EFFECTS OF SOME HYDROCOLLOIDS ON SOME PHYSICOCHEMICAL AND SENSORY PROPERTIES OF GLUTEN FREE BREAD MADE FROM CORN STARCH

A THESIS SUBMITTED TO THE GRADUATE SCHOOL OF APPLIED SCIENCES OF NEAR EAST UNIVERSITY

By
KABOLOBARI BARIELNU BORNU

In Partial Fulfillment of the Requirements for the Degree of Master of Science in Food Engineering

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Approval of Director of Graduate School of
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I hereby declare that, all the information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all materials and results that are not original to this work.

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ACKNOWLEDGEMENTS

This thesis wouldn't have been possible without the patience of my principal supervisor, Prof. Dr. Babak GHANBARZADEH and co-supervisor Assist, Prof. Dr. Perihan ADUN. I am very thankful and indebted to the Nutrition and Dietetics Department for allowing me to use their kitchen during the preliminary phase of this work. To the crew of lecturers at the NEU Engineering department, I say great thanks. My gratitude to some of my course mates, who collaborated with me, especially during periods of group assignments and Examination. Their directives were never in any way minimal to my success at NEU.

My unlimited thanks and heartfelt love is dedicated to my parents Mr. and Mrs. Godswill Menedubabari Bornu, my brothers Barikuula Bornu, Nornubari Bornu, my sister Joy Baridilo Bornu and my friends.

I also wish to thank my special friends, Miss, Hadiza Bako, Mr. Hafizu Kademi, Najat and Asheal Musara for their love and friendship in Cyprus. Their ideas towards my success are unlimited.

To my parents and siblings…

ABSTRACT

Bread being a unique cereal based product is viewed as a staple food prepared from the flours of cereals by the composition of yeast, liquids (milk or water), oil, salt, sugar, eggs, and other ingredients. Intolerance of some individuals to gluten (gliadin and glutenin) found in wheat bread has given to rise to the production of gluten free bread. Celiac disease is said to be initiated by the consumption of gluten related products of barley, wheat, oats and rye which results in destroying the linings of the small intestinal wall and culminates in malabsorption of nutrients.

The formulation used for the gluten free bread were mixtures of corn starch and rice flour along with other bread additives. Hydrocolloids such as the K-carrageenan, Xanthan gum, Guar gum, and Carboxy methylcellulose were used as gluten alternatives. The result revealed bread crumb firmness changed in a range of 10900-17610. While the control bread firmness was 5445, it showed a softer texture and crumbliness. The protein in the bread showed a range of 4.80%-6.22% and ash 1.48%-1.69%. The sensory evaluation report revealed the xanthan gum and K-carrageenan gained higher acceptance based on the 5-point hedonic scale.

Keywords: Bread; Gluten; Gluten free bread; Celiac disease; Hydrocolloids

ÖZET

Benzersiz bir hububat ürünü olan ekmek maya, su yada süt, yağ, tuz, şeker, yumurta ve diğer gıda katkıları ile hazırlanan temel bir gıdadır. Buğday ekmeğinin içerdiği glutenin bazı bireylerde yol açtığı intolerans glutensiz ekmek üretiminde artışlara yol açmıştır. Çölyak hastalığı, gluten içeren arpa, buğday, yulaf ve çavdar ürünlerinin tüketilmesiyle ince bağırsağın çeperlerinin zarar görmesi ve besin elementlerinin absorpsiyonundaki bozuklukların artması şeklinde ortaya çıkmaktadır.

Bu çalışmada glutensiz ekmek yapımında mısır nişastası, pirinç unu, şeker, tuz, mahlep, jelatin, kabartma tozu, zeytin yağı, tereyağ, süt ve glutene alternatif olarak da k-karragenan, ksantan gum, guar gum ve karboksimetilselüloz kullanılmıştır. Hazırlanan glutensiz ekmek örnekleri sertlik, protein, kurumadde, kül, yaş gluten ve duyusal özellikler bakımından incelenmiştir. Gum ilave edilmiş glutensiz ekmeklerin sertlik değerleri 10900-17610 arasında değişirken, gum ilave edilmeyen kontrol ekmek sertliği 5445 olarak bulunmuştur. Kontrol ekmek daha yumuşak olsa da kolayca ufalanan, dağılan bir yapı göstermektedir. Ekmeklerin protein içerikleri % 4.80-6.22 arasında değişirken; kül oranları % 1.48-1.69 olarak bulunmuştur. Glutensiz ekmeklerin duyusal değerlendirilmeleri sonucunda ksantan gum ve k-karregenan gum ilave edilmiş ekmeklerin diğerlerine göre daha çok beğenildiği anlaşılmıştır.

Anahtar kelimeler: Ekmek; Glüten; Glutensiz ekmek; Çölyak hastalığı; Hidrokolloid

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LIST OF ABBREVIATIONS

USFDA:	United States for Food and Drug Administration
HLA:	Human Leukocyte Antigen
HPMC:	Hydroxypropyl Methylcellulose
CMC:	Carboxy Methylcellulose
TGase:	Transglutaminase
GERD:	Gastroesophageal Reflux Disease
IBS:	Irritable Bowel Syndrome
AACC:	American Association of Cereal Chemist
WHO:	World Health Organization

LIST OF SYMBOLS USED

T-Cells	Thymus cells
CD^{4+}	Cluster of differentiation 4
IgA	Immunoglobulin A
g/d	gram per day
g	gram
kg	kilogram
cm	Centimeter

CHAPTER 1

INTRODUCTION

Conventionally, bread has been identified as a unique cereal-based product which is popular and a staple food prepared from the flour of cereals by the composition of yeast, liquids (milk or water), oil, salt, sweeteners, eggs and other ingredients for the making of dough and finally baking to get the final product (Iwe et al., 2017). Cereal based foods have played a major role in the components of human diet by acting as a good source of energy providing approximately 10-20 times of energy than vegetables and fruits (Rosell, 2007)

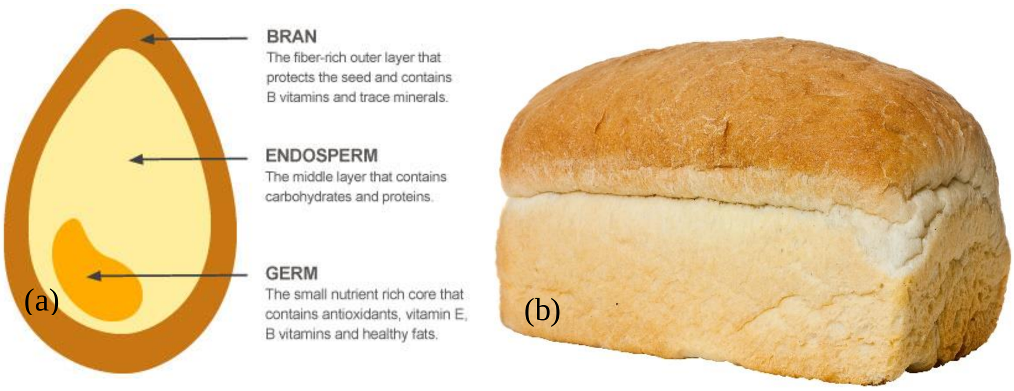


Figure 1.1: Structure of wheat cereal seed and wheat bread https://www.tes.com/lessons/QLFafINV95VCUQ/grains

Cutting across the Neolithic period, the conventional way of bread production has been the use of wheat flour and up to the present day, bread still forms the base of the food pyramid due to its global acceptance as a staple food to all class of people including the rich, poor, urban and rural class (Dooshima et al., 2014). Also, bread has been generally accepted due to its ability to be produced from a wide range of other flours which include gluten free cereals such as rice flour, with different species like *Oryza sativa* (unique with Asia), and *Oryza glaberrima* (seen cultivated mostly across Africa); (Marco et al., 2008), maize of different species including millet species and sorghum. (Schober and Bean, 2008). Pearl millet and foxtail millet has also been recognized as a type of cereals used for the production of gluten free bread (Taylor and Emmanbux, 2008). However, the intolerance of some individuals to gliadin unit found in the wheat grain, the prolamins associated with the rye, barley and oat meal has been a great concern to celiac disease patients (Murray, 1999).

Also, as substitute to wheat flour is the pseudo cereal crops such as Quinoa flour, Amaranth and Buckwheat flour which has been used for the making of gluten free bread and was found to improve the varieties of products and nutritional quality in trend of gluten free bread (Kupper, 2005). The functionality of these flours crafted from cereal grains for making of gluten free bread and the addition of pseudo cereals depends largely on their particle size and distribution, percentage volume after milling and the flour treatment. Additionally, the growing situations and the plant species have an impact on the composition of the substance and even the final product. (Schoenlechner et al., 2008).

Presently, food technologies and manufacturers are faced with the challenge of producing varieties of high nutritional value gluten free bread and products due to the absence of gluten which is needed for the unique viscoelastic nature of the dough and also induce upon the final product its chewy feeling (Demirkesen et al., 2014). This is because the absence of gluten in dough making has displayed high negativity on the dough rheology which include its texture, crumbliness, appearance etc. Also the production process has been seen to be affected leading to poor quality of the final product due to the dough being less cohesive during mixing, and loss of its viscoelastic property compared to the wheat dough. The gluten free dough are very smooth and not easy to handle; highly sticky, pasty and feels like handling the batter of a cake (Cauvain, 1998).

1.1 STRUCTURE OF PROTEIN IN CEREAL GRAINS

According to the United States for Food and Drug Administration (USFDA), gluten can be described as a unique proteinaceous material that is naturally associated with the cereal grains and have the potential to induce negative health impact on people suffering from celiac disease when they feed on gluten related products (FDA, 2013). In a research conducted by Gallagher et al. (2004), they described gluten to be a protein component of the wheat flour that is left behind after the starchy and other negligible ingredients of the flour including non-starchy polysaccharides, water soluble constituents are taken out with the help of a flowing water.

The gluten is present in the mature wheat grain endosperm and its primary function during bread production is visible in the formation of a three dimensional protein framework, structure formation of the dough texture, and also gas retention functionality (Torbica et al., 2008). It can be divided into the glutenin and the gliadin protein fractions, both of which are hydrophobic in nature and displays certain behavior in providing elasticity and strength to the dough (glutenin) and also the viscous nature of the dough (gliadin). This unique behavior of the gluten is attributable to its role in providing cohesiveness and viscoelastic properties when the glutenins and gliadins are mixed together (Anon, 1982). In a comparative study carried out to compare the chemical differences between the gliadin and glutenin, it revealed that the gliadin present in the gluten possesses a higher proline, glutamine (+glutamic acid), Isoleucine and phenylalanine than the glutenin which is said to contain glycine, lysine and tryptophan than its counterpart (Delcour et al., 2012).

It has been suggested that the proteinaceous nature of gluten is held together by the covalent bonds and a non-covalent bond which co-exist between the gliadin and glutenin network inherent in the cereal plants of rye, wheat, and barley when they interact together (Fernanda and Caroline, 2017). The development of gluten takes place as the flour and water interact together, in addition with exertion of steady power or force to provide an adhesive dough with visco-elastic behavior for the making of different cereal products like bread, biscuits and pasta products. (Xu et al., 2007). Therefore, gluten can be considered as the primary shape-forming component in bread, and in addition offers structure and texture to other bakery products. Its absence impairs the doughs potential to correctly shape itself in the course of kneading, leavening and baking to obtain the final product (Mariotti et al., 2009).

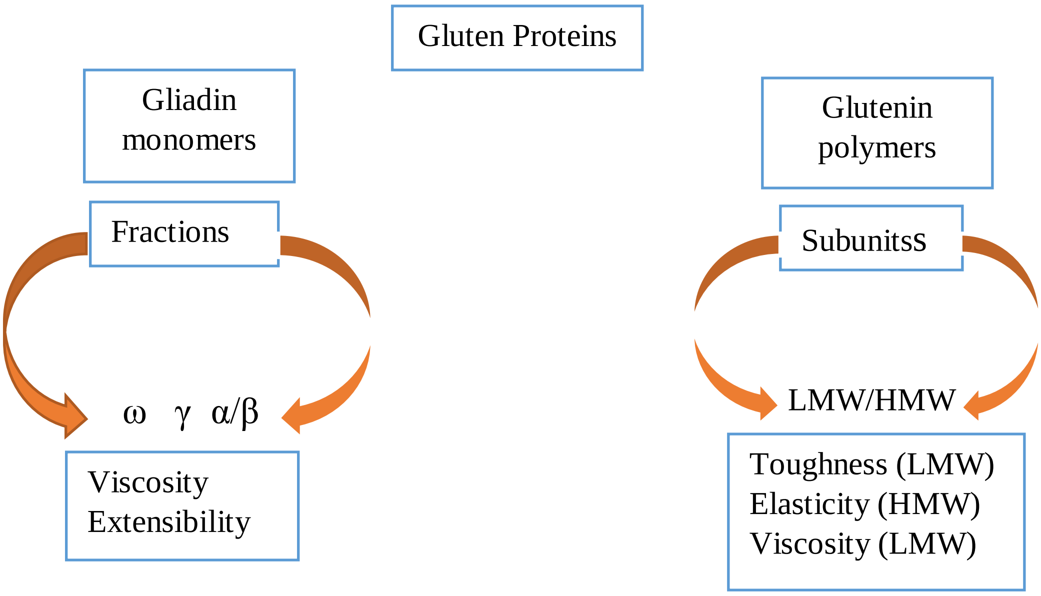


Figure 1.2: Overview of the gluten protein (Lamacchia et al., 2013)

1.2 IMPORTANCE OF GLUTEN IN BREAD MAKING

Gluten often referred to as the structural protein in the bread because of its natural abilities inherent in the flour when saturated with water and exerted upon by mechanical force displays its unique function by providing extensibility, gas retention during fermentation and provision of firmness to the crumb during bread production. (Belton, 2005). The role of gluten in bread making can be summarized under three different stages of bread making which include; Mixing process, Proofing period and finally influence of gluten on baking.

