THE UNIVERSITY OF MANITOBA

EFFECT OF FABA BEAN FLOUR ON THE NUTRITIONAL AND SENSORY PROPERTIES OF IRANIAN BARBARY BREAD

by

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"TO MY PARENTS"

A B S T R A C T

In many societies, bread represents the main dietary source of energy and protein. Hence, the nutritional quality of the overall diet can be improved by increasing the protein quality of bread. Faba beans, as a good source of protein, can play a major role in improving the protein quality of bread. The effect of faba bean supplementation on the nutritional and sensory properties of Iranian barbary bread was studied. Barbary bread is an oval, low leavened bread. Four treatments were prepared using wheat flour and three faba bean - wheat flour mixtures (30, 40 and 50% faba bean flour on a protein basis). The addition of faba bean flour to the bread increased the protein efficiency ratio, indicating improved nutritional quality as compared to wheat bread. The sensory properties including six textural and four flavor characteristics, were evaluated. Only two textural parameters (grittiness and mouthcoating) were slightly altered in supplemented breads compared to wheat bread. Slight differences were found among the breads for the four flavor characteristics evaluated.

The investigation of the microstructure employing light microscopy revealed that faba bean flour and wheat flour were well intermixed. The continuous uniform overall structure in wheat bread was ruptured in supplemented breads. The gritty texture found in faba bean bread can be explained by the washed out starch granules due to the loose interaction between starch and protein network in supplemented breads. Antinutritional factors including phytic acid, trypsin inhibitors, vicine and convicine were investigated. Levels found in the breads were lower than expected, based on the levels found in the raw materials. This indicated degradation or inactivation of these factors during dough preparation and the baking process.

The improvement of the nutritional quality of barbary bread according to the addition of faba bean flour was established in this study. The sensory properties of supplemented breads were comparable to wheat bread indicating probable high acceptability of these breads.

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Chapter 1

I N T R O D U C T I O N

In many areas of the world such as the Middle East and Northern Asia, some 75% of the available protein comes from wheat in the form of bread. Thus, the nutritional contribution of bread affects the well being of a large part of the population. However, because wheat protein is deficient in some of the essential amino acids, it is not surprising that protein malnutrition occurs in these areas. Addition of animal protein to the diet would overcome this problem. Unfortunately, the use of expensive animal protein, in the form of meat, milk and eggs, is beyond the present economic means of the great majority of the people in question. More practical is to develop high quality protein food products based on local inexpensive foods, such as cereals and legumes. These foods, already an integral part of the diet, are easily produced and processed within the existing technological base.

Several varieties of faba bean (Vicia faba) have been consumed in most Eastern European, Middle Eastern,

African and Asian countries since ancient times (Presber, 1972). They have been eaten as green beans, dried beans, and as bean flour in baked products, in any form they are valuable sources of protein. The nutritional value of faba bean protein is limited by a deficiency of sulfur containing amino acids. On the other hand, cereal proteins are, in general, of poor quality because of an inherent deficiency of certain amino acids, in particular, lysine. Two factors make bread a prime candidate for supplementation with legume proteins. The first, as mentioned, is the fact that in many areas of the world (especially in the Middle East), bread accounts for some 75% of the available protein. Second, is the knowledge that the partial replacement of wheat flour with faba bean flour or protein isolate can significantly increase the nutritional value of bread (McConnell et al., 1974; Fleming and Sosulski, 1977a and 1977b, etc.). However, the addition of non-wheat portion to bread is feasible only when the resulting product is equal to or better, in terms of sensory qualities, than the original product.

Most of the studies of faba bean protein and wheat flour have been conducted on "pan" or high leavened bread.

Figure 1

Barbary Bread

In Middle Eastern countries there is a much higher consumption of low leavened bread as a primary source of protein and calories. For the purpose of this study, an Iranian bread, known as Barbary, was chosen as a representative Middle Eastern bread. Barbary is a low leavened bread made of wheat, yeast and water. It is normally oval in shape, some 3.5 cm thick, 25 - 30 cm wide, and 70 - 80 cm long. (Fig. 1).

The purpose of the present study was to evaluate the nutritional and sensory quality of Barbary bread supplemented with faba bean flour at three different levels (30, 40 and 50% protein basis). The following criteria were investigated:

1. Nutritional value of protein by means of PER and chemical scores.
2. The level of anti-nutritional factors, namely: trypsin inhibitors, phytic acid, vicine and covicine.
3. Texture and flavor by means of sensory evaluation.
4. Microstructure of bread using light microscopy.

Chapter 2

REVIEW OF LITERATURE

2.1 Bread

Bread is a widespread food throughout the world and the staple food in many countries. Even in most high income countries, a large proportion of food protein comes from bread. In Britain, some 30% of total protein intake is derived from bread and other cereal food (Aylward, 1972). The production of bread in the United States in 1969 amounted to over 14 billion pounds and the daily contribution of bread products in terms of protein intake was about 15.2 - 18.4% (Ponte, 1971). It has been reported that wheat in the form of bread, is by far the most common cereal product consumed in the Middle Eastern countries and provides over 55% of the daily protein and calorie intake of an average person (Maleki and Djazayeri, 1968). In Iran, the rural population includes over 20,000,000 persons which is approximately 60% of the total population. Sustenance is derived almost entirely by the locally grown wheat in the form of bread, which contributes 70 to 90% of the daily calorie and protein intake (Kouhestani et al.,

1969; Ronaghi and Solter, 1973).

The chemical composition of white bread and whole wheat bread, along with the recommended daily intake of each nutrient are compiled in table (1). There are considerable differences in the composition of bread due to certain factors, such as the variety of wheat, the degree of extraction rate of flour, the different processing techniques used in bread making, etc. A general increase in the nutrient content of bread occurs with an increase in the rate of flour extraction, as is evident from the data in table (1).

2.2 Bread Making

Making bread was an early human craft. The principles underlying bread making have not changed over centuries. Unleavened bread is made by simple means of mixing flour with water to make dough, adding salt, flatening or otherwise shaping the dough, and then heating it. In making leavened bread, active yeast is kneaded into dough, which is then put in a warm place for fermentation to take place. This results in the formation of bubbles of carbon dioxide, through the action of yeast enzymes on sugar in

Table 1

Composition of Bread (in 100 gram) and the Recommended Daily Nutrient Intake

	French Bread (Unenriched) (1)	Whole Wheat Bread (1) (2% non fat dry milk)	Recommended Daily Nutrient Intake (2) (Male, 19-35 yrs.)
Water (%)	30.6	36.4	-
Energy (Cal)	290	243	3000
Protein (grams)	9.1	10.5	56
Fat (grams)	3.0	3.0	-
Carbohydrates			
Total (gram)	55.4	47.0	-
Fibre (gram)	0.2	1.6	-
Ash (gram)	1.9	2.4	-
Calcium (mg)	43	99	800
P (mg)	85	228	800
Fe (mg)	0.7	2.3	10
Na (mg)	580	527	-
K (mg)	90	273	-
Vit. A (IU) or (RE)	Trace	Trace	1000 RE
Thiamine (mg)	.08	.26	1.5
Riboflavin (mg)	.08	.12	1.8
Niacin (mg)	.8	2.8	20
Ascorbic acid (mg)	Trace	Trace	30

(1) From: Agriculture Handbook No. 8, United States Dept. of Agriculture.

(2) From: Health and Welfare, Canada, Revised 1975.

the flour. The dough is then shaped into desired forms, left to rise through further fermentation and is then baked at a high temperature. The heat makes the gas expand further, gelatinizes the starch and coagulates the proteins to produce a firm loaf, porous in texture.

Modern industrial methods of bread making are subject to continual change. An important goal is to produce methods which are rapid and automated. To achieve this, in some countries, bulk fermentation has been replaced by mechanical or chemical dough development. Preparation of dough by mechanical or chemical development takes little time, a few minutes, compared to a few hours required for bulk fermentation (Aykroyd and Doughty, 1970). Straight dough, sponge dough, liquid ferment and continuous bread making, are four baking processes used in most modern bakeries. Continuous bread making, in comparison with other methods, reduces processing time, floor space and saves labor. The continuous process shares essential baking features with other bread making processes. The ferment which is made first, is then blended with the other ingredients into a homogeneous mass. This mass is then sent through a pump which

regulates dough flow. From the pump, the dough goes to the developer for intense mixing and is extruded into baking pans (Ponte, 1971).

Along with the automization in bread making processes, the concept of enrichment comes into consideration. The loss of nutrients, during milling (table 1), losses from the action of flour improvers, and further losses incurred during baking are reasons for considering the enrichment of bread with vitamins and/or minerals. A further consideration based on the concept of fortification, is the upgrading of the protein quality of the bread by supplementation with amino acids or high quality plant proteins. Protein supplementation plays an important role in the nutritional quality of the diet, especially in countries where there is a high dependence on cereals, in the form of bread, as a main source of dietary protein.

2.3 Supplementation of Bread

Flour is the most important ingredient in the baking process, and thus determines the nutritional and overall quality of bread. Although bread has been made of different kinds of cereals, such as wheat, or mixtures of wheat and rye, corn, barley and others, wheat bread is by far the most popular throughout the

world. However, wheat alone cannot meet the protein requirements of the body, especially during the period of growth. Supplementation of wheat bread by other plant proteins could be a feasible method for overcoming protein malnutrition (Shehata and Fryer, 1970; Hussein et al., 1974; Patel and Johnson, 1975; Sarwar et al., 1975; Sarwar et al., 1977; Fleming and Sosulski, 1977a; 1977b; El-Dash and Sgarbieri, 1980). Faba bean, as a source of protein, has been widely studied. The addition of faba bean flour or protein concentrate to wheat bread is a practical way of improving the overall nutritional quality of the diet for two important reasons. First, bread is usually a popular foodstuff in areas of the world where protein shortages exist and is therefore, a good carrier for supplementation. Second, faba bean is also usually common in these areas and is an accepted and familiar food product.

Besides nutritional aspects, other important factors have to be considered. The functional properties of flours are important, since they might affect the processing and the acceptability of the product. The nutritional and functional effects of supplementation are

discussed in the following sections.

2.3.1 Nutritional Effect of Supplementation

Plant proteins are usually deficient in one or more essential amino acids. The protein quality of cereals, especially wheat, is seriously limited by its low content of lysine. However, the lysine content is relatively high in faba bean protein as it is in all legumes. It is evident from table (2) that the characteristic feature of faba bean protein is its deficiency in sulfur containing amino acids, methionine and cystine. Sulfur-containing amino acids are relatively high in wheat. Lysine and sulfur-containing amino acids play very essential roles in human nutrition (Bigwood, 1972). The combination of wheat and faba bean results in a product with good dietary supply of these important amino acids. The nutritive value of faba bean proteins was evaluated in rat diets (Sarwar et al., 1975), and a low PER value of 0.5 was obtained. On the other hand, when 15% of wheat flour was replaced by faba bean protein concentrate in the bread formula, a PER of 1.67 was obtained. The supplementation resulted in higher levels of lysine and tryptophan, but

Table 2

Amino Acid Composition of Faba Bean Protein and Wheat Protein (g/100 g Protein)

	Faba bean (1)	Wheat (2)
Lysine	6.6	1.6
Histidine	2.6	2.1
Arginine	10.5	4.3
Threonine	3.3	2.4
Serine	4.2	4.3
Glutamic acid	20.3	32.5
Proline	4.1	11.6
Glycine	4.2	3.2
Alanine	4.2	2.0
Cystine	1.7	1.7
Valine	3.9	4.3
Methionine	0.8	1.7
Isoleucine	4.3	4.2
Leucine	8.3	6.9
Tyrosine	2.8	2.8
Phenylalanine	4.4	4.9
Tryptophan	1.0	1.0
Aspartic acid	13.0	3.4

(1) Quoted from Kaldy (1978).

(2) Quoted from Kent Jones and Amos (1967).

the amount of sulfur-containing amino acids was lower compared with the control wheat bread (Fleming and Sosulski, 1977a; 1977b). Similar findings were reported (McDonald, 1979) when 9% of wheat flour was replaced by an equal amount of faba bean protein concentrate. The amount of methionine decreased to about 70% of that in wheat bread. Substantial increases in protein quality of bread supplemented with different levels of legume protein have been reported. From these investigations it is evident that there are optimal ratios of supplementation in terms of nutritional values.

2.3.2 Functional Effect of Supplementation

The functional properties of proteins play an important role in bread making. Wheat proteins have suitable elasticity for forming dough which is an essential step in bread making. Gluten particles, scattered throughout the dough, swell during the hydration and knit up with one another to form a spongy network that holds the starch granules (Sandstedt et al., 1954; Bloksma, 1971). In the bread making process, starch dilutes the gluten, improving the rheology and furnishing a surface suitable for strong

adhesion of gluten. As starch gelatinizes and swells, it provides flexibility for the gas cell walls, permitting them to stretch as the loaf expands. During baking, starch continues to dehydrate the gluten, causing the gluten film to set and giving the loaf its fine texture (Angold, 1975). The functionality of flour depends mainly on its strength. The strength of flour is related primarily to the gluten portion of the flour protein. Therefore, the strength of flour is important for both the acceptability of the final product and for the dough making capacity during processing. As defined by Pomeranz (1971), strong flour contains a higher amount of protein which forms an elastic gluten network of good gas retaining properties, and is capable of being baked into well-risen loaves with good crumb grain and texture. Strong flours require considerable amounts of water to make a dough of proper consistency which will give a high yield of bread. Doughs made from strong flour have excellent handling quality and are not critical in their mixing and fermentation requirements. In contrast, weak flours have a low protein content and form a soft, weak and relatively non-elastic gluten network with poor gas-retaining properties. They have a

low water absorbing capacity which usually yields sticky doughs of inferior handling capacity. Weak flours are very critical in their mixing and fermentation requirements.

The functionality of dough and bread can also be affected by non-wheat protein added to upgrade the nutritional quality of bread. The addition of non-wheat protein might result in restricted uptake of water, more critical mixing and fermentation requirements, or inferior handling properties during the bread processing. McConnell et al., (1974) reported a sticky nature in doughs made of blends of wheat and faba bean flour, due to the low water absorption. However, the functionality of weak doughs can be improved to a certain extent, using proper techniques during bread making.

2.4 Evaluation of Protein Quality of Bread

It is important to emphasize that the amount of protein needed for growth or other functions rises as the nutritive value of the protein falls. Thus, quality and quantity are involved in defining protein needs. In order to establish the nutritional value of a supplemented

product, the evaluation of protein quantity and quality, either by chemical analysis or biological method is essential.

2.4.1 Chemical Scores and Protein Efficiency Ratio (PER)

Protein quality is most often assessed either by chemical scores or animal bioassays. Chemical score procedures were introduced by Mitchell and Block (1946). This method requires a knowledge of the amino acid composition of the proteins as well as the amino acid requirement of the animal. Chemical scores assumes the pattern of amino acids available to animals to be the same as that obtained by chemical analysis. Bioassays for protein quality, on the other hand, are essentially a measurement of the availability of amino acids in the test animals. Therefore, the evaluation of protein quality is most accurate when it is determined with the species for which the protein is intended and under the conditions it is used. However, biological assays with humans and large animals are expensive and difficult to carry out. For this reason, biological assays are usually carried

out under standardized conditions and with laboratory animals. The protein efficiency ratio (PER) assay is the most widely used of all methods for evaluating protein quality. The PER method dates back to 1919 when it was introduced by Osborne and Mendel. It is defined as:

$$\text{PER} = \frac{\text{weight gain of test group (g)}}{\text{protein consumed (g)}}$$

The PER assay is subject to a number of criticisms. The most fundamental is that it assumes all protein is used for growth and no allowance is made for maintenance. The second limiting factor is the duration of the assay (28 days), which makes it lengthy and expensive. However, at present, the PER method as outlined by AOAC (1975) is accepted by many governments and food organizations for estimation of nutritional quality of protein.

2.4.2 Protein Rating

To determine the nutritional contributions of different foods within a diet, the protein quality as well as the daily intake must be considered. The protein rating has been defined as the product of the PER of a protein multiplied by the grams of protein in a reasonable daily intake of that food (Campbell, 1960). A food with a

rating of 20 to 39, inclusive, was considered to be a 'good dietary source of protein', and foods with ratings of 40 or above are considered'excellent dietary sources of protein.' Fleming and Sosulski (1977b) employing this method, obtained a rating of less than 20 for wheat bread, while breads supplemented with faba bean and field-pea were given protein ratings of more than 40.

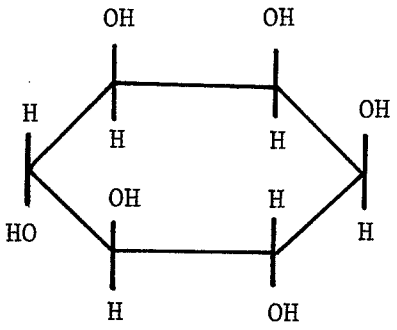
2.5 Antinutritional Factors

The nutritional quality of a product and the availability of nutrients can be decreased by the existence of some antinutritional or toxic factors. Phytic acid, trypsin inhibitors, vicine and convicine are four of these antinutritional factors discussed in the following sections. The first two factors occur in both wheat and faba beans whereas vicine and convicine occur only in faba beans.

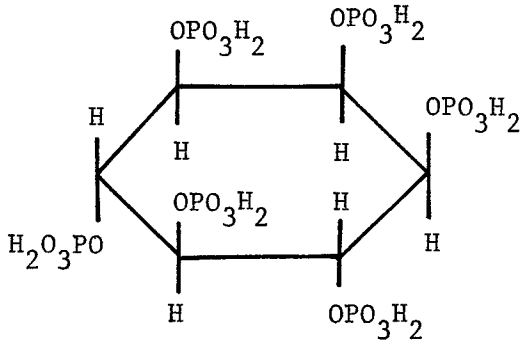
2.5.1 Phytic Acid

Phytic acid, the hexa phosphate of myoinositol (figure 2) occurs primarily in plant tissues such as seeds and whole grains. Phytic acid interferes with the

FIG (2) THE STRUCTURE OF MYOINOSITOL AND PHYTIC ACID



MYOINOSITOL



PHYTIC ACID

intestinal absorption of certain minerals, especially zinc, calcium and iron, to make them biologically unavailable. Phytic acid is present in mixed salts (phytates), which are considered the main storage form of phosphorus in almost all seeds, particularly in cereals and legumes. In grains and oilseeds, they usually occur as the calcium-magnesium salts, phytin (Lolas and Markakis, 1975; Okubo et al., 1975). Phytate concentration ranges from one percent in whole grains, legumes and oilseeds to approximately 5% for defatted sesame meal. The localization of phytates in the bran of seeds contributes significantly to the mineral deficiencies, especially zinc deficiency observed among populations with whole wheat bread as their staple food, such as in Iran (Reinhold, 1971; Reinhold et al., 1974; Reinhold et al., 1975). It would appear, therefore, that the control of extraction rate in the flour, might help in regulating the zinc (and other minerals) to phytic acid ratio, by increasing zinc availability.

Phytates occur in foods in association with proteins (Lolas and Markakis, 1975; O'Dell and deBoland, 1976; Okubo et al., 1976). There is evidence that phytate-

protein complexes are less subject to proteolytic digestion than the same protein alone (Barre, 1956). It was also suggested that indigestable phytate-protein complexes could make zinc and other minerals even less biologically available compared with zinc-phytate complex alone (O'Dell and deBoland, 1976). The interaction of phytic acid with minerals and proteins is considered to be one of the primary factors limiting the nutritive value of cereal grains and legumes. It seems likely that if the phytic acid in legumes and cereals could be substantially hydrolyzed before consumption, the nutritional value of food would be improved.

2.5.2 Trypsin Inhibitors (TI)

Trypsin inhibitors occur naturally in plants and animals. In plants, they are mainly distributed in reserve tissues of Leguminose, Solanaceae and Gramineae families (Ryan, 1973). Twelve faba bean cultivars examined by Bhatty (1974) showed different concentrations of trypsin inhibitors with different inhibitory activity. The range of inhibition of 100μg trypsin (concentration used in the study) by trypsin inhibitors varied from 2 to 32%.

However, none of the cultivars approached soy meal in its trypsin inhibitor activity (90%). The absence of trypsin inhibitor activity in Vicia faba var. baladi was reported by Hussein et al., (1974). There are only a few reports concerning cereal trypsin inhibitors. Whole wheat flour contains only 1% of trypsin inhibitor activity of raw soy bean meal (Shymala and Lyman, 1964). The average trypsin inhibitor activity of triticale flour was 1% and rye flour 2 to 3% of the trypsin inhibitor of soy flour (Chang and Tsen, 1979).

