.37^{a}	$20.41^{e\text{-}l}$	1.37^{a}
Ratios of flour 30%						
Amaranth	24.33^{bc}	1.38^{a}	24.72^{ab}	1.36^{a}	26.59^{a}	1.35^{a}
Quinoa	$20.16^{f\text{-}l}$	1.23^{a}	$20.91^{d\text{-}k}$	1.24^{a}	$21.88^{d\text{-}i}$	1.24^{a}
Chia	$19.99^{g\text{-}l}$	1.33^{a}	$20.00^{g\text{-}l}$	1.33^{a}	$19.61^{i\text{-}m}$	1.35^{a}
Mixture	$21.18^{d\text{-}j}$	1.29^{a}	$21.17^{d\text{-}j}$	1.31^{a}	$22.55^{b\text{-}f}$	1.30^{a}

Means followed by different letters within the same columns (C and h) are significantly different ($P < 0.05$).

In the same way, increasing hydrocolloid concentration significantly resulted in a lighter color (higher *L* values) for the crumb of quinoa breads ($P < 0.05$) as shown in Table 4.7. and Figure 4.5. and this effect can be observed in the formulations of 10% quinoa flour-containing breads; Q1 (70.26), Q2 (70.84), and Q3 (71.71), 20% quinoa flour-containing breads; Q4 (61.37), Q5 (62.87), and Q6 (63.80), and 30% quinoa flour-containing breads; Q7 (52.40), Q8 (53.75), and Q9 (55.27). Increasing the ratio of quinoa flour (%10, 20%, and 30%) significantly gave lower *L* values (darker bread crumb) and higher *a* and *b* values ($P < 0.05$) as shown in Table 4.7. and Figure 4.5. However, among quinoa breads, 10% quinoa flour-containing breads (Q1, Q2, and Q3) had higher *L* values and lower *a* and *b* values of the crumb color, in contrast to (30%) quinoa flour-containing breads (Q7, Q8, and Q9) which had lower *L* values and higher *a* and *b* values. Q3 (71.71) had the highest and Q7 (52.40) had the lowest *L* values of crumb color among quinoa breads in comparison to the control. The latter is due to its low hydrocolloid concentration (2%) and high ratio quinoa flour (30%), in contrast to Q3 (4% and 10%, respectively).

Also, increasing the hydrocolloid concentration significantly presented a lighter color (higher *L* values) for the crumb of chia breads ($P < 0.05$) as shown in Table 4.7. and Figure 4.6. and this effect was clear in the formulations of 10% chia flour-containing breads; C1 (63.81), C2 (68.46), and C3 (70.46), 20% chia flour-containing breads; C4 (57.65), C5 (60.79), and C6 (62.72), and 30% chia flour-containing breads; C7 (54.33), C8 (56.59), and C9 (58.30). The increase of the ratio of chia flour (%10, 20%, and 30%) significantly led to lower *L* values (darker bread crumb) and higher *a* and *b* values ($P < 0.05$) as shown in Table 4.7. and Figure 4.6. However, among chia breads, 10% chia flour-containing breads (C1, C2, and C3) had higher *L* values and lower *a* and *b* values of crumb color, in contrast with (30%) chia flour-containing breads (C7, C8 and C9) which had lower *L* values and higher *a* and *b* values. C3 (70.46) had the highest and C7 (54.33) had the lowest *L* values of crumb color among chia breads in comparison to the control. The latter is due to its low hydrocolloid concentration (2%) and the high ratio of chia flour (30%), in contrast to C3 (4% and 10%, respectively).

Also, increasing hydrocolloid concentration significantly resulted in a lighter color (higher *L* values) for the crumb of mixture breads ($P < 0.05$) as presented in Table 4.7. and Figure 4.7. and this effect is evident in the formulations of 10% mixture flour-containing breads; M1 (67.27), M2 (71.47), and M3 (72.73), 20% mixture flour-containing breads; M4 (57.3), M5 (59.98), and M6 (62.07), and 30% mixture flour-containing breads; M7 (54.32), M8 (55.03), and M9 (58.18). Increasing the ratio of mixture flour (%10, 20%, and 30%) significantly gave lower *L* values (darker bread crumb) and higher *a* and *b* values ($P < 0.05$) as presented in Table 4.7. and Figure 4.7. However, among mixture breads, 10% mixture flour-containing breads (M1, M2, and M3) had higher *L* values and lower *a* and *b* values of crumb color, in contrast to (30%) mixture flour-containing breads (M7, M8, and M9) which had lower *L* values and higher *a* and *b* values. M3 (72.73) had the highest and M7 (54.32) had the lowest *L* values of the crumb color among the mixture breads in comparison to the control. The latter is due to its low hydrocolloid concentration (2%) and the high ratio of mixture flour (30%), in contrast to M3 (4% and 10%, respectively).

