بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Agricultural Studies

Department of Food Science and Technology

Production of Biscuit from Faba beans (*Vicia faba*) as a Gluten free Product

إنتاج بسكويت من الفول المصري (*Vicia faba*) كمنتج خالي من الجلوتين

A Dissertation Submitted to Sudan University of Science And Technology in partial fulfillment for the degree of B.Sc. in food Science and Technology.

By

Mohamedalmustafa Abdullatif Osman

Fuad Suliman Adam Hamad

Supervised by:

Dr. Maha Fadul Mohammed El-Baloula

November, 2020

الآية

بسم الله الرحمن الرحيم

قال تعالى:

(فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ وَلَا تَعْجَلْ بِالْقُرْآنِ مِن قَبْلِ أَن يُقْضَىٰ إِلَيْكَ وَحْيُهُ وَقُل رَّبِّ زِدْنِي عِلْمًا).

صدق الله العظيم

سورة طه الآية (114)

Dedication

To our mothers soul

To our beloved fathers the origin of our successes

To our brothers and sisters and all our friends

To teachers in different education levels

Acknowledgement

Special praise and thanks to Almighty ALLAH for granting us health and strength to complete this work . In a special way, we would like to thank our supervisor **Dr. Maha Fadul Mohammed** for excellent guidance .

We would like to thank all staff at Department of Food Science and Technology for their Kind Cooperation .

Our thanks go to our family . and also we thank everyone who deserves our thanks .

Table of Contents

المحتويات

List of Tables

Table No. **Title** **Page N**

List of Figures

Fig. No. **Title** **Page No.**

List of Plates

Abstract

The objective of the study was to produce free gluten biscuit by using local materials (Faba beans) *Vicia faba* of high nutritional value .Faba beans biscuit were formulated from 100% faba beans flour. Different analysis were conducted for raw materials and processed faba beans biscuit including chemical composition (protein , ash , moisture , fiber , fat , carbohydrate and calories) and sensory evaluation. Faba beans biscuit contained higher levels of protein and ash 13.52 % and 3.37 % as compared to biscuit processed from wheat flour 13.33% and 3.31 % respectively. The result of sensory evaluation for biscuit (A) was 4.15, 3.75, 3.55, 4.0, 4.05 for colour , flavor, teste, texture, and overall quality respectively while the control biscuit (B) were 4.10, 3.35, 3.75, 3.35, 4.0 for colour, flavor, teste, texture, and overallquality respectively. From the results obtained in this study we can conclude that the processing of faba beans biscuit was successful. Faba beans biscuit contained higher protein and ash than control biscuit. Same time the processing faba beans biscuit has similar sensory characteristics and was very acceptable.

ملخص الدراسة

الهدف من هذه الدراسة هو إنتاج بسكويت خالي من الجلوتين بإستخدام مواد محلية متوفرة الفول المصري (*Vicia faba*) ومرتفعة القيمة الغذائية. تم تجهيز خلطة البسكويت بإستخدام 100 % طحين فول مصري. أجريت التحاليل المختلفة للمواد الخام والبسكويت شملت التحاليل التقريبية (البروتين، الرماد، الرطوبة، الألياف، الدهون، الكاربوهيدرات والطاقة) والخصائص الحسية. إحتوى بسكويت الفول المصري على مستويات عالية من البروتين والرماد 13.52 % و3.37 % مقارنة بالبسكويت المصنع من طحين القمح 13.33 % و3.31 % على التوالي. أظهرت نتائج التقييم الحسي للعينة (A), 3.75, 3.55, 4.0, 4.05, 4.15 للون والنكهة, الطعم, القوام, والجودة العامة على التوالي. بينما العينة المرجعية (B) كانت النتائج 4.0, 3.35, 3.75, 3.35, 4.10 للون والنكهة, الطعم, القوام, والجودة العامة على التوالي. من النتائج المأحوذة من هذه الدراسة يمكننا ان نستخلص ان إنتاج البسكويت من الفول المصري كان ناجحا . بسكويت الفول المصري إحتوى علي كمية عالية من البروتين والرماد مقارنة بعينة البسكويت القياسية. في نفس الوقت بسكويت الفول المصري المصنع إمتلك نفس الخصائص الحسية وكان مقبولا جدا .

CHAPTER ONE

INTRODUCTION

Food legumes, particularly dried pulses play an important role inboth crop production system and human nutrition. They from an important component of the diets of people in many developing countries of Asia and Africa.

Grain legumes are traditionally consumed as human foods along with cereals in various forms. Among food crops, legumes contain the highest amount of protein, generally twice the level formed in cereals.

Faba beans are a pulse crop in the pea family. They are utilized for human food and livestock feed. The dried seeds are cooked, canned, frozen, roasted as snacks, or ground into flour. High-protein breads can be made by adding up to 20% faba bean flour. The flour can also be used in gluten-free baking.

Faba bean is the most important pulse crop in the Sudan on basis of area cultivated and farm income. The boiled beans are considered as the main dish in breakfast and dinner meals for large population in the urban areas of the Sudan **(Ahmed, 1990).**

Around 1% of the world population is affected by celiac disease. Celiacs are constrained to follow a strict gluten free diet. Often their diet is unblanced and locks in many nutrients. In recent years, some breakthroughs have been made but there is still the need to provide better quality products to celiac people.

Biscuits represent a good vehicle to distribute nutrients to celiac patients, because they are a convenient food appreciated by all groups of population. In addition, it is easier to produce gluten free biscuits than gluten free breads.

Faba beans biscuit is a good source of protein and ash it is contain 13.52 % protein, 3.37% ash, 4.52% fat, 1.43 % fiber, 8.37 % moisture and 114.48 carbohydrates.

Objectives

1- To produce a gluten free biscuit from faba beans
2- To evaluate the chemical composition of the produced biscuit
3- To investigate the backing test of the produced biscuit
4- To estimate panel test of the produced biscuit

CHAPTER TWO

LITERATURE RVIEW

2.1 Celiac disease

Celiac disease is an immune-mediated enteropathy triggered by the ingestion of gluten in genetically susceptible individuals. The major predisposing genes are located on the HLA system on chromosome 6, namely the HLA-DQ2 and DQ8 genes found in at least 95% of patients **(Jabri *et al,* 2009)** Gluten is a complex mixture of storage proteins of wheat, a staple food for most populations in the world, and other cereals (rye and barley).

Gluten proteins have several unique features that contribute to their immunogenicproperties. They are extremely rich in the amino acids proline and glutamine. Due to the high proline content, gluten is highly resistant to proteolytic degradation within the gastrointestinal tract because gastric and pancreatic enzymes lack post-proline cleaving activity. Moreover, the high glutamine content makes gluten a good substrate for the enzyme tissue transglutaminase (tTG)**(Wouters *et al,*2009).** Gluten proteins are now known to encode many peptides that are capable of stimulating both a T cell-mediated and an innate response. The 33-mer is a gliadin peptide of 33 residues (2-gliadin 56–88) produced by normal gastrointestinal proteolysis, containing six partly overlapping copies of three T cell epitopes. The 33-mer is an immunodominant peptide that is aremarkably potent T cell stimulator after deamidation by tTG **(Fasano *et al,* 2003).**

Celiac disease is one of the most common lifelong disorders on a worldwide basis.

