EFFECT OF WHEAT GERM ADDITION ON THE SELECTED PROPERTIES OF BREAD

A MASTER'S THESIS

in

Food Engineering
University of Gaziantep

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April, 2002

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ABSTRACT

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M.Sc. in Food Engineering

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April 2002, 64 pages

Enrichment of bread with 5, 7.5, 10, 12.5, 15, 17.5 and 20 % raw wheat germ was studied by using a laboratory scale bread machine. Bread value numbers, volumes, heights, weights and general structure of the enriched bread were examined.

Bread value numbers were decreased by addition of wheat germ. To eliminate this, ascorbic acid was used and the effect of ascorbic acid on wheat germ bread was investigated. An addition of 150 mg ascorbic acid/kg was used for each formulation having 5 to 20 % wheat germ. Although negative effects of raw wheat germ was observed, bread enriched with 15 % wheat germ was found satisfactory after ascorbic acid treatment in terms of bread properties.

Key words: Wheat germ, Bread, Enrichment of bread

ÖZET

BUĞDAY RUŞEYMİ KATKISININ EKMEĞİN BAZI ÖZELLİKLERİ ÜZERİNE OLAN ETKİLERİ

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Nisan 2002, 64 sayfa

Laboratuvar tipi ekmek makinası ile % 5, 7,5, 10, 12,5, 15, 17,5 ve 20 ham buğday ruşeymi ile zenginleştirilmiş ekmek çalışıldı. Ekmek değer sayıları, hacimleri, yükseklikleri, ağırlıkları ve ruşeym ekmeğinin genel yapısı incelendi.

Ekmek değer sayılarının buğday ruşeymi eklenmesi ile düştüğü gözlendi. Bu olumsuzlukdan dolayı askorbik asit kullanıldı ve buğday ruşeymli ekmek üzerine etkisi araştırıldı. % 5-20 buğday ruşeymine sahip her formulasyon için 150 mg askorbik asit/kg kullanıldı. Ham buğday ruşeyminin ekmek özellikleri üzerine olumsuz etkilerinin gözlenmesine rağmen, askorbik asit uygulamasıyla % 15 buğday ruşeymi ile zenginleştirilmiş ekmek tatmin edici özellikte bulundu.

Anahtar kelimeler: Buğday ruşeymi, Ekmek, Ekmeğin zenginleştirilmesi

ACKNOWLEDGEMENTS

I wish to express my appreciation to my supervisor Prof.Dr. Zerrin SÖYLEMEZ for her advice, encouragement and guidance through this research.

I also wish to thank to Assist.Prof.Dr. Şenol İBANOĞLU for continuous help and suggestions. Also, heartfelt thanks are due lecturer friends, the staff of Vocational School of Higher Education in Nizip and Depertment of Food Engineering.

Finally, a special note of appreciation goes to my family for always believing in me and their continuous help.

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ABBREVIATIONS

A.A	Ascorbic acid
ANOVA	Analysis of variance
B.V.N	Bread value number
EMG	Ethoxylated monoglyceride
DATEM	Diacetyl tartaric acid esters of monoglyceride
DHAA	Dehydro-ascorbic acid
NEMI	N-ethylmaleimide
SH	Sulfhydryl
SSL	Sodium stearoyl lactylate

CHAPTER I

INTRODUCTION

1.1. Bread

Bread is the most important food in the world and especially in Turkey. It is the principle food and provides more nutrient than any other single food source. Bread supplies approximately 1/2 the total caloric intake in 53 % of the world countries, in 87 % of the countries, over 30 %. It is the source of 1/2 the carbohydrate 1/3 of the proteins, over 50 % of the B vitamins, and over 75 % of E vitamins [1].

Bread supplies a significant portion of the nutrients required for growth and maintenance of health and well-being. Although not an outstandingly good source of any single nutrient, bread is a good source of most nutrients. The potential of wheat and bread for meeting man's nutrient needs is well established. Numerous studies have demonstrated the excellent value of bread for children, adolescents, and adults. Enrichment of bread with vitamins and minerals has been instrumental in improving the nutritional adequacy of our diet. Supplementing bread with protein concentrates and pure amino acids shows great promise for improving the diet of people who depend on bread as their main source of nutrients [1]. Bread has often been described as the staff of life. Wheat and wheat foods, long recognized as a major staple and source of calories in the diets of people in many cultures, also contribute significant quantities of other nutrients to the diet [2].

Bread is the most popular yeast leavened product in the world. The amount of bread consumed in the world is truly staggering. Also staggering is the wide array of sizes, shapes, textures, and tastes. Breads vary in size from small bread sticks to loaves weighing several kilos. Crust color and texture can vary from the thick, black crust of pumpernickel to the thin, white crust of Chinese steam bread. The reasons for the large variation are complex and difficult to sort out. Many of them have to do with tradition; one likes the bread that one was raised on. Also important are the other foods in the diet, how much of the diet is bread, and many other factors [3].

Although bread is the principle food, its consumption varies widely in the world. In Europe it is highest in the southern and southeastern countries. It is low in Sweden and in the Netherlands and intermediate in Central Europe. Bread consumption in Africa is particularly wide 2/3 of the total food intake in Egypt, parts of north and West Africa, and in the South Africa. On the other hand, bread consumption is up to 75 % of total food intake in several countries in the Near and Far East, but less than 1/3 of caloric intake in Australia and New Zealand. Consumption of bread has been decreasing in most countries. Various explanations for the decrease: bread is fattening, it is not as good as in the old days, etc. In practice the decline in bread consumption is related to rising standards of living, increased buying power, and availability of more expensive and sophisticated foods.

In practice more than 1/2 the people in the world go to bed hungry. With the dramatic increase in population, the role of cereals in feeding increasing numbers of hungry in the world assumes greater importance. Without entering into a polemic on the timetable for hunger to threaten most affluent nations, most scientists agree that in the not-too-distant future it will become increasingly difficult to provide foods from animal sources; so our dependence on cereals will increase [1].

1.2. Bread Composition

1.2.1. Flour

Flour is the single most important and basic ingredient in breadmaking. While bread has been produced from meals and flours milled from most cereal grains, the type of bread accepted by the customer in the Western World is normally prepared from wheat flour, and sometimes from wheat-rye mixture.

Flour used in breadmaking is generally milled from common wheat. Flour from durum wheat is used in many parts of the world to make flat bread. In the Western World, durum is used mainly to make semolina for macaroni products. All common wheats are capable of producing some type of leavened bakery products. It is generally agreed that breadmaking quality of a flour depends on the quality and quantity of the flour proteins[1]. On the other hand, we are concerned with include flour ash and flour color. The ash content of a flour is a good indicator of the milling efficiency of a particular wheat sample under test. Protein content of the flour is very important. In the milling operation there is a reduction in protein content from wheat to flour. A reduction of more than one percent is considered undesirable in

experimental milling in the evaluation of potentially new wheat varieties. Wet gluten content is another factor that is often measured in bread flours as an indicator of flour quality [4].

Proteins of flours milled from common wheat possess the unique and distinctive property of forming gluten when wetted and mixed with water. Wheat gluten imparts to dough physical properties that differ from those of doughs made from other cereal grains. It is gluten formation, rather than any distinctive nutritive property, that gives wheat its prominence in the diet [1].

A factor of main concern in flour analysis is the amount of amylase enzyme present. In sound wheat the amount of beta-amylase is generally sufficient, however, the amount of alpha-amylase is limited. A certain amount of amylase is necessary to obtain optimum bread baking performance. For this reason either barley malt or fungal amylase is added at a flour mill to provide this enzyme. If the natural alpha-amylase activity in a flour is excessive, dough and baking properties can be adversely affected. High amounts of alpha-amylase in flour normally result from wheat that has sprouted during harvesting. This occurs as a result of very wet rainy weather. Various methods are available to measure this amylase activity. Certain methods are based on starch gelatinization followed by measurement of the change in viscosity of which the falling number and amylograph techniques are examples. Procedures based on colorimetry and nephelometry are also used.

Starch damage is another quality parameter of importance in bread flour. This starch damage is a physical damage to the starch granule during the milling of the wheat into flour and can be controlled in the milling operation. For bread baking purposes a certain amount of starch damage is desirable, as it will result in higher flour absorption and a greater percentage of fermentable sugars. Excessive amounts of starch damage will, however, result in sticky doughs and inferior bread quality [4].

1.2.1.1. Special Types of Flour

Extensive investigations of the diets of people throughout the world have established that serious and widespread deficiencies exist in regard to the intake of thiamine, riboflavin, nicotinic acid and iron. Sometimes, there are calcium and vitamin D deficiencies also.

For certain markets strong, high-protein flours containing potassium bromate are best suited for the type of bread desired and the particular baking methods

employed. For these special market requirements, standard for bromated white flour permitted adding up to 75 ppm of potassium bromate to flours that contained at least 15 % protein.

Whole-wheat flours are used for whole wheat bread; they must be made from hard or bread wheat ground to a fairly fine flour. Recombining in the proper proportions the millstreams obtained in the gradual reduction roller milling process frequently makes whole-wheat flours. The flour streams may be bleached in the usual manner and malted wheat or malted barley flour may be added where necessary to raise the amylase activity to a satisfactory level. Whole-wheat flours may be bromated, if so labelled, at levels not exceeding 75 ppm to improve their baking properties. In addition, crushing cleaned wheat other than durum or red durum produces crushed wheat and coarse ground wheat. They contain, in natural proportions, all the constituents of the cleaned wheat. Cracking or cutting cleaned wheat, other than durum or red durum, into angular fragments, prepares cracked wheat.

Phosphated flours represent a large percentage of the so-called family flours, which are marketed in southeastern United States, and comprise bleached soft wheat flours to which monocalcium phosphate, $CaH_4(PO_4)_2$ has been added. These flours are used with soda and sour milk to make sour milk biscuits. When made with plain flour, such biscuits frequently are yellow and have a soda taste because of insufficient acidity in the milk to react with all the soda; adding monocalcium phosphate provides an acid-reacting material to neutralize the excess soda. Proportions recommended in the trade are 0.5 lb monocalcium phosphate monohydrate, $CaH_4(P0_4)_2.H_2O$, (or 0.375 lb of the anhydrous salt) to each 100 lb flour.

Self-rising flours are in large demand and made from bleached, soft wheat patent flours and contain salt, sodium bicarbonate and an acid-reacting ingredient in proper proportions to produce chemically-leavened biscuits. Effective leavening requires a minimum carbon dioxide production of 0.5 % of the weight of the flour. To avoid yellow color and disagreeable "soda taste", sufficient acid ingredient must be added to react with all the soda. A common formula for production of self-rising flours contains 1.5 parts sodium bicarbonate, 1.875 parts monocalcium phosphate monohydrate and 1.75 parts by weight of salt to each 100 parts by weight of flour [1].

1.2.1.2. Flour Quality for Breadmaking

In considering bread-making flour, usually hard wheat was thought with a relatively high protein content. However, in various part of the world, breads made from both soft and durum wheat can be found. Therefore, hardness does not appear to be an absolute requirement. The protein-content requirement does appear to be more absolute. It appears to be impossible to make a good-quality loaf of bread from flour that contains a low amount of protein. On the other hand, the protein content by itself does not assure a good quality. Therefore, both a certain quantity and quality of protein is needed in the wheat flour to produce a quality loaf. The quantity of protein in flour can be determined with good accuracy by several techniques. At this time, the quality cannot be determined so easily. In fact, the only reliable test for evaluating bread-making quality is a bread-making test. The choice of bread-making test is, of course, critical. It must be not limiting in any factor, so that the true ability of the flour to retain gas and give a large volume is fully expressed. A number of such tests exist in the literature [3].

1.2.2. Water

Water is a plasticizer and solvent. Without water, we have no dough and therefore no viscous-flow properties and many of the reactions that take place during fermentation cannot occur because there is no solvent [3].

When water is added to wheat flour and mixed, the water-insoluble proteins hydrate and form gluten, a complex coherent mass in which starch, added yeast and other dough components are imbedded. Thus, the gluten is, in reality, the skeleton or framework of wheat flour dough and is responsible for gas retention which makes production of light leavened products possible. Gluten is not present, as such, in wheat flour. Gluten is formed when water is added and the dough is mechanically handled. Handling "develops" the gluten or dough and involves hydration, modification of gluten proteins and interaction with other flour components (mainly lipids). The gluten can be separated from other flour components by washing under a gentle stream of water. The thus-separated gluten is composed of 2/3 water and 1/3 dry matter. The dry matter is mainly proteins and lipids and small amounts of other ingredients. The gluten proteins can be further separated into gliadin that is soluble in 70 % alcohol and into glutenin that is insoluble in aqueous alcohol but soluble in

acids or alkali. Only a combination of gliadin and glutenin (about 1:1) gives a gluten of desirable properties in breadmaking.

The amount of water added during dough mixing depends on water absorption of the flour, the method and equipment used to make and to process the dough and the characteristics desired in the baked bread. Generally, water absorption increases with increase in protein content and increases with darkness of the flour. Basically, the baker aims at an optimum water absorption for a specified product. Excessive amounts of water make the dough sticky and difficult to handle and the bread wet, soggy and susceptible to microbial damage; additionally, the moisture content may exceed maximum levels permitted by regulatory agencies. On the other hand, dough too dry does not develop satisfactorily during mixing, fermentation by yeast is reduced, the bread stales and crumbles rapidly and the bakers return per unit of flour is reduced. Water used is generally drinking water of intermediate hardness [1].

1.2.3. Salt

Salt is added for taste, but also to improve dough handling, stability and crumb grain, and to increase loaf volume [1]. Salt is generally used at levels of about 1-2 % based on the flour weight. It appears to have two major functions. First is taste; bread made with no salt is quite tasteless. The second is to affect the dough's rheological properties. Salt makes dough stronger, presumably by shielding charges on the dough proteins [3, 5].

1.2.4. Yeast

Bread yeast (Saccharomyces cerevisiae) obtained from sugar beet and sugarcane with modern technology. Bread yeast produced four different types by modern technology. These are instant dry yeast, dry yeast, compressed yeast and yeast milk [5].

The amount of yeast is about 2 % (on flour basis) in regular white bread; for rolls, larger amounts of yeast (up to 5 %) are used. Bakers' yeast ferments the available sugars to yield carbon dioxide (and alcohol) to provide light, porous, yeast-leavened products. Leavened bread was prepared for many years by mixing a portion of leftover dough with a new dough batch and permitting the mix to ferment. A more advanced practice was to mix residual yeast, from a brewery or distillery, with bread

dough. Today, it is recognized that there are many types and strains of yeast, each possessing distinctive characteristics, properties and uses. Bakers' yeast is carefully cultured to bring about definite changes in structure and flavour of doughs [1].

Yeast is a living organism that is inactive during storage. The inactivity is caused either by drying, in the case of active dry yeast, or by low temperature, in the case of compressed or crumbled yeast. Yeast, as it is produced commercially, is always contaminated with bacteria, mainly lactobacilli, which are quite important in crackers and sourdough bread but do not appear to be important in regular bread processes. When yeast is incorporated into a dough, conditions are suitable for it to become active. Yeast is a versatile organism; it can ferment under either aerobic or anaerobic conditions. The production of yeast and the early stages of brewing are aerobic processes, whereas bread fermentation is an anaerobic process. Thus, little growth of yeast occurs during dough fermentation. The oxygen in a dough is rapidly consumed by the yeast and bacteria as fermentation starts. Thereafter, the fermentation is anaerobic unless we add oxygen to the system. The major products of yeast fermentation are carbon dioxide and ethanol [3].

1.3.Breadmaking

In yeast-leavened products, mixing dough fulfils two functions: homogenous distribution of components and development of the gluten matrix to give best bread. Mixing time varies with the flour, dough temperature, dough consistency and mixer. Each dough has an optimum mixing time. Excessive mixing yields dough with reduced elasticity and extensibility.

