EFFECT OF ALPHA-AMYLASE AND ASCORBIC ACID IMPROVER ON BREAD QUALITY

By

Elgeiliy Osman Ibrahim

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Supervisor

Dr. Hasan Ali Mudawi

Department of Food Science and Technology

University of Khartoum

Faculty of Agriculture

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Dedication

To
My family
My friends
My partners
With love

ELGEILIY
ACKNOWLEDGEMENTS

Thanks are firstly and lastly to Allah who enabled me to conduct this study by the grace of him and donated me.

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Abstract

The aim of this research was to study the effect of improver composed of alpha amylase and ascorbic acid on bread making. Samples of wheat were treated with three levels of the improver: (A: 1gm ascorbic acid-2gm alpha-amylase, 2gm ascorbic acid-4gm alpha-amylase, 3gm ascorbic acid-6gm alpha-amylase) and compared with non treated sample. Proximate composition was carried out for the sample. Gluten quantity and quality was determined by Glutomatic instrument, Falling number and sedimentation value were determined in addition of the rheological characteristics by Farinograph and Extensograph instruments for flour of the treated and non treated sample was carried out. The results showed significant differences in the quality tests for the treated sample and the breads made from it. When applying the three levels of improver, the following results were obtained: low falling number (369-539sec), sedimentation value (33cm³), water absorption (58.4-59.1%), resistance (458-1090 mm), extensibility (122-158 mm), specific loaf volume (4.32-4.93 cm³/gm) and high degree of softening (60-80) Farinograph units. Addition of improver led to increase in alpha amylase activity, degree of softening, specific volume and decreasing in percentage of water absorption. Addition of improver significantly affected the quality tests with the exception of sedimentation value(33cm³). Addition of improver increased the energy except at 135min and decreased the extensibility except at 45 min, while the ratio number increased with increased the level. Resistance was increased gradually at 45, 90 and 135 min.
Sensory evaluation of loaf bread showed that loaf bread made from the B gained the highest score of general acceptability. Generally, the level of improver B showed better results in chemical and rheological tests and higher degree of general acceptability.
المستخلص

هدف هذا البحث إلى دراسة تأثير محسن مكون من الألفا أميليوز وحمض الأسكوربيك بثلاث مستويات على صناعة الخبز.

تمت معاملة عينة الدقيق بثلاث تركيز من المحسن: A: 1جم حمض الأسكوربيك -2جم ألفا أميليوز, B: 2جم حمض الأسكوربيك -4جم ألفا أميليوز, C: 3جم حمض الأسكوربيك -6جم ألفا أميليوز. وقررت مع عينة غير معالمة. أجري تحليل تقريب لعينة الدقيق. كما تم تحديد جودة وكمية الجلوتين بواسطة جهاز جلوتروماتيك. رقم السقوط, قيمة الترسيب, درجة الخواص الريولوجية لإختبار جهاز الفارينوغراف والإكستروفغراف لعينة العينة المعالمة وغير المعالمة.

أظهرت النتائج اختلافات معنوية في اختبارات الجودة للدقيق المعالم والخبز المصنع من العينة المعالمة بالثلاثة مستويات من المحسن, وجد أن هناك قلة في رقم السقوط (369-539), الترسيب (33 سم), نسبة إمتصاص الماء (58.9-59.1%), المقاومة (1090-1258 ملم), المطاطية (125-142 ملم), الحجم النوعي (4.32-4.93 سم), وعلو في درجة النعومة (60-89) وحدة فارينوغراف.

إضافة المحسن أدت إلى زيادة نشاط الألفا أميليوز, درجة النعومة, الحجم النوعي ونقصان نسبة إمتصاص الماء.

إضافة المحسن أثرت معنويًا على اختبارات الجودة باستثناء قيمة الترسيب (33 سم).

إضافة المحسن أدت إلى زيادة في الطاقة عدا عند 135 دقيقة كما أدت إلى نقصان المطاطية عدا عند 45, بينما زاد الرقم النسبي مع زيادة تركيز المحسن. كما زادت المقاومة تدريجيا عند 45, 90 و135.

التقييم الحسي للخبز الأفريقي أظهر أن الخبز المصنوع من مستوى المحسن B أعطى أفضل درجة للفتيل العام.

عمرما مستوى المحسن B أعطى أفضل نتائج الاختبارات الكيميائية والفيزيائية وأعلى درجات التفتيض العام.
CHAPTER ONE
INTRODUCTION

Wheat ranks first among cultivated plants of the world. It is an important source of protein for the inhabitants of developing countries.

Wheat is unique among cereals, because it contains gluten which has the characteristics of being elastic when mixed with water and retains the gas developed during dough fermentation. Wheats produced in different parts of the world differ greatly in their intrinsic protein qualities and quantities, the quantity is influenced mainly by environmental factors, but the quality of protein is mainly a heritable characteristic.

Wheat is favoured for bread baking; the function of baking is to present wheat flours in attractive palatable and digestible forms. Bread is made by baking fermented dough which has as main ingredients: wheat flour, water, yeast and salt.

With no other basic food does the quality of the raw material have such an effect on the condition of the end product and its processing characteristics as with wheat flour. Factors such as grain varieties, the nature of the soil, climate, harvesting conditions and storage cause deviations from the quality specifications that may result in disruptions and problems during milling and subsequently during baking.

Although many mills rate their flours according to analytical quality data, these often supply too little information on the processing characteristics. It is the way the dough behaves during baking that shows what flour is really worth. Special attention has to be given to rheological factors such as kneading tolerance, surface moisture and fermentation stability.
Wheat flour naturally contains alpha and β- amylases, which hydrolyzes glucosidic linkage in carbohydrates. The amount of these enzymes depend on the wheat’s growing and harvesting conditions, for example, the alpha amylase content of flour increases in wet climates because this enzyme mobilizes reserved starch during germination.

Alpha amylase hydrolyzes starch into dextrins which may subsequently be hydrolyzed by β- amylase into maltose or by amylglucosidase into glucose. However, the tightly packed starch granules must first be made accessible either by damage during flour milling or gelatinization by heat and moisture. The amylase hydrolyzed sugars which are then used by yeast during the baking process. The proper amount of amylases must be present in flour to achieve the right amount of yeast fuel and thus resulting in suitable carbon dioxide generation (gassing level).

Amylases also can help prevent staling of baked goods by affecting starch retrogradation (recrystallization). Bacterial amylase is best for this purpose because it can continue after baking is complete.

The objective of this study was:

To study the effect of improvers on the rheological and bread making properties.
To study the physicochemical characteristics of dough.
To evaluate the bread loaf using sensory tests.
CHAPTER TWO
LITERATURE REVIEW

2.1 Wheat and wheat classification

Wheat is one of the most widely cultivated cereal crops in the world. Cereal, such as wheat, sorghum, millet etc. is belonging to the class Monocotyledons (Kent, 1983; Walton et al, 1988).

Wheat is a member of the family Gramineae, and has the botanical name of Triticum. Flour millers are the major wheat processor; other processors include breakfast manufactures, bulgur producer, food manufactures, and industrial users. Wheat has predominant role in the grain trade and utilise as food (67%), feed (20%), seed (7%) and industrial products (6%) as stated by Oleson (1994).