To conclude, the addition of the different pseudocereal flours produced a darker bread crumb (lower *L* values), compared to control breads ($P < 0.05$). The GF breads which were formulated with high ratios of pseudocereal flours (30%) at low hydrocolloid concentration (2%) showed the darkest color for the crumb compared to the control. Q7 (52.40) had the darkest color for the crumb followed by M7 (54.32) and C7 (54.33) ($P < 0.05$).

In general, the use of hydrocolloids significantly showed a lighter color (higher *L* values) for the crumb in all bread formulations, and this effect was clearly observed at the highest hydrocolloid concentration (4%), but did not significantly affect *a* and *b* values except in few cases. The lightening effect is attributable to the effect of the addition of hydrocolloid on the water distribution which, thus, influences Maillard browning and caramelization reactions (Mezaize et al., 2009).

Mohammadi et al. (2014) found that the inclusion of CMC and xanthan gum resulted in a lighter color for the crumb and crust in all formulations, compared to the GFC breads which does not contain gums ($P < 0.05$). The *a* and *b* scales of the crumb were not affected by the addition of gums in comparison with the control. The similar results were also found by Lazaridou et al. (2007). The inclusion of xanthan gum gave a lighter color for the crumb and crust ($P < 0.05$), compared to the GFC breads which were prepared from rice flour and starch corn, whereas the presence of CMC at 1% or 2% caused no significant increase in the lightness (*L* value) of the crumb.

Increasing the level of gums did not affect the crust color and no clear differences were observed in the crust color as presented in Table 4.9. and Figure 4.3. - 4.7. This may be attributable to the fluctuations in oven temperatures (± 5 °C) during baking. Increasing the amount of the pseudocereal flours (10%, 20%, and 30%) resulted in a darker bread crust in all pseudocereal-containing GF breads ($P < 0.05$). Gómez et al. (2003) pointed out that the original color of ingredients can slightly affect the crust color of the breads. The main reasons for the crust color are Maillard and caramelization reactions. Crumb color of breads, however, is usually similar to the color of the ingredients. This is attributed to the fact that the crumb does not reach such high temperatures as the crust.

In relation to the influence of the pseudocereal flours on the crumb and crust color of the baked breads, the addition of these flours significantly gave a darker color for the crumb and crust (lower *L* values) and higher *a* and *b* values of the bread crumb, compared to the GFC breads. This effect was more clear in the pseudocereal-containing GF breads with high ratios of flours (30%) followed by those containing medium ratios of flours (20%).

Among the pseudocereal-containing breads, quinoa breads prepared with high ratios of quinoa flour (30%) had the darkest crumb color. In contrast, chia breads made with high ratios of chia flour (30%) had the darkest crust color. Amaranth breads prepared using the lowest ratio of amaranth flour (10%) had the lightest color for the crumb and crust.

These findings are in agreement with Alvarez-Jubete et al. (2009b) who found that the replacement of potato starch by each of the pseudocereal flours (quinoa and amaranth) significantly exhibited darker color for the crumb and crust ($P < 0.05$) in comparison with the control which is prepared from 50 % rice flour and 50 % potato starch. No significant differences between quinoa and amaranth breads were determined for the crumb color, whereas quinoa breads had darker crust color. Also, Steffolani et al. (2014) reported that GF bread color was affected by the addition of 15 g chia flour and this flour led to a decrease in the *L* values of the bread crumb and an increase in the values of *a* and *b*.

Table 4.8. and 4.10. present the results of the values of chroma *C* and hue-angle *h* of the crumb and crust of breads. In the most cases, the values of *C* and *h* were not generally affected by increasing whether the level of gums or the ratio of pseudocereal flours. In some cases, the values of *C* and *h* increased slightly by increasing hydrocolloid concentration.

4.3.4. Texture Profile Analysis (TPA) of Bread Crumb

The baked bread formulations were evaluated for the crumb hardness after 24 hours of storage at 25 °C and the results are shown in Table 4.11. and Figure 4.8. Increasing hydrocolloid concentration significantly increased the crumb hardness of GFC breads, and this increase was highly observed in the samples, GFC1 (1475 g), GFC2 (1534 g), and GFC3 (1662 g) which contain different concentrations of hydrocolloids (2%, 3%, and 4%, respectively) ($P < 0.05$).