The condition can manifest with a previously unsuspected range of clinical presentations, including the typical malabsorption syndrome (chronic diarrhea, weight loss, abdominal distention) and a spectrum of symptoms potentially affecting any organ or body system. Since celiac disease is often atypical or even silent on clinical grounds, many cases remain undiagnosed, leading to the risk of long-term complications, such as osteoporosis, infertility or cancer **(Fasano *et al,* 2003).** There is a growing interest in the social

dimension of celiac disease, since the burden of illness related to this condition is doubtless higher than previously thought **(Sattar *et al.*, 2011) .**

2.1.1 Symptoms of celiac disease:

The symptoms of celiac disease (CD) vary greatly among patients and can affect almost any part of the body. One child may experience severe diarrhea and abdominal pain while another may have skin, liver, neurological, dental, or other problems. Many children with celiac disease are underweight and have short stature compared to their non-celiac peers. Patients can also have the intestinal inflammation with no overt manifestations **(Mustaiahl, *et al*).**

The most common typical and atypical celiac disease symptoms include:

(diarrhea, intolerance, abdominal pain, distention, wasting , change in appetite, constipation, dyspepsia, bacterial overgrowth, malabsorption bloating, vomiting, depression, anxiety, neurosis, moodiness) **(Wouter, 2009).**

2.1.2. Epidemiology

2.1.2.1. In the general population:

In the past, celiac disease was considered a rare disorder, mostly affecting children of European origin. Indeed, this idea is still widespread, so much that in many European countries celiac disease continues to be included in the list of rare disorders protected by specific regulations of the healthcare system. On the other hand, a huge number of studies have recently shown that celiac disease is one of the commonest lifelong disorders affecting humans in many areas of the world. Currently most cases remain undiagnosed, due to the lack of typical symptoms, and can be recognized only through serological screening by sensitive tools (e.g. serum IgA class antitransglutaminase and anti-endomysial antibodies determination) . Serological screenings performed on general population samples have confirmed that the prevalence of celiac

disease in Europe is very high, ranging between 0.75 and 0.4% of the general population, with a trend toward higher figures (1% or more) in younger subjects and among groups that have been more isolated genetically .

celiac disease was generally perceived to be less common in North America than in Europe Should the frequency of celiac disease be lower in the USA, the existence of a protective environmental factor in that country should be postulated, since Americans and Europeans largely share a common genetic background. This epidemiological “dilemma” has recently been answered by our large US prevalence study including 4126 subjects sampled from the general population **(Dalgic *et al*, 2011)** . The overall prevalence of celiac disease in this US population sample was 1:133, actually overlapping the European figures **(Fasano *et al*, 2003)**

2.1.2.2. In at-risk groups:

Studies all over the world have shown that the prevalence of celiac disease is definitely increased in specific population subgroups .

The risk of celiac disease in first-degree relatives has been reported to be 6–7% on average, mostly ranging from 3 to 10% In a Finnish study on 380 patients with celiac disease and 281 patients with dermatitis herpetiformis, the mean disease prevalence was 5.5%, distributed as follows: 7% among siblings, 4.5% among parents and 3.5% among children**(Rubio *et al*, 2008)**The prevalence of celiac disease is also increased in second-degree relatives **(Fasano *et al*, 2003),** highlighting the importance of genetic predisposition as a risk factor.

Celiac disease prevalence is increased in autoimmune diseases, especially type 1 diabetes and thyroiditis, but also in less common disorders (e.g. Addison’s disease or autoimmune myocarditis). The average prevalence of celiac disease among children with type 1 diabetes is 4.5% (0.97–16.4%) **(Frost *et al*., 2009)**.

Usually diabetes is diagnosed first, while celiac disease is often subclinical and only detectable by serological screening. The increased frequency of celiac disease in several thyroid diseases (Hashimoto's thyroiditis, Graves' disease, and primary hypothyroidism) is well established **(Lenhardt *etal.*, 2004)** A 3- to 5-fold increase in celiac disease prevalence has been reported in subjects with autoimmune thyroid disease.

On the other hand, celiac disease-associated hypothyroidism may sometimes lack features of an autoimmune process. Interestingly, treatment of celiac disease by gluten withdrawal may lead to normalization of subclinical hypothyroidism. The causal relationship between celiac disease and other autoimmune disorders is still a controversial issue. The two most accredited theories propose: (1) this association is secondary to a common genetic background predisposing to both celiac disease and the associated autoimmune disease or (2) untreated celiac disease leads to the onset of other autoimmune disorders in genetically susceptible individuals. This second hypothesis is supported by the evidence that tTG seems to be only one of the autoantigens involved in gluten-dependent autoimmune reactions. Other autoantigens which are normally "cryptic" can be unmasked and cause a self-aggressive immunological response following the gliadin-initiated inflammatory process **(Fasano *et al,* 2003).**

2.1.3 Diagnosis

2.1.3.1 Serologicaltesting:

Although an intestinal biopsy is still considered necessary to confirm the diagnosis of celiac disease, serological tests are frequently used to identify individuals for whom the procedure is indicated Commercially available **(Schuppan *et al.,* 2009)** tests include IgA- and IgG-AGA, EMA, anti-tTG, and anti-actin antibodies. These tests are particularly helpful in individuals

without gastrointestinal symptoms and those with conditions associated with celiac disease, as well as for screening asymptomatic firstdegree relatives of known cases. They have also been widely used in epidemiologic studies to determine the prevalence of celiac disease.

2.1.3.2 Small intestinal biopsy:

Small intestinal biopsy is the cornerstone of diagnosis and should be undertaken in all patients with suspected celiac disease. Biopsies can be obtained using capsules with a suction-guillotine mechanism (e.g. Watson capsule). Nowadays, most biopsies in both children and adults are taken at the time of upper gastrointestinal endoscopy using standard fiber-optic instruments. Endoscopy allows multiple biopsies to be taken, which minimizes sampling error **(Lenhardt *et al.*, 2004)**.

2.1.3.3 HLA testing:

Polymerase chain reaction sequence-specific oligonucleotide typing methods are now available for the determination of alleles encoding HLA-DQ2 and DQ8. The entity of the HLA-related risk (high or low) can be quantified using second-generation commercial kits allowing the complete characterization of the HLA-DQ2 and DQ8 genotype. Currently two major clinical applications of this test can be considered:

(1) to rule out the possibility of celiac disease in at-risk subjects (e.g. first-degree relatives and patients with type 1 diabetes). Since the HLA predisposing genotype is a necessary (but not sufficient) factor for disease development, the negative predictivevalue of HLA typing is very high (i.e. the vast majority of subjects who are DQ2- and DQ8-negative will never develop celiac disease); (2) to rule out celiac disease in doubtful cases (celiac disease can be excluded with a 99% confidence in DQ2- and DQ8-negative subjects). to 75% of patients with refractory sprue, a condition that is currently classified as cryptic enteropathy-associated T-cell lymphoma. These

patients typically undergo pharmacologic therapies, including treatment with steroids, or immunosuppressants, such as azathioprine and cyclosporin . If patients do not respond to these treatments, the ultimate treatment is total parenteral nutrition. However, none of these therapies have been subjected to rigorous controlled studies **(Rubio, *et al* 2008)** Celiac disease is associated with intestinal lymphoma and other forms of cancer, especially adenocarcinoma of the small intestine, of the pharynx, and of the esophagus. Enteropathy-associated T-cell lymphoma (EATL) is a rare form of high-grade, T-cell non-Hodgkin lymphoma (NHL) of the upper small intestine that is specifically associated with celiac disease. This NHL subtype arises in patients with either previously or concomitantly diagnosed celiac disease. In a subgroup of patients, there

is progressive deterioration of a refractory form of celiac disease. EATL derives from a clonal proliferation of IELs and is often disseminated at diagnosis. Extraintestinal presentations are not uncommon in the liver/spleen, thyroid, skin, nasal sinus, and brain.