Trypsin inhibitors have been investigated widely, because of possible adverse effects they have on protein digestion when ingested by animals. It has been suggested that trypsin inhibitors affect the utilization of protein in two ways, depending on the experimental conditions and species of animals. In chicks, it inhibits the intestinal proteolysis by reducing the effective level of trypsin to form an inactive trypsin-trypsin inhibitor complex. In rats, however, it increases the requirements of sulfur containing amino acids, especially cystine, due to an increased synthesis and fecal excretion of pancreatic enzymes, thus accentuating the deficiency of these amino

acids which already exist in plant proteins (Kakade et al., 1969; Liener and Kakade, 1969; Kakade, 1974).

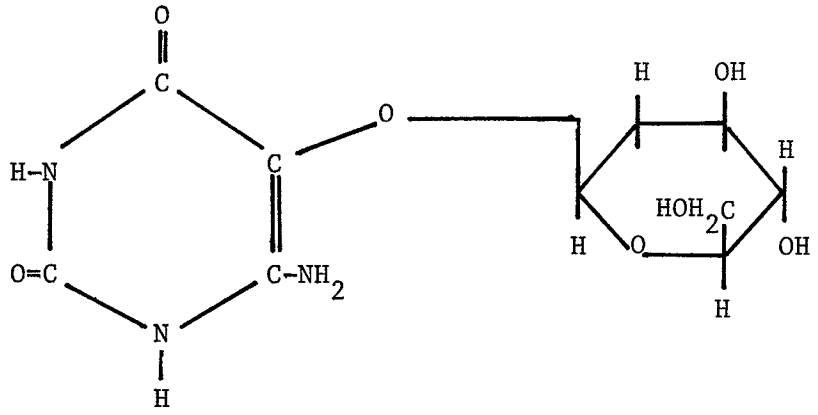
2.5.3 Vicine and Convicine

Vicine and convicine occur naturally in seeds of certain legumes, especially in the species of vicia, including Vicia faba. Vicine and convicine are glucosidase of a pyrimidine whose structures are shown in figure (3). These toxic agents can induce the haemolytic disease favism in individuals with a hereditary deficiency of red cell glucose-6-phosphate dehydrogenase (G-6-PD) Jamalian et al., 1976; Jamalian, 1978). One of the difficulties in the studies of favism lies in the fact that it has not been possible to reproduce this disease in experimental animals. However, vicine at a level of 0.6% of the diet inhibited growth in rats (Liener, 1973).

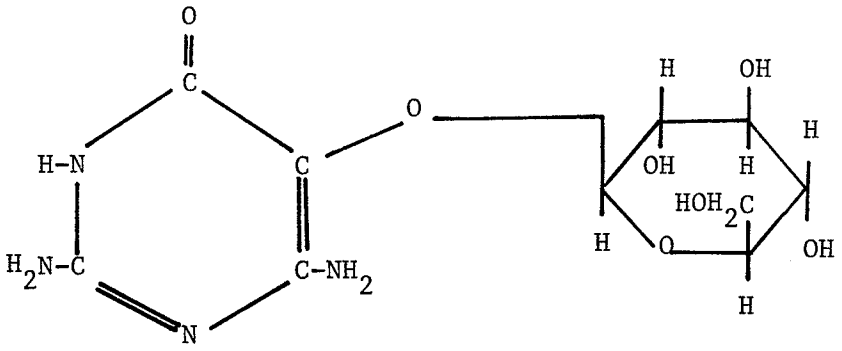
2.6 Effect of Bread Making Process on the Nutritional Quality of Bread

The nutritional as well as the anti-nutritional factors of the food can be affected during processing. According to Liener (1958) and many other investigators,

FIG (3) THE STRUCTURE OF VICINE AND CONVICINE



CONVICINE



VICINE

the nutritive value of proteins of many sources is increased by proper heat treatments. Such improvements are now recognized as being partly due to the heat inactivation of some naturally occurring anti-nutritional factors such as trypsin inhibitors or some other deleterious factors. Severe heat, however, can lead to a destruction of a number of amino acids.

In bread making, the components of flour go through different changes during the fermentation and baking of bread. Some of these changes are desirable and necessary in bread making, while others might have undesirable effects.

2.6.1 Effect on Proteins and Amino Acids

Heat, by increasing the thermal molecular ossiliation, tends to disrupt the binding forces and causes an unfolding of the protein molecules, which is followed by a disintegration due to the disruption of the disulfide bridges. This phenomena is referred to as heat denaturation. Since the first step in protein digestion is a denaturation by proteolytic enzymes, denaturation is considered a positive factor in nutrition (Mauron, 1972). Denaturation alters

only the secondary, tertiary and quaternary but not the primary structure of protein molecules.

Mechanisms which cause the deterioration of protein molecules and impair amino acid availability when proteins in food are intensely heated, are the deleterious heat effects. Susceptability of proteins and amino acids to heat damage varies among different protein sources. It is increased in the presence of various carbohydrates and other food constituents. Little precise information is available on the nature of the chemical changes that take place. There are many possibilities of interaction between the peptide chains. These linkages usually are resistant to hydrolysis by proteolytic enzymes (Ford, 1973). Availability of lysine, methionine and tryptophan was reduced to almost zero by heating in the presence of glucose mostly due to the so called Maillard reaction (Morrison and McLaughlan, 1972).

The effects of heat during baking, on the amino acid content of wheat bread and supplemented bread, have been widely studied. Slower rate of growth have been observed in rats fed on bread crust compared with those fed on bread crumb. This fact is believed to be related

to higher rate of lysine destruction in the crust than in the crumb during baking (Hutchinson et al., 1960). The findings of Horn et al., (1958) and Jansen (1978), also indicated a significant loss of lysine, methionine and sulfur-containing amino acids during baking and that the losses of these amino acids were higher in the crust than in the crumb of bread.

The effect of fermentation on the content of amino acids was also studied by several investigators. Horn et al., (1958) reported no destruction of amino acids during fermentation of the dough prior to baking. In addition, the amount of threonine increased in yeast fermented dough. The influence of yeast fermentation on the content of free amino acids of wheat bread was investigated by El-Dash and Johnson (1970). Their findings showed that with the addition of yeast, free amino acid content increased some 400%. However, during the fermentation the amount of free amino acids reduced to 50% of that in unfermented dough, and more than two thirds of the free amino acids were destroyed during baking. Considering the very low concentration of free amino acids compared to total amount of amino acids in wheat protein, this

reduction during fermentation and baking is not of importance. However, the role of free amino acids in Maillard reaction, and their effect on the flavor and color of bread, should not be ignored. In a subsequent study by El-Dash and Sgarbieri (1980), a 20% increase in the amount of lysine was reported in fermented dough compared to unfermented dough. A reduction of 27% in lysine and 14% in arginine occurred after baking of fermented dough. All the results indicate that fermentation does not seriously alter the amount of amino acids in flour and that the reduction in amino acid content occurs mainly during the baking process.

2.6.2 Effect on Antinutritional Factors

2.6.2.1 Phytic Acid

The hydrolysis of phytic acid to inositol and phosphoric acid is of importance in human nutrition for several reasons. First, most of the phosphorus in plant material is in the form of phytic acid which is not available to the body unless it is cleaved from inositol. Second, the minerals, zinc, iron, calcium, etc., in the form of phytate, are not available during digestion. Third, it is believed that proteins in the form of protein-phytate

complexes, are less accessible to proteolytic enzymes and unavailable to the body (Fontaine et al., 1946). Therefore, elimination of phytic acid in foods is an important step in food processing. There are two possibilities of preventing the harmful effects of phytic acid in bread. One possibility is to use lower extraction rate flours, since most phytic acid is located in the bran of the seed. The second possibility is to cleave phytic acid into phosphoric acid and inositol, by the action of enzyme phytase.

Phytase is present in cereals and legumes (Peers, 1953; Chang and Schwimmer, 1977). Phytase in yeast and in flour itself hydrolyses phytic acid during the leavening of bread. This markedly increases the physiological availability of minerals, especially zinc (Reinhold et al., 1974; Reinhold, 1975). However, it has been reported that the hydrolysis of phytic acid might be diminished by several factors. The addition of calcium prevents the splitting of phytic acid by precipitating the phytic acid as insoluble calcium phytate (Mollgaard, 1946).
It has been reported that the production of lactic acid during fermentation by yeast might promote the

hydrolysis of phytic acid and therefore, the absorption of calcium and phosphorus (Mollgaard, 1946). Determining the optimal conditions for the splitting of phytic acid in dough is of practical importance. The optimum pH for the activity of phytase of wheat is 5.15, with an optimum temperature of about 55°C (Peers, 1953). There are no data concerning the optimum pH or temperature for phytase of faba beans. The optimum pH for phytase activity ranges from 4.8 for soybeans (Mayer et al., 1961), 5.2 for beans (Phaseolus vulgaris) (Chang and Schwimmer, 1977), to 7.5 for mung beans (Mandal et al., 1972). The maximum phytase activity occurred at approximately 60°C in beans (Phaseolus vulgaris) (Chang and Schwimmer, 1977), 50°C in navy beans (Lolas and Markakis, 1976) and 57°C in mung beans (Mandal et al., 1972).

The relatively high temperature optima are of practical interest, since appreciable phytase activity may occur during food processing operations, such as pan proofing and early oven stage, where the temperature of the dough rises to be closer to the optimum temperature for phytase activity (Peers, 1953).

2.6.2.2 Trypsin Inhibitors

The presence in faba bean of trypsin inhibitors which were not destroyed by heat treatment was reported by Nitsan (1971). Wilson et al., (1972) reported the presence of a heat-labile trypsin inhibitor in both cotyledons and testa of faba beans, which was also confirmed by Bhatty (1974). However, in subsequent publications, Bhatty (1975, 1977), reported the trypsin inhibitors of faba beans to be heat stable. Warsy et al., (1974) reported that faba bean contains four highly heat-stable trypsin inhibitors. They purified two of these and studied some of their properties. Both trypsin inhibitors were heat-stable at 100°C for one hour in an acidic medium (pH 2.5), but at pH 12.0 they were rapidly inactivated at 75°C and above. Some of the above discrepancies may be due to different cultivars having little or no trypsin inhibitor activity. The effects of heat and processing on trypsin inhibitors needs further investigation because there is no clear cut correlation between the trypsin inhibitor content of different plants and the effect which heat has on their inactivation.

2.6.2.3 Vicine and Convicine

The effect of heat or the baking process on the destruction of vicine and convicine has not been investigated. Although heating improved the nutritional value of the beans for rat growth, no symptoms resembling human favism have been observed with the raw beans in rats (Liener, 1973).

2.7 Sensory Evaluation

The understanding and analysis of sensory characteristics of foods are important, especially when it comes to developing new foods or introducing new raw materials into food products to upgrade the nutritional quality. The main sensory properties of food are appearance, flavor and texture (Von Sydow, 1971).

The first impression of a food is visual and a major part of acceptance of a food depends on its color and appearance. Color in food can be measured by an analysis of the light reflected from a surface or transmitted through the food.

The concept of flavor consists of at least two phenomena, taste and aroma. Using the gustatory receptors

in the mouth, one can distinguish between sweet, sour, salt and bitter tastes. On the other hand, olfactory acceptors in the nasal cavity can be reached only by compounds which are volatile.

In some food, texture is considered the most important sensory property, especially in products such as bread which are generally bland in flavor (Szczesniak, 1977). Texture has been defined by Sherman (1970) as the composite of those properties which arise from the structure elements of a food and the manner in which these register with the physiological senses in the mouth.

Textural characteristics can be grouped into three main classes (Civille and Szczesniak, 1973): (1) Mechanical, those characteristics related to the reaction of food to stress, such as hardness, cohesiveness, elasticity, viscosity, chewiness, etc., (2) Geometrical, those characteristics related to the arrangement of physical constituents of a food product, such as size, shape and arrangement of particles in a food; (3) Those properties related to the moisture and fat content of a food product.

2.7.1 Sensory Properties of Supplemented Bread

The sensory properties of bread differ from country to country for different economical, cultural and social reasons. Bread has been made in a variety of shapes, from flat circular pancakes, to high leavened loaves. Changes in sensory properties can be expected when a foreign material, such as a plant protein is added to bread. In some cases, it is a matter of the flavor of the added protein, which may differ from the original protein. In other cases, however, changes in the texture of bread appear due to differences in functionality of different proteins. The taste of bread containing 20% faba bean flour has been described as flat, tasteless, or possessing a bitter after taste. The mouth-feel was variously described as gummy, doughy or pasty. In spite of these adverse comments, panelists did not dislike the bread (McConnell et al., 1974). Patel et al., (1977) reported differences in flavor and textural quality of bread supplemented with horse bean protein. Bread supplemented with horse bean flour was found to be sweeter, beanier, more bitter and sour and less wheaty than bread containing an equivalent amount of horse bean protein isolate. The

flavor of control bread was described as a complex of sweet and sour tastes, wheaty and yeasty aromatics. The amber color of crust in control bread changed to dark reddish brown with the addition of 15% and 20% horse bean flour. But with up to 20% supplement of protein isolate, bread remained soft and moist with fine crumb and grain similar to control wheat bread. Shehata and Fryer (1970) studied the sensory properties of bread supplemented with 5 to 20% chick pea flour. No significant differences were found in color, texture, flavor or overall acceptability. The sensory properties of bread supplemented with different plant proteins have also been studied by Bass and Caul (1972). All the studies indicate that by controlling the source and amount of protein supplement, and with proper processing techniques, acceptable supplemented bread can be prepared.

2.7.2 Sensory Evaluation Technique (Magnitude Estimation)

Sensory evaluation is the method concerned with the measuring and evaluating sensory properties using humans as instruments. In recent years, the methods of sensory

analysis of foods have changed to a scientific system based on psychophysics. Psychophysics is the relation between chemical and/or physical stimuli and perceived sensations. The emergence of psychophysics into the sensory field has brought with it a powerful measuring technique known as 'magnitude estimation'. It allows the panelist to judge a sample on his own sensory continuum which is a significant advance over the traditional methods of category scaling, wherein the panelist is forced to select a number from a limited scale to match graduation in perceived stimuli intensity (Moskowitz and Chandler, 1977).

Perhaps the greatest contribution that magnitude estimation has made is that it permits the prediction of the sensory responses to a physical stimulus. This relationship is defined by Stevens Power Function, $S=KC^n$, where S is sensory intensity, given by the magnitude estimate, C is physical response, K is the constant and n is the rate of growth of sensory response (Stevens, 1960). The power function can be transformed to logarithms, to the equation $\log S = \log K + n \log C$. From this equation, n becomes the slope of the regression line relating log S

to log C.

The sensory evaluation of foods is very critical. Psychological implication, the conditions of food at the time of evaluation, the manner in which the food sample is presented, and many other factors affect the response of the panelists. In the case of bread, the freshness at the time of evaluation is very important, since a number of changes occur in the flavor and texture of bread with age. These changes usually decrease the palatability and therefore, the acceptability of bread.

2.8 Microstructure of Bread

Sensory properties, especially textural characteristics of bread can be correlated to the microstructure of bread, which is the interaction between different ingredients and their changes during the baking procedure. In microscopic examination of dough and bread, three phases can be distinguished, namely protein, starch and gas cells. The starch granules usually retain their identity in dough while they are embedded in the protein network (Bloksma, 1971; Sandstedt et al., 1954). The well defined protein starch complex in wheat bread was disrupted when wheat flour was partially replaced by different plant proteins.

Microscopic evaluation indicated that added protein caused a ruptured cell structure in bread which might affect the textural properties of bread (Fleming and Sosulski, 1978). However, the effect of a foreign protein on the texture, flavor and overall acceptability of bread can be regulated by proper processing techniques and by controlling the source, amount and type of protein supplemented.

Chapter 3

MATERIAL AND METHODS

3.1 Materials

3.1.1 Raw Materials (Basic Flours)

The wheat flour used in this study was supplied by Soo-Line Mills Ltd., 7 Higgins Avenue, Winnipeg. It was an unbleached and untreated commercial flour of about 13% protein, milled from Canadian hard red spring No. 2 wheat, with 70% extraction rate. The faba bean flour (Vicia faba, L., Var. Diana) was supplied by Glenlea, University of Manitoba research farm, 1978 crop. The dehulled faba beans (cotyledon) were ground in a pinmill and were stored at $4^{o}C$ during the study.

3.1.2 Chemicals and Other Materials

The chemicals used in this study were of reagent grade, unless stated otherwise:

For PER assay:

- Casein: ANRC reference from Humko Scheffield Chemical, Toronto, Ontario.

- Salt (USP XIX salt mixture) and vitamin diet according to AOAC, were supplied by ICN Pharmaceuticals, Inc., Cleveland, Ohio, 44128
- Purina Labs rat chow from Victor Fox Foods, Winnipeg, Manitoba

For trypsin inhibitor assay:

- Trypsin (2X, crystallized, salt free) from Sigma Chemical Company, St. Louis, Mo., U.S.A.
- BAPNA (α-N-Benzoyl-DL-Arginine-p-nitroanilide Hcl) from Sigma Chemical Company

For vicine and convicine determination:

- Diastase (clarase, standardized) from Fisher Manufacturing Division, Fair Lawn, New Jersey, 07410

3.1.3 Barbary Bread

The barbary bread was prepared in the baking laboratory of the Grain Research Institute, 303 Main Street, Winnipeg. The bread making formula is given in table (3), and the procedure is outlined in figure (4). The mixer used was an open bowl, single arm, Kemper mixer. The oven was a Nicholson electric rotary oven with six shelves, with the

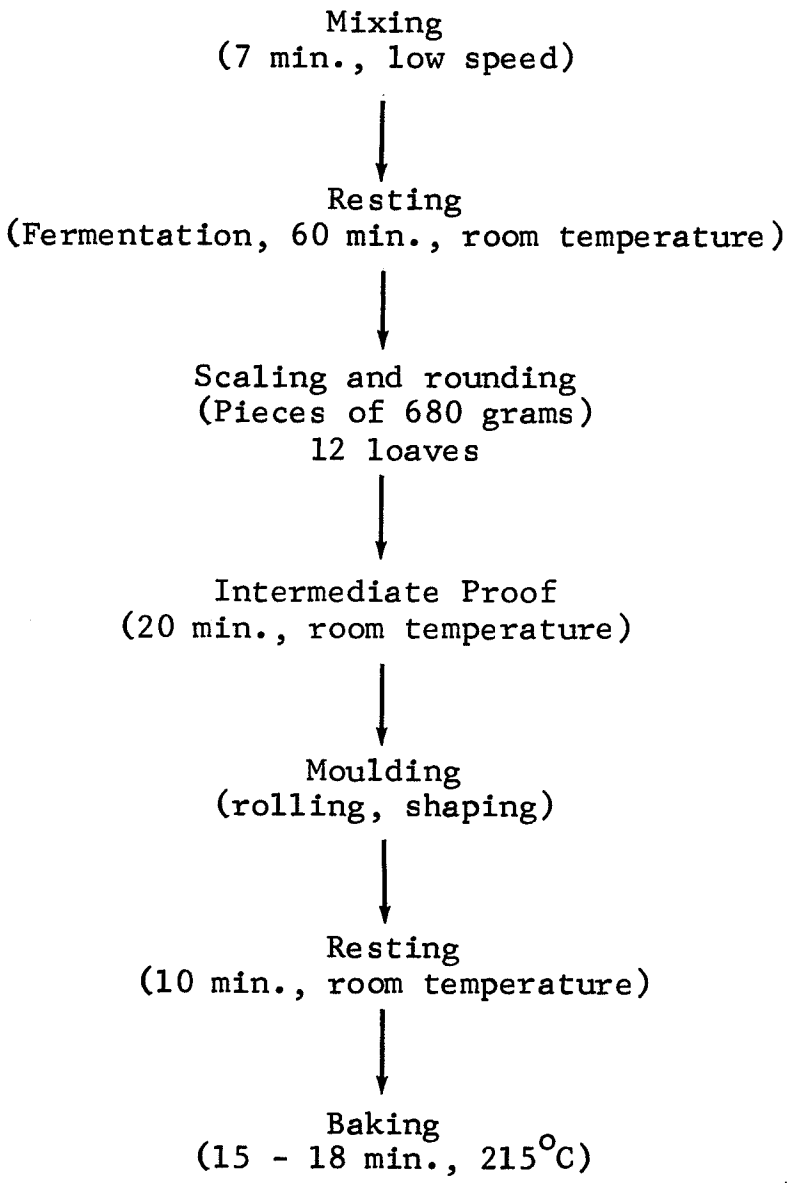
Table 3

Basic Bread Making Formula

Ingredients	Amounts (gram)	%
Flour	5680	58
Water	3860	39.6
Salt	113	1.2
Fresh Yeast	85	0.87
Sugar	14	0.14

Figure 4

Barbary Bread Making Procedure



capacity of 120 loaves of about 500 grams. The same procedure was applied for all breads. However, it was necessary to adjust the amount of water used in the bread formula according to the level of faba bean added. The amount of water and the ratio of wheat and faba bean flour used in each type of bread are given in table (4).