Wheat is considered as one of the main food crops in Sudan. It ranks after sorghum as staple diet especially in urban centres. FAO (1999) reported that wheat could be grown in relatively wide range of climatic conditions especially in temperate climate and susceptible to disease in warm, humid regions. In 1999 and 2000 Sudan production of wheat was 172,000 and 241,000 metric tons, respectively (FAO, 2000).

The wheat is divided into three groups:

1. *Triticum aesvum*; include the varieties milled mainly to produce flour that is used mainly for breads, sweet yeast goods, cakes, pastry products, blended mixed, cookies and crackers.

2. *Triticum durum*; it is used extensively for the manufacture of macaroni. Its principal use is in production of semolina, coarsely ground and highly purified. Durum wheat is used for pasta products (macaroni, spaghetti, vermicelli, noodles, etc).

3. *Triticum compactum*, or club wheat; flour milled from this class is too soft for bread making.
Wheats are classified according to the season in which the seed is sown into winter and spring wheat. Wheat may also be classified by the colour of their seed coat, conventionally known as red, yellow or white (Hoseney et al., 1988).

Wheat grain may further be considered as being strong/hard, medium or soft. Wheat kernel texture differentiates cultivars of hard and soft wheat classes. Such classification is useful since it predicts milling times and energy requirements as reported by Miller et al. (1984); Pomeranze et al. (1985) and possibly by loaf volumes (Symes, 1969). Pomeranze et al. (1985) stated that many ways can be used to determine hardness and softness of bulk wheat. Location of growth also affects texture and individual kernels from cultivar grown in one location, also vary in hardness. A hard wheat kernel requires greater force to cause it to disintegrate as does soft wheat kernel. The flour obtained from hard wheat has coarser particle size than soft wheat. Also hard wheat grinds much faster than soft Wheats (Miller et al., 1991).

Structurally, wheat grain can be divided into three main parts; the bran coatings constitute approximately 14% of the wheat grain as reported by Anon (1987). The germ is approximately 2.5% of the grain and is the wheat plant embryo. The endosperm, which is approximately 83% of the grain, from which the white flour is extracted.

2.2 The role of wheat in human nutrition

Wheat and wheat foods are major source of nutrients for people in many regions of the world. Wheat is a source of carbohydrates, proteins, vitamins and minerals when consumed as a major component of the diet. Betchart (1982) reported that the endosperm consist mainly of starch and significant proportion of many minerals and vitamins. Nutrients are generally found in the highest concentrations in the germ and in the aleurone cells surrounding the starchy endosperm. Significant quantities
of minerals and vitamins are lost when whole wheat is milled to produce white endosperm flour because the outer layers of bran are removed along with aleurone cells and germ. Extraction flour of 72-75% contains from as little as 20% to about 60% of the B vitamins originally present in whole-wheat flour (FAO, 1970).

2.3 Flour additives:

2.3.1 Bleaching agents

Wheat flour can be bleached chemically, by such materials as chlorine, chlorine dioxide, acetone peroxide and benzoyl peroxide. This type of treatment has been carried out by flour millers to improve the colour of their flour. However, treatment may not always be desirable.

2.3.1.1 Chlorine

Chlorine is widely used in the treatment of soft wheat flours to improve cake making properties. It has some colour removing effect, indicating action on flour pigments, but the creamy yellow pigments can not be removed completely at the treatment levels used to achieve optimum performance (Pomeranz, 1988).

2.3.1.2 Chlorine dioxide

Chlorine dioxide has similar improving and bleaching action. It is no longer used in Europe for their possible harmful effects on health and technical risks involved. There is no doubt that with certain baked goods chlorination of the flour that can only be carried in the mill produces best result (Anon 1998).

2.3.1.3 Acetone peroxide


2.3.1.4 Benzoyl peroxide
Benzoyl peroxide reacts with the carotenoid pigments in flour to render them white, whereas their natural colour is creamy yellow to yellow if flour is aged under ideal storage conditions, the same chemical change takes place through natural oxidation (Pomeranz, 1988).

2.3.2 Dough conditioners

Dough conditioners have been used as additives in baking products for about 50 years to improve dough characteristics, eating quality and shelf life. Dough conditioners may be grouped into four categories: oxidants, reductants, surfactants, and mixing time reducers (Stauffer, 1983).

2.3.2.1 Oxidants

Oxidants are compounds added to dough formulations to change the characteristics of the gluten matrix. These changes are commonly called ‘strengthening agents’. Oxidants enhance the rate at which cross-links are formed, building up and strengthen gluten-protein structures (Allen, 1999).

Mac Ritchie (1987) observed that oxidants increase the average molecular weight of the glutens and strengthen the dough. Generally, most bakers consider oxidants or oxidizing agents the most important class of dough conditioners because the high speed production of quality baked goods would be virtually impossible without their use.

2.3.2.1.1 Potassium bromate

Potassium bromate was first introduced as bread improver in 1916 (Fitchett and Frazier, 1986). It is slow acting oxidizing agent that work during fermentation, proofing, and baking. The oxidation process affects the dough structure and rheology. It improves dough handling, properties contributing to loaf volume and texture (Giesecke, 2000).
Potassium bromate had been used extensively until recent years after tests on laboratory animals raised question as to its mutagenicity (Kurokawa, et al. 1983). In (1992) JECFA recommended that bromate be excluded from the flour standards because of safety points of view. Consequently, this very efficient and cheap bread improver is being banned in most countries for health reasons.

2.3.2.1.2 L- Ascorbic acid (LLA)

The compound L-ascorbic acid (vitamin C) was first isolated from fruit juices in 1928 and chemically synthesized five years later. It’s a crystalline solid which is readily oxidized in solution by atmospheric oxygen. In 1935 Jorgensen recognized the improving action of (LAA) on bread dough, contrary to the action of other chemical reducing agents which tend to soften wheat flour dough (Blanshard, 1985). In 1938 Melville and Shattock noticed that oxidized LAA from old lemon juice was also very effective as a bread improver, which led to the discovery of dehydro-L-ascorbic acid (DHLAA) and the conclusion that it was this oxidized form that was active improving agent.

In the absence of air, LAA acts as a reducing agent and is used as such in continuous dough making processes (Domaker and Amflow). LAA, is of major importance in the Chorleywood bread process and if it is used at an addition level of 75 p.p.m., the baker can be quite confident of achieving a good improvement in bread quality. Indeed, it’s a characteristic feature of LAA that addition level is not critical and it’s virtually impossible to over-treat bread doughs, unlike the situation with other oxidants. Clearly LAA is a most acceptable additive in bread since it’s a vitamin, although it’s used as a bread improver confers no additional nutritional benefit since the vitamin is destroyed in the finish loaf (Thewlis, 1971). L-ascorbic acid (LAA) functions as a reducing agent in
the absence of oxygen and is used to reduce mixing time in continuous and vacuum mix systems, in the presence of oxidant (Allen, 1999). Qarooni, et al., (1989) reported that, the addition of ascorbic acid to the formula of pita bread produced doughs with higher resistant to sheeting and consequently thicker products, and the internal quality parameters significantly deteriorated with this addition. However, opposite results were found by Rubenthaler and Faridi (1981) who reported improvement in the quality of five breads by the addition of malted barley and ascorbic acid. All treated breads differed significantly from the control in their ability to roll and fold and quality of tearing.