The outlook for patients with EATL is poor. Recent studies indicated that:

(1) celiac disease is associated with a significantly increased risk for NHL, especially of the T-cell type and primarily localized in the gut (EATL);

(2) the celiac disease–lymphoma association is less common than previously thought, with a relative risk close to 3;

(3) celiac disease screening is not required in patients with NHL of any primary site at the onset, unless suggested by specific findings (T-cell origin and/or primary gut localization); (4) the risk of NHL associated with clinically milder (or silent) forms could be lower than in typical cases of celiac disease. Several follow-up studies suggest that the GFD protects from cancer development, especially if started during the first years of life. Strict

adherence to the GFD seems to be the only possibility of preventing a subset of rare but very aggressive forms of cancer **(Green and Cellier, 2007)**.

2.1.4. Celiac disease in Sudan

The disease was first reported in Sudan in 1978 when 7 children were diagnosed . Since then, many adult and paediatric cases have also been reported. The disease may in fact be under-diagnosed and not well documented because of more prevalent conditions such as malnutrition, diarrhoeal diseases and intestinal parasitic infections (**Suliman,1978**).

2.2 Faba Beans:

Faba beans *Vicia faba* are a pulse crop in the pea family. They are utilized for human food and livestock feed. The dried seeds are cooked, canned, frozen, roasted as snacks, or ground into flour.[1] High-protein breads can be made by adding up to 20% faba bean flour. The flour can also be used in gluten-free baking **(Aykroyd *et al.*, 1982) .**

Faba bean is the most important pulse crop in the Sudan on basis of area cultivated and farm income. The boiled beans are considered as the main dish in breakfast and dinner meals for large population in the urban areas of the Sudan . Faba bean is grown as a winter crop under irrigation mainly in the Northern State in about 70% of the total cultivated area and the River Nile State in about 30% of the total cultivated area in the Sudan. It is also grown to a limited extent in Khartoum State and Jabel Marra in Western Sudan due to the suitability of the environmental conditions .

Lately, it was introduced to the larger irrigated schemes of Gezeira, Rahad and New Halfa Faba beans are also known as: (Fava bean - Field bean - Broad bean - Bell bean -English bean).

Faba beans can be ground into flour and used in gluten-free baking applications. For added protein, faba bean flour can used in formulas for pizza dough, bread, pancakes, cookies and muffins. Substituting ingredients may affect flavor, texture, and volume **(Ahmed, 1990).**

2.2.1 Origin

Beans are an important food crop consumed globally. They were first domesticated around 10,000 BC.2 In 2013 faba bean production reached 4.56 million tonnes.[2] Faba beans are native to the near east but have extended to North America, South America, Burma, China, Sudan, and Uganda **(Guillon and Champ, 2002)** .

2.2.2 Function:

Faba bean flour and faba bean protein powder are used in gluten-free baking applications. The flour can be used up to 50% in combination with of starches and gums commonly used in gluten-free formulas. In conventional baking, it can be added to increase the protein content of the baked good **(Hermsdorff *et al.*, 2011)**

Faba bean flour provides the following:

- Texture and volume
- Additional protein
- Dough strengtheners
- Crumb whitening
- Thickener

2.2.3 Nutrition:

Faba beans proteins are a rich source of lysine. Using 25% faba bean flour adds protein, fiber, and iron to a baked good.

Table 2.1: Faba Bean Flour

Calories	330	Sodium	0 mg
Total Fat	2 g	Potassium	0 mg
Saturated	0 g	Total Carbs	48 g
Polyunsaturated	0 g	Dietary Fiber	10 g
Monounsaturated	0 g	Sugars	3 g
Trans	0 g	Protein	27 g
Cholesterol	0 mg	Calcium	0%
Vitamin A	0%	Iron	0%
Vitamin C	0%		

Percent Daily Values are based on a 2000 calorie diet.

2.2.4 Application:

Dried faba beans can be ground into a flour and used in the following applications:

- Protein bars
- Gluten-free coatings
- Pasta
- Gluten-free bread
- Protein enriched bread

For a high-protein bread, faba beans can be used at 20% (flour weight). The protein value will be about 7 grams per 50 gram serving, so this may potentially meet a ‘Good Source of Protein’ claim **(Massen, 1980) .**

2.2.5. Faba beans in Sudan

In Sudan, faba bean is one of the primary grown and consumed legume crops. It constitutes to the primary human nutrition, supplying high-quality proteins essential for a balanced diet for the daily breakfast and dinner of the millions of people who cannot afford meat as a source of protein in both rural and urban area **(Osman *et al*.2014).** In many parts of Sudan, faba beans are

served in several types of dishes such as stewed faba bean (Fuel Musalah), deep fried cotyledon paste with some vegetables and spices (Taamia or Falafel), and faba bean soup with bread and cheese whey (Fata). Additionally, the crop is an imperative source of income for the farmers in the country **(Salih and Mohamed, 1992)**. However, the demand for this nutritious legume crop is growing, fuelled by rapid population growth in the country, which led to an enormous gap between the supply and demand. In Sudan, faba bean is traditionally cultivated in the banks of the Nile River north of latitude 18.50°N in the Northern and Nile States, where temperature is moderately cooler and winter longer (**Salih and Mohamed, 1992).** However, to meet the ever-increasing demand for faba bean in Sudan, its production was extended into the warmer areas at latitudes lower than 15°N, where the climate is marginally suitable. In these areas, faba bean yield is far below the potential **(Gasim *et al*.2013),** mainly because of the biological limitations of the traditional cultivars and poor management practices as well as the effect of abiotic (especially temperature) and/or biotic (diseases and pests) stresses. Improving seed yield and quality of faba bean under stress conditions are important priorities to meet the increasing demand and feed a growing population. Thus, the breeding objectives for this crop have always been and still are to improve the resistances to drought, heat, diseases, and pests, as well as to enhance the grain yield and quality. However, evidence on the yield and nutritional quality of newly developed faba bean inbred lines under marginal environment of Sudan is scarce. Detailed information about the productivity and seeds quality of faba beans inbred lines grown in nontraditional areas of Sudan will enhance our knowledge and contribute to the food security and income of the growing population in semiarid areas. Therefore, the primary aim of the present study was to investigate the seed yield and quality of five faba bean inbred lines grown under the marginal environmental conditions of Sudan.