3.2 Methods

3.2.1 Sample Preparation

The fresh bread samples were air dried (about 7% moisture) and then ground and sifted to pass through 12 mesh sieve in Fitzpatrick Mill, Model M, Comminuting machine. The samples were then stored in air tight containers at 4°C until subsequent analysis.

3.2.2 Proximate Analysis

Proximate analyses including the measurement of protein, moisture, ash, fibre and fat content, were performed according to AOAC methods (1975).

Table 4

Wheat Flour: Faba Bean Flour Ratio and the Amount of Water (%) Used in Bread Making Formula

Materials	Abbreviation	Wheat: Faba bean composition (%)		% Water in Formula
		Protein Basis	Flour Basis	
Wheat Bread	W	100:0	100:0	(39.6)
Bread Sample A	A	70:30	84:16	(37.2)
Bread Sample B	B	60:40	77:23	(36.0)
Bread Sample C	C	50:50	70:30	(34.8)

3.2.3 Determination of Minerals

Zinc, magnesium, calcium and iron were determined in the ash collected from each sample, according to the AOAC methods, using Atomic Absorption Spectrophotometer, Model A 3000, Southern Analytical. Total phosphorus was determined according to Chen et al., (1956).

3.2.4 Amino Acids and Chemical Scores

3.2.4.1 Amino Acids Analysis

The amino acid analyses were carried out on a Beckman 119 C analyzer, using the standard hydrolysis procedure (6 N HCL; Vacuum; 24 hr; 110^{o}C). The Beckman modification of the single column procedure of Spackman et al., (1958) was applied. Tryptophan was measured according to Messino and Musarra (1972). The amount of cystine was measured according to Pieniazek et al., (1975). Protein recovery in cystine assay was determined employing the Lowry et al., (1951) method.

3.2.4.2 Chemical Score

Chemical score was calculated by dividing the essential amino acid content in grams of amino acid per 100 grams

of protein, by the corresponding value for that amino acid in the suggested pattern of requirements recommended by FAO (1957), and multiplying by 100.

3.2.5 Feeding Trial - Nutritional Evaluation of Bread Using PER Method

The experimental procedure for protein efficiency ratio (PER) was performed according to AOAC (1975). Sprague Dawley male rats, each weighing approximately 40 - 50 grams, at the age of twenty-one days, were purchased from Bio-Lab Corporation, St. Paul, Minnesota. The rats were placed on an adaptation diet for four days. They were fed rat chow pellets and water which were given ad libitum. At the end of the adaptation period, the rats wereweighed and divided into four groups of 24 by randomization, such that the average weights of the groups differed by no more than five grams. Each rat was placed in an individual cage (24 x 17 x 17 cm), containing a glass feeder and a glass bottle for water. All four groups were fed every 2 days and water containers were washed and refilled with fresh water every day.

The diets were prepared according to the bio-assay

diet of AOAC (1975). The protein level was adjusted to ten percent as shown in table (5). The sourcesof protein were bread samples A, B and C. The reference group was fed on casein as the source of protein. Diet ingredients were mixed thoroughly in a Hobart mixer for 5 - 7 minutes. Diets for each rat were placed in air-tight plastic containers and stored at 4°C during the feeding trial. The feeding continued for 28 days under controlled environment conditions (22°C, automatic light control, twelve hours on and twelve hours off).

A weekly record of body weight gain was kept for each rat. The feed consumption of each rat was measured at the end of the experiment (28 days). Individual PER was calculated from the feed consumption and weight gains of rats of each group using the formula below:

$$\text{PER} = \frac{\text{weight gain in 28 days (gram)}}{\text{Protein consumed (gram) in 28 days}}$$

The values obtained were adjusted, using the casein standard value (2.5) as shown below:

$$\text{Adjusted PER} = \frac{\text{PER of diet}}{\text{PER of casein}} \times 2.5$$

Statistical analysis of variance and Tukey's test were applied to assess the differences among PER's of the diets.

Table 5

Basal Bioassay Diet*

Sample (S)	=	$\frac{1.60 \times 100}{\%\ N \text{ of sample}}$
Oil (Safflower Oil)	=	$8 - \frac{S \times \%\ \text{ether extract}}{100}$
Salt mixture USP	=	$5 - \frac{S \times \%\ \text{ash}}{100}$
Vitamin mixture	=	1%
Cellulose	=	$1 - \frac{S \times \%\ \text{crude fiber}}{100}$
Water	=	$5 - \frac{S \times \%\ \text{moisture}}{100}$

Corn starch to make 100%

* AOAC (1975)

3.2.6 Protein Rating

The protein ratings of bread samples were calculated as the product of PER of the bread multiplied by the grams of protein in a 'reasonable daily intake' of bread (150 grams) (Campbell, 1960).

3.2.7 Determination of Trypsin Inhibitors

Trypsin inhibitors were determined according to Kakade et al., (1974) with the following modifications: 0.001 N NaOH was used for extraction of the samples; the trypsin solution was made by dissolving 6 mg trypsin (2X, crystallized, salt free, Sigma Chemical Company, St. Louis, Mo., U.S.A.) in 200 ml of .001 M HCL, and substrate solution was made with 60 mg BAPNA (α-N-Benzoyl-DL-Arginine-p-nitroanilide HCL, Sigma Chemical Company) dissolved in 1.5 ml dimethyl sulfoxide and diluted to 100 ml by Tris buffer.

3.2.8 Determination of Phytic Acid

Phytic acid was determined according to the method of Harland and Oberleas (1977) and Latta and Eskin (1980) using an ion-exchange procedure. The hexa phosphate

equivalent was calculated from the hydrolyzed phosphate using the method of Chen et al., (1956).

3.2.9 Determination of Vicine and Convicine

Determination of vicine and convicine content in the faba bean flour and supplemented breads, was performed by the titanium tetrachloride ($TiCl_4$) method (Kim, 1980). All the samples were extracted by 2% TCA. Bread samples were treated with enzyme diastase prior to extraction with 2% TCA.

3.2.10 Sensory Evaluation

3.2.10.1 Panel Training and Parameter Selection

A nine member panel consisting of four male and five female graduate students and staff members of the Department of Food Science (aged 22 - 40), participated in this study. Eight of the panel members had previous experience in sensory evaluation. Panelists met for six one-hour training sessions, over a period of two weeks, to become familiar with the method of magnitude estimation and to establish suitable textural and flavor parameters and definitions to be used in evaluating the bread samples.

During the first two sessions, the principles of magnitude estimation were discussed. For the next two training sessions, different bread samples (wheat barbary bread and commercial breads) were evaluated using the magnitude estimation technique and a set of defined textural and flavor parameters. During the discussion after each session, revisions were made pertaining to definitions and techniques of evaluating each parameter. In the last two sessions, the different reference samples were evaluated along with bread samples (wheat barbary bread and bread C). At the end of the session, a total of 6 textural and four flavor by mouth properties, with the appropriate reference samples for each attribute, were agreed upon. Table (6) gives the final set of textural and flavor parameters with the references chosen. The techniques followed throughout the remaining study for each textural parameter are presented in figure (5). In addition, the panelists were asked to use the value of 20 for all the references. If the parameter could not be perceived in the sample, panelists were allowed to score not present (NP).

Table 6

Sensory Characteristics and Reference Samples

Characteristics	Parameters	References
Texture:		
	Chewiness	Hot dog
	Springiness	Angle food cake
	Firmness	Olive
	Adhesiveness	Velveeta cheese
	Gritty	Pears (pureed)
	Mouthcoat	Flour slurry*
Flavor:		
	Sweet	2% sucrose solution
	Sour	0.03% citric acid
	Bitter	.05% caffein
	Beany	5% faba bean flour slurry

* 20% wheat flour slurry

Table 7

The Sequence of Replications for Sensory Evaluation of Bread

Bread Sample	Date
Bread A	Nov. 29/1979
Bread B	Nov. 30/1979
Bread W	Dec. 3/1979
Bread C	Dec. 4/1979
Bread B	Dec. 5/1979
Bread W	Dec. 6/1979
Bread C	Dec. 7/1979
Bread A	Dec. 10/1979

3.2.10.2 Sample Presentation

The sessions were held in a sensory panel room with individual booths. Only one bread sample was evaluated at each panel session. In order to prevent panelists from being biased by possible color or appearance differences in bread samples, two replications for each bread were completed during eight panel sessions held over a period of 12 days. The sequence is shown in table (7). Bread samples were ready for evaluation 2 hours after baking. The samples were cut into 1 inch cubes and served at room temperature. Crackers and water for rinsing were available in each booth. The ballot used is shown in figure (5).

3.2.10.3 Power Function of Basic Tastes

The purpose of this part of the study was to express the overall intensity of three basic tastes, sweet, sour and bitter, as an equivalent of pure chemicals. The panel members estimated intensity of a series of sweet, sour and bitter solutions with different concentrations, in order to derive a power function for each of the three basic tastes. Concentrations used for establishing the power function are given in table (8). In each case, the reference solution

Figure 5

Questionnaire for Sensory Evaluation of Bread

"Magnitude Estimation"

Name_____

Date_____

1. Taste the reference and assign it a score of 20.

2. Evaluate each sample in relation to the reference.

   For example, if the sample seems twice as intense as the reference, give it a value of 40.

3. Use 'np' if the parameter is not present in the sample.

4. Evaluate the bread for all the parameters.

5. Rinse between each evaluation.

TEXTURE:

1. Chewiness:

   Place the sample in the mouth and evaluate the amount of chewing required to reduce sample to the state ready for swallowing.

Reference (Hot Dog)	20
_____	_____

2. Springiness:

   Place the sample between the front upper and lower teeth and gently compress. Remove the force and evaluate the degree and quickness of recovery of the sample.

Reference (Angel Food Cake)	20

3. Firmness:

a) Place the sample between front upper and lower teeth. Measure the force required to compress the sample.

b) Bite through the compressed sample and evaluate the force required to shear the sample.

	a	b
Reference (Olive)	20	20

4. Adhesiveness:

Place the reference in the mouth and chew 3 to 5 times. Give score of 20 to the force required to remove the material from the palate. Then place the sample in the mouth and chew to the state ready for swallowing. Measure the force required to remove the material from the palate.

Reference (Velveeta cheese)	20

5. Gritty:

Place the sample in the mouth. Masticate and spread over the tongue. Evaluate the amount of distinct particles present.

Reference (Pears)	20

6. Mouth-coat:

Place the sample in the mouth. Masticate and swallow. Measure the amount of coating of the sample in mouth.

Reference (Flour Slurry)	20

FLAVOR:

1. Sweetness:

Reference	20

2. Sourness:

Reference	20

3. Bitterness:

Reference	20

4. Beany:

Reference	20

Table 8

Concentrations (w/v) of the Taste Stimuli Used in Determination of Power Functions for Sweetness, Sourness and Bitterness

% Sucrose	% Citric Acid	% Caffein
0.5	0.015	0.013
1.0	0.03*	0.026
2.0*	0.06	0.05*
4.0	0.12	0.10
8.0	0.24	0.20
16.0	0.48	-

* Concentrations used as reference.

had the same concentration as the reference used for the magnitude estimation of flavor in bread samples. The value of 20 was assigned to each reference.

3.2.10.4 Statistical Analysis of Data

Since the panelists were not restricted in their choice of numbers they used for the ratio estimation, high panelist variability resulted. In order to eliminate panelists effect, a normalization procedure was applied, dividing each panelists' estimates, in two replicates, by the geometric mean of his/her estimate. Geometric mean was calculated as G.M. = $\sqrt[n]{(X1)\ (X2).....(Xn)}$, where X is the panelist's score and n is the number of responses given by each panelist. The normalized scores retain the ratio properties of the original magnitude estimates. These normalized values were then transformed to normal logarithms and the data analyzed using analysis of variance. Tukey's test (Snedecor, 1956) was applied to determine significant differences among means. A value of 1/8 of the lowest value assigned by a panelist for the same parameter, replaced the notation "NP" in order to compute the geometric mean (Fabro, 1979). In the case of power

function determination, once the normalization procedure was completed for all panelists, the geometric mean of each concentration (for sucrose, citric acid and caffein solutions) over all panelists was calculated. The geometric means and the concentrations of the solutions were converted to logarithms. Statistical analysis, using linear regression equation ($Y=a + bx$) was applied.

3.2.11 Color Measurements

Color measurements of the crust and crumb were carried out using a Hunter-lab color difference meter D25-2 with a D25 optical sensor. The white tile with the tristimulus values L = 9.38, a = -1.1 and b = 2.3 was used as colaboration standard.

3.2.12 Microscopic Studies

The microstructure of bread samples was examined using light microscopy according to the method described by Fleming and Sosulski (1978). The fixed bread cubes were frozen (at -20^{o}C) and then sectioned at a thickness of 5 μm with a cryo-cut microtome (American Optical Corporation), and were transferred to glass slides and air-dried. The

proteins were stained with 0.1% Panceau 2R, leaving the proteins colored red. The starch granules were stained with iodine. The samples were observed with a Reichert photomicroscopic system. The images at 125X were recorded on Kodak high speed Ektachrome film and printed on Kodak photographic paper with a total magnification of 613X.

Chapter 4

RESULTS AND DISCUSSION

4.1 Proximate Analysis of Ingredient Flours and Bread Samples

The results of the proximate analysis of ingredient flours and bread samples are shown in table (9). The amounts of protein, fat, ash and fibre in wheat flour and faba bean flour are in agreement with reported values. The bread samples were all similar in composition, however, the protein content of supplemented bread did increase slightly as the amount of supplementation (faba bean flour) was increased. The addition of faba bean flour tends to increase the mineral levels, as is shown in table (10). This is because of the higher amounts of calcium, magnesium, iron, zinc and phosphorus in faba bean flour compared to wheat flour (table 10). The level of mineral content of wheat flour was found to be similar to that obtained by Tabekhia et al. (1978).

Proximate analysis indicated an increase in the nutritive value in terms of protein and mineral content of the supplemented bread samples corresponding to increased

Table 9

Proximate Composition of Ingredient Flours and Bread Samples (% on Fresh Basis)

Samples	Moisture	Protein*	Fat	Ash	Fibre
Wheat flour	13.4	13.0	1.92	0.38	-
Faba bean flour	11.3	27.4	1.67	2.95	-
Wheat bread	25.7	11.3	-	2.1	-
Bread A	25.8	12.7	1.1	2.1	0.46
Bread B	25.4	14.0	1.0	2.2	0.51
Bread C	24.3	14.8	1.0	2.4	0.58

* protein = %N x 5.7

Table 10

Mineral Composition of Flours and Bread Samples

(g/100 g Dry Basis)

Samples	Ca	Mg	Fe	Zn	P
Faba bean flour	.0555	0.110	.0078	.0057	0.54
Wheat flour	.0150	0.044	.0019	.0015	0.15
Bread A	.0245	0.057	.0030	.0025	0.24
Bread B	.0258	0.059	.0035	.0024	0.27
Bread C	.0277	0.062	.0039	.0026	0.27

supplementation with faba bean flour.

4.2 Nutritional Quality of Bread Protein

4.2.1 Amino Acid Composition

Table (11) represents a comparison between the essential amino acid content of raw materials and bread supplemented with 30, 40 and 50% faba bean flour (protein basis). Complete amino acid compositions are reported in appendix (1). As can be seen from table (11), the main limitation of wheat flour is the low content of lysine. Increasing the amount of the first limiting amino acid of a food reduces the total amount of protein required to meet the protein needs of an individual. Total protein content, as well as the level of lysine, leucine, isoleucine and threonine increased with the amount of faba bean flour added. On the other hand, as expected, the level of tryptophan and sulfur containing amino acids decreased. Sulfur-containing amino acids became the first limiting amino acids in supplemented breads, mainly due to the low level of these amino acids in faba bean protein.

Heat has a pronounced effect on protein and amino acids. One of the most important factors involved in food

Table 11

Essential Amino Acids in Ingredient Flours, Crust and Crumb of Bread Samples, and the Recovery Rate of Essential Amino Acids of Crust and Crumb in Bread Samples (Amino Acids g/100 g Protein)

Essential Amino Acids	Wheat Flour	Faba Bean Flour	Bread A						Bread B						Bread C					
			Total	% Rec.	Crust	% Rec.	Crumb	% Rec.	Total	% Rec.	Crust	% Rec.	Crumb	% Rec.	Total	% Rec.	Crust	% Rec.	Crumb	% Rec.
Lysine	2.32	6.57	3.28	91	3.07	85.5	3.60	100	3.57	86	3.34	84	3.92	98	3.92	88	3.63	82	4.36	98
Methionine	1.57	0.54	1.28	102	1.32	106	1.23	99	1.04	111	1.02	88	1.08	93	1.11	97	1.07	101	1.16	110
Cystine	1.29	0.36	0.84	83	-	-	-	-	0.83	90	-	-	-	-	0.69	83	-	-	-	-
Threonine	2.95	3.77	3.35	105	3.38	106	3.30	103	3.37	104	3.38	103	3.35	102	3.45	103	3.46	103	3.43	102
Isoleucine	3.74	4.16	3.96	102	3.99	103	3.92	101	3.96	103	3.95	101	3.97	102	4.08	104	4.11	104	4.03	102
Leucine	7.51	8.01	7.72	101	7.79	102	7.63	100	7.77	102	7.77	101	7.77	101	7.92	102	7.99	103	7.81	101
Valine	4.32	4.73	4.53	88	4.54	102	4.51	102	4.45	98	4.52	101	4.34	97	4.68	103	4.70	104	4.65	103
Phenylalanine	5.44	4.32	5.17	101	5.17	101	5.16	101	5.03	102	5.03	101	5.03	101	5.92	104	5.16	106	4.99	102
Tyrosine	2.98	3.47	3.25	104	3.27	104	3.22	103	3.36	106	3.35	105	3.38	106	3.37	104	3.39	105	3.34	104
Tryptophan	1.28	1.13	1.07	87	0.88	71	0.99	80	0.75	61.5	0.73	60	0.84	69	0.71	59	0.67	65	0.74	83
Protein % d.b. (%N x 5.7)	15	30	17.2	88	-	-	-	-	18.8	89.5	-	-	-	-	19.6	87	-	-	-	-

processing is the application of heat. The susceptability to heat differs for different amino acids. The effect of the baking process on the amino acid content of bread was reflected in the recovery rate of amino acids. Amino acid recovery rates are presented in table (11). Tryptophan, lysine, and cystine showed the lowest rate of recovery. This reflects the sensitivity of these amino acids to heat. The Maillard reaction, which takes place mainly in the crust of the bread, is partially responsible for the destruction of amino acids. Table (11) reveals that more pronounced losses of essential amino acids occurred in the crust of the bread in comparison with the crumb. The marked decrease of amino acids in the crust was to be expected since crust is exposed to higher temperatures for longer periods than the crumb. Concurrent with this loss of amino acids, is the increased non-enzymic browning reaction (Maillard) as reported by El-Dash and Johnson (1970). The loss of lysine was found to be approximately 15% higher in the crust than in the crumb for all three supplemented breads. This figure is somewhat lower than the 20% figure reported by Hutchinson et al. (1960).

The recovery rates of amino acids in the crusts of all supplemented breads were similar. The recovery rates of amino acids in the crumbs were also similar (table 11). This indicates that the loss of amino acids was not dependent upon the level of faba bean supplementation.