1.2.1 MIXING PROCESS

In the context of mixing, flour is mixed with various ingredients in their right proportions to obtain an optimum quality and is enabled with the addition of liquid (water or milk). Energy is used to break apart the earlier arrangement of the gliadin and glutenin network which enables the development of a massive protein chain (Tatham and Shewry, 2012). During this mixing phase, the dough displays its unique viscoelastic behavior which becomes more visible as a result of the gradual sheer and tensile force being applied which causes the gluten proteins to stick together and create a stable chain in the dough (Janssen et al., 1996). Due to the applied force exerted on the flour which creates a significant change in the earlier conformation of the gliadin and glutenin, the water binding ability of the flour also changes thereby giving access to increase flow of water within the flour and also allowing the gluten perform its role of binding and retaining the water molecules needed for mixing of the dough (Boch and Damodaran, 2013).

In a study conducted by Wang et al. (2015), they reported that the ability of the dough to form its 3-dimensional protein chain structure is based on the conformation of the amount of di-sulfide bonds and sulfhydryl groups contained in the gluten found in the interactions between the gliadin and glutenin subunit of the gluten protein. Another scientific research explained that the protein behavior during the dough mixing reveals three significant changes as a result of the influence of the gluten on the dough formation which includes; disentanglement, changes to the protein chain network and bond breakdown between the gliadin and glutenin (Macritchie, 1999). This changes that occur creates isolation of the individual components of the gluten. This is observed in the functionality of the gliadin subunit when reacted with water and starch, it provides the dough with its viscous behavior whereas the glutenin displays a rubbery substance with reduced extensibility that gives the dough its elastic potential during mixing (Shewry et al., 1997).

1.2.2 PROOFING PERIOD

The proofing time has been known to be a period when the dough is allowed to relax after undergoing the rigorous activity of mixing and molding. In this phase, the dough undergoes several reactions at room temperature of 24-26^{o}C, which include production of carbon dioxide gas as a result of fermentation by the action of yeast, and accompanied by the breakdown of starch. (Canvain, 2015). Canvain, further noted that the gluten is important in bread making especially at this stage because the released carbon dioxide gases are trapped in pockets by the gluten network thereby allowing the gluten protein chain to expand as a result creating a rise in the dough during proofing.

Further research has it that flours with a high gliadin and glutenin amount gives maximum bread quality. This is referred to the fact that the development of gluten in the dough during mixing gives the gluten the ability to retain the produced gas, therefore the increment noted in volume during proofing is directly proportional to the gluten viscoelastic behavior in the dough (Barak et al., 2014).

The importance of the gluten is also seen in the viscous nature of the gliadin which possesses the ability to keep firm of the released gases during fermentation of the dough, which is viewed as their surface-active characteristics (Wang et al., 2015).

1.2.3 INFLUENCE OF GLUTEN ON BAKING

The gradual rise of temperature in the oven makes it easy to observe the slow conversion of the dough from a foam like manner to a spongy form during baking. Various changes initiated by the heat occurs at this stage which includes denaturation of the proteins that introduce changes to the protein conformations, physicochemical changes that affect the rheology and thermal properties of the bread in the oven (Ortolan and Steel, 2017).

In an investigative study, it was noted that at a controlled temperature, adjustment in surface hydrophobicity of the dough which commenced at 45^{o}c resulted in the realignment of the gluten polymer thereby exposing the hydrophobic groups and also reducing solubility. This however, weakens the gluten elastic behavior but enables the gluten to determine the structure of the bread loaf and its volume. The emergence of the final product after cooling is a solid textural framework and fine loaf of bread (Dobraszczyk, 2004; Guerrieri et al., 1996).

1.3 CELIAC DISEASE

Celiac disease, also referred to as gluten enteropathy and celiac sprue has been known globally to be one of the most unusual food related disease and is caused by feeding on a gluten related food products in rye, barley, wheat and possibly oats (Sham et al., 2002). It is an unusual gluten related food induced disease in humans strongly linked to people with HLA genotypes, as it has been revealed in various research that mostly individuals possessing the DQA1*0501 and DQB1*0201(DQ2), or DQA1*0301 and DQB1*0302 (DQ8) alleles are linked to the disease (Paparo et al., 2005).

Series of research carried out in different parts of the world has accepted celiac disease to be an immune response problem where intestinal $CD4^{+}$ T-cells of people with the disease when reacted with gluten food products leads to development of the disease (Meresse et al., 2012). Current studies have also confirmed the fundamental role of inherent immune cells and adaptive $CD8^+$ T-cells in destroying the mucosal surface of the intestinal walls when reacted with gluten related foods (Mazzarella et al., 2008). Therefore, celiac disease is an autoimmune enteropathy initiated by the consumption of gluten related products of barley, wheat, oats and rye in response to the innate prolamins of their amino acid chains and result in destroying the linings of the small intestinal wall which culminates in malabsorption of nutrients (Feighry, 1999; Catassi and Fasano, 2008).

According to scientific investigation, various serological screening accompanied with the use of endoscopy has further revealed that celiac disease is present in at least 1% of the global population (Kang et al., 2013). This is followed up by a study carried out by Fasano et al. (2003), in which they confirmed the sensitivity of the serological tests used to evaluate the prevalence of celiac disease as being present in 1 of 130-300 of the world population.
Because of the growing challenges faced in dealing with this disease, celiac disease has brought tremendous concerns to the medical and food engineering society. This is because there is presently no medical report indicating a direct cure for the disease. More so, it has been found to be a lifelong threatening disease in patients suffering from it, in which a complete abstinence from gluten related food has emerged to become the only solution in combating the disease (Heikkila et al., 2015).

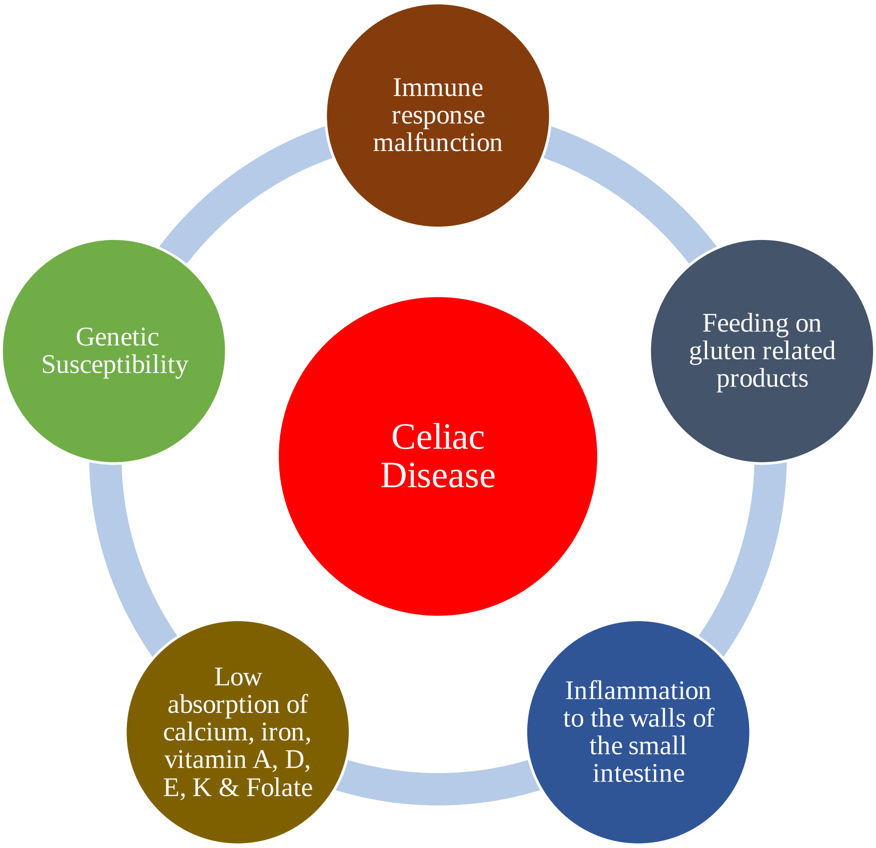


Figure 1.3: Description of the celiac disease (Cranney et al., 2007)

The only solution as revealed in several research for the cure of the disease has been a complete lifelong abstinence from gluten products and strict dependence on gluten free foods. This is because the T-cells which reacts with the gluten are resident in the ileum of celiac disease patients even after many years of complete abstinence from gluten products, intake of gluten foods reactivates the immune response which results in the damage of the mucosal surface of the ileum and causing malabsorption of nutrients, e.g., folic acid, iron, calcium and fat soluble vitamins (Koehler et al., 2014).

The technology for producing different varieties of gluten free bread has brought about a significant challenge in the food and medical industry. This is because celiac disease patients needs to feed daily on a gluten free food to maintain a healthy life. As a result, the need to utilize gluten free flours like corn flour, millet, rice flours, sorghum, pseudo cereals (amaranth, quinoa, buckwheat flours) and the addition of additives like hydrocolloids in order to replace the gluten and exhibit the same quality of flavor, appearance and mouth feel is a vital need that must be met (Gallegher et al., 2004).

Table 1.1: Pooled Seroprevalence and prevalence of celiac disease in accordance with geographical location (Prashant et al, 2018)

Geographical location	Sero prevalence (n)	Population screened	+ve subjects for CD	95% sero prevalence pooled	Prevalence based on biopsy	Population screened	Subjects with biopsy	Prevalence of biopsy proven cases
Europe	49	163,700	2340	1.3 (1.1–1.5)	33	98,391	1119	0.8 (0.6–1.1)
Asia	20	68,632	2607	1.8 (1–2.9)	12	18,052	114	0.6 (0.4–0.8)
South America	11	20,245	280	1.3 (0.5–2.5)	5	16,550	69	0.4 (0.1–0.6)
North America	7	17,778	200	1.4 (0.7–2.2)	1	200	01	0.5
Africa	7	15,775	253	1.1 (0.4–2.2)	4	7902	42	0.5 (0.2–0.9)
Oceania	2	4075	59	1.4 (1.1–1.8)	2	4075	27	0.8 (0.2–1.7)
Specific geographic regions								
Middle East	17	41,750	847	1.6 (1.2–2.1)	11	15,063	89	0.6 (0.4–0.8)
South East Asia	4	28,382	1784	2.6 (0.3–7.2)	2	4489	59	0.8 (0.4–1.4)
North Africa	6	14,275	229	1.0 (0.2–2.3)	3	12,686	27	0.4 (0.2–0.6)

1.3.1 SIGNS AND SYMPTOMS OF CELIAC DISEASE

The signs and symptoms of celiac disease has been found to develop at any stage of the life of the individual carrying the disease as long as gluten diet are included in the patient's food. Clinical investigation has however revealed that the signs and symptoms of celiac disease manifest in a classic form which include malabsorption of nutrients such as folates, fat soluble vitamin, calcium, iron etc., occurrence of diarrhea, weight loss in the patient or stunted growth in a growing child and a non-classical and symptomatic or asymptomatic forms which include gastrointestinal and extra-intestinal symptoms (Ludvigsson et al., 2013).

With the passing of age, the awareness of celiac disease and its prevalence is becoming a serious global issue as it has been established to be a food sensitivity problem attributed to consumption of gluten related products (Sicherer and Sampson, 2014).

Table 1.2: Other forms of manifestation of the signs and symptoms of celiac disease in an individual (Kelly et al., 2015)

Gastrointestinal	Intestinal disorders	Associated conditions
Persistent diarrhea	Iron-insufficiency anemia	Past family record of celiac disease
Recurring abdominal pain	Other deficiency states (vitamin B_{12}, vitamin D, folate, Zínc, Vitamin B_6)	Type 1 diabetes
Malabsorption	Fatigue	Autoímmune thyroid disease
Bloating	Chronic aphthous stomatitis	Autoímmune liver disease
Abnormal bowel habit, similar to inflamed bowel syndrome	High hepatic transaminase levels	Selective IgA deficiency
Constipation (more commonly in children)	Decrease in stature	Sjögren syndrome
Failure to thrive/weight loss	Delayed puberty/menarche	Down syndrome
Malnutrition	Amenorrhea	Turner syndrome
Vomiting	Early menopause	Willíams syndrome
GERD (gastroesophageal reflux disease)	Dermatitis herpetíformis	
	Osteopenia/Osteoporosís	
	Dental enamel hypoplasia	
	Perípheral neuropathy	
	Hyposplenísm	

Though some symptoms and signs of celiac disease has been easy to identify, some symptoms have not shown itself early in life, which has led to delay and wrong diagnosis in patients with the disease; this has resulted to poor health in patients, chronic cases of anemia, osteoporosis, risk of infertility in both male and females and also giving birth to some cancers that occur in the gastrointestinal tract of individuals (Julio et al., 2015).

1.4 GLUTEN FREE BREAD PRODUCTION

Absence of gluten which provides the viscoelastic property of the bread and the addition of several ingredients to mimic the appearance of a conventional gluten bread using wheat flour has made it difficult for bakers to produce a gluten free bread with the same quality and nutritional value for consumption by patients suffering from celiac disease. (Demirkesen et al., 2014). The rise in diagnosis and awareness of celiac disease across the world has inspired extensive research to find various technological methods and processes for the making of safe and high nutrient based gluten free bread for celiac disease patients (Houben et al., 2012).