2.2.6. Present status of faba bean production and consumption

Presently, faba beans are major crop in many countries including China, Ethiopia and Egypt, and are widely grown for human food throughout the Mediterranean region and in parts of Latin America **(Razia Akbar,2000; Naqvi, 1984).** China is major share holder in production with 60% **(FAO, 2009).** Other important producers are northern Europe, The Mediterranean, Ethiopia, Central Asia, East Asia and Latin America. In the United States and northern Europe, faba beans are not grown in large quantities and are used almost exclusively for livestock pasturage, hay, and silage **(Singh and Bhatt, 2012a; Oplinger, 1982).** World production of dry faba bean seeds in 1999 to 2003 amounted to 3.90 million tones/year from 2.60 million ha. The main producing countries are China (1.9 million tones/year from 1.2 million ha) (**FAO, 2009**). The annual production in sub-Sahara Africa in 1999 to 2003 was estimated at 510,000 tones, almost entirely from Ethiopia (405,000 tones) and Sudan (100,000 tones). It is worth to mention here the annual production during 2000 (data available) in sub-Sahara Africa increased from 230,000 tones (250,000 ha) to 540,000 tones (450,000 ha) (**Mihailovic *et al*., 2005**). The annual world production of dry faba bean seeds declined from about 5 million tons (from 5 million ha) in the early 1960s to about 4 million tons (from 2.7 million ha) in the early 2000s. The reduction in area under cultivation in China from about 3.5 million ha in the early 1960s to about 1.25 million ha in the early 2000s accounted for the largest share of the reduction in production. In contrast, the annual production in sub-Sahara Africa increased during the same period from 230,000 tones (250,000 ha) to 540,000 tones (450,000 ha). The world production of green faba bean seeds in 1998 to 2003 was estimated at 940,000 t/year from 2.6 million ha, with Algeria (118,000 tones/year), China (114,000 tones/year) and Morocco(112,000 tones/year) as the largest producers (FAO,2009). The production of green faba bean seeds in tropical Africa and Asia is negligible (**Mihailovic *et al*.,**

2005). Egyptians are leader in consuming the faba bean and about 75% of daily per capita protein intake of Egyptians is of vegetable origin, mostly cereals and beans. Mediterranean's and Chinese may depend upon faba beans to supply much of their dietary protein (**Hawtin and Hebblethpiait, 1983; Razia Akbar, 2000**). At present, world average of faba bean productivity is 1.5 t/ha, though Egypt ranked first with 2.96 t/ha Indian productivity is 1.2 t/ha (FAO, 2009). The world production of green faba bean seeds in 1998 to 2003 was estimated at 940,000 t/year from 2.6 million ha, with Algeria (118,000 tones/year), China (114,000 tones/year) and Morocco (112,000 tones/year) as the largest producers. The production of green faba bean seeds in tropical Africa and Asia is negligible.

2.3. Biscuit industry:

Biscuit is apopular item in the diet of weaned infants and young children the word (biscuit) is derived from the biscoctus or the old French –bescoit- meaning twice cooked- a reference to the practice of first baking the product in a hot oven then transferring it to a cooler oven to complete the drying out process . the product known as (biscuit) in the U.S.K **(Whiteley *et al,* 1971) .**

2.3.1 Production and consumption of biscuits in Sudan:

The biscuit industry started in sudan in the early sixties with threepioneer factories namely (snnar) karam and kambal in sudan the estimated biscuit production was 12500 tones in the year1996.

2.3.2. Biscuit raw material:

2.3.2.1. Wheat flour:

Finny 1994 mentioned that the international commercial wheat crop is limited for all preactical purpose to tow species triticum and aestivum which include the commen hard soft wheat used to tproduce bread and many product and triticum and trugidum which include the durum or macaroni wheat fat Lorenz

1994 reported that edible fats and oil are complex mixture of triglycerides and small amount of other substances occurring naturally or derived through processing and storage of the fat a triglycerides is composed of glycerol and there fatty acid sugar the majority of sweeteners are obtained from sugar in one from or another and sugar (sucrose) is derived from to sources .the main sources sugar cane and the secondary is sugar deet **(Whitelely *et al*,1971).**

2.3.2.2. Sweetening agents:

THE majority of sweetening agents are obtained from sugar in one form or another, and sugar (sucrose) is derived from two sources. The main source is sugar cane and the secondary source is sugar beet.

2.3.2.3. Aerating agents:

THE basic ingredients for biscuits have now been dealt with. To increase the palatability and to improve texture, bite, and appearance, it is necessary to achieve some form of aeration. Methods of aeration can be classified broadly into three groups, namely: mechanical (without the use of ingredients but by the method of handling the product), biological (Yeast) , and chemical (Ammonium bicarbonate) .

2.3.2.4. Dairy products:

Milk is the most popular product ' Milk can be used to advantage in most biscuit doughs, and in biscuit fillings and coatings. Milk can be used in form of : Condensed milk , Dried milk powders , Reconstitution of milk powders **(Whitelely *et al*,1971).**

2.3.2.5. Setting materials :

Required to hold fine bubbles of air and cause aeration and thickening

Setting materials include: GELATINE, PECTIN, CARRAGHEEN

2.3.2.6. Flavouring materials:

THE buildup of flavour in biscuits is a very complex matter, depending on many ingredients which give 'background' flavour in addition to those used to give the definite dominant flavour.

Flours from different sources and wheat strains have varying flavours, and as flour is the main ingredient, this must influence the final biscuit flavour. The use of dark sugars in place of refined sugars; of butter or margarine in place of a neutral blend of vegetable oils; the inclusion of fruit or nuts, of cocoa, of egg or cheese or milk, have already been discussed and all play their respective parts in producing flavour. It is the usual practice, however, to include salt to draw out flavour, and also to complement, boost, or change the natural flavour by the addition of some strongly flavoured substance such as a spice or an essence.

Flavouring materials include: SPICES , ESSENCES , Vanilla , Lemon , Orange , Fruit flavours , Coffee extract

Coffee extra , Chocolate .

2.3.2.7. Colouring materials:

THE use of colours in foodstuffs is almost as important as the use of flavours, and the two are closely associated with each other. Colour should be used to enhance the eye appeal of the product, and the use of correct colour to suggest flavour should be linked directly to the type of flavour in use. Colour should be used at all times with discretion, and it is better to use no colour than too much. If a product has ample materials present to give colour there is no point in adding it, but in most biscuits this is not so, and a small amount of added colour is beneficial. The colouring materials that are available fall into two groups: natural and artificial. The natural colours are those obtained from animal and vegetable sources such as (carmine, Annatto , Caramel), whereas the artificial colours are aniline dyes obtained from coal tar such as (Tartrazine , Sunset yellow , and Indigo carmine) **(Whitelely *et al*,1971).**

2.3.2.8 Fats and oils:

In biscuit we have two main groups:

ANIMAL FATS (Butter, Lard, Beeffa, Whale oil) , and VEGETABLE OILS (Coconut oil, Palm oil, Palm kernel oil, Groundnut oil) .

2.3.3. Classification of biscuit types:

BISCUITS are broadly classified as being of hard dough or soft dough origin. The hard dough group are savoury, unsweetened, or semi~sweet, and include all types of crackers, puff dough biscuits, and the semi-sweet varieties such as Marie, Rich Tea, and Petit Beurre. In addition to having a low sugar content, or none at all, the fat content rarely exceeds 22·0% of the flour content, except in the case of puff doughs (but even these have a very low fat content at the mixing stage). The soft dough group includes all the sweet biscuits, whether they are plain biscuits, shells, or flow type such as gingernuts. Soft dough biscuits all have many factors in common, but hard dough biscuits fall naturally into three sections: fermented doughs, puff doughs, and the semi-sweet doughs **(Whitelely *etal*,1971).**

2.3.4. Quality control:

FOR quality control to be effective, there must be standards laid down which are efficiently supervised and adhered to. This applies to every stage of biscuit making, from the receipt of raw materials, through mixing, machining, baking, processing, packing, and storage. It is the concern of all supervisory staff and production operatives, as well as the department that bears the name quality control. The extent to which quality control is carried out naturally depends upon the policy of the company, but as biscuit manufacturing becomes increasingly mechanised and automatic, the need for effective quality control becomes more and more obvious, and the standards become less flexible. Although standards must necessarily be exacting to promote and

maintain the desired quality, they should not be completely rigid. They should specify limits according to the tolerance permitted by the subject under surveillance. This is particularly true of raw materials **(Whitelely *et al*,1971).**

CHAPTER THREE

MATERIALS AND METHODS

3.1. Raw Materials

Faba bean was purchased from the local market (omdurman). Glucose and other ingredients (sugar powder, sodioum bicarbonate, Sodium chloride, Shortening, skim milk powder, Amonium bicarbonate, Cysteine and water) was obtain from local market (Bahri). All the materials are of analytical grades.