4.2.2 Chemical Scores and Protein Efficiency Ratio (PER)

The efficiency of the utilization of protein is dependent on the amino acid which is limiting to the greatest extent in a food. The assumption is that the amount of amino acid found to be present in the smallest proportion relative to the amount of that amino acid in the reference protein is the limiting amino acid. As is evident from table (12), methionine and cystine are the first limiting amino acids in all three supplemented breads with chemical scores of 50, 44 and 42% for breads A, B and C, respectively. Tryptophan is the second limiting amino acid with chemical scores of 76, 53 and 51 for breads A, B and C, respectively. As expected, the chemical score for lysine increased as the amount of faba bean flour increased. The deficiency of lysine is only 7% in bread C, while in

Table 12

Chemical Scores of Limiting Amino Acids in Bread Samples

Essential Amino Acids	Suggested Pattern of Requirement*	Bread A		Bread B		Bread C	
		a.a. %	C.S.	a.a. %	C.S.	a.a. %	C.S.
Lysine	4.2	3.28	78	3.57	85	3.92	93
Met. & Cys.	4.2	2.12	50	1.87	44	1.80	42
Threonine	2.8	3.35	-	3.37	-	3.45	-
Isoleucine	4.2	3.96	94	3.96	94	4.08	97
Leucine	4.8	7.72	-	7.77	-	7.92	-
Valine	4.2	4.53	-	4.45	-	4.68	-
Phenyl & tyr.	5.6	8.42	-	8.39	-	9.29	-
Tryptophan	1.4	1.07	76	0.75	53	0.71	51

* FAO patterns (1957)
a.a. = amino acid
C.S. = chemical score

Table 13

PER Values of Bread Samples and Casein

Diet	Uncorrected PER	Adjusted PER*
Casein	2.2	2.5 a
Bread A	0.9	1.0 b
Bread B	1.25	1.4 c
Bread C	1.35	1.5 c

* a b c treatments with same letter are not significantly different ($p \leq .05$).

bread A, it is 22%. This indicates the improvement of the lysine content due to the addition of faba bean flour. Fleming and Sosulski (1977b) found a high correlation between lysine content and the PER. The biological availability of proteins and amino acids of breads was determined by PER assay using rats as test animals. As is shown in table (13) the adjusted PER values obtained for breads B and C were significantly higher than the PER of bread A. Table (13) reveals a 50% increase in the PER of bread C compared to bread A. No significant difference was found between the PER's of bread B and C. The levelling off of the PER value in bread C indicated that the level of wheat flour and faba bean flour in bread C was very close to the optimum ratio for supplementation of bread.

The PER values of all supplemented breads were found to be greater than previously reported values for the PER of wheat bread which ranged from 0.4 to 1.09 (Maleki and Djazayeri, 1968; Jansen, 1969; Shehata and Fryer, 1970; Fleming and Sosulski, 1977b). However, the PER values obtained were lower than the PER value of 1.67 reported by Fleming and Sosulski (1977b) for faba bean supplemented bread, and lower than the PER of 1.80 reported for blends

Table 14

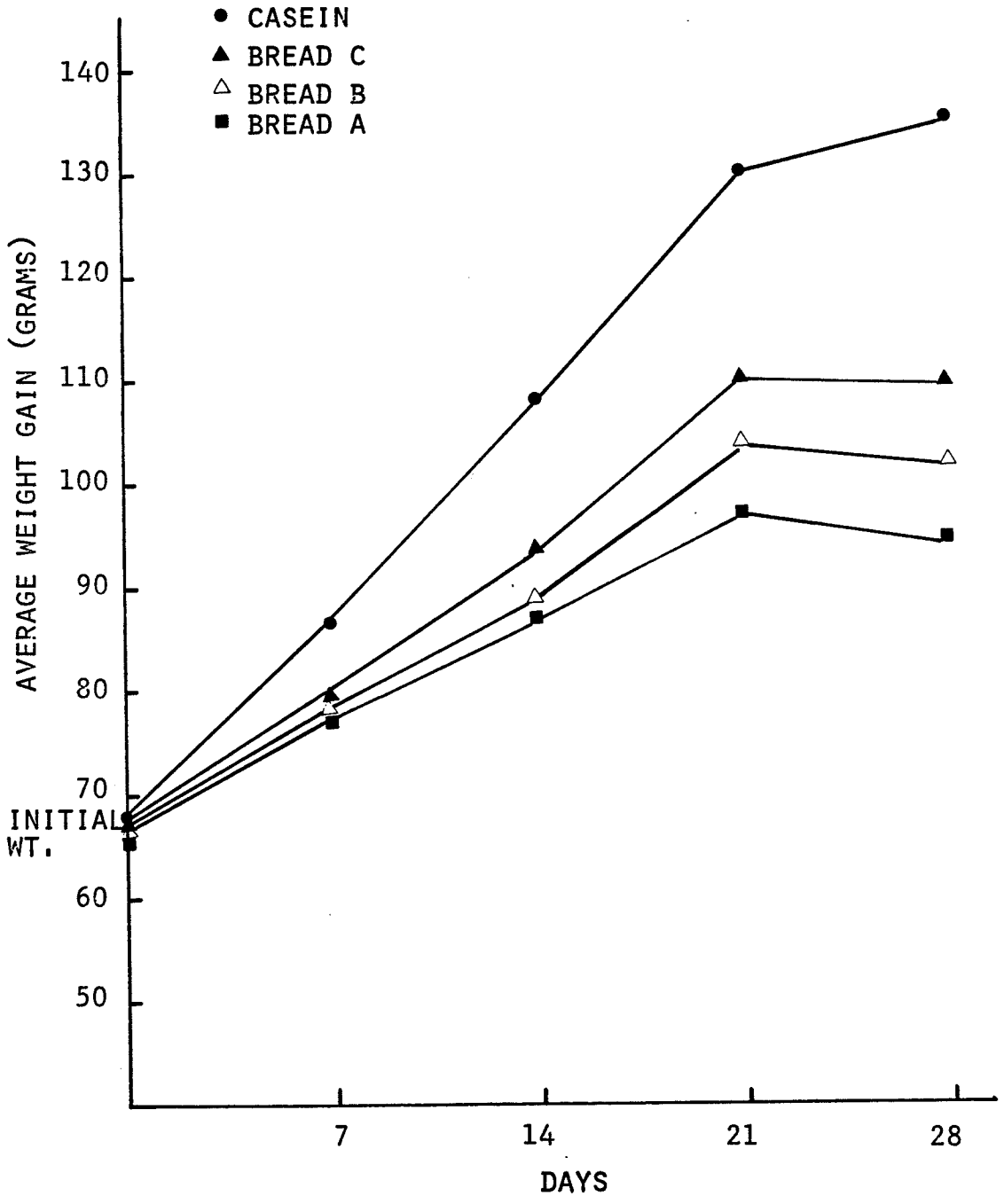
Deficiency of Amino Acids in Bread Diets Compared to Their Daily Requirements for Rat Growth

Amino Acids	Requirements for Growth as % of Diet (1)	Bread Diets A % in Diet	A Deviation from Requirement	B % in Diet	B Deviation from Requirement	C % in Diet	C Deviation from Requirement
Lysine	1.0	0.33	-67	0.35	-65	0.40	-60
Methionine	0.6	0.13	-78	0.10	-83	0.10	-82
Tryptophan	0.2	0.11	-45	0.08	-60	0.07	-65
Phenylalanine	0.7	0.52	-26	0.50	-28	0.59	-16
Leucine	0.9	0.77	-14	0.78	-13	0.79	-12
Isoleucine	0.5	0.40	-20	0.40	-20	0.40	-20
Threnonine	0.6	0.34	-43	0.34	-43	0.34	-43
Valine	0.7	0.45	-36	0.44	-37	0.47	-33
Arginine	0.2	0.60	-	0.67	-	0.37	-
Histidine	0.4	0.24	-40	0.24	-40	0.25	-38

(1) Source: Rose (1937)

of equal proportions of wheat flour and faba bean flour (Sarwar et al., 1975). On the other hand, a PER value of 1.1 was reported by Hussein et al. (1974) when 50% of the protein in the diet of rats was obtained from wheat bread and the other 50% from autoclaved faba beans. One explanation for the discrepancies in the PER values of similar diet mixtures could be the degree of availability of amino acids in a diet or the rate of growth of rats during the assay. The growth curves showing average weekly weight gain of four groups (24 rats in each group) fed on bread samples and casein are presented in figure (6). As can be seen from figure (6), increases in the average weight gain of the rats correspond to increased supplementation of bread with faba bean flour. However, a slow down in the growth rate of the rats was observed during the last week of the assay (figure 6). This could be attributed to the amino acid requirements of rats and also the availability of the amino acids present. The amount of 10 amino acids in the bread diets was compared with their daily requirements for rat growth (table 14). As is apparent, none of the diets provided sufficient amounts of these amino acids (except arginine) at a 10% protein level, to

FIG (6) GROWTH CURVES SHOWING AVERAGE WEEKLY WEIGHT GAIN OF 4 GROUPS OF RATS FED ON BREAD SAMPLES AND CASEIN.



meet the minimum daily requirements for growth of rats. However, deviation from requirements was higher for lysine, methionine and tryptophan than the other amino acids. Growth response has been found (Hutchinson et al., 1960; Fleming and Sosulski, 1977b) to be limited by and proportional to the intake of lysine. According to the chemical scores (table 12) increasing the amount of faba bean flour raised the level of lysine in bread samples, and the lysine deficiency in bread C was reduced to only 7%. On the other hand, it has been established that the availability of amino acids can be greatly reduced by prolonged heat treatments without causing any appreciable destruction of amino acids (Morrison and McLaughlan, 1972). The unavailability of lysine in the crust as a consequence of the baking treatments could be one more reason for the slow down in the growth rate of rats. The unusually large ratio of crust to crumb (60% to 40%) in barbary bread would make this condition more pronounced. The ratio of crust to crumb in normal white pan bread ranges from 15 - 32% to 68 - 75% (Wassermann, 1979). Controlling the degree and the duration of heat during baking is therefore, an important factor in bread processing.

4.2.3 Protein Rating

The quality as well as quantity of protein must be considered in the evaluation of a food as a source of protein. Three factors are involved, the percent of protein in the food, the biological value of food (PER) and the amount of the protein consumed in a reasonable daily intake of that food. On this basis, using the PER method as a measure of biological value, the protein ratings for bread samples were calculated as the product of PER of the bread multiplied by the grams of protein in a 'reasonable daily intake'. Table (15) represents the protein ratings of barbary bread supplemented with 30, 40 and 50% faba bean flour (protein basis). The protein ratings of egg, an excellent source of protein, common white and whole wheat bread, are compared in table (15). A food with a protein rating of 20 to 39 inclusive, is considered a "good dietary source of protein", and a food with a protein rating of 40, or above, is considered an "excellent dietary source of protein". As can be seen from table (15), white bread and whole wheat bread are not good dietary sources of protein, supplementation of bread with faba bean flour, however, improved the quality

Table 15

Protein Ratings of Bread Samples Supplemented With Faba Bean Flour vs. the Protein Ratings of White Bread, Whole Wheat Bread and Egg

Source	% Protein Content	Reasonable Daily Intake (grams)	Protein Intake (grams)	PER	Protein Rating
White bread*	8.4	150	12.6	0.77	9.7
Whole Wheat Bread*	10.5	150	15.5	1.1	17.1
Egg*	12.5	100 (2 eggs)	12.8	3.5	44.8
Bread A	12.7	150	19.05	1.0	19.0
Bread B	14.02	150	21.03	1.4	29.4
Bread C	14.83	150	22.24	1.5	33.4

* Source: Campbell (1960)

of bread. Bread B and C with protein ratings of 29.4 and 33.4 were considered good dietary sources of protein (table 15).

4.3 Antinutritional Factors in Bread

The digestability and nutritive value of foods can be influenced considerably by the presence of antinutritional factors such as inhibitors of digestive enzymes, or some toxic factors present in foodstuffs. Attempts to eliminate antinutritional factors during processing of foodstuffs is therefore, necessary and desirable.

The amounts of trypsin inhibitor activity, phytic acid, vicine and convicine were determined in the raw materials and in the bread samples. The levels obtained are presented in tables (16), (17) and (18). The degree of degradation of these factors during bread processing was calculated based on their level in ingredient flours before processing (appendix 2).

The degrees of inactivation of trypsin inhibitors during the bread making process are presented in table (16). About 80% of trypsin inhibitors in faba bean supplemented

Table 16

Trypsin Inhibitor Activity (TUI) in Ingredient Flours and Bread Samples and the Degree of Inactivation During Processing

Source	TUI/mg * (d.b.)	Expected Value	Inactivation %
Wheat flour	0.48	-	-
Faba bean flour	6.20	-	-
Bread A	0.30	1.35	77.7
Bread B	0.34	1.74	80
Bread C	0.49	2.13	77
Wheat bread	0.19	0.48	60

* d.b. = dry basis

Table 17

Phytic Acid in Ingredient Flours and Bread Samples and the Degree of Degradation During Processing

Source	Phytic Acid (%d.b.)*	Expected Value	Degradation %
Wheat flour	0.214	-	-
Faba bean flour	1.57	-	-
Bread A	0.30	0.418	28
Bread B	0.32	0.510	37
Bread C	0.47	0.609	22
Wheat bread	0.06	0.214	72

* d.b. = dry basis

Table 18

Vicine and Convicine in Ingredient Flours and Bread Samples and the Degree of Degradation During Processing

Source	Vicine and Convicine (% d.b.)*	Expected Value	Degradation %
Wheat flour	-	-	-
Faba bean flour	0.816	-	-
Bread A	0.098	0.126	22
Bread B	0.142	0.182	22
Bread C	0.183	0.237	23
Wheat bread	-	-	-

* d.b. = dry basis

breads and 60% in wheat bread were inactivated during bread making. This indicated heat lability in faba beans and to a lesser degree in wheat. These results support the findings of Wilson et al. (1972) and Bhatty (1974), that trypsin inhibitors in faba bean are heat-labile.

Table (17) shows the amount of phytic acid determined in bread samples and ingredient flours. Some destruction occurred during the processing of bread. The rate of the destruction of phytic acid falls in the range of 15 - 25% in whole wheat bread reported by Reinhold et al. (1974). The lack of a higher rate of hydrolysis of phytic acid during fermentation or baking can be attributed to the conditions (pH, temperature, time, ionic strength, etc.) which were not optimal for the phytase activity. It has been reported that phytic acid interfers with mineral nutrition when it comprises 1% or more of the diet (deRham and Jost, 1979). Since no details concerning the conditions of the above study were given, this finding is questionable as far as its application to food systems. However, in determining mineral availability, both mineral and phytic acid contents should be considered. Assuming that each phytic acid molecule can complex six molecules

of a divalent mineral, under ideal conditions, a phytate/mineral ratio of 0.166 would indicate that all the mineral in question is bound to phytic acid. Therefore, 0.166 is the turning point ratio. Phytate/mineral molar ratios larger than 0.166 indicate the unavailability of that mineral. Ratios smaller than 0.166 indicate that there is not enough phytic acid to complex all of the mineral in question. The values in columns (1) of table (19) indicate that the molar ratios of all minerals in breads A, B and C, are higher than 0.166 and therefore, calcium, magnesium, zinc and iron became unavailable. Based on the assumption that each phytic acid molecule can complex six molecules of a divalent mineral, the values in columns (2) of table (19) indicate the amount of phytic acid required to bind the mineral present. The total values in columns (2) indicate that the amount of phytic acid in bread C was high enough to bind all the minerals present. However, in a complex food system such as bread, many other factors influence the activity of phytic acid. This estimation of the availability of minerals, as affected by phytic acid level, is only a very general approximation. Data presented in table (19) would

Table 19

The Phytate/mineral Molar Ratio of Bread Samples

Phytate/Mineral	Bread A		Bread B		Bread C	
	(1) Molar Ratio	(2) Phytic Acid Required (a) %	(1) Molar Ratio	(2) Phytic Acid Required	(1) Molar Ratio	(2) Phytic Acid Required
Phytate/Ca	0.76	0.060	0.72	0.070	0.66	0.074
Phytate/Mg	0.20	0.248	0.20	0.250	0.18	0.280
Phytate/Fe	8.5	0.006	7.4	0.007	6.7	0.007
Phytate/Zn	12.0	0.004	12.4	0.004	11.5	0.004
Total		0.318		0.331		0.361
Phytic Acid (%)		0.3		0.32		0.47

(a) = phytic acid required to bind minerals.

indicate that, for example, zinc and iron availability was very likely low and that breads A, B and C were rather poor sources of iron and zinc. However, a proper evaluation of mineral values requires determination of their availability through biological testing.

The amount of vicine and convicine in bread samples, faba bean flour, and the degree of their degradation during bread making process are shown in table (18). The data indicate a high stability of vicine and convicine during processing. It has been established that vicine and convicine are stable at high pH's but hydrolyze to aglycones at lower pH conditions (Mager et al., 1969).

The above findings indicate that trypsin inhibitors, phytic acid, vicine and convicine, were all degraded to a certain extent during the bread making process. More research is needed to establish at which stage of the bread making process (fermentation and/or baking), inactivation of the antinutritional factors takes place. Furthermore, the optimal conditions (pH, temperature, etc.) necessary for inactivation of these factors must be determined. Once the optimal conditions are achieved, processing techniques leading to the greater destruction

of antinutritional factors can be suggested.

4.4 Sensory Evaluation of Bread

The wheat bread and faba bean supplemented bread samples A, B and C were evaluated for six textural and four flavor characteristics. Table (20) presents the panel magnitude estimation mean scores for the six textural properties evaluated. No significant differences were found among the bread samples for chewiness, springiness, adhesiveness or firmness (firmness A equals the force required to compress the sample and firmness B equals the force required to shear the sample) (appendix 3). Grittiness and mouthcoating were the only textural characteristics found to be significantly affected by the addition of faba bean flour. Grittiness became evident after the addition of faba bean flour, but did not seem to increase along with increased faba bean supplementation. On the other hand, the perception of mouthcoating did increase with a corresponding increase of faba bean flour.

The panelist's scores for flavor characteristics (appendix 4) contained a large number of "np" responses. This indicates that the bland flavor of bread was retained

Table 20

Panel Magnitude Estimation Mean Scores of Texture Characteristics of Bread Samples

Treatments	Chewiness	Springiness	Firmness A	Firmness B	Adhesiveness	Gritty	Mouth coat
Reference	0.7899[a]	1.1030[a]	1.271[b]	0.775[a]	2.376[b]	1.117[b]	1.639[c]
Wheat bread	1.0906[b]	0.9170[a]	0.797[a]	1.145[b]	0.702[a]	0.402[a]	0.604[a]
Bread A	1.0556[b]	0.8373[a]	1.030[ab]	1.097[ab]	0.748[a]	1.263[b]	0.866[ab]
Bread B	1.0127[b]	1.136[a]	0.976[ab]	1.005[ab]	1.099[a]	1.364[b]	1.008[b]
Bread C	1.0854[b]	1.038[a]	0.982[ab]	1.019[ab]	0.727[a]	1.291[b]	1.154[bc]

a b c scores within a column with the same letters are not significantly different ($p \leq .05$).

Table 21

Panel Magnitude Estimation Mean Scores of Flavor Characteristics of Bread Samples

Treatment	Sweet	Sour	Bitter	Beany
Reference	3.985^{c}	5.589^{c}	8.061^{c}	7.380^{c}
Wheat bread	1.036^{b}	0.382^{a}	0.454^{a}	0.346^{a}
Bread A	0.695^{ab}	0.872^{b}	0.494^{ab}	0.596^{ab}
Bread B	0.757^{b}	0.870^{b}	0.759^{b}	0.725^{b}
Bread C	0.458^{a}	0.615^{ab}	0.727^{b}	0.902^{b}

a b c scores within a column with the same letters are not significantly different ($p \leq .05$).

to a great extent, in supplemented bread samples. Statistical analysis of raw data (appendix 5) generated the data in table (21). Bread C was found to be the least sweet. Wheat bread was perceived as about half as sour as the supplemented breads. Significant differences were also found between the perceived bitterness in wheat bread and breads B and C. Breads B and C were each judged to be about 1.5 times more bitter than wheat bread. All bread samples were much less bitter than the reference solution (0.05% caffein). The perception of beany flavor did appear to increase as the amount of faba bean flour was increased.