In yeast- and bacteria-leavened dough, the products of microbial metabolism modify the dough and are essential for production of light, well aerated and appetizing bread. The two main changes occurring during fermentation involve:

- Fermentation of carbohydrates into carbon dioxide, alcohol and small amounts of other compounds that affect the proteins and act as flavour precursors and
- Modification of the proteinaceous matrix for optimum dough development and gas retention during the baking stage. At the same time, in yeast-leavened bread the number of yeast cells increases.

The properly fermented dough is cut and scaled and the pieces are worked mechanically and shaped. The mechanical action removes large gas bubbles and

homogenizes the dough. The dough pieces are allowed a final fermentation (proof) after panning or in free form. The final fermentation lasts about 30 min in small baked products and up to 1 hr in large loaves, but depends also on flour quality, yeast level, dough composition, and temperature. Generally, the proof temperature increases with flour darkness (extraction) from about 28 °C in white to 30-32 °C in whole meal bread [1].

The processing of bread can be divided into three basic operations: mixing or dough formation, fermentation and baking. The simplest bread-making procedure is a straight-dough system (Figure 1.1.). In such a system, all the formula ingredients are mixed into developed dough that is then allowed to ferment. During its fermentation, the dough is usually punched one or more times. After fermentation, it is divided into loaf-sized pieces, rounded, molded into the loaf shape and placed into the baking pan. The dough is then given an additional fermentation to increase its size. After reaching the desired size, it is placed in the oven and baked. In the straight-dough system, the fermentation time may vary quite widely, from essentially no time to as long as 3 hr.

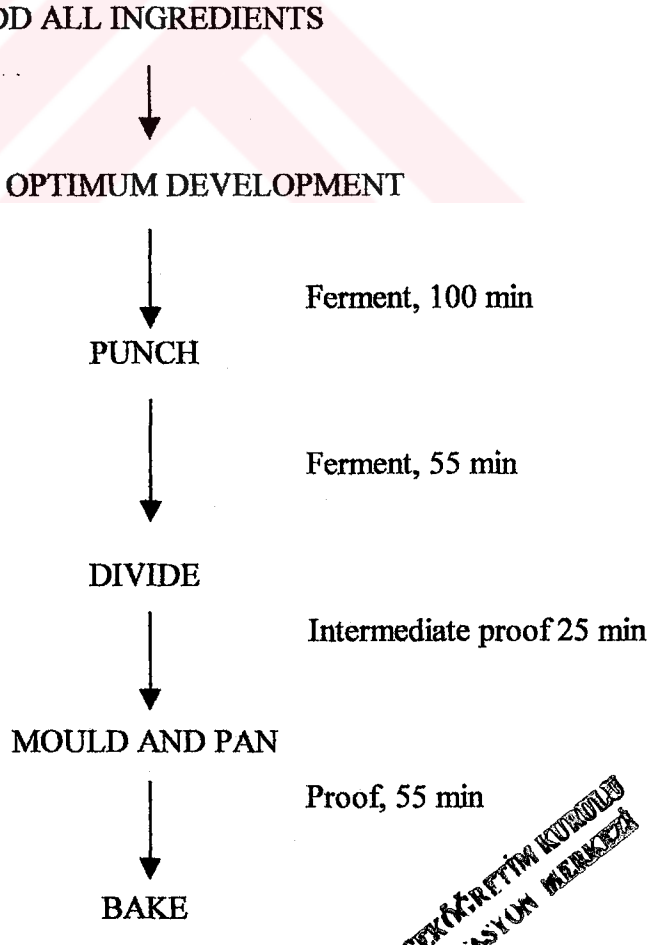

Figure 1.1. Outline of a straight-dough baking process.

In general, straight-dough bread is chewier than bread made by other techniques; it has a coarser cell structure and it is generally considered to have less flavor. The quality of the procedure is quite sensitive to the timing between individual process steps. With larger batches, its time sensitivity can be a problem: if the first of the batch receives optimum fermentation, the end of the batch becomes quite overdeveloped.

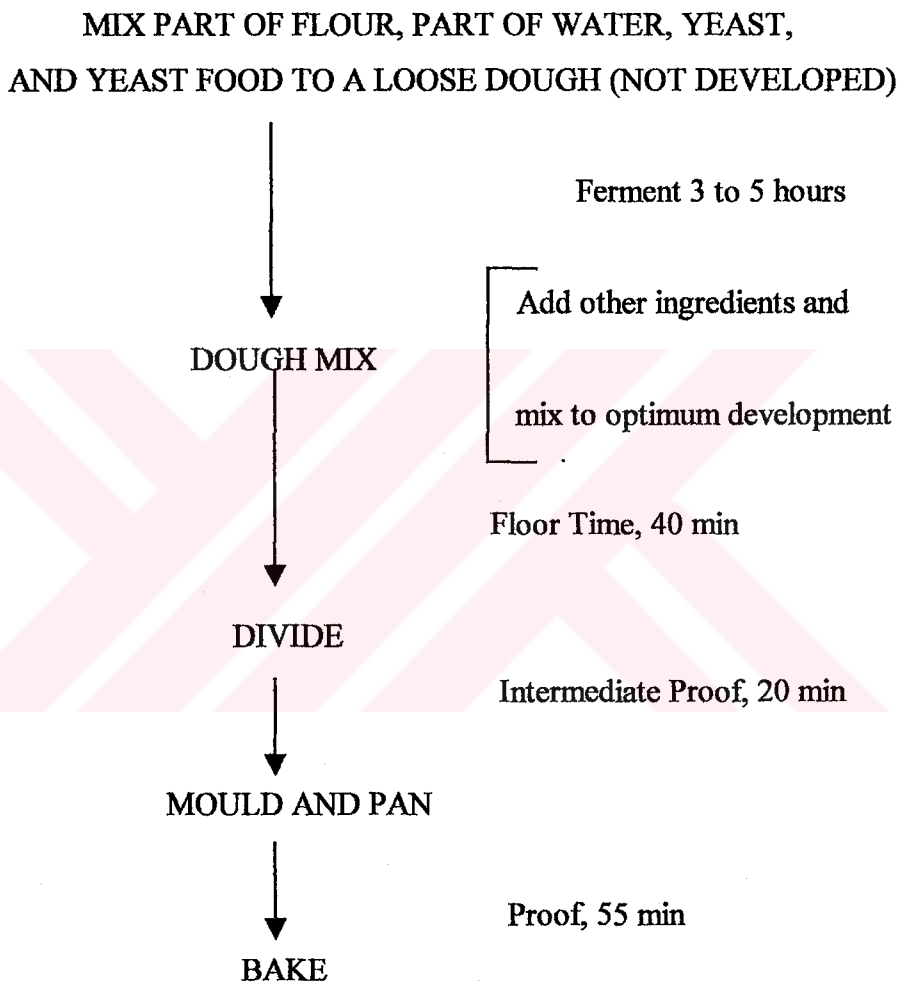

Figure 1.2. Outline of a sponge-and-dough baking process.

The most popular baking process in the United States is the sponge-and-dough procedure (Figure1.2.). In this procedure, part of the flour, part of the water and the yeast are mixed just enough to form a loose dough (sponge). The sponge is allowed to ferment for up to 5 hr. Then it is combined with the rest of the formula ingredients and mixed into developed dough. Alter being mixed, the dough is given an intermediate proof (referred to a "floor time") of 20-30 mm so that it can relax, and then it is divided, molded, and proofed as is done in the straight-dough

procedure.

The sponge-and-dough procedure gives soft bread with a fine cell structure. It is generally considered to have well-developed flavor and is the basis for comparison for U.S. breads. One of the great advantages of the sponge-and-dough procedure is its tolerance to time and other conditions.

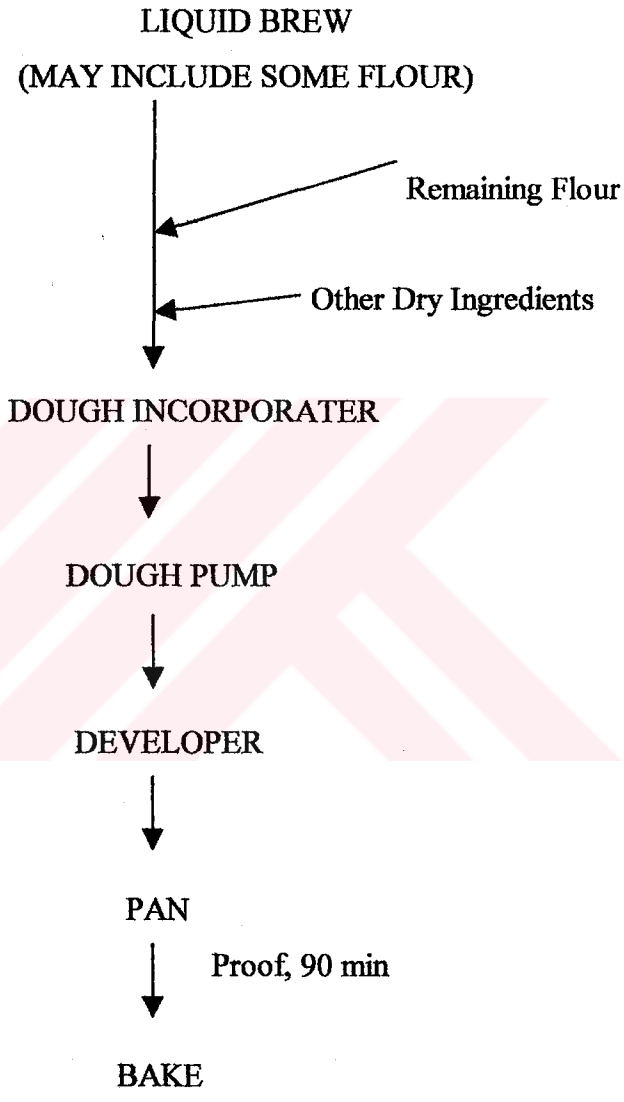

Figure 1.3. Outline of a continuous-baking process.

The numerous other bread-making systems that exist can be viewed as either modifications of one of the above two procedures or as new procedures, depending upon one's point of view. Among the other procedures are the liquid sponge, or preferment, systems, where the fermentation is performed on a liquid in a tank instead of on a sponge. In this case, part or all of the flour is held out of the fermentation. Such procedures appear to be based upon the assumption that the

metabolic products resulting from fermentation are what are beneficial to the dough properties. This is apparently not true and may be responsible for the limited success of these procedures. The continuous bread-making procedure that became popular in the United States a few years ago was, in part, such a procedure (Figure 1.3.). It used a preferment, after which the dough was mixed into developed dough and extruded into the pan, proofed, and baked. The procedure was economical; fewer personnel and less time were required to produce the same amount of bread. However, the bread produced was different from sponge-and-dough bread and not well accepted by consumers. The procedure is essentially no longer used.

Short-time baking systems have become popular in England and Australia. In the United Kingdom, the Charleywood procedure is used for about 80 % of total bread production. This procedure mixes the dough under a partial vacuum, after which it is essentially a no-fermentation time straight-dough system. It is economical and produces bread that is desirable in the United Kingdom. In Australia, a short-time procedure is also used; however, it is different from the Charleywood. It is also a no-time procedure but uses more chemicals for development [3].

1.3.1.Formulation

The minimum formula for bread is flour, yeast, salt, and water. If any one of these ingredients is missing, the product is not bread. Other ingredients that are often found in the formula are fat, sugar, milk or milk solids, oxidants, various enzyme preparations, surfactants and additives to protect against molds. Each of the components in the formula performs a function in producing the finished loaf.

The flour, of course, is the major component and is responsible for the structure of the bread. It allows the formation of viscoelastic dough that retains gas.

Yeast is one of the fundamental ingredients; its major role is to convert fermentable carbohydrates into carbon dioxide and ethanol.

Salt is generally used at levels of about 1-2 % based on the flour weight. It appears to have two major functions. First is taste; bread made with no salt is quite tasteless. The second is to affect the dough's rheological properties. Salt makes dough stronger, presumably by shielding charges on the dough proteins.

The last fundamental ingredient is water, which is a solvent. Without water, we have no dough and therefore no viscous-flow properties.

In bread that is to be stored for any significant period of time after baking,

shortening is an essential ingredient. In addition to its antistaling properties, shortening has other functions in bread baking. It gives bread with increased volume compared to bread made without shortening. The increase in volume is significant, usually about 10 %. The mechanism of the volume increase is still being studied. Fat or shortening also acts as a plasticizer in dough. Thus, if one increases the amount of shortening in the dough, one must decrease the amount of water and vice versa to maintain equal dough consistency.

Sugar also is added to the formula, for two reasons. It is a source of fermentable carbohydrate for the yeast and it provides a sweet taste to the bread. With the proper enzymes in the dough, sufficient sugar is produced from the flour to maintain fermentation and added sugar is not necessary for gas production. However, under most production conditions, added sugar is used for fermentation.

Milk or milk solids in U.S. bread were a common ingredient a few years ago. The nutritional advantages of milk are well known. It also had widespread consumer appeal and because the United States was producing a large excess of milk, it was a relatively cheap ingredient. The supply situation has now changed dramatically and milk is an expensive ingredient. Therefore, it is not used much, if any, today. More common are milk replacers or whey mixtures.

Oxidants such as ascorbic acid, potassium bromate, azodicarbonamide and calcium peroxide, at levels of parts per million, improve dough strength and result in bread with better loaf volume and texture.

The level of a-amylase is very low in wheat flour, and it is common practice to add malted wheat or barley flour to bread flour. The addition of malt increases loaf volume to a small extent, improves bread texture, The often-stated advantage of having malt produce sugar is true but is only of importance if no, or little, sugar is added in the formula. In recent years, the trend has been to use fungal a-amylase rather than malt flour. The economic advantages are obvious. However, the thermal stabilities of the two types of α-amylase are quite different. This would appear to be important if the desired action of the amylase is on the starch gelatinized during baking. Recently, a trend toward using bacterial a-amylase has developed. It appears to have advantages in keeping the bread from firming during storage.

Bread produced in the United States that must remain soft for a number of days usually contains a surfactant. Most common compounds for this usage are α-monoglycerides; a common level of usage is 0.5 % based on the flour weight. Other

surf actants are used as dough strengtheners. These are helpful in allowing the dough to withstand the mechanical abuse of the bakery's processing lines. Examples of this type of surfactant are sodium stearoyl lactylate (SSL), ethoxylated monoglycerides (EMG), diacetyl tartaric acid esters of mono- and diglycerides (DATEM) and others. They also are used at about 0.5 % of the flour weight. The additive most commonly used to stop mold growth is calcium propionate [3].

1.3.2. Dough Formation

Mixing flour, water and various other ingredients to form dough starts wheat flour. Dough is more than just a flour-water system. When wheat flour and water are mixed in various proportions, they form everything from slurry when water is in large excess, to a dry but slightly cohesive powder when flour is in large excess. At intermediate levels, they are more likely to produce a sticky mess. When such a system is continuously stirred or mixed, remarkable changes occur. The system appears to become less wet and sticky and dough that is cohesive and partially elastic is formed. As the dough is mixed for longer periods of time, it becomes more resistant to extension. The dough then is said to be "developed".

When water is added to the flour particles, the surfaces of the particles rapidly hydrate, because water is in large excess compared to the surface area of the particles. Because of the large excess of water, the system is quite fluid and not much resistance to extension occurs [3].

1.3.3. Mixing

Mixing, however, provides an additional mechanism. As the hydrated particles are rubbed against each other, the mixer bowl, or the mixer blades, the hydrated surface is removed, exposing a new layer of particles to the excess water in the system. As this is repeated many times, the flour particles slowly become completely worn away or hydrated. As more and more of the free water is used to hydrate the protein and starch, resistance of the system to extension is increased progressively.