3.2. Methods

3.2.1. Prepration of faba beans flour

The faba beans were soaked in water for five hours ‘ after that their shell was removed and boiled in water for ten minutes and dried in the sun for two days and then grinded ‘ so that the faba bean flour is ready for use.

3.2.2 Processing of biscuit

Biscuit were prepared according to Vatsala and HaridsRaO (1991) method. The formula used in biscuit processing was as follows:

Table 3.1: Ingredients quantity of biscuits formula

Faba beans flour	100
Sugar powder	30
Shortening	30
Skim milk powder	2
Sodium chloride	1
Sodium bicarbonate	0.4
Ammonium bicarbonate	1.5
Glucose	2
Cysteine	0.02
Water	15 ml

The ingredients were weighed for 200g of faba bean flour . sugar powder ’shortening ’skim milk powder ’ and glucose were creamed in Hobart N-50 mixer with a flat beater for three minutes add 61rpm. Salt ’ammonium bicarbonate and sodium bicarbonate and sodium bicarbonate were dissolved separately in part of required water and added to the cream . mixing was continued for 8 minutes at 125 rpm to obtain a homogenous cream . finally ’ flour was added and mixed for three minutes at 61 rpm ’ and then the dough was sheeted to a thickness of 3.5 mm with the help of an aluminum plate form and frame . the piece of dough was transferred to an aluminum tray .the biscuit were baked in an electric oven maintained at 205 c° for 8.5 minutes ’ the baked units were cooled and analyzed .

3.2.3. Determination of chemical compositions

3.2.3.1 determination of moisture content

Moisture content was determined according to the Association of official's analytical chemists AOAC (1990) as follows: Two grams of each sample were weighed in clean dry and pre-weighed crucible and then placed in an oven at 105C˚ and left overnight. The crucible was transferred to desiccators and allowed to cool and then weighed. Further placement in the oven was carried out until constant weight was obtained. Moisture content was calculated using the following formula:

$$\mathbf{MC\% = (W2-W1)-(W3-W1)\ /W2-W1 \times 100}$$

Where:

Mc: moisture content.

W1: weight of empty crucible.

W2: weight of crucible with the sample.

W3: weight after drying.

3.2.3.2 . Determination of fat content

Fat content was determined according to the official method of AOAC (1990) . A sample 5 grams was weighed into an extraction thimble and covered with cotton, and then extracted with the hexane . thimble containing the sample and a pre-dried weight extraction flask containing about 100 ml hexane was attached to the extraction unit . the extraction process was conducted for 16 hours . at the end of the extraction period' the flask was disconnected from the unit and the solvent was evaporated . later, the flask with the remaining crude hexane extracted was put in an oven, cooled to room temperature reweight and the dried extract was registered as fat content .

$$\mathbf{Crude\ fat\ content\ (\%) = (W2 - W1) / (weight\ of\ sample) \times 100}$$

Where :

W1 = the weight of empty extraction flask

W2 = the weight of the extraction flask after the extraction process

3.2.3.3 Determination of protein content

Crude protein of the sample was determined by using the micro-Kjeldahl method according to AOAC (1990) as follows:

Digestion:

0.2 gram of sample was weighed and placed in small digestion flask (50 ml). About 0.4 gram catalyst mixture (96% anhydrous sodium sulphate and 3.5% copper sulphate) was added, 3.5 ml of approximately 98% of H2SO4was added. The contents of the flask were then heated on an electrical heater for 2 hours till the color changed to blue-green. The tubes were then removed from digester and allowed to cool.

Distillation:

The digested sample was transferred to the distillation unit and 20 ml of NaOH (40%) were added. The ammonia was received in 100 ml conical flask containing10 ml of 2% boric acid plus 3-4 drops of methyl red indicator. The distillation was continued until the volume reached 50 ml.

Titration:

The content of the flask were titrated against 0.02 N HCL. The titration reading was recorded. The crude protein was calculated using the following equation;

$$\mathbf{CP\% = (T - B) \times N \times 14 \times 100 \times 6.25/Ws \times 1000}$$

Where:

CP = crude protein

T = Titration reading

B = Blank titration reading

N = normality of HCL

Ws = sample weight

1000 = to convert to mg

3.2.3.4. Determination of ash content

Ash content of the sample was determined according to the method of AOAC (1990) as follows: Tow grams of sample were placed in a clean dry pre-

weighed crucible, and then the crucible with its content ignited in a muffle furnace at about 550c for 3hours or more until light gray ash was obtained. The crucible was removed from the furnace to a desiccators to cool and then weighed. The crucible was reignited in the furnace and allowed to cooling until a constant weight was obtained. Ash content was calculated using following equation:

$$\mathbf{AC\% = W2\text{-}W1/W1 \times 100}$$

Where:

Ac: ash content.

W1: weight of empty crucible.

W2: weight of crucible with ash.

W3: weight of sample.

3.2.3.5. Determination of fiber

Crude fiber was determined according to **AOAC (1990).** Two grams of defatted sample were treated successively with boiling solution of H2SO4 and KOH (0.26 N and 0.23 N, respectively). The residue was then separated by filtration, washed and transferred into a crucible then placed into an oven adjusted to 105°C for 18 – 24 hours. The crucible then with the sample was weighed and ached in a muffle furnace at 500°C and weighed. The crude fiber was calculated using the following equation:

$$\mathbf{CF\ (\%) = W1 - W2\ /Ws \times 100}$$

Where:

CF = Crude fiber.

W1 = Weight of crucible with sample before ashing .

W2 = Weight of crucible with sample after ashing .

Ws = weight of sample.

3.2.3.6.Calculation of carbohydrates

Carbohydrates were calculated by difference according to the following :

Total carbohydrates = 100% - (moisture % + protein % + fat % + ash %) .

3.2.3.7. Calculation of Energy

The energy were calculated by difference according to the following :

Total Energy = (protein × 4 + carbohydrates ×4 + fat × 9) .

3.2.4 Evaluation of biscuit Quality(spreading ratio)

3.2.4.1 Biscuit weight

Biscuit were weighed (3 biscuits) and the weights were recorded .

3.2.4.2 Biscuit width and thickness

Diameter of biscuits was determined by placing Three biscuit samples edge to edge and measuring with a digital vernier caliper . an average of three values was taken for each set of samples. Average value for diameter was reported in millimeter .

Thickness of biscuits was determined by measuring the diameter of Three biscuit samples placed adge to adge with a digital vernier caliper . an average of the three values was taken for each set of samples . average value for thickness was reported in millimeter .

3.2.4.3 Biscuit spread ratio

Biscuit were evaluated for the spread ratio according to the following equation:

Spread ratio = width of the biscuit / thickness of the biscuit

3.2.5. Sensory evaluation of biscuits

Evaluation of biscuit made from faba beans flour and wheat flour were carried out.

