بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

Sudan University of Science and Technology

College of Graduate Studies

Effect of Transglutaminase Enzyme on the Preparation and Quality of Gluten Free Composite Flour Bread

تأثيرانزيم الترانسقلوتامنيز في تحضير و جودة خبز الدقيق المخلوط الخالي من الجلوتين

A Thesis Submitted to Sudan University of Science and Technology in Partial Fulfillment for the Requirements of Master Degree in Food Science and Technology

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قال تعالى

(وَهُوَ الَّذِيَ أَنزَلَ مِنَ السَّمَاء مَاء فَأَخْرَجْنَا بِهِ نَبَاتَ كُلِّ شَيْءٍ فَأَخْرَجْنَا مِنْهُ خَضِرًا نُّخْرِجُ مِنْهُ حَبًّا مُّتَرَاكِبًا وَمِنَ النَّخْلِ مِن طَلْعِهَا قِنْوَانٌ دَانِيَةٌ وَجَنَّاتٍ مِّنْ أَعْنَابٍ وَالزَّيْتُونَ وَالرُّمَّانَ مُشْتَبِهًا وَغَيْرَ مُتَشَابِهٍ انظُرُواْ إِلَى ثَمَرِهِ إِذَا أَثْمَرَ وَيَنْعِهِ إِنَّ فِي ذَلِكُمْ لَآيَاتٍ لِّقَوْمٍ يُؤْمِنُونَ).

صدق الله العظيـــــم

سورة الأنعام الآيه (99)

Dedication

I dedicate my dissertation work to my family and friends. To my father who taught me that the best kind of knowledge to have is that which is learned for its own sake.It is also dedicated to my mother, who taught me that even the largest task can be accomplished if it is done one step at a time.

I dedicate this dissertation to my husband, Bader-Aldin Mohammed Ali Agab, who have supported me throughout the process.

My daughters Mozn and Maab,who have never left my side and they are very close to my heart. Both of you have been my best Encouragers. I will always appreciate all they have done.

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I also thank my family who encouraged me and prayed for me throughout the time of my research.

ABSTRACT

The aim of this work was to determine the impact of adding transglutaminase (TGase), to sorghum, corn and rice composite flour on the physicochemical and sensory properties of gluten-free breads. Samples were produced from flour containing of 0.0 (A), 5.0 (B), 10.0 (C) and 15.0 (D) µg TGase /g of protien.

The chemical composition of gluten free bread flour has moisture, ash, fat, fiber, protein and carbohydrate contents of 9.66, 1.93, 2.09, 7.28, 11.35 and 67.60 % respectively. Also the gluten free mixture was found to be good in magnesium (17.04 mg), calcium (12.73 mg) and low in potassium (0.519 mg) and phosphorus (0.658 mg) while it was free of Iron.

The samples were weighed in the following portions to prepare gluten free bread: White rice flour 34.5 %, Sorghum 30 % ,Corn flour 22 % ,Skimmed milk 12.5 % ,Xanthan gum 1%, Yeast .5% , Salt .25% and Sugar .25%.

The functional properties of gluten free bread were determined and the water absorption capacity of the samples (control (A) 0.0µg TGase /g of protien, (B) 5.0µg TGase /g of protien, (C) 10µg TGase /g of protien ,(D) 15µg TGase /g of protien and (E) wheat flour were 81, 79, 80, 81 and 78 % respectively, The emulsification capacity results were 40.9, 40.9 ,41.9 , 42.9 and 42.9 % respectively . Foaming capacity results were 6, 9.6 , 7.5 , 5.35 and 5 , Foaming stability results were 1, 1.9 ,0.95 , 0.54, and 5.0 % respectively. Bulk density results were 0.84, 0.83, 0.82, 0.81 and 0.82 % respectively. Swelling capacity results were 6, 7, 8, 10 and 8 % respectively. TGase has an impact on the water-holding capacity. It is presumed that the protein network formed by TGase has the ability to trap water and, hence, cause an increase in the water-holding capacity.

The protein fractions were determined and the albumin content of the samples A,B,C,D and E were 2.70, 2,20, 2.10, 2.80 and 1.80 % respectively,

Prolamin were 1.30, 1.0 , 1.9, 1.30 and 1.90 % respectively. Globulin were 2.70, 1.70, 2.90, 3.00 and 4.60 % respectively. Glutelin were 1.70, 2.60, 2.80, 2.30 and 2.40 % respectively.

The protein solubility were determined and the solubility of the samples A, B, C, D, and E at pH 4 were 4.00 , 3.1 , 4.9, 3.5 and 2.2 % respectively, while, at pH 6 were 3.00 , 6.30, 5.30, 3.10 and 2.20 % respectively.

Addition of TGase in different percentages to gluten free flour significantely changed the values of farinograph . The water absorption of the samples A,B,C and D were 39.2, 39.2, 38.0 and 39.4 % respectively. The dough stability values of the samples A,B,C and D blend were 1.1, 0.1 , 0.9 and 0.0min respectively. The dough development time of the samples A, B, C and D were 10.2, 8.6 9.9, and 9.5min respectively.There is positive correlation between addition of TGase and water absorbtion when compared with wheat flour.

Sensory evaluation of loaf bread showed that loaf bread made from gluten free flour with 15µg/g of protein gained the highest score of general acceptibility among the GF bread 3.05 .

ملخص الأطروحة

أجريت هذه الدراسة لمعرفة تأثير إضافة أنزيم الترانسقلوتاميناز بنسب 0.0، 5، 10 و 15 ميكروجرام/ جرام البروتين لخلطة الدقيق الخالي من الجلوتين والمكون من دقيق الذرة الرفيعة والذرة الشامية والأرز.

لقد أجري التحليل التقريبي على الدقيق للعناصر الغذائية وكانت قيم (الرطوبة، الرماد، الدهون، الألياف، البروتين والكاربوهيدرات)، 9.66، 1.93، 2.09، 7.28، 11.35 و 67.70 % على التوالي.

كما احتوى الخليط على نسب جيدة من المعادن التالية (المغنسيوم 17.04 ملجم)، (الكالسيوم 12.73 ملجم) ونسب منخفضة من (البوتاسيوم 0.519)، (والفسفور 0.658). وقد خلت العينة من عنصر الحديد.

تم تجهيز العينات المخصصة لانتاج الخبز الخالي من الجلوتين بالنسب التالية : الارز الابيض 34.5 %, الذرة الرفيعه 30 %, الذرة الشامية 22 %, اللبن المنزوع الدسم 12.5 % الصمغ 1%, الخميرة 5. % و الملح 25. % و السكر 25. %

كذلك تم تقدير الخصائص الوظيفية للدقيق الخالي من الجلوتين حيث كانت قيم امتصاص الماء للعينات المحتوية على 0.0 ميكروجرام/جم من البروتين (A) , 0.5 ميكروجرام/جرام من البروتين(B) ,10ميكروجرام /جرام من البروتين (C) ,15 ميكروجرام/جرام من البروتين (D) و دقيق القمح (E) (81%، 79%، 80%، 81% و 78%) على التوالي.

كذلك تم تقدير السعة الاستحلابية للعينات A، B، C، D و E حيث كانت ، 40.9, 40.9، 41.9، 42.9 و 42.9 % على التوالي، وقيم القدرة على تكوين الرغاوي للعينات A، B، C، D و Eحيث كانت 6%، 9.6%، 7.5%، 5.35% و 5% على التوالي، وكذلك قيم ثباتية الرغاوي للعينات A، B، C، D و E.فكانت 1%، 1.9% 0.95%، 0.54% و 5% على التوالي، وقيم الكثافة الكلية للعينات A، B، C، D و E،كانت 0.84% 0.83%، 0.82%، 0.81% و 0.82 على التوالي، وقيم القدرة على الانتفاخ للعينات .A، B، C، D و E كانت 6%، 7%، 8%، 10% و 8% على التوالي، حيث نلاحظ وجود تأثير لأنزيم الترانسجلوتاميناز على هذه الخصائص الوظيفية.

كذلك تم تقدير مكونات البروتين للدقيق الخالي من الجلوتين للعينات A، B، C، D و Eوقد كانت القيم،البيومين 2.7، 2.2، 2.1، 2.8 و 1.8% على التوالي. و البرولامين 1.3، 1، 1.9، 1.3، و 1.9 %على التوالي. و للجلوبيولين 2.7، 1.7، 2.9، 3، و 4.6 %على التوالي.الجلوتلين 1.7، 2.6، 2.8، 2.3 و 2.% على التوالي.

كذلك تم تقدير ذوبانية البروتين عند الأس الهيدروجيني 4 وقد كانت القيم 4%، 3.1%، 4.9%، 3.5% و 2.2% على التوالي. وعند الأس الهيدروجيني 6 كانت القيم 3%، 6.3%، 5.3%، 3.7% و 2.2% على التوالي.

كذلك نجد أن إضافة أنزيم الترانسجلوتاميناز بنسب مختلفة للدقيق الخالي من الجلوتين أدت إلى اختلافات معنوية في قيم الفارينوغراف و مقدرة العينة على امتصاص الماء عند مقارنتها بالعينات الاخرى.
حيث كانت قيم امتصاص الماء للعينات (A) (B) (C) و(D) هي 39.2 , 39.2 , 38 و 39.4على التوالي وكذلك قيم ثباتية العجينة للعينات (A) (B) (C) و(D) هي 1.1 , 0.1 , 0.9 و0.0 دقيقة على التوالي اما قيم تكون العجينة للعينات (A) (B) (C) و(D) هي 10.2 , 8.6 , 9.9 و 9.5 دقيقة على التوالي حيث نجد ان هنالك علاقة طردية بين اضافة انزيم الترانسجلوتاميناز وامتصاص الماء .

أخيرا توصلت نتائج التقييم الحسي للخبز المنتج من الدقيق الخالي من الجلوتين والمضاف إليه أنزيم الترانسجلوتاميناز بنسبة 15 ميكروجرام/ جرام من البروتين حازت على أعلى تقييم في القبول العام بتقدير 3.05.

Table of Contents

List of Tables

Table Number **Page No.**

List of Figures

List of Plates

List of Symbols /Abbreviations

Abbreviation	Definition
CD	Celiac Disease
TGase	Transglutaminase
EATL	Enteropathy
AG	Anti Gliadin
ATTG	Anti Tissue Transglutaminase
IgG and IgA	Type of Antibodies
ELISA	Enzyme Linked Immuno Sorbent Assay
GFD	Gluten Free Diet
HPMC	Hydroxyl Propyl Methyl Cellulose
CMC	Carboxy Methyl Cellulose
GI	Glycemic Index
AOAC	Association of Official Agricultural Chemists
SPSS	Statistical Package for Social Science
ANOVA	Analysis of Variation
DMRT	Duncan’s Multiple Range Test
g	gram
µg	microgram
mg	milligram

CHAPTER ONE
INTRODUCTION

Gluten is the major structure-forming protein present in wheat bread and is responsible for the viscoelastic properties, it is a challenge to produce high-quality gluten-free bread. Therefore, ingredients that have the ability to mimic the properties of gluten are generally used. Recently, there are also spreading in the celiac disease in Sudan, and the gluten free bread is produced on the research level only, because cereal products, especially breads, are the basic components of the diet in many countries, there is a high demand for gluten-free breads from people with CD.

The production of gluten free bread differs significantly from standard wheat bread production . Although the same general steps are carried out times and conditions usually change considerably.Traditionally, wheat dough is mixed, bulked, fermented, divided, molded, proofed and finally baked . Usually gluten free breads have liquid batters that make difficult to follow a wheat bread process. Since gluten free batters form a weak, unstable and porous matrix therefore mixing and proofing times are shorter than in wheat bread .

Enzymes such as transglutaminase, amylases, proteases, and xylanases were reported (Harada *et al*.,2000; Guarda *et al.,* 2003) to create positive effects on the quality of wheat bread.

Transglutaminase (TGase) catalyzed an acyl transfer reaction between a γ-carboxyamide of peptide or protein-bound gluta- mine and a primary amine. When TGase acts on protein molecules, (γ-glutamyl) lysine cross-links are formed (Kuraishi, *etal.*, 2001). Microbial TGase has great potential to be used in the food industry as an agent to improve firmness, elasticity and water-holding capacity of

food through the mild enzyme reactions (Li *et al.*, 2013). Recently, the application of TGase in gluten free systems modified the viscoelastic properties of the dough, improving the quality of resulting gluten-free breads by promoting a protein network (Gujral and Rosell, 2004; Shin, *et al*., 2010; Song and Shin, 2007). The additions of TGase, protein and gum improved rheological properties of the dough for making gluten-free bread.

Celiac disease is a chronic disorder resulting from an immune reaction to certain cereal proteins. Most toxic for celiacs are wheat proteins: α-, β- and γ–gliadin, low molecular weight and high molecular weight glutenins, but secalin from rye, hordein from barley and avenin from oat may also be problematic (Skerritt *et al*., 1990).

The ingestion of gluten induces an inflammatory response resulting in the destruction of the villous structure of the small intestine causing flattening and breaking down of the small intestine's normal function (Shan *et al*., 2002). This leads to deficiencies in vitamins, minerals and sometimes protein, carbohydrates and fats. Currently, the only effective treatment for CD is the strict lifelong diet of gluten-free foods (Feighery, 1999).

Objectives of this study are:

1- To develop formulations to produce high quality gluten free bread.
2- To evaluate the chemical composition of the gluten free flour blends.
3- To determine the effect of (Tgase) on the dough rheological properties.
4- To evaluate the characteristics and functional properties of proteins in the gluten free flour blends
5- To study the effect of (Tgase) on the quality of the gluten free bread produced.

CHAPTER TWO

LITERATURE REVIEW

2.1Celiac Disease

Celiac disease is an autoimmune disorder of the small intestine, occurring in genetically predisposed people of all ages from middle infancy onward, that might cause severe malnutrition (Windt *et al*., 2010).

Celiac disease is caused by a reaction to gliadin, a prolamin (i.e., gluten protein) found in wheat. Upon exposure to gliadin, and specifically to peptides found in prolamin, the enzyme tissue transglutaminase modifies the protein, and the immune system cross-reacts with the small- bowel tissue, causing an inflammatory reaction . (Heel and West, 2006).

2.1.1 Signs and Symptoms

Severe celiac disease leads to the characteristic symptoms of pale, loose and greasy stool (steatorrhoea) and weight loss or failure to gain weight (in young children). People with milder celiac disease may have symptoms that are much more subtle and occur in other organs than the bowel itself. It is also possible to have celiac disease without any symptoms whatsoever . Many adults with subtle disease only have fatigue or anaemia. (Sabatino and Corazza,2009).

The diarrhea that is characteristic of celiac disease is chronic, pale, voluminous and abnormally malodorous. Abdominal pain and cramping, bloatedness with abdominal distension (thought to be due to fermentative production of bowel gas) and mouth ulcer may be present (Ferguson *et al*., 1976). As the bowel becomes more damaged, a degree of lactose intolerance may develop (Sabatino and Corazza, 2009).

Celiac disease leads to an increased risk of both adenocarcinoma and lymphoma of the small bowel (enteropathy-associated T-cell lymphoma (EATL) or other non-Hodgkin's lymphomas.(Freeman, 2009).

This risk is also higher in first-degree relatives like siblings, parents and children, whether or not a gluten-free diet brings this risk back to baseline is not clear. Long-standing and untreated disease may lead to other complications, such as ulcerative jejunitis (ulcer formation of the small bowel) and stricturing (narrowing as a result of scarring with obstruction of the bowel) (Gujral *et al*., 2012).

The changes in the bowel make it less able to absorb nutrients, minerals and the fat-soluble vitamins (A, D, E, and K). (Presutti, 2007)

- The inability to absorb carbohydrates and fats may cause weight loss (or failure to thrive/stunted growth in children) and fatigue or lack of energy.
- Anaemia may develop in several ways: iron malabsorption may cause iron deficiency anaemia, and folic acid and vitamin B_{12}, malabsorption may give rise to the megaloblastic anaemia.
- Calcium and vitamin D malabsorption (and compensatory secondary hyperparathyroidism) may cause osteopenia (decreased mineral content of the bone) or osteoporosis (bone weakening and risk of fragility fractures).
- A small proportion have abnormal coagulation due to vitamin K deficiency and are slightly at risk for abnormal bleeding.
- Celiac disease is also associated with bacterial overgrowth of the small intestine, which can worsen malabsorption or cause malabsorption despite adherence to treatment.(Tursi *et al*., (2003)
- Celiac disease is associated with a number of other medical conditions, many of which are autoimmune disorders: diabetes mellitus type1,

hypothyroidism, primary biliary cirrhosis, and microscopic colitis. (Schuppan *et al*., 2009).

2.1.2 Celiac Disease in Sudan

Celiac disease is probably uncommon in Sudan. The feeding habits are certainly different from the European ways and this may explain partly the low incidence of this syndrome, but constitutional, genetic, and environmental factors may be involved. Kisra, a local bread made from Dura or Sorghum Vulgaris constitutes a stable diet in many Sudanese homes; however, bread biscuits and other foods made of wheat are also used. In other study done by (Ajeep, 2012) in Red Sea State. 172 patients suspected to have celiac disease, were examined at the Red Sea Medical Center laboratory, Port Sudan, Sudan, from 2008 to 2011. All clinical data were collected using questionnaires. Serum samples were obtained from all patients for serological detection of anti-gliadin (AG) and anti-tissue transglutamase (ATTG) antibodies (IgG and IgA) using the enzyme-linked immuno-sorbent assay (ELISA).He found that 128 patients to have celiac disease. The commonest presenting symptom was chronic diarrhea (20.3%) followed by weight loss (14%). Males and females were nearly equally affected. All large prospective studies are needed to assess the true incidence, the clinical course, the efficiency of treatment modalities employed, patient compliance, disease complications and response to treatment in eastern Sudan.

