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PROTEIN QUALITY AND FUNCTIONALITY OF NAVY BEAN AND SESAME FLOUR IN BAKED PRODUCTS

BY

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ABSTRACT

PROTEIN QUALITY AND FUNCTIONALITY OF NAVY BEAN AND SESAME FLOUR IN BAKED PRODUCTS

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The effect of substitution of wheat flour with navy bean and sesame flours, alone or in combination, in bread and cookies were studied. Chemical analyses of sugar snap cookies and bread showed that protein content increased as the level of navy bean and sesame flour increased.

Cookies spread and top grain scores were reduced as the percentage of navy bean and sesame flour increased. Cookies with 20 and 30% of sesame were more tough and more force was needed to compress and break them. The force required to break and shear the cookies decreased with increasing level of navy bean substitution. Sensory data showed that cookies containing up to and including 20% navy bean substitution were scored as acceptable by most panelists. The panelists did not like the flavor of cookies when more than 10% sesame was used in cookies. Cookies with 20% substitution had a better baking properties and

organoleptic characteristics than those with 30% substitution level.

Farinograph studies showed increase in the water absorption and arrival time as well as decrease in stability as the level of navy bean and sesame flour increased from 10 to 20% in dough system. The bread flour substituted with 20% navy bean had the highest absorption among the flour blends.

The bread baked with navy bean and sesame flour showed no significant difference in volume and specific volume at 10% level of substitution. But they decreased as the level of substitution increased from 10 to 20%. Results for the compressability of the bread showed that by increasing the level of sesame flour from 10 to 20% breads were firmer in comparison with the control bread and those containing the other protein. Based on rheologic, baking and taste panel studies acceptable bread could be produced at the 6% or even up to 20% level of navy bean flour, but in case of sesame flour even 10% substitution were objectionable.

The lysine content of bread increased by increasing the navy bean flour and methionine content increased by increasing sesame flour. As the amount of navy bean flour in the bread increased the availability of lysine was also increased.

Protein quality of the diets were evaluated by Protein Efficiency Ratio (PER). Greater nutritional responses were obtained when bread was supplemented with navy bean than with sesame flour. The increase in growth rate and PER were in linear relationship with the navy bean content of the bread. The addition of sesame flour did not have much effect on improving the nutritional quality of the bread.

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INTRODUCTION

The supply of dietary protein must be increased to meet the requirements of the world's increasing population. Proteins of either vegetable or animal origin can be used. Although vegetable proteins are less expensive and more abundant than animal protein, the quantity and quality of proteins from vegetable sources are usually inferior to those from animal sources.

World food needs and supplies for the future invariably stress increasing dependence upon food grains. Grains supply more than 50% of all human energy in the world and in many countries provide nearly 70% of the total food intake. Cereal grains are often inadequate in quantity of protein as well as quality. The quality of cereal protein can be improved by supplementing them with synthetic amino acid(s), animal or vegetable proteins.

Wheat is a common cereal in much of the world. For human consumption, wheat flour is usually processed into baked products. Such wheat based baked goods as cakes, cookies and specially breads are popular foods and provide an excellent means of improving nutritional quality through incorporation of vegetable proteins.

A significant proportion in the diet of large segments

of the world's population comes from legumes. They constitute an excellent source of protein and energy to improve diets based on cereals and roots in low income countries. Common beans (Phaseolus vulgaris) are among the most important legumes in human nutrition in Latin America, Far East and parts of Africa and Asia. Two major nutritional problems have been associated with ingestion of beans. The first one is their deficiency in sulfur-containing amino acids and the second one is the presence of antinutritional factors such as trypsin inhibitors and hemagglutinins. Moderate heat treatment usually inactivate these deleterious substances and the nutritive value is improved. Moreover bean protein is rich in lysine and because of its high lysine content, beans exhibit a beneficial complementary effect when consumed with cereal proteins which are low in lysine.

Sesame (Sesamum indicum) seeds are rich source of oil, protein, calcium, phosphorous and oxalic acid. Sesame is rich in sulfur containing amino acids, particularly methionine, but they are low in lysine.

The objective of this study was to evaluate the effect of supplementation of wheat flour with navy bean and sesame flours. To achieve this, bread and cookies were baked from wheat flour with up to 30% of flour replaced by navy bean and sesame flour in cookies and up to 20% was replaced in bread. The chemical composition, baking properties and organoleptic characteristics of bread and cookies as well as the nutritive value of supplemented bread were determined.

REVIEW OF LITERATURE

Amino Acids and Protein Requirements

Protein is an essential component in the diet. The greatest single problem in the world today is insufficient distribution of protein foods. Without sufficient protein human beings have impaired physical and mental health (Jones, 1974).

People must consume sufficient protein to provide nitrogen and certain amino acids for the synthesis of new tissue during growth, gestation, lactation and for the maintenance of existing tissue. Tissue protein synthesis is a process which requires needed amino acids to be present simultaneously to form each specific protein. If a single amino acid is missing, the protein can not be synthesized.

Many studies have been carried out to establish definite requirements for the essential amino acids for both animal and man. Rose (1938, 1949) first classified the amino acids in a protein as essential and nonessential. Those amino acids that can be synthesized in the body are called "dispensable" or "nonessential" and those that can not be synthesized in the body are called "indispensable" or "essential". Classification of amino acids in this way

apply only to dietary need since all are necessary for the synthesis of proteins.

Histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine are not synthesized by mammals and are therefore essential for man (Rose, 1957). Arginine, which is synthesized by mammals but not in amount sufficient to meet the needs of most species, is not required by the human infant for normal growth (Holt & Snydermen, 1965), although, under some special circumstance, it may be limiting (Heird et al., 1972). Histidine is considered essential for the infant (Holt & Snyderman, 1965); moreover, recent studies (Weller et al., 1971; Kopple and Swend Seid, 1975) suggest that some dietary histidine may also be required by the adult.

Some of the dispensable amino acids can be synthesized only from specific essential amino acids. Cystine can be formed only from methionine, so when cystine is present in the diet in adequate amounts, less methionine is required (Rose and Wixon, 1955).

The other dispensable amino acids can be synthesized in the body from organic acids that are intermediates in carbohydrate metabolism, e.g. alpha-ketoglutarate and pyruvate (Stecle, 1952) and from the nitrogen of other amino acid or from such compounds as ammonium citrate (Rogers et al., 1970).

Because protein is the main source of nitrogen in the

human's diet, the requirement for protein is thus a requirement for amino acids. The body is in a dynamic state in which proteins and other nitrogenous compounds are being degraded and resynthesized continuously (Food and Nutrition Board, 1980).

The amount of protein required per unit of body weight depends on the "quality" of the protein, i.e., on the efficiency it can be utilized. For example, twice as much of a protein would be required if it were used only half as efficiently as the reference standard which is usually considered to be whole egg protein (Jansen, 1974).

Estimates of desirable protein intakes may be obtained in two ways. They may be based on observation of the minimum amount of protein that will promote growth in children and maintain nitrogen balance in adults. Alternatively, they may be based on calculations of losses of nitrogen that occur through the urine and feces on a protein-free diet together with allowances for the obligatory losses through the skin, sweat and loss of cells as well as the amounts needed to take care of the increase in body mass during growth as assessed by changes in the body composition (Guthrie, 1975).

Establishment of the recommended dietary allowances (RDA) for protein follows essentially three steps. The first one is the estimation of minimum requirement of good quality protein for maintenance of nitrogen equilibrium in practically all healthy persons. The second step is the

adjustment of the requirement to allow for poor utilization of proteins from a mixed diet as compared with a diet containing good quality protein. The third step includes the adjustments in protein allowance to meet added need of growth, pregnancy and lactation.

In 1945 the Food and Nutrition Board adopted 1.0 g protein/kg body wt/day as its RDA. Hegsted et al. (1946) suggested values of 0.46 g protein/kg body wt/day on a diet of mixed vegetables and 0.38 g protein/kg body wt /day when the diet supplied one third of protein as meat.

The committee on Amino Acids of the Food and Nutrition Board (1959) estimated that an intake of 0.31 to 0.34 g/ day/kg would meet the minimal adult requirement. These values were derived for a protein of high nutritional value, such as those contained in milk, eggs and meat (FAO, 1957).

In 1973 both the Food and Agriculture Organization (FAO) and the Food and Nutrition Board of the National Research Council (NRC) published RDAs for protein. Their recommendations for protein are essentially the same for all ages and sex categories except for pregnant women. The values reflect a safe level of intake or one adequate for maintenance of good nutrition. Since there is no evidence of adverse effects from the intakes above minimal requirements both groups have set their recommended levels at two standard deviations above the minimal requirement. According to the latest edition of the Food and Nutrition

Board 1980, the allowance for the mixed proteins of the United States diet is 0.8 g/kg of body weight per day. Thus the allowance for a 70 kg man is 56 g of protein per day, and for a 55 kg woman, 44 g.

Protein Quality and Its Determination

Protein quality is a term used to denote the efficiency with which a protein is utilized for growth and maintenance (Jansen, 1978). The lower the level at which a dietary protein can support maximum growth or nitrogen balance, the higher in quality that protein is said to be.

A complete protein is defined as a protein which contains all of the essential amino acids, it may or may not contain all the other amino acids as well. Complete proteins are more commonly found in animal sources (meat, milk, cheese, eggs) than in vegetable sources (Guthrie, 1975).

A second characteristic of dietary proteins that affects their quality as amino acid sources for humans is the ratio of the amino acids to each other or the amino acid balance. Ideally dietary protein will supply each amino acid in the amount needed for protein synthesis in the body. If one amino acid is supplied in an amount smaller than that needed, it will limit the total amount of protein that can be synthesized from the remaining amino acids.

Proteins differ in nutritive value because they differ in their amino acid composition and less importantly in

their digestibility (Block & Mitchell, 1946). Amino acid availability also varies with protein source, processing treatments (especially heating) and interaction with other components. Such differences should be taken into account in attempting to set a protein allowance and to assess the protein value of diets. Several methods are avaliable for determination of protein quality. These include: a) Biological methods, b) Chemical methods, c) Microbiological methods and d) Enzymatic methods.

Biological Methods

The protein to be tested can be fed to animals and its capacity to maintain the nitrogen balance or to promote growth measured. The methods used are not fully standardized and there is no complete agreement about the best ways in which to express the findings.

Protein quality bioassays with rats are subdivided into those based on weight gain and those based on nitrogen retention in the whole body either by direct carcass analysis or by nitrogen balance techniques.

Biological Value: The biological value of a protein was defined by Thomas (1909) as the percentage of absorbed nitrogen retained by the body. It is estimated from measurements of nitrogen intakes and losses made under carefully standardized conditions using a protein intake which is below the requirement level. Although a wide

variety of methods have been proposed for a number of reasons, the most widely used methods and the methods of choice for protein quality evaluation are all based on growth of the weanling rat.

A complete evaluation of the dietary protein includes measurements of the Biological Value and the digestibility. These are obtained by measuring the fecal and urinary nitrogen of a subject fed a test protein diet and then correcting for the amounts excreted when a nitrogen-free diet is fed (Hegsted 1974). True digestibility is defined as the percentage of food nitrogen absorbed from the gut.

$$\text{Digestibility} = \frac{I-(F-F_o)}{I} \times 100$$

The Biological Value (BV) is calculated as the ratio of the retained N over the absorbed N and is usually expressed by the following formula.

$$BV = \frac{I-(F-F_o)-(U-U_o)}{I-(F-F_o)} \times 100$$

Where:

I = Nitrogen intake of test protein

F = Fecal nitrogen

F_o = Fecal nitrogen when a nitrogen free diet is fed (metabolic N)

U = Urinary nitrogen

U_o = Urinary nitrogen when a nitrogen free diet is fed (endogenous N)

Mitchell (1923) reported that the BV was affected by the level of protein in the diet. He provided data which showed that an increase in the protein content from five to ten percent of the diet, when the protein sources were milk, corn, oats or potatoes, resulted in a decrease in BV on the average by eleven percentage points.

Since the BV is based on the amount of nitrogen absorbed, it does not take into account differences in digestibility from one protein to another. Thus other biological parameters have been devised.

Net Protein Utilization: The measurement of protein quality by an index known as Net Protein Utilization (NPU) has been introduced to express in a single measurement both the digestibility of the protein and the biological value of the amino acid mixture absorbed from the intestine.

NPU is defined by the FAO/WHO expert group (1965) as the proportion of nitrogen intake which is retained, i.e., the product of BV and digestibility.

$$NPU = \frac{I-(F-F_o)-(U-U_o)}{I} \times 100$$

Since both NPU and BV are based upon estimates of "retained N" they should measure similar parameter except that the calculation of BV is based on the amount of N absorbed rather than consumed. Thus BV would be expected to be somewhat higher than NPU.

Protein Efficiency Ratio: Qualitative differences in protein quality can be demonstrated by many methods. The PER, because of its relative simplicity, has been the method most widely used. Osborne et al. (1919) observed that young rats fed certain poor-quality proteins gained little weight and ate little protein, whereas those that were fed better quality proteins gained more weight and consumed more protein. In an attempt to compensate for the difference in food intake, the investigators calculated the weight gain per gram of protein eaten, which has come to be called PER. The PER for any protein is dependent upon the amount of protein incorporated in the test diet.

Derse (1962) proposed a set of standardized conditions for measuring PER. These include the use of 10 weanling rats per test group, the use of diets containing 9.09 percent protein (N x 6.25), a test period of 4 weeks duration, and the inclusion in each experiment of a group receiving standardized casein. PER is calculated as the average total weight gain divided by the average amount (grams) of protein consumed.

$$\text{PER} = \frac{\text{Gain in body wt (g)}}{\text{Protein Intake (g)}}$$

It was recommended that a corrected value be calculated on the basis of an assumed PER of the standardized casein of 2.50 (corrected PER = 2.50 x PER /PER of reference casein).

Protein efficiency ratios appear to be related

reasonably well to other methods of evaluating protein quality. Block & Mitchell (1946) found that there was a good relation between PER's and Biological Values and also between PER's and chemical score based on amino acid composition. PER shows good correlation with the other biological methods. Hackler (1977) computed the correlation coefficients for the BV, Digestibility (D), NPU and PER bioassays from data on plant and animal proteins published by FAO. With this particular set of data the correlation coefficient (r) for NPU and PER was the highest (.973). For BV:NPU r=0.899, BV:PER r=0.885, BV:D r=0.649, D:NPU r= 0.576, D:PER r=0.479.

In spite of its simplicity, the PER has been severely criticized as a measure of protein quality (Mitchell, 1944; Hegsted and Worcester, 1947; Hegsted and Chang, 1965). It has been criticized on the basis that (a) gain in body weight may not be constant in composition for different proteins, (b) results may vary with protein level, and (c) the determination makes no allowance for the maintenance requirement. The literature indicates that in a four-week assay important differences have not been encountered in body composition, and that variation that may exist in food intake or protein level do not constitute a serious criticism of the method as carried out under standardized conditions (NAS - NRC, 1963). PER does not provide protein evaluations that are directly proportional to quality, because no account is taken of loss of weight when animals

are given no protein in the diet. This defect is corrected in Net Protein Ratio measurements by inclusion of a control group receiving no dietary protein (Food and Nutrition Board, 1980).

Net Protein Ratio: The Net Protein Ratio (NPR) was described by Bender and Doell (1957). They suggested that the problem with PER can be avoided by including in each test a group of animals fed a protein-free diet. NPR was then calculated as the overall difference in gain (gain in weight of the test group plus loss in weight of the protein-free group) divided by the amount of protein eaten.

$$NPR = \frac{wtgt + wtle}{wtct}$$

Where: wtgt = weight gain of the group on test protein

wtle = weight loss of non protein group

wtct = weight of protein consumed

This method takes into account the maintenance requirements of the rats. It also assumes that the body composition is constant, an assumption which may not be valid when widely different test and protein-deficient diets are fed to the animals.

Slope Ratio Techniques: The relative protein value (RPV) or Slope Ratio Technique was proposed by Hegsted and associates (Hegsted and Chang, 1965; Hegsted et al., 1968). In this method the slope of the regression line relating

body weight or body protein or body water of young rats fed a standard protein, either egg protein or lactalbumin which is assumed to have maximal nutritive value, was compared to that of young rats fed the test protein at various protein levels.

The use of a standard protein under the same conditions as the test proteins aims at eliminating the causes of variability which can not be clearly identified and controlled. The slope of the test protein is expressed as a percentage of the slope of the standard protein. A valid slope-ratio assay requires that the response curves for sample and standard be linear and that they meet at the zero dosage level. This does not always happen, in which case only values falling on the linear portion of the curve are used in computation of the slope assay value. However, the slope may not always be a valid index of protein quality. For example, lysine deficient proteins yield a lower slope: while threonine deficient proteins tend to yield higher slope values (Hackler, 1977).

In all bioassay methods, the rat is the most common animal used in assessing the nutritional quality of food proteins for human consumption. Although rats have many similarities to the protein needs of man, they do differ. The growth rate, maintenance needs and consumption of variety of foods containing various proteins by humans at each meal are very different from the procedures used in rat bioassays. The need for essential amino acids for

maintenance are much lower than those for growth in the rat. Also, protein requirements for growth or maintenance are different in the rat from that in the human in all stages of life (Bodwell, 1977). Since there is no bioassay which we can achieve absolute accuracy in determining the nutritional value of protein for human, the selection of the methods is based on criteria such as simplicity of the test economics, labor and reproducibility within and between laboratories.

Chemical Methods:

In addition to the biological methods of evaluating protein quality, there are several chemical methods that are based on the determination of the amino acid pattern of a particular food and a comparison of this to a reference protein. The essential amino acid showing the greatest deficit is considered to be the amino acid limiting utilization of the protein. The amount of this amino acid present, expressed as a percentage of that in a standard provides the chemical score.

In 1957 the FAO proposed a reference protein based on the amino acid requirements of man but in 1965 decided to abandon this use in favor of an amino acid pattern of whole egg or human milk as a standard. The whole egg protein is chosen as reference protein because the amino acid requirements of the rat are not well known and also because its amino acid mixture is highly digestible and almost

perfectly utilizable in the rodent metabolism, being better than milk protein in this respect (Bricker and Mitchell, 1947).

Table 1 shows the essential amino acid composition of whole egg protein. The excellent protein quality of the whole egg was demonstrated by Mitchell (1950) when he showed that the growth-promoting value of this protein was not improved for the growing rat by supplementation with any of the essential amino acids except lysine, which induced a three percent increase in body weight in the 28 day feeding period.

In 1951 Oser developed an integrated index in which he used the geometric mean of the egg ratios to estimate the BV of a protein. This method of rating protein quality is based not only on one essential amino acid, as the chemical score, but on the contribution of all the essential amino acids. Oser felt that each essential amino acid is specific in its own right and all are equally essential.

The essential amino acid index was defined as the geometric mean of the "egg ratio" (the ratio of the essential amino acids in a protein relative to their respective amounts in whole egg protein). The EAAI method was modified by Mitchell (1954) by adjusting to 100 the egg ratios that happens to have values higher than that. The antilogarithm of the average of the egg ratio logarithms that includes adjusted values is called Modified Essential Amino Acid Index (MEAAI).

Table 1. Essential amino acid composition of the whole egg protein and the reference pattern (FAO/WHO, 1973)

Amino Acids	Whole Egg (g/16g N)	FAO/WHO Reference Pattern (g/16g N)
Lysine	7.8	5.4
Methionine + Cystine	5.3	3.5
Phenylalanine & Tyrosine	9.3	6.1
Leucine	8.8	7.0
Isoleucine	5.9	4.0
Valine	7.1	4.9
Threonine	4.9	4.0
Tryptophan	1.4	1.0
Histidine	2.6	-
Total	53.1	36.0

The major problem with the chemical methods for protein quality evaluation is the assumption that all the amino acids are biologically available. This assumption is not always valid, especially with proteins that have been heat treated or otherwise processed. On the other hand, the advantages of these indexes are a) the small size of the sample required b) the short time of analysis required compared to bioassays and c) the limiting amino acid is identified.