Twenty semi-trained assessors were provided coded samples and ask to evaluate the general appearance – colour – flavor – teste – texture – and overall quality of the biscuits according to the scoring (Hedonic) scale of 5

points described by Ihekoronye and Ngoddy (1985). A key table was given to the panelists guided them to score accordingly.

3.2.6. Statistical analysis

Statistix 8.0 were preformed to examine significant differences between normally distributed data of replicated measurement . probability less than 0.05 was considered significant ($p \leq 0.05$). All data was analyzed using vision 17MINITAB statistical software for windows (2007).

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1. Approximate composition of raw materials

From the result in table (4.1),Faba beans flourcontained moisture 6.16 % , ash 2.52%,protein 31.16%,fat 0.94%,fiber 1.9% and total carbohydrates 48% .

while the wheat flour contained moisture 10 % , ash 0.50 %,protein 11%,fat 0.10%, fiber 0.36% and total carbohydrate 78%.

Faba bean flour containing 31.16% protein, 0.94% fat, 0.09 % fiber higher than that of wheat flour . and average rice flour is composed of 7.09 % protein, 0.6 % fat , 0.93 % ash , 1.94 % fiber and 89.38 % total carbohydrates **ALY,M. (2015).**

Table 4. 1: Approximate Composition of Raw Materials

Parameter (%)	Faba bean Flour	Wheat Flour
Moisture	6.16	10
Ash	2.52	0.50
Protein	31.16	11

Fat	0.94	0.10
Fiber	1.09	0.36
Total carbohydrate	48	78

Values are mean for replicate analysis

4.2. Chemical composition of different biscuits

The results were shown in Table (4.2).Protein and ash of faba bean biscuit were higher than their level in wheat biscuit

While fat, carbohydrate and fiber of wheat biscuit were higher than in faba bean biscuit.

Faba beans biscuit was containing 13.52% protein, 3.37% ash, 1.14% fiber and 114.48 total carbohydrates these result was higher than that reported by **ALY,M. (2015).**

Table 4. 2: Chemical composition of different biscuits

Parameter (%)	**A**	**B**
Moisture	8.37	5
Ash	3.37	3.31
Protein	13.52	13.33
Fat	4.52	5.47
Fiber	1.14	1.22
Total carbohydrates	114.48	118.33
Calories	552.75	575.87

Value mean for replicate analysis .

A= 100% Faba bean flour

B= 100% Wheat flour

4.3. Physical properties of Biscuit

Physical properties of biscuit such as thickness , spread ratio , weight , and width were studied and given in table (4.3).

Spread ratio of sample (A) was 5.5 higher than that reported by **ALY,M.e (2015)** which were 4.4 .

Table 4. 3: Biscuit spread ratio

Sample	Width(cm)	Thickness(cm)	Spread ratio
A	4.87	0.88	5.5
B	3.45	0.9	3.8

Value mean for replicate measurement .

A= 100% Faba bean flour biscuit

B= 100% Wheat flour biscuit

4.4. Nutritional composition of different biscuits

The result presented in Table(4.4) showed that faba bean biscuit contained higher level of moisture than wheat biscuit.

Wheat biscuit contained carbohydrate and calories higher than faba bean biscuit.

While ash, protein , fat and fiber was the same level.

Table 4. 4: Nutritional composition of different biscuits

Sample	Moisture	Ash	Protein	Fat	Fiber	Carb	Calories
A	$8.3^{a} \pm 0.03$	$3.3^{a} \pm 0.12$	$13.5^{a} \pm 0.14$	$4.5^{a} \pm 0.78$	$1.43^{a} \pm 0.18$	$114.4^{b} \pm 0.76$	$548.8^{b} \pm 4.14$
B	$5.03^{b} \pm 0.57$	$3.3^{a} \pm 0.11$	$13.5^{a} \pm 0.01$	$5.4^{a} \pm 0.26$	$1.2^{a} \pm 0.41$	$118.5^{a} \pm 0.6$	$577.6^{a} \pm 0.10$
LSD	0.92	0.26	0.2	1.3	0.72	1.56	6.65
CV	6.1	3.5	0.74	11.6	24.1	0.59	0.52

Values are mean ± SD, means carrying the same superscript letters in the same column are for significantly different P≤ 0.05 using Minitab.

A= 100% Faba bean flour

B= 100% Wheat flour

LSD = Less significant different

CV =Coefficient of variation

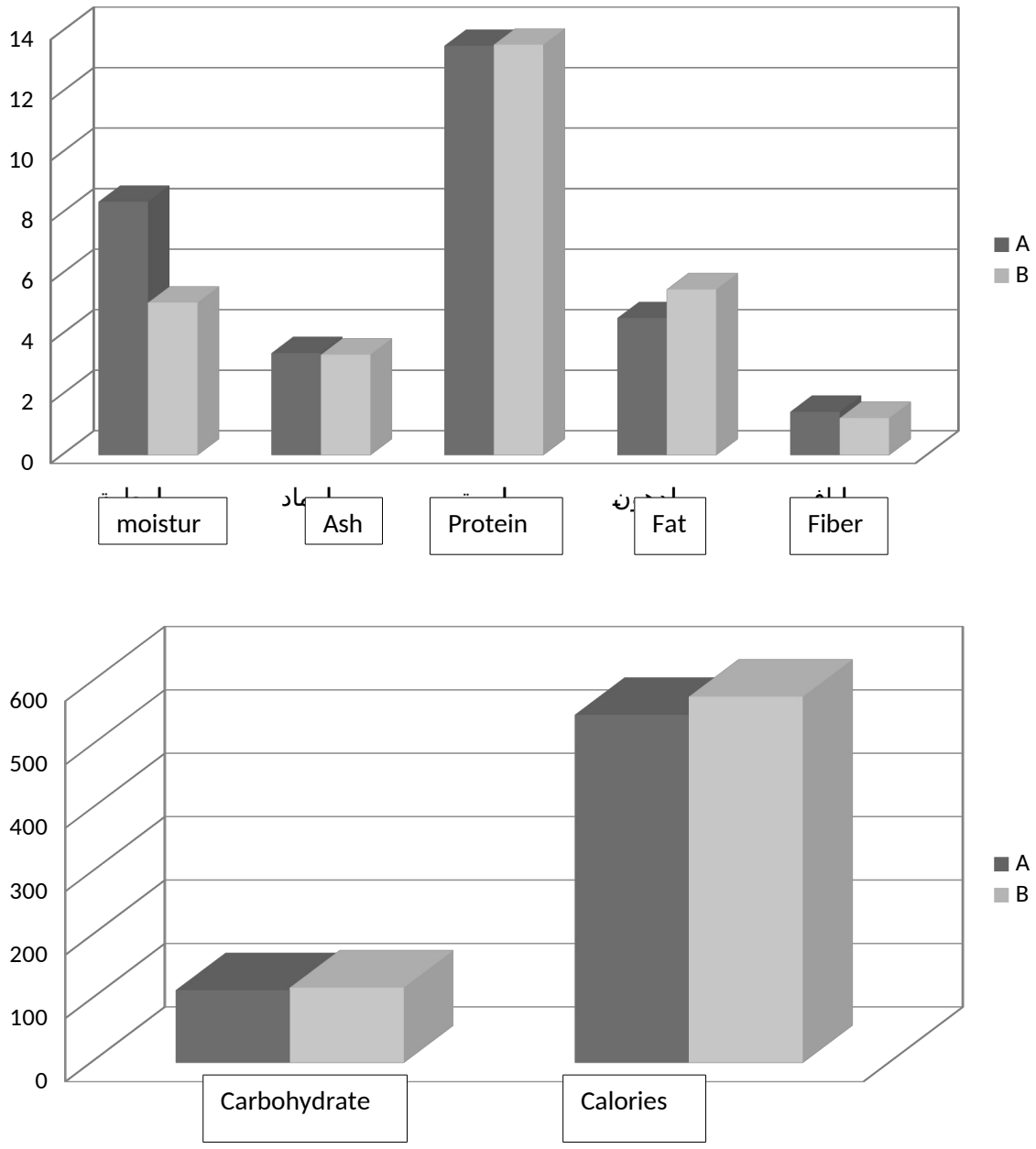

Figure 1: Nutritional composition of different biscuits

4.5. Sensory evaluation of biscuits

The result of panelists on sensory characteristics was presented in Table (4.5) there were no differences in colour, flavor, taste, texture and overall acceptability between the control and faba bean biscuit.