2.2 Gluten Free Diet

It is a diet that excludes gluten so far. A gluten-free diet is the only medically accepted treatment for celiac disease (Hischenhuber *et al*., 2006).This means the elimination of products containing wheat, rye and barley following a gluten-free diet presents significant challenges and many barriers to compliance. One of the most significant challenges patients face is the cost of certain

components of the diet. Great tasting gluten-free alternatives are essential to help patients comply with the diet, but come at a much higher cost than their gluten containing counterparts. Helping patients manage the cost of the diet is as important as understanding the basic concepts of the diet. (Parrish, 2007)

Some people believe that there are health benefits to gluten-free eating for the general population, but there is no published experimental evidence to support such claims (Gaesser and Angadi , 2012).

2.3 Gluten-Free Bakery Products

As it is necessary to remove wheat flour from gluten- free formulations, it has to be replaced with flours naturally free of gluten such as maize or rice, or starches of various botanical origin (Sanchez *et al*., (2002) and Dar (2013).

The latter group includes also wheat starch, which can be used only after careful removal of protein traces. In traditional bakery products, gluten provides a network, which is responsible for water binding and viscoelastic properties of the dough, allows gas retention and supports final porous structure of the crumb (Zghal *e.t al*., 2002; Gallagher *et al*.,2003and Moore *et al*.,2004). After its removal these properties have to be supplied by other components of the dough, which are present in native flours or could be added to starch as admixtures (Gallagher *et al*.,2004 and Gambus *et al*., 2007).

Gluten traditionally gives cakes their structure. Without gluten, cakes are drier and more crumbly but there are a number of products you can use to try and counter this. Trying new flours such a rice, tapioca and potato are great ways to add texture to cakes in the absence of gluten. (Sleet, 2005)

Gluten-free biscuits, their diverse combinations of texture and taste have given biscuits and cookies universal appeal. The three principal ingredients in

these products are wheat flour, fat and sugar. In different combinations, they form the basis of a full range of biscuit products. In gluten-free biscuits the wheat flour, which originates from soft winter wheat, is replaced by other ingredients. These do not only replace the starch, which is normally delivered by the wheat flour, but also the protein fractions. An advantage in developing gluten-free biscuits (in comparison with gluten-free breads) is that gluten network formation is unwanted in many biscuit products. (Gallagher *et al*., 2004).

Gluten-free pizza bases, the quality of gluten-free pizza products is generally poor and closer to a cake product than to a wheat dough pizza. The criteria for a good quality pizza are sheetable dough, which rises on proving and holds the gas produced by the yeast, and also good textural and sensory attributes. A combination of gluten-free flours and starches, protein sources (egg, soya), hydrocolloids (guar gum), and a microencapsulated high fat powder delivered all these requirements.

Tests on dough hardness, pizza base hardness, and color and pizza volume confirmed that it is possible to produce gluten-free pizza products with similar attributes to wheat flour pizzas. (Gallagher *et al*., 2004).

2.3.1 Gluten Free Bread

Traditionally wheat dough is mixed, bulked, fermented, divided, molded, proofed and finally baked. Usually gluten free breads have liquid batters that make it difficult to follow a wheat bread process. Since gluten free batters form a weak, unstable and porous matrix, mixing and proofing times are shorter than in wheat bread.

The production of gluten-free breads is required utilization of polymeric substances such as xanthan gum or hydroxyl propyl methyl cellulose (HPMC).

According to (Toufeili *et al* 1994 and Guarda *et al* 2003) several studies have been conducted by (O'Brien *et al* (2002), Gallagher *et al* 2003), Moore *et al* (2004); and Schober *et al* 2003) whereby novel ingredients such as dairy powders, sorghum, rice, starches, pseudo cereals, etc., in combination with hydrocolloids replaced gluten. Another approach is the addition of different enzymes. Enzymes such as amylases, proteases, and xylanases to create positive effects on the quality of gluten bread (Harada *et al* (2000) and (Guarda *et al* 2003).

Moore *et al*., (2004) developed a procedure to manufacture gluten free breads with modified conditions especially during mixing, moulding and proofing steps. This method was successfully applied by other researchers.

2.3.2 Gluten Free Bread Characteristics and Challenges

In Gluten free breads the lack of gluten is countorbalanced by complex formulations based in gluten free flours, starches and hydrocolloids . The aim of reproducing the viscoelastic properties of wheat dough . however, GF breads are usually still of poorer quality than wheat bread (Stauffer, 1998).

Removal of gluten and consequently, lack of strength of the protien matrix ability to expand and retain gas, results in weak batters with high permeability to carbon dioxide and big difficulties to maintain the structure, which decreases the volume of baked goods (Stauffer, 1998). The absence of gluten also impairs the water holding capacity of the bread which shows an early crumbly structure and quick staling. In addition, due to the common use of starches in formulations and the short proofing times, gluten free breads are pale and have poor flavor and taste. Commercial GF breads are mainly starch based and therefore lack fibre, vitamins and nutrients (Arendt, 2004).

2.3.3 Quality of Gluten Free Bread

Production of gluten free bread is much more complicated than traditional baking, because of time consuming, adjustment of raw materials and processing method. The wide range of possible ingredients, including starches of various botanical origin, and hydrocolloids, make it difficult to compare various formulations, which should be further adjusted with suitable amounts of water to give the dough appropriate viscosity and stability, and provide the bread with good volume and a well structured crumb (Arendt *et al.* , 2008). Even more difficult is the choice of nutritional additives which would be most suitable for celiacs, and could be applied to bakery products at reasonable amounts.

2.3.4 Gluten Free bread Ingredients

The increasing number of diagonosed celiac patients and the poor quality of GF products has encouraged researchers to investigate new ingredients and technologies that reproduce gluten properties and improve the qualtiy of GF baked goods. The development of GF bakery has involved the use of starches, gums, GF flours, animal and vegetable protien supplements and alternative technologies such as sourdough, fermentation or high hydrostatitic pressure processing.

2.3.4.1 Starch

Starch is the main component of the dry substance and plays an important role in establishing it’s structure and mechanical properties. It’s role is even greater in the case of gluten free bread where the elastic wheat protien is replaced with mixture of different hydrocolloids.

The addition of starch to protien free bread improves consistency during mixing and enhances softness of the crumb. Including corn starch, rice and sorghum.

2.3.4.1.1 Sorghum

Sorghum mainly contains carbohydrates of which starch and dietary fiber are the main components. Starch is absorbed by the body and converted into glucose.

Glucose is taken up in the blood stream to provide the body with energy for essential functions of the body. Dietary fiber promotes healthy digestion and combats diseases of the digestive tract.

The protein in sorghum contributes largely to the total protein intake. It is important to remember that the protein which occurs in grain lacks the essential amino acids and cannot replace animal protein. Sorghum is rich in iron, magnesium, potassium, calcium and phosphorous. (FAO, 2004).

It is one of the most important cereal crops. Sorghum's GF nature allows it's inclusion in GF bakery but some disadvantages like high gelatinization temperature or the tendency to form coarse grifts in. A sandy mouth feel has limited its application in gluten free bread. Sorghum (dura) is the staple food for most people living in the Sudan, except for the northern states (Nahr al-Nil and Northern states) where wheat is the traditional staple (Schober *et al*, 2003).

2.3.4.1.2 Rice

Rice (*Oryza sativa)* is one of the most important foods of the human diet and one of the most extended cereal crops, 9% of the total cultivated soil has probably fed more people in history than any another crop. Rice flour is colorless, has a soft taste, hypo allergic properties, low level of sodium and easily digested carbohydrate, because of these properties rice flour is the most suiitable cereal to make gluten free bread (Gujral and Rosell, 2004)

2.3.4.1.3 Corn

Corn also known as Maize (*Zea mays*), is one of the world leading cereal grain along with rice and wheat. It provides 15% of the world protien and 20% of the world calories, and is a dietary stable for more than 200 million people. Maize is a rich source of energy provided by highly digestable carbohydrates, high protien content composed of essential amino acid, free oil and good qualtiy of trace minerals. It contains about 72% starch, 10% protien, 4% lipid, 7.3% fiber. It contains important vitamins such as vitamin A and vitamin E but lacks vitamin B12. These fat soluble vitamins found in maize play important role as antioxidants among other functions.

Corn starch is one of the main components in the gluten free bread formulation. It absorbs up to 45% of water and is considered as inert filler in the continuos matrix of the dough (Schober *et al*, 2003).

2.3.4.2 Skim Milk Powder

Gluten-free bread recipes often contain skim milk powder. It adds protein and also has beneficial effects on the end product. The casein in milk makes it a good emulsifier, and it makes for a finer crumb and better consistency. People who are allergic to dairy can try substituting soy milk powder, but the results will not be as good (Mohamed *et al.*,2006).

2.3.4.3 Water

Water is one of the main components of gluten free bread. It allows the solubilaization rehydration and interactions of the ingredients and also plays an important role in physical and chemical interactions (De La Hera *et al*., 2014)

2.3.4.4 Salt

Salt is a source of low levels of sodium in wheat dough which help obtaining an easy workable dough (Mohamed *et al.*, 2006).

Salt is added to devolop flavor, it also gives a less sticky dough. Salt slows down the rate of fermentation and it's addition is sometimes delayed until the dough has been partly fermented.

2.3.4.5 Yeast

Yeast is a leavening organisim belonging to the fungi kingdom. Yeast is the leavening agent in bread that transforms sugar into carbon dioxide and ethanol during fermentation. The trapt carbon dioxide makes the dough rise while the alcohol evaporates during baking. Shorter fermentation in gluten free bread prevents yeast to fully develop organic substance thus only acting as a leavening agent (Sleet, 2005).

2.3.4.6 Hydrocolloides

Main bread hydrocolloides starch which is a part of the flour and acts as a filler and a gelling agent but other hydrocolloids particularly gums, are being used, jargum has been employed for improving the volume and texture of frozen dough bread (Ribotta *et al*. 2004). The use of hydrocolloids with thickeninig and stabilizing properties such as arabic gum, guar gum and xathan gum seem suitable for gluten free bread as the lack of gluten in the gluten free batter which requires the develpoment of complex material with sufficient visco elastic properties for holding both the carbon dioxide released during fermentation and the mixed matrex structure expansion along baking.

Xanthan gum is a useful ingredient for baking with gluten-free mixes as it improves the texture and shelf-life of your baked products. It works very much like

gluten by binding ingredients during the baking process to give a conventional texture. When added to gluten-free flour mixes, it replaces the gluten ‘ stretch factor ’. Xanthan gum comes in a powder form which dissolves easily in water. Mix it with the gluten-free mix before adding any liquid. You may need to use a bit more liquid as the xanthan gum thickens the mixture quite a lot. It does not need heat to thicken like flour does, but it is not affected by oven temperatures either, making it quite versatile (Sleet, 2005).

2.3.5 Production of Gluten Free Bread

Baking without gluten (as found primarily in wheat flour) can be challenging because gluten contributes important properties to various types of baked products like cookies, cakes, pastries and breads. Gluten development is not as important for cookies as it is for cakes, so gluten-free flours can be substituted with similar results. Cakes and other types of batter-based products, like pancakes, need gluten for its gas-retaining ability that produces a light and airy interior structure and a tender crumb (Belton and Taylor, 2002).

In addition to replacing the wheat flour with gluten-free flour, other additives can hold gas. These products include xanthan gum and guar gum that can be found in the baking or natural food section of the grocery store. Bread is perhaps the most challenging gluten-free baked product to make because gluten provides structure, creates a tender crumb, and retains gas. With experimentation and practice, a combination of gluten-free flours and gums can be used to create a loaf with good volume, softness and texture. Although it is not a baked product, pasta is usually made from hard wheat flour. The gluten component not only gives structure to the noodles but also keeps the starch in the flour from leaching into the cooking water or becoming too sticky. These properties can be approximated with

the use of gluten-free flours in combination with eggs and xanthan gum (Case, 2006).

2.3.6 Nutritional Value of Gluten Free Bread

The nutritional evaluation of different commercial gluten free breads revealed that they are mainly starchy foods with great divergences in fat and protein composition. In consequence, these products have very low contribution to the recommended daily protein intake, but higher contribution to the carbohydrate dietary reference intake than their gluten containing counterpart. The majority of gluten free breads evaluated contained a good amount of dietary fiber. The estimated glycaemic index (is a number associated with a particular type of food that indicates the food's effect on a person's blood glucose) of the gluten free breads could be considered as food with high glycaemic index. Overall, gluten free breads shows great variation in the nutrient composition, being starchy based foods low in proteins and high in fat content (Segura and Rosell 2011).

Nutritional value of gluten free bakery products depends mostly on the source of raw materials. Due to the presence of large quantities of starch caloric value of gluten free bread is usually comparable with traditional products. However the level of other components highly vary between different formulations, which should be taken into account by people following gluten free diet. This is especially important in the context of obesity problems among celiacs (Murray *et al*. (2004); West *et al*.(2004) and Aurangzeb *et al*., 2010). Properly balanced gluten-free diet should have appropriate energetic value, but also provide certain levels of all necessary macro- and micronutrients. Gluten free bread is often low in protein, dietary fiber and minerals in comparison to traditional wheat bread. At the same time it could be high in fat, especially of plant origin, which is used to provide porosity for the crumb, and slow down rapid stalling, caused by starch

retrogradation. Bakery products are good mediums for nutritional supplements, which could make them functional foods.

However such additions are difficult from technological point of view, and could negatively affect quality and product acceptance (Morias *et al*,. 2014 and Hager *et al*., 2011). Low nutritional value of gluten free bread in comparison to traditional bakery products prompts to search the ways of its supplementation. It could be done by the addition of whole and ground seeds of gluten free plants (legumes, oil producing plants, nuts), flours from edible tubers, as well as fiber and protein preparations, vitamins and minerals (Houben *et al*. 2011); (Krupa-Kozak *et al*. 2013). Supplements could modify the quality of bread in a number of ways, depending on their quantity and properties. The application of flour-like additives is usually much more valuable than modification of starch blends, because they usually provide substantial amounts of protein, fiber and antioxidants. Addition of fiber is usually limited to several percent, because of its negative impact on quality parameters of the crumb. In the case of gluten-free bread it is especially important, as the structure of such products is normally weaker than of their wheat-based equivalents (Gallagher *et al*, 2003).

According to the rules of rational nutrition, every diet should be balanced and supply all necessary products: energetic, building and regulatory.

There could be many forms of food pyramid, but its basis is often build by non-refined cereal products. (Davis *et al*., 2001).

Consumption of bread supplies organism with important nutrients and dietary constituents, because it contains not only necessary calories but also protein, dietary fiber and fat rich in unsaturated amino acids, as well as vitamins (especially B), minerals, antioxidants and phytoestrogens. (Dewettinck *et al*., 2008 and Gellynck *et al*., 2009). A decrease in consumption of cereal products, including

bread, seems to be inevitable in the case of celiacs, because of the limited availability, higher price and varying quality of gluten-free cereals. (Dennis *et al*, 2004 and Cureton, 2007).

2.3.6.1 Protein in Gluten Free Bread

The content of protein may significantly vary. Generally it cannot exceed values characterizing whole grains of gluten-free plants, such as amaranth (12–17% protein), buckwheat (11–12%), corn (10%), millet (8–19%), quinoa (16–20%), rice (6–7%), and sorghum (11–13%) and decreases with the removal of outer layers and embryo. Similar variability could be observed in the content of selected protein fractions: albumins, globulins, prolamins and glutelins, which depends on the botanical origin of the plant (the latter two groups are typical for most cereals, while pseudocereals and legumes are rich in soluble fractions), and the place of protein biosynthesis e.g. cereal endosperm contains mostly storage proteins, while aleuronic layer and embryo are rich in enzymes and regulatory proteins (Guerrieri *et al*., 2004).

As a result, proteins present in various plants have different amino acid composition and structure which could influence human organism in a variety of ways, including immunological activity. Apart from proteins provided with the flours, gluten free bread contains a small amount of microbial proteins, added with yeasts and sour dough starters or synthesized during fermentation. Proteins and raw materials rich in them could also be added as improvers or technological aids (milk powder, gelatin) as well as for nutritional reasons, to overcome the deficiencies of gluten-free diet (Ziobro *et al*., 2013). Properties of proteins in gluten-free dough could be modified by hydrolyzing and cross-linking enzymes, which has a direct impact on rheology. Specifically the presence of glutamic acid

residues allows to use transglutaminase as a structure forming agent (Smerdel *et al*., 2012).

2.3.6.2 Carbohydrates in Gluten Free Bread

Starch is the most important component of bread, which could not be replaced by other substances. Depending on biological origin it could significantly vary in its granular structure, molar mass and amylose content, which has a direct impact on functional properties and digestibility. Apart from native starches also physically or chemically modified starch could be used in bread formulations. Their technological parameters, such as water binding, gelatinization temperature, gelling properties are optimized so as to facilitate dough preparation and baking or improve dietary properties of the products. Typical gluten-free dough formulations contain polysaccharide hydrocolloids, such as arabinoxylans, guar gum, Arabic gum, xanthan gum, locust bean gum, and cellulose derivatives, namely hydroxypropylmethylocellulose (HPMC) and carboxymethylcellulose (CMC) (Witczak *et al*., 2010).