In recent years, different approach has been applied for the making of gluten free bread with the use of naturally gluten free flours such as rice flours, corn flour, sorghum, millet, potato, cassava etc. (Sciarini et al., 2010) and the inclusion of hydrocolloids such as guar gum, xanthan gums, alginate, carrageenan, hydroxypropyl methylcellulose (HPMC) (Lazaridou et al., 2007). This ingredients and additives has been used in order to obtain the same viscoelastic property of the gluten bread and also a quality nutrient gluten free bread for patients suffering from celiac disease (Gujral and Rosell, 2004).

Zea maize popularly known as corn, has been viewed to be a gluten free cereal, possessing the attributes suitable for the preparation of a gluten free bread. This is attributed to its content of 75-87% of starch and 6-8% of protein (Shukla and Cheryan, 2001). Also, its unique flavor, distinctive colour attributes and ability to easily bind with hydrocolloids when used as a substitute for the production of gluten free bread (Arendt and Dal-Bello, 2008). Scientific literature has described corn to be a good source of energy, but possesses proteins with reduce biological quality which include the amino lysine and tryptophan (Losak et al., 2010). Further research also proved that the maize kernel is not a good source of numerous essential vitamins such as the B group vitamins, and contains insignificant traces of niacin (Vitamin B_3), which is a vital vitamin for human health (Zang, 2010).

Also, *zea maize* being considered a potential ingredient for the production of gluten free bread is due to its hypoallergenic qualities, the flavor it exhibits when blended, and its availability in commercial quantity (Kadan et al., 2001). More so, appearance of the final product, being one of the important perimeter used by consumers when making food choices; the corn starch gluten free bread proved to display a yellow color, firm and dense crumb for the final product (Renzetti et al., 2008).

Hydrocolloids are known for their multifunctional group of long network of polymers of polysaccharides with high molecular weight and proteins with the ability to form viscous dispersions and able to change starch gelatinization process when miscible with water (Rojas et al., 1999). It comprises of a large and heterogeneous amount of extracellular materials commonly sourced from microorganism of Xanthomonas compestris, algae, bacterial, fruit and plants derivatives, which contain a high volume of dietary fiber varying between 60-90% and are regarded as soluble dietary fiber (Viebke et al., 2014).

Based on the established fact that gluten is lacking in corn starch used for the gluten free bread production, the viscoelastic behavior associated with the conventional wheat flour which provides the ability to retain gas, enhance structure formation in the gluten bread is lacking. This results to reduced volumes and firmer crumb in the final product of gluten free bread as compared to the wheat gluten bread (Hager et al., 2012). Commonly used hydrocolloids for the development of gluten free bread includes Hydroxypropyl methylcellulose (HPMC), Xanthan gums, Guar gum, Carboxymethyl cellulose (CMC) etc. It serves to improve the rheology of the final product and water-binding ability of the dough of gluten during mixing (Casper and Atwell, 2014).

Appearance, quality and nutrient of a product are perimeters used by food producers and consumers to quality-check to make food choices. Gluten free bread has shown to display low quality due to the absence of gluten. Utilization of hydrocolloids as a substitute of gluten has been observed to perform same functions of gluten by improving gas retention in the dough, bread batter modification, high-water binding capacity, acceptability and prolonging of shelf-life of the gluten free breads (Lazaridou et al., 2007). Further research also suggested that the use of one or two hydrocolloids to help impact on the organoleptic and rheology properties of the gluten free bread enables the gluten free bread to imitate the gluten found in the wheat flour, thereby providing stability during processing, causes gelatinization of gas granules and also promotes cohesiveness in the dough. (Onyango et al., 2009).

In the research conducted by Mariotti et al. (2013), they observed the performance of the Hydroxypropyl methylcellulose (HPMC) for its ability to disperse gas cells, lowering the movement and reduction of water molecules from bread crumb. More so, they revealed the ability of HPMC to be able to inhibit the binding of starch and proteins which resulted to a soft crumb, reduced staling, raise specific bread volume, enhance sensory properties and increments in the shelf-life of the bread. Guarda et al, (2004), discussed the water binding ability of the hydrocolloid which they observed that the hydroxyl groups associated with the xanthan gum created room for water movement due to the hydrogen linkage around them. Xanthan gum being a member of the extracellular polysaccharides sourced from the popular Xanthomonas campestris is also known for its improvement of the gluten free bread rheology, its sensory and organoleptic acceptance by consumers and its provision of pseudo elastic property for the dough during production of gluten free bread (Moreira et al., 2011).

In view of the above characteristics attributed to the functions of hydrocolloids to further enhance the making of gluten free bread, hydrocolloids has been seen to be the primary source of improving batter viscosity, due to their hydrophilic behavior in binding with water molecules, promoting bread texture and acceptability among consumers. (Rosell et al, 2001; Lazaridou, 2007).

This study is aimed at discussing the technology and the effects of hydrocolloids in producing gluten free bread made from a composite mixture of corn starch and rice flour. The primary focus is to proffer a lifelong solution to patients suffering from celiac disease, by providing an alternative away from the conventional wheat bread since it has been discovered that exposure to the gluten in the wheat bread or related products causes a dysfunction in the system of celiac patients which only a lifelong abstinence is the solution.

CHAPTER 2

THEORETICAL FRAMEWORK

2.1 Diet Management for Celiac Patients

Due to the reduced value of essential nutritional requirement contained in the diet of celiac disease patients, it has become a necessity to initiate a diet plan to balance the required nutrients value. However, different countries have demonstrated nutritional habit for persons living with the disease and as a result suggesting a diet plan fit for celiac patients has been a major challenge (Letizia et al., 2010).

2.1.1 Carbohydrates

According to Krauss et al. (2011), they suggested the complex and simple forms of carbohydrate ingestion for celiac patients should contain about 55% of the total calories. Although, there is a shortage of carbohydrate in some gluten free foods and some contain high glycemic index, legumes and some forms of grains product have been seen to be good form of carbohydrate and therefore should be considered as an inclusion in the diet of celiac patients (Coulter and Lorenz, 1990).

2.1.2 Dietary Fiber

Dietary fiber are polysaccharides components of plants that are resistant to breakdown in the intestine. Due to their composition, some have shown significant physiological properties when they are finally broken down by normal flora of the intestine and have shown significant assistance in preventing colon diseases, cardiovascular diseases and diabetes (Anderson et al., 2009). A daily intake of 20-35g/d of dietary fiber has been suggested to be vital for daily nutritional balance of celiac patients. This is also to accommodate some of the report in some research concerning low content of dietary fiber (Thompson, 1999). An insight into the amount of fiber present in some cereal foods is displayed in Table 2.1

Table 2.1: Amount of fiber present in some cereal foods (Letizia et al, 2010)

Cereals	Fiber g/100g
Oat	10.3
Wheat	9.5
Barley	9.2
Teff	8.0
Corn	7.3
Spelt	6.8
Rice	2.8
Pseudo-cereals	
Buckwheat	10.0
Quinoa	7.0
Amaranth	6.7
Fruit and vegetable	0.5-5.0
Nuts	4.0-12.0
Pulses	5.0-18.0

2.1.3 Protein

Several research has reported animal protein like meat, milk, dairy sources, eggs and fish to be higher than plant protein base. 15% of total calories have also been suggested to be involved in the gluten free food of celiac disease, therefore the need for daily protein intake is a primary necessity (Gorinstein et al., 2002). Pseudo cereals have also been proven to contain high level of methionine and cysteine which are needed for nutritional stability and health of celiac disease patients (Adel-Aal et al., 2002).

2.1.4 Lipids

Celiac disease patients has been advised to maintain 25-30% or lower content of all calories involving lipids and fats. The consumption of mono-unsaturated and poly-unsaturated fatty acids have been suggested to give about 15% and 10% of the total calories. Mono-unsaturated and Omega-3 fatty acids are commonly related to foods such as vegetable oils, nuts, salmon fish, trout etc. and has been linked with the prevention of cardiovascular disease in celiac patients. Therefore the need to include them in the diet plan is vital (Temple, 1996). Furthermore, the need to also limit the use of trans-fatty acids is vital due to negative role in causing atherosclerosis, and as a result their ingestion should be at a level less than 1% of total calories (5g/d) (Judd et al., 1994).

2.1.5 Vitamins and Minerals

Proper consumption of vitamins and minerals have been viewed as a substitute for celiac disease patients due to the damage done on the intestinal wall of celiac patients which has caused the inability of the intestine to absorb significant trace elements such as iron, zinc and selenium. The intake of calcium, phosphorus, sodium, potassium, chloride, and magnesium has been prescribed as a major substitute for a healthy dietary plan for celiac patients (Adeyeye and Ajewole, 1992). According to scientific literature, pseudo cereals such as amaranth and quinoa has shown to contain minerals and vitamins twice the portion present in cereal grains and also contain increase amount of riboflavin, vitamin C and E. therefore, celiac patients should explore the use of this items for their dietary plan (Dyner et al., 2007; Fabjan et al., 2003).

2.1.6 Phytochemical

Food sources of phytochemicals have been known for their significant role in preventing risk of heart diseases, type II diabetes, colon cancers etc. (Anderson et al., 2009). Further research has also confirmed they possess antiviral effect, anti-allergic, anti-platelet, antioxidant functions, anti-inflammatory response and antitumor properties (Stevenson and Hurst, 2007). Yao et al., (2004), suggested that plant based food such as fruits, vegetables, wines and teas are commonly associated with phytochemical properties and their food sources contain high level of antioxidant properties. However, further research on pseudo cereals has confirmed buckwheat and quinoa to contain higher antioxidant, antitumor, anti-inflammatory, hepatoprotective and antidiabetic. The globulins from the wheat flour has been known to incur inflammatory effects on the celiac disease patients. However, the globulins in buckwheat provide a bioactive effects that gives it anti-inflammatory abilities. Therefore, consumption of this gluten free products could help to maintain a healthy balance for celiac disease patients (Zielin and Kozlowska, 2000; Zdunczyk et al., 2006).

2.2 Allowed and not Allowed Foods for Celiac Patients

As summarized in Table 2.2, the foods not allowed for celiac disease patients include foods made with the inclusion of gluten which include wheat, its derivatives, such as kamut, spelt, rye, barley, triticale, and thickeners which are associated with gluten e.g., hot dogs, medicinal ingredients that uses gluten as binders in the production of pills or tablets (Ellis et al., 1994). Malt and beer drinks has also been viewed to be harmful to celiac disease patients due to the presence of hordein in the beer and the malt being a partial hydrolysate of the barley prolamins, as a result, it syrup, extract, flavorings and derivatives has been deemed to be avoided by celiac disease patients. (Ellis et al., 1994).

Over the years there has been differs views over the addition of oats in the diet of celiac patients but significant studies has revealed that daily consumption of oat of about 25-60g/day is healthy for celiac disease patients. However, oats was found to be removed from the diet meant for celiac disease patients because of traces of appreciable quantities of avenin found in oats. The avenins in oats are found to look like to those in cereal prolamins, as their polypeptides contain proline and glutamine. This two protein region containing these amino acids have been found to induce celiac disease when the patients feed on their food related product (Real et al, 2012). Therefore the celiac organization in the United States and Canada have initiated a shutdown on the use of oats as a member of the food chain for patients suffering from celiac disease (Lohiniemi et al., 1988; www.celiac.ca).

Further studies by other scientist carried out to determine the long term safety for the possible use of oats have also suggested that when oats are taken in moderate quantities, oats not contaminated with gluten derivatives are well tolerated by celiac disease patients even in the long term (Haboubi et al., 2006). Therefore pure oats, undefiled with gluten have been suggested to be part of the food menu plan for people suffering from celiac disease due to their palatability and increased nutritional value (Lee et al., 2009).

Table 2.2: Some allowed and not allowed foods for patients suffering from celiac disease. www.massgeneral.org

ALLOWED FOODS	NOT ALLOWED FOODS
Grains & Starches	**Grains & Starches**
This includes all grains that do not contain gluten naturally or as an additive. Soybean flours, potatoes flour, Pseudo cereals such as quinoa, amaranth, buckwheat flours. Millet, teff, sourghum, flax rice; puffed rice, corn starch. Gluten free oats may be allowed.	Wheat flour, its components and its derivatives. Durum flour, Semolina, kamut, wheat berries, couscous, spelt, faro, triticale, dinkel. Rye including its flour and derivatives. Barley, Oats that contains gluten.
Beverages	**Beverages**
Coffee and teas that do not contain grains with gluten. Milk, chocolate milk prepared with cocoa 100%. Fruit juices, soda, Wine; distilled alcohols and cordials (check labels for preservatives and dyes); gluten free beers.	Instant coffee; instant tea; some herbal teas; instant cocoa with grains containing gluten or its derivative. Rice or soy beverages that uses barley enzymes. Beverages containing flavored syrups that is not properly labelled.
Soups and Casseroles	**Soups and Casseroles**
Soups made with rice or gluten-free pasta and gluten-free stock; creamed soups and chowders thickened with cream, cornstarch, potato flour or other allowed special flours.	Bouillon-based broths; creamed soups or chowders thickened with flour. Prepared soups with prohibited cereal grains or thickeners.
Fats and Oil	**Fats and Oil**
Butter, Lard, vegetable oil, margarines that is well labelled and do not possess gluten and its derivative. Pure mayonnaise (and other salad dressings that are thickened with egg, cornstarch or allowed special flours)	Wheat germ oil, margarines and spreads made with prohibited stabilizers. Most fried and breaded foods Low calorie mayonnaise made with prohibited cereal thickeners.
Meat or Meat Substitutes	**Meat or Meat Substitutes**
Fresh meat; poultry; fish and shellfish; eggs Edamame (soy beans); tofu; beans; nuts that contain gluten protein or its derivative.	Most meats such as sausages and hot dogs containing prohibited grains. Animal proteins that is marinated using prohibited ingredients. Dry roasted nuts containing prohibited ingredients

2.3 Problems Associated With Gluten Free Bread

Absence of gluten in breads without gluten is viewed as a significant problem to the quality of the bread, nutritional properties and its rheological performance. Gluten free bread is also accompanied with issues of insufficient gas during fermentation process which culminates in reduced loaf volume in the final product, possession of liquid batter other than the dough like in the gluten contained bread and leads to distorted texture in the crumb, which results in negative color and finally display poor baking properties. (Matos and Rosell, 2012; Onyango et al., 2009).