2.3.2.1.3 Azodicarbonamide

Azodicarbonamide (ADA) is a rapid acting reagent that reduces the thiol content of dough and increases its resistance to extension. It has no bleaching action (Joiner; 1962, Tsen, 1963, 1964, Fitchett and Frazier, 1986). It also shortens the dough development time and decreased tolerance to over mixing (Fitchett and Frazier, 1986). Allen (1999) reported that (ADA) exhibits rabidly rate of reaction and extended period of oxidation; it also facilitates setting of the basic dough structure.

2.3.2.1.4 Lipoxygenase

Lipoxygenase usually added to flour in the form of enzyme active soy flour (Barret, 1975). Lipoxygenase exhibits a gluten strengthening effect and increases the mixing tolerance of the dough. It oxidizes carotenoids and chlorophyll pigments in flour to their colorless form, which result in bleaching action to whiten flour (Mathewson, 2000). However, Hoseney, et al.,(1980) found that lipoxygenase contributes to stronger mixing characteristics and more tolerance to cover mixing.
2.3.2.2 Reductants

Reductants are compounds used to inhibit the formation of disulfide bonds between gluten subunits and consequently make dough mix faster and handle more easily. The most commonly used chemical reductants are the sulfhydryl amino acid, cysteine and the inorganic compound, sodium meta bisulfite. Reductants (reducing agents) enhance the rate at which cross links are broken or reduced, which degrades and weakens gluten protein structure (Allen, 1999). Mac Ritchie (1987) found that reductants decrease the average molecular weight and weaken the dough.

2.3.2.2.1 Cysteine

Cysteine shortened the required mixing time, as determined by the mixograph (Weak, et al., 1977). It reduced the energy requirement to mix the doughs to optimum development (Fitchett and Frazier, 1986).

2.3.2.2.2 Sodium meta bisulfite

The mode of action of sodium meta bisulfite is similar to cysteine. The overall effect is that the elastic strength of the gluten matrix is decreased, so it becomes more pliable and extensible. They are commonly used where strong gluten characteristics are not wanted, such as in soda crackers certain types of hard cookies (Stauffer, 1983).

2.3.2.3 Surfactants

Surfactants are added to soften the crumb or make the dough more resistant to work input during processing. They are used by food manufacturers to improve the dough strength, volume, and texture of baked goods. Researchers (Knightly 1973, Hoseney, et al., 1970, Ckek, 1986) found surfactants to be effective in increasing dough strength and mixing tolerance, extensibility and water absorption.
2.3.2.3.1 Sodium stearoyl lactylate (SSL)

Mixing time of doughs containing (SSL) tended to increase with increased concentration, but stability decreased (Watson and Walker, 1986). Contradicting results reported by Tsen and Weber (1981) who found an increase in the curves stability in solution to yeasted doughs.

2.3.2.3.2 Monoglyceride (MG)

Monoglycerides in bread formula extend shelf life, strengthen the dough, and retarded crystallization (Morad and ,Appolonia, 1980). However, Al-Eid (2000) found that MG has futhermorean effect in pita thickness, and very good rolling and folding ability. Moreover crust color was uniform and moderate brown.

2.3.2.3.3 Sucrose esters (SE)

Sucrose esters added to bread flour in powder form increase the mixing time (Watson and Walker, 1986). While Farvili et al., (1995) found that low concentration (0.25%) of sucrose esters and sodium stearoyl lactylate produced high quality Arabic (pita) bread than the control.

2.3.2.4 Mixing time reducers

Mixing time reducers are additives that decrease the number of disulfide bonds formed during mixing (Stauffer, 1983).

2.3.2.4.1 Fumaric and sorbic acid

Sidhu, et al., (1980) found that, the energy input during mixing breaks disulfide links to form two thiol-free radicals, which react with the double bond in fumaric or sorbic acid, forming a covalently bonded additional product that can not form any linkages between the proteins molecules. The difference between this reaction and that of reducing agents is that when mixing stops, the action of fumaric or sorbic acid also stop. A reducing agent continues to weaken the gluten sheet unit the reducing agent is depleted.
2.3.2.4.2 Proteases

Proteases are enzymes that hydrolyze peptide bonds in proteins. Some proteases are present in flour, but their activity is generally low. Supplementation with proteases helps to break down the gluten protein so that the dough is softened and become more extensible (Mathewson 2000).

Barrett (1975) reported that, protease breaks a limited number of peptide links in the protein backbone, decreasing the elasticity of the gluten and bringing the development of gluten strength to a maximum in a shorter time. Like reductants, however, proteases continue to work even after mixing is completed, and so can soften the dough during resting and proofing periods.

2.3.3 Shortening

A major effect of adding shortening to bread dough is an increase in the volume of the bread. Dough made with and without shortening typically proofs the same height but differ greatly in final loaf volume (Elton and Fisher, 1966). However, Junge and Hoseney (1981) found that, s doughs made with shortening have been shown to continue to expand for longer time (to a higher temperature), thus attaining a larger volume than doughs made without shortening. Faridi and Rubenthaler reported that, shortening at the level of 1% improved the physical quality.

2.3.4 Enzymes

Dough prepared with insufficient enzyme activity tend to be tight and over elastic. They are difficult to machine because they resist being molded and shaped. They may not fill the shape of the pan because of their resistance to flow. The sheeted dough shrinks back too fast, increasing the individual piece weight.
In general, several effects can be expected as a result of specific enzymatic modification of the flour components. Keeping in mind that commercial enzymes contain multiple forms of enzymatic activity, one can expect that amylases will produce additional fermentable sugars and will alter the dough consistency, making it softer. Proteases will also soften the dough as a result of gluten protein modification and can produce enough new amino groups to effect product colour and flavour through the Millard reaction. Pentosanases will significantly affect dough rheology and depending on the moisture content and type of product produced, can adversely affect product texture (Mathewson, 2000).

2.3.4.1. Amylases

2.3.4.1.1 Historical

The amylase of wheat was probably the first enzyme to be discovered. It was observed by Kirchhoff in (1811). Leach's (1898) discovered the digestive action of saliva upon starch in 1831. In 1876 O'sullivan (1876) demonstrated that when amylase acts upon starch the chief end products i maltose. In (1878) stated that malt amylase is composed of two different enzymes.

2.3.4.1.2 Sources of amylases

Amylases are found in nearly all plants, animals and microorganisms. They occur in starchy seed and the amount increases when the animal’s amylases occur in high concentration in the pancreas. Starchy substances constitute the major part of the human diet in the world, as well as many other animals. They are synthesized naturally in variety of plants. Similar to cellulose, starch molecules are glucose polymers linked together by alpha 1-4and alpha 1-6 glucosidic bonds, as opposed to the beta 1-4 glucosidic bonds for cellulose.
Since a wide variety of organisms including humans, can digest starch, alpha amylase obviously synthesized in nature as opposed to cellulose for example human saliva and pancreatic secretion contain large amount of alpha amylase for starch digestion.