Dough that has been mixed to a peak can be referred to by a number of terms, for example, mixed dough, a dough with minimum mobility, or an optimally mixed dough. All of these imply that an end point has been reached. They also imply that this is the point to which a dough should be mixed for producing a loaf of bread.

Development occurs as the dough is mixed to obtain optimum hydration. One

way of examining this is to take advantage of the dough's tendency to relax. If we mix dough to optimum, then allow time to pass after mixing, the dough relaxes. When we start to mix it again, the dough does not immediately give a height equal to the peak height that occurred during the initial mixing. Some mixing is necessary to restore the resistance to extension to its original value. It does, however, reach that point with additional mixing. The mixing required could be viewed as the energy necessary to develop the dough. So, then development is a reversible process. We can develop dough, allow it to relax, and then develop it again. This implies that the bonding involved is not covalent but probably hydrogen or hydrophobic bonding or both.

Another important aspect of dough mixing is the incorporation of air. As dough becomes cohesive, it starts to incorporate air and thus decreases in density. At the point of optimum mixing, about half the total amount of air that is possible to incorporate has been incorporated. This air, particularly nitrogen, is important in most baked products because it produces the cells to which the CO_2 diffuses [3]. As carbon dioxide diffuses from the yeast cells into the aqueous phase of the sponge or dissolves in liquid ferments, it forms carbonic acid, which, although weakly ionizable, alters the acidity of the medium. The pH changes are affected not only by the amounts of generated carbon dioxide but also by the buffering capacities of the sponge-dough or liquid ferment systems. Excess carbon dioxide then collects as gas bubbles around loci in the aqueous phase. These loci originate from small air bubbles incorporated during mixing [6] or perhaps from air absorbed by the flour; about one fifth of the volume of flour is air entrapped among the flour particles [7]. The significance of air bubbles was also demonstrated by mixing dough in oxygen. The oxygen formed bubbles in loci; these bubbles were metabolized by yeast during fermentation, and the resulting structure of the breadcrumb lacked the normal porosity [8]. Yeast cannot produce new gas cells. Because of this fact, if there were no air cells, the grain would be very coarse with only a few large cells. Stated in different terms, the nitrogen gas trapped during mixing provides the nuclei for subsequent gas expansion, or leavening of the dough.

Some dough are mixed under vacuum. At first glance, this would appear to be detrimental to their quality. However, the vacuum is not complete. High vacuum cannot exist because dough contains water. Actually, the partial vacuum is beneficial because the small bubbles created by the mixing expand under the reduced pressure and thus can be subdivided into more bubbles as the mixing proceeds. The more bubbles created, the finer the grain of the baked product will be [3].

1.3.3.1. Mixing Time

The mixing time of various wheat flours is under genetic control and like any other character, can be selected for by breeders. The protein content of the flour also can affect mixing time. It has been shown that low-protein flours require longer mixing times simply because they contain less protein. Protein content above 12 % does not affect mixing time. Another factor affecting mixing time is the environment under which the wheat was grown. The control of mixing time appears to be associated with the glutenin protein fraction of wheat flour.

Certain chemical agents, particularly reducing agents such as cysteine, sodium bisulfite and related compounds, are quite effective in shortening mixing time. These reagents apparently work by breaking disulfide bonds in the glutenin proteins, thus making the proteins smaller. These smaller proteins hydrate more easily and thus lead to shorter mixing times. The pH of the dough also affects mixing time, with lower pH giving a shorter mixing time and higher pH (up to pH 10) giving longer times. This can be explained as an effect upon the charge of the proteins. The effect of low pH can be overcome with salt [3].

1.3.3.2. Overmixing

After reaching the optimum development, continued mixing produces wet, sticky dough with an "overmixed" sheen. This is generally spoken of as the dough being broken down. One explanation of overmixing is that shear thinning occurs. If we continue to mix the long protein molecules, they line up in the direction of flow and thus offer less resistance to mixing. Although this is a good explanation, it apparently is not correct because doughs do not overmix in a nitrogen atmosphere or if the water-soluble fraction is removed. There is no reason to believe that either of these two factors would influence shear thinning.

The fact that doughs mixed in a nitrogen atmosphere do not overmix implies that overmixing is an oxidation process. The fact the doughs do not overmix if the water solubles are removed implies that something in the water-soluble fraction is involved in the process. The clue to what was important in the water-soluble fraction came from the finding that fumaric acid and related compounds reduced mixing time and also greatly increased the rate of dough breakdown. Certain α-or β-unsaturated carbonyl compounds, such as fumaric acid, maleic acid, or ferulic acid have effects on overmixing similar to those of the sulfhydryl (SH-blocking reagent N-ethylmaleimide

(NEMI). However, NEMI and cysteine interact during dough mixing, whereas fumaric acid and cysteine do not. Thus, the effect of the activated double-bond compounds cannot be explained by the SH-blocking.Surprisingly, the effect of the activated double-bond compounds on dough breakdown during overmixing can be reversed by lipoxygenase or by free radical scavengers. This fact led to the hypothesis that the activated double-bond compounds have their effect by reacting with free radicals created in the gluten protein during dough mixing.

Interestingly, fast-acting oxidants, such as potassium iodate and azodicarbonamide, induce a rapid dough breakdown that appears very similar to that produced by activated double-bond compounds. One explanation of the effect of fast-acting oxidants is that they oxidize SH groups in the flour to disulfides. Because of the resulting lack of SH groups, no thiol-disulfide interchange reactions occur between protein molecules. These theoretically could reduce the stress on large glutenin molecules as they are sheared. Thus, the gluten proteins become more stressed, which results in more disulfide bonds being broken, forming more thioyl radicals that could react with the activated double bonds. Therefore, breakdown occurs more rapidly even though the number of activated double bonds remains the same. An alternate and perhaps better explanation is that the strong oxidants oxidize something in the flour to produce additional activated double-bond compounds [3].

1.3.4. Fermentation

There are distinctions between straight and sponge fermentation. Straight fermentation is quite popular as it is the simplest and best suited for processing soft, weak, low-protein flours. Its simplicity makes it attractive to use in processing doughs that are made from relatively strong flours. In a straight fermentation all ingredients-flour, liquid, yeast, malt, salt, sugar and other optional ingredients-are mixed in one process. Generally, a suspension of yeast, malt, sugar, and salt solutions are added to the sieved flour and mixed by hand or in a mechanical mixer. The dough is mixed to optimum consistency and allowed to rest for about 2-2.5 hr. The fermented dough is punched 3 times at about 50-mm intervals and scaled. Leavening of the dough depends on the amount of yeast; regular doughs contain about 30-40 gm of yeast per liter of water or 1.5-2 % on flour basis. Too little yeast causes dragging of fermentation, sticky dough, and poor crumb grain and texture. Too much yeast results in an overly porous dough and bread that stales rapidly.

There are several modifications of sponge. The classical sponge method; bread baked from doughs containing brewer's yeast had little "ovenspring". If, however, a sponge is used, fermentation proceeds satisfactorily and the indigenous dough yeasts which resemble bakers' yeast, can multiply and act in the oven. Though bakers' yeast is available today, the sponge method is still useful. The sponge gives better results with strong flours that are modified and mellowed in the sponge stage. Many bakers feel that the sponge method yields better and more uniform bread than the straight method. Saving in yeast from the two stage fermentation method is apparently small [1].

1.3.4.1. Gas Production and Retention

The process of anaerobic fermentation of carbohydrates by yeast to the end products, ethanol and carbon dioxide:

$$C_6H_{12}O_6 + \text{yeast} \rightarrow 2\ C_2H_5OH + 2\ CO_2$$

Glucose Ethanol Carbon dioxide

Sucrose or glucose is utilized at a rate of 0.77-3.0 g of sugar per hour per gram of yeast solids. In a 3-hr fermentation and 55-min proof, with compressed yeast (29 % solids) added at 2 % flour weight, which calculates to be 1.75-6.82 g of sugar. Estimates of the sugar consumed, based on the fermentation products and the volume of CO_2 necessary for dough leavening, have been reported as approximately 3.5 % sugar (based on the flour weight) utilized during the 4-hr fermentation and proof. This value, however, does not consider loss of CO_2 to the atmosphere. Other estimates have been made that during fermentation of straight dough, approximately 2.0 % sugar (based on flour weight) is consumed from mixing to the end of proof. That value accounts only for the disappearance of sucrose and does not include fermentable carbohydrates naturally present in the flour. Sucrose depletion by yeast in a synthetic fermentation broth during 3 hr is about 4 %. In straight dough, the yeast is in a stressed environment and would not be expected to maximize substrate utilization.

Carbon dioxide is retained in the bread dough in two phases:

1. As a gas contained within the gas cells
2. Dissolved in the aqueous phase.

Carbon dioxide solubility in water is inversely related to the temperature and affected by pH. At the pH of bread doughs, most of the carbon dioxide is present as CO_2 and little of it is as H_2CO_3, HCO_3^-, or CO_3^{2-}.

The amount of carbon dioxide present in fully proofed dough is only about 45 % of the total produced by fermentation. The balance is lost during fermentation, punching, molding, and proofing. The expansion of CO_2 in the air cells and the CO_2 that comes out of the aqueous phase are insufficient to completely account for the increased volume of dough in the oven. Vaporization of the water-ethanol azeotrope during heating in the oven contributes greatly to the overall expansion of the dough [3].

1.3.4.2. Gas Retention

In a mixed bread dough, an insoluble but highly hydrated gluten protein system constitutes the continuous phase, with starch and air bubbles as the discontinuous phases. Also dispersed throughout the aqueous system are yeast cells, which ferment sugar and produce, among other things, carbon dioxide. The CO_2 is produced in the aqueous phase and saturates the water. Once the water is saturated, newly produced CO_2 must find a place to go. As discussed above, it cannot form new bubbles; thus, the preexisting air bubbles appear to be a logical choice. The CO_2 enters the bubble and increases the pressure. Dough has viscous-flow properties and therefore allows the bubble to expand to equalize the pressure. The total volume of the dough mass is increased or, in other words, the dough is leavened. Carbon dioxide is retained by the gluten proteins, which form a sheet or membrane [3].

1.3.4.3. Role of Pentosans

Wheat flour contains gums, the water-soluble and water-insoluble pentosans. The water-soluble fraction of wheat flour has been shown to be important in producing an optimum loaf volume. The mechanism of its action is not clear but may be just an increase in the viscosity of the aqueous phase. Rye flour is second only to wheat flour in its ability to retain gas and produce a light loaf of bread. Rye flour contains a much greater amount of pentosans than does wheat flour. Recent studies have shown that the pentosans are the most important fraction in rye flour baking quality [3].

1.3.4.4. Role of Lipids

If flour is defatted in such a way as not to damage the gluten, the flour still produces a reasonable loaf of bread. However, if the lipids are added back to the flour, the bread volume is larger. Research has shown that the polar lipids, and particularly the glycolipids, are most important in this phenomenon [3].

1.3.4.5. Role of Gluten

The total amount of gluten protein in flour is important, greater protein content gives greater loaf volume. Recent work has suggested that the gluten protein can be extended only to a certain level before reaching its elastic limit. As the loaf expands during baking, that elastic limit and the amount of protein in the dough together determine the size of the loaf [3].

1.3.5. Baking

The baking stage is essential to the conclusion of the breadmaking process. The extent to which fundamental qualities determined by ingredients, biochemical reactions, and processing is built into the dough is finally revealed in the oven. Proofed loaves going into the oven have a skin, formed during proofing; the skin soon thickens and becomes elastic in the oven, depending to some extent on the moisture content of the oven atmosphere-high humidity improves crust formation [9].

The water content of the baked products is below 5 % in freshly baked rye crisp and over 40 % in large, dark bread. The water content in the whole loaf increases with the size of the baked product and darkness (extraction) of the flour used, and decreases with increase in crust: crumb ratio. The water content of the crust in a freshly baked bread is practically nil, but after some time moisture increases by absorption from the surroundings and by migration of water from crumb to crust [1].

During the first few minutes in the oven, the dough expands in size rapidly. This is called the oven-spring. Several factors are responsible for the oven-spring. Gases heat and increase in volume; carbon dioxide becomes less soluble as the temperature is raised; yeast becomes quite active as the temperature is raised; and other materials are vaporized. [3]

During baking several changes take place in both the crumb and crust. The so-called browning reaction that involves both caramelization of sugars and interaction between sugars and proteinaceous materials miparts a deep color to the crust; termal

decomposition of starch and formation of dextrins contribute to crust luster [1]. Watching a loaf bake in an oven with a glass door is an interesting experience that is highly recommended if one is interested in the process. Browning occurs very late in the system because it takes place much faster in a dehydrated system. The browning on the bread surface results from a Malliard-type reaction. Sucrose is a nonreducing sugar, but yeast contains invertase, which rapidly produces glucose and fructose, both of which are reducing and brown quite readily. Caramelizatjon is occasionally mentioned as a mechanism of browning in bread; however, bread produced by chemical leavening does not brown to any extent. Thus, the role of caramelization in yeast-leavened products appears to be minor [3].

Other important changes occurring in the oven include physicochemical reactions of flour proteins, which undergo denaturation, and thermal changes of starch, generally designated gelatinization and swelling. Both reactions, conformational reorientation in the structure of starch granules and proteins, cause important moisture interchange among dough components. Starch, present originally in the form of semicrystalline granules, changes its order by undergoing gelatinization and swelling. There is no general agreement among starch chemists on the definition of gelatinization and swelling. The degree of both changes is dependent on the amount of available water and levels of sugars and salts in the system. In this discussion, gelatinization of wheat starch refers to changes of order that occur within the temperature range of 55-63 °C. Their initiation is detectable by microscopy as a loss of birefringence of starch granules in polarized light. Swelling, which can also be observed, starts at the same time as gelatinization and proceeds further throughout the baking process. The water in bread doughs limits the degree of swelling, but it is not sufficiently low to alter the gelatinization temperature range [10, 11, 12]. During both processes, amylose is leached from the granules. This linear starch component undergoes a rapid retrogradation upon the cooling of baked bread and contributes to the initial crumb firmness of freshly baked bread. Starch granules in their native state are resistant to the action of amylases. When heated, they become susceptible to amylases and start absorbing water after reaching the gelatinization temperature. Since the cereal amylases are still active at that point, an excessive degradation of starch may occur, resulting in adverse properties of baked bread: sticky crumb, low loaf volume, and open crumb grain. This condition may occur when the flour has been supplemented with excessive amounts of barley malt at the mill or when it has been

milled from sprouted wheat. This damage is attributed to the excessive level of α-amylase, but other enzymes may also contribute to the deleterious effects [13, 14].

Wheat protein denaturation starts at about 70 °C and is of major importance in establishing bread structure. Another functional feature of wheat protein is its hydration during dough formation and the transfer of water from gluten to the starch component during baking, to support the swelling of starch granules. The denaturation of gluten is accompanied by decreased solubility and proceeds to a point where the gas vesicle walls are fixed and expansion is terminated. Denaturation is more extensive in the crust regions than in the crumb; the temperature rise is much faster in the crust, and much higher temperatures are reached there. Crumb temperatures do not exceed 1000 °C, whereas crust temperatures reach 1950 °C [15].

1.4. Sensory Attributes of Bread

Sensory attributes are most important properties for many food products.The selection, acceptance, and digestibility of a food are governed by its sensory attributes. Important sensory attributes of bread include: appearance , texture, and flavor.

Gloss and color of the crust of a loaf of bread, important appearance factors, depend on dough composition and breadmaking procedure. Crumb color, crumb texture, and crumb softness are among criteria most used by the consumer to evaluate bread's acceptability. Undesirable changes that take place in bread after baking have increased in significance under modern, large scale methods of bread production and distribution [1].

Bread loses its desirability progressively with the time it is out of the oven. Those undesirable changes that occur with time are collectively called "staling." They include toughening of the crust, firming of the crumb, a loss of flavor, an increase in the opaqueness of the crumb, and a decrease in soluble starch [3].