Their addition is usually low (a few percent), which makes gluten free bakery products deficient in dietary fiber in comparison to typical bread, especially based on whole meal. It is possible to supplement them in this dietary constituent by adding various types of soluble and insoluble polysaccharides such as inulin (Juszczak *et al*. , 2012 and Ziobro *et al*. , 2013) , and byproducts obtained during processing of fruit and vegetables (Korus *et al*. 2012). Such additions seem to be especially important in the context of observed deficiencies in nondigestible carbohydrates in the diet of celiacs (Hager *et al*., 2011).

Nutritional status of bread depends highly on the type of carbohydrates it supplies, as they could highly differ in their rate of absorption and metabolism. A useful tool to monitor this phenomena is glycemic index (GI), which allows to

classify food based on carbohydrates on the basis of postprandial glucose level in blood (Cummings and Stephen , 2007).

2.3.6.3 Lipids in Gluten Free Bread

Gluten free products usually contain high levels of fat, sugar and salt. Their presence is to some extent caused by technological reasons (application of raw materials and additives reach in above mentioned compounds), but also reflects tendency of the manufacturers to increase attractiveness of their products. People with celiac disease tend to compensate limitations caused by gluten-free diet by eating food with distinctive and attractive flavor, which contain high amounts of fat and carbohydrates (Saturni, 2010). Addition of fat to gluten-free dough facilitates its mechanical processing, by decreasing adhesion to metal surfaces, and causes positive changes (small and evenly distributed pores) in the structure of bread crumb. It also interacts with amylose molecules and thus retards bread staling caused by starch retrogradation. The amount of fat in gluten-free bakery products is often high and may exceed 10% (Segura *et al*., 2011). The type of applied fat (usually of plant origin) is decisive for nutritional quality of the lipids present in gluten-free bread.

2.3.6.4 Minerals in Gluten Free Bread

Diagnosis of celiac disease is often accompanied with observed deficiencies in mineral components, caused by the damage of intestinal epithelium. It is usually most evident in the levels of calcium and iron. Although the adherence to gluten-free diet restores proper absorption of minerals, they could be lacking in many of gluten-free products (Wild *et al*., 2010).

Supplementation of gluten-free bread seems to be a good way to provide appropriate levels of minerals (Gambuś *et al*., 2009), especially those which are typically associated with the consumption of wheat bread.

2.3.7 Rheological Properties of Gluten Free Bread

Hydrocolloids have been essential ingredients in the formation of gluten free cereal products. Hydrocolloids or gums are substances consisting of hydropholic long-chain, high molecular weight molecules, usually with colloidal properties, which in water based system produce gels, that is highly viscous suspensions or solutions with low dry substance content.

Hydrocolloids have been found to affect dough rheological performance, as they mimic the visco-elastic properties of gluten in bread doughs (Toufeili *et al*., 1994 and Collar *et al*,. 1998) and also swelling, gelatinization, pasting properties, and staling of the starch. According to (Toufeili *et al*., 1994) and (Guarda *et al*., 2003), the utilization of polymeric substances such as xanthan gum or hydroxypropymethylcellulose (HPMC) are required for the production of gluten free bread. The lack of a gluten network determines the properties of the gluten free dough, which is more fluid than wheat doughs and closer in viscosity to cake batters (Cauvain, 1998 and Moore *et al*., 2004) and thus has also to be handled in a similar manner to cake batters rather than doughs.

Most gluten free breads are formulated using gluten free starches and require the addition of hydrocolloids to provide srtucture and gas retaining properties in the dough. For gluten free bread production,the most commonly used hydrocolloids are pectin, guar gum, xanthan gum and locust bean gum (Ylimaki *et al*., 1988 and Moore *et al*., 2004).

2.3.8 Functional Properties

Functional properties are the fundamental physico-chemical properties that reflect the complex interaction between the composition, structure, molecular conformation and physico-chemical properties of food components together with the nature of environment in which these are associated and measured (Kinsella,

1976 Kaur and Singh, 2006 and Siddiq *et al*., 2009). Functional characteristics are required to evaluate and possibly help to predict how new proteins, fat, fibre and carbohydrates may behave in specific systems as well as demonstrate whether or not such protein can be used to stimulate or replace conventional protein. The food property is characterized of the structure, quality, nutritional value and/or acceptability of a food product. A functional property of food is determined by physical, chemical, and/or organoleptic properties of a food. Example of functional properties may include solubility, absorption, water retention, frothing ability, elasticity and absorptive capacity for fat and foreign particulars. Typical functional properties include emulsification, hydration (water binding), viscosity, foaming, solubility, gelation, cohesion and adhesion.

2.3.9 Bread Making Procedure

The following steps are generally considered essential for the production of good quality bread (Belton and Taylor , 2002).

Sieving

The flour is generally sieved before using in bread primarily for following reasons:

- To aerate the flour.
- To remove coarse particles and other impurities.
- To make flour more homogeneous.

Weighing

The next step is weighing of different ingredients as per formulation. Minor ingredients have to be weighed more precisely. Salt, sugar, oxidizing agents and yeast are added in solution form. Yeast is added as a suspension, which is mixed well each time before dispensing.

Mixing

Mixing of flour and ingredients involves i.e. hydration & blending, dough development and dough breakdown. The process of mixing begins with hydration of the formula ingredients.

The dough system subsequently becomes more coherent, losing its wet and lumpy appearance, and it achieves a point of maximum consistency or minimum mobility. This is the point to which dough should be mixed for producing bread of superior loaf quality. If mixing is continued beyond this point, mechanical degradation of the dough occurs resulting in the breaking down of the dough network. The mixing time varies with the type of flour, type of mixer, speed of mixing arm, presence of salt or shortening, additive, particle size.

Fermentation

Optimally mixed dough is subjected to fermentation for a suitable length of time to obtain light aerated porous structure of fermented product. Fermentation is achieved by yeast. The yeast in dough breaks down the sugars to carbon dioxide and ethanol. The gas produced during fermentation leavens the dough into foam. The foam structure of dough is discrete and has stability during fermentation. When fermented dough is baked, the foam structure gets converted into sponge structure that is responsible for aerated structure of bread crumb.

Dividing

The dough is divided into individual pieces of predetermined uniform weight and size. The weight of the dough to be taken depends on the final weight of the bread required.

Proofing

It is necessary to let the dough piece rest while fermentation proceeds. Average time at this stage ranges from 5 to 20 min.

Baking Process

After proofing the dough is subjected to heat in a baking oven. Baking temperature generally varies depending on oven and product type but it is generally kept in the range of 220-250ºC. During baking the temperature of dough centre reaches about 95ºC in order to ensure that the product structure is fully set. When the dough is placed in the oven, heat is transferred through dough by several mechanisms such as convection, radiation, conduction, and condensation of steam and evaporation of water. The baking time of bread may range from 25 to 30 minutes depending up on size of bread loaf. After baking, bread is cooled prior to packaging to facilitate slicing and to prevent condensation of moisture in the wrapper.

2.3.10 Bread Quality

Bread quality is usually judged by:

2.3.10.1 Loaf Volume

Soulaka and Morrison (1985) reported that loaf volumes obtained fom reconstituted flours were larger and the crum was softer. As the gelatinization temperature of the starch fraction increased ,however Hoseney *et al*., (1971) did not find a relation between the gelatinization temperature of starches from various plants and their baking quality.

Cauvin and Chamberlain, (1988) stated that loaf volume increase is attributed to improved gas retention and to extending the period of dough expansion during the baking stage.

Perten,(1995) stated that, quality factors such as loaf volume and water absorption are related to gluten quality and quantity. Higher gluten quantity values generally give a greater bread volume.

Basically, strong flours must be used for making good bread. If weak flour is used, loaves of small volume are produced.

2.3.10.2 Crumb Texture

Dough is a complex system, and many problems associated with the poor texural quality of a final product can result from a dificiency in one or more of the following dough charecterisitics: gas generation, gas retention, and seting of the structure in expanded state.

The texture may be too soft, sometimes " gummy ". This retention of moisture in the crumb results from the production of too many dextrins from the starch and the loss of gluten structure. (Mathewson, 2000).

Kaldy and Rubenthler, (1987) reported that a fine uniform crumb texture that is tender and moist is one of the main criteria for good bread quality. Generally,flour with high protein content or strong gluten or both, produces a course and heavy crumb texture.

2.3.10.3 Aroma

Aroma is important factor governing food acceptability. The aroma of bread results from the interaction of reducing sugars and amino compounds, accompanied by the formation of aldehydes. Also aroma is affected by the

products of alcoholic and in some cases lactic acid fermentation (Kent 1983 and Lyla 2002)

Matz, (1968) mentioned that yeast consumes sugar and prdoduces carbon dioxide and alchol, the former reaction is responsible for raising the dough, while alchol is party responsible for the aroma of the baked product.

2.3.10.4 Color

Golden brown color of the crust is one of the most obvious trait of a baked product. This color results from polymerization reactions known as Millard browning and caramelization. Millard browning occurs when amine groups on amino acids combine with carbonyl groups of reducing sugar molecules. It is temperature and PH dependent, with higher PH increasing the reaction rate. The reaction continues and colored pigment known as melnoidins are eventually formed. Caramelization involves only the sugar in the system, and although it is fostered by condition of higher temperature and lower moisture than millard browning, it likely contributes to the appearance as well.

Mathewson, (2000) reported that,amylases and proteases can contribute to Millard reaction which requires a reducing sugar and amino group by making these compounds available.

2.4 Transglutaminase

Transglutaminase (TGase) is a relatively new tool used in the manufacture of baked goods (Diez Poza, 2002). TGase is an enzyme that catalyzes an acyl-transfer reaction between the γ -carboxyamidegroup of peptide-bound glutamine residues and a variety of primary amines Motoki and Seguro, (1998). When protein-bound lysyl residues act as acyl receptors, intra- and intermolecular isopeptidebonds are formed within the enzyme reaction. According to (Motoki and Seguro, 1998) in the

absence of primary amines in the reaction system, water is used as an acyl acceptor leading to a deamination of glutamine residues.Thus, the TGase can modify proteins by amine incorporation, cross-linking, and deamination.

Transglutaminase is widely used in different branches of the industry because of its ability to modify the physical and chemical properties of proteins. To date, a bacterial expression system with *Streptoverticillium mobaraensis* has mainly been used to biosynthesise transglutaminases. This system,however, has some drawbacks, involving, e.g. problems related to post-translational protein modification (Griffin *et al*., 2002). Research should thus be pursued to develop a cheaper and more efficient system that will allow reduction of costs associated with the distribution, storage, extraction and purification of recombinant proteins.

2.4.1 Comparison of Transglutaminase from Different Sources

Transglutaminases are enzymes that are commonly found inside and outside of a cell. This determines the versatility and diversity of their functions. Enzymatic activity was observed in many microorganisms and in plant and animal tissues as well. It is noteworthy that the presence of different transglutaminase forms is observed in one organism. Animal and plant transglutaminases manifest catalytic activity and biochemical properties similar to those of microbiological transglutaminases, despite having a lack of homology in the amino acid composition . Animal transglutaminases are involved in a number of physiological processes, e.g. they participate in spermatogenesis and blood coagulation. Many forms of transglutaminases have also been identified in plants. It was also confirmed that more than one transglutaminase may function in one plant, or even in one organelle (Sobieszczuk-Nowicka *et al*., 2008). These enzymes play a role in plants' processes of growth and development (Samelak *et al*., 2010). A specific feature of a plant is transglutaminase enzyme sensitivity to light. This property applies especially to chloroplast transglutaminase (TGase), which has been

confirmed by many studies Campos *et al*., (2009) and Sobieszczuk-Nowicka *et al*., 2008).

The application of isolated transglutaminase enzymes from a microbiological source has allowed for simplification of certain processes and has provided energy and economical savings. Thanks to established transgenesis procedures, gene transfer became possible and the expression of genes gave rise to transglutaminase production. The transfer of genes to expression systems such as *Escherichia coli* has remarkably increased production efficiency. It should also be stated that these enzymes are safe for consumers and easily biodegradable which, in contrast to chemical substances, is a great advantage.

2.4.2 Functional Properities of Transglutaminase

The ability of transglutaminase (TGase) to modify the functional properties of food proteins has been extensively reported(Lorenzen, 2000; Kuraishi *et al*., 2001; Lauber et al.2003; Bruno *et al*. 2008 and Ribotta *et al*., 2004). By acyl group transfer between the ε-amino group of lysine and the γ-carboxyamide group of glutamine residues in proteins/peptides, TGase catalyses the formation of an ε-(γ-glutamyl) lysine isopeptide bond. In the absence of free ε-groups, water acts as the acyl acceptor, resulting in the deamidation of glutamine to glutamic acid.

Food proteins are often denatured during processing, so there is a need to understand the protein both as a biological entity with a predetermined function, and as a randomly coiled biopolymer. Protein cross-linking has profound effects on their structure which affects the functional attributes of these proteins. Food processing often involves high temperature as in baking and low PH as in beverage industry. Such conditions can result in the introduction of protein cross links producing substantial changes in the structure of proteins and which can be reflected in the final product profile (Gerrard, 2002). The formation of this cross link does not reduce the nutritional quality of the food, as the lysine residue

remains available for digestion. Chemical and physical methods are commonly used. Food proteins can have their functionality altered by temperature and other chemical means. Specific functional attributes could be obtained by enzymatic polymerization of proteins and such enzymatic reaction could be controlled for desired time to enhance the functionality to the desired level (Singh, 1991). Work on enzymes, especially mammalian and microbial transglutaminases have been employed to modify proteins for functionality. The covalent cross linking of proteins catalyzed by transglutaminases can cause significant changes in the size, conformation, stability and other properties of the proteins by enhancing protein–protein interaction.

Transglutaminases are currently being used in baking technologies to form links between polypeptide prolamin chains. Transglutaminase was found to have a positive impact on the stability and volume of dough as well as on the improvement of the baking quality of poor flour and, consequently, the texture of the bread (Marco and Rosell, 2008). Kuraishi *et al*,. (2001) reported that transglutaminase improved the rheological properties of dough and ensured proper pore size and bread elasticity after baking. In addition, transglutaminases were shown to improve water absorption by dough modification of wheat flour proteins with transglutaminase increases the elasticity and resilience of dough as well as the volume of bread by 14 % in comparison with pastry made from traditionally prepared dough (Gerrard *et al*., 2001).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Materials

Sorghum, rice, corn, skimmed milk powder, and xanthan gum, yeast, salt, sugar were purchased from local market (Omdurman). Transglutaminase was obtained from (Shanghai Seebio Biotech. inc). All chemicals and reagents were of analytical grade.

3.2 Methods

3.2.1 Preparation of the Flour Blend

Sorghum, rice and corn grains were cleaned and milled to flour at a local stone mill.

3.2.2 Transglutaminase (TGase) Preparation

The addition of TGase was calculated on the basis of the amount of crude protien present in the recipe.

The stock solution was prepared for 0.0, 5.0, 10.0, 15.0 µg of TGase.

3.2.3 Blend of Samples

The samples were weighed with the following portions to prepare gluten free bread.

White rice flour	34.5 %
Sorghum	30 %
Corn flour	22 %
Skimmed milk	12.5 %
Xanthan gum	1 %
Yeast	.5 %

Salt	.25 %
Sugar	.25%

The previous portions were well mixed and sealed in polyethylene bags and kept in refrigerator at 5^0C for further use.

3.3 Preparation of Bread Sample

The precedure described by (Badi *et al*., 1978) was modified for this type of bread . Bread improvers transglutaminase were added at 0.0 , 5.0 , 10.0 , 15.0 µ/g of protein (where 0 gram refers to the control for the bread).

Dry ingredients (dry yeast 10g, salt 5g , sugar 5g, and the required amount of white rice flour, sorghum, cornflour, skim milk powder and xanthan gum) were mixed for one minute using Hobart N-50G mixer. Cold water was added and mixed for three minutes at low speed and then gradually to high speed, and then batter was poured in pans and transfered into fermentation cabinet for 45 minutes.

The fermented batters were then baked in the baking oven at 230°C for 30 minutes.The loaves were depanned and allowed to cool on cooling racks at room temperature.

3.4 Analytical Methods

3.4.1 Chemical Analysis

3.4.1.1 Moisture Content

The moisture content in each sample was determined following the standard method described by the(AOAC, 2003).

Two grams of well mixed sample was weighed accurately in cleaned, dried Petri dishes using a sensitive balance (Item No: AR2140, Made for OHAC,S CORO. USA). Then, the samples were placed in an oven (Carblite, sheffield, England) at

105°C for five hours. After that the Petri dishes were transferred to adesiccator and re -weighing after cooling to room temperature. Again, the dishes were transferred to the oven and weighed after two hours and this was repeated till a constant weight was obtained. Then, the moisture content (M.C) as percent was calculated as the loss in weight after drying:

$$\text{Moisture content (\%)} = \frac{(Ws - Wd)}{\text{Sample weight (g)}} 100\%$$

Where:

Ws =weight of sample before drying.

Wd=weight of sample after drying.

3.4.1.2 Crude Protein

The crude protein content in the sample was determined by the micro-Kjeldahl method following the method of (AOAC, 2003).