The basic tastes, sweet, sour and bitter, were expressed as equivalent concentrations of sucrose, citric acid, and caffein based on power functions. The linear regression analysis was conducted on data resulting from panel magnitude estimation of different concentrations of pure solutions of sucrose, citric acid and caffein. Power functions plotted on semi-log paper (figures 7, 8 and 9) yielded slopes of 1.24 for sucrose, 0.96 for citric acid and 0.99 for caffein. These slopes are comparable with the ones obtained by Malcolmson and McDaniel (1980a

FIG (7) SWEETNESS POWER FUNCTION

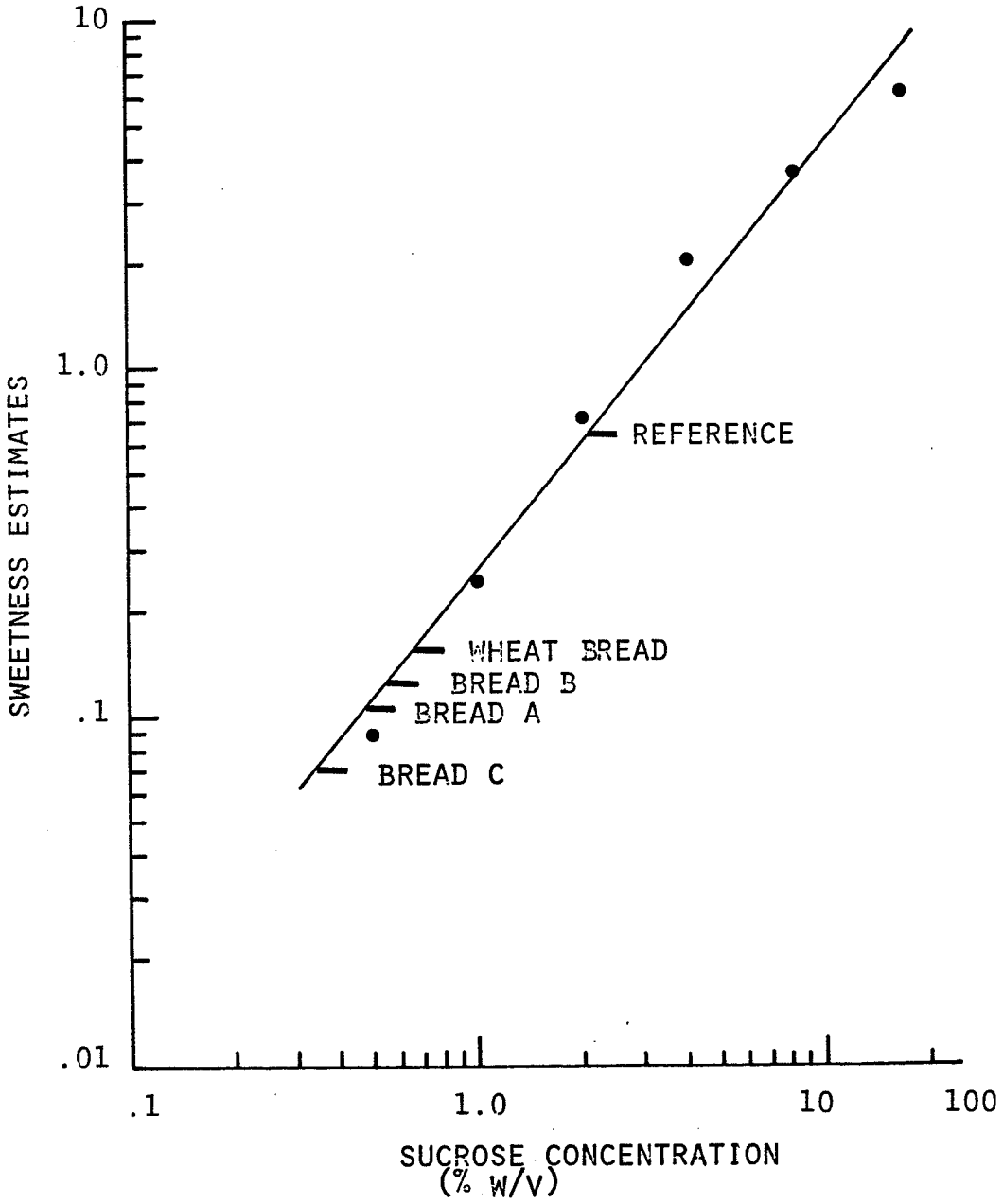


FIG (8) SOURNESS POWER FUNCTION

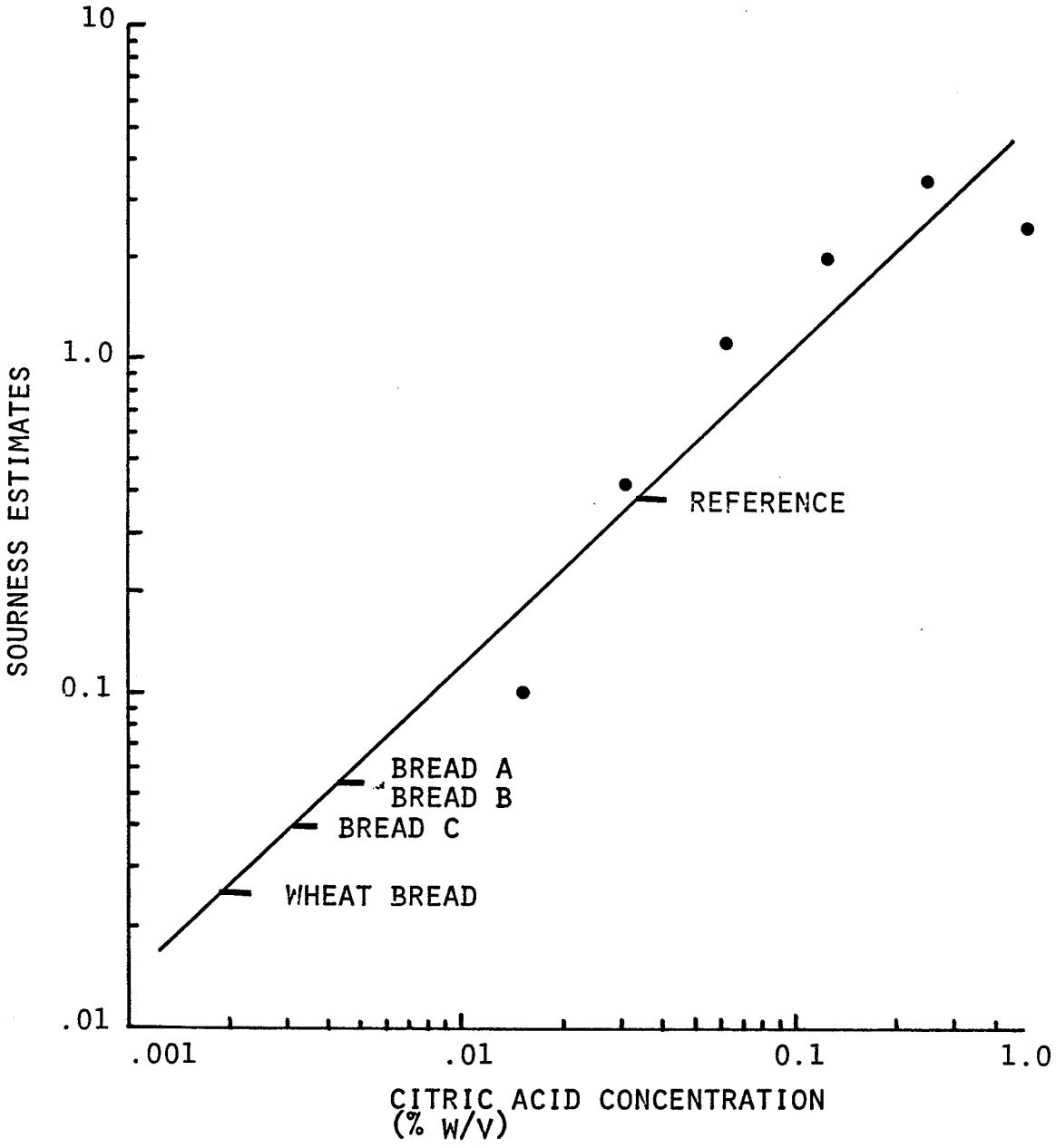
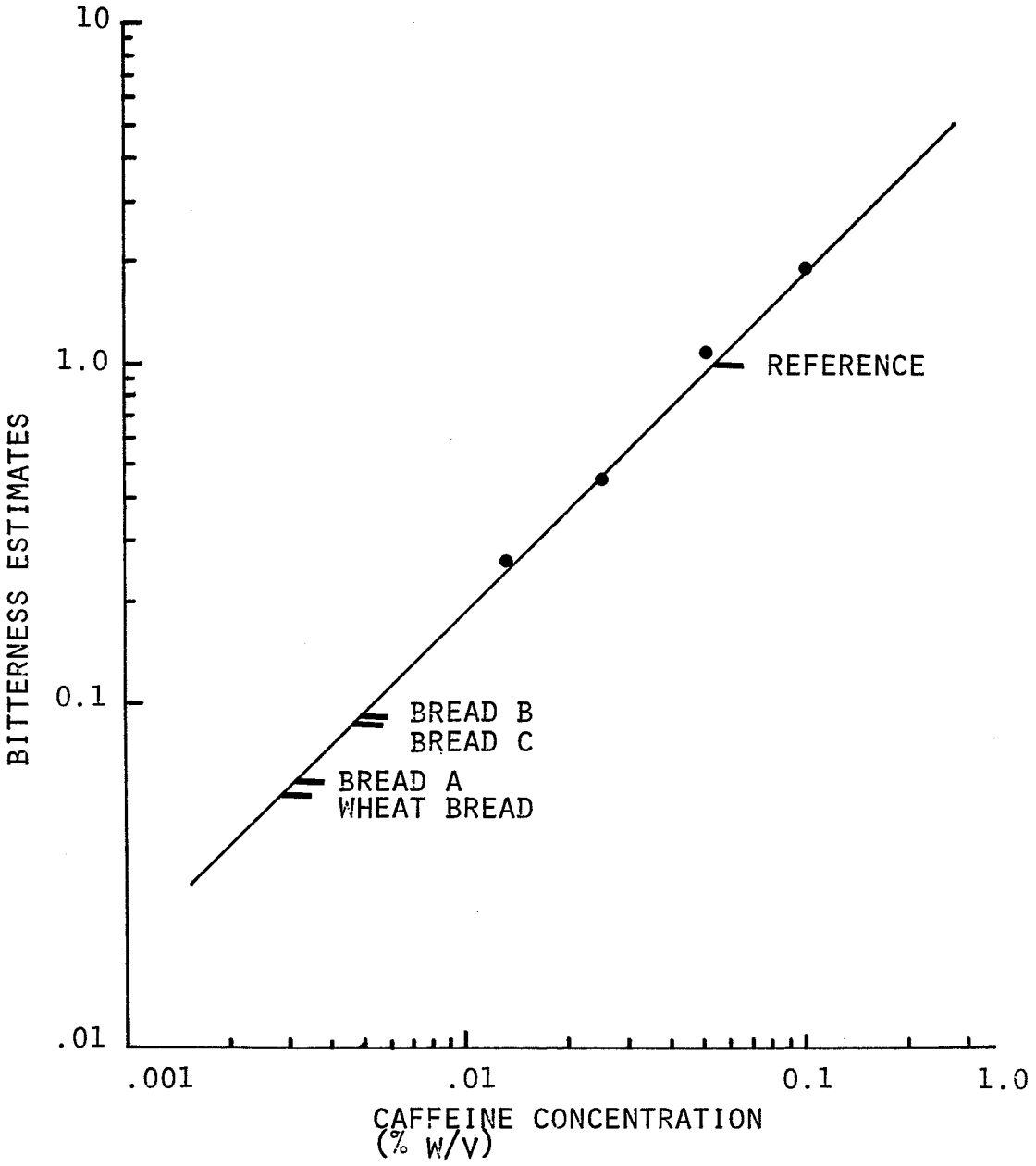


FIG (9) BITTERNESS POWER FUNCTION



and 1980b). The power function is considered to be relatively constant for sweet, sour and bitter intensities, and can therefore, be used to compare intensities from separate taste sessions (Moskowitz and Chandler, 1977). Since all bread samples were compared to reference solutions on a ratio basis, the bread samples can be placed directly on the power functions of the corresponding stimuli (Malcolmson and McDaniel, 1979). The sweet, sour and bitter intensities of bread samples were expressed as concentrations of sucrose, citric acid and caffein, respectively (figures 7, 8 and 9). The equivalent concentrations found using power functions, and the threshold ranges for corresponding stimuli, are compiled in table (22). Threshold levels are the minimum detectable concentrations of pure solutions. It is evident that the equivalent concentrations of all flavor intensities found in bread samples are at threshold levels. This indicates that the bland flavor of wheat barbary bread was retained in all supplemented breads.

Although the texture and flavor properties are of primary importance in sensory evaluation of foods, the importance of appearance and color should not be ignored,

Table 22

Comparison of Thresholds and the Equivalent Concentrations of Sucrose, Citric Acid, and Caffein Solutions

Stimuli	Bread				Threshold Ranges*
	W	A	B	C	
	% w/v				
(Sucrose) sweetness	0.68	0.49	0.52	0.35	0.274-0.753
(Citric Acid) sourness	0.002	0.004	0.004	0.003	0.00096-.0223
(Caffein) bitterness	0.003	0.003	0.005	0.004	0.0078-.035

* Source: Amerine et al. (1965)

for these characteristics also influence the acceptability of food. Color measurements of the crust and crumb of bread samples were made using a Hunter-lab color difference meter. The results are presented in tables (23) and (24). The predominant visual difference between bread samples was lightness (figure 10), and this is reflected in the Hunter readings. As is shown in tables (23) and (24), the L value, which reflects lightness, in both crust and crumb, decreased with increasing supplementation. All faba bean supplemented breads were darker than wheat bread. The darkness in the crust of supplemented bread samples could be attributed in part, to the Maillard reaction, which occurred during baking. But the increasing trend in the degree of darkness in supplemented breads corresponding to increased supplementation, is most likely because of the hull remnant in faba bean flour (Hoehn et al. 1976) rather than the Maillard reaction. The rate of recovery of amino acids in the crust of bread which is an indication of Maillard reaction, did not increase with increasing supplementation.

The results of sensory evaluation on bread samples indicate only slight differences in the texture,flavor,

Figure 10

Color Differences in Bread Samples

w - wheat bread
A - bread A
B - bread B
C - bread C

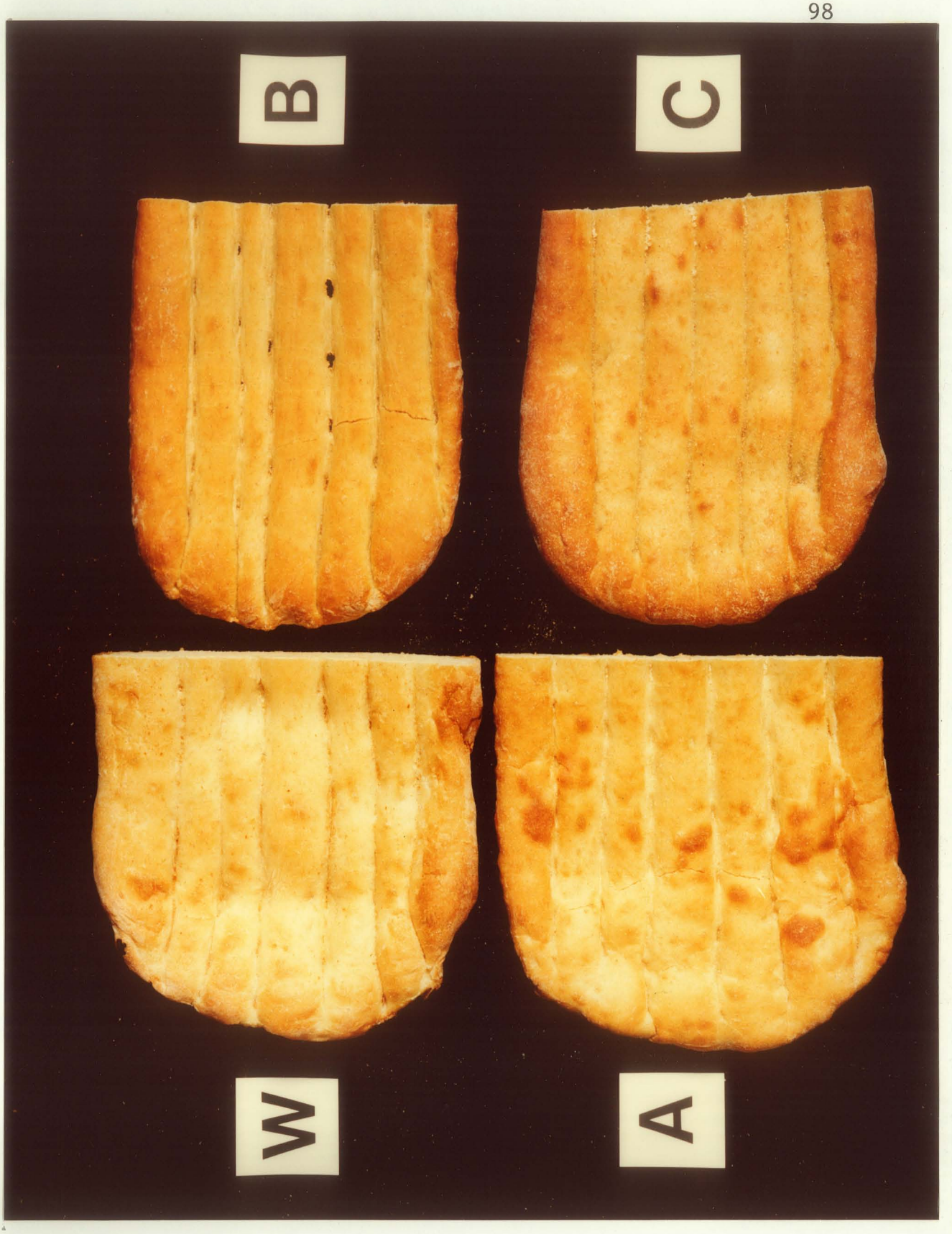

B
C
W
A

Table 23

Color Measurements of the Crust of Bread Samples

Bread Samples	Hunter Values		
	L 100 = white 0 = black	a + = red - = green	b + = yellow - = blue
Wheat Bread	66.6 [a]	3.6 [a]	23 [ab]
Bread A	59.5 [b]	5.4 [a]	23.8 [a]
Bread B	58 [bc]	6.3 [a]	23.7 [a]
Bread C	54 [c]	7.5 [a]	21.9 [b]

a b c values not followed by the same letter are significantly different ($p \leq .05$).

Table 24

Color Measurements of the Crumb of Bread Samples

Bread Samples	Hunter Values		
	L 100 = white 0 = black	a + = red - = green	b + = yellow - = blue
Wheat Bread	72.1 [a]	-0.8 [a]	18.3 [a]
Bread A	65.4 [b]	0.4 [b]	18.0 [a]
Bread B	64.9 [b]	0.7 [c]	17.6 [b]
Bread C	62.0 [c]	0.82 [c]	17.4 [b]

a b c values not followed by the same letter are significantly different ($p \leqslant .05$).

and color of supplemented breads as compared to wheat bread. The bland flavor, the texture and the shape and appearance of wheat barbary bread, were not seriously altered by faba bean flour supplementation. On the basis of the data presented here, one would expect that faba bean supplemented barbary bread would be an acceptable product, even at the highest level of supplementation (50% protein basis).