Microbiological Methods

Many microorganisms require the amino acids that are essential for monogastric animals. If it were possible to find microorganisms that required amino acids not only in the same pattern, but also in the same relative amounts, their growth response when supplied with limited amounts of various proteins, or protein hydrolysates would provide a simple and efficient assay of nutritive value. The growth response of Streptococcus zymogenes (Ford, 1960) and the protozoa Tetrahymena pyriformis (Rosen and Fernell, 1956; Pilcher and Williams, 1954) were used for the determination of the biological value of proteins. Using Streptococcus zymogenes, Ford (1960) obtained protein quality values for several meat meals. The results which he obtained were closely correlated with the available lysine content. Some work has been done in this area (Horn and Warren, 1961, 1964; Ford, 1964; Baum and Haenel, 1965), and

the results obtained with some organisms resemble those obtained in some of the rat assays experiments. Nevertheless, Menden and Cremer (1970) showed there was a poor correlation between microbiologically obtained values for the BV of cereal products and the results obtained with animal experiments. If accurate microbiological tests could be developed they would have the advantages of shorter duration and lower cost than the animal experiments.

Enzymatic Methods

Enzymatic methods are an improvement over chemical methods because they determine only amino acids that can be freed from the protein molecules and thus considered as available under physiological conditions. They can be used to measure amino acid availability or to estimate the BV of a protein.

Sheffner et al., 1956, used a peptic (100:2.5 substrate: Enzyme, S:E) digest which was analyzed microbiologically for available essential amino acids to formulate his Pepsin Digest Ratio (PDR). Akeson and Stahmann (1964) used Pepsin (100:1.5 S:E) at 37°C for 3 hours, followed by pancreatin (25:1 S:E) at 37°C for 24 hours, and analyzed the digest with an amino acid analyzer. They calculated the Pepsin Pancreatin Digest Index (PPDI) for ten food proteins.

An enzyme score, which is calculated like the chemical score, comparing the essential amino acids released from

the test protein to those released from egg protein, was determined on the heat processed casein (Stahmann and Woldegiorgis, 1975). Both the chemical score and enzyme score compared well with the PPDI.

The major problem with the use of *in vitro* digestion methods for protein quality is the incomplete digestion of the test protein. Therefore, a reference protein is always assayed in conjunction with test protein. The advantages of enzyme digest methods are: 1) lower cost and shorter analysis time; 2) less variation in the results than found with rat bioassays; and 3) generation of information on amino acid availability and relative nutritional adequacy (Stahmann and Woldegiorgis, 1975).

Cereal Proteins

Substantial segments of the world's population do not receive adequate amounts of protein due to inequitable distribution of protein resources (Rosenfield, 1969a). It is now generally accepted that the primary cause of this uneven distribution and subsequent protein malnutrition is poverty. The classical approach has been to attempt to increase supplies of meat, eggs, milk and other dairy products. However, since those suffering from protein deprivation can not afford high-priced products, this approach has not had any significant effect in those poverty areas (Kent, 1970).

Cereals account for about two-thirds of the caloric

intake in the Far East and more than half in North Africa, West Asia, and East Europe. They are also the major source of protein. Even in areas where animal products are relatively plentiful, such as the United States, Canada, and Northern Europe, cereals supply over 20% of the total protein (West 1969). The amounts of protein supplied to growing children if they are fed their entire calorie requirements in the form of the major cereals are compared with FAO protein required (FAO/WHO, 1965) in Table 2. All of the cereals including rice would meet the quantitative protein requirements even for the youngest infants, provided calorie requirements were met.

Cereal protein is distributed non-uniformly among the morphological tissues of the grain with the highest concentrations occuring in the outermost, or subaleurone, part of the so-called starchy endosperm (Kent 1966), in the germ as well as in the aleurone layer of the endosperm. The inner endosperm has a lower protein content than that of the whole grain. Very little protein is found in the pericarp.

The protein content of all of the common cereals averages around 10% at natural moisture content. Nevertheless, individual samples of particular cereals might contain as little as 6% or more than 20% (Kent, 1970). Table 3 shows the effect of milling and extraction on protein content of wheat and rye.

Table 2. Theoretical protein intakes in children fed caloric requirement in form of cereals[a]

Grain	Protein Intake (g/Kg body wt/day)		
	1-12 months old	1-3 years old	4-6 year old
Rice	2.3	2.2	1.9
Wheat	3.3	3.2	2.8
Corn	2.6	2.6	2.2
Millet	2.8	2.7	2.3
	Protein Requirement[b]		
	1.2-2.3	1.1	1.0

[a] Protein intake assuming FAO Caloric Requirements Completely met by Cereal Consumption (FAO nutrition studies No. 15, Rome, 1957).

[b] Requirement for completely utilizable protein with an NPU equal to 100 (FAO-WHO 1965).

Table 3. Effect of milling and extracting on protein content of wheat and rye.

	extraction rate of				
	100%	85%	80%	75%	70%
	of the whole grain				
Wheat flour	% protein				
- low protein	8.9	8.6	8.2	8.0	7.9
- high protein	13.6	13.6	13.2	13.1	12.8
Rye	8.0	7.3		6.7	

Jones, 1974.

Lockwood (1960) stated that the protein content of wheat in typical British bread wheat blends varies between 7.5 and 18%. Jansen (1974) cited the work of Johnson (1971), who in his analysis of 144 samples of wheat from the world collection, found protein contents from 6.5 to 21.5%.

The primary problem with cereals as suppliers of dietary protein is that they contain poor quality protein, not that they contain inadequate amounts. Another problem with some cereals is poor digestibility which compounds the poor quality problem. A deficiency of one or more essential amino acids, especially lysine, is the cause of poor quality of cereal proteins (Jansen, 1974).

Wheat grown for flour can be classed according to color of the surface of the Kernel (white or red), season when planted (winter or spring), and relative amounts of protein (hard or soft). The soft wheats are higher in starch and lower in protein with the reverse true for the

hard wheats (Jansen, 1974).

The hard spring and hard winter wheats are the types most desirable for bread production. They mill well and yield good quantities of flour that is high in good quality protein, from which strong, elastic doughs can be made. These doughs have good tolerance with respect to mixing, fermentation, temperature, etc, and have excellent gas-holding properties essential to yield a bread with good volume and texture (Schopmeyer, 1960).

The wheat proteins include water soluble proteins (albumen) salt-soluble proteins (globulins), alcohol soluble proteins (prolamines or gliadins), and acid- and alkali- soluble proteins (glutenins). The wheat endosperm proteins (gliadin and glutenin) form a colloidal complex known as gluten, when water is added to them (Pomeranz, 1980).

The amino acid composition of gliadin and glutenin are relatively similar. Both have unusually high glutamic acid (present largely as glutamine) and proline contents. Relatively large numbers of non-polar amino acids contribute to apolar bonding. Few of the carboxyl groups of glutamic and aspartic acid are free to ionize and the low content of lysine, histidine and arginine result in a low ionic character for the gluten proteins (Pomeranz and Finney 1973).

Osborne and Mendel (1914) showed that the wheat proteins are generally of poor biological value as compared

with animal proteins. Vaghefi et al. (1974) showed that young adults can maintain nitrogen balance by eating only wheat protein, but large amounts of additional calories were required to obtain the necessary nitrogen balance. The studies of Howard et al. (1958) and Bender (1958) suggest that the protein content of wheat flour is inferior to animal protein for growth and maintenance of rats. These studies have shown that the lysine is the primary deficient amino acid as far as growth of rats is concerned (Mitchell and Block, 1946; Block and Weiss, 1956). When the lysine has been added to wheat flour to the point of maximum nutritional efficiencies, the next amino acid to be added for improving BV is threonine. Deshparde et al. (1955) presented results which showed that with a given protein content in the diet the results obtained were influenced by the level of supplementation with lysine and threonine. It seems that the effect of threonine depends on the level of lysine added at any level of dietary protein. The results obtained with experimental animals have also been found working with adults (Jansen, 1962; Barness et al. 1961; Hoffman and McNeil 1949), and children (Bressani et al. 1960, 1963).

Wheat Proteins Contribution to Dough and Bread

Dough is one of the principal intermediate stages in the transformation of wheat, through flour, to bread. Elasticity is developed in a wheat-flour and water paste

when the hydrated particles of flour are manipulated. A complex known as "gluten" forms as the mass is worked. The viscous and elastic properties of dough are primarily due to the properties of its continuous phase or gluten phase. Knowledge of the molecular organization of gluten proteins and their physical behavior permits greater manipulation of dough systems to achieve desired end products (Wall and Beckwith 1969). Wheat flours that contain the same quantity of protein may present far different physical properties (Pomeranz, 1966).

Gluten proteins are characterized by high concentration of glutamic acid (about 32%) and proline (about 10%). Many studies have shown that both ionic and nonionic groups govern the unique viscoelastic properties of wheat gluten. Types of bonds which contribute to viscoelastic properties of dough are 1) amide groups 2) sulfhydryl groups and disulfide bonds, 3) hydrogen bonds and 4) hydrophobic interactions.

Different chemical treatments apparently modify the rheological properties of dough by affecting different types of cohesive bonds. Many of the studies on gluten structure have involved disulfide linkages. In gliadin the disulfide bonds are primarily intramolecular, while in glutenin inter and intramolecular linkages both occur. Some disulfide bonds are broken during mixing; some are formed. The relative extents of cleavage and formation of disulfide bonds depends on the presence of reducing and oxidizing

agents. For a given protein level, optimum baking results are obtained with a total SS/SH ratio around 15 (Bilderok, 1967). The SS/SH ratio increases with flour storage (Wehril and Pomeranz 1969). The ratio of reactive to total SS groups increase with decreasing mixing strength, and the ratio of reactive to total SH groups also increases with decreasing flour strength. Thus mixing strength appears inversely related to reactive SH and SS contents (Tsen and Bushuk, 1968).

The distinctive property of wheat gluten makes possible the production of bread dough. Wheat gluten is a viscoelastic mass which can be stretched and molded into a variety of shapes and which will "rise" as the gases entrapped within it expand. When the dough is baked, the protein coagulates and the starch is gelatinized to produce the cellular crust-covered material known as bread. For a single wheat variety, it is well known that loaf volume is directly proportional to the protein content, however, there is disagreement as to which wheat protein fraction is responsible for the variation in loaf volume among wheat varieties (Khan and Bushuk, 1978).

Finney (1943) found that the water soluble protein material is responsible for differences in baking quality. Moreover, the water soluble proteins were necessary for normal baking characteristics in two of his three reconstituted flours. Pence et al. (1951) also found that soluble components were required for maximum performance in

all glutens studied, except for a durum wheat. A crude albumen fraction isolated from the water solubles was responsible for the largest volume response. Orth and Bushuk (1972) concluded that glutenin is responsible for the variation in loaf volume (at constant protein content), while Hoseney et al. (1969) had concluded that gliadin portions control the loaf volume potential of wheat flour. Thus, this apparent controversy remains unsolved.

Formation of a properly developed dough, essential in producing an acceptable loaf of bread, involves the interaction of protein with lipids. Available information indicates that dough lipids interact mainly with gluten protein rather than with the soluble wheat flour proteins.

Hoseney et al. (1970) found that free polar lipids (principally glycolipids) are bound to the gliadin proteins by hydrophilic bonds and to the glutenin proteins by hydrophobic bonds. In unfractionated gluten, the lipid apparently is bound to both protein groups simultaneously. Such binding may contribute structurally to the gas-retaining complex in gluten.

Dough handling properties can be modified either beneficially or adversely, by the addition of minute amounts of reducing agents or sulfhydryl-blocking reagents. Also the performance of a flour in bread making can be improved significently by addition of an appropriate amount of oxidizing agent, such as 20-50 ppm potassium bromate (Pomeranz, 1980). The oxidation requirement (the amount of

oxidant needed to produce the best loaf of bread in terms of volume, crumb, texture and freshness retention) is related to total protein content and to protein sulfhydryl groups and disulfide linkages.

Physical Dough Testing

Because of the extreme importance of physical dough characteristics of wheat flour doughs in bake-shop performance and in bread quality, many efforts have been made to evaluate flour quality in terms of physical measurements on flour doughs to give an indication of their hydration and mixing characteristics. For this purpose numerous devices have been developed.

The farinograph and extensigraph are the most commonly used instruments in measuring and recording the physical properties of dough. The farinograph measures dough viscoelasticity as a function of continuous mixing. It outlines the mixing characteristic of a flour with respect to absorption, dough development and the ability of the flour to produce a maximum consistency in mixing and stability to prolonged mixing (Holmes, 1966; Bushuk et al., 1968; Bloskma, 1971; Bloskma 1972).

The extensigraph measures the extensibility of dough and resistance to extension, it also shows the effect of flour improvers such as bromate or iodate which can scarcely be observed from farinograph data.

Farinograms and extensigrams measure functional

characteristics and baking quality of flour gluten proteins. With increases in protein content there are increases in farinograph absorptions, mixing requirements, tolerance to mix and extensigram dimensions (Aitken et al., 1944; Geddes et al., 1940; Markley et al., 1936).

Experiments with flours with various protein contents (Merritt and Stamberg 1941), flour gluten mixtures (Aitken and Geddes 1938, 1939), flour starch mixtures (Markley, 1938) and air classified flour fractions (Gracza, 1959, 1960), showed that the water absorption, dough development time, stability and extensibility increase with protein content.

The mixograph is a recording dough mixer that is somewhat similar to the farinograph. Its graphs (mixograms) provide information similar to that obtained with the farinograph. Johnson et al. (1943) in a study of the relationships of mixograms to baking results concluded that the mixograms were the greatest value in supplying supplementary information with regard to mixing requirements, mixing tolerance, etc., which may or may not be related to the actual baking quality of flour itself.

The amylograph is a recording viscometer which provides a continuous automatic record of the viscosity changes which occur in a flour-water suspension being subjected to a uniform increase in temperature to a final end temperature of 98^{o}C. The viscosity of the sample of flour dough or suspension in water tends to increase as the

starch gelatinizes while the alpha-amylase present tends to liquidify the gelatinized starch. Thus evaluation of changes in viscosity provide a measurement of the extent of enzyme activity.

Legumes

Leguminosea comprise approximately 6oo genera with around 13,000 species. Only a few of this number, about 20, are commonly consumed by humans and have been analyzed for nutrient content (Haytowitz et al., 1981). Grain legumes are normally consumed as whole or split legumes with only a small quantity being processed into flour (Sosulski and Youngs, 1979).

Edible legume seeds contain relatively large amounts of protein that vary considerably in nutritional value. Except for soybean and ground nuts (peanuts), the crude protein content (Kjeldahl Nitrogen x 6.25) of most legumes calculates to 18-32%.

The protein is located in the cotyledons and embryonic axis of the beans, with only small amounts present in the seed coat (Singh et al., 1968). The seed coat of the navy bean seed contains 4.8% crude protein, while the cotyledons and the embryonic axis have 27.5 and 47.6% respectively. Since the cotyledons represent the greater part of the whole seed, they contribute the major amount of protein to the whole seeds (Singh et al., 1968; Varner and Schiolouwsky, 1963).

The essential amino acid pattern of legume protein is exceptionally good when compared to reference pattern or to animal protein sources, except that legume protein are deficient in sulfur containing amino acids with a secondary deficiency in tryptophan. In most of the legume foods, lysine is present in amounts which approach and sometimes surpass the level established for the reference protein. The essential amino acid content of selected beans is shown in Table 4 and is compared with the FAO pattern of amino acid requirements (FAO/WHO, 1973).

Knowledge of the sulfur amino acid deficiency in beans is quite old, dating as far back as 1920, when various investigators (Liener, 1962, Klose et al 1949, Johns and Finks, 1921) demonstrated that cooked, but not raw, legume foods induced normal growth in rats when the protein was supplemented with cystine. These results were not understood completely at the time until Rose (1938) showed that cystine stimulates growth when methionine is present in sub-optimal quantities, but not when methionine is entirely absent from the diet. Richardson (1948) added 0.2% methionine as a supplement to heated lima and pinto beans, and reported an improvement in protein quality.

The high content of lysine and low content of sulfur amino acids makes leguminous proteins natural complements to cereal based diets because cereals are, in general, lysine deficient, but have adequate amounts of sulfur-containing amino acids. Soy flour and several other legume

Table 4. Essential amino acid content of selected beans compared with the FAO/WHO pattern (g/16g N)

Amino Acid	FAO/WHO Reference Pattern[a]	Navy Beans[b]	Kidney Beans[c]	Black Beans[c]	Red Beans[c]
Histidine	--	2.4	2.6	2.9	3.2
Lysine	5.4	5.7	6.7	6.7	7.2
Methionine & Lysine	3.5	1.7	1.9	3.0	2.2
Phenylalanine & Tyrosine	6.1	8.4	9.8	9.6	9.9
Leucine	7.0	6.7	8.1	7.7	7.7
Isoleucine	4.0	3.7	4.2	4.1	3.8
Valine	4.9	4.4	5.1	4.9	5.0
Threonine	4.0	4.1	4.2	3.1	3.5
Tryptophan	1.0	1.2	1.5	1.5	1.7

[a] FAO/WHO (1973) reference pattern

[b] Boloorforooshan (1977)

[c] Evans and Bandemer (1967b)

flours are effective fortifiers of wheat flour products not only because of their high protein contents but also because they contain more lysine than wheat flour does.

It has been recognized for many years that the nutritive value and protein digestibility of legumes are very poor unless subjected to cooking or some other form of heat treatment (Liener, 1962). The depression in protein value and digestibility has been generally attributed to the presence of certain antinutritional factors, including the trypsin inhibitors, hemagglutinins and flatulence factors (Liener, 1962: Liener and Kakade, 1969; Rackis, 1974).

Read and Haas (1938) were among the first to recognize that a trypsin inhibitor can occur in plant material. Bowman (1944) described trypsin inhibitors in navy bean, soy bean and corn. Wagner and Riehm (1967) isolated an inhibitor from navy bean (California small white) by gel filtration and ion exchange on diethyl amino ethyl (DEAE) cellulose. Bowman (1971) and Whitley and Bowman (1975) isolated from the seeds of navybean (sanilac) a trypsin inhibitor with 7,900 molecular weight, as estimated from the inhibitor-trypsin interaction.

Mature bean seeds, like many other seeds, contain relatively large amount of phytate (inositol hexaphosphate) (Makower, 1969; Kon et al., 1974; Lolas and Markakis, 1975; Chang et al., 1977). Phytate serves as a ready source of phosphate (Mayer, 1956) and inositol (Darbre and Norris, 1957) during germination. Its decomposition may also

release certain metals which are essential to the developing plant. Nutritionally, phytate is probably unavailable to humans (McCance and Widdowson, 1935) due to the lack of an endogenous enzyme system (Rapoport et al., 1941) that can catalyze the hydrolysis of the molecule to its moieties. The presence of undegraded phytate in the intestines may render less available for absorption some essential di-and tri-valent cations such Zn^{++}, Mg^{++} and Fe^{+++}.

Following ingestion, phytate and oligosacharides which are not hydrolyzed by gastrointestinal secretions pass into the large intestine where they are fermented by bacteria with formation of gas and generation of flatus (Bergey 1957; Speck et al., 1970; Rackis et al., 1970; Steggerda, 1961; Hellendorn, 1969). This is commonly associated with the ingestion of cooked legume seeds. The specific factor or factors in beans responsible for flatulence have not been established as yet, even though Murphy (1967, 1969) found that the flatulance factor in beans can be extracted from cooked beans with 60% ethyl alcohol. He emphasizes that the factor is low in molecular weight and also reports that the protein, starch polysaccharides and lipids of beans, are not responsible for gas production.

Sesame

Sesame, Sesamum indicum L., a member of the pedaliacene family, has been called the "queen of the oilseed crops" because of the high yield of oil obtained and the good

qualities of the seed, oil and meal (Eckey, 1954). Theplant, cultivated in India for several thousand years, is grown extensively in tropical and subtropical areas of Asia, Mediterranian countries, and South America.