Principle:

2 gm of Flour sample was digested with a concentrated sulphuric acid so that the sample release its nitrogen content which can be determined by a suitable titration technique. A conversion factor of 5.8 (equivalent to .16 g nitrogen per 100 grams of protein) was used in this method to calculate the sample protein content. The kjeldahl method is divided into three steps which can be summarized under the following:

A) Digestion

The Flour sample (2grams) was transferred into a digestion flask and then digested by heating for 2-3 hours in (3.5N) sulphuric acid. The digestion process was catalyzed by a mixture 0.4 of 10 parts K_2SO_4 to one part of $CuSO_4$. The

heating was continued till the black colour turned to pale blue and the fumes disappeared which indicate that the digestion process was completed.

B) Distillation

After the digestion has been completed the digestion flask was cooled and transferred to a distillation unit using a minimum volume of water. The solution in the distillation unit was then turned alkaline by addition of 20 ml of sodium hydroxide (40%) to release the ammonia. Then, the released ammonia was distilled into 20ml of 2% boric acid in a conical flask with 2 to 3 drops of Bromochresol Methyl red as indicator.

C) Titration

The nitrogen content in the sample was then estimated by titration of the ammonium borate formed with a standard hydrochloric acid (0.1N). The titrations continued till the colour of the solution turned to red (pink). Then, the following formula was used to determine the protein concentration as per-cent:

$$\text{Nitrogen Content\%} = \frac{(TVxNx\ 14.00\ )}{1000 \text{ x sample weight (g)}} \times 100$$

$$\text{Protien Content \%} = (\text{Nitrogen Conten \%}) \times F$$

Where:

TV: actual volume of HCl used for sample titration (ml HCl – ml blank).

N: normality of HCl.

F: protein conversion factor = 5.8

3.4.1.3 Fat Content

The sample oil content was determined by using a continuous extraction apparatus (Soxhlet type), as described by (Pearson, 1970).

About five grams (5±1) samples were weighed and transferred to an extraction thimble covered with a piece of glass wool and then placed in the Soxhlet apparatus. After that, the solvent (petrolum ether) was added into a dried weighted Soxhlet flask and the extraction process was continued for about six hours. Then, the oil sample was dried in an oven (Carblite, sheffield, England) for a 30 minutes to eliminate any remaining amounts of the solvent and the flask was reweighted. The fat percent was calculated by using the following equation:

$$\% \text{ Crude fat} = \frac{(W_2 - W_1)}{\text{sample weight (g)}} \times 100$$

Where**:**

W_1=weight of the empty soxhlet flask (g).

W_2=weight of soxhlet flask with oil content (g).

3.4.1.4 Crude Fiber

The crude fiber was determined according to the (AOAC, 2003). About two grams sample were weighted and two hundred ml of sulphuric acid (0.26N) were added,boiled for 30 minutes and then filtered. The residue was washed three times by using hot water and after that 200ml of NaOH was added, boiled again for 30 minutes and filtered. Then, the residue was carefully washed three times with hot water until it was free from alkali. After that, the sample was transferred to an oven (Carblite, sheffield, England) at 105° C (overnight) and reweighted. The residue was ached in a muffle furnace (LEF- 103S, watts: 2KW10A serial no: 07033002, Korea) at 550° C for three hours till a light gray ash was formed and a constant

weight was obtained. Then, the total crude fiber percent was calculated using the following equation:

$$\text{Crude fiber \%} = \frac{(W_1 - W_2)}{\text{sample weight(g)}} \times 100$$

Where:

W_1= weight of the sample before ignition (g).

W_2= weight of sample after ignition (g).

3.4.1.5 Ash Content

The ash content of the sample was determined according to (AOAC, 2003). The empty crucibles were accurately weighed and then two grams of flour were transferred to each crucible by using a sensitive balance. Then, the crucibles and their content were placed in a muffle furnace (LEF- 103S, watts: 2KW10A serial no: 07033002, Korea) at 550° to 700°C for more than 6 hours until white to grey ash was obtained. After that, the crucibles were transferred from thefurnace to a desiccator to cool to room temperature and re-weighed. The ash content was calculated by using the following equation:

$$\text{Ash content (\%)} = \frac{(Wt_1 - Wt_2)}{\text{Sample weight(g)}} \times 100$$

Where:

Wt_1 = weight of crucible with the remaining ashed sample (g).

Wt_2 = weight of the empty crucible (g).

3.4.1.6 Mineral Content

Ten milliliters (10 ml) of HCL (2N) were added to the remaining ash sample and placed in a hot sand bath for about 10-15 min. After that, the sample was

filtered and diluted to 100 ml in a volumetric flask. Then, the trace elements ferrous (Fe^{++}) was determined according to (Perkin Elmer, 1994) by using Atomic Absorbance Spectroscopy (JENWAY 3110, UK). and potassium (K) were determined by using Flame Photometer (Model PEP7 JENWAY). While, calcium (Ca), magnesium (Mg) and phosphorus (P) were determined as described by (Chapman and pratt, 1961).

3.4.1.7 Determination of Protein Fractions

Protein fractions were determined by the method of (Landry and Moureaux 1970) with some modifications. One gram of the mixed sample (gluten free flour) was sequentially extracted with 10 mL of each, distilled water (albumin), 1 mol/l NaCl (globulin), 70% (v/v) aqueous ethanol (prolamin), and 0.1 mol/l NaOH (glutelin) for 2 h at 25C° under continuous stirring, each extraction step was performed twice. The extraction was centrifuged at 6000g at 4 C° for 30 min. Protein contents of each extraction step were determined with some modifications by using the macro-kejeldahl methods (AOAC,1990).

3.4.2 Determination of Functional Properties of Protein

3.4.2.1. Water and Oil Absorption Capacity

The water and oil absorption capacities were determined by the method of (Sosulski *et al*., 1976). The sample (1.0 g) was mixed with 10ml distilled water or refined soybean oil, kept at ambient temperature for 30 min and centrifuged for 10min at 2000g. Water or oil absorption capacity was expressed as percent water or oil bound per gram of the sample.

3.4.2.2 Bulk density

The bulk density was determined according to the method described by (Okaka and Potter, 1977). The sample (50 g) was put into a 100 ml graduated cylinder and

tapped 20-30 times. The bulk density was calculated as weight per unit volume of sample (g/cm3).

3.4.2.3 Swelling capacity

The method of (Okaka and Potter, 1977) with some modifications was used for determining the swelling capacity.

The sample filled up to 10 ml mark in a 100 ml graduated cylinder was added with water to adjust total volume to 50 ml. The top of the graduated cylinder was tightly covered and mixed by inverting the cylinder. The suspension was inverted again after 2 min and allowed to stand for further 30 min. The volume occupied by the sample was taken after 30 min.

3.4.2.4 Foaming capacity and foaming stability

Foaming capacity and foaming stability were determined as described by (Narayana and Narasinga Rao, 1982) with slight modifications. Sample (1.0 g) was added to 50 ml distilled water at 30±20 C in a graduated cylinder. The suspension was mixed and shaken for 5 min to foam. The volume of foam after whipping for 30 sec was expressed as foaming capacity. The volume of foam was recorded 1h after whipping to determine foaming stability as percent of the initial foam volume.

$$\text{FC} = \frac{Volume\ of\ foam\ (AW) - Volume\ of\ foam\ (BW)}{\text{Volume of foam (BW)}} \times 100$$

Where:

(AW) = After whipping

(BW) = Before whipping

$$FS = \frac{Volume\ of\ foam\ 1h\ (AW)}{\text{Initial foam volume}} \times 100$$

3.4.2.5 Emulsion Activity

The emulsion activity and stability were determined by the method of (Yasumatsu *et al*., 1972). The emulsion (1g sample, 10ml distilled water, 10ml soybean oil) was prepared in a calibrated centrifuge tube. The emulsion was centrifuged at 2000 × g for 5 min. The ratio of the height of the emulsion layer to the total height of the mixture was calculated as the emulsion activity expressed in percentage.

$$\text{Emulsion activity} = \frac{Height\ of\ emulsion\ layer}{\text{Height of whole layer}} \times 100$$

3.4.2.6 Solubility of Proteins

The solubility of proteins is considered as that proportion of nitrogen in a protein product which is in the soluble state under specific conditions. Solubility is the amount of protein in a sample that dissolves into solution. Proteins recommended as food additives can be partly or completely soluble or completely insoluble in water.

Procedure:

Protein solubility was determined by the method of (Sathe *et al*., 1982) with some modifications. The suspensions (2%) of the flour in distilled water were adjusted to pH 4-6 using 1 m HCl and 1 m NaOH. The amount of nitrogen in each supernatant was determined by macro Kjedahl method according to the method already described in the AOAC (1990). Percent soluble protein was calculated as percent nitrogen multiplied by 6.25 on wet basis.

Soluble protein % = Nitrogen % × protein factor (6.25).

3.5 Dough Characteristics

3.5.1 Farinogram Value

The physical dough characterisitics of the dough's prepared from wheat flour and the gluten free mixture was determined using the Brabender Farinograph according to the methods of AACC (2000).

3.5.2 Titration Curve

Brabender Farinograph was operated as described in AACC method (2000). Titration curve was used for the assesment of water absorption for samples which were in different levels of transglutaminase (0.0, 5.0, 10.0, 15.0)µg/g of protien.

- A quantity of 300g of flour sample was weighed and transferred into a cleaned farinograph mixer.
- The farinograph was switch on at a 63 rpm for one minute, then the distilled water was added from special burette (the correct water absorption can be calculated from the deviation, 20 units deviation correspond to 0.5% water, if the consistency is higher than 500 F.U. more water is needed and vice-versa).
- When the consistency is constant, the instrument was switched off and the water drawn from the burrete indicates water absorption of the flour in percentage.

3.5.3 The Standard Curve

The significant readings taken from a farinograph were:

1. Water Absorption: is the quantity of water, which made a curve reached a 500 FU line and made a defined consistency expressed as a percentage of the flour at 14% moisture.
2. Dough Development: describes the time (minutes) in which the curve reaches its maximum dough consistency.
3. Arrival time: is the difference between zero minutes and the point at which the top of the curve first intersects the 500 FU line.

4. Departure time: is the time between the origin and the point where the top of the curve falls below the 500 FU line.
5. Dough stability: is the difference in minutes between the departure time and the arrival time.
6. Dough weakening: is the difference of the dough strength between the moment dough weakening begins and after 12 minutes dough kneading (measured in FU).
7. Farinograph quality number is the length of the curve in (mm) from the beginning to the point at which the curve has decreased by 30 Farinogram units.

3.6 Physical Characteristics of Bread

3.6.1 Bread Weight

The weight of bread loaf was taken in grams

3.6.2 Bread Volume

The bread volume was determined by the seed displacement method according to Pyler (1973). The bread was placed in a container of known volume into which small seeds (millet seeds) were run until the container is full. The volume of seeds displaced by the bread was considered as loaf volume . Loaf volume could then be calculated and recorded according to : VL (cc)= Vc – Vr.

Where (VL) = Loaf Volume

(Vc)= Container Volume.

(Vr) = Rapeseed Volume.

3.6.3 Bread Specific Volume

The specific volume of the bread was calculated according to (AACC Methods, 2000) by dividing loaf volume (cc) by its weight (gram) .

3.7 Sensory Evaluation of Bread

The bread were sliced with an electric knife and prepared for sensory evaluation on same day using the ranking method . The sensory evaluation of

bread samples was carried out by 20 panelists from M.Sc. students majoring in food science were asked to evaluate (colour, odor, taste, texture and general acceptability) of the bread samples.

Before the actual evaluation sessions started , panelists were familiarized with terminology and test techniques and procedures , and the samples were coded with letters and presented to each panelist. The surrounding conditions were kept the same all through the panel test.

The ranking test gives; (1) as excellent, (2) as very good , (3) as good , (4) as acceptable and (5) as bad in quality.

3.8 Statistical Analysis

The data obtained in this study were subjected to statistical analysis by using the Statistical Package for Social Science (SPSS). Mean values were obtained by the Analysis of Variation (ANOVA). Probability of 5% was used to indicate the significances according to Duncan's Multiple Range Test (DMRT) as described by (Mead and Gurnow, 1983)

CHAPTER FOUR
RESULTS AND DISCUSSION

4.1 Chemical Composition of Gluten Free Flour

Chemical composition of gluten free mixture was shown in table (1).The results are expressed on dry matter basis per 100g of material.

4.1.1 Moisture Content

The moisture content of the mixture was 9.66% , and this result disagreed with Hegazy *et al*., (2009) who reported that the moisture content of a mixture made of rice flour, corn starch, defatted soy and chickpea in different levels ranged between 10.05 to 10.22%. this may be due to the dryness of the content of the mixure and the weather.

4.1.2 Ash Content

Ash content of gluten free mixture flour was 1.93%, and this result was similar to Hegazy *et al*., (2009) who reported that the ash content of a mixture made of rice flour, corn starch, defatted soy and chickpea in different levels was 1.29-1.90%. On the other hand Elhasan (2007) also reported that ash content of mixture of corn starch and soybean were 1.22-2.57 % . Differences in ash content could be attributed to differences in the recipes and the degree of decortication .

4.1.3 Protein Content

protein content of gluten free mixture flour was 11.35% , this result was nearly in agreement with Hegazy *et al*., (2009) who reported that the protein content of a mixture made of rice flour, corn starch, defatted soy and chickpea in different levels with addition of xanthan gum were 11.78 – 12.94%. Elhasan (2007) reported that protein content of mixture of corn starch and soybean were in the

range of 4.80 to 13.40%. Differences in protein content could be attributed to differences in the recipes.

4.1.4 Fat Content

Fat content of gluten free flour from sorghum, rice, corn flour and skimmed milk was 2.09% , this result disagreed with Elhasan (2007) who found that the fat content of former mixer was in the range of (3.1 to7.53 %). The low fat content may be due to the low fat of rice and sorghum grains. On the otherhand Hegazy *et al.*, (2009) reported that the fat content was in the range of 1.72 to1.75 %.

4.1.5 Fiber Content

Fiber content of the mixture of gluten free flour was 7.28% ,and this result disagreed with Elhasan (2007) and Hegazy *et al*., (2009) who reported that the fiber of the mentioned mixture made of rice flour corn starch, defatted soy and chickpea in different levels with addition of xanthan gum was in the range of (0.22to1.33%) and (0.95 to 0.99%) respectively. The increase of fiber content may be due to the high fiber content of sorghum grain .

Table (1): Proximate Composition (%) of Gluten Free Flour

Moisture content	Ash content	Oil content	Crude fiber	Crude protein	CHO*
9.66 ± 0.08	1.93 ± 0.02	2.09 ± 0.05	7.28 ± 0.16	11.35 ± 0.14	67.70 ± 0.42

Values are mean ±SD.
*Carbohydrates by difference

4.2 Minerals Content of Gluten Free Flour

Table (2) shows the minerals content of gluten free flour blends expressed; as mg/100g. From the results, the gluten free flour was found to be good in magnesium (17.04 mg), calcium (12.73 mg) and low in potassium (0.519 mg) and phosphorus (0.658 mg) while it was free of Iron. Generally, these results are nearly similar to those obtained by Wild *et al*., (2010). Differences in minerals content could be attributed to differences in the recipes.

Table (2): Minerals Content of Gluten Free Bread

Minerals	**(mg/100g)**
Calcium (Ca)	12.73 ± 0.04
Magnesium (Mg)	17.04 ± 0.22
Iron(Fe)	ND
Potassium (K)	0.519 ± 0.45
Phosphorus (P)	0.658 ± 0.23

Values are means ± standard deviation

4.3 Farinograph Results

Table (3) and figures (1- 4) show the farinograph values of doughs prepared from gluten free flour blends.

The water absorption of the samples A,B,C and D (A=Sample with 0.0µg of TGase. B=Sample with 5.0µg/g of TGase. C=Sample with 10.0µg/g of TGase. D=Sample with 15.0µg/g of TGase) were 39.2, 39.2, 38.0 and 39.4 respectively.These results were in a good agreement with those mentioned by (Kuraishi *et al*., 1996) who stated that there is positive correlarion between addition of Tgase and water absorbtion when compared with wheat flour.The reduction in water absorption is the result of gluten free recipe (the main protein of flour and flour strength factor). Part of reduction of water absorption was due to

damaged starch hydrolysis by increased amylolitic activity (damaged starch has more water absorption capacity compared to undamaged starch, this is due to the fact that damaged starch can swell at room temperature). The farinograph curve obtained for the flour blends used in this study has shown the water absorption of 39% and it was not possible to obtain consistency of 500 BU.

The dough development time of the samples A, B, C and D were 10.2, 8.6, 9.9 and 9.5min respectively, development time of gluten free flour decreased with the increased level of Tgase. The dough stability values of the samples A, B, C and D blend were 1.1, 0.1, 0.9 and 0.0 % respectively. Low stability could be attributed to the lower protein content of the mixture compared to that of wheat flour only.

The degree of softening of the samples A, B, C and D were 1, 18, 9 and 10 FU respectively. The softening of the gluten was caused by the reduction of disulfide cross-links present in the protein.

The application of TGase in gluten-free systems modified the viscoelastic properties of the dough, improving the quality of resulting gluten-free breads by promoting a protein network. The additions of TGase, protein and gum improved rheological properties of the dough for making gluten-free bread. But the level and type of additives were different by researchers and treated conditions (Gujral and Rosell, 2004; Shin, Gang, and Song, 2010; Song and Shin, 2007).