Gluten free bread also proved to contain reduced protein and low level of lysine, increased amount of carbohydrate and fat. Describing the nutritional value of the gluten free bread shows the amount of proteins ranges between 0.90-15.5g/100g, 2.00-26.1g/100g of fat and 42.4-75.9g/100g of carbohydrate which therefore contributes little or excess nutritional requirement for celiac disease patients (Matos and Rosell, 2011). The absolute removal of gluten has also revealed the low level of fibers, minerals, calories, irons, vitamins, and poor organoleptic properties of the gluten free bread (Yazynina et al., 2008).

Further research showed the nutritional ingredient of gluten free bread, pastas and other cereal derivatives compared with gluten contained products proved the gluten free bread and other gluten free products revealed reduced level of folic acid and iron, which will lead to poor minerals in celiac disease patients (Thompson, 2000). The elimination of gluten from gluten free bread also displayed poor taste and aroma as a result of volatile compounds present in the gluten contained breads being absent. Therefore, it has been a major problem to mimic the aroma, taste and texture of the gluten contained bread since this natural occurring aroma compounds are only present in the gluten containing breads. (Pacynski et al., 2015).

2.4 Parameters for Making Gluten Free Bread

Based on celiac disease awareness across the world, consumer’s interest in maintaining a healthy and balanced eating pattern has also increase. This has led many food scientist to look for alternative source of physiologically induced food with the ability to satisfy the mental wellbeing of celiac patients (Pang et al., 2012). Apart from satisfying hunger status of the patients, but also impact nutritional value and help in preventing health related problems that could arise due to malabsorption of nutrients in celiac patients (Roberfroid, 2000; Menrad, 2003).

Due to the absence of glutenin and gliadin component of the gluten in the gluten free bread, technological replacement of the gluten protein fraction has been a major challenge because of its significant effect in maintaining the viscoelastic properties and water binding capacity of the dough (Gallagher et al., 2004). Having these in mind, the need for developers to produce gluten free bread based on adherence to strict parameters for the safety of the bread, acceptability and affordability by consumers and also in line with the set standards proposed by regulated authorities needs to be adhered to by producers (Prakriti et al., 2016).

2.4.1 Removal of Gluten-Related Sources

The primary factor to consider in the gluten free bread production for celiac patients is the complete removal of gluten related sources. This is due to complication that accompanies the ingestion of gluten food sources in celiac disease patients. These include the gluten protein fractions in wheat and its derivatives, horedins in barley, the secalins component of rye and the possible presence of avenins in oats (Moreno et al., 2014). The European regulatory authority have proposed the avoidance of oats used in the production of gluten bread, this is due to diverse debates by researchers on the possible inclusion of oats in celiac disease patients due to the presence of avenins in oats. (Comino et al., 2015). However, the use of wheat and its derivatives has been completely avoided in the gluten free bread formulation for celiac disease patients.

2.4.2 Producing Gluten Free Bread with Quality Sensory Properties

The absence of gluten in bread formulations is a major concern due to the positive effects its presence gives to the bread in sustaining quality appearance in the final product, cohesive crumb, chewy nature and desired mouth feel (Gallagher et al, 2004). As a result, the sensory properties of the bread is a major factor to consider during gluten free bread production because in the absence of gluten, an alternate source of gluten should be introduced as an additive for the enhancement of gluten free bread, so as to maintain same sensory and organoleptic properties found in the gluten containing bread which include the texture, cohesive nature of the bread and enriched mouth feel (Ylimaki et al., 2006; Sanchez et al., 2002).

Rotsch, et al. (2013), suggested that a bread without the presence of gluten protein fractions cannot retain gas (CO_2) trapped during fermentation and responsible for dough rising, unless there is a replacement with a gel-like substance in the form of gluten. Different scientific research has proposed the use of hydrocolloids which include xanthan gum, hydroxymethyl cellulose, guar gum etc. (Moreira et al., 2013; Mahmoud et al., 2013).

2.4.3 Maintaining Nutritional Quality during Production

Investigative studies has revealed that celiac disease patients suffer malabsorption of nutrients due to damages done on the intestinal wall when they take in gluten related products. As a result, celiac patient's strict adherence to gluten free bread show signs of nutritive deficiencies and weight loss (Hallert et al., 2002; Ciacci et al., 2002). Further research proved that the nutritional quality of the diet of celiac disease patients was found to contain increased amount of calories from fats and reduce form of carbohydrate. This was a direct effect of the gluten free breads produced majorly from the use of refined gluten-free flours that were enriched or fortified (Moreno et al., 2014). As a result various supplements in the form of enzymes, proteins and hydrocolloids has been incorporated in the gluten free bread production so as to enhance acceptability and promote nutritional value of the gluten free bread (Matos and Rosell, 2015).

2.4.4 Regulations for Gluten Free Bread

Due to the complications associated with the foods of celiac disease patients in order to stay healthy, it has become necessary for producers of gluten free breads and products to obey and adhere strictly with the laws of different national and international regulatory bodies during production (Prakriti et al., 2016). This is in accordance with the Codex set laws for gluten free food which was introduced and accepted by the Codex Alimentarius Commission (CODEX) arm of the World Health Organization (WHO) and the FDA (Food and Agricultural Organization) in 1976 (Saturni et al., 2010).

According to the set law, gluten free foods can be said to be foods that do not consist of any prolamin fraction of wheat or triticum species which include spelt, kamut and durum, secalins of the rye, hordeins from the barley or its derivative and possibly oats and its constituents or their cross breed containing gluten not more than 20ppm (Arendt and Moore, 2016). In the remark of the Food and Drug Administration of the United States (FDA) in

2013, they stated the need for labelling of gluten free foods, in which they proposed the term to be,

- Gluten-free
- Free of gluten
- No gluten
- Without gluten

Figure 2.1: Labels describing gluten free products

http://www.bamco.com/blog/gluten-free-biola

The labelling of gluten free products has played a major role in the health of celiac disease patients by indicating safe foods for consumption and contributing to their satisfaction of making quality choices of products (Zou and Hobbs, 2010).

2.5 Technological Enhancement of Gluten Free Bread

Due to the use of an alternate flour for gluten free bread production, clinical investigation has proved gluten free bread is always characterized by reduced level of dietary fiber in comparison with the conventional gluten containing bread (Penagini et al., 2013). Gluten free bread has also been viewed to be rheological poor which include definite volume of the bread, softness and possessing increased staling when compared with the conventional wheat gluten contained breads (Arendt et al., 2007).

Scientific investigation into the use of corn starch for producing zero gluten bread noted the presence of micro and macronutrients associated with corn starch, but confirmed the low level of adequate essential nutrients such as proteins, minerals and dietary fibers. As a result, celiac disease patients stands the risk of nutritional deficiency (Mastromatteo et al., 2011; Schober et al., 2008). Further investigation into corn flour for gluten free formulations also, proved that pasta production using maize flour and 15% chickpea as an additive to enhance its nutritional value gained a significant increase in the dietary fiber, proteins and lipids content (Padalino et al., 2014). Different non-gluten ingredients has been identified as an additive for the nutritional quality enhancement of zero gluten bread in order to produce a safe and nutrient enriched gluten free bread, and also mimic the same gluten properties found in the gluten contained bread (Marrioti et al., 2009).

2.5.1 Addition of Dietary Fiber

The use of dietary fiber for enrichment purposes is mostly based on its nutritional and functional property in the gluten free bread, which include its ability to absorb water, formation of gel and inducing textural and thickening properties etc. (Tsatsaragkon et al., 2016). Penagini et al. (2013), described gluten free bread to be one commonly identified for its low form of dietary fiber. Furthermore, they suggested the ingredients used in gluten free bread making which include refined flour to be low in dietary fiber due to lack of fortification, as a result not fulfilling the nutritional content of celiac disease patients. Lack of adequate fiber could lead to poor food digestion, increased risk of cardiovascular disease, lead to weight gain and poor blood sugar control.

Inulin, which is a plant based, naturally occurring polysaccharide with prebiotic effect has been found to be a significant dietary fiber for gluten free bread enrichment due to its functional properties in increasing loaf volume, enhance dough stability and improving crumb textural properties (Korus et al., 2006). Also, the use of soluble fiber with the inclusion of resistant starch for enrichment purposes has served to help in the reduction of glycemic index, a response which is highly needed for the health stability of celiac disease patients. (Gunners and Gidley, 2010). According to a scientific research, the use of resistant starch for enrichment purposes did not only impact positively on the digestive functions of celiac patients, but also improved the rheological properties of the bread by increasing the elasticity and porosity of the gluten free bread (Witczak et al., 2016; Tsatsaragkou et al., 2014).

2.5.2 Using Different Protein Sources

The use of protein based composition from various sources such as legumes, egg and dairy for the enrichment of gluten free bread has noted significant increase in the nutritional value by making it a functional food and also impacting positively on the organoleptic quality of the bread by enhancing maillard browning reaction and flavor (Deora et al., 2015). However, care should be taken so as to avoid unwanted compounds such as acrylamide and furfurals produced by maillard reaction which is harmful for human consumption. The presence of the gluten protein has not only enabled the decrease in water circulation of the bread due to its water binding property, but also generated a soft crumb. Its absence has negatively impacted the bread, because the water movement now occurs in a manner that promotes a rigid crumb and a soft crust (Lazaridou et al., 2007).

The application of whey protein for functional purposes of bread enrichment, improving water retention and nutritional quality has been identified for its vital role in improving nutritional quality of zero gluten bread (Kenny et al., 2000). In a scientific research carried out by Gallagher et al. (2003) on whey protein, they revealed that the presence of whey protein in breads without gluten was able to enhance the protein content without having any additional effect on it dietary fiber composition. The incorporation of whey protein into gluten free bread has also displayed positive potentials in the bread rheology by adding to the size and bulk of the bread loaf, rigid structure and mixing tolerance of the dough (Indrani et al., 2007). One promising attribute of applying whey protein in gluten free bread is also to promote mesoscopic network in the batter and create a well-defined rigid strain which is also needed for a dough-like property (Riemsdijk et al., 2011).

Furthermore, in a novel research, the use of bovine plasma proteins for enriching the gluten free bread revealed inducement of positive impacts on the rheological attributes of zero gluten bread (Furlan et al., 2015). Food science literature also explained the use of albumín, collagen, pea, lupíne and soy proteín. It revealed that the inclusion of lupine and albumin enhanced the specific loaf and bulk size of the zero gluten bread, reduced the rigidity and chewy texture of the crumb and created anti-stalling functions in breads without gluten (Ziobro et al., 2013).

2.5.3 Addition of Sourdough

Due to the growing need to produce functional foods that can satisfy the shortage of essential vitamins in celiac disease patients, incorporation of sourdough for gluten free bread production has proven to be a better solution (Di-Cagno et al., 2008), this is because sourdough comprises of fermented flour by cultured lactic acid bacteria, yeast and other ingredients, which aid in the acidification process of the gluten free batter and also enhances the production of aroma aggregates (Gobbetti, 1998).

Due to severe impairment of the immune system of celiac disease patients, the incorporation of sourdough starter culture to the gluten free bread was found to help improve the immune functions of celiac disease patients by releasing high content of proline/glycine peptide through proteolytic functions (Rollan et al., 2005).

Several research revealed sourdough shows significant contribution in impacting positively in the appearance of breads without gluten, its texture, the nutritional value and also in extending the shelf-life of gluten free breads. This is however, viewed to be the metabolic function of the lactic acid bacteria (Racyts et al., 2012). Sourdough addition to breads without gluten also proved to decrease starch breakdown, thereby acting as an anti-stalling agent in zero gluten bread (Rojas et al., 1999).

2.5.4 Combination of Gluten Free Flour or Starch

In line with consumer's view, gluten free bread acceptability is based on appearance, sensory properties and nutritional value. Conducted research has indicated the use of soy flour together with buckwheat as additives of rice flour and corn starch. It revealed the final product to gain better acceptance based on quality and satisfaction (Moore et al., 2004). The research further proved soy flour had a major impact on the batter and the gluten free bread properties which culminated in increase in bread volume and improvement in batter firmness. It also produced softening effect in the crumb, and reduced bread staling rate due to water retention ability of the soy protein and inhibition of starch regression. Studies also confirmed this positive impacts by soy flour can be attributed to the presence of the lecíthin present in the soy flour, which contributes to the regression of the starch by stopping the movement of water, thereby promoting gas molecules balance in the dough due to formation of soluble lamellar films around the gas molecules (Eduardo et al., 2016). Nunes et al. (2009), researched further to confirm the increment in the bread size of the gluten free bread and weight of the dough was due to the soy lecithin present as an ingredient.