In all ratios of amaranth flour, the crumb hardness of the amaranth breads significantly increased ($P < 0.05$) by increasing hydrocolloid concentration (2%, 3%, and 4%), and the differences were observed in the formulations of 10% amaranth flour-containing breads; A1 (956 g), A2 (1073 g), and A3 (1351 g), 20% amaranth flour-containing breads; A4 (1052 g), A5 (1273 g), and A6 (1682 g), and 30% amaranth flour-containing breads; A7 (1582 g), A8 (1834 g), and A9 (1913 g). Increasing the ratio of amaranth flour (10%, 20%, and 30%) led to an increase in the crumb hardness of amaranth breads ($P < 0.05$). The highest values of the crumb hardness was found for the 30% amaranth flour-containing breads (A7, A8, and A9) followed by those containing medium ratios (20%) (A4, A5, and A6) and low ratios (10%) (A1, A2, and A3) of amaranth flour. Among amaranth breads, A9 (1913 g) showed the highest crumb hardness and A1 (956 g) showed the lowest crumb hardness (softest crumb). Thirty percent amaranth flour-containing breads (A7, A8, and A9) had the highest values of the crumb hardness in comparison to their counterparts of control breads (GFC1, GFC2, and GFC3, respectively), while 10 % amaranth flour-containing breads (A1, A2, and A3) and 20% amaranth flour-containing breads (A4 and A5 with the exception of A6) exhibited lower hardness values, compared to the GFC breads (GFC1, GFC2, and GFC3, respectively). The value of crumb hardness of A6 (1682 g) was higher than that of the control (GFC3) ($P < 0.05$).

Table 4.11. Hardness (g) of gluten-free breads

Ratios of pseudocereal flour %	Control (100% starch)			10			20			30		
Levels of gums %	2	3	4	2	3	4	2	3	4	2	3	4
CGF	1475^{efg}	1534^{def}	1662^{bc}	-	-	-	-	-	-	-	-	-
Amaranth	-	-	-	956^{q}	1073^{op}	1351^{hij}	1052^{p}	1273^{jkl}	1682^{b}	1582^{cd}	1834^{a}	1913^{a}
Quinoa	-	-	-	847^{rs}	1051^{p}	1127^{nop}	734^{t}	860^{r}	1053^{p}	644^{u}	764st	1031^{pq}
Chia	-	-	-	1159^{mno}	1209^{lmn}	1231^{klm}	1204^{lmn}	1440^{fgh}	1549de	1472^{efg}	1521^{def}	1645^{bc}
Mixture	-	-	-	1074^{op}	1305^{ijk}	1382^{ghi}	1475^{efg}	1648^{bc}	1709^{b}	1542de	1687^{b}	1886^{a}

Means followed by different letters are significantly different ($P < 0.05$).