Table (3): Farinograph Readings of Gluten Free Flour

Flour blends	Water absorption (%)	Dough stability (min)	Dough development time (min)	Degree of softening (FU)
A	39.2	1.1	10.2	1
B	39.2	0.1	8.6	18
C	38.0	0.9	9.9	9
D	39.4	0.0	9.5	10

A=Sample with 0.0µg/g of TGase.
B=Sample with 5.0µg/g of TGase.
C=Sample with 10.0µg/g of TGase.
D=Sample with 15.0µg/g of TGase.

4.4 Functional Properties for Gluten-Free Flour

The results of functional properties of gluten free bread flour where shown in Table (4). and fig. (5- 10)

4.4.1 Water and Oil Absorption Capacity

From the results water absorption capacity of the samples A, B, C, D and E were 81, 79, 80, 81 and 78 % respectively, Though there were significant differences (p <0.05) in water absorption capacity among flour samples, a slightly increased trend was obvious (Fig. 5). An increase of water absorption capacity (WAC) on gluten free flour could be attributed to an increase in protein content and change in the quality of protein and also breakdown of polysaccharide molecules. Hence, the sites for interacting with water and holding water would be increased by Tgase level.

Water binding capacity is a useful indication of whether flour or isolates can be incorporated into aqueous food formulations especially those involving dough handling. Higher water absorption capacity results obtained suggested that sorghum flour could be useful in food systems such as bakery products, which requires hydration to improve handling characteristics.

4.4.2 The emulsification Capacity

Emulsification capacity were 40.9, 40.9 ,41.9 , 42.9 and 42.9 % respectively . There were significant differences (p<0.05) in EC values. The emulsification properties of protein-containing products like cereal and legume flours may result from both soluble and insoluble proteins, as well as other components, such as polysaccharides. Protein can emulsify and stabilize the emulsion by decreasing surface tension of the oil droplet and providing electrostatic repulsion on its surface, while some types of polysaccharides can help stabilize the emulsion by increasing the viscosity of the system. Soluble proteins are surface active and known to promote oil-in-water emulsion. Emulsion capacity of a product depends

on its oil content and protein concentration (Nkonge ,1984). The capacity of proteins to enhance the formation and stabilization of emulsion is important for many applications in cakes, coffee whiteners, and frozen desserts. In these products, varying emulsifying and stabilizing capacities are required because of different compositions and stresses to which these products are subjected.

The improvement in the emulsifying properties is likely due to an increase in the negative charges which result from the hydrolysis of the amide groups in glutamine and asparagines (Hassan *et al*., 2007).

Table (4): Functional Properties (%) For Gluten-Free Flour

Samples	Water absorption capacity	Emulsifying activity	Foaming capacity	Foaming stability	Bulk density	Swelling capacity
A	81.00 ± 1.00 ^{a}	40.90 ± 0.10 ^{c}	6.00 ± 1.00 ^{c}	1.00 ± 0.01 ^{c}	0.84 ± 0.01 ^{a}	6.00 ±1.00 ^{c}
B	79.00 ± 1.00 ^{bc}	40.90 ± 0.10 ^{c}	9.60 ± 0.10 ^{a}	1.90 ± 0.10 ^{b}	0.83 ± 0.01 ^{b}	7.00 ± 1.00 ^{bc}
C	80.00 ± 1.00 ^{ab}	41.90 ± 0.10 ^{b}	7.50 ± 0.10 ^{b}	0.95 ± 0.01 ^{c}	0.82 ± 0.01 ^{c}	8.00 ± 1.00 ^{b}
D	81.00 ± 1.00 ^{a}	42.90 ± 0.10 ^{a}	5.35 ± 0.01 ^{c}	0.54 ± 0.01 ^{c}	0.81 ± 0.01 ^{d}	10.00 ± 1.00 ^{a}
E	78.00 ± 1.00 ^{c}	42.90 ± 0.10 ^{a}	5.00 ± 1.00 ^{c}	5.0 ± 1.00 ^{a}	0.82 ± 0.01 ^{c}	8.00 ± 1.00 ^{b}
$Lsd_{0.05}$	1.819*	0.1819*	1.156*	0.8177*	0.0005753*	1.819*
SE±	0.5774	0.05774	0.367	0.2595	0.0001826	0.5774

Values are mean±SD.

Mean value(s) bearing same superscript(s) are not significantly different (P≤0.05).

Key

A ≡ sample as control (without enzyme)

B ≡ sample containing 5 µg/g protein

C ≡ sample containing 10 µg/g protein

D ≡ sample containing 15 µg/g protein

E ≡ sample containing wheat flour without enzyme (blank)

4.4.3 Foaming Capacity and Foaming Stability

The foaming capacity (FC) and foaming stability (FS) are generally determined by loss of liquid resulting from destabilization that is measured as volume decrease. Foam formation is governed by three factors, including transportation, penetration and reorganisation of the molecule at the air-water interface. Therefore, to exhibit good foaming, a protein must be capable of migrating at the air-water interface, unfolding and rearranging at the interface (Padmashree,1987).

The results of FC are presented in table 4. Foaming Capacity were 6, 9.6 , 7.5 , 5.35 and 5, Foaming stability were 1, 1.9, 0.95, 0.54, and 0.50%. There were significant differences (p <0.05) in foaming capacity values among flour samples and were proportional to Tgase level. The foaming capacity of a food material depends on the surface-active properties of its protein (Udensi,2006) .

The higher emulsion and foaming attributes of the treated protein could have been due to increased ability to form an interfacial protein film, since its high molecular size and cross linked structure are more resistant to excessive denaturation than the native protein at the high speed of the homogenization used to make emulsions and foams (Aluko and Yada, 1995).

4.4.4 Bulk Density

Bulk density results were 0.84, 0.83, 0.82, 0.81 and 0.82% respectively, there were decrease in bulk density with increasing Tgase level. Bulk density gives an indication of the relative volume of packaging material required. Generally, higher bulk density is desirable for the greater ease of dispersibility and reduction of paste thickness, which is an important factor in convalescent child feeding due to the physiology of the alimentary canal and stomach of the infant.

4.4.5 Swelling Capacity

Swelling capacity were 6, 7, 8, 10 and 8%. The results shows that there were significant differences p≥0

These results disagreed with those reported by Suresh and Samsher, (2013) who reported that the water absorption, swelling capacity, emulsification activity and foaming capacity of rice flour were 192% , 15.20 %, 37.31 % and .98 % respectively.

TGase has an impact on the water holding capacity. It is presumed that the protein network formed by TGase has the ability to trap water and hence cause an increase in the water holding capacity.

4.5 Protein Fractions

A change in the protein fraction contents of gluten free flour at different level of Tgase were shown in table 5.

From the results the albumin content of the samples A, B, C, D and E were 2.70, 2,20, 2.10, 2.80 and 1.80 % respectively, It was clear that the most abundant storage proteins in sorghum are Albumin**.**

Prolamin were 1.30, 1.0, 1.9, 1.30 and 1.90 respectively. Globulin results were 2.70, 1.70, 2.90, 3.00 and 4.60 respectively. The glutelin fraction increased during increasing the level, of TGase until it reached 2.40. There was significant difference (p <0.05)

Glutelin were 1.70, 2.60, 2.80, 2.30 and 2.40% respectively. These results were in a good agreement with those reported by Demirkesen (2013).

Table (5): Protein Fraction (%) for Gluten-Free Flour

Samples	Albumin	Prolamin	Globulin	Glutelin
A	2.70 ± 0.10^{a}	1.30 ± 0.10^{a}	2.70 ± 0.10^{b}	1.70 ± 0.10^{d}
B	2.20 ± 0.10^{b}	1.00 ± 1.00^{a}	1.70 ± 0.10^{c}	2.60 ± 0.10^{b}
C	2.10 ± 0.10^{b}	1.90 ± 0.10^{a}	2.90 ± 0.10^{b}	2.80 ± 0.10^{a}
D	2.80 ± 0.10^{a}	1.30 ± 0.10^{a}	3.00 ± 1.00^{b}	2.30 ± 0.10^{c}
E	1.80 ± 0.10^{c}	1.90 ± 0.10^{a}	4.60 ± 0.10^{a}	2.40 ± 0.10^{c}
$Lsd_{0.05}$	0.1819^{*}	$0.8297^{n.s}$	0.8297^{*}	0.6164^{*}
SE±	0.05774	0.2633	0.2633	0.2195

Values are mean±SD.

Mean value(s) bearing same superscript(s) are not significantly different (P≤0.05).

Key
A ≡ sample as control (without enzyme)
B ≡ sample containing 5 µg/g protein
C ≡ sample containing 10 µg/g protein
D ≡ sample containing 15 µg/g protein
E ≡ sample containing wheat flour without enzyme (blank)

4.6 Protein Solubility

Proteins solubility is probably the most critical among the functional properties because it affects other properties such as emulsification, foaming and gelation. Protein solubility characteristics are influenced by factors such as origin, processing conditions, pH, ionic strength and the presence of other ingredients (Kinsella , 1976).

Variation in protein solubility with PH values (4-6) are presented in Table (6) and figures (15-16).

Generally, solubility increases as the PH values increase. From the results the solubility of the samples A, B, C, D and E at PH 4 were 4.00 , 3.1 , 4.9, 3.5 and 2.2 respectively, while at pH 6 the results were 3.00 , 6.30, 5.30, 3.10 and 2.20 respectively.

Flanagan *et al*., (2003) reported that limited and extensive TGase cross linking resulted in significant improvements in solubility at low PH (PH 2.0 and 3.0). Improvements in solubility were also observed at PH 5.0 for the minimal and extensively crosslinked samples. These results were in agreement with those reported by Demirkesen (2013).

Table (6): Protein Solubility (%) for Gluten-Free Flour

Samples	pH_4	pH_6
A	4.00± 1.00 ^{b}	3.00± 1.00 ^{cd}
B	3.10 ± 0.10 ^{c}	6.30± 0.10 ^{a}
C	4.90± 0.10 ^{a}	5.30 ± 0.10 ^{b}
D	3.50± 0.10 ^{bc}	3.10± 0.10 ^{c}
E	2.20± 0.10 ^{d}	2.20 ± 0.10 ^{d}
$Lsd_{0.05}$	0.8297 *	0.8297 *
SE±	0.2633	0.2633

Values are mean±SD.

Mean value(s) bearing same superscript(s) are not significantly different (P≤0.05).

Key

A ≡ sample as control (without enzyme)

B ≡ sample containing 5 µg/g protein

C ≡ sample containing 10 µg/g protein

D ≡ sample containing 15 µg/g protein

E ≡ sample containing wheat flour without enzyme (blank)

4.7 Specific Volume of Bread Made from Gluten Free Flour

Table (7) shows the specific volume of gluten free bread from (mixture) with TGase, at four different levels (0,5,10,15 µg of transglutaminase /g of protien).

Specific volume (cm^3/g) ranged from (1.046-1.435). The wheat flour gained the highest value for specific volume (2.810)cm^3/g. Where sample A with the 0.0µg TGase gained the lowest volume 1.046 and sample D gained the best value with 15µg TGase is (1.435)cm^3/g. From the results, it is clear that specific volume of gluten free bread was positively affected by the amount of TGase. (Research by Basman *et., al* (2002)) found a decrease in loaf volume for wheat bread with the addition of TGase which was probably due to excessive cross-linking within the system.

The dark color and the bitter taste are attributed to Maillard reactions which are accelerated at higher temperature (Yokoyama and Kikuchi , 2004).

Table (7): Specific Volume of Bread Made from Gluten Free Flour

Samples	**Loaf specific volume**
A	1.05 ± 0.01^{c}
B	1.23 ± 0.02^{bc}
C	1.21 ± 0.01^{bc}
D	1.44 ± 0.44^{d}
E	2.81 ± 0.04^{a}
$Lsd_{0.05}$	0.36^{*}
SE±	0.11

Values are mean ±SD.

Mean value(s) bearing same superscript(s) are not significantly different (P≤0.05).

E=Sample of TGase free bread (control).
A=Sample with 0.0µg/g of protien.
B=Sample with 5.0µg/g of protien.
C=Sample with 10.0µg/g of protien.
D=Sample with 15.0µg/g of protien.

4.8 Sensory Evaluation of Bread Made from Gluten Free Flour

Table (8) show the scores of colour for wheat bread (E) and gluten free bread made from mixture of sorghum, rice and corn flour, with improver TGase levels 0.0,5,10,15 µg/g.The colour of bread scores ranged from 1.10-3.85. The colour score of wheat bread gave the highest value 1.10 with signifecant difference ($p<0.05$) compared with gluten free bread. While the colour score at GF bread sample C gave the lowest value 3.85. The results showed that the sample D with improver TGase level 15 µg/g of protien gave the highest score of colour among the gluten free bread.

The scores of flavour of wheat bread and gluten free bread made from sorghum, rice, cornflour, milk powder with T.G level 0.0, 510,15 µg/g of protien.The score of bread flavour are found to range from 1.60-3.80.The flavour score of wheat bread gained the highest score 1.60 with significant difference ($p<0.05$) compared with gluten free bread, whereas GF bread with level of 0.0 µg/g TGase (sample A) gave the lowest score 3.80.Among the GF bread the bread with level 15 µg/g gave the highest value 3.10.

The score of bread taste are found to range from 1.50-3.55.The taste scores of wheat bread gained the highest score 1.50 with significant difference ($p\leq0.05$) compared with GF bread whereas GF bread with level 0.0 µg of TGase/g gave the lowest score 3.55.Among the gluten free bread the bread with level of 15 µg/g TGase gained the highest value 3.00

The crumb texture score at wheat bread and GF bread made from mix of sorghum, rice, cornflour with different levels of T.G 0.0,5.0,10.0,15.0 µg/g of protien.The score of bread crumb texture were found to range between 1.55-3.05.The texture

score of wheat bread gained the highest score 1.55 with significant difference ($p \leq 0.05$) compared with gluten free bread whereas the GF bread with level 0.0 and 5 µg/g gained the lowest score 3.05.Among the gluten free bread the bread with level of TGase 15µg/g gained the value 2.50.

The general acceptibility score of wheat bread and gluten free bread made from sorghum, rice and corn flour with T.G levels 0.0,5,10,15µg/g of protien. The scores of bread general acceptibility were found in range of 1.45–3.55.The general acceptibility score of wheat bread gained the highest score 1.45 with significant difference ($p \leq 0.05$) compared with GF bread while the GF bread with level 0.0 µg/g T.G gained the lowest value 3.55. Among the GF bread, the bread with level of TGase 15µg/g gained the highest value 3.05. (Basman *et al*., 2003) found similar results for wheat bread, where the addition of low levels of TGase led to a finer crumb. Overall quality, TGase does have an impact on gluten-free breads systems, however the types of protein source and enzyme addition level are limiting factors.

Table (8): Sensory Evaluation of Gluten Free Bread

Samples	Colour	Flavour	Taste	Texture	General acceptability
	Scores				
A	3.40 ± 0.82^{a}	3.80 ± 0.95^{a}	3.55 ± 1.15^{a}	3.05 ± 1.1^{a}	3.55 ± 0.89^{a}
B	2.80 ± 0.83^{b}	3.45 ± 1.00^{ab}	3.20 ± 1.1^{a}	3.05 ± 0.8^{a}	3.30 ± 0.80^{a}
C	3.85 ± 1.04^{a}	3.25 ± 1.071^{ab}	3.05 ± 0.94^{a}	3.00 ± 0.8^{a}	3.15 ± 0.99^{a}
D	2.75 ± 1.21^{b}	3.10 ± 1.02^{b}	3.00 ± 1.0^{a}	2.50 ± 1.1^{a}	3.05 ± 1.15^{a}
E	1.10 ± 0.31^{c}	1.60 ± 0.68^{c}	1.50 ± 0.61^{b}	1.55 ± 0.9^{b}	1.45 ± 0.69^{b}
$Lsd_{0.05}$	0.5619^{*}	0.5992^{*}	0.618^{*}	0.6164^{*}	0.5747^{*}
SE±	0.2001	0.2134	0.2201	0.2195	0.2047

Values are mean ±SD.

Mean value(s) bearing same superscript(s) are not significantly different ($P \leq 0.05$).

E=Sample of TGase free bread (control).

A=Sample with 0.0µg/g of protien.

B=Sample with 5.0µ/g of protien.

C=Sample with 10.0µ/g of protien.

D=Sample with 15.0µ/g of protien.

Plate (1- A): Gluten Free Breads with Different Levels of Tgase

Plate (1- B): Gluten Free Breads With Different Levels of Tgase

Key

A ≡ sample as control (without enzyme)

B ≡ sample containing 5 µg/g protein

C ≡ sample containing 10 µg/g protein

D ≡ sample containing 15 µg/g protein

E ≡ sample containing wheat flour without enzyme (blank)

CHAPTER FIVE
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

- From the results obtained in this study, it can be concluded that the gluten free flour blend were found to be of good nutritional value and suitable for gluten free bread production.
- The gluten free bread produced in this study is also found to contain appreciable amounts of calcium and magnesium.
- The bread sample 15µg/g of protien (D) was highly accepted by the panelists, while the sample 0µg/g of protien (A) was less acceptable by the panelist.
- We can conclude that TGase dose (15µg) have a positive impact on gluten-free bread's quality, however the types of protein source and enzyme addition level may be limiting factors.

5.2 Recommendations

- The use of transglutaminase enzyme in the production of gluten bread is recommended.
- It is recommended to use the level of 15µg of transglutaminase enzyme /g of protein in the composite flour (white rice, sorghum and corn flour) of gluten free bread.
- Further investigations are needed to produce gluten free bread with better quality.