In another effort, Elgeti et al. (2014), replaced the rice and corn flour with quinoa flour in order to verify the impact of the quinoa flour on free gluten bread rheology. They noted a significant increase in the loaf volume of the bread, which they proposed it is due to lack of bran properties and an improved presence of α-glucosidase functions. Furthermore, the crumb of the bread fared well by sticking together and evenly allowed the passage of gas bubbles without altering the taste of the bread. As a result, they concluded the possibility of utilizing quinoa white flour for promoting the rheological parameters of gluten free bread, thereby adding quality.

2.5.5 Addition of Enzymes

As a result of gluten absent in the dough, formation of protein network chain which is responsible for the viscoelastic characteristics is lacking. Transglutaminase (TGase) enzyme have been found to significantly change the protein functions, increase cross-joining among gluten free breads, and also promoted their baking abilities (Renzenti et al., 2008). Renzenti et al. (2008), further carried out a study on the use of transglutaminase (TGase) in the absence of gluten and hydrocolloids on gluten free buckwheat and brown rice breads. It was revealed the enzyme was able to introduce batter pseudo plasticity, thereby increasing the rheological structure of the bread and its water-retention ability which culminated in promoting the crumb structure of the bread.

Further research carried out on addition of enzymes to formulations of gluten free breads, confirmed the use of protease enzyme as an ingredient in breads without gluten which also helped to promote the batter qualities of the bread. Some of the proteases applied according to the study includes bacillolysin, papain and subtilisin, which revealed 30-60% improvement in the gluten free rice bread specific volume, reduced 10-30% of crumb firmness, initiated protein compound formation in the batter of the bread when studied using optical microscope, produced bubbles of gas cells which helped in trapping the CO_2 gas molecules during fermentation process, and amounting to a total improvement in the specific loaf size and reduction of crumb firmness when compared to the total control bread (Hatta et al., 2015).

In an attempt to expatiate on the need for the incorporation of enzymes into gluten free bread, Renzenti and Arendt (2009a) further conducted a research to measure the effects it has on gluten free sorghum and corn breads when glucose oxidase is added, and also gluten free rice bread. They found out the addition of glucose oxidase produced a significant positive effect by improving the loaf size and reducing the crumb firmness.

2.5.6 Addition of Hydrocolloids

Application of hydrocolloids in the food industry for gluten free bread production has been very successful due to its extensive properties which include gelling, thickening effect, ability to hold water, enhance dough viscosity and stabilization of the textural structures (Balaghi et al., 2011). Mostly derivatives of plants, microorganisms, seaweeds and fruits. They are commonly found in the form of polysaccharides or protein (Mollakhalili et al., 2014).

Based on a research carried out on gluten free bread production, it suggested hydroxypropyl methylcellulose and xanthan gum are the highly accepted hydrocolloids due to their consistent performance in increasing the rheological parameters of the gluten free bread (Hager and Arendt, 2013). However, other forms of hydrocolloids like the guar gum, Carboxyl methylcellulose, Agarose etc. has also proved to be successful when applied for gluten free bread production (Naji-Tabasi and Mohebbi, 2015).

The use of corn flour for gluten free bread production revealed that xanthan gums, guar gums, locust bean gum, and tragacanth showed significant success due to their binding property and ability to act as gluten replacement in dough without gluten (Acs et al., 1996). Furthermore in the history of hydrocolloids, studies has revealed the use of inulin for production of functional foods due to its prebiotic effect, global acceptability as a fundamental hydrocolloid and its incorporation into production of gluten free bread as dietary fiber enrichment, and improving rheological properties (Kolida et al., 2002; Korus et al., 2006).

Friend et al. (1993), suggested an improvement in water retention capacity when hydrocolloids were introduced during bread making. They further noted that the influence is as a result of the Hydroxyl groups (-OH) present in the hydrocolloids network which created enough space for water movement across the hydrogen bonds. In a way of extending their work, they went further to propose the stability of the dough when 0.5% amount of hydrocolloids were used, it resulted in enhanced stability of the dough. Rosell et al. (2001) also discussed xanthan gum addition during the dough processing to improve the viscosity which as a result induced stability functions to the dough. Viscosity can be modified due to changes in concentratíon, temperature and shear straín stress in a way based on the hydrocolloids and other additives present. Therefore applying different hydrocolloids may help to improve the viscosity or act to lower it. (Marcotte et al., 2001).

Studies on the effect of a combined hydrocolloids in which guar gum and pectin were used to act on different gluten free flour including potato starch, corn starch and corn flour revealed that the bread with guar gum proved the highest quality in terms of volume, moisture present in the crumb and the baking ability (Gambus et al., 2001). Therefore, the effect of the hydrocolloid may change from one bread type to another, depending on their chemical functions, compositions and origin (Rojas et al., 1999).

Further studies carried out on hydrocolloids for gluten free bread production from corn starch proved their binding properties and suitable use as gluten replacement. Acs et al. (1997) discussed application of xanthan gum, guar, locust bean and tragenth to gluten free bread production. The effect was displayed based on their differences, as it proved the bread produced using xanthan gum proved to be most successful with compared qualities as it is in gluten contained bread.

2.6 Nutritional and Health Impact of Corn Starch

Phytochemical properties inherent in the corn (*Zea Maize*) flour are bioactive chemical compounds that resides in the plants that are responsible for human health benefits and serves in lowering chronic illness (Liu, 2004). According to Kopsell et al. (2009), they conducted a research in which they proved that utilizing corn for various purposes provides different phytochemicals like carotenoids, phenolic compounds and phytosterols. The carotenoid found in the corn belong to the red family, orange and the yellow pigments. However, the yellow corn grains commonly used for the gluten free bread production has been found to contain a higher value of carotenoid pigments which by nature are higher source of anti-oxidants. Also present in the maize plant are the phenolic compounds commonly termed ferulic acids, flavonoids, stilbenes, coumarins and tanins (Liu, 2004). Furthermore, it has been proven that among the cereal grains the corn flour possesses an increase amount of ferulic acid followed by barley and wheat flour (Zhao and Maghadasian, 2008).

The presence of anthocyanin in the corn can greatly influence the health of celiac patients due to their anti-carcinogenic properties, lowering the risk of diabetes, anti-inflammatory properties, inducing immune system response and lowering the risk of platelet coagulation which is due to the high anti-oxidant nature of the corn grains (Ghosh and Konishi, 2007). Renzentti et al. (2008), confirmed in their research that the maize flour contains considerable amount of protein, lipids, fibers, carbohydrate etc., and when used in gluten free bread production, they displayed a solid crumb which feels heavy to handle.

The maize kernel which is also blended for its flour purpose has been found to contain considerable amounts of Vitamin C, E, K, B_1 (Thiamine), B_3 (Niacin), B_2 (Riboflavin), also the Vitamin B_5 (Pantothenic acid), B_6 (Pyridoxine), folic acid, selenium and also potassium (Kumar and Jhariya, 2013). A research conducted at the Colorado State University stated that carbohydrate is a significant component of the corn starch. A cup is viewed to contain about 94g of the total carbohydrates, thereby making up to 76% of corn caloric content, and also said to contain dietary fiber that helps in prevention of constipation. In addition, this dietary fibers which plays significant physiological roles and are broken down by gut bacteria in the intestine of celiac patients has been researched upon and it revealed that consumption of 20-35g/d in celiac patients could help to prevent colon cancer, cardiovascular disease and diabetes. (Anderson et al., 2009; Thompson, 2000).

As a result of the damage caused by the gluten in destroying linings of the celiac patients, it has culminated in malabsorption of vital nutrients like iron, calcium, vitamins such as A, D, E, K and folate, corn grain used in producing gluten free products has also been revealed to contain appreciable amounts of iron as its components, therefore can be beneficial when used in producing gluten free bread for celiac disease patients (Cannon et al., 2011).

Kumar and Jhariya (2013), further argued that the consumption of maize kernel which can also be used for the production of corn starch contained sufficient amount of potassium of about 153-163mg/100g. According to their research, they confirmed that potassium in maize has positive diuretic functions when consumed in the right quantity. Sen et al. (2006) suggested that the maize oil extract was linked with fatty acids components and indicated the presence of 54-60% linoleic acid which is responsible for regulating blood pressure, maintaining cholesterol levels and serves in inhibiting cardiovascular problems.

Gluten free bread produced for celiac disease patients has been compromised with dietary fiber deficiency when correlated with normal diet containing gluten (Penagin et al., 2013; Kupper, 2005). In a research conducted by Murphy et al. (2008), they described soluble dietary fiber to have positive impacts on human diet due to their ability to reduce cholesterol level, enable fecal excretion, improve fermentation and the presence of short chain fatty acid in the large intestine, lowers the chances of intestinal cancer, atherosclerosis and issues of obesity.

According to Jiang. (2010), the maize endosperm carries about 39.4mg/100g of resistant starch which when ingested helps to reduce intestinal transit time and culminates in early removal of wastes content through feces from the intestine. Johnson et al. (2010) described the resistant starch present in maize to be significantly helpful in increasing insulin sensitivity in human. Based on the significant nutritional value of maize in possessing different phytochemical properties, its utilization for the gluten free bread production will go a long way in advancing the nutritional desires of celiac disease patients (Kopsell et al., 2009).

CHAPTER 3

RELATED RESEARCH

This chapter contains background information detailing similar and related research studies on the subject of producing gluten free bread for celiac disease patients using corn flour and hydrocolloids as a gluten substitute.

Generally, gluten free bread production using zero gluten flours involves replacement of gluten by using different hydrocolloids and suitable ingredients in order to imitate the elastic behavior of gluten in dough containing gluten, thereby providing elasticity to the dough (Demirkesen et al., 2014).

Investigative studies of celiac disease in the early 1990s described celiac disease to be an irregular disorder, which was attributed to the absence of diagnostic materials (Carlos et al., 2014); however, current epidemiological studies confirmed celiac disease to be a disease found in at least 1% of the global population (Fasano et al., 2012). Kennedy and Feighery (2000), proposed that apart from the use of biopsy diagnosis to evaluate the presence of celiac disease in a patient, Anti-gliadin antibody serological test have proved to be a more definitive and accurate test to detect the presence of celiac disease in an individual.

Further studies has revealed that one of the problems encountered during celiac disease diagnosis involves irritable bowel syndrome (IBS), this is because the signs and symptoms of IBS appear in the same form as that of celiac disease patients and non-celiac patients which include abdominal disorder, swelling of the bowel, gas etc. (Whitehead et al., 1980).

The diet of celiac patients have been found to be deficient of trace elements such as iron, zinc, magnesium, folates, fiber and as a result celiac patients are faced with possible chances of developing obesity and metabolic syndrome, osteoporosis due to calcium deficiency, anemia as a result of insufficient iron in the diets (Gokmen et al., 2011, Krupa et al., 2000).

Research into the use of corn flour for gluten free bread production revealed the increased content of energy after consumption of maize, possess proteins with reduce traces of vital amino acids such as lysine and tryptophan, also deficient is vital minerals, deficient source of vitamin B and responsible for little quantity of vitamin B_3 (Niacin) (Losak et al., 2010;

Zeng, 2010). However, further studies for gluten free bread production indicated rice flour (*Oryza Sativa*) and corn flour (*Zea mays*) has been highly suitable when blended together for use, due to the flavor they emit, low allergenic effects on people and are found in commercial quantity (Kadan et al., 2001). Based on this understanding, the use of hydrocolloids has been used as an additive for promoting the virtue of zero gluten bread, increase gas retention, binding of the water molecules and stability of the gluten free batter (Casper and Atwell, 2014).

Further investigation into promoting the totality of zero gluten bread suggested the use of dietary fiber as an ingredient due to their health gain which involves blood sugar levels reduction, risk of constipation reduction and enabling good bowel functions. They have also been viewed based on their physicochemical functions which include their capacity to increase viscosity and promote the textural structure of the overall product. (Kaczmarcyzk et al., 2012; Collar et al., 2007).

In a similar research carried out on the effect of dietary fiber addition, it confirmed that Psylium fiber, β-glucan, corn fiber and locust bean gum when included for gluten free bread production, it produced an increased volume and soft crumb when compared to gluten free bread that possess no fiber material (Perez-Quirce et al., 2014; Martinez et al., 2014).

The moisture present in the gluten free bread has great effect on the crumb and is usually based on the influence of the flour specificity, additives, and the method of baking (Barcenas and Rosell, 2005); this confirmed the research conducted by Mandala et al. (2007) on the use of different hydrocolloids such as xanthan, hydroxylpropyl methylcellulose, guar gum for the production bread, found out the sample containing hydroxylpropyl methylcellulose possessed reduced crust size and also involved in the lowering of water movement, thereby maintaining moisture in the crumb.

Constantini, et al. (2014) studied the use of 10% whole chia flour as a substitute of buckwheat flour for gluten free bread production. They noted tremendous result in protein yield, lipids, dietary fiber, ash, α-linoleic acid which culminated in higher antioxidant functions and increase in phenolic compounds. Investigative studies has been conducted on legume flours for the gluten free bread production and in order to improve its technological and organoleptic properties, composite flours of soy, chickpea, carob germ and vinal has been incorporated (Minarro et al., 2012). More research also revealed 15% of the carob germ incorporated to rice flour for producing a gluten free bread amount to a better physicochemical functions with the bread displaying high potential of dietary fiber to the range of 6.1%, protein 8.4% and the presence of essential minerals (Shin et al., 2013).