Types alpha amylase depends on the sources of the enzyme. Currently, two major classes of alpha amylases are commercially produced through microbial fermentation. Based on the points of attack in the glucose polymer chain, they can be classified into two categories, liquefying and saccharifying. The bacterial alpha amylase attacks only alpha 1-4 bonds and it belongs to the liquefying category. The hydrolysis reaction catalyzed by this class of enzyme is usually carried out only to the extent, for example, the starch is rendered soluble enough to allow easy removal from starch-sized fabrics in the textile industry. The paper industry also uses liquefying amylases on the starch used in paper coating where breakage into the smallest glucose subunits is actually undesirable.

On the other hand fungal alpha amylase belongs to saccharifying category and attacks the second linkage from the non reducing terminals (i.e. C4 end) of the straight segment, resulting in the splitting of two glucose units at a time, of course, the product is a disaccharide called maltose. Nam S.W. (undated).

Alpha amylases from cereal grains have been one of the most widely studied groups of plant enzymes. The role that they play during seed germination and technological processing of cereals has been the subject of much research interest for over 100 years (Brown and Morris, 1890).

2.3.4.1.3 Mode of action of alpha amylase

The hydrolysis of soluble substrates such as amylose and amyllopectin by alpha amylase has been described in detail (Greenwood and Milne, 1968; Thoma et al., 1971; Robyt 1984). Amylase is often used as substrate for studies of alpha amylase action patterns because the linear
Dextrins formed can be identified and quantitated more easily than the complex mixture of linear and branched dextrins formed during hydrolysis of amylopectin soluble starch (Manners 1985). Alpha amylolysis of amylose can be considered to take place in two stages (Myrback and Neumuller, 1950). Initially, rapid breakdown of macromolecules to short-chain dextrins is accompanied by a large decrease in viscosity, loss in iodine staining power, and limited formation of reducing sugars. Three mechanisms can be postulated to describe dextrinization, this initial phase of amylose hydrolysis (Robyt, 1984). In the single chain mechanism, the enzyme forms an active complex with the substrate and hydrolysis it completely in one direction a "Zipper"-type manner to form large amounts of small dextrins throughout hydrolysis.

There is also no evidence that cereal alpha amylase react in this way (Banks et al., 1970), although it is difficult to eliminate the possibility of small amount occurring. Strong evidence shows, however, that the alpha amylases from other sources may utilize this mechanism (Robyt and French, 1967; Thoma, 1976). Completely random hydrolysis of internal bonds of amylase is achieved in the multichain process, in which the enzyme hydrolyses one bond per encounter with a substrate molecule. This mechanism best describes the behaviour of cereal alpha amylases.

Enzyme hydrolysis slows down markedly after only limited amylase degradation (Myrback and Neumuller, 1950). This indicates the beginning of the slow second stage, or saccharification stage, of hydrolysis, which is characterized by further degradation of amylose dextrin to low-molecular-weight products.

An important property of alpha amylase is their ability to hydrolyze intact starch granules. Hydrolysis is slow, however, compared to that of starch in solution (Sandstedt and Gates, 1954), probably because of the
low effective substrate concentration in starch granule digest. The rate and extent of granule hydrolysis depends not only on the experimental conditions but also on the source of enzyme and starch (Sandastedt and Gates, 1954, Sandastedt and Ueda, 1969). For example, root starches such as potato and sago are particularly resistant to malt alpha amylase compared to starches from cereal grains. In general waxy starches appear to be more susceptible to hydrolysis by cereal alpha amylases than normal starch from the same cereal. This difference must reflect a difference in the granular structure of the two types of starch. Millet and sorghum starches are equally susceptible to degradation by malt alpha amylase but are less susceptible than wheat starch (Rasper et al., 1974). Similarly millet starch was hydrolyzed more slowly than wheat starch by millet alpha amylase (Beleia and Varriano-Marston, 1981).

2.4 Bread

All over the world, bread means food and life. It requires no further preparation once purchased. Bread, a nutritionally dense food, is high in complex carbohydrates, which give the body a sustained energy (Bennion, 1967). Herbst (1995) reported that, bread is a staple food since prehistoric times. It has originated in Egypt about 3500 BC as reported by Joswellman (2003). The migration from countries site inward the urban has imposed the spread out of the habit of eating bread and thus increases the consumption (Dendy, 1992). The large consumption of bread led to the development of a well-organized baking industry. Bread acts as a vehicle for protein and vitamin-rich materials such as meat and cheese. In addition to that, the solid state of bread facilitates its transportation.

2.4.1 Bread making

Bread making industry is not modern discipline, but the improvement of its techniques was slow. From the nineteenth century and
forward, with evolution in food science and technology it would be possible to control its different operation. Bread making process is a multi step process which involves many tasks to be performed before the final product is obtained (Dendy, 1992).

The main factor, which places wheat in the front position among the world crops, is its bread making quality. Wheat is used for several purposes but the traditional staple food is bread, which is produced in many forms by different processes. Flour suitable for bread making in one country may not be acceptable in others (Anon, 1987).

Hoseney, et al., (1988) stated that, soft wheat flours with low protein content are used for pastry products rather than for bread making, where hard wheat flour with higher protein content is used for bread making. However, Blachman and Payne (1987) reported that, the protein content of wheat used for bread making may vary from 11-15%.

Generally, good quality flour for bread making should have high water absorption, medium, medium-long mixing requirement, satisfactory mixing tolerance, dough handling properties and good loaf volume. Williams (1970) mentioned that, the general methods of making bread is to prepare a dough of flour, yeast, salt and water, the yeast acts on the sugars present in the dough, either in the form of added glucose or sucrose or those produced by natural enzymatic action on the flour, producing carbon dioxide gas which distends the dough causing it to rise. At the same time by the initial mixing operation and during the period of fermentation the gluten of the dough is developed and mellowed. When the dough is considered to be in the right condition (ripe) at the end of the fermentation period the dough is divided into pieces of the correct weight, molded to the shape required and allowed to recover and ferment further in the pieces. This part of the process is known as the proof. The pieces are then passed into oven for baking.
2.4.2 Rheology of the Bread making process

The bread making process is divided into its three main stages: mixing, fermentation and baking. Changes in the rheological properties of the dough in each of these stages are the consequences of changes in structure at both the molecular and microscopic levels (Bloksma, 1990a).

2.4.2.1 Mixing

Mixing transforms the combination of powder-the flour and liquid - the water - into a cohesive visco – elastic dough. This dough is capable of retaining the carbon dioxide produced by the yeast during fermentation and the carbon dioxide, water and ethanol evaporating during oven rise. Gas retention is an essential property of dough for bread making. The transformation into cohesive dough can be followed by observing change in the resistance to mixing or in the microscopic structure of the dough. For proper dough development, both mixing energy and mixing power must be above a critical level, for processing with short fermentation times, high level of both are required.

2.4.2.2 Fermentation

The objective of fermentation is to bring the dough to an optimum condition for baking. During fermentation the carbon dioxide produced by the yeast is collected in the gas cells which have been formed during mixing, and their volume increases. This expansion requires an excess pressure in the cells, estimated to be small compared to atmospheric pressure. A major part is thought to be due to the surface tension in the gas – dough, interface. The result of the expansion of the gas cells is a deformation of the dough phase, but there is little evidence that this contributes significantly to dough development.