The seeds are a rich source of oil, protein, calcium, phosphorus and oxalic acid. They are often decorticated by lightly pounding, or rubbing on a stone or wooden block. White seed is preferred when there is a choice. Decortication produces a less bitter and therefore more palatable foodstuff. It does not have any effect on the fat and protein but it reduces the mineral, calcium and oxalic acid contents. The whole seed may be eaten raw, either roasted or parched, or fed to birds and stock. In industrial countries it is mainly a speciality food, and the whole seed is used in large amounts in various sweets.

Sesame oil is used directly in cooking wherever it is produced, and is often the favored oil. The refined oil is used commercially as a bland salad oil and for those purposes where an edible oil of exceptionally good keeping qualities is required.

In general, sesame meal is considered to be equal to cottonseed or soybean meal as a protein supplement for livestock and poultry. It is especially high in certain amino acids, such as methionine, which are low in soybean meal.

The valuable components of sesame seed are the oil and protein and the contents of these have been determined for

several varieties of sesame which were grown in the Southern and Southwestern parts of the U.S. (Kinman and Stark, 1954). Oil content varied from 45-63% and averaged 54%. Protein contents varied from 17-32% and averaged 26%. The average protein content of the oil-free meals was 57%.

Being a good source of methionine, sesame meal offers a great advantage as a natural supplement to many legume proteins deficient in this amino acid. The supplementary value of sesame to soya, groundnut, chickpea or mixtures of these legumes in different proportions has been demonstrated in several investigations using rats as experimental animals (Krishnamurth et al., 1960; Tasker et al., 1960; Guttikar et al., 1965).

Evans and Bandemer (1967a) demonstrated the effect of fortification on the nutritive value (relative to casein) of sesame meal. Sesame alone had a protein nutritive value of 47%. Fortification with 0.2% lysine raised this to 94%. A 1:1 mixture of soybean and sesame gave the protein nutritive value about the same as casein.

High protein vegetable mixtures for human feeding which contain 35% sesame flour have been developed (Scrimshaw et al., 1961) by the Institute of Nutrition of Central America and Panama (INCAP). These low cost mixtures were readily accepted and well-tolerated as the chief protein source of a needy population.

Availability of the amino acids in sesame is affected by the processing method used on the seed (Villegas et al.,

1968). Enzyme digestibility was affected very little, but methionine availability was increased by heat treatment. In the sample that was heated after oil extraction, it was 2.33%, close to an average literature value. Lysine availability was not affected consistently by heat treatment and in all cases was less than 50% of an average literature value for lysine content. Sesame meal contains an average of 1.0-1.3% of phytic acid (Carter et al., 1961).

Supplementation Value of Plant Protein on Cereal

The concept of enrichment or fortification of plant proteins is not new. The observation that wheat protein can be nutritionally improved by the addition of lysine goes back over a half century to the work of Osborne and Mendel (1914). Since that time, there have been many studies investigating the myriad of technical, medical and technological aspects of upgrading the biological quality of plant proteins in general and cereal proteins in particular. There are several review papers in this area (Howe et al., 1967; Harris 1968; Hegsted, 1969; Jansen 1969, and Rosenfield, 1969a).

Vegetable protein typically supplies over 80% of the dietary protein in the developing countries of the world. The major source of vegetable protein are cereal grains, pulses, and oilseeds (Mitchell, 1954).

The greater proportion of protein in a diet that is supplied by cereals, the poorer will be the protein value

of that diet and the more chance that the limiting amino acid will be lysine. In contrast, if enough animal, pulse, or oilseed protein is consumed along with cereals to make sulfur amino acids limiting, the overall diet that results will be adequate in protein value, even for growing infant.

Many studies carried out in man as well as in experimental animals confirm the improvement in B.V of poor quality cereal proteins that results from the addition of the limiting amino acid. Cereals are especially prime candidates for amino acid fortification because they generally supply adequate amounts of dietary protein, but the protein is poor in quality. The role of cereals as prime suppliers of dietary protein in the developing countries has been emphasized (Howe et al., 1967; Jansen, 1970).

Lysine addition to most cereals should be of practical value in improving poor diets in which most of the protein is supplied by cereals. This is especially true for wheat millet and sorghum. Methionine addition should be beneficial when the diet is primarily non cereal, but is based on root crops and pulses.

A number of studies have been carried out with infants and small children receiving lysine supplemented wheat protein, with nitrogen retention in some cases approaching or equalizing those obtained with milk protein (Albanese 1953; Bressani et al., 1960; Barness et al., 1961; Bressani et al., 1963; Graham et al., 1969). Comparable data for

adult humans have been lacking, however, except for an observation on a single individual by Bricker et al. (1945) showing increased nitrogen retention when a diet based on white flour was supplemented with 13% soyflour, or a report by Hoffman and McNeil (1949) that the nitrogen balance index (Allison method) of wheat gluten could be markedly elevated for adults by 4% lysine supplementation.

Sure (1953) reported an increase in the PER of white flour from 0.68 to 2.32 by the addition of a combination of 0.4% L-lysine monohydrochloride, and 0.5% DL-threonine. Jansen (1974) reported an increase in PER values from 1.6 for unfortified wheat to 2.0 and 3.0 for wheat fortified with 0.36% L-lysine monohydrochloride and this amount of lysine together with 0.5% DL threonine, respectively. Graham et al. (1971) reported that lysine-fortified white flour was capable of supporting normal growth in human infants for a 6 months period when fed as the sole source of protein in the diet. These workers recommended that wheat be enriched with at least 0.12% lysine and in areas where wheat is the main source of protein for infants and small children and where dilution with nonprotein calories is the rule, a 0.2% enrichment with lysine was recommended.

Studies carried out by several investigators have indicated that the protein value of a mixture of bean and cereal grain is superior to that of each of these components fed individually (Adolph et al., 1955; Phansalkar, et

al., 1957). They emphasize that the maximum effect occurs when about 50% of the legume protein is replaced by cereal protein.

The supplementation of legume proteins with cereals and millets has been very successful. Since legume proteins have high amounts of lysine and threonine, they complement to a marked extent those of cereals and millets, which are characteristically low in these amino acids. On the other hand, the low methionine content of legumes is compensated by a higher content of this amino acid in cereals and millets. Phansalkar et al. (1957) showed a good supplementary effect of chick peas, black gram, green gram and red gram proteins to wheat, soybean and pearl millet. They believe that the maximum combined effect occurs when about 50% of the legume protein is replaced by cereal protein.

Bressani et al. (1962) reported that the best combination of cooked black beans and lime treated corn was one in which each component contributed 50% of the total protein of the diet. In terms of weight, this would be 72% of corn and 28% beans.

Calloway et al. (1965) reported that the improved protein quality obtained from a mixture of wheat and peas was comparable to egg protein. The nitrogen contributed by ingredients in the different protein mixtures studied was distributed as follows: wheat:pea:ragi, 50:35:15; wheat:pea :corn gluten, 50:30:20; wheat:pea:Brazil nut, 60:25:15; wheat:pea:egg, 50:30:20; wheat:pea, 60:40. The authors

indicate that when appropriate levels of both essential amino acids and protein are provided, the nutritive value of the vegetable protein mixture is equal to that of egg protein.

Supplementation of Bread with Oilseed and Pulse Proteins

Protein malnutrition is a serious nutritional problem facing people whose diets consists mainly of cereals or starchy products. Because of the universal acceptance of bread and the potential of its fortification with proteins, amino acids, vitamins and minerals, greater emphasis has been placed on the use of composite flour in bread baking during the last 10-15 years. In general, composite flour refers to flour containing blends of wheat flour with non wheat flours, although in some instances non-wheat flours have been used as a total substitute for wheat (D'Appolonia, 1977). The effect of added proteins on dough formation and bread's physical and nutritional quality have been extensively studied.

Effect of Protein Supplementation on Physical Dough Properties

Many investigators have studied the effects on bread quality of supplementing wheat flour with proteins from plant and animal sources. The plant proteins studied include protein rich residues from oilseed processing (soybean, cottonseed, sunflower, sesame seed and peanuts)

and legumes of non oil type (navy bean, faba bean...). Among the oilseed flours, soya products have increased most rapidly in availability and use.

The maximum level of replacement depends on the type of non wheat flour, the strength of wheat flour, the baking procedure and the dough stabilizing compunds used (Dendy et al., 1970; Pringle et al., 1969). Researchers studying flours from soybean (Kim and DeRuiter 1968, Finney and Shogren 1971), faba beans (McConnel et al. 1974), pinto beans, navy beans, mung beans and lentils (D'Appolonia 1977), mung beans (Thompson 1977), peanut protein concentrate (Khan et al. 1975), cow pea powder (Okaka and Potter 1977), Four concentrated plant proteins - soy beans, sunflower, faba bean and field pea - (Fleming and Sosulski 1977), and pea and cooked yellow pea flours (Jeffers et al. 1978) have found that with increased levels of legume additives in breads, loaf volume and crumb grain quality deteriorated. Generally, wheat flour containing 10-15% legume flour can be used to make acceptable fortified bread.

If a high protein bread is intended to have a significant influence on the supply of protein and the protein quality of the diet, this bread must be both visually and organoleptically acceptable. It was suggested by Matthews (1969) that the particle size of oilseed flours was important when used in bread doughs. She reported that better quality loaves of yeast bread were produced when 25%

coarse soy flour (60 to 80 mesh) was substituted for wheat flour than when a finer soy flour (100 mesh) was substituted at the same level. The coarse particles of soy flour absorbed water more easily than did the finer particles.

Jeffers et al. (1978) reported that yellow pea flour was superior to soy flour in mixing and bread making properties at supplementation levels of 5-20%. Hsu et al. (1980) used wheat flour blends containing ungerminated and germinated legume flours at 5-10 and 15% levels in baking studies. Addition of 15% legume flours to bread resulted in only small deleterious effects on loaf volume, crumb grain and flavor. Germination adversely affected the baking properties of peas and lentils, but not faba beans.

Rooney et al. (1972) compared the functional bread making properties of heat treated and non heat treated flour from cottonseed, peanut, sesame and sunflower. The oilseed flours replaced wheat flour to produce blends at two protein levels, 17.5 and 20.5%. These researchers reported that water absorption increased with increased levels of replacement of the wheat flour with oilseed flours. They also showed that properties of sunflower and cottonseed flours responded positively to heat. Sesame and peanut properties were slightly impared for baking, although mixing properties were slightly improved by heat.

D'Appolonia (1978) used 5, 10 and 15% of untreated and roasted navy bean in bread. He concluded that bread containing roasted navy bean flour had a higher volume than did

bread containing untreated flours at the same level of incorporation. The only bread quality factor that would be affected adversely would be crumb color.

Finney (1946) showed that bread of good loaf volume and crumb grain could be made from blends containing up to 8% of defatted soy flour with hard red spring wheat flour provided that the quantity of potassium bromate was increased in parallel with the amount of soy flour used. Tsen and coworkers (1971) used sodium and calcium stearoyl-2 lactylate to produce sponge dough bread with 12% soy flour. These dough conditioners increased loaf volume, and the organoleptic properties of soy-wheat blend bread were comparable to bread with 100% wheat flour. Adler and Pomeranz (1959) reported that loaf volume and bread baking quality could be maintained when defatted soya flour was added to wheat flour by adding lecithin and increasing the level of added oxidant.

Effect of Protein Supplementation on Nutritional Value of Bread

Grain legumes and oilseed meals represent probably the most valuable potential source of protein for the less developed countries since in many cases they are nutritionally complementary to wheat protein. Far greater use could be made of these sources as protein supplements for bread and other cereal foods.

Addition of soya meal containing approximately 55%

protein to bread at levels between 2.5 and 5% was reported in Germany (Hulse, 1974). At 2.5% the protein content was increased by 1.1% and at the 5% level by 2.2% on a day weight basis. Shehata and Fryer (1970) measured protein quality of Arabic bread supplemented with 20% chickpea flour by a rat-growth study and they concluded that percent weight gain of rats fed 20% chickpea flour diets was significantly higher than that of rats fed diets with 0, 10 and 15% chickpea flour. There were significant increases in feed consumption at the 15 and 20% levels of chickpea flour. Rats fed diets with 10 and 20% chickpea flour had measured PER's of 1.46 and 1.56, significantly better than that of rats fed diets with 0% chickpea flour.

Womack et al., (1954) fed young rats bread containing 10 parts of cottonseed flour per 100 parts of wheat flour. They found that bread with cottonseed flour gave a significantly higher rate of gain per 100 gram of nitrogen consumed than bread without cottonseed flour. A comparison was also made between bread containing skim milk powder and bread with skim milk powder and cottonseed flour (4 Lbs of milk solid and 8 lbs of cottonseed flour per 100 lbs of white flour) no significant difference was found in weight gain of rats when the two breads were fed at the same protein intake level. However, when the breads were fed at the same percentage by weight, the higher protein content in cotten seed flour bread brought about significantly higher rates of weight gain.

Guggenheim and Friedman (1960) studied 20 kinds of bread prepared from flours varying in extraction rate from 74 to 95% and fortified with soya flour up to 12% of the wheat flour weight. Lysine per gram of nitrogen increased both with increasing extraction rate and with percentage of soya flour added.

Breads made with 18.8% LCP cottonseed flours substituted for wheat flour were tested for their nutritional value by amino acid analysis, and biological tests (Harden and Yang, 1975). Amino acid analyses of breads indicated that lysine content was higher than the comparable wheat breads. Fleming and Sosulski (1977) fortified bread with vital gluten and sufficient amounts of soy flour, sunflower concentrate, faba bean concentrate, and field pea concentrate to bring up its protein level to 14%. The FAO provisional pattern (1973) was used as reference protein for calculating chemical score and essential amino acid index. Soy, faba bean, and field-pea bread gave PERs ranging from 1.7 to 1.8, whereas sunflower and wheat bread had values of 1.3 and 1.1, respectively. Protein quality evaluations were conducted on blends of wheat flour: concentrated plant protein: vital gluten of 100:0:0 for the control wheat flour bread, 83:15:2 for soy, faba bean, and field pea bread, and 86:12:2 for sunflower bread.

Mizrahi et al. (1967) fortified bread with 2, 4, 6, 8 and 10% isolated soybean protein and they observed that the PER of bread increased with the percentage of soybean

protein. The increase in PER wasin linear relationship with the lysine content of the bread. It is concluded that isolated soybean protein may be mixed with wheat flour up to the level of 6% as a means of successful nutritional supplementation without significant impairment of over-all acceptability.

Effect of Protein Supplementation on Cookie

Although cookies are not a staple food like bread, they have some attractive features, including long shelf life, which makes large scale production and distribution possible, as well as good eating quality, which makes it attractive for protein fortification and other nutritional improvements. High protein cookies would be particularly useful for child feeding programs. These features enhance the value of producing cookies from composite flours for organoleptic, economic and nutritional reasons (Tsen et al., 1973).

Nutritionally, cookies can be easily fortified with protein rich flours to provide a convenient high protein supplement for children because they accept cookies readily Tsen (1976). Protein fortified cookies can be prepared from composite flour such as wheat flour fortified with soy flour (Kim, 1972; Kwon et al., 1976; Tsen et al. 1973; tsen, 1974), cottonseed (Fogg and Tinklin, 1972; Bresseni et al., 1966; Bacigalupo 1969), peanut (Subrahmanyan, 1958) or corn germ flour (Blessin et al., 1972).

Fortification of wheat flour with non wheat flour causes adverse changes in such physical qualities as dough character and baking properties. For example non wheat proteins used in cookie formulas have exhibited greater water retention properties than has wheat flour and thus have possessed a greater capacity for competing for the limited free water in cookie dough (Kissel and Yamazaki, 1975). Consequently, typical spread and top grain characteristics of cookie containing these types of proteins fail to develop during baking. Matz (1962) indicated that cookie texture is dependent upon forces that hold the particle together, when the piece breaks apart, mouth feel is affected by the size, shape, consistency and degree of uniformity of resulting particles. Overall texture is affected by smoothness or roughness of surfaces and hardness of the individual particles.

Soft wheat flour is particularly good for making cookies. Tsen et al. (1975), by using a farinograph, showed that soft wheat flours developed and broke faster than hard wheat flours and their absorption was lower than hard red winter (HRW) flours. This difference in absorption is the result of differences in gluten quality and quantity between hard and soft wheat flours. So they concluded that because of these differences and also because the cookies posses a better, spread ratio and top grain score, soft wheat flour is superior to hard wheat flour for making sugar cookies.

Organoleptic properties and nutritional quality of cookies are expected to vary widely with different composite flours and with percentages of additive used. Kwon et al. (1976) reported that cookies fortified with 5 to 10% defatted soy flour did not differ from control in flavor and mouth feel. Tsen (1974) tested protein fortified sugar cookies containing 12 or 24% defatted soy flour with grade school students and found that all protein fortified cookies were rated acceptable by children. They could not differentiate between fortified and regular cookies. Kim (1972) reported that cookies could be prepared from wheat flours supplemented with 25% non wheat flour such as cassava starch or flour, corn flour or rice flour and 5% defatted soy flour or fish meal without modifying dough composition or procedure. Badi and Hosney (1976) found that sugar cookies made from grain sorghum or millet flour, like cookies from other non wheat flours, did not spread during baking and also had poor top grain characteristics. Tsen et al. (1974) found that good quality cookies could be prepared from hard wheat flour fortified with up to 50% with soy flour or protein isolate by adding surfactants such as Sodium-Stearoyl Fumarate (SSF) and Sodium Stearoyl-2 Lactylate (SSL). They also concluded that effects of SSF and SSL are more desirable at lower levels than higher levels of incorporation.

Lorenz (1978) made cookies with 5, 10 and 15% of roasted shelled and unshelled sunflower meal. Cookies baked

with the shelled sunflower meal had a greater cookie spread than the control and the cookie baked with the unshelled product. The flavor of all cookies was very pleasant. Vecchionacce and Setser (1980) fortified cookies with liquid cyclone-processed cottonseed flour and they also used SSL and xanthan gum at the level of 1% of the combined flour weight to improve the cookie quality. The results of their investigation indicated that acceptable cookies could be made with up to 48% cottonseed flour substituted for wheat flour, using a stabilizing agent such as SSL or xanthan gum.

Cookies are not considered to be major source of nutrients, but they could complement other foods such as milk. Protein efficiency of a regular cookie is low. However their PER's can be greately raised with protein fortification. Protein fortified cookies can carry nutrients in concentrated forms for feeding programs at such institutes as day-care centers and schools. Morck et al. (1976) reported that wheat cookies could be fortified with some protein combinations of texturized soy protein, whole fish protein and milk protein to produce cookies with PERs equal to or exceeding that of casein. Protein fortified cookies are convenient and economical items which can be used effectively to increase dietary protein of children and adults in need.

Effect of Processing on Protein Quality

Susceptibility to heat damage varies among different protein sources. There are numerous reports which indicate that amino acids present in the protein molecule of foods are inactivated and even destroyed by various factors during processing. These factors can be of at least two kinds: (a) Those concerned with the composition of the food such as the presence or absence of reducing sugars, level of moisture, free fatty acids; and (b) Enzymatic activity of the food (Bressani, 1975).

Evidence of the effect of heat on the nutritive value of protein was obtained as early as 1917 by Osborne and Mendel in their attempts to destroy the toxic pigments gossypol in the cottonseed by steaming the seeds for a long period of time. They also reported the effect of heat on reducing the nutritive value of seeds. When food products are heated, their proteins are known to undergo certain changes in nutritional value. Thus changes could be beneficial or deleterious to nutritive value of proteins. The beneficial effects are due to the inactivation by heat of the trypsin and growth inhibitor, hemagglutinins and other toxic factors present. The adverse effects are due to a decrease in availability of certain essential amino acids, such as lysine and methionine, as a result of reaction with reducing sugar and carbonyl compounds present in the food.

The damaging effect of heat processing on the availability of lysine is of special nutritional importance, thus many researchers have investigated lysine availability in heated products. It has been suggested that the reaction between the epsilon-amino group of lysine and carboxyl groups renders lysine unavailable. Although free alpha-NH_2 N-terminal groups are at least as reactive as the epsilon-NH_2 groups of lysine (Schwartz and Lea, 1952), the latter provides the great majority of the reactive groups in most proteins.