Commercially produced gluten free formulations has demonstrated poor crumb and crust properties, low quality of flavor and poor mouth feel due to the abundance of starch associated with gluten free bread (Moroni et al., 2009). However, research proved that with the addition of pseudo-cereals such as amaranth, buckwheat, quinoa and grains such as millet, brown rice, sorghum and teff, the nutritional and rheological constituents of the zero gluten bread increased very well (Alvarez-Jubete et al., 2009); In addition, the viscoelastic nature of the gluten free formulation were found to increase when hydrocolloids were added (Kittisuban. et al., 2014).

Research into the use of hydrocolloids has revealed various hydrocolloids applied in the food industry have produced a broad result based on their nutritive and physiological properties. Some of them include β-glucan, pectin, inulin, guar gum, gum Arabic, carboxyl methylcellulose, hydroxylpropyl methylcellulose, psyllium, carrageenan etc. (Pentikainen. et al., 2014).

In a scientific research carried out it was noted that dissolved xanthan gum distribution is time-dependent and can be viscous when applied in small concentrations, therefore can be used in the thickening and stabilizing of gluten free batters (Morris, 1995). This fractions is as a result of the xanthan particles forming compounds through hydrogen linkage and polymer associations which enables the xanthan gum to possess high viscous properties when applied at low concentration under low shear rate (Sworn, 2000).

Based on these findings, further research proved xanthan gum to be viscous in hot or cold water solutions even at small quantities and demonstrate better stability also in acidic solutions (Dreher, 1999), therefore when it is used in the absence of gluten to produce gluten free bread, increasing its concentration produced significant effect of pseudo plastic behavior and also stabilizing the batter quality of gluten free bread (Morris, 1995).

In an investigative research on the use of hydroxylpropyl methylcellulose, it found the hydrocolloid to be a cellulose derivative gotten by binding hydroxypropyl and methyl groups together usually to a β-1,4-D-glucan cellulosic backbone which enables it to be soluble in water and possess an increase surface activity regardless of the modification in temperature (Sarkar and Walker, 1995). The influence of hydroxylpropyl methylcellulose was tested on the gluten free teff breads, rice breads, buckwheat and maize bread. It revealed a linear effect with a level of linear positivity size of the teff breads, negative linear display on rice breads, and no significant change on the buckwheat bread and on corn breads (Anna-Sophie and Elke, 2013).

Addition of hydroxylpropyl methylcellulose proved to contribute sameness around the batter of gluten free bread due to its ability to bond with the aqueous and non-aqueous state of the batter, thereby creating an artificial coatings around the edges of gas cells and gives support to the gas cells during fermentation (Bell, 1990).

Hydrocolloids has also been used for producing chapatti breads using rice flour and it revealed a significant positive effects on the bread due to the ability of the hydrocolloids to regulate water activity by binding with the water molecules thereby causing a distortion in the plastic nature of the crumb and causing moisture movement from the crumb to the crust (Mandala et al., 2007). This has impacted the crumb positively in starch retro gradation of the bread by inhibiting flow and loss of water molecules from the crumb. (Elke and Dal-Bello, 2008).

CHAPTER 4
MATERIALS AND METHOD

4.1 Materials

4.1.1 Gluten Free Bread Ingredients

The gluten free bread was formulated using the mixture of corn starch, rice flour and other additives which include sugar, salt, gelatin, Turkish mahlep, baking powder, yeast, olive oil, butter and milk. All these ingredients were bought from a local supermarket in Lefkosa, North Cyprus. The Hydrocolloids used in making the bread are food grade which include the K-Carrageenan, Carboxyl methylcellulose, Guar gum and Xanthan gum. All the hydrocolloids used was brought in from Tabriz University, Iran. The effects of this hydrocolloids on the dough and final bread product were determined for their texture, protein, ash, wet gluten and sensory properties.

4.1.2 Preliminary Bread Making Experiments

Preliminary studies were carried out on the gluten free bread in the kitchen of Nutrition and Dietetics Department of Near East University, Nicosia, North Cyprus, to ascertain the proper amount of the ingredients that will be suitable for the preparation of the gluten free bread. Various equipments were also employed for method validation of the effect of the hydrocolloids on the dough.

The preliminary experiments was carried out using corn starch as the flour and other ingredients including salt, sugar, gelatin, yeast, olive oil, butter and milk (See Table 4.1). The bread samples were analyzed with different hydrocolloids including guar gum, carboxy methylcellulose, carrageenan and xanthan gum.

The gums were used separately and also as mixtures of them (Table 4.1). After the ingredients for the bread dough were combined, the dough was kneaded by hand until it was smooth and elastic. After kneading, the dough was allowed to rest and ferment for at least a minimum of 1 hour. Thereafter, the dough was splitted into smaller portions and then again allowed to rest for about 10-15 minutes before shaping. After the bread have been proofed, they were baked at 150-200ºC for 25-30 minutes.

Table 4.1: Amount of ingredients used in preliminary gluten free bread experiments

Ingredients	I (g)	II (g)	III (g)	IV (g)	V (g)	VI (g)
Corn starch	100	100	100	100	100	100
Salt	1	1	1	1	1	1
Sugar	5	5	5	5	5	5
Gelatine	1	1	1	1	1	1
Gums(X, G, K, CMC)	0	1gX	1gG	1gK	1gC	Mix. of gums (2g)
Yeast	4	4	4	4	4	4
Olive oil	5	5	5	5	5	5
Butter	5	5	5	5	5	5
Milk	80	80	80	70	80	80

The hydrocolloids includes X (Xanthan gum), G (Guar gum), K (K-Carrageenan) and CMC (Carboxy methycellulose). The control dough using only the corn starch without any hydrocolloids gave a fluid and shapeless dough and even after baking, the dough was scattered as seen in Figure 4.1

Figure 4.1: Image showing the fluid nature of the control dough

The addition of gums helped to obtain a smooth dough and better shape. However, there was no rise after fermentation in all experiments due to the absence of gluten protein.

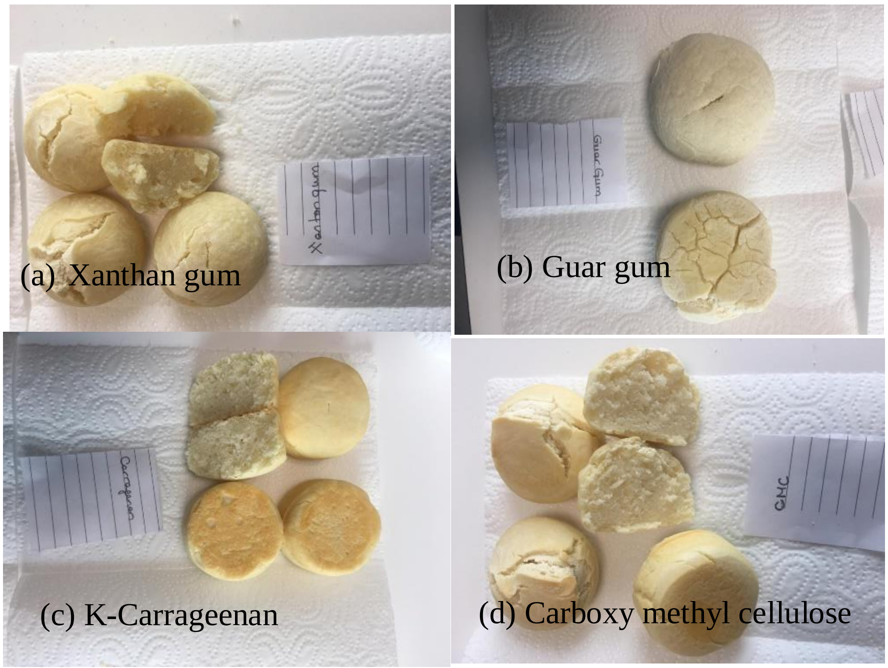


Figure 4.2: Image displaying different bread sample using only corn starch and hydrocolloids.

The next step was followed with the addition of rice flour to the corn starch as seen in Figure 4.3. Although, the end result gave rise to a flexible, softer and better dough, still there was no good fermentation and rise in the dough.

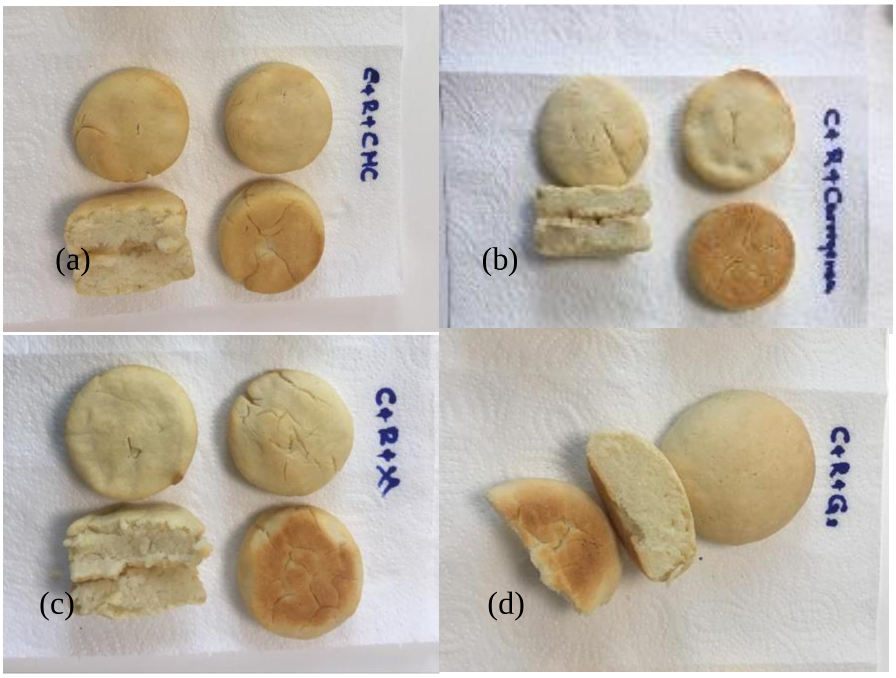


Figure 4.3: Image displaying different bread samples with the addition of rice flour to the corn starch

Further experiments were carried out with the addition of garri, chickpea and pounded yam (composite flour of potatoes flour, corn flour and yam flour) to the corn starch. Other ingredients were also added along with yeast for proper fermentation.

Finally, after observing no significant rise in dough using the yeast, a decision to add baking powder as a yeast substitute along with a Turkish traditional spice (Mahlep) to improve the taste was carried out. A good result was obtained regarding the textural properties and flavor. The final experiment was therefore carried out based on this recipe.

Figure 4.4: Final preliminary bread sample using baking powder

4.1.3 Characterization of the Bread Formulation

Combining the formulations used in preparing the bread, baking powder was viewed as a replacement for the yeast based on the quality of the bread produced when it was used as an additive. The chickpea being a good replacement for protein in the formulation was not used for the final preparation as a result of sour taste associated with it when used in the preliminary stages of the bread making. Furthermore, the garri flour which is locally produced from the cassava proved not to be a suitable flour due to the wetness revealed in the bread after its final production. The gluten free bread was finally prepared using some selected ingredients as seen in Table 4.2 that provided better quality of the bread.

4.1.4 Gluten Free Bread Experiment

After several trials carried out in the preliminary stages with different combinations of the gluten free flours, 2g of baking powder combining with the hydrocolloid and other ingredients displayed optimum sensory properties.

The final experiment was carried out at a bread institute in Turkey. All ingredients used for the preparation of the bread was bought at a local market in Ankara, Turkey. The formulation includes corn starch, rice flour and other additives. The control bread was prepared without the addition of hydrocolloids, only the corn starch; rice flour and two commercial flours along with other ingredients was used for the control. The gluten free bread formulation was finally prepared using 1g of the xanthan gum, guar gum, and k-carrageenan and carboxy methylcellulose. The breads were baked immediately after adding the baking powder at 215ºC for 25 minutes in the oven. The weights of each sample was recorded 2 hours after the bread has been brought out of the oven and allowed to cool down.

Table 4.2: Gluten free bread formulation

Ingredients	Amount (g)
Corn starch	50
Rice flour	50
Turkish mahlep	1
Salt	1
Sugar	7
Gelatin	1
(K-carrageenan, Xanthan, Guar and Carboxyl methylcellulose gums)	1
Baking powder	2
Olive oil	5
Butter	5
Milk	55-70

With the addition of milk to increase the protein content of the bread, the experiment was carried out according to the flow chart shown in Figure 4.5.