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Appendices

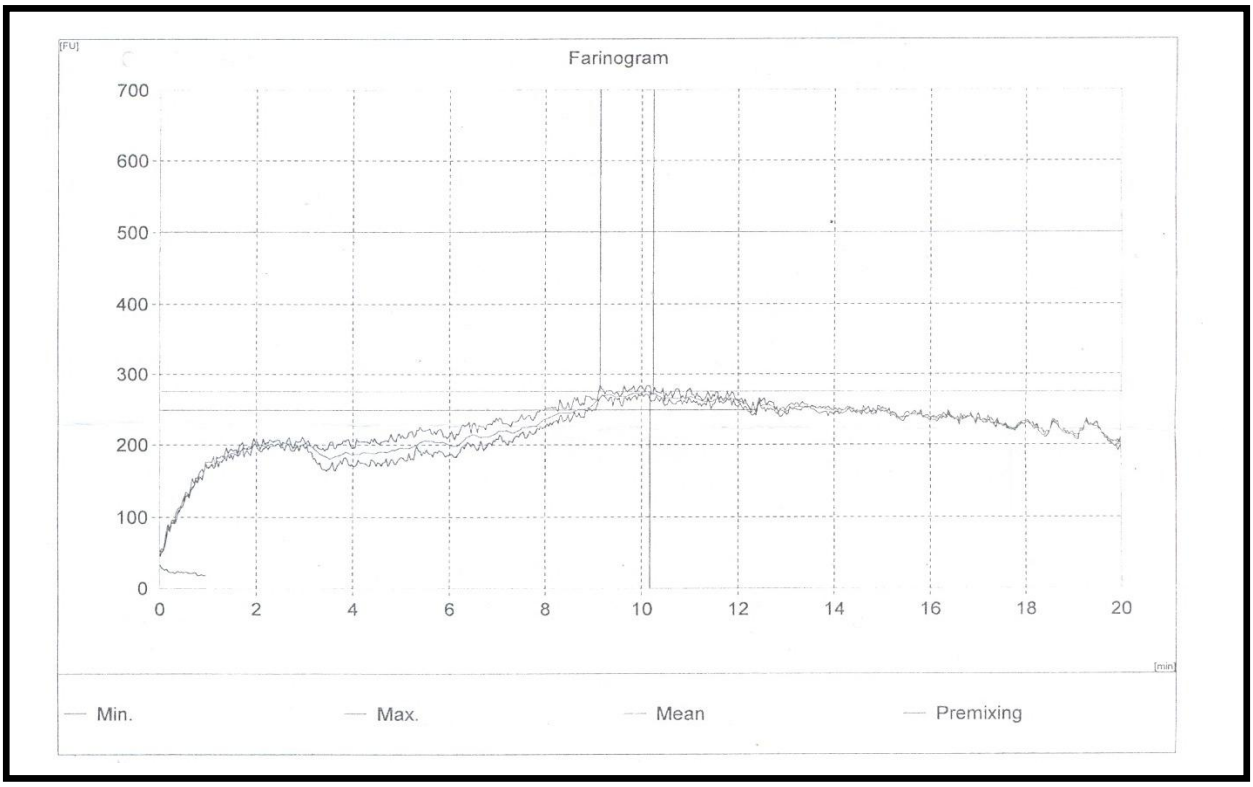


Figure (1) Farinogram of Dough Prepared from Gluten free Flour with 0 μg TGase

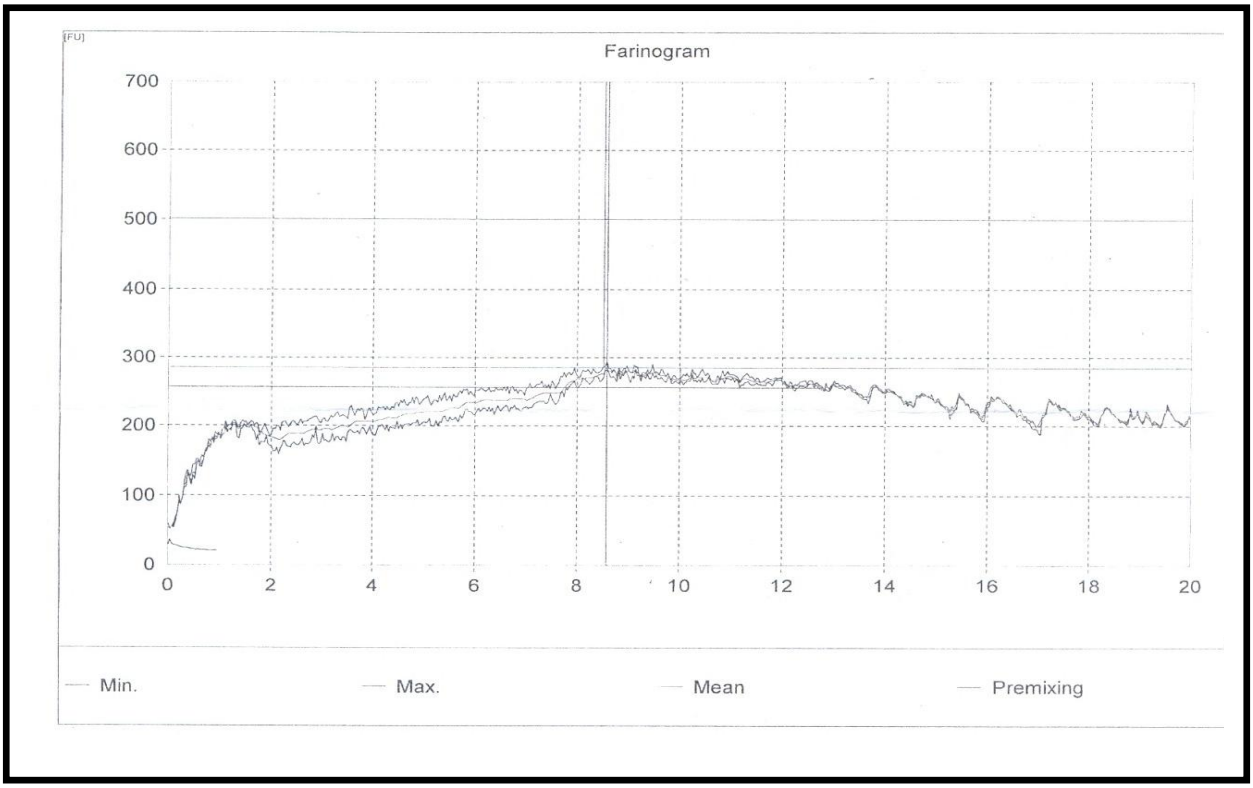


Figure (2) Farinogram of Dough Prepared from Gluten free Flour with 5 μg TGase

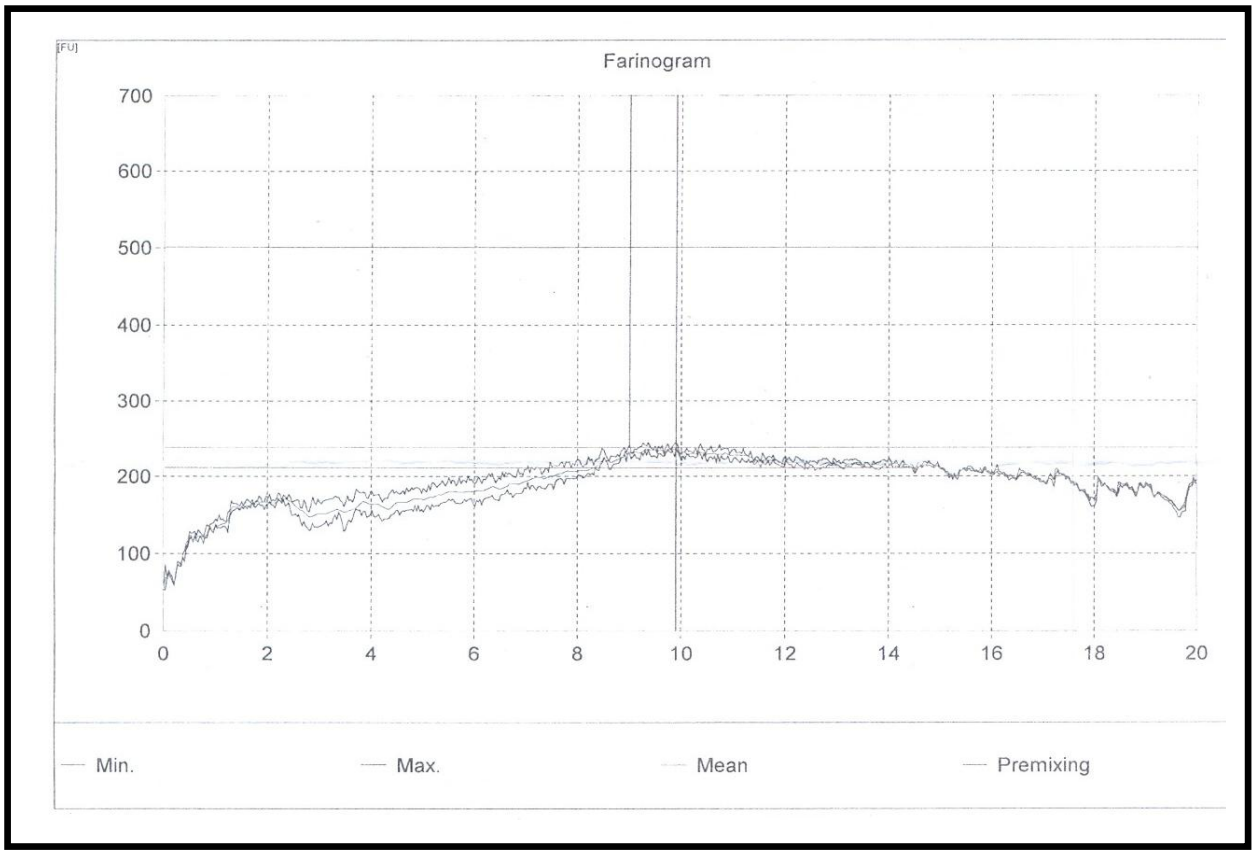


Figure (3) Farinogram of Dough Prepared from Gluten free Flour with 10 μg TGase

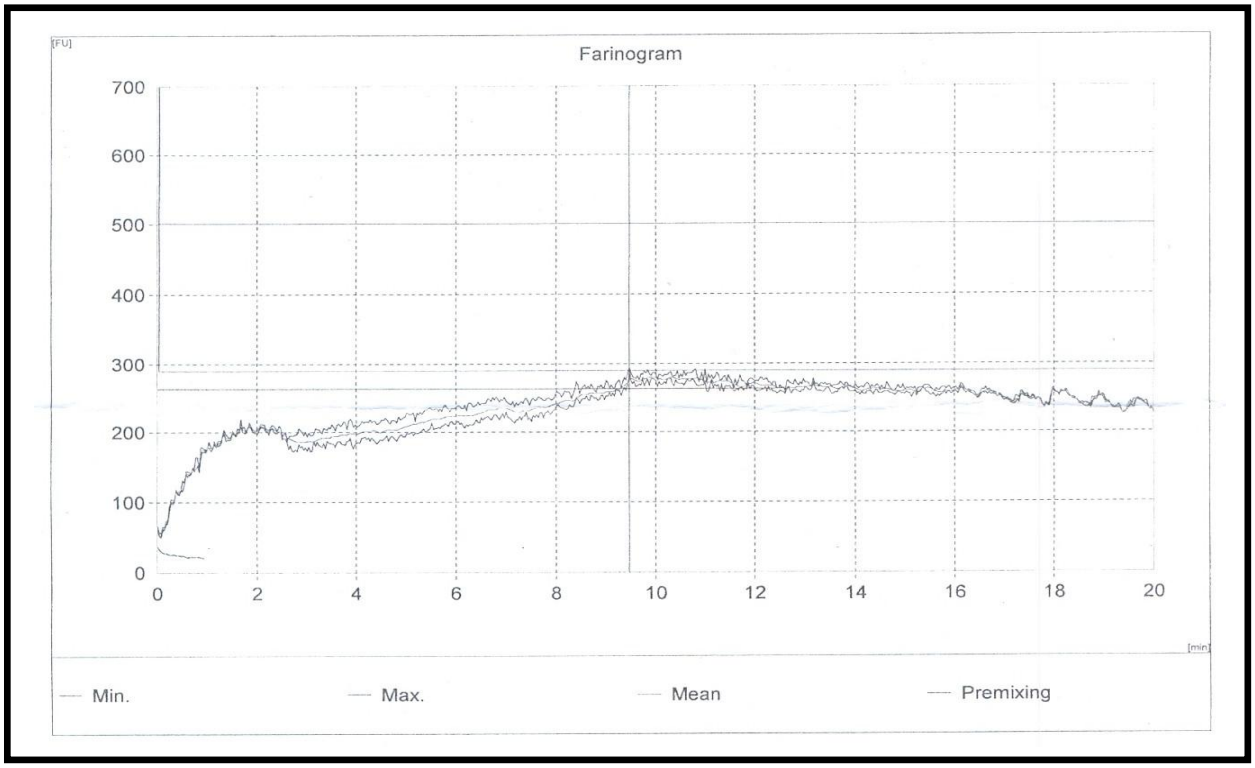


Figure (4) Farinogram of Dough Prepared from Gluten free Flour with 15 μg TGase

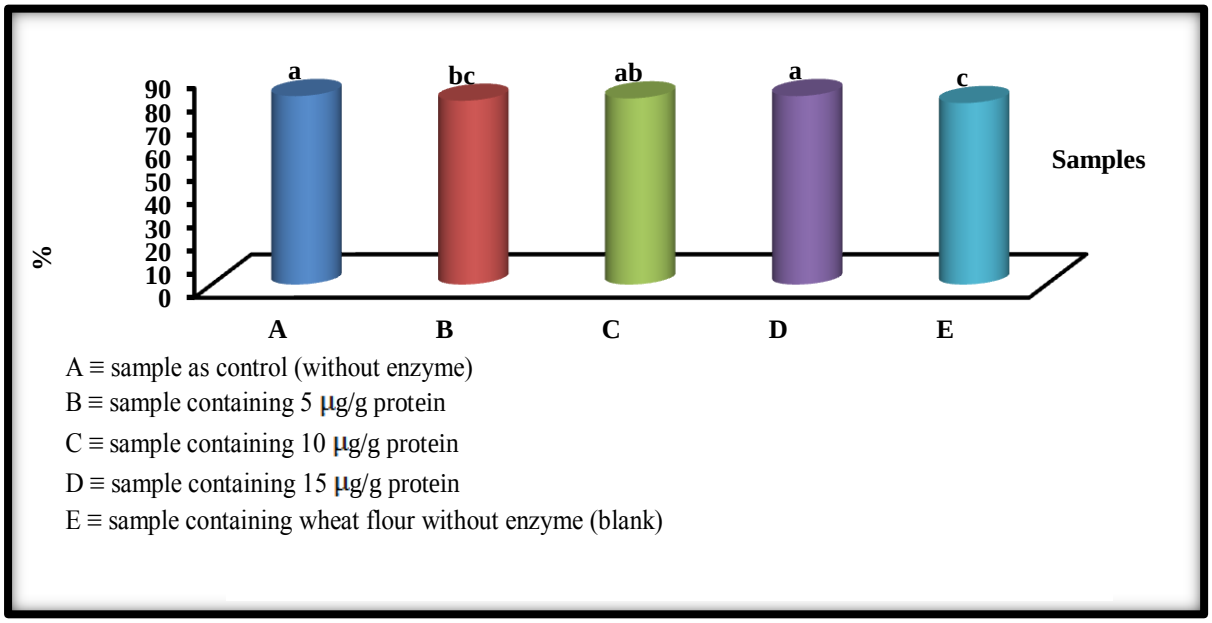