It has been shown that excessive heating results in a decrease in fluorodinitrobenzene (FDNB) - available lysine (Carpenter et al., 1957; Lea et al., 1960; Carpenter et al., 1962). There is indirect evidence that excessive heat also decreases the level of available sulfur amino acid.

Jansen et al. (1964a, b) demonstrated that when the baking time was increased from 0 to 50 min, the PER values of both unsupplemented bread and bread supplemented with 0.3 g L-lysine hydrochloride per 100 g flour was reduced. Of the added lysine hydrochloride, 30% became nutritionally unavailable in bread baked for 30 min at 450°F. When the baking time was reduced to 20 min no significant nutritional loss was found by rat growth assay though an 18% loss was indicated by extraction and ion exchange chromatography. Murata et al. (1979) measured the loss of lysine and threonine during the baking of bread and they concluded that 14±8% of lysine and 15±5% of threonine were lost

in the whole loaf after baking.

Several methods of analysis for available lysine are possible. It can be measured by animal growth assay or chemical methods. A commonly used chemical method involves forming a chromagenic derivative of lysine by reacting it with either 2, 4, 6-trinitrobenzene sulfonic acid or 2, 4-dinitrofluorobenzene. Once the lysine derivative is formed it can be quantitated with the use of a spectrophotometer. High-pressure liquid chromatography represents a more rapid and efficient method for separating lysine derivatives in studies involving nutritional availability of lysine.

EXPERIMENTAL DESIGN

This reasearch project was divided into three sections. The first one was preparation of the navy bean and sesame flours. The second section was the evaluation of physical and chemical as well as the sensory characteristic of cookies which were supplemented with navy bean or sesame protein, as well as a mixture of both. The third one was the evaluation of physical dough properties and baking potential of navy bean and sesame flours blended at different levels with hard red wheat flour. In this latter study, the nutritional improvement of the supplemented bread also was evaluated.

Navy bean flour was prepared by using drum drier (Counter, 1969). Sesame was prepared by extracting with hexane. These two flours were stored for later evaluation in sugar snap cookies and in bread. Different combinations of navy beans and sesame seeds were used in these sugar snap cookies which were evaluated objectively for texture and color, subjectively using panel and chemically for proximate analyses of protein, fat, ash and moisture.

The third section was divided to two different parts. The first part consisted of baking bread for objective and subjective evaluations and the second part was baking for

feeding rats to measure the nutritional quality by Protein Efficiency Ratio determination. The bread in both cases was supplemented with 10 and 20% of both navy bean and sesame flour. Dough rheology was measured by farinograph and bread was evaluated for objective measurements of texture, and color and subjectively using a sensory panel. Amino acid, available lysine as well as Protein Efficiency Ratio of the breads were measured. The results of these three sections were analyzed for variance.

Materials and Methods

Navy Bean Flour

Navy beans (Phaseolus Vulgaris) obtained from food stores at Michigan State University. To prepare the flour, the drum dry method was used (Counter, 1969; Bakker-Arkema, et al. 1967).

Beans were soaked in water at 210°F for 45 min., placed in a wire basket and retorted in steam at 220°F for 30 min. These cooked beans were macerated by Qualheim-Electro-Cut vegetable cutter and slicer, model 101. An American 12" x 19 1/18" double drum drier, manufactured by the Overton Machine Company of Dowagiac, Michigan, was used. This drier was set up to simulate a single drum operation by running one drum cold (Bakker-Arkema, et al., 1966). Drying conditions of 11 r.p.m., one layer, and 43 p.s.i.g. were used. After drying by drum drier, the bean

flour was left at room temperature for a week, during which time the powder was intermittently stirred to further reduce the moisture after which they were ground by Model D Comminuting mill using a .027" screen and thoroughly mixed to obtain a homogeneous product. This flour was stored in an air tight plastic container at room temperature (20-23^{o}C) for further use.

Sesame Seed Flour

Dehulled sesame seeds were obtained from the food stores, Michigan State University. The dehulled seeds were ground into flour in a double volume of commercial hexane to extract the oil using an Osterizer^{R} blender under the hood. After five minutes of blending, the slurry was filtered under vacuum in a Buchner funnel. This extraction with hexane was repeated four times. After the last extraction the flour was left as a thin layer in a ventilated hood for 24 hours at room temperature for final complete removal of solvent. This flour was ground by Cyclone Mill (UD Corporation, Boulder; Colorado) to give a finer and more uniform particle size. This flour was then stored at 5^{o}C for later use.

Bread and Cookie Flour

Straight grade hard wheat flour was purchased from the Department of Grain Science, Kansas State University, Manhatan, Kansas. This flour was malted (0.25%) with barley

malt, bleached with benzoyl peroxide and given the normal enrichment. Cookie flour milled from a soft red winter wheat and representing a 90% patent flour was obtained from Mennel Mills of Fostoria, Ohio.

High Protein Sugar Snap Cookies

General Cookie Formulation

Micro III method formula which was modified by Finney et al., (1950a) was followed. The method was modified to include soy lecithin based on the recommendations of Kissell and Yamazaki (1975). The formulation was changed to prepare 6 cookies per bake. The formula for making sugar snap cookies is shown in Table 5.

Four replications of each variable were prepared to be evaluated by both objective and subjective measurements. The variables prepared included control cookies and those which were substituted with 20 and 30% navy bean and sesame. For 20% substitution variables the ratio of navy bean to sesame were 20:0, 15:5, 10:10, 5:15 and 0:20. In case of 30% substitution these ratios were 30:0, 20:10, 15:15, 10:20 and 0:30. The baking order was completely randomized.

Sugar Snap Cookies Preparation

The preparation of the navy bean, sesame and wheat flour mixture at each of the five levels of substitution

Table 5. Formulation for sugar snap cookies.

Ingredients	Level of cookie flour substituted with navy bean and sesame		
	0 (control)	20	30
		%	
Cream mix, g[1]	112.8	112.8	112.8
Soy lecithin g	2.2956	2.2956	2.2956
Solution A, ml[2]	12.0	12.0	12.0
Solution B, ml[3]	9.0	9.0	9.0
Flour, g	114.78	91.82	80.35
Navy bean and/or Sesame flour	-	22.95[4]	34.43[5]
Deionized H_2O (ml)	6	6	6

1 Cream mix was made up of 594.0 g sugar, 297.0 g shortening, 9.9 g sodium bicarbonate and 29.7 g non-fat dry milk solids.

2 Prepared by dissolving 39.9 g of sodium bicarbonate in 500 ml deionized water.

3 Solution B was prepared by dissolving 33.8825 g ammonium chloride and 29.6175 g Nacl in 500 ml deionized water.

4 These included the following ratios of navy bean to sesame flour: 20-0, 15-5, 10-10, 5-15, and 0-20.

5 These included the following ratios of navy bean to sesame flour: 30-0, 20-10, 15-15, 10-20, and 0-30.

involved mixing of the navy bean, sesame and wheat flour for 10 minutes in a Kitchen Aid Mixer, Model K 5-A at medium speed to insure an even distribution of these three components. Enough flour mixture per level of substitution was prepared at the same time so that the ingredients for four replications, could be portioned from one mixture.

Humidities on each day of baking were recorded by a weather measure meterograph, Model M701-E and are presented in Table 6. The first step in the Micro III method involved preparation of sufficient quantity of creamed mass for one day of baking. Sugar, sodium bicarbonate and non-fat drymilk solids were weighed to the nearest 1.0 g and were sifted together five times. The shortening, also weighed to the nearest 1.0 g, was added to the sifted dry ingredients and all ingredients were creamed in a kitchen aid mixer at low speed (57 r.p.m.) for five min., followed by scraping of the bowl and mixing five additional minute at medium speed (120 rpm). After scraping of the bowl, the mixture

Table 6. Humidity values during baking days for sugar snap cookies.

Baking Day	% Humidity
1	27
2	29
3	31
4	33

was mixed a final 5 minutes at high speed (200 rpm) with scraping down of the bowl being done after each one minute of mixing. The creamed mass was then weighed into 112.8 g portions, which was the quantity required for preparation of 6 cookies.

One portion of creamed mass was transferred with a rubber spatula to the bowl of a National Nonrecording Micromixer, Model 100-200 A, with a head speed of 86 rpm. Soy lecithin in a quantity representing 2% of the flour blend by weight was added to the creamed mass (Kissell et al., 1971). To this mixture, 12 ml of solution A and 9 ml of solution B and 6 ml water was added (Table 5). Solution A and B and water were dispensed from automatic pipettes.

These liquids were mixed with the creamed mass for 3 minutes. The preweighed flour or flour, navy bean, sesame blend was added to the ingredients and mixed for 10 seconds with the bowl being tapped eleven times during the last 5 seconds of mixing. After the mixer was switched off and the pins lifted out of the dough, the dough was scraped into the bowl. The dough was then dislodged from the bottom and sides of the bowl by being pushed across the bowl with a spatula using seven double strokes. The dough was then mixed 5 seconds, scraped down, mixed 5 more seconds, scraped and mixed a final five seconds. After scraping the dough from the upper prongs of the mixer with a spatula, the bowl was removed from the mixer, the dough removed from the bowl and divided into six approximately equal parts

which were placed on a stainless steel baking sheet, 17 x 10 x 1/8" (42.50 x 25 x 0.31 cm). The portions of dough were rolled with one movement using a wooden rolling pin, and cut with a stainless steel cutter. The dough around the cutter should be removed before lifting the cutter. The cookie cutter was made from stainless steel tubing having an inside diameter of 2 13/32 inches (6.03 cm), a thickness of 3/64 inch (0.12 cm) and was 1 1/2 inches (3.75 cm) in height. The thickness to which the dough was rolled was determined by stainless steel riders 1 inch wide, 18 inches long and 0.25 inch thick (2.50 x 45 x 0.63 cm) which were placed along either side of the baking sheet.

The excess dough was reserved at 5°C for moisture analysis. Some of the excess dough also was used each day for two prebakes to condition the oven before any variables were baked.

Immediately following rolling and cutting of the dough, the cookies were baked for 10 minutes at 400°F (204°C) in a National Reel Type Test Baking Oven. After removal from the oven, the cookies were allowed to cool for 5 minutes on the cookie sheet and then were transferred to wire racks and cooled an additional 30 min. The cookies were bagged in polyethylene bags and kept in the freezer at -23°C for later evaluation.

Cookie Evaluation

Top grain-surface characteristics: Using a series of cookies from the Soft Wheat Quality Laboratory in Wooster, Ohio, as reference standard. The top grain of a selected cookie representing each bake was scored from 0 for a compact cookie with no surface breaks to 9 for a well-broken top containing numerous small "islands" characteristic of an optimal sugar-snap cookie (Finney et al., 1950a).

Spread factor: Spread factor is the ratio of width of cookie to the height on each bake (W/T). Width was determined by measuring the length of six cookies to the nearest mm. After rotating each cookie 90^{o}, another measurement was taken and the average of these two values were recorded as width. The height or thickness determination was made by stacking the six cookies, one on top of the other against a ruler, which was taped vertically to a ring stand and measuring to the nearest mm. Spread factor was calculated according to the AACC method 10-50 (AACC, 1962).

Color: The color of the cookies was determined by using a Hunter Color Difference Meter Model D-25. A yellow tile ($L=78.4$, $a_L=-1.9$, $b_L=25.0$) was used to standardize the instrument. Three reading L = lightness, a = redness and greenness, and b = yellowness and blueness were taken for each cookie with 60^{o} rotations between readings. The average of these readings were recorded as the color values for the cookie.

Tenderness: An Allo-Kramer shear press equipped with

a TR-3 recorder was used to evaluate the tenderness of the cookies. Cookie halves were weighed to the nearest 0.01 g before being positioned in the bottom of the standard shear-compression cell so that no overlapping of the cookie occurred. The 3000 pounds transducer with a range of 1000 (1/3 of full scale) was used for each measurement. The tenderness value expressed as pounds of force per g was calculated according to this formula

$$\text{Tenderness} = \frac{1000 \times \text{reading}}{\text{Sample wt} \times 100}$$

Breaking Strength: The breaking strength of each cookie was determined using Allo-Kramer Shearpress, Model SP12 equipped with an Electronic Recorder, Model E2EZ. The height and width of the cookie halves were determined to the nearest 0.01 cm using a Vernier Caliper. The sample was placed at the base of the shear press column and broken with a single blade cell at the mid point which had been measured. A 100 pound ring with a range of 20 was used for each measurement. Each breaking strength was calculated from a single determination according to this formula.

$$\text{Breaking Strength} = \frac{\text{Ring} \times \text{Range} \times \text{Reading}}{\text{Cm}^2 \times 100 \times 100}$$

The breaking strength was expressed as pounds of force per square centimeter.

Sensory Evaluation: Control cookies and those cookies which were substituted with navy bean and sesame flour were judged for surface appearance, interior appearance and eating characteristic by six trained taste panelists. The sample score card is shown in the Appendix.

High Protein Bread

Farinograph Testing

To measure dough rheology a C.W. Brabender Instruments, Inc. Farinograph was used. It was equipped with a type Pl-2H Dynamometer and a type 3-S-300 measuring head. Temperature of the instrument was kept at $30\pm0.1^{\circ}C$ by a Heat-Transfer Circulator Waterbath, type T-60-B.

The AACC constant dough weight procedure 54-21 B (1962) was followed using a 300 g bowl. Flour was weighed to the nearest ±0.5 g before it was premixed in the bowl for 1 min. starting at the 9.0 min. mark on the chart paper. At zero minute, the water was added from a fast delivery burette within 20 seconds. Bowl sides were scraped and the cover was replaced. When the peak was centered on the 500 Brabender Unit (BU)-line, the farinograph was run for 20 minutes. If the curve was not centered in the 500 BU line, readjustment was done on the basis of 20 BU=0.6 ml H_2o until a curve centering on the 500 B.U. line was achieved.

Variable weights of flour and water, which correspond

to the estimated "as-is" absorption were obtained from AACC farinograph table 54-28A (1962). These values were used to run triplicate farinograms for each level of supplementation with navy bean and sesame flour. The following parameters were obtained from the curve:

Water Absorption: The amount of water necessary to center the peak on the 500-BU line.

Arrival Time: The difference between zero time and the point where the top of the curve first intersects the 500 BU-line.

Peak Time: The interval from the first addition of water to that point in maximum consistency range immediately before first indication of weakening.

Stability: This is defined as the time difference, to closest 0.5 min., between point where top of curve first intersects 500 BU-line (arrival time) and point where top of curve leaves 500 B.U-line (departure time).

Departure Time: The time from zero to the point where top of curve leaves 500 B.U-line.

Mixing Tolerance Index (M.T.I.): This value is difference in B.U from top of curve at peak to top of curve measured at 5 min. after peak is reached. A related measurement called "drop off" refers to difference in B.U. from 500 B.U-line center of curve measured at 20 min. from the addition of water.

Bread Preparation

The formula which was used for making bread included 100 g flour, 3 g yeast, 1.5 g salt, 5 g sugar, 0.5 g sodium stearoyl lactylate (SSL), and in addition, 3 g fat and the amount of water used was the farinograph absorption for each mix. Baking procedure was the one which was optimized for the zero percent formula according to the AACC method 10-10 (1962).

The yeast was hydrated in the Kitchen-Aid, Model K5-A, mixing bowl for 5 minutes, then sugar, salt, flour, SSL& shortening were added. The ingredients were blended at low speed for one min. after which the dough was mixed at speed 8 (150 rpm) for 4 more minutes. The dough was transferred to the flourboard, where it was shaped into a ball by folding in half for 6 times. The dough ball was transferred to a lightly greased stainless steel bowl and fermented for 60 minutes in Cres-cor model 210-1828 fermentation cabinet at $87^{o}F \pm 2$ and 85% relative humidity (R.H.). Dough were scaled to 150 g, then degassed with a dough sheeter set at 7/32 ", molded into loaves and panned in a 9 x 5.3 x 5.8 cm pan. A National Manufacturing Company sheeter and roller was used for shaping, After shaping the loaf was proofed for 40 minutes at $87 \pm 2\ ^{o}F$ and 85% R.H. Then the bread was baked in a Reel Type Test Baking Oven at $425^{o}F$ for 20 minutes. The bread was cooled to room temperature before being weighed and wrapped in plastic wrap. Volume was measured by rapeseed displacement (National Manufacturing

Company Loaf Volumeter). The bread was kept in the freezer at -23°C for later evaluation.

Bread Evaluation

Specific Volume: Specific volume of the bread was determined by measuring the weight and volume of the bread before putting the bread in the freezer.

Compressibility: To measure the compressibility, a 2.5 cm height slice of each loaf was cut with a round cutter of 5.0 cm diameter and was compressed by using a Food Technology Corporation texture test system. A 100 pound transducer at range of 10 was used and compressibility was expressed as pounds force.

Tenderness: To measure the tenderness a 1.5 cm height slice of each loaf was cut with a 5.1 cm square cutter and tenderness was measured by using the same instrument as for compressibility. A 100 Lb ring operating on full scale was used. The values were expressed as pounds of force per gram.

Color: Color values were determined as outlined for the sugar snap cookies. One slice of each bread variable was measured for lightness, redness and yellowness.

Sensory Evaluation: Control and those breads which were supplemented with navy bean and/or sesame flours were judged for color, crust and crumb character, grain texture and for overall acceptability by eight trained taste panelists. A linear scale was applied, where the score card

was designed so that a 10 cm line represents each character to be tested. Panelists were asked to put a check mark along the line indicating their evaluations for each character. The sample score card is shown in the appendices. The sensory evaluation was repeated 5 times before the data were subjected to statistical analyses.

Nutritional Evaluation of Bread

Bread was also evaluated to see the effect of protein supplementation on its nutritional quality. For this reason another series of breads were baked to feed the rats. The procedure for baking was the same as it was mentioned before except fat and SSL were omitted for rat feeding so that the only fat source in the diet would be corn oil as outlined by AOAC (1980). Baking was done in one pound loaf pans (9.50 x 5.50 x 2.50 inches) to simulate a commercial crust to crumb ratio and this increased loaf size necesitated that fermentation time be increased to 2 hours and proofing time 1 hour.

Preparation of Diet: To prepare diet for rat growth study, bread loaves were sliced, while still frozen, by meat slicer (Model 410 Hobart Manufacturing Company, Troy, Ohio) into slices of 1.5 cm thickness. These slices were dried in a single layer on trays at room temperature for 72 hours. The dry bread was ground by meat grinder (Model 84181 D Hobart MFG. Co Troy, Ohio). The ground bread was stored in plastic bags in the refrigerator ($4^{o}C$) until

diet was prepared. Moisture by vacuum, ash, and fat of these breads were determined according to AOAC (1980) and protein was measured according to AACC (1962). Diets made with the bread were adjusted to contain 9% protein, 8% fat, 5% ash, 1% vitamin mix, 1% fiber and the rest was corn starch (Table 7). A control diet with casein as the protein source was also prepared.

Protein Efficiency Ratio Determination: PER was measured according to AOAC (1980). Weanling male rats of the Sprague Dawley strain, 21 days of age, 10 for each diet were used throughout the experiment. Rats were housed individually in stainless steel cages in a room at 23°C. They were given the appropriate assay diet and water ad libitum. Prior to the 28 days of test period, the animals were fed a standard rat diet for 3 days in order to adapt the animals to their new environmental condition. Then they were randomly divided into groups. The animals were weighed twice a week and their food intake and waste measured every three days.

The materials under test were fed as the sole source of protein at the 9% protein level. The diets were adjusted according to the proximate analysis of the test materials so that all diets, samples and reference had the same composition.

The average 28 day weight gain and protein (N x 6.25) intake per rat for each group was calculated. Protein Efficiency Ratio (PER) (weight gain/protein intake) was

Table 7. Composition of Basal Diet for the PER method

Ingredient	Amount %
Protein Source[1]	9
Corn oil	8
Salt mixture[2]	5
Vitamin mixture[3]	1
Non-nutrition fiber[4]	1
Corn starch	To complete 100

1 Vitamin-free casein; purchased from Tecklad Test Diets, P.O. Box 4220, Madison, Wisconsin. Percent nitrogen of casein was 14.4. All rations contained the equivalent of 9% protein (N x 6.25).