1.5. Wheat Germ

Wheat grain consists of the endosperm, the bran, and the germ, which account for 81 to 84 %, 14 to 16 %, and 2 to 3 % of the grain, respectively [16,17]. The germ is composed of two major parts, the embryonic axis and the scutellum, which functions as a storage organ. The germ is relatively high in protein (25 %), sugar (18 %), oil (16 % of the embryonic axis and 32 % of the scutellum are oil), and

ash (5 %). It contains no starch but is rather high in B vitamins and contains many enzymes. The germ is quite high in vitamin E (total tocopherol), with values ranging up to 500 ppm. The sugars are mainly sucrose and raffinose [3].

1.5.1. Chemical Composition of the Germ

The exhaustive studies reported on the chemical composition of the germ cover mainly commercial mill germ that is admixed with impurities like bran and endosperm. Separation of dissected germ of high purity is feasible only on a laboratory scale. Information on its composition is scanty and can be considered to be only of academic interest. Dissected germ naturally contains comparatively larger amounts of proteins, sugars, lipids, thiamine, and tocopherols, and lesser amounts of carbohydrates and phytic phosphorus, compared with mill germ [17].

Compared with wheat flour, the germ is unique in that it provides three times as much protein, seven times as much fat, and about fifteen times as much sugar. In addition, the high sugar content makes it one of the most acceptable foods in its natural form. It is interesting to note that, unlike flour protein, wheat germ lacks in gluten and is rich in salt-soluble proteins. In spite of the high nonprotein nitrogen content of about 15 %, the conversion factor used by most of the workers for determining protein remains 6.25. Among vegetable proteins, mill germ has probably the best essential amino acid make-up, which compares well with that of egg protein. It is a rich source of lysine, unlike other cereal proteins. Mill germ has also proved to be an excellent source of B-group vitamins and tocopherols, which enhances the value of the germ as a food supplement. The similarity between the physical properties of germ oil and edible vegetable oils makes it suitable for food uses. Fatty acids of germ lipids are predominantly unsaturated, and over 70 % of these are essential ones. However, the high lipase and lipoxidase activities coupled with unsaturated fat pose problems regarding an adequate shelf life of the germ [17].

1.5.2. Germ Separation

Wheat germ is a by-product of the roller flour milling industry. Its separation from other milled products is important for the following reasons:

1. It adversely affects the keeping quality of flour and other mill feeds. 23 to 34 % of the oil in flour originated from the germ, owing to expression of the oil during rolling [18]. The presence of highly unsaturated germ oil in

flour decreases its storage life because of oxidative rancidity [19].

2. The presence of the germ in flour was reported to affect the baking quality and color of the flour [20].

Wheat germ is a rich source of protein of high nutritive value, B-group vitamins, and tocopherols, and a potential nutritious food supplement. It thus has great commercial value, fetching more than double the price of other mill feeds, and can be used with advantage in the preparation of speciality breads and also as a vitamin E concentrate.

Even though wheat contains 2 to 3.5 % of germ, only about 1 % is recovered during normal milling operations for the following reasons:

1. Most of the scutellum portion goes into the flour stream, as it is more friable, like the endosperm. The embryonic axis gets flattened between the rollers and can be recovered by proper sieving techniques.
2. Breakage of some of the germ portions into small pieces occurs in between the rollers. The broken portions escape into the floury portion and are not recovered.
3. Also, some of the germ portion is lost during screen-room operations such as handling, scouring, washing, and brushing.

There are two ways by which the germ can be separated in the mill. One method is to separate it in the break system itself, in the form of whole germ, by using a "germ separator" as ancillary equipment. In the other method the middling containing the germ particles are passed through the reduction rolls, where the germ gets flattened and is removed by sieving [17].

1.5.3. Nutrition Value of the Germ

In human nutrition, cereals, including wheat, have been reported to be poor sources of quality protein. Wheat germ, however, is unique in containing high proportions of protein, edible oil, sugars, certain B-group vitamins, and tocopherols. The biological value of wheat germ proteins has been reported to equal that of highly rated animal proteins. For the nutritional evaluation of different foods, several workers have reported different methods [21], including chemical methods, rat and chick growth methods, and supplementary value studies. They have also studied the toxic or inhibitory factors present and the effect of processing on the nutritive value of wheat germ [17].

Generally, protein-rich foods are used as supplements to improve the nutritive value of different staple diets based on cereals and millets. Several workers have studied the supplementary value of wheat germ to wheat flour, gluten, barley, corn, rice, etc [17].

1.5.4. Toxic Factors in the Germ

If the germ diet was continued for long, the coat color returned to normal. They inferred that wheat germ contained a toxic element that was neutralized by feeding the whole grain. They also assumed the development of immunity to the toxicity after long-term feeding with the germ. The germ did not make a complete food for rats, as the sexual functions did not develop adequately when only the germ was fed.

Wheat germ proteins have a very well balanced amino acid make-up. The amino acid composition is quite similar to that of egg proteins as indicated by its chemical score. When cereals in general are deficient in lysine, wheat germ is a rich source of lysine. The only limiting amino acid reported was isoleucine.

Nutritionally, wheat germ is much superior to other milled products of wheat. Wheat germ has excellent supplementary value when added to other cereal proteins and compare favorably in this respect with nonfat dry milk solids. Mild heat processing or light toasting of germ improves its nutritive value, probably owing to an improvement in the digestibility of the proteins or to destruction of antinutritional factors like hemagglutinin or trypsin inhibitors, whose presence in the germ is well established. Severe heat processing lowers the nutritive value considerably, depending on the extent of the heat treatment [17].

1.5.5. Storage of the Germ

Only a few researchers have systematically studied the shelf life of differently packed wheat germ as influenced by various conditions such as moisture and temperature. The spoilage of the germ during storage has been attributed to different enzymes. Various methods have been suggested to evaluate the freshness or spoilage of stored germ [17].

The shelf life of whole wheat germ packed in vacuum or with inert gases like nitrogen or carbon dioxide at various temperatures. Alcoholic acidity was used as a measure of the freshness or the spoilage of the germ during storage. Even when

samples were stored under the same condition, the acidity changes of vacuum-packed germ were dependent on the purity of the germ. Considering acidity changes as well as organoleptic quality, vacuum-packed germ was found to be better than that packed under inert atmosphere. Packing under nitrogen or pelleting of the germ was desirable for a longer shelf life.

The increase in germ acidity was a function of temperature. Acidity increased eight times as fast at 29 °C as at -10 °C. In the majority of cases, no unpleasant flavor was observed until the acidity exceeded 0.25 %. The germ packed either under vacuum or under nitrogen kept well for over 338 days at -10 °C and only for 90 days at 29 °C [17].

Stability of the germ at different moisture levels ranging from 8.0 to 26.5 % and observed an increased shelf life for the germ when it was stored at a low moisture level. The germ developed an undesirable odor within 6 weeks even when it was stored at a moisture level of 3 % in a closed glass jar. The development of acidity in the various mill fractions was directly proportional to their lipid contents. After a one-year storage period, the acidity of the germ containing 12.9 % lipids was about 2.0 %, whereas that of patent flour with 1.3 % lipids was as low as 0.03 % [22]. Germ samples stored at a higher moisture content showed a marked decrease in the alcohol-ether extractives, and an increase in the ether or acetone extracts. This was due to the solubility differences of lecithin and its split products-that is, fatty acids, choline, and glycerophosphoric acid. The nitrogen and phosphorus contents also decreased in all the extractives. Storage at low moisture levels showed relatively slight changes in germ lipids. The changes observed were explained as being due to enzyme hydrolyses and were correlated with increasing moisture and acidity of the samples.

Rancidity and peroxide values in germ oil increased concurrently during storage, whereas in whole germ the rancidity was detectable even when the peroxide values were relatively low [17].

1.5.6. Uses of the Germ in Food Industry

Until recently, wheat germ was almost wholly disposed of as an animal feed. However, after it was realized that wheat germ contains a significant quantity of proteins of superior nutritive value and vitamins, several workers investigated various aspects of its utilization as a human food. The unique characteristics of

palatability and high nutritive value place wheat germ on a par with other foods based on animal proteins. Source of energy and nutrients as the germ should not go unutilized for human consumption. The addition of the germ to wheat flour would considerably improve its nutritive value. Durum semolina had a significantly lesser nutritive value, as a result of loss of the germ during milling and purification. The nutritive value of milled products for human consumption would certainly increase if the germ could be retained during the milling operation.

The poor stability of the germ had restricted its wide usage as a human food, however. But today, various methods are available for stabilization of the germ to prevent its deterioration during storage [17].

1.5.6.1. Germ Oil

Wheat germ is a good source of edible salad oil that is classified as semidrying oil. In view of the shortage and high cost of edible oils in many developing regions, the extraction of oil from wheat germ will go a long way in augmenting the supplies of edible oils. In addition to its use in the preparation of food products and vitamin concentrates, germ oil is also used in cosmetics. Wheat germ oil has the potential for use in the preparation of margarine [23].

1.5.6.2. Fermented Foods

Wheat germ can be used in the preparation of miso and koji. Replacing portions of rice and soybean with germ prepared germ miso. The chemical characteristics as well as the organoleptic qualities of germ miso were similar to those of miso prepared from soybeans. Koji was prepared from wheat germ by inoculating with Aspergillus oryzae [17].

1.5.6.3. Vitamin Concentrates

Different methods have been described for separating vitamin E or vitamin B complex concentrates from the germ. The presence of high amount of vitamins in the germ, particularly vitamin E, thiamine, riboflavin, and pyridoxine, makes it a very suitable raw material for the enrichment or preparation of vitamin concentrates. The multiple uses of wheat germ suggested [17] included the preparation of

- Pyridoxine concentrate
- Concentrate of the B-group vitamins

- Thiamine concentrate from the water-soluble fractions

and the preparation of

- Vitamin E concentrate
- Sitosterol concentrate
- Food fat
- Raw fat from the fat-soluble fraction.

1.5.6.4. Animal Feeds

The by-products of the roller flour milling industry in general form very useful and popular constituents of animal feeds. Wheat germ is mostly used as a feed for pigs, poultry, and cattle, since it is rich in protein and thiamine. When compared with wheat and rice bran, wheat germ was a superior cattle feed in increasing the milk yield. The butter of these cows had a better storage life, possibly owing to the antioxidant effect of tocopherols.

The mixture of wheat germ, rice bran, and rice hulls was extracted with acidulated water containing alcohol and chloroform. The extract was neutralized with chalk and filtered. The filtrate was then concentrated to a syrupy consistency [17].

1.5.6.5. Bakery and Pastry Products

Biscuits and Cakes: Biscuits and cakes made from self-rising flour containing toasted germ were highly acceptable and were comparable to products containing no germ. The incorporation of 12 to 15 % of toasted germ in the self-rising flour was found to be optimum. The recipe proposed for self-rising flour was: patent flour, 850 gr; toasted germ, 125 to 150 gr; baking soda, 15 gr; sodium acid pyrophosphate, 16.8 gr; calcium acid phosphate, 8.1 gr; common salt, 16 to 18gr; and dextrose, 15 gr. The keeping quality of this mix was found to be excellent, as no off-flavor developed during storage at 120 to 130 °F for 25 days in a closed jar. Up to 15 % of toasted germ could be used in biscuits without any adverse effect on its quality. An improvement in the quality of biscuits when only 0.25 % of stabilized and lyophilized, wheat germ was included in the biscuit recipe [24]. Deterioration of sponge cake can be prevented by incorporation of wheat germ [25].

Pastry Products: Wheat germ has also been used as one of the optional ingredients for the enrichment of noodles or macaroni products. In addition to

improving the nutritive value, the addition of wheat germ to the pastry products was reported to reduce the cost of the product.

Among bakery products, germ bread has been studied extensively. Information on other products is scanty [17].

Germ Bread: Germ is not an intentional component of white wheat flour, though some germ remains in flour. The amount of germ in a flour increases with increased flour-extraction rate. Because even the most refined flour contains some germ, the effects of germ in breadmaking interest cereal chemists. Furthermore, the high protein content and excellent amino acid balance make it attractive to supplement wheat flour with germ. About 0.5 % of fairly pure germ can be isolated during conventional flour milling. Supplementing cereals with 10-15 % defatted wheat germ strikingly improves nutritive value of cereals used as feedstuffs. Meaningful improvement of nutritional value of white bread requires about a 10 % germ addition.

The germ, including scutellum, comprises 1.5-3.3 % of the wheat kernel. Purified germ contains about 27 % protein, 9 % fat, 46 % carbohydrates, 2 % crude fiber, and 4 % minerals. The minerals include 5 mg sodium, 837 mg potassium, 69 mg calcium, 8 mg iron, and 1100 mg phosphorus. Among the vitamins are 0.2 mg carotene, 27.5 mg vitamin E, 2.0 mg thiamine, 0.7 mg riboflavin, 4.5 mg nicotinamide, 1 .0 mg pantothenic acid, 3.0 mg vitamin B_6, and 0.5 mg folic acid; but vitamin C is absent. The embryo and the scutellum, which comprise only 1.2 and 1.5 % of the kernel weight, contain respectively 3 and 59 % of the kernel's thiamine. The germ is the main source of tocopherol in the wheat kernel and wheat germ contains more tocopherol than found in other cereal grains. Germ fat is rich in polyunsaturated fatty acids. Wheat germ proteins are rich in essential amino acids. The proteins are particularly rich in lysine, arginine, aspartic acid, threonine, alanine, and valine (Table 1.1.). The concentration of glutamic acid and proline in the germ proteins is much lowers an in protein of wheat flour or wheat gluten. The amounts of sulfur-containg amino acids in proteins of wheat germ and wheat flour are comparable.

Table 1.1. Amino acid composition of wheat flour and whole germ

Amino acid	Flour	Whole Germ
Lysine	1.78	7.76
Histidine	1.82	2.65
Ammonia	3.01	1.71
Arginine	3.23	8.86
Aspartic acid	3.81	10.21
Threonine	2.31	4.82
Serine	4.43	4.62
Glutamic acid	37.19	15.45
Proline	11.55	4.37
Glycine	3.37	6.54
Alanine	2.87	7.00
Cystine	1.44	0.66
Valine	3.99	5.65
Methionine	1.45	1.88
Isoleucine	3.80	3.91
Leucine	6.64	6.79
Tyrosine	2.15	3.12
Phenlylalanine	5.16	4.07

Freshly prepared germ becomes rancid in a few days at room temperature. Action of wheat lipases that results in forming oxidizable free fatty acids is most important in deterioration of germ in storage. Shelf life can be increased by; storing at low temperatures (around 4^0C). Most commonly, the germ is stabilized by wet or dry heat treatment to inactivate enzymes responsible for rancidity. Drying at low temperatures to 5 % moisture and storing the dried product in waterproof containers

can obtain similar results [1].

High levels of raw germ deleteriously affect breadmaking. Long before the deleterious components were identified, several methods were proposed to counteract their undesirable effects. Heat treatment, increasing fermentation length, and increasing oxidant levels, all decrease the harmful effect. The deterious effects of glutathione in germ were demonstrated; recommended eliminating the deleterious effects by heating wheat germ at elevated temperatures to oxidize the glutathione thiol groups [26, 27]. Increasing fermentation time, oxidant level, or combination of both decreased harmful effects of 5-10 % germ [28].