Therefore is possible to develop faba bean biscuit for people with celiac disease.

Table 4. 5: Sensory evaluation of biscuits

Sample	Colour	Flavour	Taste	Texture	Overall quality
A	$4.15^{a} \pm 0.81$	$3.75^{a} \pm 1.01$	$3.55^{a} \pm 1.09$	$4.00^{a} \pm 0.97$	$4.05^{a} \pm 0.82$
B	$4.10^{a} \pm 0.19$	$3.35^{a} \pm 1.13$	$3.75^{a} \pm 1.44$	$3.35^{a} \pm 1.08$	$4.00^{a} \pm 0.79$
CV%	20.94	30.41	35.19	28.11	20.13
SE±	0.27	0.34	0.4062	0.33	0.26
$LSD_{0.05}$	0.55^{NS}	0.69^{NS}	0.822^{NS}	0.66^{NS}	0.52^{NS}

Values are mean ± SD , means carrying the same superscript letters in the same column are for significantly different P≤ 0.05 using Minitab.

A= 100% Faba bean flour

B= 100% Wheat flour

LSD = Less significant different

CV =Coefficient of variation

SE = Standard error

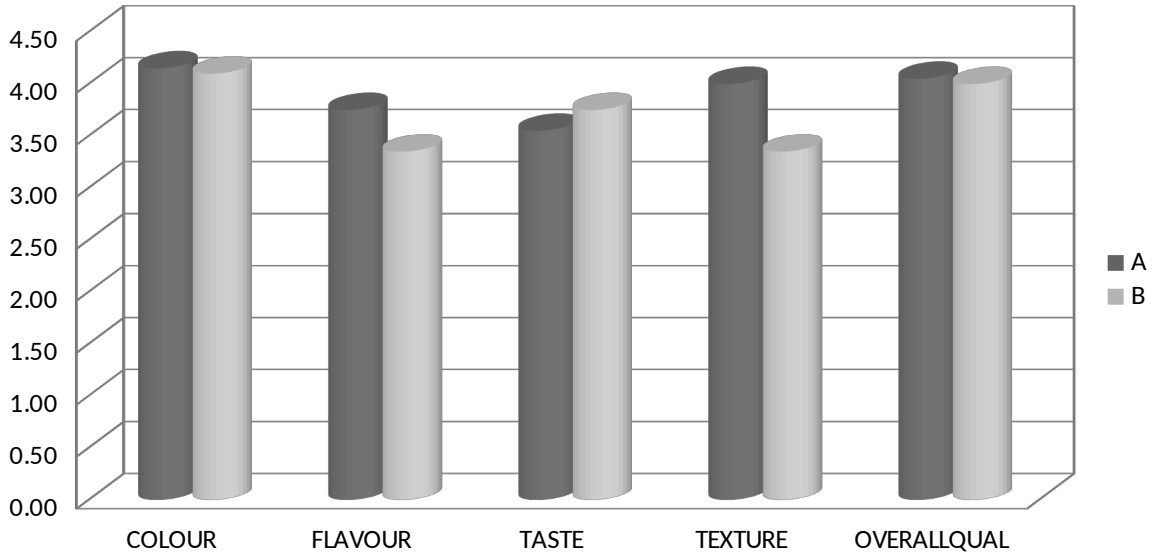

Figure 2: Sensory evaluation of biscuits

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1. Conclusions

From the results obtained in this study we can conclude that the processing of faba beans biscuit was successful.

Faba beans biscuit contained higher protein and ash than control biscuit.

Same time the processing faba beans biscuit has similar sensory characteristics and was very acceptable.

Further it fulfills the requirement of free gluten foods containing free gluten ingredients and higher nutritional value.

5.2. Recommendations

1. Highlight the high nutritional value of faba beans biscuit.
2. Increase protein and ash content in free gluten products.
3. Expansion of the cultivation of faba beans in the Sudan as it has a higher nutritive value.
4. Commercially production of faba beans biscuit as a free gluten product for Sudanese people.

References

ALY,M. M. & SELEEM, H. A. (2015). Gluten-free flat bread and biscuits production by cassava,extruded soy protein and pumpkin powder. Food and Nutrition Sciences, 6, 660.

AOAC, C. (1990). Xanthophylls in Dried Plant Materials and Mixed Feeds.Method 970.64. Official Methods of Analysis of the Association of Official Analytical Chemists, 15th ed.,Association of official Analytical Chemists, Inc. Arlington, VA, USA, 1048-1049.

AYKROYD, W. R., DOUGHTY, J. & WALKER, A. F. (1982). *Legumes in human nutrition*, Food & Agriculture Org.

DALGIC, B., SARI, S., BASTURK, B., ENSARI, A., EGRITAS, O., BUKULMEZ, A., BARIS, Z. & GROUP, T. C. S. (2011). Prevalence of celiac disease in healthy Turkish school children. *American Journal of Gastroenterology,* 106**,** 1512-1517.

FAO STAT (2009). Production stat: crops. FAO statistical databases (FAO stat), food and agriculture organization of the United Nations

FASANO, A., BERTI, I., GERARDUZZI, T., NOT, T., COLLETTI, R. B., DRAGO, S., ELITSUR, Y., GREEN, P. H., GUANDALINI, S. & HILL, I. D. (2003). Prevalence of celiac disease in at-risk and not-at-risk groups in the United States: a large multicenter study. *Archives of internal medicine,* 163**,** 286-292.

FROST, A. R., BAND, M. M. & CONWAY, G. S. (2009). Serological screening for coeliac disease in adults with Turner's syndrome: prevalence and clinical significance of endomysium antibody positivity. *European journal of endocrinology,* 160**,** 877-881.

Gasim, S. Hejien H., Khalifa J., and Abdelmula A..(2013).Effect of self-fertilization in performance,breeding and germplasm management of four local faba bean cultivars. J. Agric. Sci. Technol. B 3:182-188.

GREEN, P. H. & CELLIER, C. (2007). Celiac disease. *New england journal of medicine,* 357**,** 1731-1743.

GUILLON, F. & CHAMP, M.-J. (2002). Carbohydrate fractions of legumes: uses in human nutrition and potential for health. *British Journal of Nutrition,* 88**,** 293-306.

GUPTA, R., REDDY, D. N., MAKHARIA, G. K., SOOD, A., RAMAKRISHNA, B. S., YACHHA, S. K., THAPA, B. R., BANERJEE, R., ANURADHA, S. & DUTTA, U. (2009). Indian task force for celiac disease: current status. *World Journal of Gastroenterology: WJG,* 15**,** 6028.