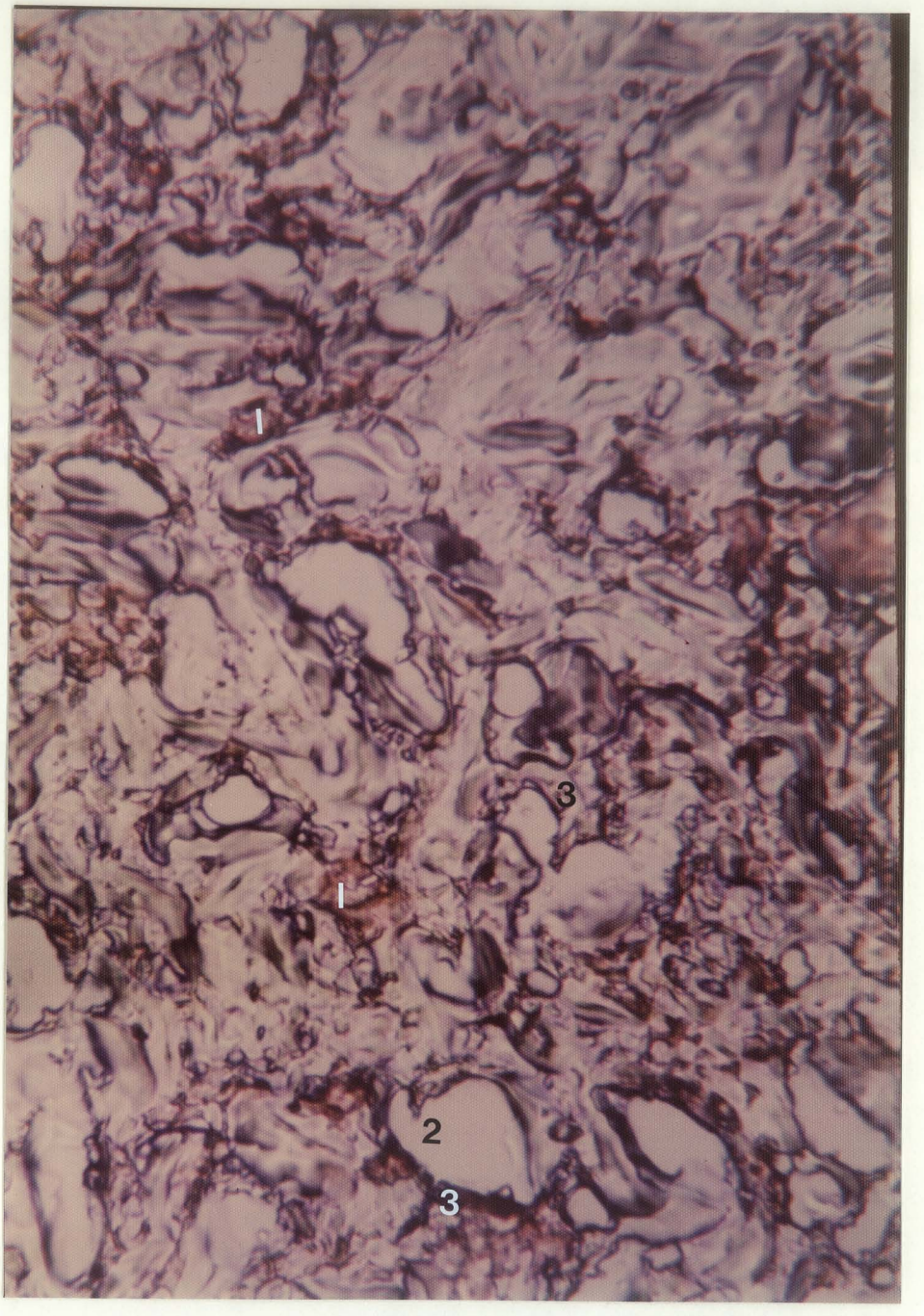
4.5 Microstructure of Bread

Protein, starch and their interaction in bread samples were examined using light microscopy. These examinations were made in order to reveal, where possible, the relation between the microstructure of bread and its textural quality. Wheat gluten (figure 11) showed a weak staining reaction with panceau 2R (pale red), while faba bean protein particles appeared an intense red color (figures 12, 13 and 14). Conditions for staining the samples, taking and developing the photomicrographs for all treatments were identical. Therefore, variations in color are due to the different reactions of wheat protein and faba bean protein to panceau 2R. Both gluten and faba bean protein in supplemented breads were intermixed well and evenly distributed in the mixture. A compact overall structure with uniform continuous cell walls and small gas cells was characteristic of the wheat barbary bread (figure 11). Compared with wheat

Figure 11

Light Micrograph Showing Protein Network in Wheat Bread

1 - protein mass
2 - gas cells
3 - cell wall



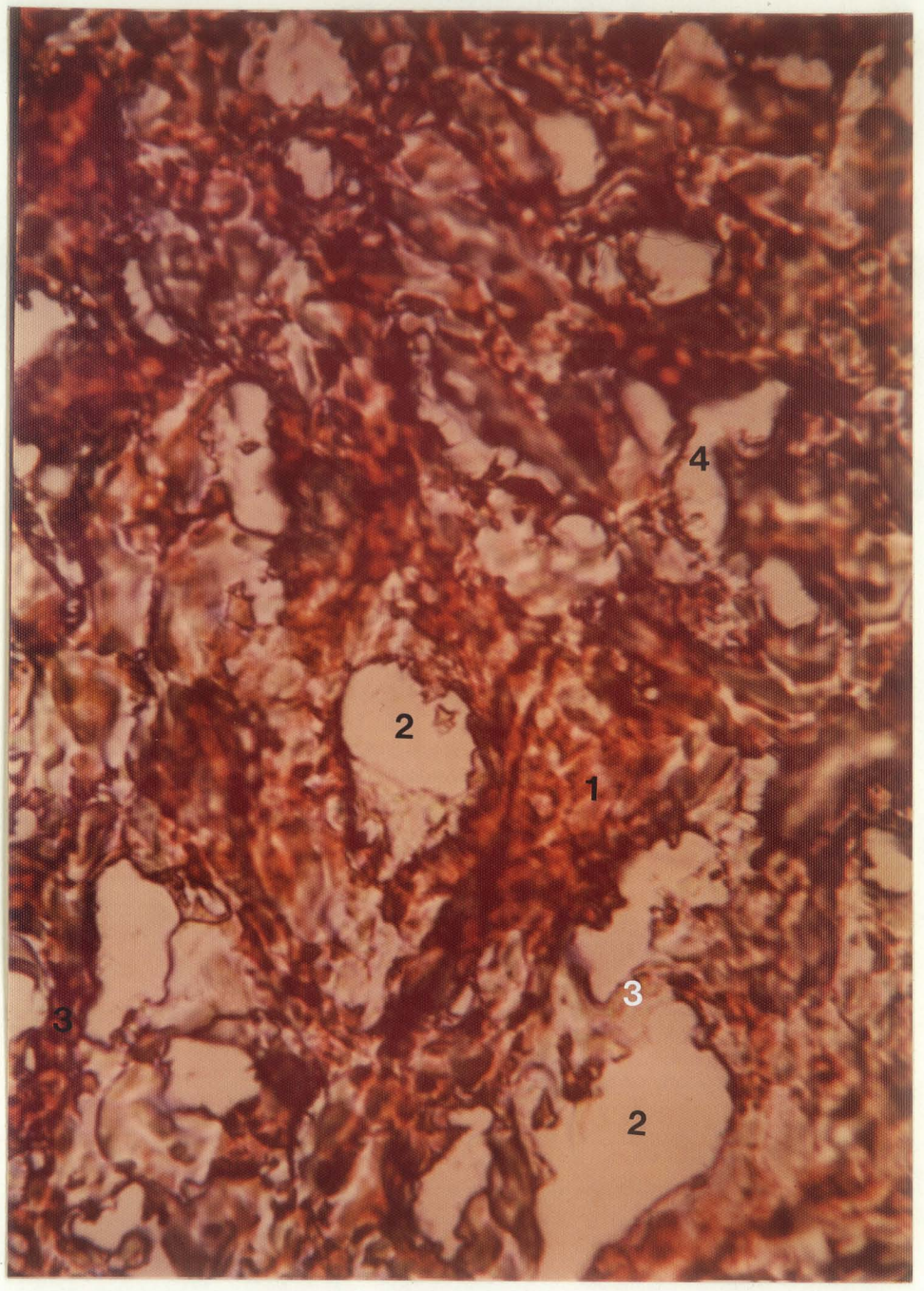
613 X

⊢ 10 μ

Figure 12

Light Micrograph Showing Protein Network in Bread A

1 - protein mass
2 - gas cells
3 - cell wall
4 - washed out starch



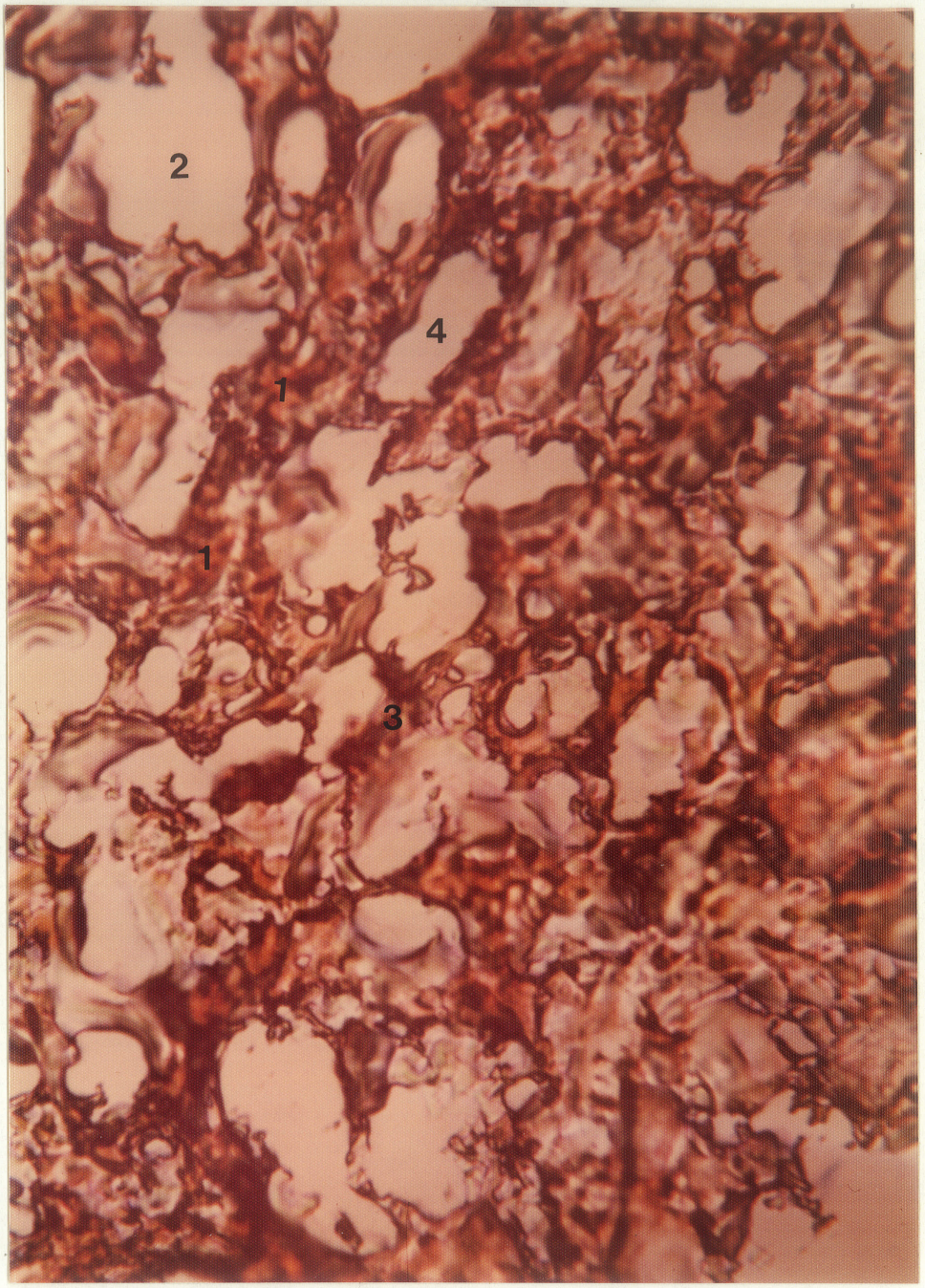
613 X

⊢ 10 μ

Figure 13

Light Micrograph Showing Protein Network in Bread B

1 - protein mass
2 - gas cells
3 - cell wall
4 - washed out starch



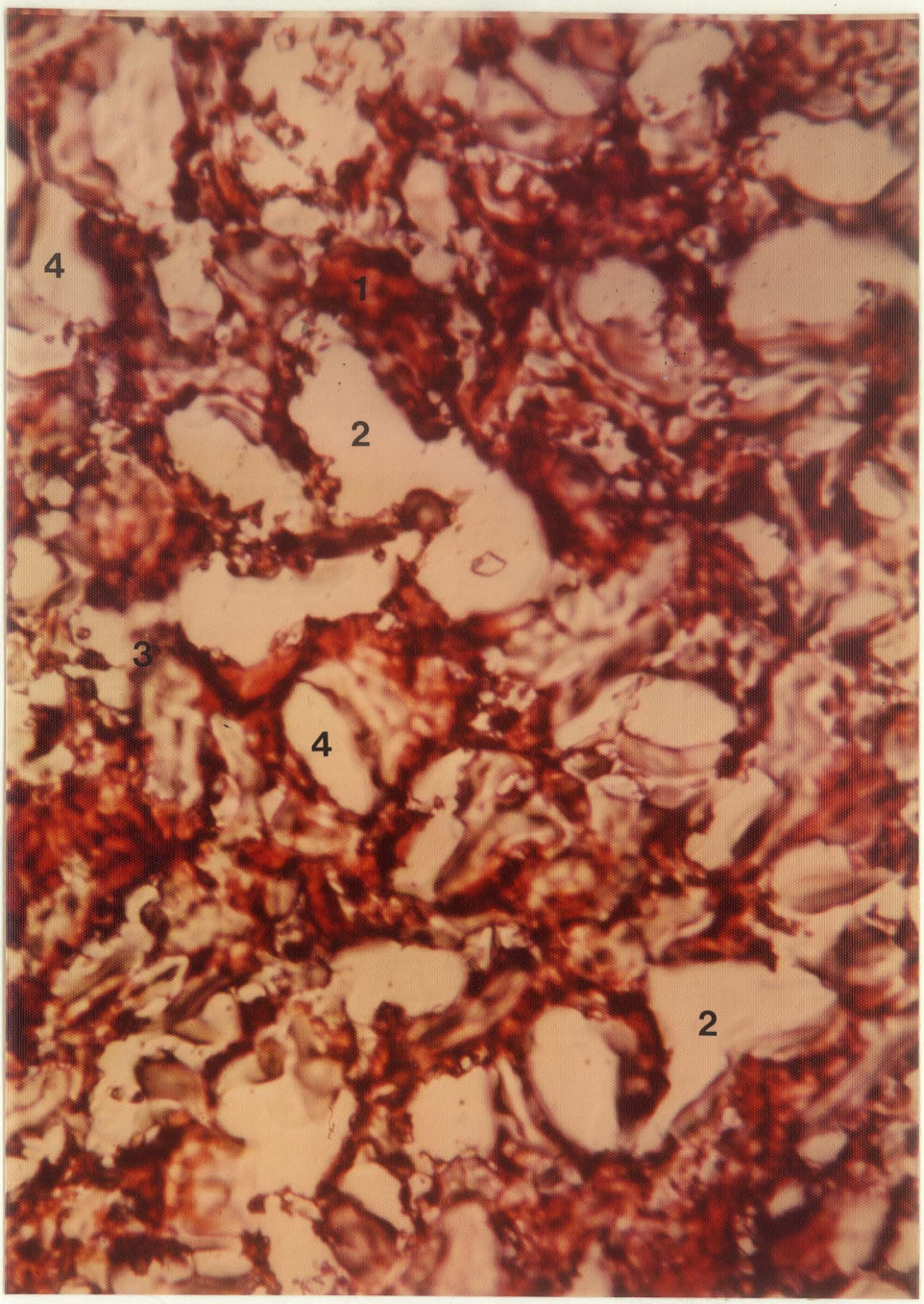
613 X

⊢ 10 μ

Figure 14

Light Micrograph Showing Protein Network in Bread C

1 - protein mass
2 - gas cells
3 - cell wall
4 - washed out starch



613 X

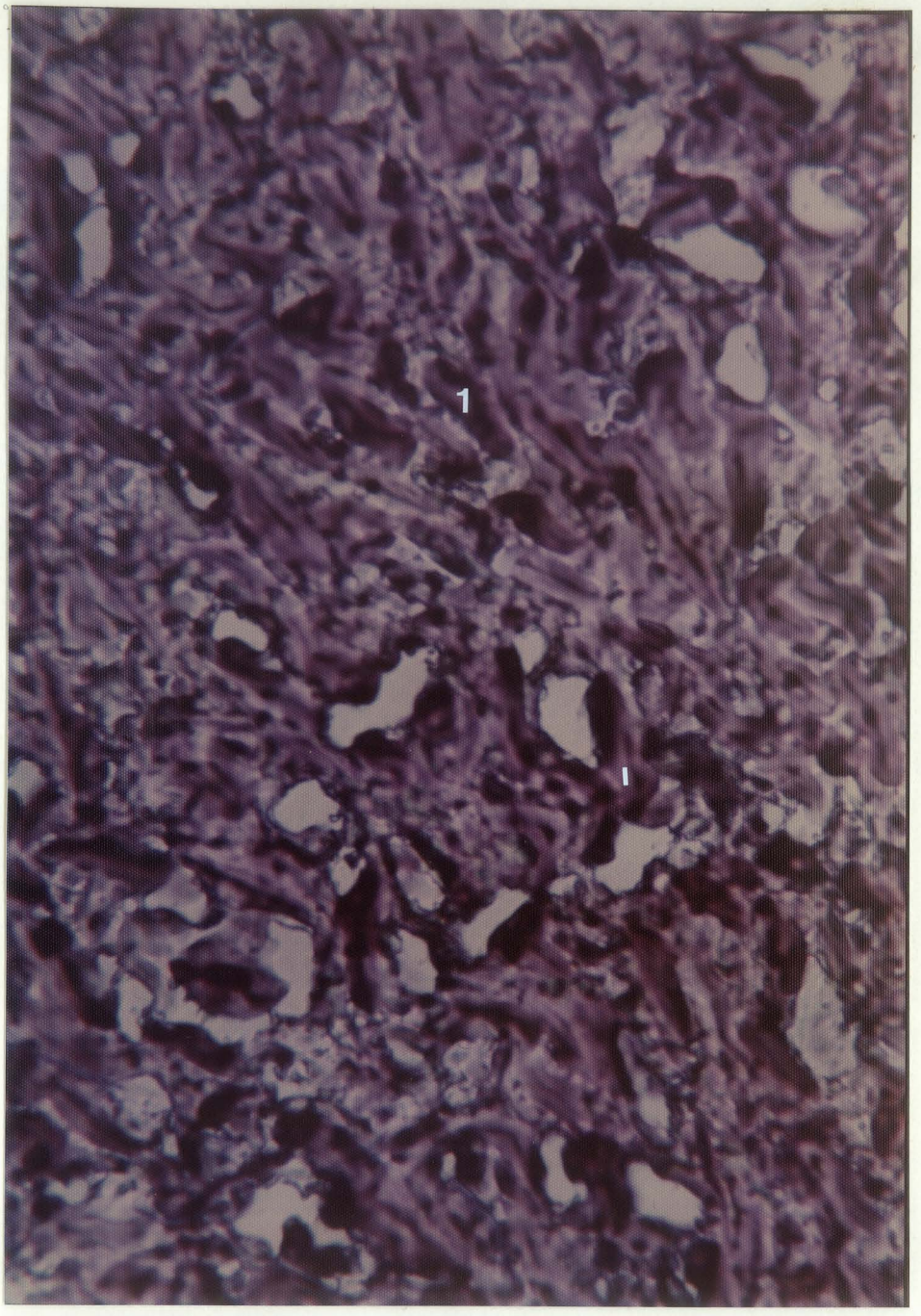
⊢⊣ 10 μ

bread, a discontinuous structure with broken cell walls and large gas cells was found in the supplemented breads (figures 12, 13 and 14). Some textural characteristics of bread appear to correspond to the thickness of the walls between gas cells. The thinness of the cell walls depends on the number of gas cells and the uniformity of the protein network. The larger the number of gas cells, the thinner will be the walls between the cells and the softer the crumb and grain of bread (Burhans and Clapp, 1941). The thin cell walls with a large number of small gas cells are the characteristics of high leavened bread. The strength of a dough is due to the strong adherence of protein to starch (Sandstedt et al., 1954). The protein network and starch in supplemented breads A, B and C did not adhere well. Some of the large pores in the sections of breads A, B and C (figures 12, 13 and 14) resulted when starch granules were washed out of the protein film during the staining procedure. This indicates the loose interaction between the starch granules and the protein network in the supplemented breads, as compared to the strong adherence of protein and starch in wheat bread. If starch is not adhered to the protein network, during gelatinization, it will become separate granules in the film, causing a gritty texture in bread (Murray,