2 USP XVII mineral mix; purchased from Tecklad Test Diets. Composition (%): Sodium chloride (NaCl) 13.93; potassium phosphate, monobasic (KH_2PO_4) 38.90; Magnesium sulfate ($MgSO_4$) 5.73; Calcium carbonate ($CaCO_3$) 38.14; Ferrous sulfate $FeSO_4.7H_2O$, 2.7; Manganese sulfate ($MnSO_4.H_2O$), .40; Potassium iodide, (KI) .079; Zinc sulfate ($ZnSO_4.7H_2O$), 0.0548; Cupric sulfate ($CuSO_4.5H_2$), 0.0477; Cobalt chloride ($CoCl_2.6H_2O$), .0023.

To compensate for the different amount of ash which was present in different samples, salt mixture was added to the diets at a level to make the sum of the mineral mixture and the ash in the test materials equal to 5%.

3 Vitamin mixture was prepared in the laboratory and contained (mg/100 g diet): Vitamin A, 2000 (IU); Vitamin D, 200 (IU); Vitamin E, 10 (IU); Menadione, 0.5; Choline, 200; P-Amino- benzoic acid, 10; Inositol, 10; Niacin, 4; Ca-D-pantothenate, 4; Riboflavin, 0.8; Thiamine, HCl, 0.5; Pyridoxine. HCl; 0.5; Folic acid, 0.2; Biotin, 0.04; Vitamin B_{12}, 0.003.

4 Microcrystalline cellulose was used as non-nutritive fiber.

then determined for each group and the values were adjusted to 2.5 for the reference casein.

Chemical Analyses

Protein Determination

Total protein content of the flours, cookies and breads was determined according to the AACC (1962) Micro-Kjeldahl method. Reagents prepared for this method included:

Methyl red-methylene blue indicator: 2 parts of 0.2% alcoholic methyl red solution were mixed with 1 part 0.2% alcoholic methylene blue solution.

Sodium hydroxide-sodium thiosulfate solution: 50 g NaOH and 5 g $Na_2S_2O_3$, 5 H_2O were dissolved in water and then diluted to 100 ml.

Boric acid solution: 4 g H_3BO_3 was dissolved in 100 ml.

0.02 N Hydrochloric acid solution: This solution was prepared diluting 1.65 ml of 37% hydrochloric acid to 1000 ml.

About 30 mg of dried sample, 1.3 g K_2SO_4 and 40 mg HgO were weighed and added to a narrow mouthed, 100 ml non transfer micro kjeldahl flask. Two ml of H_2SO_4 was added to this mixture after which they were digested on a Lab Con-Co digestion rack model number 21621. Flasks were cooled after digestion and the digest was diluted with

about 15 ml deionized water before distillation.

For distillation, a Scientific Glass Associates Micro-Kjeldahl distillation apparatus was used. The mouth of each digestion flask was greased before being attached to the apparatus. When the boiling water started to distill over, 10 ml of NaOH $Na_2S_2O_3$ solution was added. The steam distilled mixture was collected in a 50 ml beaker containing 5 ml of 4% boric acid solution and 4 drops indicator (methyl red-methylene blue). Fourty five ml of distillate was collected and after rinsing the tip of the condenser 5 more ml of distillate was collected. Then combined distillates were titrated with 0.02 N HCl to a gray color end point. A blank was run to correct for nitrogen contamination.

The percentage protein was calculated by multiplying the percentage nitrogen by 5.7 for wheat and 6.25 for navy bean and sesame flours.

$$\% N = \frac{(\text{corrected ml HCl})(\text{N HCl})(\text{equivalent wt. N})}{\text{wt of sample mg}} \times 100$$

Nitrogen recoveries were determined with Dl-Tryptophan that had been dried in a dessicator.

Lipid

Lipid content in flours, cookies and bread were determined according to AOAC (1980) the following reagents

were used for fat analysis:

- Ethyl alcohol 95%.
- Ethyl ether.
- Hydrochloric acid solution 25 + 11 (V/V).
- Petroleum ether b.p below 60°C.

A two g sample was weighed into a 50 ml beaker. To this sample 2 ml alcohol was added and it was stirred to moisten all particles to prevent lumping on addition of acid. Ten ml hydrochloric acid (25+11) was added and after mixing, the mixture was held in a water bath at 70-80° for 40 minutes, stirring frequently during the incubation period. The sample was then cooled and 10 ml alcohol were added to each mixture. These samples were transferred to Majonnier fat extraction flasks by washing each beaker with 25 ml ethyl ether, added in 3 portions. After stoppering, the flasks were shaken vigorously for 1 min. after which 25 ml redistilled petroleum ether were added and again the flasks were shaken for 1 min. The flasks were kept at room temperature for 1 hour until the upper fat-ether layer cleared. This clear layer was then filtered through a glass wool packed on a filter paper in a funnel into a dried preweighed 125 ml flask.

The liquid which remained in the flasks was reextracted twice, each time with only 15 ml of each ether. The upper layer was again filtered into the same flask, then the funnel and tip of its stem were washed with a mixture

of both ethyl ether and petroleum ether. The fat was dried in a vacuum oven at 70°C for 3 hours. After drying,flasks were cooled for 30 minutes in air to constant weight and then reweighed. The weight was corrected by running a blank. The fat was expressed as percentage fat by acid hydrolysis.

Moisture

The moisture contents of flours, cookies, cookie dough and bread were determined according to the AOAC (1980). A well mixed sample of 2 g was weighed accurately into a predried tared aluminum dish. These samples were dried at 90°C under vacuum equivalent to 25-30 mm Hg in a Hot Pack #633 Vacuum Drying Oven overnight. Samples were cooled in a desicator and reweighed soon after attaining room temperature. The percentage loss in weight was recorded as percentage moisture.

Ash

The ash contents of all samples were determined according to AOAC (1980). Approximately 2-3 g of well mixed samples were weighed into dried preweighed porcelain ashing dishes. The dishes were put in a Temco Muffle Furnace at approximately 550°C until light gray ash resulted. Ashing dishes were cooled in a dessicator to room temperature to constant temperature before reweighing ash was expressed as percentage.

Hydration Capacity

Hydration capacity of flours was determined according to AACC 56-20 (1962). A 2 g sample was weighed into a 100-ml centrifuge tube. Forty ml of water was added to this sample, then stoppered and shaken vigorously to suspend sample completely. These samples were kept in room temperature for 10 min., during which time they were mixed by inverting 3 times at the end of 5 min and 10-minute periods. After removing the stopper samples were centrifuged for 15 minutes at 1,000 x g. (2400 rpm). After centrifugation, the supernatant was decanted and the tubes were inverted to drain (Suction is better for loosly packed samples). Then the tube and contents were weighed and hydration capacity was determined according to this formula:

$$\text{Hydration Capacity} = \frac{(\text{wt of Tube + Sediment}) - (\text{wt of Tube})}{\text{wt of sample (dry basis)}}$$

Amino Acid Analyses

Amino acid analyses were done only for wheat flour and bread samples for rat feeding. These analyses were performed by a qualified department technician on 24 hour hydrolysates of the protein in the samples. These analyses were carried out on a Beckman Model 120C Amino Acid Analyzer in which the amino acids were separated by column chromatography and quantitatively determined by automatically recording the intensity of the color produced by their

reaction with ninhydrin (Moore and Stein, 1948, 1951, 1954; Moore et al., 1958; Spackman et al., 1958). Samples were prepared according to the method described in the Beckman manual for amino acid analysis (Toeffer, 1965).

Approximately 4-5 mg of protein was weighed into 10 ml ampules. Five ml of 6 N HCl was added to the ampules. The contents of the ampules were frozen in a dry-ice-alcohol bath, evacuated by vacuum pump and allowed to melt slowly to remove gases. After evacuation, the ampules contents were refrozen in the dry-ice alcohol bath and then sealed with a propane flame. The sealed ampules were placed in an oil bath (or oven) at 110°C for 24 hours. After hydrolysis, the ampules were removed from the oven and cooled to room temperature.

To the hydrolysates, 1 ml solution containing a 2.5 µmole N-leucine was added as a standard to measure mechanical losses during transfer. The content of each ampule was transferred to a pear-shaped evaporating flask and evaporated to dryness under vacuum on a rotary flask evaporator immersed in a 45-50°C water bath. After evaporation a small amount of distilled water was added and residue was redried until all remaining HCl was removed. Finally the sample was transferred from the flask into a 5 ml volumetric flask with a citrate-HCl buffer, pH 2.2. In order to separate solid particles, the 5 ml hydrolysate was filtered and the filtrate was transferred into a small vial and stored at 4°C. An aliquot of 0.2 ml was applied to

the analyzer for amino acid analysis. The amino acid analyzer was operated at 57°C. The running time was 4 hours 55 min. for the short and 185 min. for the long column. The chromatograms were compared to the one obtained from a standard amino acid calibration mixture.

Sulfur Containing Amino Acids: In order to protect methionine and cysteine, since these two amino acids are instable during acid hydrolysis, a preliminary oxidation with performic acid is necessary. The methods of Schram et al. (1954) and Lewis (1966) were used. These methods involve performic acid oxidation of methionine and cysteine to methionine sulfone and cysteic acid, respectively.

The performic acid solution was prepared by mixing one volume of 30% (w/w) hydrogen peroxide with nine volumes of 88 percent (w/w) formic acid. This mixture was allowed to stand for one hour at room temperature. The amount of sample representing 4-5 mg protein was weighed into a 25 ml pear-shaped flask. Ten mls. of performic acid, which was previously cooled to 0°C, were added to the samples. Oxidation was carried out at 4°C for 15 hours. The acid was evaporated on the rotary evaporator, with a cold finger trap for the very corrosive performic acid. After evaporation to a syrup-like consistency, 5 ml 6 N HCl was added and flask was closed with a specially designed pressure top. To remove the air, vacuum or nitrogen can be used. Following hydrolysis at 110°C for 24 hours, 1 ml of nor-leucine (2.5 µmole) standard was added and the sample

was treated exactly the same as in the procedure for the acid-hydrolysis. The chromatograms were compared to those of the standard methionine-sulfone and cysteic acid.

Tryptophan: The tryptophan content of a protein must be determined separately from the rest of amino acids since tryptophan is labile to acid hydrolysis. Tryptophan was determined colorimetrically after hydrolysis with enzyme pronase as described by Spies (1967).

A 3 mg sample of protein was weighed directly into a small glass vessel. To each sample 100µl of freshly prepared pronase solution was added. Pronase solution contained 10 mg of pronase per milliliter of 0.1 M phosphate buffer, pH 7.5, and a drop of toluene (as a pre servative). Each vessel was placed in a vial, and the vials were stoppered and then incubated for 24 hours at 40°C. After incubation, 0.9 ml of 0.1 M phosphate buffer, pH 7.5, was added to each vial. The uncapped vials were placed into 50 ml erlenmayer flasks containing 9.0 ml of 21.2 N sulfuric acid and 30 mg of p-dimethylaminobenzaldehyde prepared immediately before use. The vials were tipped over and the contents were quickly mixed by rotating the erlenmeyer flasks. Sample were cooled to room temperature and kept in the dark at 25 °C for six hours. After the addition of 0.1 ml of 0.045 % sodium nitrate solution, the reaction mixtures were shaken and the color was allowed to develop for 30 minutes in the dark at room temperature. The absorbance was measured at 590 nm, using a Beckman DU

Spectrophotometer.

Duplicate samples of the pronase solution, without protein were treated and analyzed as described above. The tryptophan content of pronase solution was subtracted from the total tryptophan content. The blank solution contained everything but protein and pronase. A standard curve from Zero to 120 µg of trypotophan was prepared according to the procedure described by Spies and Chambers (1948).

D, L-Tryptophan (2.4 mg) was dissolved in 200 ml 21.2 N sulfuric acid containing 600 mg of dimethylamino benzaldehyde (DAB). Quantities of 0, 1, 2, 4, 6, 8 and 10 ml of this solution were made up to 10 ml with solutions of 21.2 N sulfuric acid containing 600 mg DAB/200 ml, and placed in 50 ml erlenmeyer flasks. The mixture were kept in the dark at 25 ^{o}C for six hours, then 0.1 ml of .045% sodium nitrate was added to each flask. The flasks were allowed to stand for 30 minutes for color development, and absorbance was measured at 590 nm, using a Beckman DU Spectrophotometer. A straight line curve was obtained between absorbance and tryptophan content.

Available Lysine

The method used to determine the available lysine was that described by Peterson and Warthesen (1979). Ten ml of $NaHCO_3$ solution (8% w/v) were added to approximately 1.0 g of sample placed in a round bottom flask. The flask was shaken gently to allow the sample to be wetted. The mixture

was left to stand for 8-10 minutes.

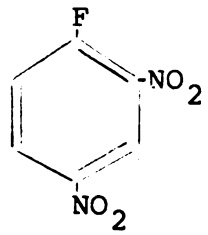
A 0.5 ml of 1-fluoro, 2,4-dinitrobenzene already dissolved in 15 ml of ethanol was then added to the flask containing the sample. The mixture was then shaken on a Burrell wrist-action shaker at room temperature for 4 hours. After the shaking period, the ethanol was evaporated in a rotary evaporator. To assure complete evaporation of ethanol a 12.5 g weight loss was checked. The mixture was then cooled to room temperature and mixed with 30 ml of 8.1 N HCl and refluxed for approximately 16 hours with 4 antibump glass beads.

After 16 hours and while still hot, the condenser was washed with a little warm water and the flask then disconnected. The content were filtered while still hot through a Whatman #2 filter paper and rinsed repeatedly with hot water until a 250 ml volume was reached. The hydrolyzates were stored at $4^{o}C$, in the dark, until used for further analysis. Approximately 1 ml of this sample was then filtered through a 0.2 μm membrane filter. Separation and quantitation using a standard dinitrophenylL-lysine (DNP-lysine) was then accomplished by high pressure liquid chromatography (HpLc).

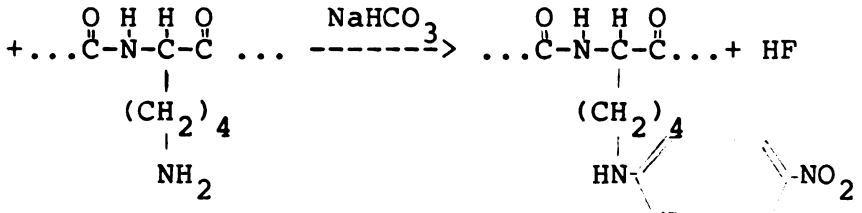
The liquid chromatograph used in this study consisted of a Waters Associates model-6000 A pump, U6K injector and 400 absorbance detector fitted for determination of wave length of 436. The wavelength used to detect DNP-lysine was

436 nm. A standard curve was prepared for peak identification and quantitation by injecting known quantities of DNP-lysine standard onto a Bond Pack C_{18} column, with a mobile phase of 20% nanograde acetonitrile and 80% 0.01 M acetate buffer pH 4.0.

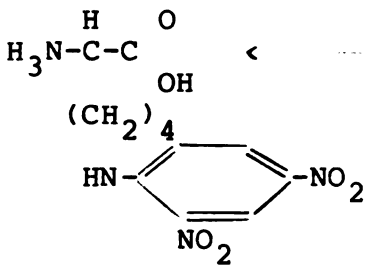
The principle behind the above procedure lies in the conversion of lysine residues, with reactive epsilon-amino groups, in protein to dinitrophenyl-L-lysine (mono-epsilon DNP-lysine) by treatment of the protein containing sample with 2,4-dinitrofluorobenzene in a basic environment at room temperature. Subsequent hydrolysis with acid at high temperature yields amino acid moieties of the sample protein, including the lysine derivative.



2,4 Dinitro-
flurobenzene



epsilon-amino of lysine
with peptide chain



mono-epsilon-DNP-lysine

Analysis of Data

Data were analyzed for variance using the STAT package with the Michigan State University Cyber, Model 750 computer. When significant difference were found between the two extreme means, Duncan's Multiple Range Test (Duncan, 1957) was used to determine differences among means.

RESULTS AND DISCUSSION

Flour Analyses

The breads, cookies, navy beans and defatted sesame flours were analyzed for moisture, protein, fat, ash and hydration capacity. Table 8 presents the results of these analyses as well as the percentage carbohydrate which was obtained by difference. Bread flour had more moisture than the other flours. Defatted sesame flour still had the highest percentage of oil, and it also had the highest percentage of protein and ash. Bread and cookie flour had similar percentages of fat, ash and protein. The protein content of navy bean flour was almost two times more than wheat protein; but it was less than half of the protein in sesame flour. The hydration capacities of navy bean and sesame flours were greater than that of bread and cookie flours.

The total amino acid contents of bread, navy bean and sesame flours expressed as grams of amino acids per 16 grams of total N are given in Table 9. The reference pattern of essential amino acid requirements of the Food and Agricultural Organization (1973) is also given for comparison. It is evident that the total sulfur containing amino acids in the sesame flour (methionine + cysteine =

Table 8. Composition of bread, cookie, navy bean and sesame flour.

	Bread Flour	Cookie Flour	Navy Bean Flour	Sesame Flour
Moisture (%)	12.8	10.5	6.9	9.1
Fat (%)	1.7	1.5	3.1	3.6
Total Protein[1] (%)	11.3	10.8	23.0	52.6
Ash (%)	0.4	0.4	3.3	7.4
Carbohydrate[2] (%)	73.7	76.9	63.7	27.2
Hydration Capacity (%)	1.5	1.6	3.2	3.0

[1] For bread and cookie flour (N x 5.7), for navy bean and Sesame flour (N x 6.25).

[2] Obtained by the difference.

Table 9. Total amino acid composition of wheat, navy bean and sesame flours.

Amino Acid	Wheat Flour g/16 g Total N	Navy Bean Flour[a] g/16 g Total N	Sesame Flour[a] g/16 g Total N	FAO/WHO[b] Reference Pattern
Lysine	2.15	5.7	2.3	5.4
Histidine	2.16	2.4	2.2	
Arginine	3.74	5.1	11.6	
Aspartic Acid	3.88	10.6	6.7	
Threonine	2.57	4.1	3.0	4.0
Serine	4.25	5.1	3.6	
Glutamic Acid	34.98	12.8	17.1	
Proline	11.59	3.3	3.0	
Glycine	3.03	3.1	3.6	
Alanine	2.57	3.3	3.5	
Cysteine	2.22	0.7	1.6	
Methionine	1.60	0.98	3.1	
Total Sulfur Amino Acid	3.82	1.68	4.7	3.5
Valine	5.27	4.4	3.9	4.9
Isoleucine	4.09	3.7	3.0	4.0
Leucine	7.27	6.7	5.4	7.0
Tyrosine	2.82	3.1	2.9	
Phenylalanine	4.96	5.3	3.9	
Total Aromatic Amino Acid	7.78	8.4	6.8	6.1
Tryptophan	0.85	1.2	1.5	1.0

[a] Boloorforooshan (1977)

[b] FAO/WHO (1973) Reference Pattern

4.7 g per 16 g of total N) is approximately 2.8 times that of the navy bean flour (1.7 g per 16 g total N) and 1.2 times that of the wheat flour (3.82 g per 16 g total N). On the other hand the lysine content of protein in the navy bean flour is 2.5 times higher than the lysine content of the protein in the sesame flour and 2.65 times higher than the lysine content of the protein in the wheat flour. This result agrees with the result which was obtained by Evans and Bandemer (1967). They measured the nutritive value of some oil seed proteins and their results showed that sesame seed was deficient in lysine and isoleucine, but it was a good source of sulfur-containing amino acids. In another experiment, Evans and Bandemer (1967b) found that all legume seeds were deficient in the sulfur-containing amino acids, but they were a good source of lysine. They also mentioned that if all of the lysine is available, legumes could be a good supplement for lysine deficient proteins.

As it is shown in Table 9, wheat flour is not a good source of lysine and is deficient in lysine as compared to the FAO pattern as a guide for requirements. Bressani et al. (1963), by comparing the amino acid pattern of wheat to FAO reference protein, found the order of limiting amino acids in a wheat basal diet was: lysine, tryptophan, methionine, isoleucine, valine and threonine.