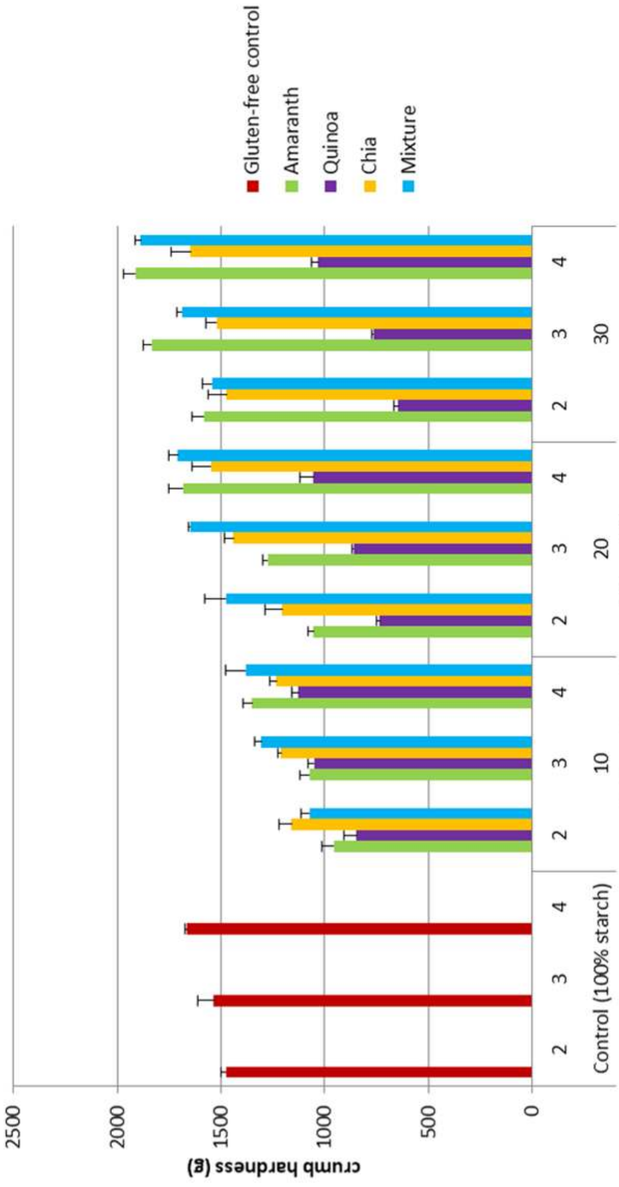

Figure 4.8. Hardness (g) of gluten-free breads

In relation to the influence of hydrocolloid concentration on the crumb hardness of quinoa breads, increasing hydrocolloid concentration (2%, 3%, and 4%) also significantly increased ($P < 0.05$) the hardness of quinoa breads in all ratios of quinoa flour, and the differences can be observed in the formulations of 10% quinoa flour-containing breads; Q1 (847 g), Q2 (1051 g), and Q3 (1127 g), 20% quinoa flour-containing breads; Q4 (734 g), Q5 (860 g), and Q6 (1053 g), and 30% quinoa flour-containing breads; Q7 (644 g), Q8 (764 g), and Q9 (1031 g). Unlike the effect of increasing ratio of amaranth flour in the formulations, increasing ratio of quinoa flour (10%, 20%, and 30%) led to a reduction in the crumb hardness values of quinoa breads ($P < 0.05$). The highest values of the crumb hardness was found for the 10% quinoa flour-containing breads (Q1, Q2, and Q3) followed by those containing medium ratios (20%) (Q4, Q5, and Q6) and high ratios (30%) (Q7, Q8, and Q9) of quinoa flour. Among quinoa formulations, Q3 (1127 g) showed the highest crumb hardness and Q7 (644 g) exhibited the lowest crumb hardness. All of quinoa breads had lower crumb hardness values than those of GFC breads ($P < 0.05$).

In all ratios of chia flour, the crumb hardness of chia breads slightly raised ($P < 0.05$) by increasing hydrocolloid concentration (2%, 3%, and 4%), and the differences were found in the formulations of 10% chia flour-containing breads; C1 (1159 g), C2 (1209 g), and C3 (1231 g), 20% chia flour-containing breads; C4 (1204 g), C5 (1440 g), and C6 (1549 g), and 30% chia flour-containing breads C7 (1472 g), C8 (1521 g), and C9 (1645 g). Increasing the ratio of chia flour (10%, 20%, and 30%) (like amaranth flour) showed an increase in the crumb hardness of chia breads ($P < 0.05$). The highest value of the crumb hardness was found for the 30% chia flour-containing breads (C7, C8, and C9) followed by those containing medium ratios (20%) (C4, C5, and C6) and low ratios (10%) (C1, C2, and C3) of chia flour. Among chia breads, C9 (1645 g) showed the highest crumb hardness and C1 (1159 g) showed the lowest crumb hardness (the softest crumb). All of chia breads had lower values of crumb hardness than those of GFC breads ($P < 0.05$).

In all ratios of mixture flour, the crumb hardness of mixture breads significantly increased ($P < 0.05$) by increasing hydrocolloid concentration (2%, 3%, and 4%). The differences can be observed in the formulations of 10% mixture flour-containing breads; M1 (1074 g), M2 (1305 g), and M3 (1382 g), 20% mixture flour-containing breads; M4 (1475 g), M5 (1648 g), and M6 (1709 g), and 30% mixture flour-containing breads; M7 (1542 g), M8 (1687 g), and M9 (1886 g). Increasing the ratio of mixture flour (10%, 20%, and 30%) (like amaranth and chia flours) led to an increase in the crumb hardness of mixture breads. The highest values of crumb hardness was found in the case of breads containing 30% mixture (M7, M8, and M9) followed by those containing medium ratios (20%) (M4, M5, and M6) and low ratios (10%) (M1, M2, and M3) of mixture flour. Among mixture breads, M9 (1886 g) showed the highest crumb hardness and M1 (1074 g) had the lowest crumb hardness (the softest crumb). Thirty percent mixture flour-containing breads (M7, M8, and M9) had the highest values of crumb hardness in comparison with the control breads (GFC1, GFC2, and GFC3, respectively) followed by 20% mixture flour-containing breads (M4, M5, and M6) (which had closer hardness values to those of GFC breads (GFC1, GFC2 and GFC3, respectively)), while 10 % mixture flour-containing breads (M1, M2 and M3) exhibited lower values of crumb hardness than those of GFC breads (GFC1, GFC2 and GFC3, respectively) ($P < 0.05$).