Figure 15

Light Micrograph Showing Starch Granules in Wheat Bread

1 - starch granules



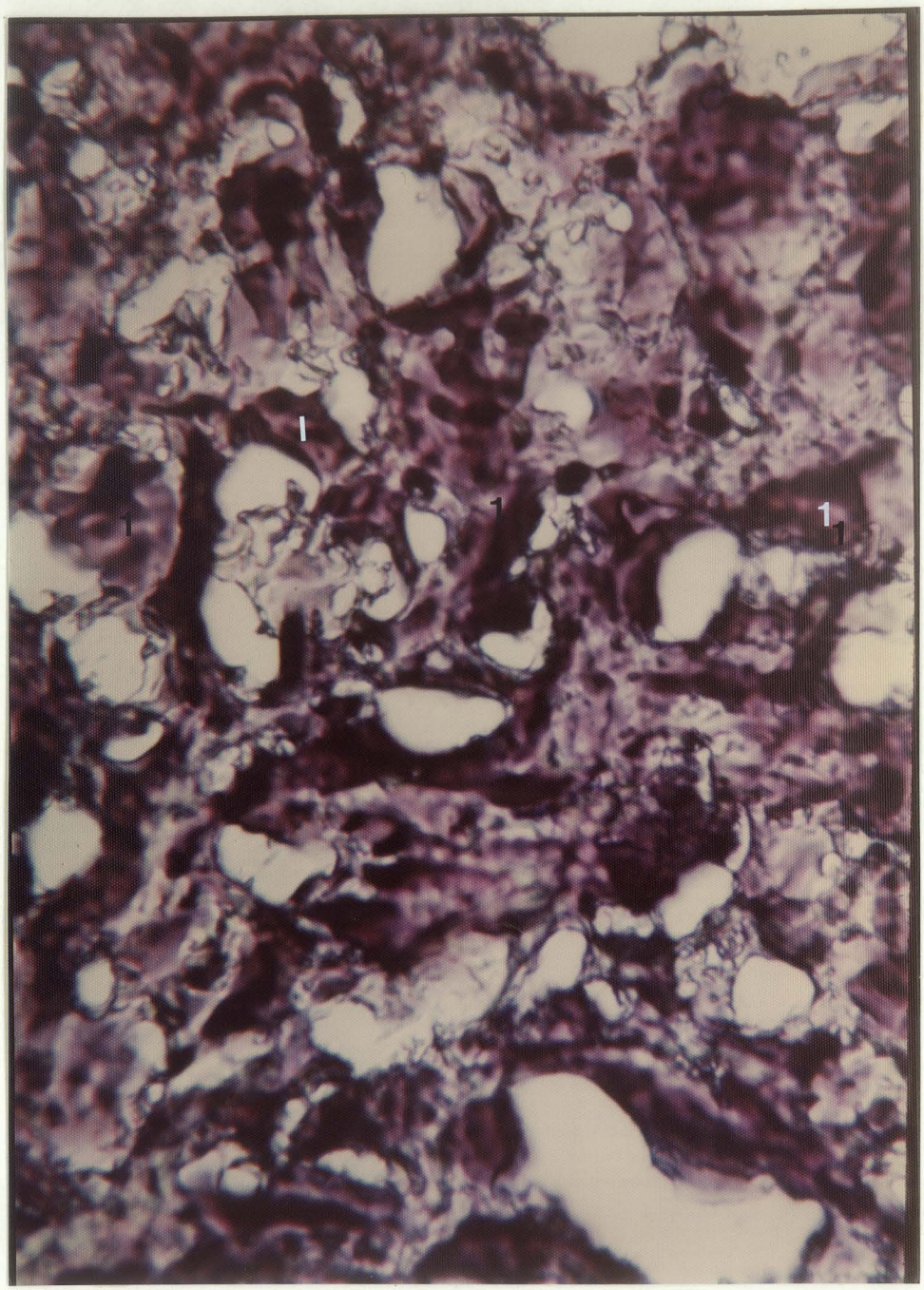
613 X

⊢⊣ 10 μ

Figure 16

Light Micrograph Showing Starch Granules in Bread A

1 - starch granules



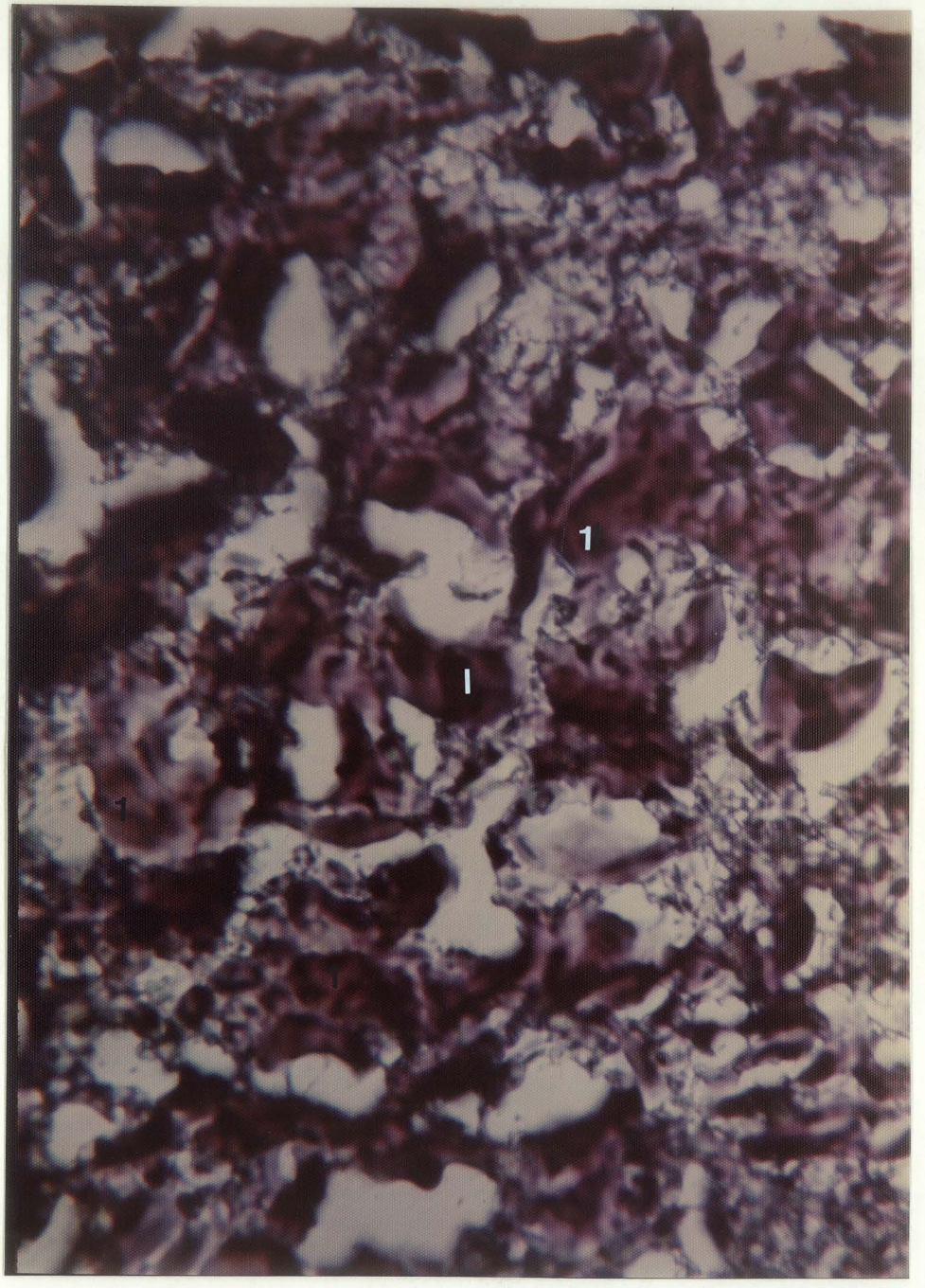
613 X

⊢ 10 μ

Figure 17

Light Micrograph Showing Starch Granules in Bread B

1 - starch granules



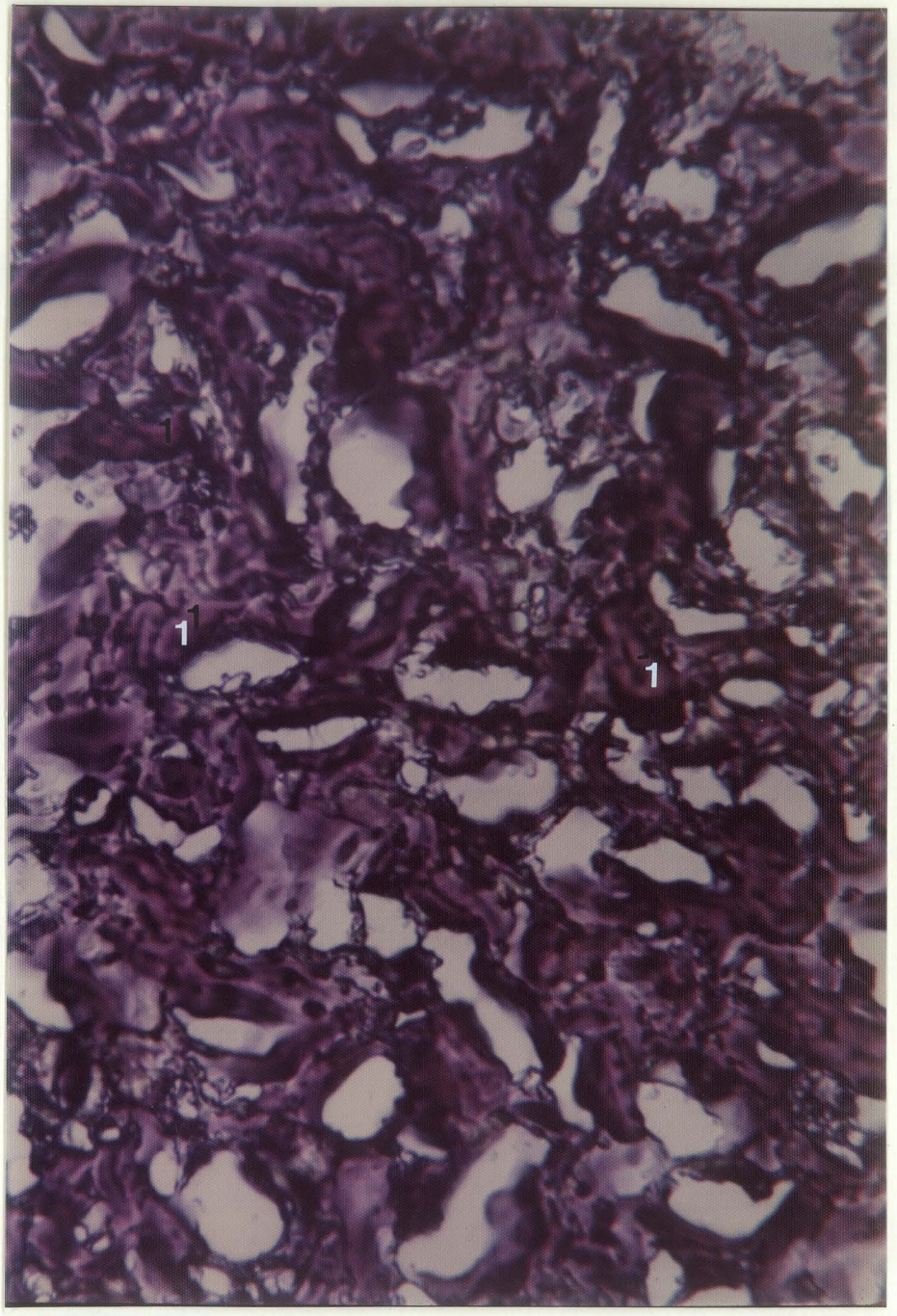
613 X

⊢⊣ 10 μ

Figure 18

Light Micrograph Showing Starch Granules in Bread C

1 - starch granules



613 X

⊢⊣ 10 μ

1980). This occurred in faba bean supplemented breads A, B and C and gritty texture was perceived during the evaluation of bread. Only granules which are 30 microns or greater in diameter may be detected in the mouth as grit. The size of starch granules in faba beans range from 10 to 40 microns (McEwen, 1974).

The photographs in figures 15, 16, 17 and 18, represent the sections of bread samples stained with iodine to identify the starch granules. The starch granules were uniformly distributed in the protein network both in wheat bread and supplemented breads. The shape of some of the starch granules had been altered due to partial gelatinization during baking. In all bread samples, the partially gelatinized starch granules retained their identity and did not disintegrate.

4.6 General Discussion

The beneficial role that faba bean supplementation can play in improving the protein quality of bread has been shown in this study. Because of the high quantity and quality of protein, faba bean will play a major role in alleviating the protein shortages, both for nutritional

and economical reasons. The nutritional value (PER) significantly increased in bread C compared to that of bread A. The level of supplementation in bread C (50:50 protein basis) is considered very close to the optimum. Only very slight differences in the sensory properties of wheat bread and supplemented bread were detected. It is expected that faba bean supplemented bread will meet with consumers acceptance. Supplementation of barbary bread with faba bean flour did introduce some antinutritional factors. These factors were partially inactivated during bread processing. The more complete inactivation of antinutritional factors would, of course, be advantageous. More research into the basic biochemical conditions involved in the destruction of these antinutritional factors is needed. However, in many cases, studies on experimental animals alone are not sufficient to eliminate all possibilities of adverse reactions in man. Symptoms which may appear in humans, such as favism, may not arise in experimental animals. Therefore, basic biochemical research into the properties of antinutritional factors should be followed by clinical trials. This would lead to possibilities of adopting

processing techniques resulting in greater degradation of antinutritional factors in the diet.

Chapter 5

SUMMARY AND CONCLUSIONS

Wheat bread as a source of protein and calories plays an important role in the diet of a large portion of the world's population. However, the protein in wheat bread is of low nutritional quality because it is deficient in certain amino acids, especially lysine.

The main objective of this study was to evaluate the nutritional and sensory properties of faba bean supplemented Iranian barbary bread. Wheat flour was supplemented with faba bean flour in order to replace 30, 40 and 50% of the wheat protein with equal amounts of faba bean protein. The following factors were investigated:

1. Nutritional value of protein by means of PER and chemical scores.
2. The level of antinutritional factors, namely: trypsin inhibitors, phytic acid, vicine and convicine and the degree of their degradation during processing.
3. The texture and flavor by means of sensory evaluation.
4. The microstructure of bread using light microscopy.

The following conclusions can be drawn from this study:

1. The addition of increasing amounts of faba bean flour to wheat flour in the bread formula caused an increase in both protein content and PER values.
2. Bread supplemented with 40% and 50% faba bean (protein basis) with protein ratings of 29.4 and 33.4, are considered good dietary sources of protein.
3. The content of the amino acid lysine, increased in supplemented bread due to the addition of faba bean flour.
4. Sulfur-containing amino acids became the limiting amino acid in supplemented breads.
5. The recovery rate of amino acids was higher in the crumb than in the crust of supplemented bread.
6. The levels of antinutritional factors (trypsin inhibitors, phytic acid, vicine and convicine) were lower in bread samples than the levels in raw materials, indicating some degradation during the bread making process. More research is needed in order to determine the optimum processing conditions for more complete inactivation of these factors.

7. The sensory properties of bread were retained to a large degree in the supplemented bread.
8. Some textural properties can be accounted for by an examination of the microstructure. For example, the gritty texture in supplemented bread perceived by panelists, was explained by the loose bonding between the starch granules and protein network.

It can be concluded that barbary bread supplemented with 40 and 50% faba bean flour (protein basis) is a good dietary source of protein and is of higher nutritional quality than wheat bread. The overall acceptability of supplemented breads is expected. It is hoped that the data presented in this manuscript will be helpful in assessing the utilization of faba bean in bread production in countries where bread is the main source of dietary protein.

B I B L I O G R A P H Y

Agriculture Handbook No. 8 (Revised 1963). Composition of food. United States Department of Agriculture.

Amerine, M.A., Pangborn, R.M., and Rosessler, E.B., (1965). Principles of sensory evaluation of food. Academic Press, New York, San Francisco, London.

Angold, R. (1975). Wheat starch (structure aspects). In: Bread. Arnold Spicer (editor). Applied Science Publishers Ltd., London.

AOAC (1975). Official Methods of Analysis. 12th ed. Assn. Offic. Anal. Chem. Washington, D.C.

Aykroyd, W.R., and Doughty, J. (1970). Wheat in human nutrition. Food and Agriculture Organization of the United Nations (FAO). Rome.

Aylward, F. (1972). Proteins and nutrition policy. In: Protein in Human Nutrition. Porter, J.W.G., and Rolls, B.A. (editors). Academic Press, London, New York.

Bass, J.L., and Caul, J.F. (1972). Laboratory evaluation of three protein sources for use in chapati flours. J. Food Sci. 37:100-102.

Barre, R. (1956). Influence of phytic acid on peptic digestion of various proteins. Ann. Pharm. Fr. 14:183-193.

Bhatty, R.S. (1974). Chemical composition of some faba bean cultivars. Can. J. Plant Sci. 54:413-421.

Bhatty, R.S. (1975). Trypsin inhibitors of faba bean (Vicia faba L. var. minor). 1. Preliminary studies. Can. J. Bot. 53:3075-3077.

Bhatty, R.S. (1977). Trypsin inhibitor activity in faba bean (Vicia faba var. minor), changes during germination and distribution. Can. J. Plant. Sci. 57:979.

Bigwood, E.J. (1972). Amino acid patterns of animal and vegetable proteins. In: Protein and Amino Acid Functions. Bigwood, E.J. (editor) Pergman Press, Oxford, New York, Toronto.

Bitar, K., and Reinhold, J.G. (1972). Phytase and alkaline phosphatase in intestinal mucosa of rat, chicken, calf and man. Biochem. Biophys. Acta 268:412.

Bloksma, A.H. (1971). Rheology and chemistry of dough. In: Wheat, Chemistry and Technology. Pomeranz, Y. (editor). American Association of Cereal Chemists. St. Paul, Minnesota.

Burhans, M.E., and Clapp, J. (1941). A microscopic study of bread and dough. Cereal Chem. 19:196-216.

Campbell, J.A. (1960). Evaluation of protein in foods for regulatory purposes. J. Agric. Food Chem. 8(4):323-327.

Chang, C.R., and Tsen, C.C. (1979). Note on trypsin inhibitor activity in the acetate extract of cereal samples. Cereal Chem. 56(5):493-494.

Chang, R., and Schwimmer, S. (1977). Characterization of phytase of beans (phaseolus vulgaris). J. Fd. Biochem. 1(1):45-56.

Chen, P.S. Jr., Toribara, T.Y., and Huber, W. (1956). Microdetermination of phosphorus. Analytical Chem. 28(11):1756-1758.

Civille, G.V., and Szczesniak, A.S. (1973). Guidelines to training a texture profile panel. J. of Texture Studies. 4:204-223.

deRham, O., and Jost, T. (1979). Phytase - protein interactions in soy bean extracts and low - phytate soy protein products. J. Fd. Sci. 44(2):596-600.

El-Dash, A.A., and Johnson, J.A. (1970). Influence of yeast fermentation and baking on the content of free amino acids and primary amino groups and their effect on bread aroma stimuli. Cereal Chem. 47:247-259.

El-Dash, A.A., and Sgarbieri, V.C. (1980). Sweet-lupine fortified bread: nutritional value and amino acid content. Cereal Chem. 57(1):9-11.

Fabro, J.M. (1979). Infant acceptance and trained panel evaluation of model taste and texture systems. M.Sc. Thesis, University of Manitoba, Winnipeg, Manitoba.

FAO Pattern (1957). In World Health Organization Technical Report. Series No. 522. Report of Joint FAO/WHO. Geneva (1973).

Fleming, S.E., and Sosulski, F.W. (1977a). Bread making properties of four concentrated plant proteins. Cereal Chem. 54(5):1124-1140.

Fleming, S.E., and Sosulski, F.W. (1977b). Nutritive value of bread fortified with concentrated plant proteins and lysine. Cereal Chem. 54(6):1238-1248.

Fleming, S.E., and Sosulski, F.W. (1978). Microscopic evaluation of bread fortified with concentrated plant proteins. Cereal Chem. 55:373-383.

Fontaine, T.D., Pons, W.A. Jr., and Irving, W.G. Jr. (1946). Protein-phytic acid relationship in peanuts and cotton seeds. J. Biol. Chem. 164:487-507.

Ford, J.E. (1973). Some effects of processing on nutritive value. In: Proteins in Human Nutrition. Porter, J.W.G., and Rolls, B.A. (editors). Academic Press, London and New York.

Harland, B.F., Oberleas, D. (1977). A modified method for phytate analysis using an ion-exchange procedure: Application to textural vegetable proteins. Cereal Chem. 54(4):827-832.

Health and Welfare Canada, Revised (1975). Nutrient Value of Some Common Foods.