2.4.2.3 Baking

When the fermentation process is finished the dough is baked in an oven. During the first stage of baking, the dough expands further, mainly
as a result of evaporation of water, carbon dioxide, and ethanol from the dough phase. Gelatinization of starch markedly increases the viscosity at temperature above 60ºC; this cause’s marked increase in the tensile stress in the dough membranes. During baking they rupture, and the foam is transformed into sponge. A possible explanation of the rupture is that the increase of the tensile stress in the membranes results in a value that exceeds the tensile strength of the liquid dough phase.

2.4.3 Bread ingredients

Bread is made of the four essential ingredients, flour, water, yeast and salt, to which others may be added depending on the type of bread desired.

The optional ingredients used most frequently are sugar, milk and shortening, all of which improve the quality of bread, in addition, small amount of dough conditioners are often used by bakers.

2.4.3.1 Flour

Flour is the major ingredient in bread formula. Wheat flour, and more specifically the protein of wheat flour, is unique in its ability to form dough that retains gas. This is fundamental property required for the production of all leavened dough based products.

American hard red winter and red spring wheat and Australian prime hard wheat with 12.5% protein content at least and 62.65% water absorption is suitable for bread making (NCFM, 2003).

2.4.3.2 Water

In the dough, water comes next in importance to the flour. It is responsible for the formation of the gluten which gives the dough its rheological characteristics. The amount of water used for making a sac of
flour into dough varies and depends on the type of flour used and the type of bread being produced.

In dough the starch took up 46% of the water, gluten 31% and pentosans 23% (Bushuk, 1966).

2.4.3.3 Yeast

The baker’s yeast (Saccharomyces cervisia) is the best microorganism adapted for leavening of baker’s product (Banwart, 2002). It needs sugar for producing carbon dioxide and alcohol. If no sugar is available in the dough-no fermentation occurs in spite of an excess of yeast.

2.4.3.4 Salt

Salt is added to every bread formula as a flavouring agent, and control the rate of fermentation. Its retarding effect on yeast activity can be demonstrated by using an excessive amount of salt. Roach (1989) observed increasing mixing time with the addition of sodium chloride, this mean salt also strengthen the gluten.

2.4.4 Bread improvers

Bread improvers encompass a large group of dough additives that serve to alter the handling properties of dough or the sensory properties of bread or both. Its designs are constantly changing to meet the rabid advance in food ingredient technology and demand for higher quality bakery product.

There are many types of commercial bread improvers manufactured for variety of applications, whether it is for pan bread, pizza bases or fermented dough. Each bread improver type is specifically tailored to enable the desired characteristic of dough or bread type to be achieved. The usage of bread improver can vary widely, often reflecting the quality level or type of the improving ingredients that it contains.
Bread improvers provided better gas retention, resulting in lower yeast requirements, short proof time and larger finish product volume. It also improved tolerance to variations in the quality of flour and other ingredients, and gave drier dough that can be mechanically processed more easily and have greater resistance to abuse.

2.4.5 Bread quality

Bread quality is usually judged by:

2.4.5.1 Loaf volume
Soulaka and Morrison (1985) reported that, loaf volumes obtained from reconstituted flours were larger and the crumbs 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.

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

2.4.5.2 Crumb texture

Dough is a complex system, and many problems associated with the poor textural quality of a final product can result from a deficiency in one or more of the following dough characteristics: gas generation, gas retention and setting of the structure in the expanded state.
The texture may be too soft, some times "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 Rubenthaler (1987) reported that, 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 or both, produces a coarse and heavy crumb texture.

2.4.5.3 Aroma

Aroma is an important factor governing food acceptability. The aroma of bread results from the interaction of reducing sugars and amino compound, accompanied by the formation of aldehydes. Also aroma is affected by the products of alcoholic and in some cases, lactic acid fermentation (Kent, 1983, Lyla, 2002).

Matz (1968) mentioned that, yeast consumes sugars and produces carbon dioxide and alcohol is partly responsible for the aroma of the baked product.

2.4.5.4 Color

Golden brown color of the crust is one of the most obvious traits 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 sugars molecules. Temperature and pH increase the reaction rate. The reaction continuous and colored pigments, known as melanoidins 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.
CHAPTER THREE
MATERIALS AND METHODS

3.1 Materials:

3.1.1 Wheat sample:

Four samples of bread flour wheat were brought from Seen Company.

3.1.2 Improvers:

Bread improver from the local market contains (Baobab-Malt-Emulsifiers-Guar Gum-Lcystine-Ascorbic Acid-Enzymes), and three levels (A,B,C) of improver contain ascorbic acid and alpha-amylase enzyme: (A: 2 gm ascorbic acid – 1gm alpha-amylase, B: 2gm ascorbic acid - 4gm alpha-amylase, C: 3 gm ascorbic acid - 6gm alpha-amylase).

3.1.3 Chemicals:

All chemicals and reagents were of analytical grade.

3.2 Methods:

3.2.1 Proximate composition:

The determination of moisture, crude fiber, crude protein and ash were carried out on the samples according to AOAC (1984) methods.

3.2.1.1 Moisture content:

Two grams of well-mixed samples were weighed accurately in clean preheated crucible of known weight by using sensitive balance. The uncovered sample and dish were kept in an oven provided with fan at 105°C and let to stay overnight. The crucible was covered and transferred to desiccator and weighed after reaching room temperature. The crucible was heated in the oven for another two hours and was weighed. This crucible was heated in the oven for another two hours and was re-weighed. This was repeated until constant weight was obtained.
The loss of weight was calculated as percent of sample weight and expressed as moisture contents.

Moisture content % = \( \frac{Wt1 - Wt2}{\text{Sample weight}} \times 100 \)

Where: 

- \( Wt1 \) = Weight of sample + crucible before oven dry.
- \( Wt2 \) = Weight of sample + crucible after oven dry.

### 3.2.1.2 Ash content

The ash content was determined according to AOAC method (1990) using muffle furnace.

A 2g sample was weighed into porcelain and placed in a temperature controlled furnace of 600°C for complete ashing. The ash crucible was transferred directly into desiccator, cooled, weighed immediately and the ash was calculated as a percentage of original weight of the sample.

Ash content was calculated using following equation:

\[
\text{Ash content %} = \frac{Wt1 - Wt2}{\text{Sample weight}} \times 100
\]

Where: 

- \( Wt1 \) = Weight of crucible with sample.
- \( Wt2 \) = weight of empty crucible.