While most investigators studied deleterious effects of germ in breadmaking and methods of counteracting, or minimizing, undesirable changes, at least two reports deal with the beneficial effects of germ or germ components in breadmaking. [1] Adding small amounts of steeped germ and potassium bromate improves bread [29]. In yeast-fermented germ two benzoquinone derivatives that act as improvers. [30]

Recently, extensive studies were conducted on use of wheat germ and protein concentrates from germ in breadmaking. The proteins in wheat germ are mainly of the globulin type and over 86 % can be extracted with dilute (3 %) salt solutions. The extract, from which no protein components can be removed by dialysis, contains, after lyophilization, over 94 % protein. The proteins in the concentrate are comparable in amino acid composition to proteins of the whole germ. The extracted proteins can be added at high levels (about 6 %) with little damage to breadmaking. However, the high cost of extracting, dialyzing, and lyophilising the extract limits the usefulness for large-scale bread supplementation. Nutritive value of bread also can be improved, without impairing breadmaking quality, by adding 10-30 % heat-treated wheat germ in combination with phospholipids. Loaf volume was highest if the phospholipid to germ ratio was from 1:10 to 1.5:10 [1].

Formulation of Germ Bread: A process for the preparation of bread containing 10 % of germ has been described [31]. Commercial germ samples from soft red winter or durum wheats were found to be better than samples from hard spring varieties [32]. When flour is to be used within a short time after milling, the germ could be retained in the flour. If the flour is to be stored for longer duration, it is desirable to add freshly extracted germ, just before using the flour to make bread [17].

Nutritive Value of Germ Bread: Bread containing the germ had better odor, flavor, digestibility, and biological value. Bread containing 2 to 5 % of wheat germ was nutritionally superior to that made from the same flour without any germ. When fasting rats were fed, the gain in weight was 5 % greater with germ bread than with the same quantity of control bread [17].

Bread containing wheat germ from which the fat was extracted was definitely better than bread from the same wheat flour enriched with gluten. The gain in weight, the consumption index, the protein efficiency ratio, the ratio of total nitrogen to creatine nitrogen, the amount of xanthine oxidase of the liver, and the formation of new liver proteins were significantly higher in rats fed on germ bread. Wheat germ bread represents special bread enriched with important vitamins. Especially vitamins A and E and the B-group vitamins are present in wheat germ in a well-balanced form, and also beneficial effects [17].

In bread made from first grade flour enriched with 5 % wheat germ the amounts of protein, lysine and essential amino acids were higher by 4, 17, and 6 %, respectively, as compared to the control. In addition, the enriched bread had better taste with pronounced flavor. Use of 1.5 % freeze-dried germ has been reported to bring about considerable improvement in flavor as well as yield of bread [24].

Speciality Breads: For a bread to be labeled germ bread, it must contain at least 10 % (calculated on a dry-weight basis) of processed wheat germ [33]. At least 8 to 10 % of germ was required to effectively improve the nutritional value of bread. The best-known types of commercially produced germ bread are Hovis, Daren, Vitbe, and Turog. These breads are prepared by using different germ meal formulations, and they contain the germ cooked with known amounts of salt, soy flour, and wheat flour. Only Turog bread is quite different. It contains smaller percentages of the germ together with caramel, which imparts a characteristic color to the bread. [17]

1.6. The Aim of this Thesis.

The aim of this thesis was to study the enrichment of bread with wheat germ. Different percentages of wheat germ were added to determine the effect of germ on bread quality. The effect of ascorbic acid on germ bread was also studied.

In fact, germ is not an intentional component of white wheat flour since some germ remains in flour after milling. Germ is a rich source of polyunsaturated fatty

acids and proteins. Wheat germ proteins are rich in essential amino acids. Therefore nutritive value of bread can be improved by adding wheat germ to the wheat flour [1, 24]. However, high levels of raw germ deleteriously affect breadmaking. In order to prevent such undesirable changes in bread quality several improvers were used [26, 30]. The ascorbic acid as an oxidant was recognized in this study, as an agent that might eliminate or minimize the changes due to the addition of wheat germ.

CHAPTER II

MATERIALS AND METHODS

2.1. Materials

2.1.1. Wheat Flour

Wheat flour used was kindly provided from Fedaioğlu Gıda San. & Tic. Ltd. Şti. (Gaziantep). Ash content of wheat flour was 0.550 % (w/w, dry basis) and was suitable for breadmaking.

2.1.2. Salt

Salt was purchased from a local market in Gaziantep (Billurtuz).

2.1.3. Water

Tap water was used.

2.1.4. Yeast

Active fresh yeast was used which was provided from local market (Pakmaya). It was 42 gr and has 45 days shelf life. Yeast was not used after 30 days.

2.1.5. Wheat Germ

Commercial wheat germ was kindly provided from Fedaioğlu Gıda San. & Tic. Ltd. Şti. (Gaziantep).

2.1.6. Ascorbic Acid

Ascorbic acid ($C_6 H_{12} O_6$) used was 150 mesh in particle size and 99 % pure in crystal form and obtained from Yılmaz Kimya (İstanbul).

2.1.7. Breadmaking

A laboratory scale bread machine was used (Belmo, Model BL – 100)

2.2. Methods

2.2.1. Bread Formulation.

Flour, salt, yeast and water were used for normal bread formulation. On the other hand, different percentages of wheat germ were added in place of flour for germ bread. Besides, 150 mg ascorbic acid/kg was used for each formulation in order to eliminate problems which come from germ. All of the bread formulation was shown in Table 2.1.

Table 2.1. Bread formulation used in this study

	% Wheat germ (w/w) based on wheat flour							
Bread ingredients	0	5	7.5	10	12.5	15	17.5	20
Flour (gr)	400	380	370	360	350	340	330	320
Wheat germ (gr)	0	20	30	40	50	60	70	80
Yeast (gr)	12	12	12	12	12	12	12	12
Water (ml)	250	250	250	250	250	250	250	250
Salt (gr)	6	6	6	6	6	6	6	6

2.2.2. Size Reduction of Wheat Germ

The particle size of raw wheat germ was reduced to <120 μm using hammer mill.

2.2.3. Breadmaking Procedure

All the ingredients were mixed and following procedure was followed to breadmaking (Figure 2.1.).

2.2.4. Measurement of Bread Height

Bread height was determined by using micrometer.

2.2.5. Measurement of Bread Volume

Bread was put into thin plastic bag and wrapped tightly. The air between bread and bag was vacuumed. The bread was immersed into the container that was full of water. The volume of bread was determined by the amount of overflowing water.

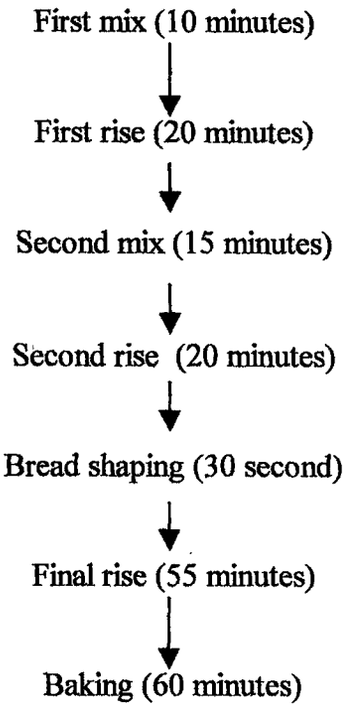

Figure 2.1. Bread machine- standard program [34]

2.2.6. Determination of Pore Factor.

Pore number was determined by dividing the bread into two pieces and by comparision of pore situation with Dallman pore scale. Dallman scale is numbered from 1 to 8 and a pore factor is given for each of the number. Pore factor was obtained from Table 2.2.

Table 2.2. The relationship between pore number and pore factor [35, 36].

Pore Number	Pore Factor
1	30
2	40
3	50
4	60
5	70
6	80
7	90
8	100

2.2.7. Determination of Bread Crumb Properties

Bread crumb properties are texture and elasticity of bread crumb and homogeneity of pores. Evaluations of texture and elasticity were obtained manually and evaluation of homogeneity was obtained visually. Bread crumb factors were determined from Table 2.3 [35, 36].

Table 2.3. Evaluation of bread crumb properties [35, 36]

Characteristic	Evaluation	Factor
Texture	Rough	0
	Quite rough	10
	Quite fine	15
	Fine	20
	Quite soft	30
	Silky soft	40
Homogeneity	Homogenous	5
	Quite homogenous	0
	Non homogenous	-5
Elasticity	Good	0
	Quite good	-5
	Acceptable	-10
	Imperfect	-75
	Sufficient	-100

2.2.8. Determination of Volume Factor.

Volume factor can be calculated with the following equations:

If bread volume (obtained from 100 gr flour) is under 400 ml

$$\text{Factor} = \text{Volume} - 300$$

If bread volume (obtained from 100 gr flour) is above 400 ml

$$\text{Factor} = [(\text{Volume} - 400)/2] + 100$$

Using these equations, volume factor was obtained with respect to bread volume (Table 2.4.).

2.2.9. Measurement of Bread Weight

Bread weight was determined with 1-gram sensitivity balance after 30 minutes later after baking.

Table 2.4. Bread volume – volume factor relationship [35, 36]

Bread Volume (ml)	Volume Factor
300	0
350	50
380	80
400	100
420	110
460	130
500	150
600	200
700	250

2.2.10. Determination of Bread Value Number.

Bread value number (B.V.N) was related with volume factor, pore factor and bread crumb properties. It can be determined with the following equation:

B.V.N = [(Volume Factor * Pore Factor)/100] + Bread crumb properties

2.2.11. Sensory Analysis

Bread was analysed by untrained five housewives according to colour, texture and flavour.

2.2.12. Statistical Analysis.

One-way ANOVA, Completely Randomised Block Design and Duncan's Multiple Range Test were carried out using CoStat statistical programme (Version 2.10, CA and U.S.A)

CHAPTER III

RESULTS & DISCUSSION

3.1. Wheat Germ Bread

Bread is the main food and provides more nutriment component than any other food. It supplies over 50 % of total caloric intake in 50 % of the countries [37, 38]. In Turkey, 66 % of total caloric intake per person is supplied from cereal; 56 % of consumption of cereal is supplied from only bread [37]. Enrichment of bread is attractive and necessary in the world because of consumption of bread. Enrichment of bread with thiamine, riboflavin, niacin and iron is a legal necessary in United States from 1971, addition of calcium and vitamin D is related to company request. Although enrichment of bread is legal necessary in developed countries, it is desirable in Turkey. Generally, enrichment process was used to prevent some negative effect from poor quality of ingredient [39].

Experts generally recommend enrichment of bread with B-complex group vitamins, Vitamin E, niacin, lysine and iron [40]. Wheat germ is enrichment material for bread. It is a good source for high protein content and excellent amino acid balance [41]. In addition, it is the richest source of vitamin E, also a rich source of thiamine, riboflavin and niacin and also other vitamins. Large amounts of fats and sugars make wheat germ highly palatable [17]. On the other hand, amino acid composition and amino acid balance of wheat germ also provides an advantage for usage in foods as improver. Especially proteins in the germ contain large amount of lysine, arginine, aspartic acid, threonine, glycine, alanine, and valine (Table 1.1.) [41, 17].

Wheat germ is very rich for vitamins and amino acids. Wheat germ addition in flour is very attractive [41]. Although the composition of wheat germ is rich, depending on the amount of wheat germ added to the bread, negative effects on characteristics of bread appears [42]. On the other hand, the use of wheat germ was limited because of its short shelf life. In this study, raw wheat germ was used to

prepare bread in the absence and presence of ascorbic acid. All of the experimental data with or without A.A was shown in Table 3.1 and Table 3.2.

Table 3.1. Experimental data for bread with raw wheat germ (Table 2.2, 2.3 and 2.4 for factor)

% Wheat germ	Texture factor	Homogeneity factor	Elasticity factor	Height (cm)	Volume (ml)	Pore factor	Weight (gr)
0	40	0	-5	11.6±0.2	1854±5	70	581±5
5	40	0	-5	9.9±0.1	1824±7	70	579±2
7.5	30	5	0	9.6±0.1	1778±3	70	572±4
10	30	5	-10	9.0±0	1723±2	70	572±4
12.5	30	5	-10	8.1±0.1	1492±2	70	570±3
15	30	-5	-10	7.5±0.1	1456±2	70	568±3
17.5	20	-5	-10	7.0±0.1	1429±3	70	568±3
20	20	-5	-10	6.6±0.1	1358±4	70	568±4

Table 3.2. Experimental data for bread with raw wheat germ + 150 mg A.A/kg (Table 2.2, 2.3 and 2.4 for factor)

% Wheat germ	Texture factor	Homogeneity factor	Elasticity factor	Height (cm)	Volume (ml)	Pore factor	Weight (gr)
0	40	0	-5	11.9±0.1	1873±3	70	581±4
5	40	0	-5	11.0±0	1839±5	70	580±3
7.5	40	0	-5	10.5±0	1827±2	70	580±4
10	30	0	-5	9.7±0.1	1818±5	70	579±2
12.5	30	5	-5	9.5±0.1	1691±4	70	575±0
15	30	5	-5	8.9±0.1	1628±3	70	572±3
17.5	20	-5	-10	8.0±0	1547±3	70	574±3
20	20	-5	-10	7.3±0.1	1388±5	70	570±5

3.2. Determination of Bread Volume

In this study, the bread was put into thin plastic bag. The air between bread and bag was vacuumed. So bread was wrapped tightly to plastic bag. Water was put into a basket. The bread prepared as above was immersed into the container that was full of water. The bread was protected from getting wet by the help of thin plastic bag wrapping it. After determining the amount of overflowing water from the basket, the volume of bread was determined. Standard deviation and average values were determined (Table 3.1, Table 3.2), by using Microsoft 2000-Excel software.

According to the Figure 3.1, by increasing the amount of raw wheat germ, the volume of bread decreased. In the same amounts of wheat germ, in the presence of ascorbic acid, an improvement was seen in the volumes of bread. The usage of ascorbic acid, for the bread containing 0 %, 5 % and 20 % raw wheat germ, a limited effect of A.A was observed on the volumes of bread.

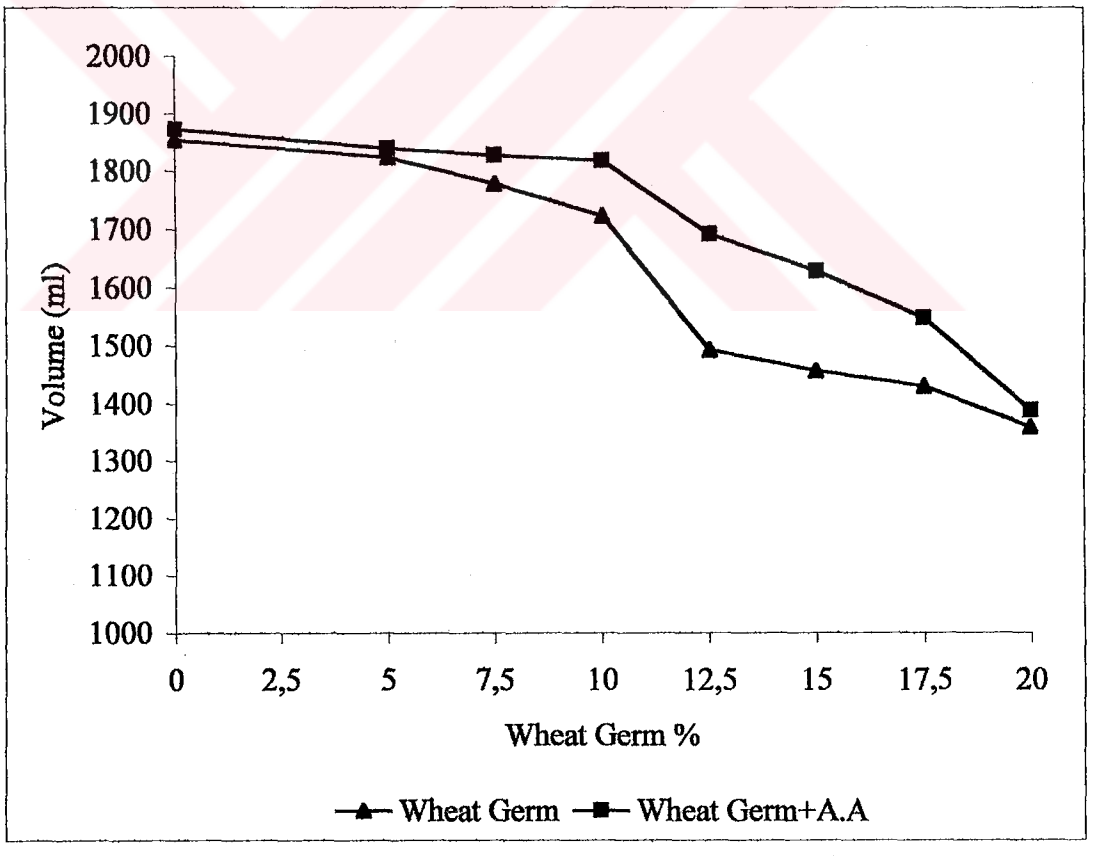

Figure 3.1. Volume variation of bread with wheat germ and wheat germ + A. A

By using ascorbic acid in the bread containing up to 10 % raw wheat germ, a very small increase in the volume was seen. The reason of very little increase, is

the limited amount of raw wheat germ in the bread. Because of this, the effect of the ascorbic acid was thought to have a little effect.