It is reasonable to assume at this point that navy bean flour is a good supplement for sesame flour and wheat flour. The amino acid content of a combination of different

Table 10. Amino acid composition of different combinations of wheat[a], navy bean[b] and sesame[b] flours (expressed in g amino acid per 16 g of total nitrogen).

Amino Acids	90% Wheat Flour			80% Wheat Flour		
	10%Nb	10%S	5%Nb & 5%S	20%Nb	20%S	10%Nb & 10%S
	g/16 g total N					
Lysine	2.51	2.17	2.35	2.86	2.18	2.52
Histidine	2.18	2.16	2.17	2.23	2.17	2.19
Arginine	3.88	4.53	4.21	4.01	5.31	4.66
Aspartic Acid	4.55	4.16	4.36	5.22	4.44	4.83
Threonine	2.72	2.61	2.66	2.88	2.66	2.77
Serine	4.34	4.19	4.27	4.44	4.12	4.27
Glutamic Acid	32.76	33.19	32.98	30.54	31.40	30.97
Proline	10.76	10.73	10.75	9.93	9.87	9.90
Glycine	3.04	3.09	3.06	3.04	3.14	3.09
Alanine	2.64	2.66	2.65	2.72	2.76	2.74
Cysteine	2.07	2.16	2.12	1.92	2.10	2.01
Valine	5.18	5.13	5.16	5.10	5.00	5.05
Methionine	1.54	1.75	1.65	1.48	1.90	1.69
Isoleucine	4.05	3.98	4.02	4.01	3.87	3.94
Leucine	7.21	7.08	7.15	7.16	6.90	7.03
Tyrosine	2.85	2.83	2.85	2.88	2.84	2.86
Phenylalanine	4.99	4.85	4.93	5.03	4.75	4.89
Tryptophan	0.89	0.92	0.91	0.92	0.98	0.95

[a] This study

[b] Bloorforooshan (1977)

percentages of these flours are presented in Table 10. Wheat flour, which was substituted with 20% navy bean hadthe highest amount of lysine and the one with 10% sesame had the lowest amount. The highest methionine content was obtained with a combination of 20% sesame and 80% wheat flour. Supplementing wheat flour with navy bean without adding sesame resulted in a composite with low methionine content. The wheat flour which was substituted with 10% navy bean and 10% sesame flour had the best result as far as the balance of lysine and methionine is concerned.

Sugar-Snap Cookies

Protein fortified cookies can carry nutrients in concentrated forms for feeding programs. However the acceptance of these cookies depends on their quality. Research to determine properties which contribute to cookie quality has been two-fold. It has included an investigation of the individual constituents of flour and their relation to cookie quality as well as understanding of the interactions of various components in the dough system which lead to the finished product. The quality criteria for sugar-snap cookies include the diameter of the cookie and "islanding" or surface cracking in which the surface of the cookie is broken by fairly wide cracks somewhat evenly spaced to give uniformly sized "islands" (Finney et al., 1950b). In addition to appearance, flavor and texture have been known to affect the quality of a cookie, and are

important factors considered in acceptability.

Baking Study

Spread factor and Top grain: Different combinations of defatted sesame and navy bean flours were used to replace 20 and 30% of wheat flour in sugar cookies. Cookies prepared with navy bean flours exhibited dough handling properties much like those of the 100% wheat flour controls, especially those with 20% navy bean substitution. As the amount of sesame flour in the cookie formula increased the doughs became more sticky and were more difficult to handle. Spread ratio and top grain of cookies substituted with 20% and 30% navy bean sesame flour blends are presented in Tables 11 and 12, respectively.

Control of cookie spread has been indicated as one of the most serious problems confronting commercial cookie producers. Fortifying wheat flour with navy bean and sesame flour influenced the cookies width and thickness, especially those with 30% substitution. Width of cookies were reduced, while thickness increased. As a result of decreasing diameter and increasing height the spread ratio (width/thickness) was significantly higher for the control than the substituted cookies. As the percentages of sesame flour increased in the cookie formulation, the spread ratio decreased. Those cookies with 20 and 30% sesame flour had significantly lower spread ratios than that of the other variable. There were no significant differences among the

Table 11. Means and standard deviations[1] of objective measurements of cookies substituted with 20% navy bean and sesame flour.

W:B:S %	Spread Ratio	Shear Compression lb/g	Breaking Strength lb/cm^2	Top Grain
100:0:0	13.8^{a} ± 0.5	12.0^{b} ± 0.9	3.2^{a} ± 1.0	9.0^{a} ± 0.0
80:20:0	11.1^{bc} ± 0.4	8.6^{d} ± 0.3	2.0^{c} ± 0.3	7.0^{bc} ± 1.4
80:15:5	11.3^{b} ± 0.3	10.4^{c} ± 0.5	2.3^{bc} ± 0.5	6.3^{bc} ± 1.0
80:10:10	11.4^{b} ± 0.1	11.7^{b} ± 0.3	2.5^{abc} ± 0.6	5.3^{c} ± 1.5
80:5:15	10.7^{cd} ± 0.3	11.8^{b} ± 0.2	3.0^{ab} ± 1.0	3.8^{d} ± 0.5
80:0:20	10.3^{d} ± 0.6	12.7^{a} ± 0.5	2.5^{abc} ± 0.7	3.3^{d} ± 1.0

[1] Mean and standard deviations of the mean based on four replications.

Means followed by the same letters are not significantly different at $p \leq 0.05$ (Duncan, 1957).

Table 12. Means and standard deviations[1] of objective measurements of cookies substituted with 30% navy bean and sesame flour.

W:B:S %	Spread Ratio	Shear Compression lb/g	Breaking Strength lb/cm^2	Top Grain
100:0:0	13.8^{a} ± .05	12.0^{b} ± 0.9	3.24^{a} ± 1.0	9.0^{a} ± 0.0
70:30:0	9.70^{b} ± 0.2	7.2^{d} ± 0.1	1.6^{b} ± 0.5	7.0^{b} ± 0.0
70:20:10	10.0^{b} ± 0.2	9.3^{c} ± 0.3	2.4^{ab} ± 0.4	6.2^{a} ± 0.05
70:15:15	10.1^{b} ± 0.3	11.5^{b} ± 0.4	2.7^{a} ± 0.6	5.0^{d} ± 0.0
70:10:20	10.0^{b} ± 0.3	12.4^{ab} ± 0.8	2.4^{ab} ± 0.8	5.0^{d} ± 0.0
70:0:30	8.60^{c} ± 0.2	13.2^{a} ± 1.1	3.3^{a} ± 0.5	1.0^{e} ± 0.0

[1] Mean and standard deviations of the mean based on four replications.

Means followed by the same letters are not significantly different at $p \leq 0.05$ Duncan's Multiple Range Test (Duncan, 1957).

spread ratios of the other combinations of navy bean, sesame and wheat flours. Comparing the results of cookies prepared with 20% substitution level and those with 30% substitution level showed significant difference in spread ratio. Cookies substituted with 30% navy bean and sesame flour had significantly ($P \leq .05$) lower spread ratios than those with 20% (Table 13). Tsen et al. (1973) fortified wheat flour with up to 50 percent of soy flour and they concluded that fortifying wheat flour with soy flours and protein isolates drastically reduced cookie width (spread) and increased thickness (height). Both effects were enhanced progressively as fortification increased.

Top grain scores were determined using a series of cookies from the Soft Wheat Quality Laboratory in Wooster Ohio, as a reference. A score of 0 represented a compact cookie with no surface breaks and 9 represented a well broken surface containing numerous small "Islands" characteristic of an optimal sugar-snap cookie (Finney et al., 1950a). A comparison of variable means for the top grain score revealed that cookies prepared with 100% wheat flour had significantly higher scores than those which were fortified with different combinations of sesame and navy bean flours (Table 11 and 12). Significant differences were also found among different combinations of navy bean and sesame flours in both 20 and 30% substitution levels. As the amount of sesame in the flour blend increased the top grain decreased. The cookies with 20 and 30% sesame had the

lowest top grain value. There was no significant decrease between 20 and 30% substitution level (Table 13).

The mechanism by which cookie spread is reduced by certain wheat flour supplements is not understood completely. Kissel and Yamazaki (1975), enriched cookie flours with wheat gluten and soy flour derivatives. They reported that, non wheat proteins used in cookie formulas have exhibited greater water retention properties than has wheat flour and thus have possessed a greater capacity for competing for the limited free water in cookie dough. Consequently, typical spread and top grain characteristic of cookies containing these types of proteins fail to develop during baking. Yamazaki (1955) also showed that such conditions reduce cookie spread and limit top grain formation.

McWatters (1978) fortified cookies with defatted peanut, soybean and field pea flours and concluded that high water-absorptive properties exhibited by soybean flours used at 20 and 30% replacement leaves restricted cookie spread and development of a typical top grain during baking. Yamazaki et al. (1977) also found that cookie spread was depressed by increasing the relative quantity of hydrophilic additives in cookie dough. The use of dough conditioners delays the gelatinization of flour starch, thereby reducing dough viscosity and allowing cookies to spread before becoming firm or set. McWatters (1978) reported that the addition of water to the cookie formula containing 30% soy flour substitution could improve dough

Table 13. Spread ratio, top grain, breaking strength, and color means of cookies substituted with 20 & 30% navy bean and sesame flour.

	Substitutional Level		Level
	20%	30%	of significance
Spread Ratio	11.4 ± 1.18	10.4 ± 1.6	*
Top gain	5.8 ± 2.2	5.5 ± 2.5	
Breaking Strength (lb/cm^2)	2.6 ± 0.8	2.6 ± 0.8	
Tenderness (lb/g)	11.2 ± 1.4	10.9 ± 2.2	
Color (L)	59.1 ± 1.9	58.3 ± 2.3	
Color (A)	2.4 ± 0.9	2.4 ± 1.1	
Color (B)	21.6 ± 0.9	21.1 ± 1.1	*

* Analyses of variance showed significant differences at the 5% level of probability.

handling, spread characteristics, top grain and certain sensory quality attributes of sugar cookies.

Breaking Strength and Shear Compression: Means and standard deviations for breaking strength and shear compression values are presented in Tables 11 and 12 for 20 and 30% substitution levels, respectively. Breaking strength was used as a measure of the crispness of the cookie and shear compression as indicative of the cookie tenderness. The force required to break and shear the cookies decreased with increasing level of navy bean substitution, indicating a less crisp and more tender cookie was formed as the level of navy bean flour increased. The cookies with 20 and 30% navy bean had significantly higher tenderness and lower crispness than the cookies with 20 and 30% sesame. The cookies with 20 and 30% sesame were tough and difficult to break, so the higher value for breaking strength in these cookies does not mean they were crisper.

Tsen et al. (1975) reported that incorporation of a protein-rich flour or additive in a composite flour generally demands more water to obtain a machinable cookie dough, and those cookies prepared from a high absorption dough tend to be extremely hard. Kim (1972) suggested mixing the protein-rich flour or additive with a part of the shortening before adding it to the previously mixed dough. The protein additive is thus coated with shortening which reduces its water absorption. There were no significant differences at 5% level of probability between cookies

substituted with 20% or those which were substituted with 30% sesame and navy bean flour for breaking strength or shear compressibility (Table 13).

Color: Means and standard deviations for the lightness (L), redness (a) and yellowness (b) are shown in Table 14 for cookies with 20% substitution level and in Table 15 for cookies with the 30% substitution level.

Cookies prepared with 30% navy bean and 20% sesame substitution were significantly darker than the control. The redness tended to decrease as the percentage of sesame flour increased with the exception of cookies with 15% navy bean and 15% sesame flour substitution. Yellowness was also decreased as sesame flour increased. Cookies with 20 and 30% sesame substitution had significantly lower b values than the control and other substituted cookies. There was no significant differences between the L and a values of cookies prepared with 20 and 30% substitution level, but cookies prepared with 20% protein substitution were more yellow ($P \leq .05$) than those with 30% protein substitution (Table 13).

Sensory Evaluation: Quality characteristics evaluated included shape, surface color and surface characteristics, distribution of cells, shape and size of cells, interior color, texture, mouth feel and flavor. Results for sensory evaluation are shown in Tables 16 and 17 for cookies containing the 20% substitution level and in Tables 18 and 19 for cookies containing the 30% substitution

Table 14. Means and standard deviations[1] of color difference in cookies substituted with 20% navy bean and sesame flour.

W:B:S %	Color[2] L	a	b
100:0:0	60.9 ± 3.8^{a}	3.0 ± 1.5^{a}	22.5 ± 0.7^{a}
80:20:0	58.9 ± 1.5^{ab}	2.8 ± 0.5^{ab}	22.2 ± 0.2^{ab}
80:15:5	59.0 ± 1.0^{ab}	2.4 ± 0.7^{ab}	21.9 ± 0.3^{bc}
80:10:10	58.7 ± 1.9^{ab}	2.4 ± 1.0^{ab}	21.8 ± 0.1^{c}
80:5:15	59.3 ± 0.7^{ab}	1.7 ± 0.1^{b}	20.9 ± 0.3^{d}
80:0:20	58.2 ± 1.0^{b}	2.1 ± 0.4^{ab}	20.2 ± 0.2^{e}

[1] Based on four replications.

[2] L: Lightness a: greenness b: yellowness

Means followed by the same letters are not significantly different at $p \leq 0.05$ (Duncan, 1957).

Table 15. Means and standard deviations[1] of color difference in cookies substituted with 30% navy bean and sesame flour.

W:B:S %	Color[2]		
	L	a	b
100:0:0	60.9 ± 3.8^{a}	3.0 ± 1.5^{a}	22.5 ± 0.7^{a}
70:30:0	57.1 ± 1.8^{b}	2.9 ± 1.6^{a}	21.4 ± 0.8^{b}
70:20:10	58.4 ± 2.3^{ab}	2.0 ± 1.0^{ab}	21.2 ± 0.8^{b}
70:15:15	56.9 ± 0.8^{b}	3.1 ± 0.4^{a}	21.2 ± 0.4^{b}
70:10:20	58.5 ± 1.3^{ab}	2.2 ± 0.5^{ab}	21.0 ± 0.3^{b}
70:0:30	58.2 ± 0.6^{ab}	1.2 ± 0.3^{b}	19.3 ± 0.4^{c}

[1] Based on four replications.

[2] L: Lightness a: greenness b: yellowness

Means followed by the same letters are not significantly different at $p \leq 0.05$ (Duncan, 1957).

Table 16. Means and standard deviations[1] for surface appearance[2] and interior appearance[2] of cookies substituted with 20% navy bean and sesame flour.

W:B:S %	Surface appearance			Interior appearance		
	Shape	Surface Color	Surface Character	Dist. of cells	Size of cells	Interior color
100:0:0	6.3^{a} ± 0.5	6.3^{a} ± 0.2	5.6^{ab} ± 0.9	6.0^{a} ± 0.3	6.3^{a} ± 0.4	6.6^{a} ± 0.2
80:20:0	5.5^{b} ± 0.7	6.2^{a} ± 0.4	5.1^{b} ± 0.6	5.1^{bc} ± 0.4	5.1^{b} ± 0.4	6.0^{b} ± 0.5
80:15:5	6.4^{a} ± 0.4	6.2^{a} ± 0.3	5.9^{a} ± 0.5	5.5^{ab} ± 0.6	5.5^{b} ± 0.8	6.0^{b} ± 0.3
80:10:10	6.4^{a} ± 0.3	6.2^{a} ± 0.4	5.9^{a} ± 0.5	5.2^{bc} ± 0.4	5.3^{b} ± 0.3	5.9^{b} ± 0.5
80:5:15	6.1^{a} ± 0.4	6.0^{ab} ± 0.4	5.4^{ab} ± 0.4	5.5^{ab} ± 0.4	5.3^{b} ± 0.7	5.7^{b} ± 0.4
80:0:20	5.8^{ab} ± 0.3	5.6^{b} ± 0.2	4.6^{c} ± 0.2	4.8^{c} ± 0.5	5.1^{b} ± 0.5	5.6^{b} ± 0.2

[1] Based on four replications.

[2] Total score of 7, with 7 representing the most desirable.

Means followed by the same letter are not significantly different at $p \leq 0.05$ (Duncan, 1957).

Table 17. Means and standard deviations[1] for eating characteristics[2] of cookies substituted with 20% navy bean and sesame flour.

W:B:S %	Tenderness	Mouthfeel	Flavor
100:0:0	6.2 ± 0.7^{c}	6.2 ± 0.5^{a}	6.5 ± 0.2^{a}
80:20:0	6.2 ± 0.4^{c}	6.3 ± 0.5^{a}	5.2 ± 0.4^{b}
80:15:5	6.3 ± 0.5^{bc}	6.1 ± 0.7^{a}	5.4 ± 0.6^{b}
80:10:10	6.8 ± 0.2^{a}	5.7 ± 0.5^{b}	5.8 ± 0.3^{ab}
80:5:15	6.5 ± 0.4^{abc}	5.4 ± 0.4^{c}	4.1 ± 0.7^{c}
80:0:20	6.6 ± 0.3^{ab}	4.9 ± 0.5^{d}	3.4 ± 0.6^{c}

[1] Based on four replications.

[2] Total score of 7, with 7 representing the most desirable.

Means followed by the same letter are not significantly different at $p \leq 0.05$ (Duncan, 1957).

Table 18. Means and standard deviations[1] for surface appearance[2] and interior appearance[2] of cookies substituted with 30% navy bean and sesame flour.

W:B:S %	Surface appearance			Interior appearance		
	Shape	Surface Color	Surface Character	Dist. of cells	Size of cells	Interior color
100:0:0	6.3^{a} ± 0.5	6.3^{a} ± 0.3	5.6^{a} ± 0.9	6.0^{a} ± 0.3	6.3^{a} ± 0.5	6.6^{a} ± 0.2
70:30:0	5.1^{c} ± 0.4	5.8^{ab} ± 0.6	4.3^{b} ± 0.8	5.1^{b} ± 0.4	5.3^{b} ± 0.4	6.0^{bc} ± 0.4
70:20:10	5.9^{ab} ± 0.5	5.7^{ab} ± 0.6	5.2^{a} ± 0.6	5.5^{ab} ± 0.8	5.4^{b} ± 0.5	6.1^{b} ± 0.1
70:15:15	5.8^{abc} ± 0.3	5.9^{ab} ± 0.3	5.3^{a} ± 0.8	5.1^{b} ± 0.3	5.3^{b} ± 0.3	6.0^{bc} ± 0.2
70:10:20	6.3^{a} ± 0.3	5.9^{ab} ± 0.4	5.3^{a} ± 0.8	5.0^{bc} ± 0.2	5.2^{b} ± 0.4	5.8^{c} ± 0.3
70:0:30	5.2^{bc} ± 0.5	4.8^{b} ± 0.5	2.6^{c} ± 0.5	4.3^{c} ± 0.4	4.4^{c} ± 0.6	5.4^{d} ± 0.4

[1] Based on four replications.

[2] Total score of 7, with 7 representing the most desirable.

Means followed by the same letter are not significantly different at $p \leq 0.05$ Duncan's Multiple Range Test (Duncan, 1957).

Table 19. Means and standard deviations[1] for eating characteristics[2] of cookies substituted with 30% navy bean and sesame flour.

W:B:S %	Texture	Mouthfeel	Flavor
100:0:0	6.2 ± 0.7^{a}	6.2 ± 0.5^{a}	6.5 ± 0.2^{a}
70:30:0	6.2 ± 0.6^{a}	5.9 ± 0.3^{abc}	4.0 ± 0.5^{bc}
70:20:10	6.6 ± 0.1^{a}	6.1 ± 0.3^{ab}	4.8 ± 1.0^{b}
70:15:15	6.7 ± 0.3^{a}	5.5 ± 0.4^{bc}	3.9 ± 0.4^{bc}
70:10:20	6.8 ± 0.1^{a}	5.4 ± 0.7^{c}	3.6 ± 0.8^{c}
70:0:30	6.7 ± 0.5^{a}	3.85 ± 0.2^{d}	2.6 ± 0.4^{d}

[1] Based on four replications.