Figure (5): Water Absorption Capacity of Gluten Free Flour Blends

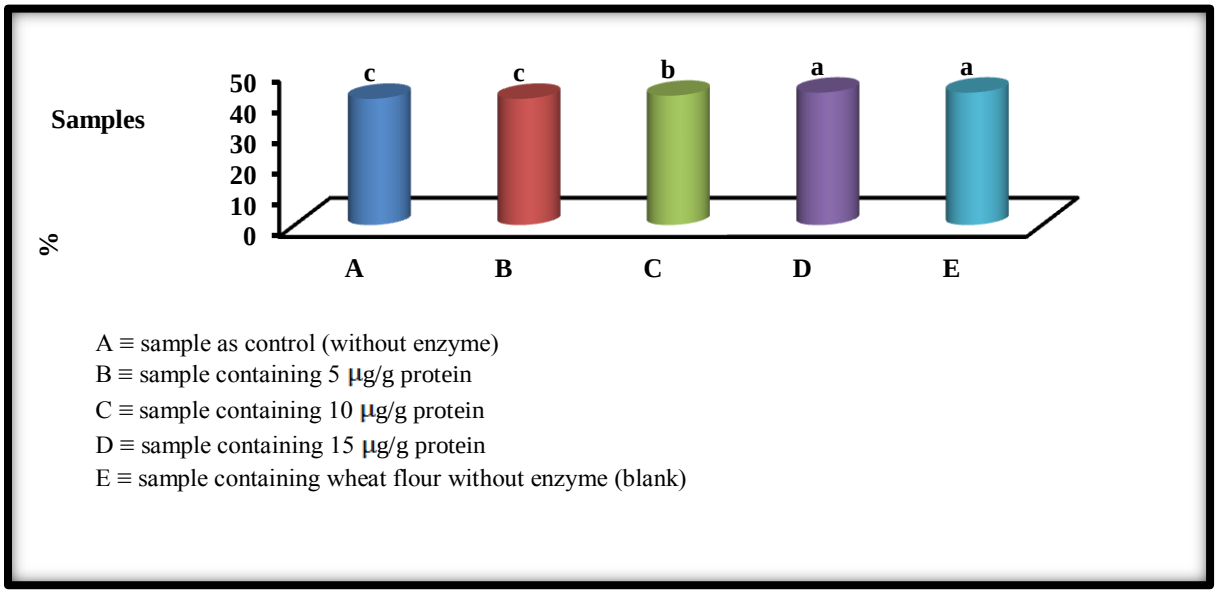


Figure (6): Emulsifying Activity Of Gluten Free Flour Blends

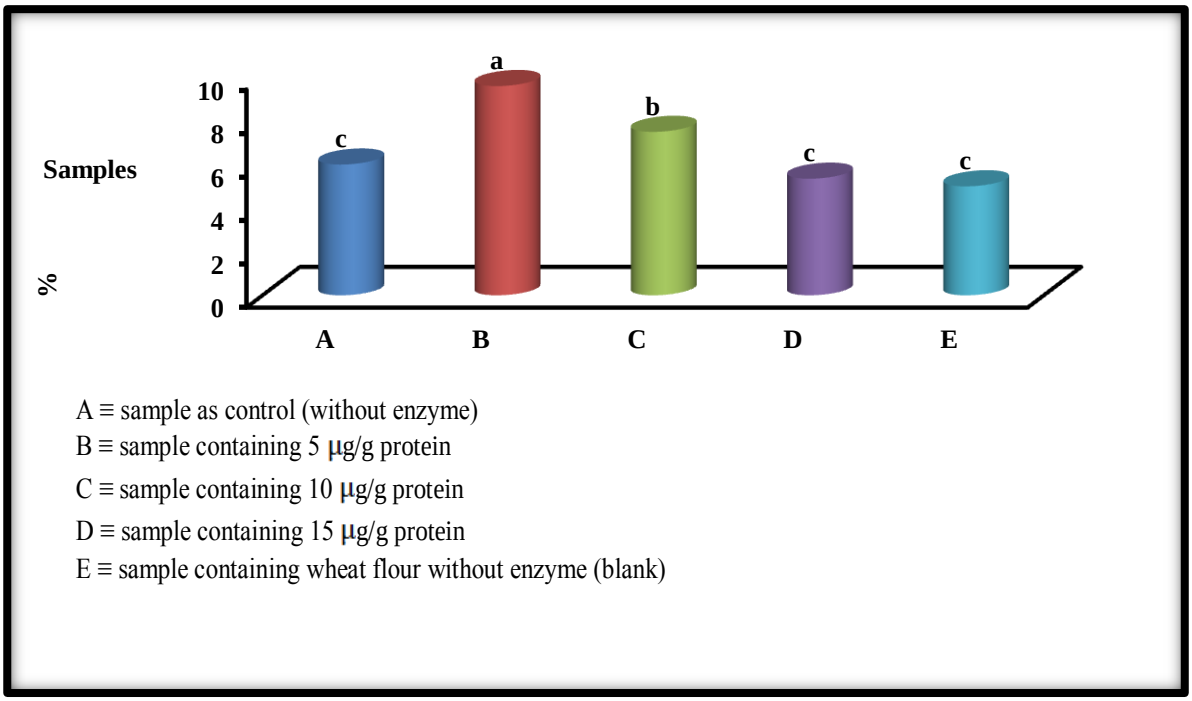


Figure (7): Foaming Capacity Of Gluten Free Flour Blends

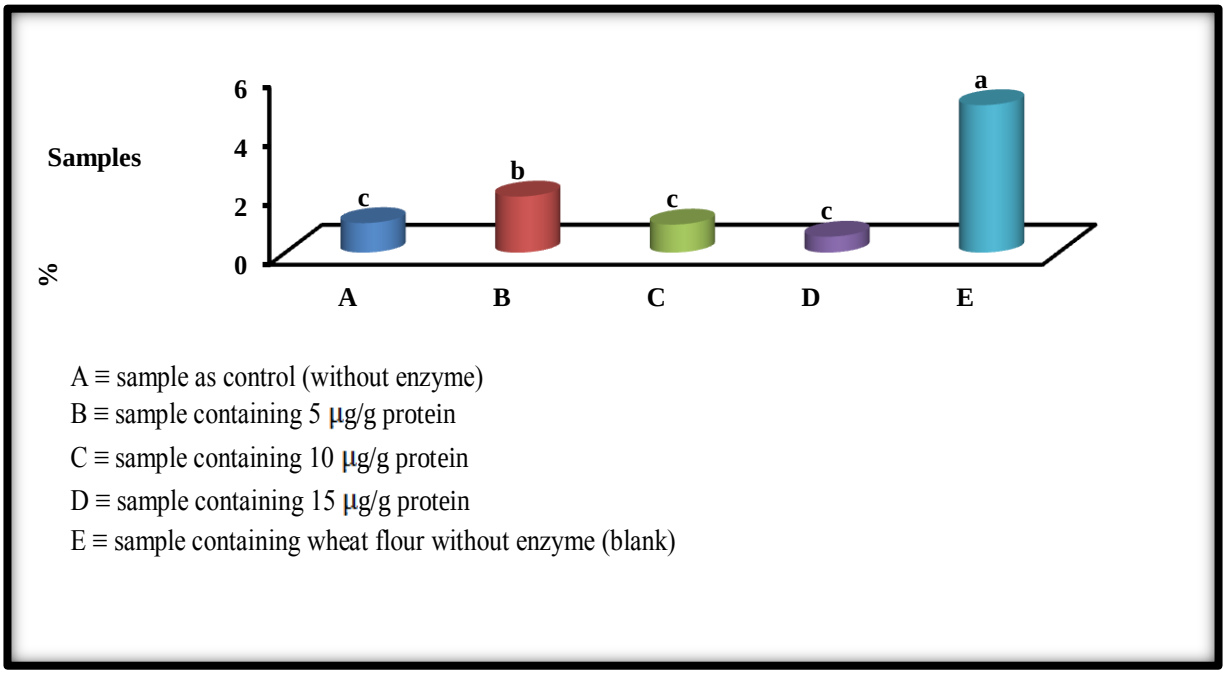


Figure (8): Foaming Stability Of Gluten Free Flour Blends

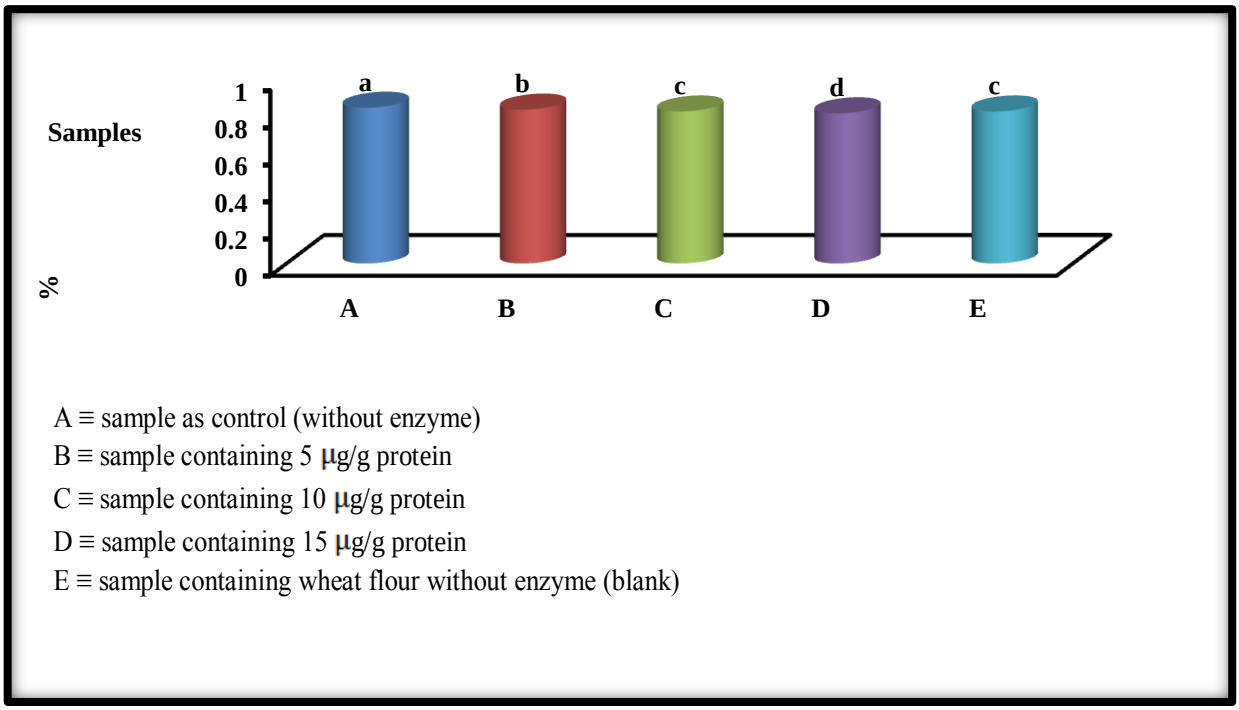


Figure (9): Bulk Density of Gluten Free Flour Blends

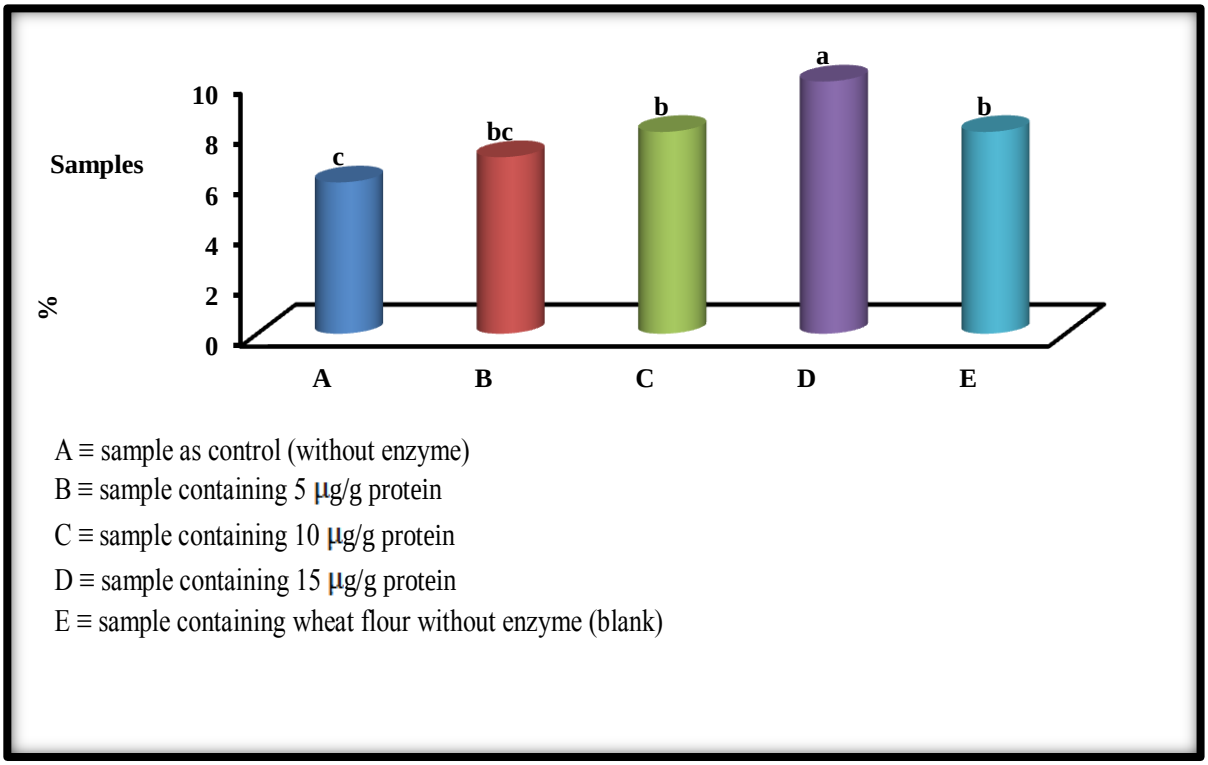
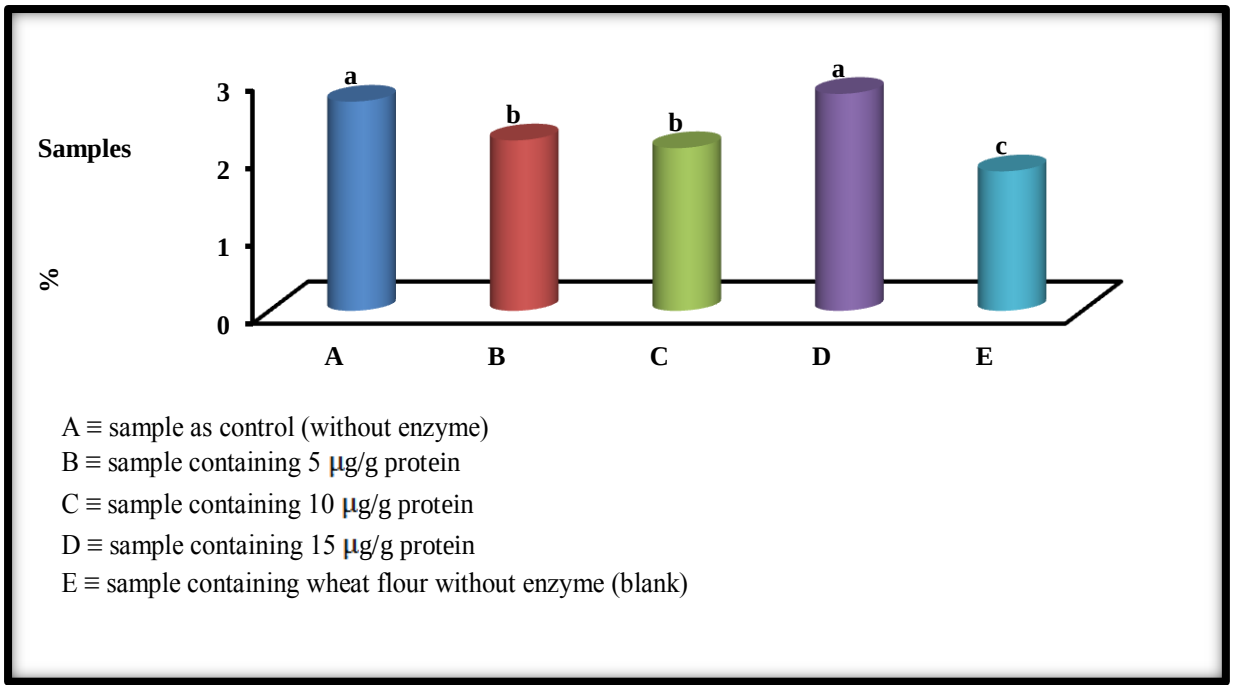
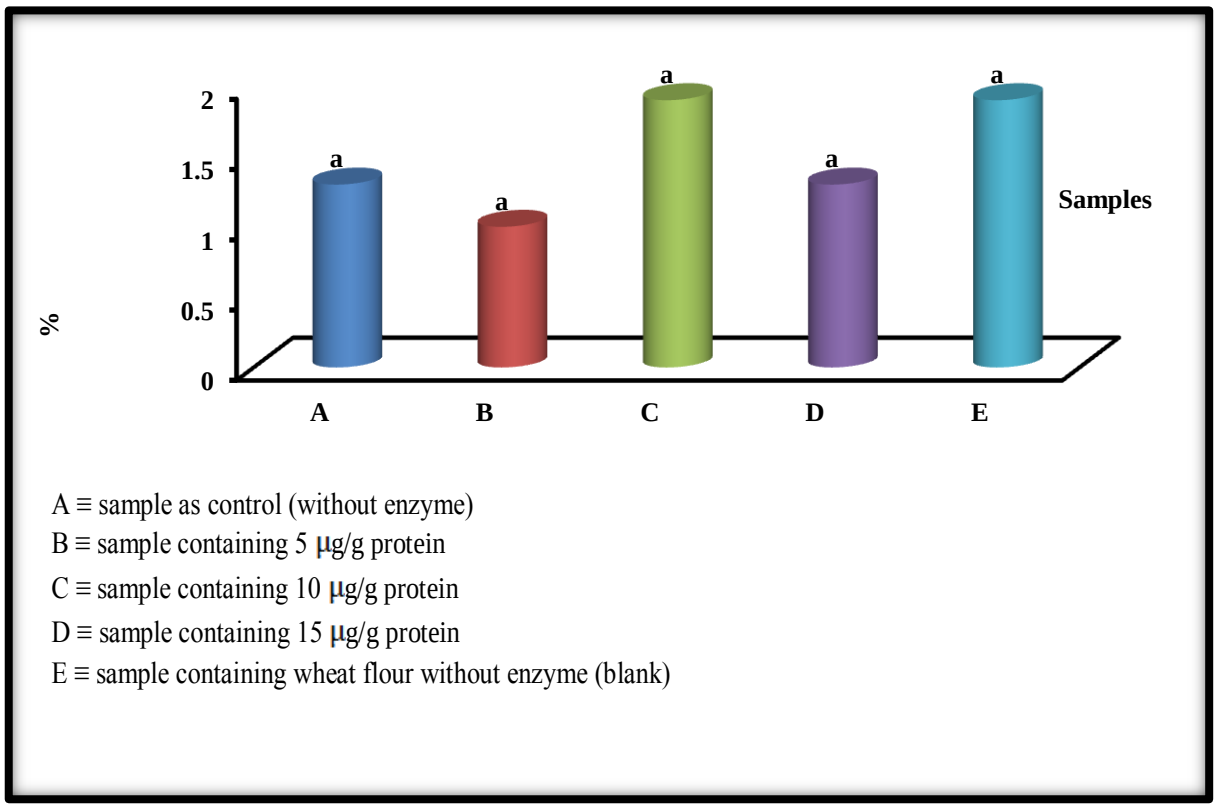