In general, the addition of hydrocolloids increased the crumb hardness in all bread formulations, and this was highly observed at the highest hydrocolloid concentration (4%). In previous studies, it was found that using several hydrocolloids, such as xanthan gum and CMC (Mohammadi et al., 2014), and xanthan gum, guar gum, LBG, HPMC and pectin (Demirkesen et al., 2010), causes crumb softening in the

GF bread, while some other studies showed that the addition of xanthan gum results in an increase in the hardness of GF bread (Lazaridou et al., 2007, Schober et al., 2005), and wheat bread (Guarda et al., 2004).

Despite there are some hypotheses about the hydrocolloids mechanism, it has not been completely understood yet. Hydrocolloids have a weakening effect on the starch structure that results in better water distribution and retention and thus, a decrease in the crumb hardness is observed (Guarda et al., 2004). The effects of hydrocolloids on the starch structure and mechanical properties are results from two opposite phenomena; (1) an increase in the rigidity as a consequence of the decrease in the swelling of the starch granules and amylose leaching, (2) a weakening effect on the complex starch network structure due to the prohibition of interparticle contacts among swollen granules. It is perhaps a combination of both factors that determine the overall influence on the mechanical properties of bread structure, however, each effect is dependent on the specific hydrocolloid used for fortification (Guarda et al., 2004; Lazaridou et al., 2007; Mohammadi et al., 2014).

Lazaridou et al. (2007) reported that the crumb firmness was not significantly affected by the addition of CMC when added at (1–2% concentration) compared to the GF control formulations prepared from rice flour and corn starch. Xanthan gum at both supplementation levels (1–2%) had an unfavorable influence on crumb firmness ($P < 0.05$). They also pointed out that the crumb firmness values ($P < 0.05$) increased by increasing storage time (hardening during storage); this is expected as a consequence of moisture loss as well as due to starch retrogradation phenomena. Similarly, Schober et al. (2005) found an increase in the crumb firmness observed by the addition of xanthan gum in GF breads prepared from sorghum ($P < 0.001$). Also, Guarda et al. (2004) reported that the hardness of the wheat bread increased by adding xanthan gum after 24 h of storage.

Contrary to these results, Mohammadi et al. (2014) found that xanthan gum and CMC significantly decreased the crumb hardness of both fresh and stored breads ($P < 0.05$) in comparison with control made from corn starch and rice flour. The reason for the softness is that water retention increases moisture content and thus, causes retrogradation of starch and bread firming is retarded (Mohammadi et al., 2014). Also Demirkesen et al. (2010) investigated the optimization of GF formulations based on rice flour prepared using different hydrocolloids and emulsifiers, they reported that the hardness of GF bread decreased with the addition of hydrocolloids (xanthan gum, guar gum, HPMC, pectin, LBG, xanthan–guar, and xanthan–LBG) and further decreased with inclusion of emulsifiers (Purawave and DATEM). Ozturk and Mert (2018) also, found that the addition of xanthan gum caused lower hardness for bread based on corn starch. This can be attributed to the water binding ability of hydrocolloids, leading the prevention of water transfer from the bread crumb to the crust and delayed starch retrogradation.

A possible explanation for these contradictory results in these studies is that GF breads were prepared by different methods. In this study, the level of water used in the preparation of GF breads was low and kept constant in all formulations, while Mohammadi et al. (2014) considered the level of water needed to maintain consistency. In addition to that, we used corn starch as a flour base to produce GFC breads and other pseudocereal-containing GF breads. We also used a combination of hydrocolloids (MC, CMC, Xanthan gum, and Guar gum; which were used in variable concentration) and emulsifier (SSL; which was used at fixed concentration).

On the other hand, the inclusion of the pseudocereal flours with low and medium ratios (10 and 20%) of flours significantly decreased the crumb hardness of the pseudocereal-containing GF breads in comparison with GFC breads, with the exception of some formulations; A6, M5, and M6 had higher values of crumb hardness than that of their counterparts of control; GFC2 and GFC3 breads, while M4 had the same value with GFC1. The inclusion of the pseudocereal flours with high ratios (30%) of flours significantly increased the crumb hardness of the pseudocereal-containing GF breads in comparison with GFC breads, with the exception of the 30% quinoa flour-containing breads (Q7, Q8, and Q9).

Alvarez-Jubete et al. (2009b) reported that the replacement of potato starch by pseudocereal flour resulted in a softer crumb in comparison with the GF control prepared from 50 % rice flour and 50 % potato starch. Amaranth breads had the softest crumb and they are followed by the buckwheat and quinoa breads. Moreover, the crumb hardness of GF breads increased with storage time.

All the pseudocereal-containing GF breads were characterized by a significantly softer crumb. This is attributed to the presence of natural emulsifiers found in the pseudocereal flours (Alvarez-Jubete et al., 2009b).