### 3.2.1.3 Protein content

Nitrogen was determined by the micro Kjeldahl technique according to the method described by Perten (1995) after digestion (Model DDS, 50, 60 H 77 Amp and distillation Model 50/60 HZ Amp), 0.2g of sample was transferred to digestion flask and 0.8g of catalyst mixture (96% anhydrous sodium sulphate, 3.5% copper sulphate and 0.5% selenium dioxide) was
added to each flask in addition to 7 ml of conc. H2SO4 and 5 ml of H2SO4 (3.5% catalyst). Contents were allowed to digest for 35 min and distilled by 50ml sodium hydroxide (40%). Collected nitrogen in boric acid was titrated against 0.1 NHCL. The crude protein was calculated from the following equation:

\[
\text{Nitrogen content\%} = \frac{T.F \times N \times 14 \times 100}{1000 \times \text{wt of sample}}
\]

Crude protein= Nnitrogen content\% \times 5.7s

Where: T.F= titer figure
N= normality of HCL
14= each wt of 0.1NHCL is equivalent to 14
1000= to convert from mg to g
5.7= Constant factor for wheat flours and wheat products.

3.2.1.4 Fat content

Fat content was determined according to the AOAC method (1990). Three grams from each sample were wrapped in filter paper, placed in a thimble, covered by a piece of absorbent cotton, and placed in extraction tube. A 50 ml of the solvent (Hexane Bp 60 -70\degree C) was added to the apparatus. The extraction was carried out for 45 min before the solvent was recovered from the oil. The oven removed races of solvent at 103± 2\degree C. the flask was then cooled and re-weighed. The oil content was calculated according to the following equation:

\[
\text{Fat \%} = \frac{W2 - W1 \times 100 \times 100}{\text{Wt of sample} \times 100 - M}
\]

Were: W1= weight of empty flask
W2= weight of the flask with oil
M= Moisture percentage of the sample
3.2.1.5 Fiber content

Crude fiber was determined according to the method of the AOAC (1984) using fiber system as follows: a 2 g of finely ground sample were accurately weighed, transferred to an extraction apparatus and extracted with hexane, the air dried fat free sample was transferred to the fiber beaker then digested with preheated KOH (1.25%) for 30 min. Again the sample was then ashed in muffle furnace at 450°C, allowed to cool and reweighed. The crude fiber was calculated using the following equation:

\[
\text{Fiber \%}= \frac{W_2-W \times 100 \times 100}{\text{Sample weight(100-M)}}
\]

Where:  
\(W_1 = \text{weight of sample and crucible}\)
\(W_2 = \text{weight of crucible with ash sample}\)
\(M = \text{moisture percentage of the sample}\)

3.3 Gluten quantity and quality

Gluten quantity and quality were determined on wheat flours with different levels of ascorbic acid and alpha amylase according to standard ICC method (1968) (revised 1996) by using Glutomatic instrument (type2200).

Ten grams of the sample was mixed into dough with 5 ml distilled water in a test chamber with bottom sieve. The dough was then washed with 2% solution of sodium chloride. The gluten ball obtained was centrifuge and quickly weighed.

The percentage of wet gluten remaining on the sieve after centrifugation is defined as the gluten index. The total wet gluten was dried in a heater (Glutork 2020) to give the dry gluten. The weight of gluten was multiplied by ten to give the percentage of wet or dry gluten.
3.4 Falling number

Appropriate flour sample weight, was weighed and transferred into falling number tube and 25 ± 0.2 ml distilled water were added, the stopper was fitted into top of the viscometer, and shaked well(20-30 times or more if necessary), until a homogenous suspension was formed. The viscometer tube was placed in the boiling water –bath, and locked into position.

The test automatically starts. The sample was stirred for 60 seconds, and then the viscometer stirrer was stopped in position, released and sanked under its own weight through the uniform gelatinized suspension. The time in seconds for the stirrer to fall through the suspension was recorded as the falling number (seconds), the required flour sample weight (R. W) is obtained from the correction Table of sample weight to 14% moisture basis (ICC 107/1, 1995) and AACC 56-81 B (1992), corresponding to the 7g at 14% moisture, no change is made in the quantity of the water used (25ml).

Calculations:

\[
(R.W.) \text{ g} = 7 \times \frac{(100-14)}{(100-m)}
\]

Where:

\(m\) = Actual moisture percentage of the flour sample.

\(R.W.) = \) The required flour sample weight used for determination.

3.5 Sedimentation value

Sedimentation values were carried on wheat flours according to AACC (2000).

Reagents:

1. Isopropyl alcohol, 99-100, nitrate- free or equivalent.
2. Water containing 4 mg bromophenol blue 1liter.
3. Lactic acid stock solution, dilute 250 U.S. Pharmacopeia 85% lactic acid 1 liter with water. Reflux diluted acid for 6 hr without volume loss.
4. Mix thoroughly 180 ml lactic acid stock solution (reagent 3), 200 ml isopropyle alcohol (reagent 1), and water to make 1 liter. Let stand for 48 hrs, before using protect against evaporation.

Procedure:

3.2 grams of sieved flour sample were placed in 100 ml glass stoppered graduate cylinder, simultaneously timing started when 50 ml containing bromophenol blue was added, then flour and water were thoroughly mixed by moving stoppered cylinder horizontally length wise, alternately right and left through space of 7 to 12 times in each direction in 5 sec, then flour was completely swept suspension during mixing.

At the end of first 2 periods, the contents were mixed for 30 sec, in this manner the cylinder was completely inverted then righted up, as if it was pivoted at center, this action was performed smoothly 18 times in the 30 sec, then was let to stand 1.5 min. after that 25 ml of isopropyl alcohol lactic acid (reagent 4) were added, mixed immediately by inverting cylinder four times as the latest step then was let to stand 1.7 min, mixed again for 15 sec, then the cylinder was immediately placed up to stand for 5 min. The factor to obtain sedimentation values was brought from Table on 14% moisture basis, (AACC, 2000).

3.6 Farinograph

Brabender farinograph method was carried out on wheat flours according to AAOC (2000).

A-The titration curve:

Brabender farinograph was operated as described in AAOC method (2000). Titration curve was used for the assessment of the water-absorption for each flour sample.
A sample of 300g (14% moisture) was weighed and transferred into cleaned mixer. The farinograph was switched on at 63 rpm for one min, then the distilled water was added from especial 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 burette indicates water absorption of the flour in percentage.

**B-The standard curve:**

The measuring mixer was thoroughly cleaned. A sample of 300g was weighed, and then introduced into the mixer; the farinograph was switched on such as before. The water quantity, which is determined by the titration curve, was fed at once. When an appreciable drop on the curve was noticed, the instrument was run further 12 min, and then shut off.

The significant readings taken from farinograph are:

A. Water absorption: is the amount of water added to balance the curve on the 500 FU line, expressed as a percentage of the flour at 14% moisture.

B. Dough development time (peak time): is the time in min between the origin of the curve and its maximum.

C. Arrival time: is the time between the origin and the point where the curve first reaches the 500-FU line.

D. Departure time: is the time between the origin and the point where the top of the curve falls below the 500 FU line.

E. Dough stability: is defined as the difference in min between the departure time and arrival time (D-C).
F.   Dough softening: is defined as the difference of the dough strength between the amounts where dough weakening begins after 12 min dough kneading (measured in F.U.).

G.   Mixing tolerance index is measured as the difference in F.U. between the top of the curve at the optimum and the point on the curve 5 min later.

3.8 Extensograph

   Extensograph method was used according to ICC (2001). The extensograph and farinograph were operated at 30°C.