Figure (10): Swelling Capasity Of Gluten Free Flour Blends



A ≡ sample as control (without enzyme)
B ≡ sample containing 5 µg/g protein
C ≡ sample containing 10 µg/g protein
D ≡ sample containing 15 µg/g protein
E ≡ sample containing wheat flour without enzyme (blank)

Figure(11): Albumin of Gluten Free Flour Blends



A ≡ sample as control (without enzyme)
B ≡ sample containing 5 µg/g protein
C ≡ sample containing 10 µg/g protein
D ≡ sample containing 15 µg/g protein
E ≡ sample containing wheat flour without enzyme (blank)

Figure (12): Prolamin Of Gluten Free Flour Blends

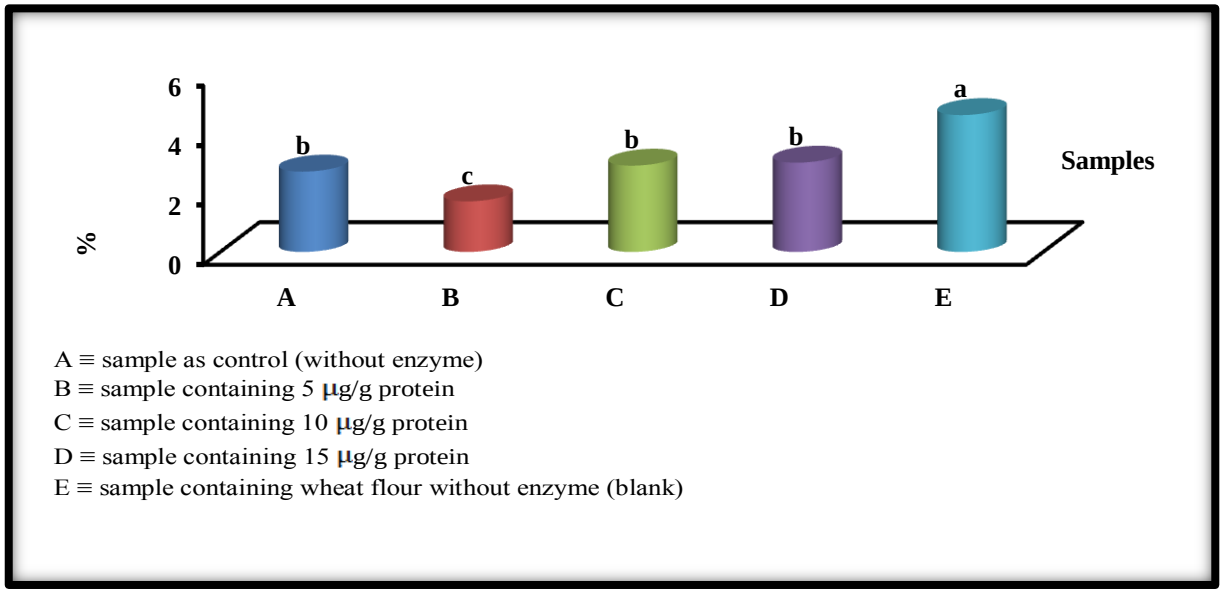


Figure (13): Globulin of Gluten Free Flour Blends

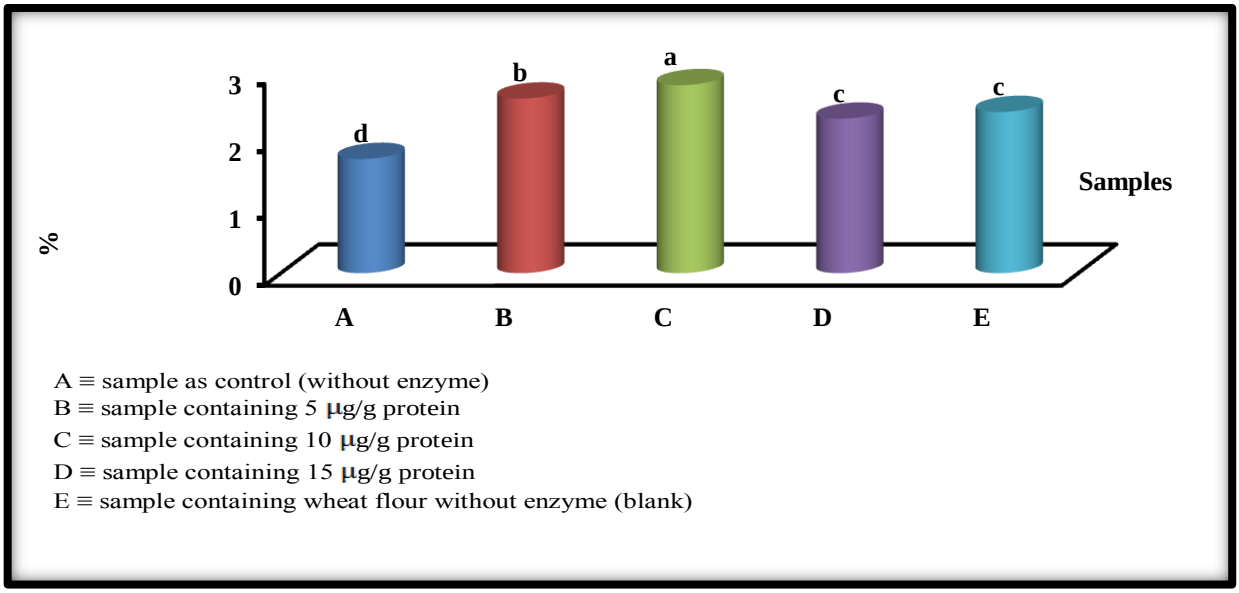


Figure (14): Glutelin Of Gluten Free Flour Blends

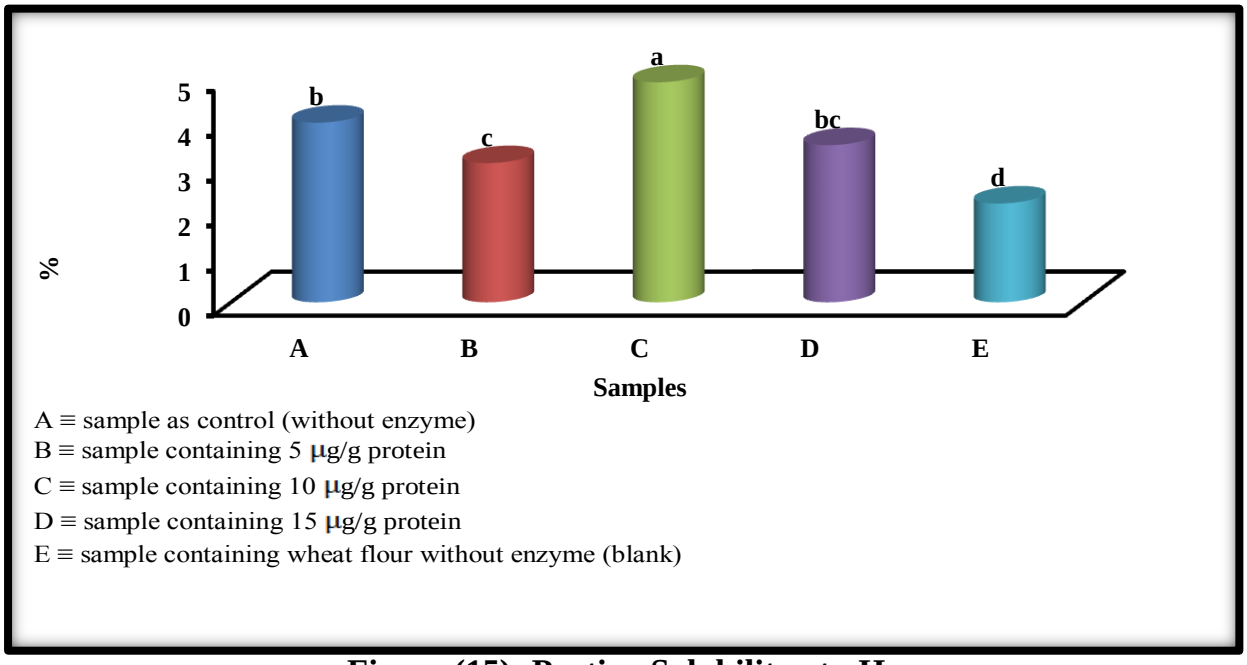


Figure (15): Protien Solubility at pH_4

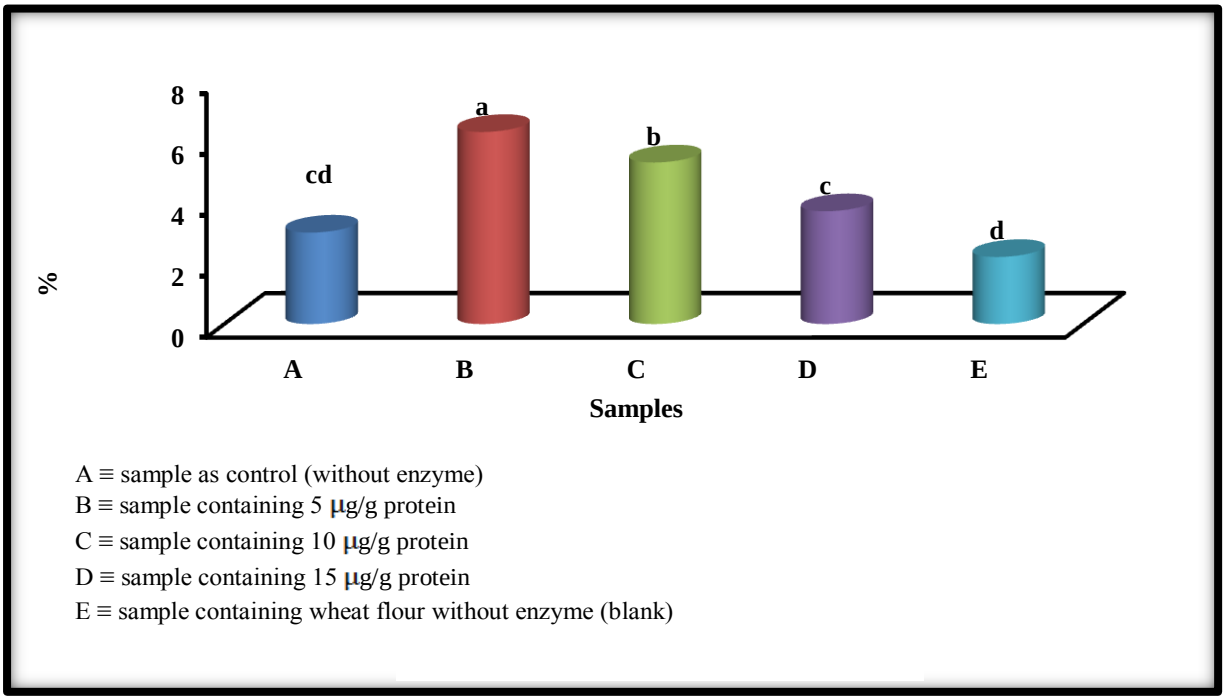


Figure (16): Protien Solubility at pH_6

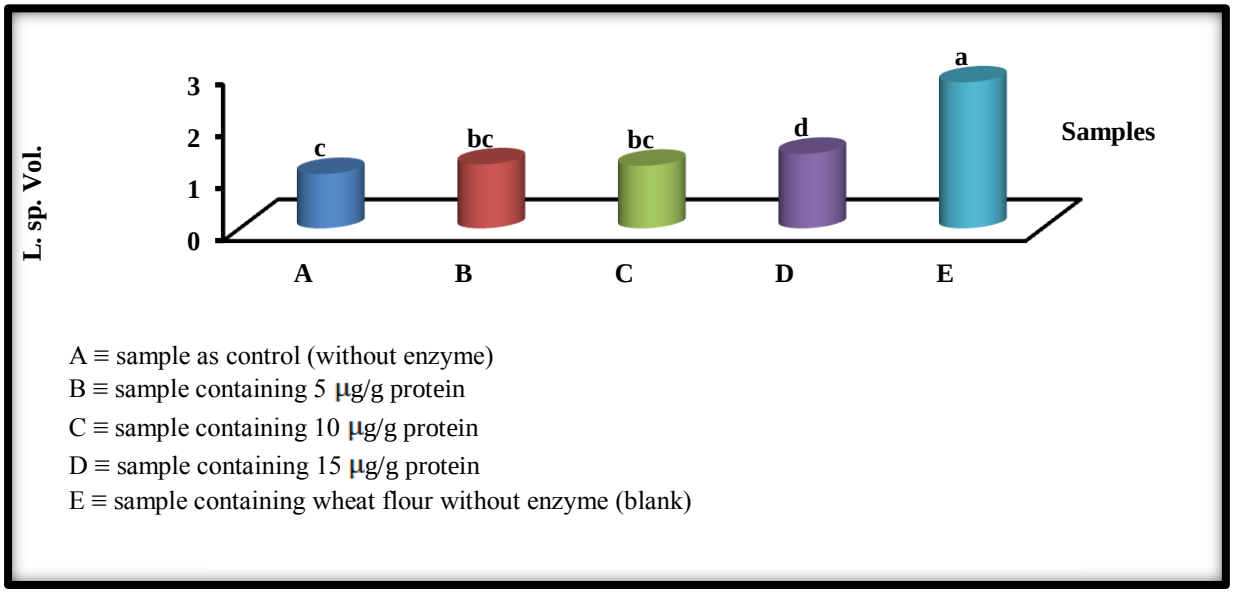


Figure (17): Specific Volume Bread Made of Gluten Free Flour

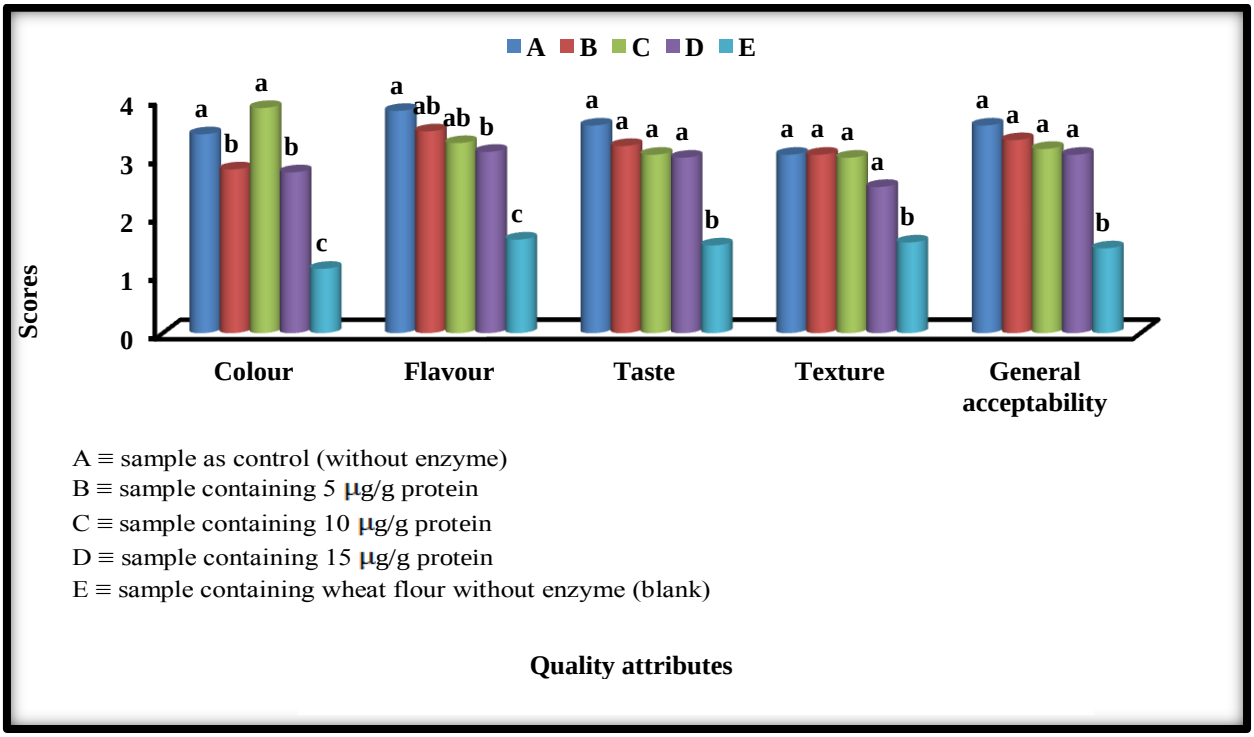


Figure (18): Sensory Evaluation of Gluten Free Bread