[2] Total score of 7, with 7 representing the most desirable.

Means followed by the same letter are not significantly different at $p \leq 0.05$ (Duncan, 1957).

level. The baking performance of cookies with navy bean up to 20% and low level of sesame up to 15% was quite similar to those with 100% wheat flour. Cookies containing these flours spread uniformly during baking and developed a typical top grain. This was reflected in sensory quality scores for appearance as no significant differences were noted among the control and these test samples. Cookies with 30% navy bean and those with 30% sesame had significantly lower scores for surface character and shape. Cookies with 30% sesame flour scored significantly lower than the control cookies for surface color too. Possibly competition of 30% substitution of navy bean and sesame proteins for moisture was a factor for poor surface appearance of the cookies made with these two plant proteins.

Control cookies were significantly more acceptable than those containing navy bean and sesame flour for their interior appearance. Significantly lower scores for interior appearance resulted when 30% of sesame flour was used for substituting wheat flour. Texture scores, especially for cookies with 20% substitution level, were variable and unordered indicating that these differences should be discounted. The differences among the control, navy bean and sesame supplemented cookies in mouthfeel and flavor were more marked. As the percentage of sesame flour increased the score for flavor and mouthfeel decreased (Fig. 1 and 2). The cookies with 15, 20 and 30% sesame had

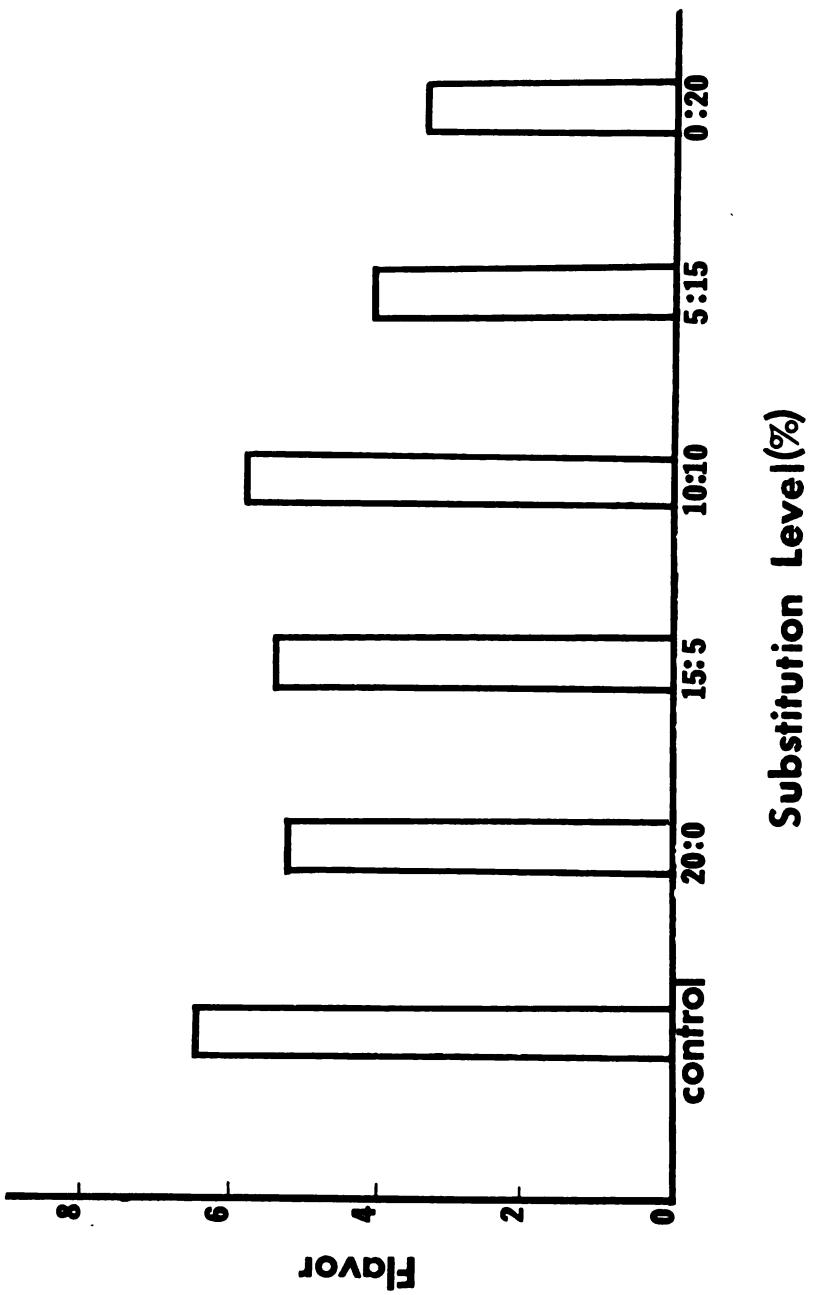


Figure 1. Flavor of cookie substituted with 20% navy bean and sesame flour.

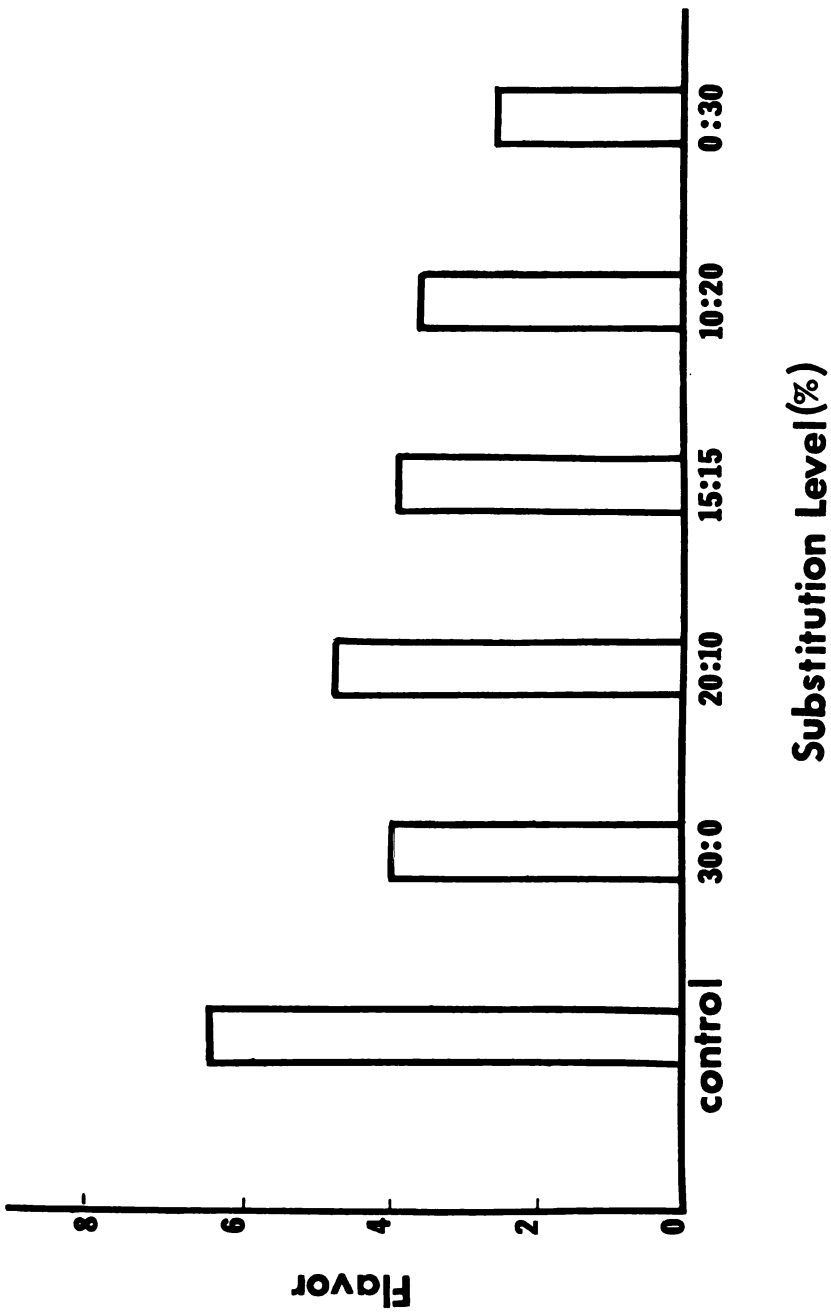


Figure 2. Effect of navy bean and sesame flour on flavor of cookie substituted with 30% navy bean and sesame flour.

significantly lower scores than the control as well as those which had navy bean substitute greater than the 20% level. Taste panelists detected an undesirable bitter taste at higher levels of sesame (15 and 20 and 30%) and they described the cookies as gummy, chalky and harsh. The panelists also could detect beany flavor when 20 or 30% of navy bean were substituted for wheat flour in cookies. McWatters (1978) suggested that one means of reducing raw, beany flavor in legume flours to be used as ingredients in sugar cookies and other baked goods might be exposing the materials to moist heat. McWatters and Heaton (1974) have shown this to be an effective flavor modifying technique for use with plant proteins. Comparing cookies with 20% and those with 30% navy bean and sesame flour showed significant differences between these two levels of substitution for surface appearance, mouthfeel and flavor. Cookies with 20% substitution level had significantly higher scores than those with 30% level of substitution at 5% level of probability (Table 20).

Looking at flavor results does not give much information about how much navy bean or sesame could be used for making sugar snap cookies acceptable for the market. The reason was variability among panelists. Some of them liked the cookies even with 20% navy bean substitution, some didn't like or were more sensitive to the navy bean flavor and gave very low scores. But from the results of preliminary testing and this test, most of the panelists liked the

Table 20. Surface appearance, interior color and eating characteristics means of cookies substituted with 20 and 30% navy bean and sesame flour.

	Substitution Level		Level
	20%	30%	of significance
Shape	6.1 ± 0.5	5.8 ± 0.6	*
Surface color	6.1 ± 0.4	5.7 ± 0.8	*
Surface character	5.4 ± 0.7	4.7 ± 1.2	*
Distribution of cells	5.3 ± 0.6	5.1 ± 0.6	
Size of cells	5.4 ± 0.6	5.3 ± 0.7	
Interior color	6.0 ± 0.5	6.0 ± 0.4	
Tenderness	6.4 ± 0.5	6.5 ± 0.5	
Mouthfeel	5.8 ± 0.7	5.5 ± 0.9	*
Flavor	4.9 ± 1.0	4.2 ± 1.3	*

* Analyses of variance showed significant differences at the 5% level of probability.

flavor of the cookies containing up to and including 20% navy bean substitution but they didn't like the flavor of the cookies when more than 10% sesame was used in the cookies. Cookies containing a combination of 10% navy bean and 10% sesame were also scored as acceptable by these panelists.

Chemical Analyses

Fat: Means and standard deviations of percentage fat were shown in Tables 21 and 22 for cookies with 20 and 30% protein substitution levels, respectively. As the amount of sesame flour in the cookies increased the fat content increased except for cookies with 30% sesame flour substitution. The reason is not known. However there was no significant difference in fat content between control and the cookies which were supplemented by different combination of navy bean and sesame at the 30% level of substitution except the one with 30% sesame flour. Significant differences were found at 5% level of probability between 20 and 30% level of substitution (Table 23).

Ash: Significant differences were present among the control and cookies substituted with navy bean and sesame at 20 and 30% level (Tables 21, 22). As the percentage of sesame increased, the ash content increased also. There also was a significant differences between 20 and 30% of substitution for ash content in cookies (Table 23).

Table 21. Means and standard deviations[1] of proximate analysis of cookies substituted with 20% navy bean and sesame flour.

W:B:S	Fat	Ash	Protein[2]	Moisture	
				Raw	Cooked
%	%	%	%	%	%
100:0:0	15.52±.70^{c}	1.08±.05^{f}	4.06±.40^{d}	15.07±.14^{a}	3.37±.10^{c}
80:20:0	15.34±.12^{c}	1.28±.03^{e}	6.28±.20^{c}	14.74±.28^{bc}	3.65±.15^{ab}
80:15:5	16.37±.10^{b}	1.39±.02^{d}	6.54±.35^{c}	14.80±.24^{b}	3.82±.10^{a}
80:10:10	16.64±.09^{b}	1.45±.04^{c}	7.84±.07^{b}	14.73±.12^{bc}	3.73±.06^{a}
80:5:15	16.65±.08^{b}	1.54±.04^{b}	8.37±.21^{ab}	14.49±.19^{c}	3.67±.04^{a}
80:0:20	17.64±.27^{a}	1.60±.01^{a}	8.84±.44^{a}	14.14±.17^{d}	3.48±.13^{bc}

[1] Based on three replications.

[2] Based on 14% moisture.

Means followed by the same letter are not significantly different at $p \leq 0.05$ (Duncan, 1957).

Table 22. Means and standard deviations[1] of proximate analysis of cookies substituted with 30% navy bean and sesame flour.

W:B:S	Fat	Ash	Protein[2]	Moisture	
				Raw	Cooked
%	%	%	%	%	%
100:0:0	15.52±.70[a]	1.08±.05[f]	4.06±.40[e]	15.08±.14[a]	3.37±.10[c]
70:30:0	15.74±.29[a]	1.48±.03[e]	6.74±.28[d]	14.25±.06[bc]	3.53±.22[bc]
70:20:10	15.65±1.02[a]	1.63±.06[d]	8.42±.12[c]	14.45±.19[b]	3.44±.10[c]
70:15:15	16.18±.12[a]	1.69±.02[c]	9.94±1.19[b]	14.47±.18[b]	3.43±.16[c]
70:10:20	16.27±.22[a]	1.79±.03[b]	9.92±.10[b]	14.31±.19[bc]	3.77±.18[b]
70:0:30	15.14±.27[b]	1.91±.02[a]	11.23±.42[a]	14.18±.11[c]	4.47±.03[a]

[1] Based on three replications.

[2] Based on 14% moisture.

Means followed by the same letter are not significantly different at $p \leq 0.05$ (Duncan, 1957).

Table 23. Moisture, fat, ash, protein and dough moisture means of cookies substituted with 20 and 30% navy bean and sesame flour.

	Level of Substitution		Level
	20%	30%	of significance
Moisture	3.6 ± 0.2	3.7 ± 0.4	
Fat	16.4 ± 0.8	15.8 ± 0.6	*
Ash	1.4 ± 0.2	1.6 ± 0.3	*
Protein	7.0 ± 1.7	8.4 ± 2.5	*
Dough moisture	14.7 ± 0.3	14.5 ± 0.3	*

* Analyses of variance showed significant differences at the 5% level of probability.

Protein: The protein content of cookies made with navy bean and sesame increased from 4.06 in the control to 8.84 for the 20% and 11.23 for the 30% level of substitution. This increase in protein is equal to 118 percent increase for 20% substitution and 177 percent increase for 30% substitution in the protein content (Tables 21, 22). There also was a significant difference between 20 and 30% substitution level for protein content (Table 23).

Moisture: Moisture was determined in raw and baked cookies (Tables 21, 22). The percentage moisture values in the raw dough was significantly higher than moisture values in the baked cookies· ($P \leq .05$). There was significant differences between the control and substituted cookie dough. Cookie dough with 20 and 30% sesame flour had the lowest moisture content. Control cookie dough showed the highest moisture percentage and the baked one showed the lowest moisture content. It seems that the control cookies lacked the water holding capacity in comparison with high protein supplement, because it lost more water during baking than the protein supplemented cookies. Thus the baked control cookies had significantly less water than cookies baked with protein substitution. There was significant differences ($P \leq .05$) between the 20 and 30% substitution level for cookie dough. But there was no significant differences between 20 and 30% level of substitution in baked cookies (Table 23).

Bread

Although the nutritional contribution of protein-rich food stuff to wheat protein has been appreciated by many, acceptance of non wheat proteins, has been limited because of their detrimental effects on dough properties, baking qualities, or flavor of baked products (Bohn and Favor, 1945; Bailey et al., 1935; Rooney et al., 1972). The acceptance of baked products supplemented with auxiliary proteins depends on source, amount, and type of supplement (Rooney et al., 1972; Marinez et al., 1969). Baking formulas or procedures usually are modified when nongluten proteins are included. Changes include using shorter fermentation times (Mathews et al., 1970), relatively strong wheat flour, and surfactants (Tsen and Hoover, 1971). The purpose of this part of study was to investigate the physical properties of dough, baking potential and nutritional characteristics of bread supplemented with 10 and 20% of navy bean and sesame flours.

Physical Dough Properties

The physical dough properties of the various wheat, navy bean, and sesame flour blends were examined with the farinograph. The farinograph is a widely used instrument based on the principle that flour quality depends on the extent of water absorption by flour during mixing, the consistency of the dough, the time required for the development of maximum dough stiffness, and the resistance

of the mixed dough to break. To work with farinograph, water should be mixed with flour under strict control of the rate of mixing and other conditions. Absorption is the amount of water added to the flour to form a dough of optimum consistency. It is usually determined to be the amount of water required to center the farinograph curve on the 500-BU line. Absorption depends on the amount of water required by the various flour components particularly by starch and protein (Bushuk and Hlynka, 1964). The increased absorptions of wheat flours substituted with different oilseed and non oilseed proteins have been reported by many researchers.

Effects of navy bean and sesame flours on physical dough properties are illustrated in Table 24. Water absorption was increased by the replacement of wheat flour with navy bean and sesame flours at both the 10% and 20% level of substitution. Bread flour substituted with 20% navy bean had the highest absorption among the flour blends. The type of protein supplement had almost no effect on water absorption when the 10% level of substitution was used. Water absorption increased as the percentage of substitution increased from 10 to 20%. The exact opposite result however, was obtained when D'Appolonia (1977) studied the effect of legume on wheat flour. His results showed a decrease in water absorption as the percentage of legume flour in the blend increased. However, in D'Applonia's study, the navy and pinto bean flour blends

Table 24. Means and standard deviations[1] of farinograph data for HRW wheat flour dough substituted with navy bean and sesame flour.

W:B:S[2] %	Arrival Time Min.	Peak Time Min.	Stability Min.	Absorption[3] %
100:0:0	1.4^{d} ± 0.2	4.5^{ab} ± 0.0	8.1^{a} ± 0.1	66.8
90:10:0	2.0^{c} ± 0.0	4.1^{b} ± 0.2	5.3^{c} ± 0.4	74.0
90:5:5	2.9^{b} ± 0.2	4.5^{ab} ± 0.0	5.4^{c} ± 0.2	73.6
90:0:10	3.0^{b} ± 0.0	4.3^{ab} ± 0.4	6.1^{b} ± 0.6	73.0
80:20:0	2.9^{b} ± 0.2	4.8^{a} ± 0.4	5.4^{c} ± 0.18	81.6
80:10:10	3.5^{a} ± 0.0	4.6^{ab} ± 0.2	4.4^{d} ± 0.2	80.8
80:0:20	3.5^{a} ± 0.0	4.3^{ab} ± 0.0	1.8^{e} ± 0.0	79.0

[1] Means and standard deviations of the mean based on duplicate determinations of the sample.
[2] Wheat:Navy bean:Sesame flour ratios.
[3] Absorption is expressed on a moisture basis and it was done once.
Means followed by the same letters are not significantly different at $p \leq 0.05$, Duncan's multiple range test (Duncan, 1957).

without dough conditioner had also a higher farinogram absorption than the control. The higher farinogram absorption values of substituted wheat compared to the control and higher absorption value of navy bean substituted flour especially at 20% substitution were in agreement with the hydration capacity values of these flours (Table 8).