Weighing of ingredients
↓
Mixture of ingredients (Kneading of dough)
↓
Division of dough
↓
Shaping to obtain a uniform size
↓
Baking/Cooking of dough
↓
Cooling
↓
Weighing after 2 hours

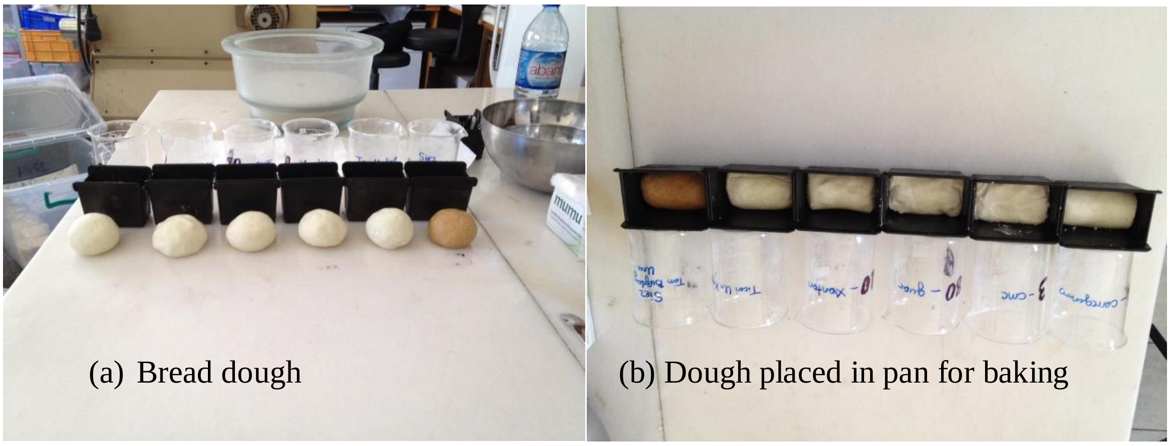
Figure 4.5: Flow chart of bread preparation

The making of the gluten free bread started by weighing all the ingredients needed for the formulation of the bread. The next step involve the mixture and kneading of the dough in order to obtain a uniform mixture of the ingredients. The addition of the ingredients was based on a specified sequence which include first adding of the dry ingredients followed by the butter, olive oil and milk for proper homogenization.

Figure: 4.6: Weighed ingredients ready for bread making

The dough was further rolled, divided and placed in a greased baking pan and transferred into the oven. The baking or cooking process was carried out in an electric oven at the specified time and temperature. The baked bread was later brought out of the oven and allowed to cool down for two hours at room temperature after which their weights was recorded.

Figure 4.7: Dough undergoing rolling for proper shaping and sizing



(a) Bread dough

(b) Dough placed in pan for baking

Figure 4.8: Displayed prepared dough ready for baking

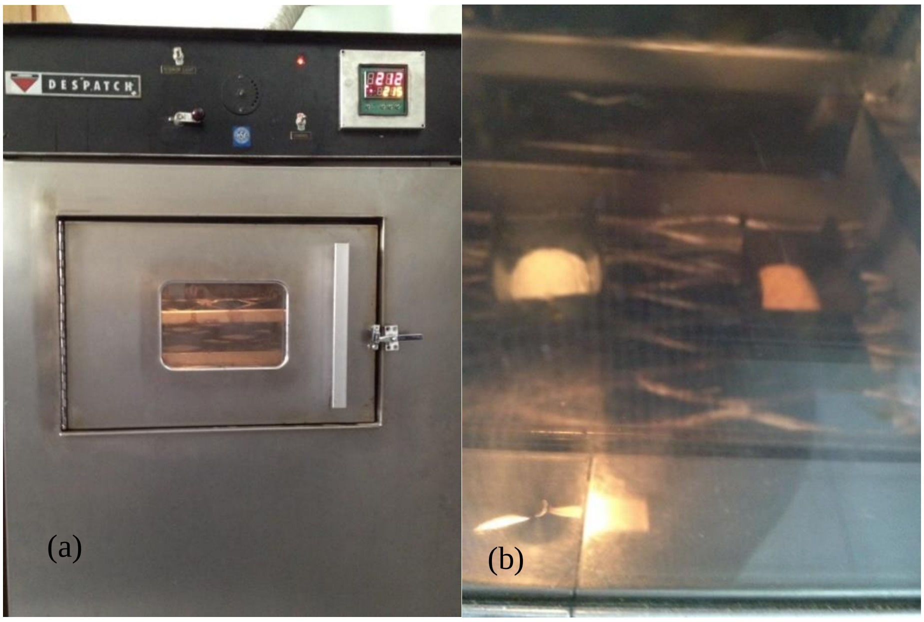


Figure 4.9: Displayed image of oven used for bread baking and bread undergoing baking

4.2 Methods

The effects of the hydrocolloids and their combinations on the gluten free breads was determined for their texture, protein, ash, wet gluten and sensory properties under specified conditions. The methods used for analyzing the samples was based on the international approved methods of the American Association of Cereals Chemist (AACC)

4.2.1 Crumb Firmness Determination

The firmness of the crumb was analyzed in line with the AACC method 74-09, using a texture analyzing machine (Stable microsystems, TA-XT plus, Godalming, Surrey, England). The primary purpose of this method is to significantly determine the force needed to compress a baked product by a preset distance. The texture analyzer was equipped with 2 kg load cell and a 36 mm cylindrical probe was utilized for the texture determination. The force (firmness) needed to compress 40 % of two bread slices (1.25 cm each) was analyzed at 1.7 mm/sec test speed.

Apparatus:

- A universal testing machine (Stable microsystems, TA-XT plus)
- A compression load cell of 2 kg
- 36 mm cylinder probe (Aluminum plunger)

Procedure:

- The total thickness of the bread used is 1.25cm each. The loaves were sliced mechanically.
- Two slices of the bread stack together was used for the test sample

Method:

- The upper head cross limit of the machine was positioned so that the compression plunger is 1mm above center surface of the bread sample
- The lower crosshead limit was positioned at 40 % compression (10 mm compression depth)
- The crosshead speed (rate of compression) was set 1.7 mm/sec
- The scale (range) was set at 2 kg
- The prepared bread sample was positioned under the compression plunger, avoiding any irregular or no representative areas of the crumb.
- The sample was compressed approximately 10 mm (40 % compression)
- The compression plunger was returned to its upper limit position
- Test slices was discarded

Figure 4.10: Stable Microsystems TA-XT plus Texture Analyzer

4.2.2 Protein Evaluation

The Dumas nitrogen analyzer Velp-NDA 701 was used in accordance with the AACC method 46-30 (2000). The method suggests a flaming method for analyzing the total protein. Flaming at an increased temperature in pure oxygen releases the nitrogen, and is then calculated by heat conductivity detection. All the nitrogen in the sample is analyzed and changed to a similar protein by multiplying with the factor of 6.25 to get the protein content.

Apparatus:

- Any instrument capable of measuring nitrogen by combustion method can be used for the analysis as long as it has the ability to maintain minimum operating temperature of 950 ºC for combustion of sample in pure oxygen (99.9%).
- Contain an isolation system with the ability to isolate free nitrogen gas from other combustion materials for further measurement by thermal conductivity detector.
- A detection system that can interpret detector response
- Finally, can calculate nitrogen in products containing 0.2-20% nitrogen.

Procedure:

- The samples were weighed into a tin capsule and then released into a combustion reactor in an automated Dumas nitrogen analyzer Velp-NDA 701, where it is measured via a built-in gas chromatograph.

Method:

- The carbon in the sample were converted to carbon dioxide as a result of flash combustion process.
- Nitrogen oxides are produced in the process and are then reduced to nitrogen in a copper reduction column at a high temperature range of about 500-600 ºC
- The total nitrogen (Nitrate and Nitrite) that is produced in the process is then carried by pure helium gas and calculated by gas chromatograph with the aid of a thermal conductivity detector.
- Finally, the nitrogen is then converted to protein content in the sample using a protein conversion factor.

Figure 4.11: Image of The Dumas nitrogen analyzer Velp-NDA 701

4.2.3 Dry Ash Analysis

Ash being the residual inorganic matter that remains after water and other organic content of the sample have been removed via heating and in the presence of oxidizing materials was carried out to provide information on the measured amount of minerals resident in the gluten free bread.

The analysis was conducted using samples of bread slices that was dried at 30 ºC and carefully grounded to expose the surface area and for maximum moisture reduction. Thereafter, the grounded samples were analyzed.

Procedure:

- A measured amount of 4-10g of grounded sample was weighed into a crucible
- The crucible was placed in a cool muffle furnace
- Heat was applied to the crucible for a period of 10-18 hours at a temperature of 450-550 ºC.
- At the end, the furnace was turned off and temperature allowed to drop to about 250 ºC or lower.

- Safety tongs was used to quickly transfer crucibles to a dessicator with a porcelain plate. The dessicator was closed and the crucibles covered to allow for cooling before weighing.

The ash content was calculated based on the percentage ash (dry basis)

$$\frac{\text{Weight after ashing} - \text{weight of crucible}}{\text{Original sample weight x dry matter coefficient}} \quad \text{x } 100$$

Where, dry matter coefficient = % solid/100

4.2.4 Wet Gluten

The analysis was carried out by weighing 20g of flour mixture into a bowl. Making a dough out of it and thereafter washing the dough with 2% of salt solution by placing it on the palm of the hand. After thorough squeezing, the flour materials were washed out leaving the gluten. The final material left on the palm of the hand is retained as the gluten. However, in our own case, all the ingredients were washed out and there was nothing on hand as gluten.

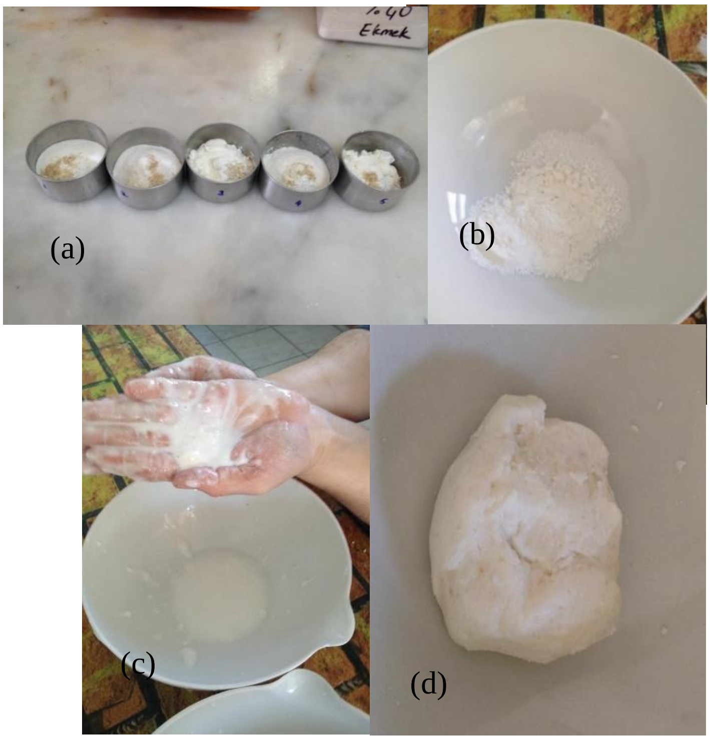


Figure 4.12: Wet gluten analysis

4.2.5 Sensory Evaluation

The bread samples of the four hydrocolloids including the control bread and commercial flour bread were evaluated by 8 semi trained panelist for the determination of sensory characteristics of the gluten free bread samples like the general appearance, odor and taste, color and mouth feel. The samples were analyzed based on a 5-point hedonic scale.

Respondents

A total of 8 semi trained panelists participated in the sensory study. They originated from different countries and were aged between 23 and 56 years, with a total of 5 females and 3 males.

Procedure:

- Accurate information regarding the samples was given to the panelist
- The samples were arranged and served on a plate
- Water was kept by the side for proper mouth rinsing
- The panelist were told to use a 5-point hedonic scale which include (1= not satisfactory, 2= fair, 3= medium, 4= good and 5= excellent) to evaluate for product general appearance, odor and taste, color and mouth feel.
- One slice was given to each taster
- Each taster gave a remark based on the 5-point hedonic scale

After the analysis, sensorial data was statistically calculated based on records given by the panelists

CHAPTER 5

RESULTS AND DISCUSSION

The analysis of the bread samples was carried out in accredited bread institute in Ankara, Turkey in accordance with standard international method of bread analysis and procedures. The equipments used for the analysis were properly calibrated to standard for machine assurance and to avoid incorrect results at the end of the analysis. Final results of gluten free bread samples are given in Figure 5.1.

Figure 5.1: Cross section of overall bread appearance after baking

Table 5.1: Recorded weights of bread sample after two (2) hours of baking

Samples	1^{st} experiment (g)	2^{nd} experiment (g)	Ave ±SD
Bread with CMC	161.97	159.92	160. 95 ± 1.45
Bread with K	171.83	166.94	169. 39 ± 3.46
Bread with G	165.93	161.77	163.85 ± 2.94
Bread with X	161.70	163.86	162.78 ± 1.53
Control bread	153.82	154.34	154.08 ± 0.37
Commercial flour	151.73	152.54	152.14 ± 0.57

5.1 Crumb Firmness

Two successive test was carried out for each bread sample and the results were recorded using the stable microsystems TA-XT plus texture analyzer.