Hawtin GC, Hebblethpiait PD (1983). Background and history of faba bean production. Pages 3-22 in The Faba Bean (Vicia faba L.) (Hebblethwaite, P.D., ed.). Buttenvorths, London, U.K.

HERMSDORFF, H. H. M., ZULET, M. A., ABETE, I. & MARTÍNEZ, J. A. (2011). A legume-based hypocaloric diet reduces proinflammatory status and improves metabolic features in overweight/obese subjects. *European journal of nutrition,* 50**,** 61-69.

KARELL, K., LOUKA, A. S., MOODIE, S. J., ASCHER, H., CLOT, F., GRECO, L., CICLITIRA, P. J., SOLLID, L. M., PARTANEN, J. & DISEASE, E. G. C. O. C. (2003). HLA types in celiac disease patients not carrying the DQA1* 05-DQB1* 02 (DQ2) heterodimer: results from the European Genetics Cluster on Celiac Disease. *Human immunology,* 64**,** 469-477.

LENHARDT, A., PLEBANI, A., MARCHETTI, F., GERARDUZZI, T., NOT, T., MEINI, A., VILLANACCI, V., MARTELOSSI, S. & VENTURA, A. (2004). Role of human-tissue transglutaminase IgG and anti-gliadin IgG antibodies in the diagnosis of coeliac disease in patients with selective immunoglobulin A deficiency. *Digestive and Liver Disease,* 36**,** 730-734.

MASSEN, L. 1980. India is the native home of the pigeonpea.

Mihailovic V, Mikic A. Cupina B, Eric P (2005). Field pea and vetches in serbia and Montenegro. Grain Legumes 44:25-26.

MUSTALAHTI, K., CATASSI, C., REUNANEN, A., FABIANI, E., HEIER, M., MCMILLAN, S., MURRAY, L., METZGER, M.-H., GASPARIN, M. & BRAVI, E. (2010). The prevalence of celiac disease in Europe: results of a centralized, international mass screening project. *Annals of medicine,* 42**,** 587-595.

Osman, A. M. A. , Hassan A. B., Osman G. A. N., Mohamed N.,Rushdi M. H., Diab E. E., et al. (2014). Effects of gammairradiation and/or cooking on nutrional quality of faba bean(*Vicia faba* L.) cultivars seeds. J. Food Sci.Technol. 51:1554-1560.

PETER R R. Whiteley(1971) Biscuit manufacture,Rush Green Technical Collage,Departement of Baking.

Razia Akbar (Tr.) (2000). Nuskha Dar Fanni-Falahat: (The Art of Agriculture); (Persion Manuscript Compiled In The 17Th Century by the Mughal Prince Dara Shikoh) (Agri-History Bulletin No. 3) Munshiram Manoharlal Publishers Pvt Ltd. P. 98.

RUBIO–TAPIA, A., VAN DYKE, C. T., LAHR, B. D., ZINSMEISTER, A. R., EL–YOUSSEF, M., MOORE, S. B., BOWMAN, M., BURGART, L. J., MELTON III, L. J. & MURRAY, J. A. (2008). Predictors of family risk for celiac disease: a population-based study. *Clinical Gastroenterology and Hepatology,* 6**,** 983-987.

Salih, F. A., and Mohamed N. B.. (1992).Shambat 75, a faba bean cultivar for El Rahad area of Sudan.Fabis Newsletter 30:17-19.

SATTAR, N., LAZARE, F., KACER, M., AGUAYO-FIGUEROA, L., DESIKAN, V., GARCIA, M., LANE, A., CHAWLA, A. & WILSON, T. (2011). Celiac disease in children, adolescents, and young adults with autoimmune thyroid disease. *The Journal of pediatrics,* 158**,** 272-275. e1.

SCHUPPAN, D., JUNKER, Y. & BARISANI, D. (2009). Celiac disease: from pathogenesis to novel therapies. *Gastroenterology,* 137**,** 1912-1933.

Singh AK, Bhatt BP (2012a). Faba Bean (Vicia faba L.): A potential leguminous crop of India ISBN 978-93-5067-773-5ICAR, RC for ER, Patna, P. 518

Suliman G. Coeliac disease in Sudanese children. Gut, (1978), 19(2):121–5.

Vatsala, C. N., & Haridas Rao, P.,(1991).Studies on invert syrup for use in biscuits. Journal of food science and technology (Mysore), 28(3), 149-152.

WANG, X.-Q., LIU, W., MEI, H., GAO, Y., PENG, H.-M., YUAN, L. & XU, J.-J. (2011). Celiac disease in children with diarrhea in 4 cities in China. *Journal of pediatric gastroenterology and nutrition,* 53**,** 368-370.

WHITELEY, P. R. (1971).Biscuit Manufacture: Fundamentals of In-Line Production, Springer.

WOUTERS, J., WEIJERMAN, M. E., VAN FURTH, A. M., SCHREURS, M. W., CRUSIUS, J. B. A., VON BLOMBERG, B. M. E., DE BAAIJ, L. R., BROERS, C. J. & GEMKE, R. J. (2009). Prospective human leukocyte antigen, endomysium immunoglobulin A antibodies, and transglutaminase antibodies testing for celiac disease in children with Down syndrome. *The Journal of pediatrics,* 154**,** 239-242.

APPENDICES

Plate 1: Preparation of faba beans

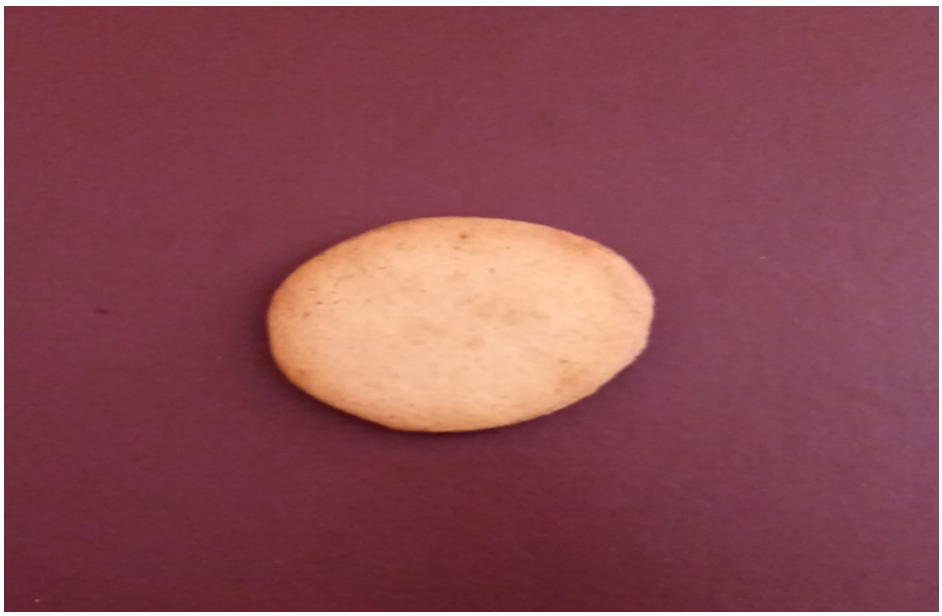

Plate 2: A=100% Faba beans flour biscuit

Plate 3: B= 100% wheat flour biscuit