Hoehn, E., Vaisey, M., and Medeiros, A.F. (1976). Note on seed coat effects on color and flavor of faba bean flours. Cereal Chem. 53(4):597-601.

Horn, M.J., Fifield, C.C., Blum, E., and Warren, H.W. (1958). The distribution of amino acids in wheat and certain wheat products. Cereal Chem. 35:411-421.

Hussein, L., Gabrial, G.N., and Morcos, S.R. (1974). Nutritional value of mixtures of baladi, bread and broad beans. J. Sci. Fd. Agric. 25:1433-1440.

Hutchinson, J.B., Moran, T., and Pace, J. (1960). The quality of the protein in germ and milk bread as shown by the growth of weanling rats: the significance of the lysine content. J. Sci. Fd. Agric. 11:576-582.

Jamalian, J., Aylwood, F., and Hudsun, B.J.F. (1976). Favism inducing toxins in broad beans (vicia faba) examination of bean extract for pyrimidine glucosides. Qual. Plant Pl. Fds. Hum. Nutr. 26(4):331-339.

Jamalian, J. (1978). Favism inducing toxins in broad beans (vicia faba). Determination of vicine content and investigation of other non-protein nitrogenous compounds in different broad bean cultivars. J. Sci. Fd. Agric. 29:136-140.

Jansen, G.R. (1969). Total protein value of protein and amino acid supplemented bread. The Am. J. of Clin. Nutr. 22(1):38-43.

Jansen, G.R. (1978). Biological evaluation of protein quality. Fd. Technol. 32(12):52-56.

Kakade, M.L., Simons, N., and Liener, I.E. (1969). Nutritional effects induced in rats by natural and synthetic trypsin inhibitors. J. of Nutr. 100:1003-1008.

Kakade, M.L. (1974). Biochemical basis for the differences in plant protein utilization. J. Agric. Fd. Chem. 22(4):550-555.

Kakade, M.L., Rackis, J.J., McGhee, J.E., and Puski, G. (1974). Determination of trypsin inhibitor activity of soy products: a collaborative analysis of an improved procedure. Cereal Chem. 51:376-382.

Kaldy, M.S. (1978). Amino acid composition and protein quality of two faba bean cultivars. Can. Inst. Food Sci. Technol. J. 11(2):97-98.

Kent-Jones, D.W., and Amos, A.J. (1967). Modern cereal chemistry. Taylor Garnett Evans and Co. Ltd., Watford, Herts.

Kim, S.I. (1980). Analytical determination and stability of vicine and convicine in food products. M.Sc. Thesis, Department of Food Science, University of Manitoba, Winnipeg, Manitoba.

Kouhestani, A., Ghavifekr, H., Rahmanian, M., Mayurian, H., and Sarkissian, N. (1969). Composition and preparation of Iranian breads. J. of Am. Diet. Assoc. 55:262-266.

Latta, M., and Eskin, N.A.M. (1980). A simple and rapid colorimetric method for phytate determination. J. Agric. Food Chem. (In press).

Liener, I.E. (1958). Effect of Heat on Plant Proteins In: Processed Plant Protein Foodstuffs. Altschul, A.M. (editor). Academic Press, New York.

Liener, I.E., and Kakade, M.L. (1969). Protease inhibitors. In: Toxic Constituents of Plant Foodstuffs. Liener, I.E. (editor). Food Science and Technol. A series of monograph. Academic Press, N.Y., London.

Liener, I.E. (1973). Toxic factors in protein foods. In: Proteins in Human Nutrition. Porter, J.W.G ., and Rolls, B.A. (editors). Academic Press, London, New York.

Lolas, G.M., and Markakis, P. (1975). Phytic acid and other phosphorus compounds of beans. J. Agric. Fd. Chem. 23(1):13-15.

Lolas, G.M., and Markakis, P. (1976). The phytase of navy beans (phaseolus vulgaris). Abstract, No. 27 - 36th annual meeting. Institute of Food Technologists. Anaheim, Calif.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. J. of Bio. Chem. 193:265-275.

Mager, J., Razin, A., and Hershko, A. (1969). Favism. In: Toxic Constituents of Plant Products. Liener, I.E. (editor). Food Science and Technology, Academic Press, New York, London.

Malcolmson, L.J., and McDaniel, M.R. (1979). Magnitude estimation of infant foods. II. Taste, texture and odor of infant formulas. Can. Inst. Food Sci. Technol. J. (In press).

Malcolmson, L.J., and McDaniel, M.R. (1980a). Magnitude estimation of infant foods. II. Taste, texture and odor of infant formula. Can. Inst. Fd. Sci. Technol. (In Press).

Malcolmson, L.J., and McDaniel, M.R. (1980b). Magnitude estimation of infant foods. III. Taste and texture of infant cereals. Can. Inst. Fd. Sci. Technol. (In press.)

Maleki, M., and Djazayeri, A. (1968). Effect of baking and amino acid supplementation on the quality of arabic bread. J. Sci. Fd. Agric. 19:449-451.

Mandal, N.C., Burman, S., and Biswas, B.B. (1972). Isolation, purification and characterization of phytase from germinating mung beans. Phytochem. 11:495-502.

Mauron, J. (1972). Influence of industrial and household handling on food protein quality. In: Protein and Amino Acid Functions. Bigwood, E.J. (editor). Pergman Press, Oxford, New York.

Mayer, F.C., Campbell, R.E., Smith, A.K., and McKinney, L.L. (1961). Soy bean phosphatase purification and properties. Arch. Biochem. Biophys. 94:301-307.

McConnell, L.M., Simmonds, D.H., and Bushuk, W. (1974). High-protein bread from wheat-faba bean composite flours. Cereal Science Today, 19(11):517-521.

McDonald, B.E. (1979). Nutritional assessment of faba bean enriched wheat-flour breads. The Dept. of Foods and Nutrition, University of Manitoba, Winnipeg. (Personal communication).

McEwen, T.J. (1974). Physiochemical properties of faba bean and its components. Ph.D. Thesis. Dept. of Plant Science. University of Manitoba, Winnipeg, Manitoba.

Messino, L., and Mussarra, E. (1972). A sensitive spectrophometric method for the determination of free or bound tryptophan. Inst. J. Bioch. 3:700-704.

Mitchell, H.H., and Block, R.J. (1946). Some relationships between the amino acid contents of protein and their nutritive values for the rat. J. Biol. Chem. 163:599-620.

Mollgaard, H. (1946). On phytic acid, its importance in metabolism and its enzymic cleavage in bread supplemented with calcium. Biochemical J. 40:589-603.

Morrison, A.B., and McLaughlan, J.M. (1972). Availability of amino acids in foods. In: Proteins and Amino Acid Functions. Bigwood, E.J. (editor). Pergman Press, Oxford, New York.

Moskowitz, H.R., and Chandler, J.W. (1977). New uses of magnitude estimation. In: Sensory Uses of Food. Birch, G.C., Brennan, J.G., and Parker, K.J. (editors). Applied Science Publishers Ltd., London.

Murray, E.D. (1980). Personal communications. Dept. of Food Science, University of Manitoba, Winnipeg, Manitoba.

Nitsan, Z. (1971). Vicia faba beans vs soy bean meal as a source of protein. J. Sci. Fd. Agric. 22:252-255.

O'Dell, B.L., and deBoland, A. (1976). Complexation of phytate with proteins and cations in corn germ and oil seed meals. J. Agric. Fd. Chem. 24(4):804-808.

Okubo, K., Waldrop, A.B., Iacobucci, G.A., and Mayers, D.V., (1975). Preparation of low phytate soybean protein isolate and concentrate by ultra filteration. Cereal Chem. 52:263-270.

Okubo, K., Mayers, D.V., and Iacobucci, G.A. (1976). Binding of phytic acid to glycinine. Cereal Chem. 53:513-524.

Osborne, T.B., Mendel, L.B., and Ferry, E.L. (1919). A method of expressing numerically the growth promoting value of proteins. J. Biol. Chem. 37:223.

Patel, K.M., and Johnson, J.A. (1975). Horse bean protein supplements in bread making. II. Effect on physical dough properties, baking quality and amino acid composition. Cereal Chem. 52:791-799.

Patel, K.M., Caul, J.F., and Johnson, J.A. (1977). Horse bean as protein supplement in bread making. III. Effects of horse bean protein on aroma and flavor profile of Moroccan-type bread. Cereal Chem. 54(2): 379-387.

Peers, F.G. (1953). The phytase of wheat. Biochemical J. 53:102-110.

Pieniazek, D., Grabarek, Z.,and Pakowska, M. (1975). Quantitative determination of the content of available methionine and cystine in food proteins. Nutr. Metabol. 18:16-22.

Pomeranz, Y. (1971). Composition and functionality of wheat flour components. In: Wheat, Chemistry and Technology. Pomeranz, Y. (editor). American Association of Cereal Chemists, St. Paul, Minnesota.

Ponte, J.G. (1971). Bread. In: Wheat Chemistry and Technology. Pomeranz, Y. (editor). American Association of Cereal Chemists, St. Paul, Minnesota.

Presber, A.A.W. (1972). An inquiry into the origin, cultivation and utilization of the small faba bean in Australia. The Federal Republic of Germany and England. A report published by the Canada Grains Council, Winnipeg, Canada.

Reinhold, J.G. (1971). High phytate content of rural Iranian bread: a possible cause of human zinc deficiency. The Amer. J. Clin. Nutr. 24:1204-1206.

Reinhold, J.G., Parsa, A., Karimian, N., Hammick, J.W., and Ismail-Beigi, F. (1974). Availability of zinc in leavened and unleavened whole meal wheaten breads as measured by solubility and uptake by rat intestine in vitro. J. of Nutr. 104:976-982.

Reinhold, J.G. (1975). Phytate destruction by yeast fermentation in whole wheat meals. J. of the Amer. Diet. Assoc. 66:38-41.

Reinhold, J.G., Ismail-Beigi, F., and Faradji, B. (1975). Fibre vs phytate as determinant of the availability of calcium, zinc and iron of breadstuffs. Nutrition Reports International 12(2):75-85.

Ronaghi, M.A., and Solter, S.I. (1973). The auxiliary health worker in Iran. Lancet 2:427.

Rose, W.C. (1937). The nutritive significance of the amino acids and certain related compounds. Science 86:298.

Ryan, C.A. (1973). Proteolytic enzymes and their inhibitors in plants. Ann. Rev. Plant Physiol. 24:173.

Sandstedt, R.M., Schoumburg, L., and Fleming, J. (1954). The microstructure of bread dough. Cereal Chem. 31:43-49.

Sarwar, G., Sosulski, F.W., and Bell, J.M. (1975). Nutritive value of field pea and faba bean proteins in rat diets. J. Inst. Can. Sci. Tech. Aliment. 8(2):109-112.

Sarwar, G., Sosulski, F.W., and Bell, J.M. (1977). Availability of amino acids in legumes and legume-wheat blends. Can. Inst. Fd. Sci. Technol. J. 10(1):31-35.

Shehata, N.A., and Fryer, B.A. (1970). Effect on protein quality of supplementing wheat flour with chick pea flour. Cereal Chem. 47(6):663-670.

Sherman, P. (1970). Industrial Rheology, Academic Press, London.

Shyamala, G., and Lyman, R.L. (1964). The isolation and purification of a trypsin inhibitor from whole wheat flour. Can. J. Biochem. Physiol. 42:1825-1832.

Snedecor, G.W. (1956). Statistical Methods. 5th ed. Iowa State College Press, Iowa State College, Ames, Iowa.

Spackman, D.H., Stein, W.H., and Moore, S. (1958). Automatic recording apparatus for use in chromatography of amino acids. Anal. Chem. 30:1190-1206.

Stevens, S.S. (1960). The psychophysics of sensory function. American Scientist 48:226-253.

Szczesniak, A.S. (1977). An overview of recent advances in food texture research. Food Technol. pp. 71-75.

Tabekhia, M.M., Toma, R.B., and D'appolonia, B.L. (1978). Macromineral and micromineral distribution in bread produced by conventional and continuous (mechanical development) method. Cereal Chem. 55(6):1050-1055.

Von Sydow, E. (1971). Flavor, a chemical or psychophysical concept. Food. Technol. 25:40-44.

Warsy, A.S., Norton, G., and Stein, M. (1974). Protease inhibitors from broad bean. Isolation and purification. Phytochemistry 13:2481-2486.

Wassermann, L. (1979). Physikolische Eigenschaften von Brot. L.T. 12(3):5-12.

Wilson, B.J., McNab, J.M., and Bentler, H. (1972). Trypsin inhibitor activity in field bean (vicia faba, L.) J. Sci. Fd. Agric. 23:679-684.

Appendix 1

Amino Acid Composition of Ingredient Flours and Bread Samples

(g amino acid/100 g protein)

Amino Acid	Wheat Flour	Faba Bean Flour	Bread A	Bread B	Bread C
Lysine	2.32	6.57	3.28	3.57	3.92
Methionine	1.57	0.54	1.28	1.04	1.11
Cystine	1.29	0.36	0.84	0.83	0.69
Threonine	2.95	3.77	3.35	3.37	3.45
Isoleucine	3.74	4.16	3.96	3.96	4.08
Leucine	7.51	8.01	7.72	7.77	7.92
Valine	4.32	4.73	4.53	4.45	4.68
Phenylalanine	5.44	4.32	5.17	5.03	5.92
Tyrosine	2.98	3.47	3.25	3.36	3.37
Tryptophan	1.28	1.13	1.07	0.75	0.71
Histidine	2.27	2.68	2.35	2.42	2.47
Arginine	4.06	11.35	6.0	6.67	7.26
Aspartic Acid	4.38	11.82	6.87	7.59	8.19
Serine	5.22	5.40	5.24	5.31	5.33
Glutamic Acid	38.91	19.25	34.28	32.18	30.68
Proline	12.61	4.69	10.19	9.68	9.36
Glycine	3.69	4.39	4.02	4.07	4.16
Alanine	3.16	4.32	3.56	3.68	3.70

Appendix 2

Calculation of Expected Values and the Degree of Degradation of Antinutritional Factors

The expected values for phytic acid, trypsin inhibitors, vicine and convicine etc., were calculated as below:

e.g.

expected value for phytic acid content in bread A

% wheat flour in bread A formula x % phytic acid in wheat + % faba bean flour in bread A formula x % phytic acid in faba bean

The percent degradation was calculated by dividing the amount of each factor in bread by expected value of that factor and multiplying by 100.

Appendix 3

Analysis of Variance for Textural Characteristics of Bread Samples

(a) Analysis of Variance for Chewiness of Bread

Source	DF	SS	MS	F
Replications	1	0.006	0.006	-
Breads	4	0.247	0.062	14.062*
Panelists	8	0.000	0.000	0.000
Breads - Panelists	32	0.367	0.011	2.609
Error	44	0.194	0.004	
Total	89	0.814		

* Significantly different at $p \leq .05$.

Appendix 3 (b)

Analysis of Variance for Springiness of Bread

Source	DF	SS	MS	F
Replications	1	0.044	0.044	-
Breads	4	0.226	0.057	1.182 ns
Panelists	8	0.000	0.000	0.000
Breads - Panelists	32	2.531	0.079	1.654
Error	44	2.104	0.048	
Total	89	4.905		

ns = not significant at $p \leq .05$.

Appendix 3 (c)

Analysis of Variance for Firmness (A) of Bread

Source	DF	SS	MS	F
Replications	1	0.018	0.018	-
Breads	4	0.376	0.094	2.660*
Panelists	8	0.000	0.000	0.000
Breads - Panelists	32	1.110	0.035	0.981
Error	44	1.557	0.035	
Total	89	3.062		

* Significantly different at $p \leq .05$.

Appendix 3 (d)

Analysis of Variance for Firmness (B) of Bread

Source	DF	SS	MS	F
Replications	1	0.001	0.001	-
Breads	4	0.314	0.079	2.830*
Panelists	8	0.000	0.000	0.000
Breads - Panelists	32	1.209	0.038	1.362
Error	44	1.221	0.028	
Total	89	2.745		

* Significantly different at $p \leq .05$.

Appendix 3 (e)

Analysis of Variance for Adhesiveness of Bread

Source	DF	SS	MS	F
Replications	1	0.002	0.002	-
Breads	4	3.625	0.906	18.140*
Panelists	8	0.000	0.000	0.000
Breads - Panelists	32	4.103	0.128	2.566
Error	44	2.198	0.050	
Total	89	9.929		

* Significantly different at $p \leq .05$.

Appendix 3 (f)

Analysis of Variance for Grittiness of Bread

Source	DF	SS	MS	F
Replications	1	0.008	0.008	-
Breads	4	3.598	0.900	35.718 *
Panelists	8	0.000	0.000	0.000
Breads - Panelists	32	4.908	0.153	6.089
Error	44	1.108	0.025	
Total	89	9.622		

* Significantly different at $p \leq .05$.

Appendix 3 (g)

Analysis of Variance for Mouthcoating of Bread

Source	DF	SS	MS	F
Replications	1	0.006	0.006	-
Breads	4	1.830	0.458	10.664 *
Panelists	8	0.000	0.000	0.000
Breads - Panelists	32	2.817	0.088	2.052
Error	44	1.888	0.043	
Total	89	6.541		

* Significantly different at $p \leq .05$.

Appendix 4

Raw Data of Panelists Scores for Flavor Characteristics of Bread Samples

(a) Panelists Scores for Flavor Characteristics of Wheat Bread

Panelists	Wheat Bread			
	Sweetness	Sourness	Bitterness	Beany
1	10	np	np	np
2	3	np	5	3
3	4	np	np	np
4	6	np	1	1
5	15	7	5	5
6	4	1	np	np
7	5	np	np	5
8	2	np	np	np
9	5	5	np	np

Appendix 4 (b)

Panelists Scores for Flavor Characteristics of Bread A

Panelists	Bread A			
	Sweetness	Sourness	Bitterness	Beany
1	1	5	2	5
2	np	np	2	3
3	3	5	1	3
4	1	np	np	2
5	15	10	5	3
6	np	10	np	np
7	np	1	np	np
8	np	5	2	5
9	5	10	np	2

Appendix 4 (c)

Panelists Scores for Flavor Characteristics of Bread B

Panelists	Bread B			
	Sweetness	Sourness	Bitterness	Beany
1	2	np	5	1
2	np	np	5	10
3	4	5	1	3
4	3	np	np	1
5	12	10	5	3
6	5	10	np	np
7	15	10	np	np
8	np	5	15	18
9	5	1	1	1

Appendix 4 (d)

Panelists Scores for Flavor Characteristics of Bread C

Panelists	Bread C			
	Sweetness	Sourness	Bitterness	Beany
1	np	np	20	10
2	np	np	2	4
3	1	3	2	5
4	2	np	4	2
5	7	3	8	12
6	np	10	np	np
7	5	np	np	np
8	np	4	8	5
9	2	5	1	np

Appendix 5

Analysis of Variance for Flavor Characteristics of Bread Samples

(a) Analysis of Variance for Sweetness of Bread

Source	DF	SS	MS	F
Replications	1	0.123	0.123	-
Bread	4	9.264	2.316	44.433*
Panelists	8	0.000	0.000	0.000
Breads - Panelists	32	2.860	0.089	1.715
Error	44	2.293	0.052	
Total	89	14.539		

* Significantly different at $p \leq .05$.

Appendix 5 (b)

Analysis of Variance for Sourness of Bread

Source	DF	SS	MS	F
Replications	1	0.049	0.049	-
Bread	4	14.121	0.530	65.711*
Panelists	8	0.000	0.000	0.000
Breads - Panelists	32	5.478	0.171	3.186
Error	44	2.364	0.054	
Total	89	22.012		

* Significantly different at $p \leq .05$.

Appendix 5 (c)

Analysis of Variance for Bitterness of Bread

Source	DF	SS	MS	F
Replications	1	0.001	0.001	-
Bread	4	19.190	4.797	119.048*
Panelists	8	0.000	0.000	0.000
Breads - Panelists	32	6.177	0.193	4.790
Error	44	1.773	0.040	
Total	89	27.140		

* Significantly different at $p \leq .05$.

Appendix 5 (d)

Analysis of Variance for Beany Flavor of Bread

Source	DF	SS	MS	F
Replications	1	0.078	0.078	-
Breads	4	18.668	4.667	55.963*
Panelists	8	0.000	0.000	0.000
Breads - Panelists	32	11.138	0.348	4.174
Error	44	3.669	0.083	
Total	89	33.553		

* Significantly different at $p \leq .05$.