Table 5.2: Crumb firmness data for each bread sample

Samples	1st experiment	2nd experiment	Average
Carboxyl methyl cellulose	10900	13159	12030
K-carrageenan	11913	10456	11185
Guar gum	11648	10392	11020
Xanthan gum	13752	17610	15681
Control bread (without gum)	5521	5445	5483
Commercial flour	12122	11381	11752

Figure 5.2: Displayed control bread undergoing textural crumb firmness analysis

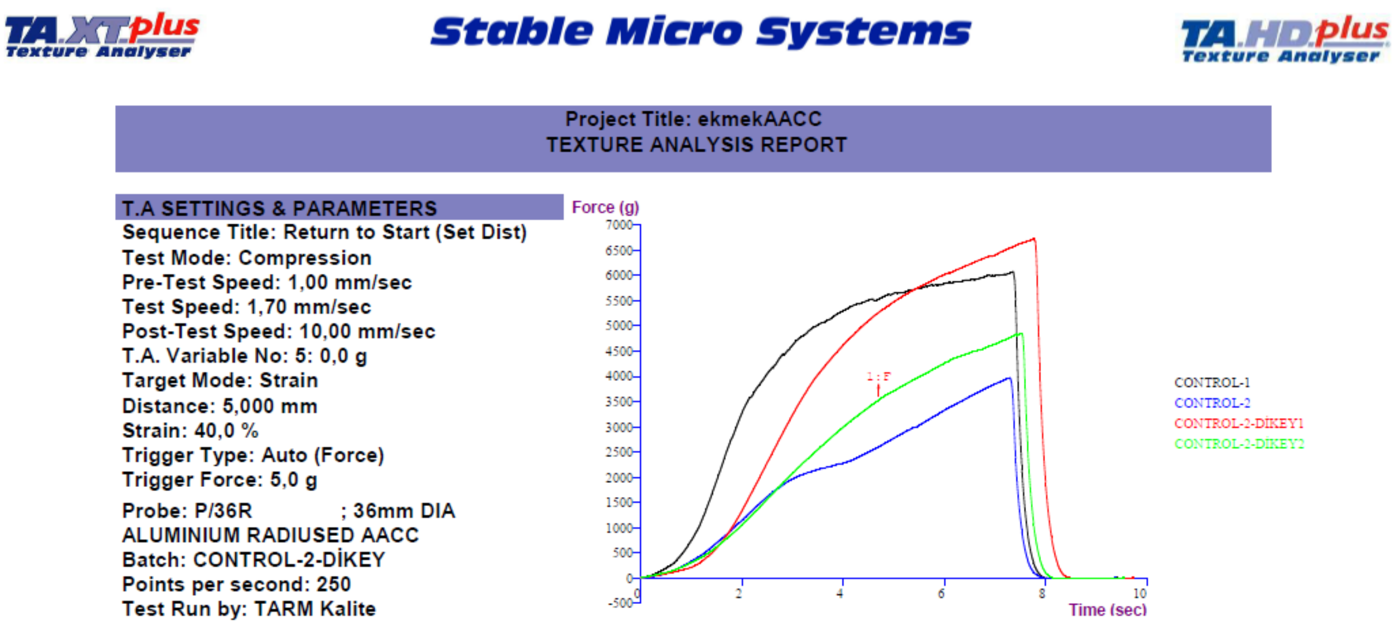


Figure 5.3: Graphical display of the Texture (crumb firmness) report for control bread

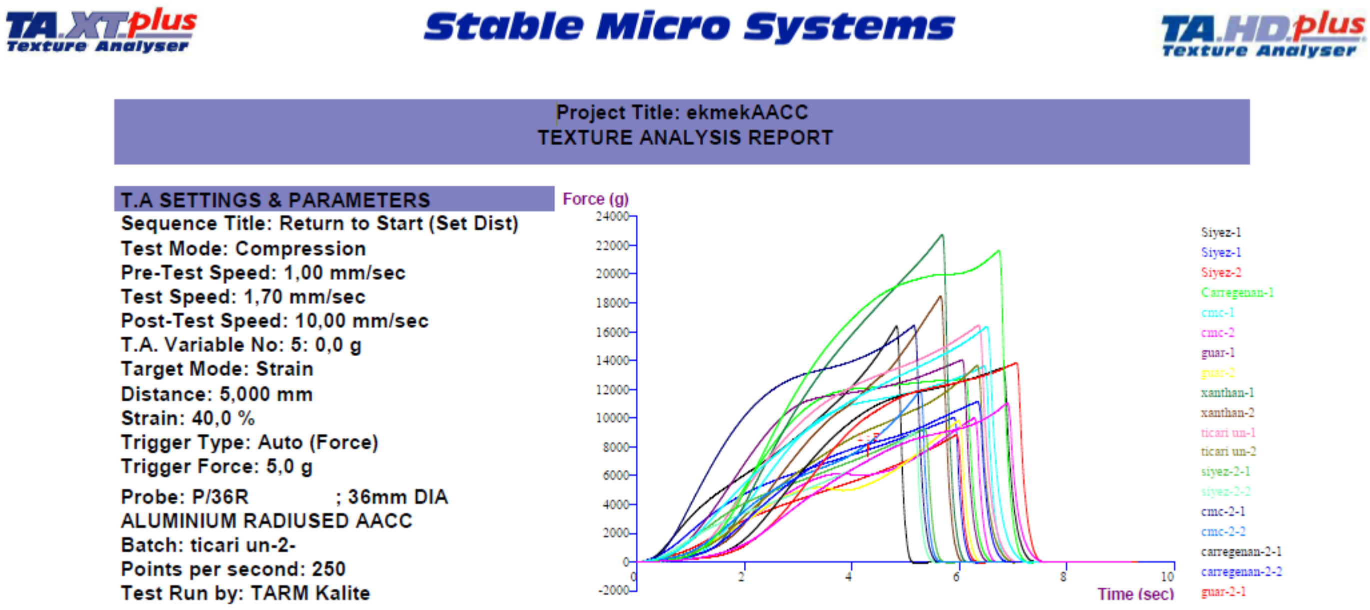


Figure 5.4: Graphical textural analysis report for the bread samples containing X, G, K and CMC

5.2: Protein and Ash analysis

The result as seen in Table 5.3 was calculated based on average of three (3) replicates of the bread sample.

Table 5.3: Protein and Ash determination result

Bread samples	Dry matter (Air dried)	Ash % 1st exp.	Ash % 2nd exp.	Protein % 1st exp.	Protein % 2nd exp.
Control bread	9.85	1.48 ±0.02	1.64 ±0.03	5.59 ±0.08	6.20 ±0.09
Xanthan bread	7.39	1.46 ±0.04	1.58 ±0.04	5.29 ±0.15	5.71 ±0.16
K-C bread	7.28	1.57 ±0.02	1.69 ±0.02	5.77 ±0.93	6.22 ±1.00
Guar bread	7.03	1.50 ±0.05	1.61 ±0.06	5.52 ±0.31	5.94 ±0.34
CMC bread	7.94	1.55 ±0.04	1.68 ±0.04	4.88 ±0.19	5.30 ±0.21
Commercial Flour bread	7.91	2.19 ±0.01	2.37 ±0.01	3.72 ±0.14	4.04 ±0.15

There is a significant increase in protein and ash content of all bread samples in the second (2nd) experiment, including their standard deviation.

5.3 Sensory Evaluation of Gluten Free Bread

The central tendency approach which averages scores for each gluten free bread sample under different quality attributes and overall acceptance based on the respondent's preferences is summarized in Table 5.4.

According to Table 5.4, the gluten free bread samples containing xanthan gum, and k-carrageenan were ranked higher by the panelist. This was followed by the bread samples containing guar gum and carboxy methylcellulose. The commercial brand and control bread (without hydrocolloids) were seen to be less accepted by the panelist.

Table 5.4: Average score of gluten free bread samples analyzed for different quality attributes and overall acceptance.

Gluten free bread samples	General appearance	Color	Odor and taste	Mouth feel	Average	RSD %	Rank
Xanthan gum	4.25	3.88	3.88	3.88	3.97	22	1
K-carrageenan	4.00	4.50	3.75	3.63	3.97	23	1
Guar gum	4.13	4.63	3.50	3.38	3.91	25	2
CMC	3.88	4.25	3.75	3.75	3.91	23	2
Commercial brand	4.38	4.38	3.00	3.50	3.81	27	3
Control bread	3.88	3.88	4.00	3.25	3.75	24	4

Based on the relative standard deviation, the uncertainty of the evaluation was 24%.

Furthermore, there is no significant difference between breads average sensory values since the F-calculated is smaller than F-critical as can be seen in Table 5.5

Table 5.5: ANOVA (Single factor)

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F-critical*
Between Groups	1.229167	5	0.245833	0.285335	0.920708	2.262672
Within Groups	160.25	186	0.861559			
Total	161.4792	191				

The information gathered by the panelist during the sensory evaluation as organized in Table 5.6 was to reveal the importance of the sensory quality attributes in general. Based on the choice of preference given by the panelist, the odor and taste was found to be the most important sensory quality attribute of the gluten free bread followed by the mouthfeel, colour and finally the general appearance.

Table 5.6: Total number of respondents giving their preferences for the quality attributes of gluten free bread in general.

Sensory quality attributes of samples	Not at all important	Somewhat important	Important	Highly important	Extremely important
General appearance	0	0	4	4	0
Color	0	0	5	3	0
Odor and taste (Flavour)	0	0	0	2	6
Mouth-feel (Texture)	0	0	1	1	6

Figure 5.2: Displayed slices of gluten free bread samples used for sensory analysis

5.4 Discussion

The control (without hydrocolloids) dough as seen in (Figure 4.1) displayed a fluid dough when compared to a wheat dough. This is due to the absence of gluten in the corn starch used for the making of the bread. This findings comply with the research work of Moroni, et al. (2009) where they stated that commercially produced gluten free bread formulations demonstrate poor crumb and crust properties, low quality of flavor and mouth feel due to the abundance of starch associated with the gluten free bread. According to Alverez-Jubete et al. (2009) addition of grains such as rice, sorghum, millet and teff to the gluten free bread improved the nutritional and rheological constituents of the bread. Therefore, rice flour was added to the formulation of the gluten free bread to further improve the textural properties of the bread.

During the preliminary phase of the analysis, the addition of yeast did not result to an increase in the dough even after one hour fermentation process. However, the addition of the hydrocolloids increased the visco-elastic properties of the dough and also reduced bubble coalescence during fermentation. According to the preliminary studies, there was no rise in the dough due to poor fermentation by the yeast and poor color of the bread made with yeast. Therefore, baking powder was used as a substitute to compare the both result. The study revealed the bread formulation baked with baking powder displayed a good brown color with satisfactory appearance and improved crumb texture as seen in (Figure 4.4) which can be compared to a normal wheat bread.

The data of the crumb firmness for the bread samples showed high level of stability after baking and the bread did not collapse after cooling, which means the hydrocolloid network was properly developed. A typical example is seen in Table 5.2, in which 40% compression for each bread sample obtained revealed crumb firmness for experiment 1 to be 10900 for carboxy methylcellulose, 11913 for K-carrageenan, 11648 for Guar gum, 13752 for Xanthan gum, 5521 for Control bread and 12122 for commercial bread. This result shows the firmness level of the bread increased with respect to time.

However, the xanthan gum at 13752 for experiment 1 and 17610 for experiment 2 proved to display a higher crumb firmness than other bread samples. Comparing the result with that of wheat bread as a reference, a previous study showed wheat bread has 5-6kg of force needed to compress 60% of bread baked on the first day (Kiskini et al, 2010).

Comparing the firmness result with the research carried out by Jai and Bawa (2000), they noted that the reduction of firmness in bread by the addition of gums could be as a result of water absorption. However, milk was used in place of water for this research thereby enabling an increase in firmness as seen in the bread samples, especially in xanthan bread sample with the highest crumb firmness result.

The result for the protein and ash properties in the bread sample is an average of three determinations of the experiment. Taking the whole wheat bread as a reference, the average protein present in a bread ranges between 8-15g protein and 0.6-1.2% ash per 100g of bread, and this is dependent on the type of bread. The results obtained from the first and second protein analysis as seen in (Table 5.3) showed a low protein content in the gluten free bread as compared to the wheat bread. This is viewed as a lack of gluten protein in the corn starch and rice flour ingredients used for the preparation of the gluten free bread. The sample with the lowest protein as seen in (Table 5.3) is the commercial flour sample with an average of 3.72 in the first experiment and 4.04 in the second experiment. The carrageenan bread sample proved to contain a higher protein when compared to other samples with an average of 5-77% in the first experiment and 6.22% in the second experiment. Based on the result obtained, none of the gluten free bread sample measured up to the amount of protein present in the wheat bread. This is a direct confirmation that the absence of gluten protein in the gluten free bread do not only affects the viscoelastic properties of the dough, but also reduces the protein content of the gluten free bread.

Comparing the data obtained for the ash analysis with that of a wheat bread, the result proved the percentage (%) ash content obtained from the first and second experiment was higher as seen in (Table 5.3) when compared to the percentage (%) ash content of a wheat bread when used as a reference.

CHAPTER 6

CONCLUSION AND RECOMMENDATIONS

6.1 CONCLUSION

- The textural analysis proved crumb firmness data of the gluten free bread samples was changed in a range of 10900-17610, while the control bread firmness was 5445. The control bread was therefore seen to be softer than the others, however it displayed signs of crumbliness as seen in Figure 5.2.
- We used milk and gelatin to supply protein to the gluten free bread. The protein and ash content revealed a range of 4.80%-6.22% for the protein and 1.48%-1.69% of ash present in the bread samples.
- The sensory evaluation of the gluten free bread was carried out to determine the sensory quality attributes and ranking of the gluten free bread sample based on the panelists decisions. According to the choice of preference by the panelists, odor and taste impacted the most, followed by mouth-feel. The colour and general appearance was found to be the least attributes. All the gluten free breads were ranked better on an approximate average value of four (4) based on the 5-point hedonic scale. Breads containing xanthan and K-carrageenan were found to be of higher acceptance.

6.2 RECOMMENDATIONS

- Due to the absence of gluten protein in the gluten free breads, protein deficiency is a major threat to consumers, therefore, protein substitutes like whey protein and chickpea protein should be recommended as additives to the gluten free bread formulations.
- Food producing companies should innovate ways of producing gluten free breads with locally made formulations other than the use of hydrocolloids

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