   The dough for extensograph was operated as for the farinograph, but the amount of water used for mixing was 2% less due to the addition of 2% salt and the dough was mixed for 5 min only.

   Two pieces of dough (150g each) were weighed, molded on the balling unit, rolled with dough roller into cylindrical test pieces, fixed in the dough holder, and stored in the rest cabinet for 45 min. The dough piece was placed on the balance arm of extensograph and stretched. The behavior of the dough was recorded on a curve via extensograph. This test was performed at 45, 90 and 135 min intervals.

3.8.1 Extensograph measurements

   A.   Energy: measure the area under the curve by means of a planimeter and indicate the value in cm². This value describes the work applied for stretching the dough and is a measure for the flour quality.

   B.   Resistance to extension: is the height of the extensogram at a constant deformation of the dough. The value is determined at the point where the paper has run 50 mm from the beginning of stretching. The result is given in EU with a precision of 5 EU.

   C.   Extensibility: is the distance in mm traveled by the chart paper from the beginning of stretching until breaking of the test piece.

   D.   Ratio: is the equation of resistance and extensibility.
Ratio  = Resistance to extension / Extensibility

3.9 Preparation of loaf bread

The procedure described by Badi, et al., (1978) was modified for this type of bread. Bread improvers levels were Ascorbic acid 1, 2, 3, alpha amylase 2.4.6, with constant bread improver were added as manufacture recommended. Dry ingredients (flour 250 g, dry yeast 2.5 g, salt 1.5 g and sugar 3 g) were mixed for 1 min using Mono- Universal Laboratory dough mixer. Water was added (based on the farinograph optimum absorption) and mixed for 3 min at medium speed. After mixing the dough was allowed to rest for 10 min at room temperature (38 ±2°C), scaled to three portions of 120, each molded into round put in pans and transferred into the fermentation cabinet for 45 min.

The fermented doughs were then baked in Simon Rotary baking oven at 250°C for 15-20 min.

3.9.1 Physical characteristics of loaf bread

The loaves were left to cool for 1 hr at room temperature (38±2°C).

3.9.1.1 Bread weight

The weight of the loaf bread was taken in g.

3.9.1.2 Bread volume

The loaves volume was determined by the speed displacement method according to Pyler (1973). The loaf was placed in container of known volume into which small seeds (millet seeds) were run until container is full. The volume of seeds displaced by the loaf was considered as the loaf volume.

3.9.1.3 Bread specific volume

The specific volume of the loaf was calculated according to the AACC method (2000) by dividing volume (CC) by weight (g).
3.9.2 Sensory evaluation of loaf bread

The loaves were sliced with an electric knife and prepared for sensory evaluation at the same day. The sensory evaluation of bread samples (aroma, color, texture, taste and general acceptability) was carried out by 10 trained panelists.

3.10 Statistical analysis

All statistical analyses done using computer software. Data subjected to Statistical Package for Social Science (SPSS). Means were tested using one – factor analysis of variance (ANOVA), and then separated using Duncan’s Multiple Range Test (DMRT) (Mead and Gurnow, 1983).
4.1 Chemical composition

4.1.1 Moisture content

The moisture content of the wheat flour sample (70% extraction rate) was about 13.38%.

This result is comparable with the result obtained by Zeleny (1971) and Pyler (1973) who reported values of moisture content of 8.14% and 13.0%, respectively.

The present result indicated that the moisture content among bread with different levels of improvers was not affected by the addition of improver.

4.1.2 Ash content

Ash content of the wheat flour was about 0.42%.

This result agreed with Zeleny (1971); Dappolonia and Young (1987) who reported that the ash content of wheat flour is 0.2, 0.5 and 0.53% respectively.

4.1.3 Protein content

Protein content of the wheat flour was about 13.15%.

This value is within the range of 10-16% reported by Haldor et al. (1982), but higher than the value obtained by Giami (2005) who gave 11.3 and lower than Doxasta kis et al. (2002) who gave 11.7% for protein content of wheat flour.

4.1.4 Fat content

The result showed that fat content of wheat flour was 1.19%. This result was lower than FAO (1999). They found that, the fat content of wheat flour ranged between 2-3%.
4.2 Gluten quality and quantity

Gluten quantity (wet and dry) and gluten quality (gluten index) percentages of doughs prepared from wheat flour sample with addition three levels of improver is shown in Table (1).

Significant differences (P ≤ 0.05) among the levels in their gluten index was found. Gluten index over 80% is an indication of good quality bread flour (ICC, 1995). The optimum range of gluten index for bread making is between 60- 90% (Perten, 1995).

The gluten index percentage of bread wheat flour was ranged from 81.35% in the level A to 77.85 in the level B.

4.3 Falling number

Addition of improver enhanced the alpha-amylase activity and reduced the falling number from 539 to 369 sec.
Falling number of most wheat flour were reported to be in range of 342-488 sec. (Pards-Lopez et al. 1978).

4.4 Sedimentation value

The sedimentation test was based on the fact that gluten in bibes water and swells greatly when treated with dilute lactic acid under standard conditions. The amount of water of flour depends on the quality of gluten.
Strong gluten swells more and occupies bigger volume. (Williams, 1970). The addition of improver has no effect on the sedimentation value which is about 33cm³.
Water absorption value of the samples ranged from 59.1 to 58.4 the highest value was observed in control while the C level gained the lowest

The falling number of wheat flour with certain levels was shown in Table (2).
Falling number values, i.e. alpha-amylase activity of bread flour with three levels of improver was found to be in range of 369 to 539 sec. wheat
flour falling number decreased with increasing the levels of improver (increasing alpha-amylase activity). For this reason the sedimentation values in the three levels of improver was found to be 33 cm³.

4.5 Farinograph results

The water absorption of the flour depends on lower moisture content, higher bran content, high protein content, high pentosan levels, more damaged starch and higher enzymatic activity.

Farinograph results of the bread wheat flour samples were shown in Table (2) and Figs (1-4).

Water absorption of different levels of improver ranged from 59.1 to 58.3. The highest value observed in control sample, while the B sample gave the lowest value.

From the results, it is obvious that the water absorption decreased with increasing the level of improver, this result maybe due to

Dough development time decreased with increasing the level of improver from 2.73 to 2.07

Dough stability decreased significantly with increasing the levels of improver

The degree of softening was increased with increasing in levels of improver.
Table (1): Gluten quality and quantity and sedimentation value of the three levels of improver:

<table>
<thead>
<tr>
<th>Dose</th>
<th>Wet gluten %</th>
<th>Dry gluten %</th>
<th>Gluten index %</th>
<th>Sedimentation Value(cm³)</th>
<th>Falling No. (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>31.15</td>
<td>10.35</td>
<td>80.70</td>
<td>33</td>
<td>539</td>
</tr>
<tr>
<td>A</td>
<td>31.15</td>
<td>10.40</td>
<td>81.35</td>
<td>33</td>
<td>502</td>
</tr>
<tr>
<td>B</td>
<td>31.40</td>
<td>10.45</td>
<td>77.85</td>
<td>33</td>
<td>407</td>
</tr>
<tr>
<td>C</td>
<td>31.05</td>
<td>10.25</td>
<td>81.10</td>
<td>33</td>
<td>369</td>
</tr>
</tbody>
</table>

(A: 2 gm ascorbic acid – 1gm alpha-amylase, B: 2gm ascorbic acid - 4gm alpha-amylase, C: 3 gm ascorbic acid - 6gm alpha-amylase).
Table (2): Farinograph measurements of dough on addition of the three levels.