Steffolani et al. (2014) found that the inclusion of chia flour at a ratio of 15 g per 100 g of rice flour resulted in a considerable increase in the crumb hardness of breads. This effect cannot be opposed to our findings for the chia breads and can be explained by the differences in the levels of water used. In our study, the amount of water used was at a fixed level (120%) in each bread formulation, while the amount of water used was 100% in the study of Steffolani et al. (2014). This agrees with McCarthy et al. (2005) who found that; the increasing levels of water used significantly increased the specific volume ($P < 0.01$) and decreased the crumb hardness ($P < 0.01$) of breads.

This study reports a negative relation between the specific volume and the crumb hardness of the pseudocereal-containing breads. In this study, the increase in the ratio of pseudocereal flours significantly decreased the specific volume and, simultaneously, increased the crumb hardness of the pseudocereal-containing GF breads with the exception of the quinoa breads. Steffolani et al. (2014) found that the inclusion of 15 g chia flour resulted in a reduction in the specific volume and a greater increase in the crumb hardness of breads. This means that decreasing the specific volume yields higher crumb hardness values.

4.3.5. Sensory Evaluation

Table 4.12. and Figure 4.9. present the organoleptic assessment of baked breads. The formulations prepared at the highest hydrocolloid concentration (4%) were selected for sensory evaluation test due to their high values in bread evaluation. The table shows that there are significant differences among the breads in terms of appearance, color, texture, taste and overall acceptability; M3 gained the highest score (3.97) of appearance followed by Q3 (3.80), while Q9 gained the lowest score (2.33) (even lower than 2.5 (neither like nor dislike)) in comparison to GFC (3) ($P < 0.05$). Q3 and M6 gained the same score of bread color (4) which was the highest score for color evaluation. They are followed by A9 (3.93), A3 (3.67), M3 (3.63), and A6 (3.60), and GFC gained the lowest score (2.53) (slightly exceed a score of 2.5) ($P < 0.05$). M3 showed the highest score (4.30) of bread texture followed by Q3 (4.06), while M9 showed the lowest score (2.63) in comparison to GFC (3.67) ($P < 0.05$). Also, M3 showed the highest score (3.62) of bread taste followed by Q3 (3.46), while GFC showed the lowest score (2.62) ($P < 0.05$). With regard to overall acceptability of breads, there are also significant differences; M3 gained the highest score (3.84) followed by Q3 (3.80), while Q9 gained the lowest score (2.83) in comparison to GFC (3.03) ($P < 0.05$).

Table 4.12. Sensory evaluation of gluten-free breads

Formulations	Appearance	Color	Texture	Taste	Overall Acceptability
GFC	3.00^{bcd}	2.53^{d}	3.67^{abc}	2.62^{c}	3.03^{bc}
A3 10%	3.67^{ab}	3.67^{ab}	3.67^{abc}	3.06^{abc}	3.40^{abc}
A6 20%	3.27^{ab}	3.60^{ab}	3.00^{cd}	3.00^{abc}	2.86^{c}
A9 30%	3.73^{ab}	3.93^{ab}	3.26^{bcd}	2.86^{abc}	3.06^{abc}
Q3 10%	3.80^{ab}	4.00^{a}	4.06^{ab}	3.46^{ab}	3.80^{ab}
Q6 20%	3.60^{ab}	3.40^{abc}	3.33^{bcd}	3.40^{abc}	3.50^{abc}
Q9 30%	2.33^{d}	2.60^{d}	2.67^{d}	3.3^{abc}	2.83^{c}
C3 10%	3.61^{ab}	3.18^{bcd}	3.54^{abc}	2.92^{abc}	3.46^{abc}
C6 20%	3.47^{ab}	3.57^{abc}	3.65^{abc}	2.86^{abc}	3.25^{abc}
C9 30%	2.93^{bcd}	2.82^{cd}	3.22^{cd}	2.67^{bc}	2.88^{c}
M3 10%	3.97^{a}	3.63^{ab}	4.30^{a}	3.58^{a}	3.84^{a}
M6 20%	3.17^{abc}	4.00^{a}	3.32^{bcd}	3.31^{abc}	3.45^{abc}
M9 30%	2.43^{cd}	3.27^{abcd}	2.63^{d}	3.26^{abc}	2.86^{c}

Means followed by different letters within a column are significantly different ($P < 0.05$).