On the other hand, the effect of usage of ascorbic acid in the bread containing 20 % raw wheat germ was occurred limited too. However being similar to the bread including 0 % and 5 %, the reason of this effect was thought to be different. By increasing the % raw wheat germ, dough has been observed to be weakening and so the effect of ascorbic acid is thought to be limited.

The bread containing 10 %, 12.5 %, 15 % and 17.5 % raw wheat germ, especially for the ones having 12.5 % and 15 % rate, the ascorbic acid was seen to be rationally quite effective on the volumes of bread. The raw wheat germ was thought to weaken the dough because of the glutathione. But using ascorbic acid compensated the decreasing volume because of the disadvantage weakening the dough by protecting against a loss of protein stability by counteracting glutathione.

The effect of ascorbic acid addition on the bread volume are given in Table 3.3 and 3.4. The differences between the bread volumes were found statistically significant ($p \leq 0.05$).

Table 3.3. Effects of wheat germ on selected properties in the absence of A.A*

% Wheat germ	Volume (ml)	Height (cm)	Weight (gr)
0	1854±5(a, A)	11.6±0.2(a, A)	581±5(a, A)
5	1824±7(b, B)	9.9±0.1(b, B)	579±2(ab, A)
7.5	1778±3(c, C)	9.6±0.1(c, C)	572±4(bc, B)
10	1723±2(d, D)	9.0±0(d, D)	572±4(bc, B)
12.5	1492±2(e, E)	8.1±0.1(e, E)	570±3(c, B)
15	1456±2(f, F)	7.5±0.1(f, F)	568±3(c, B)
17.5	1429±3(g, G)	7.0±0.1(g, G)	568±3(c, B)
20	1358±4(h, H)	6.6±0.1(h, H)	568±4(c, B)

*Small letters: Duncan's Multiple Range Test results for 1 % significance level;
Capital letters: Duncan's Multiple Range Test results for 5 % significance level

Table 3.4. Effects of wheat germ on selected properties in the presence of A.A*

% Wheat germ (with A.A)	Volume (ml)	Height (cm)	Weight (gr)
0	1873±3(a, A)	11.9±0.1(a, A)	581±4(a, A)
5	1839±5(b, B)	11.0±0(b, B)	580±3(ab, AB)
7.5	1827±2(c, C)	10.5±0(c, C)	580±4(ab, AB)
10	1818±5(c, D)	9.7±0.1(d, D)	579±2(ab, ABC)
12.5	1691±4(d, E)	9.5±0.1(e, E)	575±0(abc, BCD)
15	1628±3(e, F)	8.9±0.1(f, F)	572±3(abc, CD)
17.5	1547±3(f, G)	8.0±0(g, G)	574±3(bc, D)
20	1388±5(g, H)	7.3±0.1(h, H)	570±5(c, D)

*Small letters: Duncan's Multiple Range Test results for 1 % significance level;
Capital letters: Duncan's Multiple Range Test results for 5 % significance level

Previous studies have shown that parallel results were obtained. It is reported that volume of bread are decreased according to ratio of wheat germ in bread formulation [35, 43, 44]. On the other hand, in some studies it is observed that, using wheat germ in small ratios, like 2.5 % or 5 %, increases the volume of bread limited [44]. By adding bran to flour, the decrease occurred in the volume of bread. The problems of pore structure and the hardening of bread crumb was not important for the bread containing 10 % bran. By increasing the amount of bran, these problems grow quickly and volume of bread and pore values decrease to unacceptable levels for the bread containing 40 % bran. The crumb of bread becomes as hard, wet and dough like as not suitable to consume [45].

3.3. Determination of Bread Height

The height of bread was measured after baking. Because of the negative effect of increasing the % raw wheat germ on homogeneity, the height of bread was measured three times from three different points. The average of these measurements was registered as the height of bread.

As seen at Figure 3.2, by increasing the percentage of wheat germ, a decrease was seen near to linear line not only the heights of bread including just raw wheat germ in it but also raw wheat germ + ascorbic acid in it. The usage of ascorbic acid was seen to be effective positively to the height of bread. Although by using ascorbic acid, decreasing of height of bread was observed.

The effect of ascorbic acid addition on the bread heights are given in Table 3.3 and 3.4. The differences between the bread heights were found statistically significant ($p \leq 0.05$).

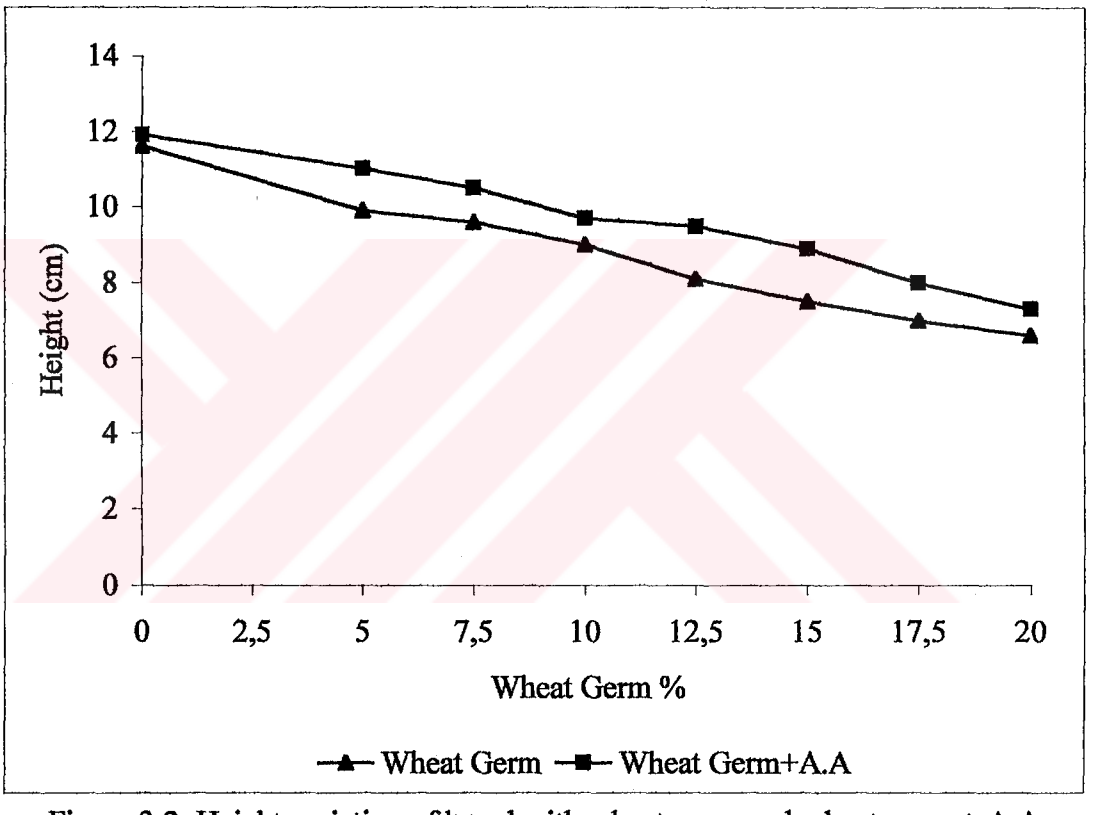

Figure 3.2. Height variation of bread with wheat germ and wheat germ + A.A

3.4. Determination of Bread Weight

The Figure 3.3 shows the weight of bread including 150 mg / kg ascorbic acid and the raw wheat germ with it. To determine the weight of bread, it is needed to weight 30 minutes after baking to standard application in this study [35, 36].

The difference between the weights of the bread containing ascorbic acid is somehow more than the bread including raw wheat germ (Figure 3.3.) Nevertheless, by increasing the amount of raw wheat germ, bread weight gets lesser. For the bread in which ascorbic acid is used, the situation is the same. But decreasing amount of bread weights is quite limited that has been occurred by increasing of % raw wheat

germ. The rate of decreasing is just 2.2 % between the bread by 0 % raw wheat germ and containing 20 % raw wheat germ. This fall is also 1.9 % for the bread used ascorbic acid in it. Although, the weight of bread decreases by increasing the raw wheat germ, there is not a certain difference as seen on the graphic.

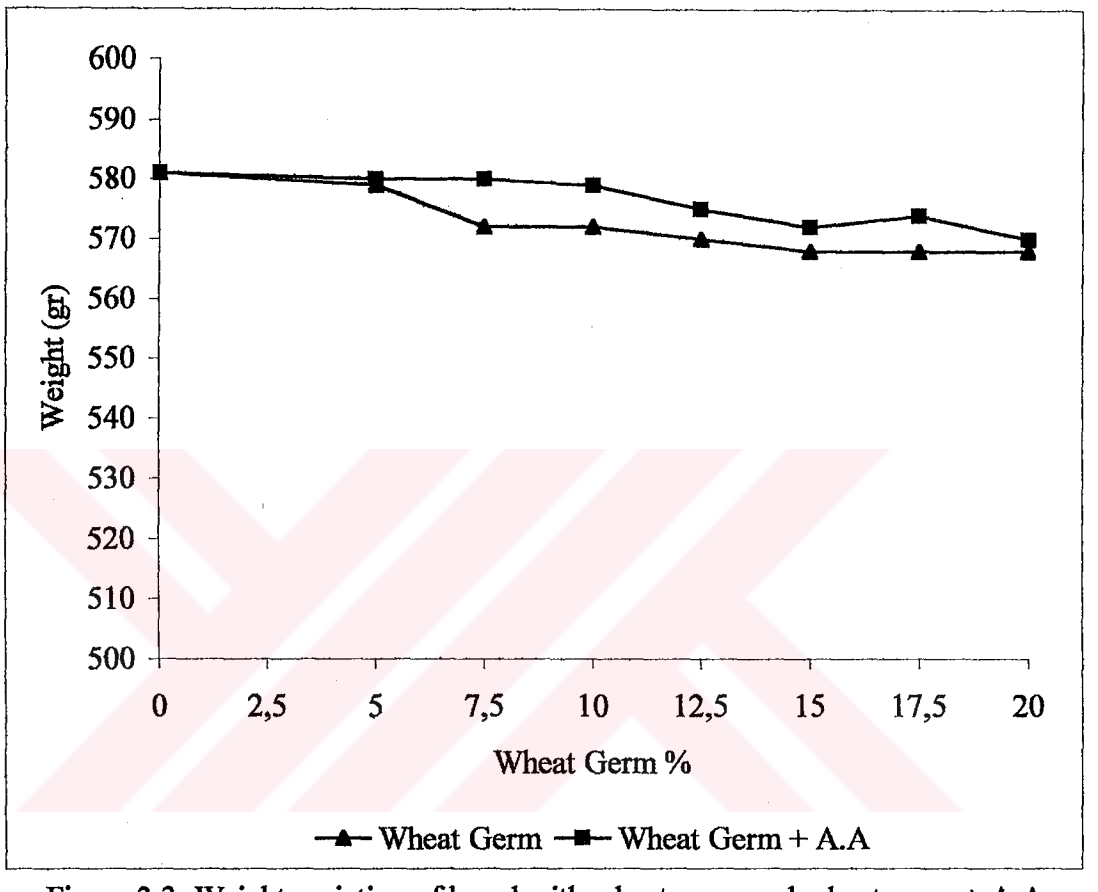

Figure 3.3. Weight variation of bread with wheat germ and wheat germ + A.A

The effect of ascorbic acid addition on the bread weights are given in Table 3.3 and 3.4. The differences between the bread weights were found statistically significant ($p \leq 0.05$).

3.5. Determination of Bread Crumb Properties

Bread crumb properties are texture, homogenous and elasticity. They were observed after 30 minutes from baking process. Texture, homogenous and elasticity characteristics of bread, their evaluation and factors were shown in Table 2.3. If percentage of wheat germ was compared with these factors, as shown in Figure 3.4, 3.5 and 3.6, bread properties are significantly affected by the addition of both wheat germ and A.A. Bread crumb properties were decreased by increasing the amount of

wheat germ in bread formulation. Ascorbic acid application positively affected the undesirable changes of bread. The effect of A.A was significant at 7.5-15 % wheat germ addition.

Figure 3.4 Texture factor variation of bread with wheat germ and wheat germ + A.A

Previous studies have shown that breadcrumb properties are significantly affected by wheat germ addition. Symmetry of the bread changes negatively due to the adding germ. On the other hand, negative changes of the texture and pore structure in the bread was seen [43]. Vital gluten, Diacetyl tartaric acid esters of monoglyceride, $KBrO_3$ and ascorbic acid provide high volume and developing bread crumb properties. They can be used for low protein content flour [43, 46].