<table>
<thead>
<tr>
<th>Level</th>
<th>water</th>
<th>Deve. time</th>
<th>Stability</th>
<th>Softening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>59.10±1.47a</td>
<td>2.73±0.25a</td>
<td>7.83±0.75a</td>
<td>60.67±4.51b</td>
</tr>
<tr>
<td>A</td>
<td>58.70±0.61a</td>
<td>2.17±0.15b</td>
<td>4.13±0.23b</td>
<td>79.33±5.03a</td>
</tr>
<tr>
<td>B</td>
<td>58.80±0.46a</td>
<td>2.07±0.12b</td>
<td>2.93±0.40c</td>
<td>89.67±5.13a</td>
</tr>
<tr>
<td>C</td>
<td>58.37±0.90a</td>
<td>2.20±0.00b</td>
<td>3.27±22.08bc</td>
<td>89.67±0.78a</td>
</tr>
</tbody>
</table>

Means values having different superscript litter in each column differ significant at (p≤0.05) using Duncan’s Multiple Range Test (DMRT).
(A: 2 gm ascorbic acid – 1gm alpha-amylase, B: 2gm ascorbic acid - 4gm alpha-amylase, C: 3 gm ascorbic acid - 6gm alpha-amylase).
Fig (1): Farinogram of dough prepared from control wheat flour.

Fig (2): Farinogram of dough prepared from A wheat flour.
Fig (3): Farinogram of dough prepared from B wheat flour.

Fig (4): Farinogram of dough prepared from C wheat flour.
4.6 Extensograph results

The Extensograph results of the three levels of improver and control were shown in Table (4) and fig. From fig 5 to 8.

Addition of improver increased the energy compared to the control bread flour except at 135 min and decreased the extensibility except at 45 min. while the ratio number increased with increase the level of improver compared with the control.
Resistance was increased gradually at 45, 90, and 135min and increased with increasing the levels of improver.
Perhaps this improver contains oxidizing agents (ascorbic acid) causing s-s groups in the dough resulting in high resistance to extension.
Energy and extensibility decrease with increase in fermentation time, while the resistance and ratio number increase with increase in fermentation time.
Table (3): Extensograph measurements of dough on addition of the three levels.

<table>
<thead>
<tr>
<th>Lev el</th>
<th>Energy</th>
<th>Extensibility</th>
<th>Resistance</th>
<th>Ratio number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>45</td>
<td>90</td>
<td>135</td>
<td>45</td>
</tr>
<tr>
<td>Control</td>
<td>132.33±4.59b</td>
<td>140.33±7.50a</td>
<td>140.00±1.00b</td>
<td>137.33±3.15a</td>
</tr>
<tr>
<td>A</td>
<td>151.00±2.00a</td>
<td>146.00±6.00a</td>
<td>138.00±2.00a</td>
<td>141.00±3.00b</td>
</tr>
<tr>
<td>B</td>
<td>143.33±5.50b</td>
<td>141.00±5.00b</td>
<td>125.00±5.00b</td>
<td>139.00±3.00b</td>
</tr>
<tr>
<td>C</td>
<td>155.00±1.73a</td>
<td>142.00±3.00a</td>
<td>134.33±1.15a</td>
<td>143.33±2.51b</td>
</tr>
</tbody>
</table>

Mean values having different superscript letter in each column differ significantly at \((p \leq 0.05)\) using Duncan’s Multiple Range Test (DMRT).

(A: 2 gm ascorbic acid – 1gm alpha-amylase, B: 2gm ascorbic acid - 4gm alpha-amylase, C: 3 gm ascorbic acid - 6gm alpha-amylase).
Fig (5): Extensogram of dough prepared from control wheat flour.

Fig (6): Extensogram of dough prepared from A wheat flour.
Fig (7): Extensogram of dough prepared from B wheat flour.

Fig (8): Extensogram of dough prepared from C wheat flour.
### Table (4): Bread specific volume

<table>
<thead>
<tr>
<th>Sample</th>
<th>Bread volume cm$^3$</th>
<th>Bread weight g</th>
<th>Bread specific volume cm$^3$/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>448.750</td>
<td>103.750</td>
<td>4.31775</td>
</tr>
<tr>
<td>A</td>
<td>481.500</td>
<td>104.750</td>
<td>4.59350</td>
</tr>
<tr>
<td>B</td>
<td>518.750</td>
<td>105.250</td>
<td>4.93100</td>
</tr>
<tr>
<td>C</td>
<td>502.500</td>
<td>105.250</td>
<td>4.77825</td>
</tr>
</tbody>
</table>

Mean Values $\pm$SD differ significantly ($P \leq 0.05$).

### Table (5): sensory evaluation of bread.

<table>
<thead>
<tr>
<th>Level</th>
<th>Aroma</th>
<th>Color</th>
<th>Texture</th>
<th>General acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.17$\pm$0.99</td>
<td>3.43$\pm$0.82</td>
<td>3.37$\pm$0.72</td>
<td>3.20$\pm$0.71</td>
</tr>
<tr>
<td>A</td>
<td>3.30$\pm$0.75</td>
<td>3.83$\pm$0.65</td>
<td>3.57$\pm$0.77</td>
<td>3.53$\pm$0.73</td>
</tr>
<tr>
<td>B</td>
<td>3.67$\pm$0.61</td>
<td>4.13$\pm$0.73</td>
<td>3.70$\pm$0.70</td>
<td>4.10$\pm$0.80</td>
</tr>
<tr>
<td>C</td>
<td>3.53$\pm$0.63</td>
<td>3.67$\pm$0.66</td>
<td>3.73$\pm$0.86</td>
<td>3.87$\pm$0.73</td>
</tr>
</tbody>
</table>

Mean Values $\pm$SD differ significantly ($P \leq 0.05$).
Plate(1): effect of improver on bread specific volume

A: Control
B: Sample with A improver
C: Sample with B improver
D: Sample with C improver

(A: 2 gm ascorbic acid – 1gm alpha-amylase, B: 2gm ascorbic acid - 4gm alpha-amylase, C: 3 gm ascorbic acid - 6gm alpha-amylase).
CHAPTER FIVE
Conclusions and Recommendations

Conclusions:
From this work it could be concluded that the addition of improver showed positive effect on rheological properties of the dough. Level B : (2gm ascorbic acid - 4gm alpha-amylase), gave the best results compared with control and the other levels.

Recommendations:
It is recommended that:
Further investigations are needed to seare bread high yielding with high quality. Using improver B: (2gm ascorbic acid - 4gm alpha-amylase), in loaf bread.
References


Banwart, G.J. (2002). Basic food microbiology. 2nd ed. CBS publishers and distributors. India.


Pagenstedt, B., (1965). In; determination of baking value of wheat by mean of physical testing method (ed., Brabender, O.H), Disburge of Rhine, Germany