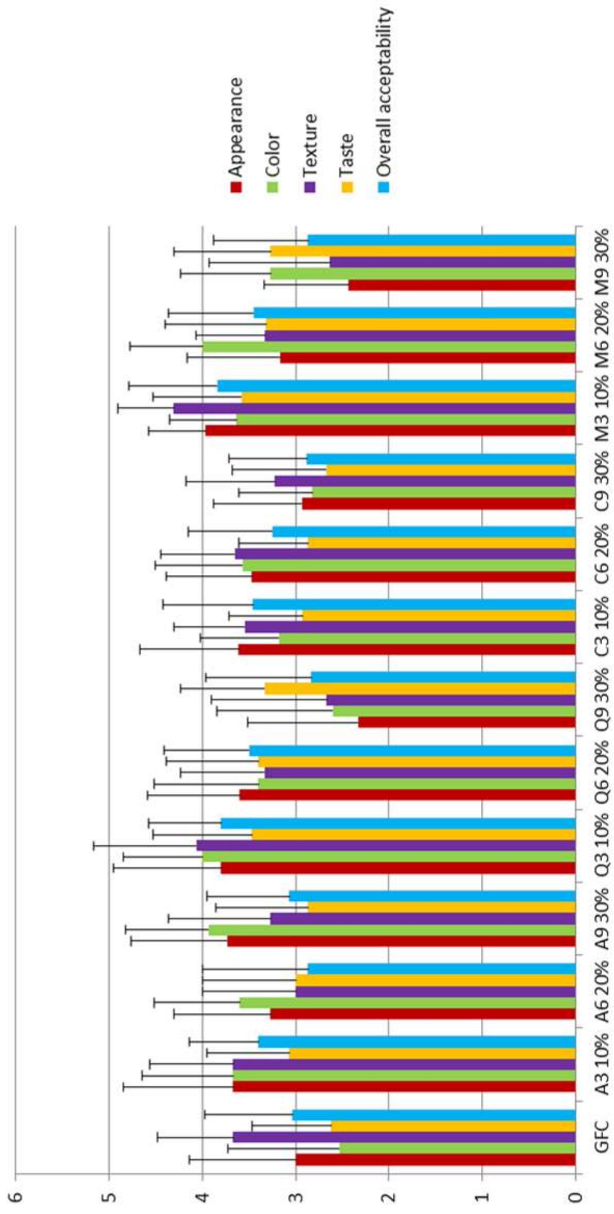

Figure 4.9. Sensory evaluation of gluten-free breads

It must be taken into account that the participants in the sensory evaluation test were not celiac patients and were accustomed to eating wheat bread. Changes both in the flavor and in the texture of the GF breads adversely influenced overall evaluation (Steffolani et al., 2014). In general, as expected, GF breads containing low ratios of pseudocereal flours (10%) gained the highest scores (M3, Q3, A3, and C3) among other GF breads.

Pseudocereal flours may be introduced into a GF bread formulation without negatively affecting the sensory properties of the loaves. Moreover, pseudocereal flours represent feasible ingredients in the production of good-quality, healthy GF breads (Alvarez-Jubete et al., 2009b). Bodroža-Solarov et al. (2008) reported that the sensory characteristics of the supplemented breads are acceptable with 10-15% amaranth grain, while supplementation of 20% resulted in loaves with lower specific volumes compared to the control. Sanz-Penella et al. (2013) reported that amaranth flour could be successfully used as a nutritious ingredient for wheat flour in bread formulations. They indicated the level of amaranth flour addition in bakery products should not exceed 20 g/100 g in order to maintain product quality and preserve the principal nutritional benefit of this ingredient. In addition, Steffolani et al. (2014) pointed out the addition of 15 % chia did not change the overall evaluation of GF breads.

In a similar way, our findings show that the GF bread formulation having pseudocereals in a ratio of 10% is superior to other formulation having 20% and 30% pseudocereals. This formulation can be used to produce GF breads with a pleasant flavor, favorable external appearance, and well homogeneously distributed cells.

5. CONCLUSIONS

Wheat is one of the most common grain in diets and bakery products since it contains gluten which is responsible for the rheological properties and for the formulation of gluten network. These properties are required to produce high-quality products. Nevertheless, it has been found that gluten consumption causes some health problems for individuals who have allergic reactions or intolerances to gluten like Celiac Disease (CD) (Mariotti et al., 2009). The only accepted and effective treatment is adherence to Gluten-Free Diets (GFD) throughout their life. Since the consumption of the gluten must be avoided, pseudocereal flours (GF flours) have been used to produce GF products as substitutes for gluten-containing cereals. These flours are called as GF flours and their high-nutritional value makes them suitable for the GFDs of celiac patients.

Increasing hydrocolloid concentration and the ratio of pseudocereal flours, generally, produced darker dough. The dark color was attributed to their natural color which is normally more darker than corn starch.