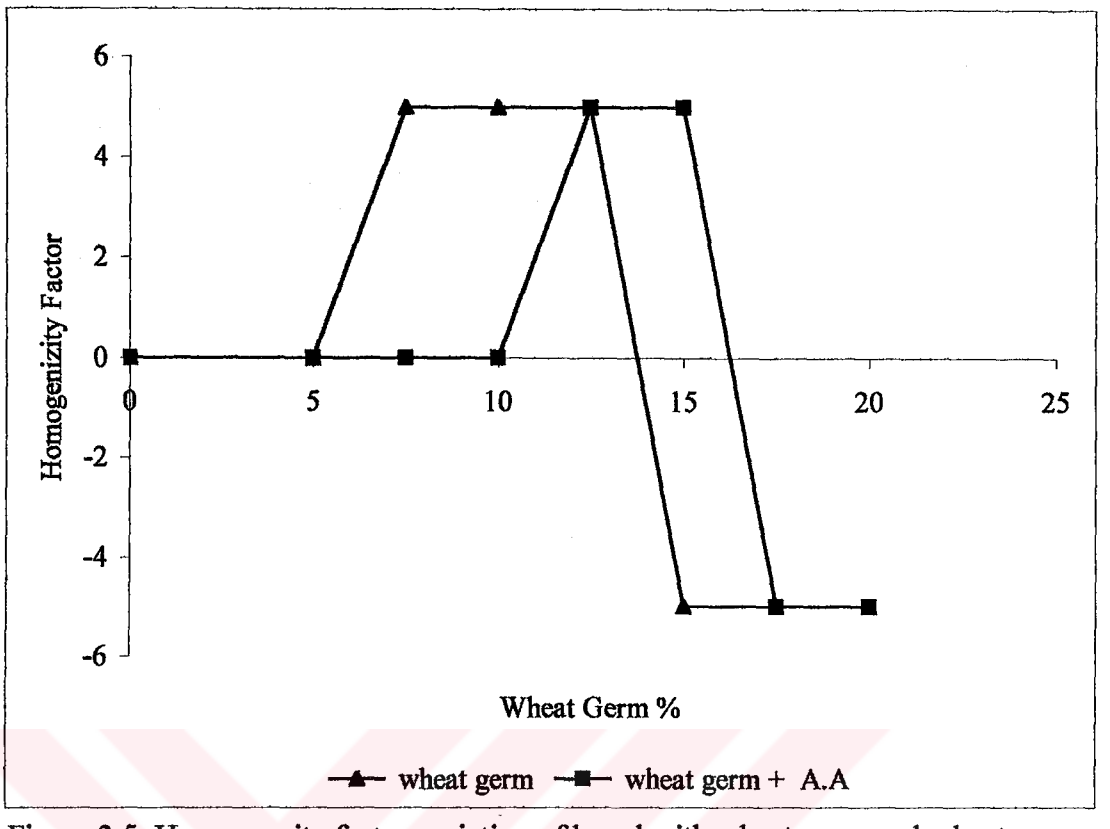

Figure 3.5. Homogeneity factor variation of bread with wheat germ and wheat germ + A.A

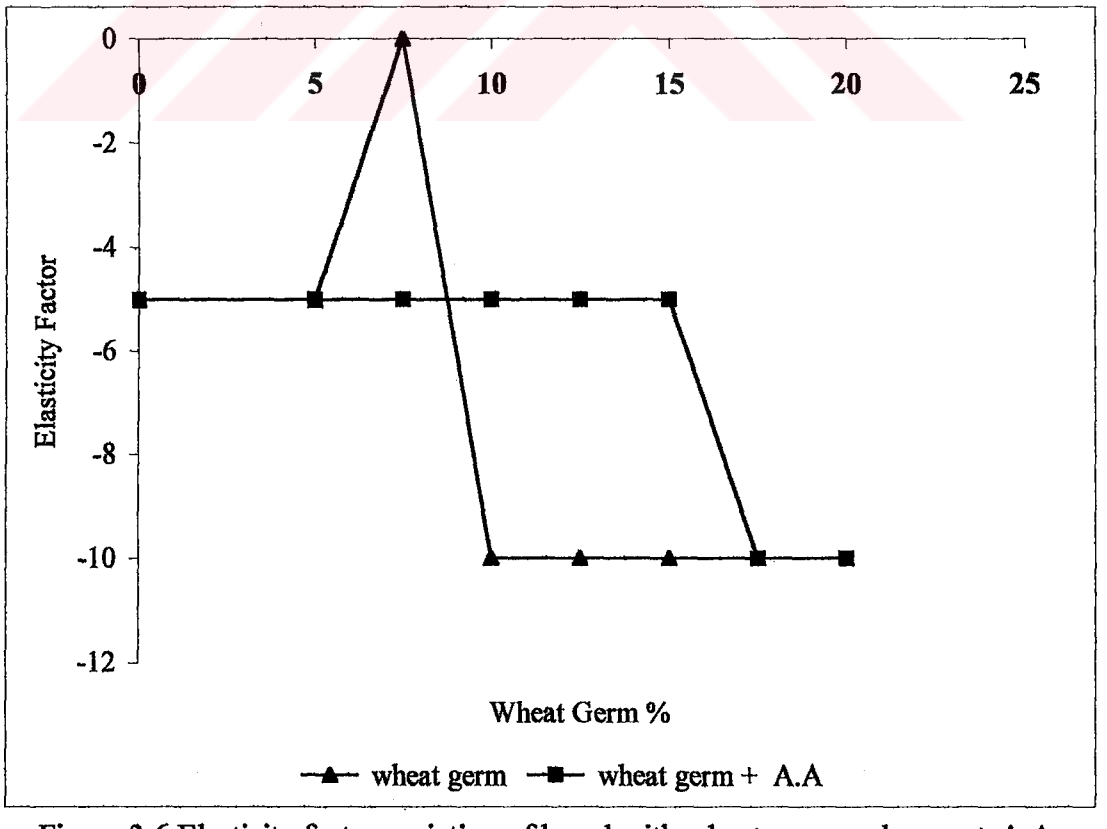

Figure 3.6.Elasticity factor variation of bread with wheat germ and germ + A.A.

3.6. Determination of Pore Number and Factor

Bread was cut from exactly middle and was compared with Dallman pore scale to determine pore number after baking. Pore factor was obtained from Table 2.2 [52,53]. There was no difference in pore structure both of wheat germ and wheat germ + A.A. (Table 3.1 and 3.2) In the previous studies, it is reported that the pores of the crumb was enlarged by increasing the amount of added germ [43].

3.7. Determination of Bread Value Number

Bread value number (B.V.N) contains volume of bread, porosity of bread and bread crumb value which contains texture, elasticity and homogeneity. Because of this, bread value number is a good indicator for completely properties of bread. As was shown in Table 3.5 bread value number was calculated from two different formulas. Increasing the percentage of raw wheat germ, similar to that obtained in height, weight and volume, a decrease in the bread value number were obtained. After a certain percentage of raw wheat germ, a sharp decrease was seen to the contrary of results obtained in volume, height and weight. A sharp decrease of B.V.N of bread containing only raw wheat germ was seen at 10 % level. On the other hand, sharp decrease of B.V.N of bread containing raw wheat germ + ascorbic acid was seen at 15 % level. After these levels, B.V.N of bread obtained by both of these formulas was decreased sharply because of the raw wheat germ addition, which causes negative effects on bread quality. (Figure 3.7.)

Table 3.5. Effect of A.A on B.V.N values

Percentage of raw wheat germ	Bread Value Numbers	
	Wheat Germ addition	Wheat Germ +A.A addition
0	127(a, A)	129(a, A)
5	125(b, B)	126(b, B)
7.5	121(c, C)	125(c, C)
10	106(d, D)	114(d, D)
12.5	76(e, E)	108(e, E)
15	60(f, F)	103(f, F)
17.5	45(g, G)	66(g, G)
20	33(h, H)	38(h, H)

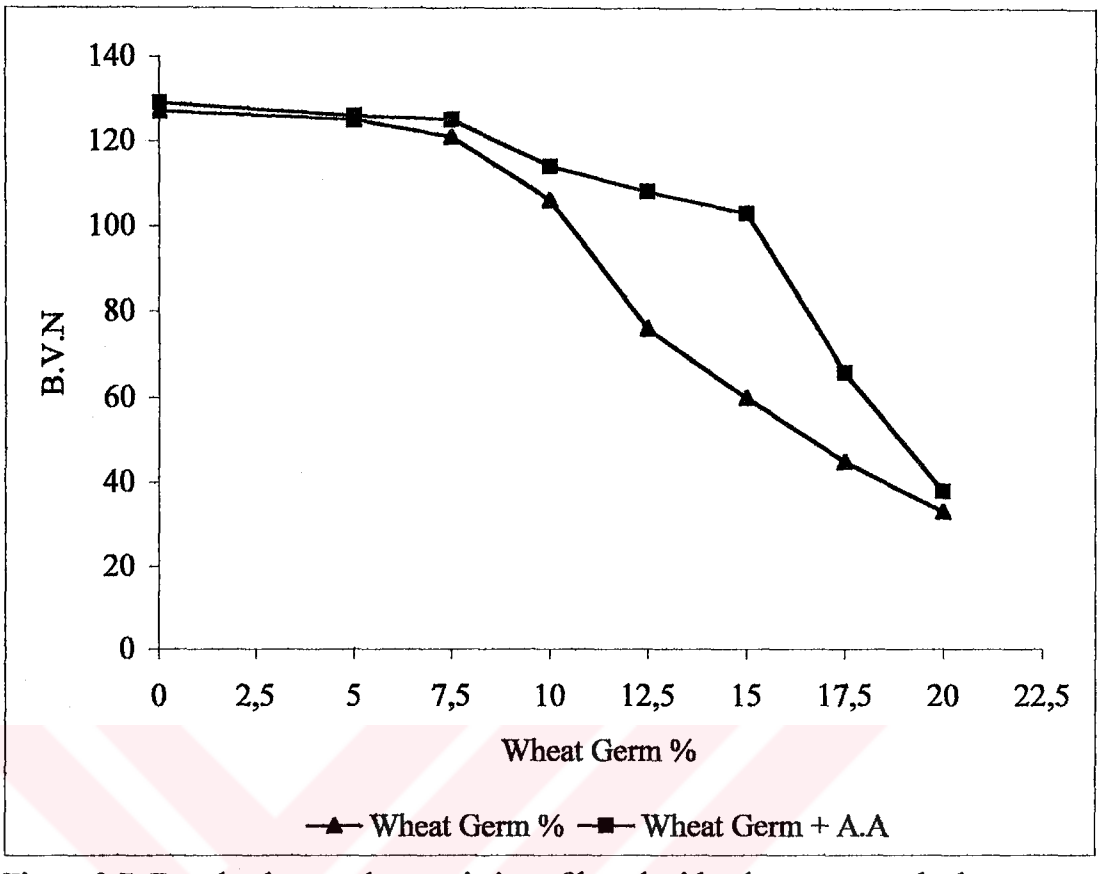

Figure 3.7. Bread value number variation of bread with wheat germ and wheat germ + A.A

Figure 3.8 shows the relationship between wheat germ bread in the absence and presence of A.A and control bread. The amount of decrease in bread value number at 15 % level (wheat germ + A.A) compared to control bread was 18.9 % but this decrease at 17.5 % level was 48 %. So 15 % raw wheat germ level was critical point for germ + ascorbic acid formulation. On the other hand, the decrease in bread value number at 10 % level (wheat germ) compared to control bread was 16.5 % but this decrease at 12.5 % level was 40.2 %. So 10 % raw wheat germ level was critical point for wheat germ bread without ascorbic acid.

3.8. Effect of Ascorbic Acid

Oxidation primarily affects sulphur-containing amino acids that are constituents of the gluten. The oxidation of two adjacent SH (thiol) groups result in the formation of a disulfide bridge between different sections of the long gluten molecule or between different gluten molecules. This causes a hardening of the protein.

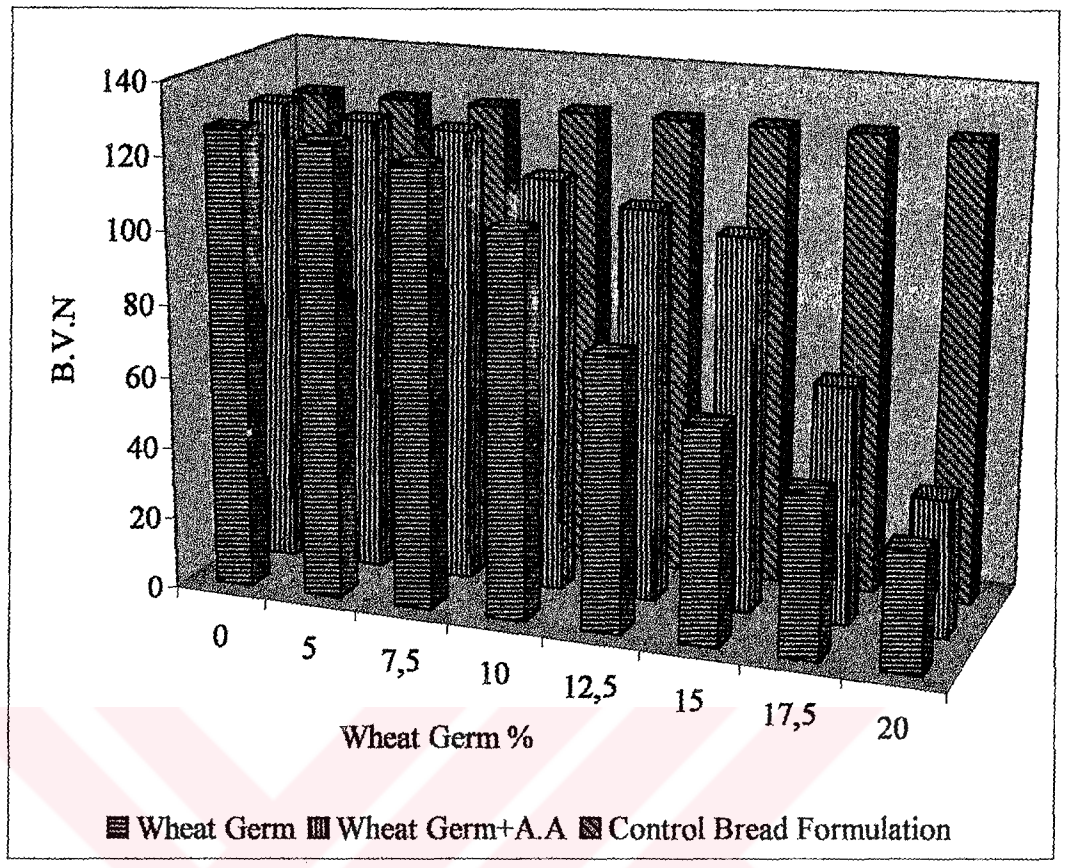

Figure 3.8. Relationship between bread value numbers of germ bread, germ bread (with A.A.), control bread(without wheat germ and A.A)

Ascorbic acid does not act on the protein directly. It protects the loss of protein stability by counteracting glutathione, that occurs in the flour naturally has the opposite effect. This is only possible if AA is oxidized to dehydro-ascorbic acid [DHAA] at the beginning of the kneading process with the aid of the flour's own enzymes [ascorbate oxidase and glutathione dehydrogenase]. In this process glutathione is oxidized to glutathione disulfide, thus eliminating the gluten softening effect of glutathione [47].

Previous studies have shown that oxidation requirement of flour must be provided by natural or by using chemical additives. Especially, flour that was rich in quality and quantity with respect to gluten was preferred to add wheat germ [43]. There are three types of flour for breadmaking purposes. There are type 550, type 650, type 850 [48]. In this study, type 550 was used because of its strength. The bread characteristics of flour affected negatively when wheat germ amount in bread formulation increases. A.A, KBrO3, vital gluten, DATEM were used for preventing these negative effects in previous studies. They provide high volume and developing

bread properties [46, 16]. Branned bread volumes can be increased approximately to the level of 80 % of control bread by using DATEM and A.A [45]. Vital wheat gluten affected the bread containing thin and thick bran considerably positive [49]. It is reported that KBrO3 and similar oxidative materials increase the elasticity and gas retention of gluten like it increases the volume, breadcrumb properties, texture, pore structure and color of bread [50].

In this study, the amount of raw wheat germ was increased 5 % more in the presence of ascorbic acid. Rate of decrease in bread value number for wheat germ + A.A at 15 % level according to the control bread was 18.9 % but this decrease at 17.5 % level was 48 %. So 15 % raw wheat germ level was critical point for wheat germ + ascorbic acid formulation (Figure 3.8) Also A.A addition increases other bread properties, namely B.V.N that contains volume of bread, pore structure and bread crumb properties.

3.9. Sensory Analysis

There were two different breads for comparing according to the color, texture and flavor. First was control bread, the second was germ bread containing 10 % raw wheat germ. They are compared by five untrained people. According to the color, they selected control bread. They could not find any significant difference according to the texture. On the other hand, germ bread was selected according to the taste and odor without any exceptions. In previous studies, it was reported that the color of the crumb turned to brown considerably due to the amount of added germ [43]. In this study, also brown color of germ bread was darkened by increasing germ ratio. Although color was found poor by five untrained people, the flavor of the germ bread were found better than control bread.

CHAPTER IV

CONCLUSIONS

- A decrease in the quality of bread was observed with the addition of raw wheat germ.

- The decrease in bread properties was found different in the absence and presence of ascorbic acid.

- The amount of decrease in bread value number at 15 % level (wheat germ + A.A) compared to control bread was 18.9 % but this decrease at 17.5 % level was 48 %. So 15 % raw wheat germ level was critical point for germ + ascorbic acid formulation.

- The decrease in bread value number at 10 % level (wheat germ) compared to control bread was 16.5 % but this decrease at 12.5 % level was 40.2 %. So 10 % raw wheat germ level was critical point for wheat germ bread without ascorbic acid.