Moisture content of bread is associated with the increasing of the levels of gums as well as in the increase of the ratios of pseudocereal flours. The increase in the crumb moisture content was attributed to constant dough consistency, and to the water binding capacity of the hydrocolloids. The effect was more evident in the formulations prepared with high ratios of pseudocereal flours (30%) at the highest hydrocolloid concentration (4%) ($P < 0.05$).

The specific volume index of all breads significantly improved by the increasing hydrocolloid concentration except in some GF breads with high ratios of flours (30%). In general, the addition of hydrocolloids significantly increased the specific volume of the GFC breads and the pseudocereal-containing GF breads with low and medium ratios (10% and 20%) ($P < 0.05$), and that increase was significantly evident at high levels of hydrocolloids (4%). No great improvements were observed by increasing the level of hydrocolloid in the specific volume of some the GF breads with high ratios of pseudocereal flours (30%). The addition of the pseudocereal flours adversely affected in the specific volume of the pseudocereal-containing GF breads in comparison with the GFC breads, with the exception of some formulations which were better than control. Among the pseudocereal-containing breads, quinoa breads had the best specific volume, followed by chia, mixture and amaranth breads.

The use of hydrocolloids significantly showed a lighter crumb color in all bread formulations, and this effect was clearly observed at the highest hydrocolloid concentration (4%). The lightening impact was attributable to the effect of the addition of hydrocolloid on water distribution which impacts Maillard browning and caramelization reactions. The incorporation of pseudocereal flours into a GF formulation produced a darker GF bread crumb than that of control bread ($P < 0.05$). The GF breads formulated with high ratios of pseudocereal flours (30%) at low hydrocolloid concentration (2%) showed the darkest color for the crumb, compared to the control breads.

No clear differences were observed in the crust color by increasing the level of gums. This was attributable to the fluctuations of oven temperatures (± 5 °C) during baking. While increasing the addition of the pseudocereal flours produced a darker color for the bread crust in all pseudocereal-containing GF breads ($P < 0.05$). This effect was mainly associated to Maillard and caramelization reactions. Crumb color which does not reach such high temperatures as the crust and was usually affected by the color of the ingredients used.

In relation to the influence of hydrocolloid concentration on the crumb hardness of breads, the addition of hydrocolloids increased the crumb hardness of all bread formulations, and this was highly observed at the highest hydrocolloid concentration (4%). The inclusion of the pseudocereal flours with low and medium ratios (10 and 20%) of flours significantly decreased the crumb hardness of the pseudocereal-containing GF breads in comparison to GFC breads, with the exception of some formulations had higher values of crumb hardness than that of their counterparts of the control breads. The inclusion of the pseudocereal flours with high ratios (30%) of flours significantly increased the crumb hardness of the pseudocereal-containing GF breads in comparison to GFC breads, with the exception of the 30% quinoa flour-containing breads. All the pseudocereal-containing GF breads were characterized by a significantly softer crumb. This was attributed to the presence of natural emulsifiers found in the pseudocereal flours.

A negative relation between the specific volume and the crumb hardness of the pseudocereal-containing breads was found in this study. Increasing ratio of pseudocereal flours significantly decreased the specific volume and, simultaneously, increased the crumb hardness of the pseudocereal-containing GF breads with the exception of the quinoa breads.

In general, among pseudocereal-containing GF breads, the highest results in terms of moisture, specific volume, color, texture, and overall acceptability of breads were found for the pseudocereal-containing GF breads with low ratios of flours (10%) at the highest hydrocolloid concentration (4 %).

Pseudocereal flours can be successfully incorporated into a GF formulation with corn starch and gums for the production of GF breads without negatively affecting the sensory properties of the loaves. Moreover, the pseudocereal flours represent feasible ingredients in the production of good-quality, healthy GF breads.

Nevertheless, the use of GF flours and starches to produce GF breads with the same quality of their counterparts of wheat breads is still a big challenge to cereal technologists and bakers.

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CURRICULUM VITAE

Ahmed I. A. ALSAIQALI was born in Khan Younis - (Palestine) on 17/11/1990. He completed his primary, secondary and high school education in Rafah (Palestine). He graduated from Al-Azhar University - Gaza (Palestine), Faculty of Agriculture & Environment, Department of Food Technology in 2012. In the same year, he worked as an agricultural inspector at The Ministry of Agriculture. In the year of 2013, he worked as an inspector at The Ministry of Labor. In the year of 2014, he was accepted in the Turkish scholarship programme to peruse his studies at Çukurova University, Faculty of Agriculture, Department of Food Engineering to have his master degree in Food Engineering in the year 2018.