- Addition of ascorbic acid has no effect on the bread weight above 7.5 % wheat germ addition ($p \leq 0.05$)

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APPENDICES

ONE WAY ANOVA RANDOMIZED COMPLETE BLOCKS
Apr 18, 1998 10:51:12 pm
Using: C:\PRJ\COSTAT\STATISTI.DT
Variable: VOLUME

Source	SS	df	MS	F	P	
Blocks	25.083333336	2	12.541666668	0.9393669194	.4142	ns
Main Effects						
GERM	840131.83333	7	120018.83333	8989.3731614	.0000	***
Error	186.91666666	14	13.351190475			
Total	840343.83333	23				

Duncan's Multiple Range Test
Factor: GERM
Error mean square = 13.351190475
Degrees of freedom = 14
Significance level = 1%
LSD .01 = 8.8811715289

Rank	Trt#	Mean	n	Non-significant ranges
1	1	1854	3	a
2	2	1824	3	b
3	3	1777.6666667	3	c
4	4	1722.6666667	3	d
5	5	1491.6666667	3	e
6	6	1455.6666667	3	f
7	7	1428.6666667	3	g
8	8	1358.3333333	3	h

ONE WAY ANOVA RANDOMIZED COMPLETE BLOCKS
Apr 18, 1998 10:52:56 pm
Using: C:\PRJ\COSTAT\STATISTI.DT
Variable: VOLUME

Source	SS	df	MS	F	P	
Blocks	25.083333336	2	12.541666668	0.9393669194	.4142	ns
Main Effects						
GERM	840131.83333	7	120018.83333	8989.3731614	.0000	***
Error	186.91666666	14	13.351190475			
Total	840343.83333	23				

Duncan's Multiple Range Test
Factor: GERM
Error mean square = 13.351190475
Degrees of freedom = 14
Significance level = 5%
LSD .05 = 6.3987990526

Rank	Trt#	Mean	n	Non-significant ranges
1	1	1854	3	a
2	2	1824	3	b
3	3	1777.6666667	3	c
4	4	1722.6666667	3	d
5	5	1491.6666667	3	e
6	6	1455.6666667	3	f
7	7	1428.6666667	3	g
8	8	1358.3333333	3	h

ONE WAY ANOVA RANDOMIZED COMPLETE BLOCKS
Apr 18, 1998 10:57:51 pm
Using: C:\PRJ\COSTAT\STATISTI.DT
Variable: HEIGHT

Source	SS	df	MS	F	P
Blocks	0.0258333333	2	0.0129166667	1.4183006536	.2749 ns
Main Effects					
GERM	59.58	7	8.5114285714	934.58823529	.0000 ***
Error	0.1275	14	0.0091071429		
Total	59.733333333	23			

Duncan's Multiple Range Test
Factor: GERM
Error mean square = 0.0091071429
Degrees of freedom = 14
Significance level = 1%
LSD .01 = 0.2319537171

Rank	Trt#	Mean	n	Non-significant ranges
1	1	11.6	3	a
2	2	9.9333333333	3	b
3	3	9.5666666667	3	c
4	4	9	3	d
5	5	8.1333333333	3	e
6	6	7.5333333333	3	f
7	7	6.9666666667	3	g
8	8	6.6	3	h

ONE WAY ANOVA RANDOMIZED COMPLETE BLOCKS
Apr 18, 1998 10:59:53 pm
Using: C:\PRJ\COSTAT\STATISTI.DT
Variable: HEIGHT

Source	SS	df	MS	F	P
Blocks	0.0258333333	2	0.0129166667	1.4183006536	.2749 ns
Main Effects					
GERM	59.58	7	8.5114285714	934.58823529	.0000 ***
Error	0.1275	14	0.0091071429		
Total	59.733333333	23			

Duncan's Multiple Range Test
Factor: GERM
Error mean square = 0.0091071429
Degrees of freedom = 14
Significance level = 5%
LSD .05 = 0.1671204323

Rank	Trt#	Mean	n	Non-significant ranges
1	1	11.6	3	a
2	2	9.9333333333	3	b
3	3	9.5666666667	3	c
4	4	9	3	d
5	5	8.1333333333	3	e
6	6	7.5333333333	3	f
7	7	6.9666666667	3	g
8	8	6.6	3	h

ONE WAY ANOVA RANDOMIZED COMPLETE BLOCKS
Apr 18, 1998 11:01:48 pm
Using: C:\PRJ\COSTAT\STATISTI.DT
Variable: WEIGHT

Source	SS	df	MS	F	P	
Blocks	60.333333333	2	30.166666667	3.8984615384	.0451	*
Main Effects						
GERM	511.29166667	7	73.041666667	9.4392307692	.0002	***
Error	108.33333333	14	7.7380952381			
Total	679.95833333	23				

Duncan's Multiple Range Test
Factor: GERM
Error mean square = 7.7380952381
Degrees of freedom = 14
Significance level = 1%
LSD .01 = 6.7612547161

Rank	Trt#	Mean	n	Non-significant ranges
1	1	580.66666667	3	a
2	2	578.66666667	3	ab
3	3	572.33333333	3	bc
4	4	571.66666667	3	bc
5	5	570.33333333	3	c
6	8	568.33333333	3	c
7	6	568	3	c
8	7	567.66666667	3	c

ONE WAY ANOVA RANDOMIZED COMPLETE BLOCKS
Apr 18, 1998 11:03:52 pm
Using: C:\PRJ\COSTAT\STATISTI.DT
Variable: WEIGHT

Source	SS	df	MS	F	P	
Blocks	60.333333333	2	30.166666667	3.8984615384	.0451	*
Main Effects						
GERM	511.29166667	7	73.041666667	9.4392307692	.0002	***
Error	108.33333333	14	7.7380952381			
Total	679.95833333	23				

Duncan's Multiple Range Test
Factor: GERM
Error mean square = 7.7380952381
Degrees of freedom = 14
Significance level = 5%
LSD .05 = 4.8714192864

Rank	Trt#	Mean	n	Non-significant ranges
1	1	580.66666667	3	a
2	2	578.66666667	3	a
3	3	572.33333333	3	b
4	4	571.66666667	3	b
5	5	570.33333333	3	b
6	8	568.33333333	3	b
7	6	568	3	b
8	7	567.66666667	3	b

ONE WAY ANOVA RANDOMIZED COMPLETE BLOCKS
Apr 18, 1998 11:05:30 pm
Using: C:\PRJ\COSTAT\STATISTI.DT
Variable: B.V.N

Source	SS	df	MS	F	P	
Blocks	0.25	2	0.125	0.5675675676	.5794	ns
Main Effects						
GERM	30366.666667	7	4338.0952381	19697.297297	.0000	***
Error	3.0833333333	14	0.2202380952			
Total	30370	23				

Duncan's Multiple Range Test
Factor: GERM
Error mean square = 0.2202380952
Degrees of freedom = 14
Significance level = 1%
LSD .01 = 1.1406607119

Rank	Trt#	Mean	n	Non-significant ranges
1	1	127.33333333	3	a
2	2	124.66666667	3	b
3	3	120.66666667	3	c
4	4	106	3	d
5	5	76	3	e
6	6	59.666666667	3	f
7	7	45	3	g
8	8	32.666666667	3	h

ONE WAY ANOVA RANDOMIZED COMPLETE BLOCKS
Apr 18, 1998 11:07:07 pm
Using: C:\PRJ\COSTAT\STATISTI.DT
Variable: B.V.N

Source	SS	df	MS	F	P	
Blocks	0.25	2	0.125	0.5675675676	.5794	ns
Main Effects						
GERM	30366.666667	7	4338.0952381	19697.297297	.0000	***
Error	3.0833333333	14	0.2202380952			
Total	30370	23				

Duncan's Multiple Range Test
Factor: GERM
Error mean square = 0.2202380952
Degrees of freedom = 14
Significance level = 5%
LSD .05 = 0.8218351215

Rank	Trt#	Mean	n	Non-significant ranges
1	1	127.33333333	3	a
2	2	124.66666667	3	b
3	3	120.66666667	3	c
4	4	106	3	d
5	5	76	3	e
6	6	59.666666667	3	f
7	7	45	3	g
8	8	32.666666667	3	h

ONE WAY ANOVA RANDOMIZED COMPLETE BLOCKS
Apr 18, 1998 11:21:49 pm
Using: C:\PRJ\COSTAT\STATIST.DT
Variable: VOLUME

Source	SS	df	MS	F	P
Blocks	12.25	2	6.125	0.4062376629	.6738 ns
Main Effects					
G+AA	615534.66667	7	87933.52381	5832.1484411	.0000 ***
Error	211.08333331	14	15.077380951		
Total	615758	23			

Duncan's Multiple Range Test
Factor: G+AA
Error mean square = 15.077380951
Degrees of freedom = 14
Significance level = 1%
LSD .01 = 9.4378531614

Rank	Trt#	Mean	n	Non-significant ranges
1	1	1873.3333333	3	a
2	2	1839	3	b
3	3	1826.6666667	3	c
4	4	1818	3	c
5	5	1691.3333333	3	d
6	6	1628.3333333	3	e
7	7	1547.3333333	3	f
8	8	1388	3	g

ONE WAY ANOVA RANDOMIZED COMPLETE BLOCKS
Apr 18, 1998 11:23:19 pm
Using: C:\PRJ\COSTAT\STATIST.DT
Variable: VOLUME

Source	SS	df	MS	F	P
Blocks	12.25	2	6.125	0.4062376629	.6738 ns
Main Effects					
G+AA	615534.66667	7	87933.52381	5832.1484411	.0000 ***
Error	211.08333331	14	15.077380951		
Total	615758	23			

Duncan's Multiple Range Test
Factor: G+AA
Error mean square = 15.077380951
Degrees of freedom = 14
Significance level = 5%
LSD .05 = 6.7998828388

Rank	Trt#	Mean	n	Non-significant ranges
1	1	1873.3333333	3	a
2	2	1839	3	b
3	3	1826.6666667	3	c
4	4	1818	3	d
5	5	1691.3333333	3	e
6	6	1628.3333333	3	f
7	7	1547.3333333	3	g
8	8	1388	3	h

ONE WAY ANOVA RANDOMIZED COMPLETE BLOCKS
Apr 18, 1998 11:27:16 pm
Using: C:\PRJ\COSTAT\STATIST.DT
Variable: HEIGHT

Source	SS	df	MS	F	P
Blocks	8.333333E-04	2	4.166667E-04	0.0985915493	.9067 ns
Main Effects					
G+AA	48.689583333	7	6.9556547619	1645.8450704	.0000 ***
Error	0.0591666667	14	0.0042261905		
Total	48.749583333	23			

Duncan's Multiple Range Test
Factor: G+AA
Error mean square = 0.0042261905
Degrees of freedom = 14
Significance level = 1%
LSD .01 = 0.158010081

Rank	Trt#	Mean	n	Non-significant ranges
1	1	11.9	3	a
2	2	11	3	b
3	3	10.5	3	c
4	4	9.7	3	d
5	5	9.4666666667	3	e
6	6	8.9333333333	3	f
7	7	8	3	g
8	8	7.3333333333	3	h

ONE WAY ANOVA RANDOMIZED COMPLETE BLOCKS
Apr 18, 1998 11:28:52 pm
Using: C:\PRJ\COSTAT\STATIST.DT
Variable: HEIGHT

Source	SS	df	MS	F	P
Blocks	8.333333E-04	2	4.166667E-04	0.0985915493	.9067 ns
Main Effects					
G+AA	48.689583333	7	6.9556547619	1645.8450704	.0000 ***
Error	0.0591666667	14	0.0042261905		
Total	48.749583333	23			

Duncan's Multiple Range Test
Factor: G+AA
Error mean square = 0.0042261905
Degrees of freedom = 14
Significance level = 5%
LSD .05 = 0.1138447504

Rank	Trt#	Mean	n	Non-significant ranges
1	1	11.9	3	a
2	2	11	3	b
3	3	10.5	3	c
4	4	9.7	3	d
5	5	9.4666666667	3	e
6	6	8.9333333333	3	f
7	7	8	3	g
8	8	7.3333333333	3	h

ONE WAY ANOVA RANDOMIZED COMPLETE BLOCKS
Apr 18, 1998 11:30:17 pm
Using: C:\PRJ\COSTAT\STATIST.DT
Variable: WEIGHT

Source	SS	df	MS	F	p
Blocks	64.083333333	2	32.041666667	3.7617051013	.0493 *
Main Effects					
G+AA	348	7	49.714285714	5.8364779874	.0025 **
Error	119.25	14	8.5178571429		
Total	531.33333333	23			

Duncan's Multiple Range Test
Factor: G+AA
Error mean square = 8.5178571429
Degrees of freedom = 14
Significance level = 1%
LSD .01 = 7.0937428005

Rank	Trt#	Mean	n	Non-significant ranges
1	1	581.33333333	3	a
2	2	579.66666667	3	ab
3	3	579.66666667	3	ab
4	4	578.66666667	3	ab
5	5	575	3	abc
6	7	574	3	abc
7	6	572.33333333	3	bc
8	8	570	3	c

ONE WAY ANOVA RANDOMIZED COMPLETE BLOCKS
Apr 18, 1998 11:31:29 pm
Using: C:\PRJ\COSTAT\STATIST.DT
Variable: WEIGHT

Source	SS	df	MS	F	p
Blocks	64.083333333	2	32.041666667	3.7617051013	.0493 *
Main Effects					
G+AA	348	7	49.714285714	5.8364779874	.0025 **
Error	119.25	14	8.5178571429		
Total	531.33333333	23			

Duncan's Multiple Range Test
Factor: G+AA
Error mean square = 8.5178571429
Degrees of freedom = 14
Significance level = 5%
LSD .05 = 5.1109737678

Rank	Trt#	Mean	n	Non-significant ranges
1	1	581.33333333	3	a
2	2	579.66666667	3	ab
3	3	579.66666667	3	ab
4	4	578.66666667	3	abc
5	5	575	3	bcd
6	7	574	3	cd
7	6	572.33333333	3	d
8	8	570	3	d

ONE WAY ANOVA RANDOMIZED COMPLETE BLOCKS
Apr 18, 1998 11:33:15 pm
Using: C:\PRJ\COSTAT\STATIST.DT
Variable: B.V.N

Source	SS	df	MS	F	p
Blocks	0.25	2	0.125	0.4666666667	.6365 ns
Main Effects					
G+AA	22220	7	3174.2857143	11850.666667	.0000 ***
Error	3.75	14	0.2678571429		
Total	22224	23			

Duncan's Multiple Range Test
Factor: G+AA
Error mean square = 0.2678571429
Degrees of freedom = 14
Significance level = 1%
LSD .01 = 1.2579456513

Rank	Trt#	Mean	n	Non-significant ranges
1	1	129	3	a
2	2	125.66666667	3	b
3	3	125	3	b
4	4	114	3	c
5	5	108	3	d
6	6	102.66666667	3	e
7	7	65.666666667	3	f
8	8	38	3	g

ONE WAY ANOVA RANDOMIZED COMPLETE BLOCKS
Apr 18, 1998 11:37:29 pm
Using: C:\PRJ\COSTAT\STATIST.DT
Variable: B.V.N

Source	SS	df	MS	F	p
Blocks	0.25	2	0.125	0.4666666667	.6365 ns
Main Effects					
G+AA	22220	7	3174.2857143	11850.666667	.0000 ***
Error	3.75	14	0.2678571429		
Total	22224	23			

Duncan's Multiple Range Test
Factor: G+AA
Error mean square = 0.2678571429
Degrees of freedom = 14
Significance level = 5%
LSD .05 = 0.9063377974

Rank	Trt#	Mean	n	Non-significant ranges
1	1	129	3	a
2	2	125.66666667	3	b
3	3	125	3	b
4	4	114	3	c
5	5	108	3	d
6	6	102.66666667	3	e
7	7	65.666666667	3	f
8	8	38	3	g