Matthews et al. (1970) studied the effects of cottonseed, peanut, safflower seed and soy on doughs and breads. In their experiments, the absorption rate for the control was 58.5% while for oilseed flours at the 25% level of replacement, absorption was higher (range 61.6 to 68.4%) than for wheat flour alone. Rooney et al. (1972) reported that the water absorption was increased significantly by replacement of wheat flour with oilseed flours at both 17.5 and 20% protein substitution levels. They also reported that heat treatment increased water absorption of the oilseed-wheat flour blends, but the type of oilseed didn't significantly influence water absorption. El-Minyawi (1980) supplemented wheat flour with cottonseed flour at 0, 4, 8, 12, and 16% level. Higher absorption was obtained with increasing the percentage of cottonseed flour. El-Minyawi (1980) cited the work of Hagenmaier (1972) who studied the water binding of some oilseed isolates and reported that there was an increase in water binding with larger values of hydrophilic groups. This could be a reason for the higher farinograph water absorption with higher levels of

oilseed and legume flours.

Arrival time is a measure of the rate of hydration of flour constituents. Bushuk and Hlynka (1964) reported that there is a decrease in the rate of hydration with the increase in protein content. The effect of substituting wheat flour with navy bean and sesame flour on arrival time is also shown in Table 24. Arrival time was increased as the level of substitution increased from 10 to 20%. The arrival time for 100% wheat flour was significantly lower than those which were substituted with navy bean and sesame flour. Wheat flour substituted with 10% navy bean, 10% sesame and the one with 20% sesame flour showed significantly higher arrival times than the other substitution levels at 5% level of probability.

The peak time is the time that dough takes to reach maximum consistency or minimum mobility. The results of the effect of navy bean and sesame flour on peak time is presented in Table 24. There were no significant differences among the control and those which were substituted with navy bean and sesame flours except the one which contained 20% navy bean substitution which had significantly higher peak time at the 5% level of probability than the one with 10% navy bean substitution.

Stability, which is the difference between arrival time and departure time was measured; these data are presented in Table 24 and Fig. 3. Subjective evaluation of the farinograph curves revealed differences in the effect

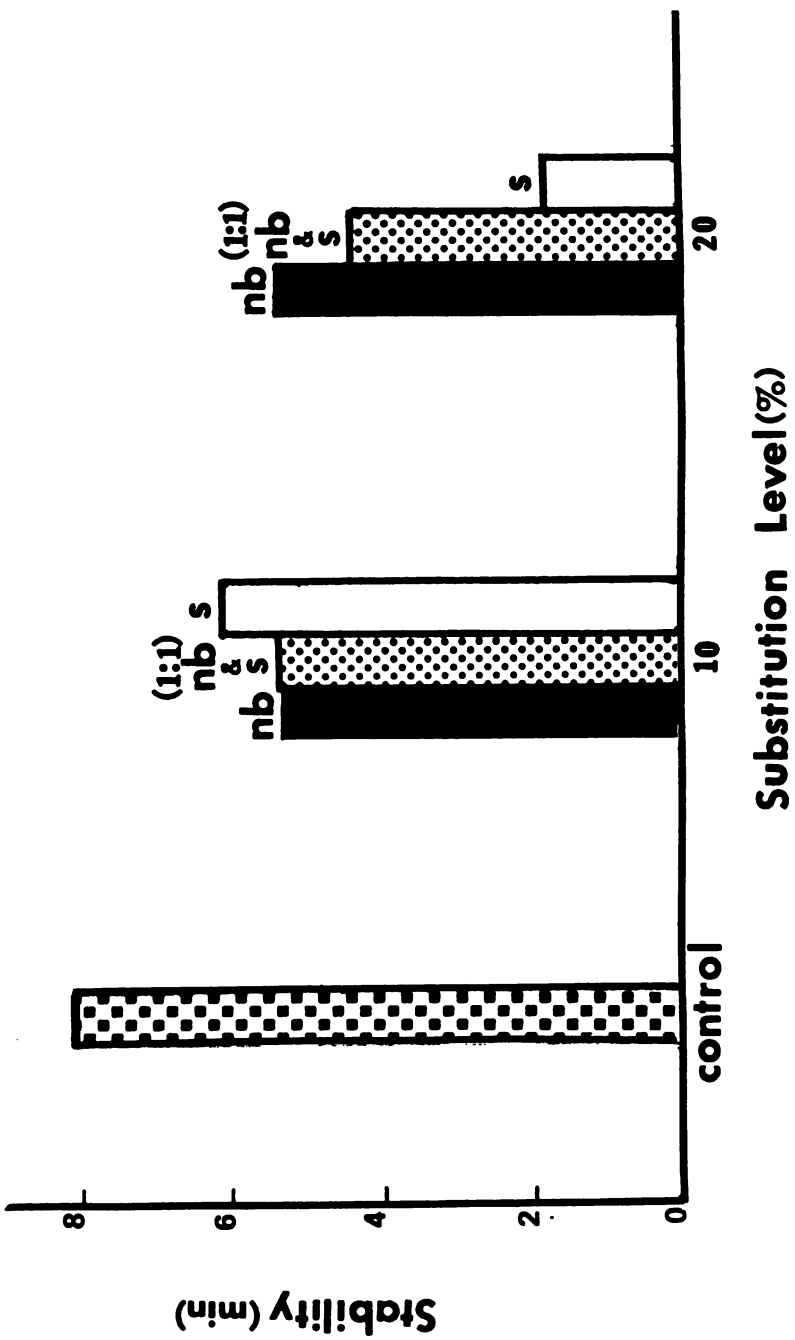


Figure 3. Effect of navy bean and sesame flour on stability of dough.

of navy bean and sesame flours on dough mixing properties. Substitution with these two plant protein weakened dough structure and all of the substituted levels had significantly lower stability than the control ($p \leq .05$). For sesame flour blends, the dough breakdown was most rapid at the 20% level of replacement as shown by the stability of 1.8 min. as compared to 8.1 min. for the control; this stability value was significantly lower than that of the control and all of the other substituted flour blends ($p \leq .05$). There was no significant difference in stability between 10% and 20% navy bean substitution. Wheat flour dough substituted with 10% sesame flour alone had significantly higher ($p \leq .05$) stability than the dough containing the other substitution levels.

Rooney et al. (1972) observed the effect of oilseed flours on dough mixing properties. They concluded that sunflower drastically weakened dough structure, while cottonseed seriously destroyed dough stability in the high protein blend. In addition peanut flour caused a noticable weakening of structure at high protein levels, whereas incorporation of sesame protein showed only a slight decrease in mixing strength. They also reported that heat treatment of oilseed flours significantly improved mixing strength and stability. They suggested that proteins or other constituents in the oilseed flours that adversely interfere with the properties of wheat proteins are denatured or rendered ineffective by heat. D'Appolonia

(1978) reported that weakening of dough was noted as the level of navy bean flour in the blend was increased. This result was not noted with roasted navy bean flours. Such a result could be attributed in part to the decrease in protease activity as a result of roasting. To compensate for weakness in flour dough substituted with oilseeds, these doughs must be made more fluid and extensible for holding the gas bubbles produced during fermentation. Less severe or shorter mixing times must be adopted to minimize damage to the gluten structure (Matthews et al., 1970).

Baking Study

Bread was baked using the straight dough procedure. The formula was based on 100 g flour was: yeast, 3 g; sugar, 5 g; salt, 1.5 g; shortening 3 g; and sodium stearoyl lactylate (SSL), .5 g. The amount of water added was exactly the farinograph water absorption, which was determined previously for each flour blend. White bread which was made from HRW wheat flour and breads substituted with different combinations of navy bean and sesame flour were baked and evaluated objectively for volume, specific volume, compressibility, tenderness and color. Breads were also evaluated by taste panelists for the organoleptic characteristics. Control and substituted breads were also analyzed for moisture, oil content, total protein and ash. In addition to physical and organoleptical characteristics, the effect of protein supplementation on nutritional

characteristic of breads was also determined.

Volume, Specific volume, Compressibility and tenderness. Data on compressibility, tenderness, volume and specific volume for control and breads substituted with 10 and 20% navy bean and sesame are presented in Table 25. Loaf volume and specific volume of bread decreased as the level of substitution increased from 10 to 20%. There was a significant difference ($p \leq .05$) in loaf volume and specific volume between the control bread and those which were substituted with navy bean and sesame. There was no significant difference in volume and specific volume among those breads which were substituted with 10% navy bean and sesame flour. Bread substituted with a combination of 10% sesame and 10% navy bean and the one substituted with 20% sesame showed a dramatic reduction in loaf valume and specific volume, thus, they had significantly lower volume than the control and other substituted levels. Rooney etal. (1972) reported the baking properties of several oilseed flours. They found sesame and peanut flours were more compatible with wheat flour for baking at 17.5 and 20% level of substitution than sunflowers and cottonseed flours and even the high protein blends containing sesame protein produced bread with good loaf volume and acceptable interior properties.

Results for compressibility of the bread (Table 25) indicated that increasing the level of sesame flour from 5 to 20% increased compressibility values. Therefore these

Table 25. Means and standard deviations[1] of objective measurements of bread supplimented with navy bean and sesame flour.

W:B:S %	Comp. lb Force	Tenderness lb/g	Loaf	
			Volume cc	Specific cc/g
100:0:0	2.1^{c} ± 0.8	8.5^{a} ± 0.7	812.5^{a} ± 30.7	6.6^{a} ± 0.2
90:10:0	2.6^{c} ± 0.8	7.0^{b} ± 1.4	743.8^{b} ± 37.5	6.0^{b} ± 0.3
90:5:5	2.7^{c} ± 1.1	5.4^{c} ± 0.8	725.0^{b} ± 35.4	5.8^{b} ± 0.3
90:0:10	3.8^{c} ± 0.5	7.2^{b} ± 0.7	706.3^{b} ± 12.5	5.7^{bc} ± 0.1
80:20:0	2.7^{c} ± 0.9	4.5^{d} ± 0.9	660.0^{c} ± 36.3	5.3^{c} ± 0.4
80:10:10	5.7^{b} ± 0.5	6.5^{bc} ± 1.1	573.8^{d} ± 70.8	4.6^{d} ± 0.6
80:0:20	8.9^{a} ± 0.4	6.7^{b} ± 1.2	543.8^{d} ± 23.9	4.3^{d} ± 0.2

[1] Means and standard deviations are based on four replications.

Values followed by the same letters are not significantly different at $p \leq 0.05$ Duncan's Multiple Range Test (Duncan, 1957).

breads required more force to become compressed and were firmer. However, the only two levels which were significantly different ($p \leq .05$) from the control and other substituted levels were bread with a combination of 10% navy bean, 10% sesame flour and the one with 20% sesame flour. The results of compressibility were in agreement with the results which were obtained by taste panel. Taste panelists rated the bread with 20% sesame very low and they indicated the texture of the bread was compact.

Tenderness of the bread is presented in Table 25. It was difficult to compare these breads by looking at their tenderness results, because they were not in order. According to the compressibility and taste panel results bread with 20% sesame flour was the toughest and the control and the bread with 10% navy bean were more tender, but the results of tenderness showed the opposite of what was expected. This may be due to the unit of tenderness which is lb/g. Dividing by the weight however may compensate for the increased denseness of these firmer breads.

Based on the results of dough physical properties, bread volume and compressibility, substitution with 20% sesame flour and with a combination of 10% sesame and 10% navy bean flours did not yield good results. It may be possible to make bread with these two levels of substitution if some modification in procedure could be arranged. Using more SSL (1% instead of .5%), decreasing the mixing

time and fermentation time are some of those modifications which have been done by other researchers to obtain better results. Matthews et al. (1970) suggested using the soft dough method instead of the straight-dough method when oilseed flours were used in breads based on microscopic examinations of the dough. Variation in distribution of starch granules occured when more water was used in the procedure. They suggested that the high concentration of starch granules in straight dough can prevent maximum gelatinization owing to limited water availability. In the soft dough method the distribution of starch granules was more disperse and it allowed maximum gelatinization and swelling to occur. They suggested greater availability of water for gelatinization of the starch granules in soft doughs may account for increased volume of these breads. Fleming and Sosulski (1977) used dough conditioners (glycolipids) in bread supplemented with oilseed flour; they suggested that, the loaf volume improvement resulting from the addition of dough conditioners indicated that they functioned, in part, to strengthen the structural frame work for holding the gas inside the dough.

Color: Means and standard deviations for the lightness (L), redness (a) and yellowness (b) are shown in Table 26. There were significant differences between the control and breads which were substituted with 10 and 20% navy bean and sesame flour except bread with 10% navy bean. Bread

Table 26. Means and standard deviations[1] of color differences in bread substituted with navy bean and sesame flour.

W:B:S %	Color[2] L	a	b
100:0:0	64.7 ± 0.9^{a}	3.4 ± 0.2^{a}	11.9 ± 0.8^{e}
90:10:0	63.3 ± 2.2^{b}	2.7 ± 0.1^{c}	12.8 ± 0.6^{de}
90:5:5	62.7 ± 0.5^{bc}	2.9 ± 0.1^{b}	13.0 ± 0.4^{cd}
90:0:10	62.2 ± 0.7^{bc}	2.9 ± 2.9^{bc}	13.7 ± 0.9^{bcd}
80:20:0	62.3 ± 1.3^{bc}	2.1 ± 0.1^{e}	13.9 ± 0.4^{bc}
80:10:10	61.1 ± 1.3^{c}	2.3 ± 0.1^{d}	14.5 ± 0.6^{ab}
80:0:20	61.8 ± 0.9^{bc}	2.3 ± 0.2^{de}	14.9 ± 0.7^{a}

[1] Based on four replications.

[2] L: Lightness a: greenness b: yellowness

Means followed by the same letters are not significantly different at $p \leq 0.05$ (Duncan, 1957).

with 10% navy bean or 10% sesame flours were slightly lighter than the other substituted bread. Control bread had significantly higher redness values than the supplemented breads. Breads with 10% substitution showed significantly higher redness than those breads with 20% substitution. Yellowness was increased as the percentages of navy bean and sesame increased from 10 to 20%. Bread with 20% sesame had significantly higher yellowness than the other substituted levels except the one with a combination of 10% sesame and 10% navy bean.

Sensory Evaluation: Taste panel results (Table 27) indicated that there were no significant difference at 5% level of probability in crust color among the control and protein supplemented breads, except the bread substituted with 20% sesame flour which obtained the lowest score by the panelists. The bread with 20% sesame was also rated the poorest in crust character, grain texture, tenderness, flavor and general acceptability compared to the breads containing the other substituting levels. Bread with a combination of 10% navy bean and 10% sesame flour was also rated low by the panelists for crust character and grain texture but was not significantly different from the bread containing a 20% sesame substitution. There was no significant difference at 5% level of probability in tenderness among control breads, bread with 10%, 20% navy bean or a combination of 5% navy bean 5% sesame substitution. Taste panel information indicated that the breads containing 10

Table 27. Means and standard deviations[1] of sensory evaluation of wheat/navy bean/sesame flour bread.

W:B:S %	Crust[2] Color	Crust[3] Character	Grain[4] Texture	Tenderness[5]	Flavor[6]	General-[7] Acceptability
100:10:0	5.3^{a} ± 0.3	6.2^{a} ± 1.0	7.4^{ab} ± 0.7	7.7^{a} ± 0.3	7.4^{a} ± 0.4	7.4^{a} ± 0.7
90:10:0	6.0^{a} ± 0.3	6.7^{a} ± 0.9	6.8^{a} ± 0.9	7.4^{ab} ± 0.9	6.7^{a} ± 0.9	7.0^{ab} ± 1.0
90:5:5	5.3^{a} ± 0.7	6.0^{ab} ± 1.5	4.6^{b} ± 1.5	6.5^{abc} ± 0.8	5.3^{bc} ± 1.0	5.8^{bc} ± 1.4
90:0:10	5.3^{a} ± 0.8	5.4^{ab} ± 0.9	5.1^{b} ± 0.7	6.2^{bc} ± 0.3	4.0^{d} ± 0.7	4.4^{c} ± 0.7
80:20:0	6.1^{a} ± 0.6	6.1^{ab} ± 1.2	4.8^{b} ± 1.5	7.2^{ab} ± 0.7	6.4^{ab} ± 1.0	6.1^{ab} ± 1.1
80:10:10	5.1^{a} ± 1.1	4.7^{bc} ± 0.6	3.6^{bc} ± 0.8	5.3^{c} ± 1.4	4.3^{cd} ± 0.1	4.3^{c} ± 0.5
80:0:20	3.9^{b} ± 1.0	3.7^{c} ± 0.2	2.4^{c} ± 0.3	3.7^{d} ± 1.0	1.8^{e} ± 0.8	1.9^{d} ± 0.5

[1] Means and standard deviations of the the means based on four replications.
[2] Scale of 0 (pale) to 10 (dark).
[3] Scale of 0 (thick rubbery) to 10 (soft tender).
[4] Scale of 0 (coarse uneven cells) to 10 (uniform cells).
[5] Scale of 0 (tough) to 10 (tender).
[6] Scale of 0 (bad) to 10 (excellent).
[7] Scale of 0 (unacceptable) to 10 (acceptable).

Means followed by the same letter are not significantly different at $p \leq 0.05$ Duncan's Multiple Range Test (Duncan, 1957).

or 20% navy bean flour were the most satisfactory among the protein substituted breads for tenderness, flavor and acceptability. Nevertheless, for the bread containing the 20% level of navy bean flour, several panel members commented that the bread had a beany taste, but it was not objectionable. No significant differences were found for flavor, tenderness and acceptability among control bread, bread with 20% navy bean or 10% navy bean at 5% level of probability. D'Appolonia (1977) reported similar results for bread substituted with 10% navy bean or lentil flour. In his study 67% of the panelists rated the bread as having a pleasant taste. The breads containing 10% pinto bean or faba bean flour were rated the poorest in taste and aroma compared to the bread containing the other legume flour. At 10% level of incorporation of sesame flour, certain panel members detected a difference in taste and they commented that the bread with 10% or more sesame had a bitter after-taste which was objectionable. Based on rheologic, baking and taste panel studies acceptable bread could be produced at the 10% or even up to 20% level of navy bean flour.

Nutritional Evaluation of Bread

Protein in wheat ranges from about 8 to 20%. During the milling of wheat into white flour, protein content decreases about 1% and the concentration of certain amino acids, including lysine, decreases substantially. Osborne and Mendel (1914) showed that wheat protein is deficient in

lysine. It is well established that adding lysine, threonine and methionine to wheat flour improves its nutritive value as feed for rats. Thus the nutritional value of bread protein can be enhanced by the addition of synthetic limiting amino acids or by supplementation with other protein sources, which compared with wheat flour, are significantly richer in those amino acids which are deficient in wheat flour.

A nutritional study was done to determine the protein quality of bread supplemented with 10 and 20% navy bean and sesame flour. The Protein Efficiency Ratio (PER), amino acid content and availability of lysine as well as the chemical composition of the supplemented bread were studied and evaluated.

Bread Composition

The chemical composition of bread supplemented with navy bean and sesame flour was measured and the results are shown in Table 28. Control bread had significantly higher moisture than the supplemented breads ($p \leq .05$). The proximate analyses of control and protein supplemented bread showed the expected trend of increasing percentage protein with increased levels of navy bean and sesame flour (Table 28). Percent of protein in control bread was significantly lower than the supplemented bread at the 5% level of probability. The protein content of bread increased significantly as the percentage of sesame increased from 10 to 20%.

Table 28. Proximate analyses[1] of bread prepared with different levels of navy beans and sesame flour.

W:B:S %	Moisture[2] %	Protein[3] %	Fat[2] %	Ash[2] %
100:0:0	12.16[a] ± 0.03	11.02[d] ± 0.5	1.64[c] ± 0.12	1.43[e] ± 0.11
90:10:0	11.29[b] ± 0.00	11.54[cd] ± 0.47	2.03[abc] ± 0.36	1.91[d] ± 0.08
90:5:5	11.42[b] ± 0.00	13.68[bc] ± 0.70	1.80[bc] ± 0.14	1.92[d] ± 0.04
90:0:10	11.36[b] ± 0.20	14.19[b] ± 1.45	2.07[abc] ± 0.03	2.05[cd] ± 0.01
80:20:0	11.40[b] ± 0.11	13.52[bc] ± 0.22	1.94[abc] ± 0.24	2.21[bc] ± 0.07
80:10:10	11.27[b] ± 0.11	15.60[b] ± 1.00	2.30[ab] ± 0.30	2.35[b] ± 0.05
80:0:20	11.46[b] ± 0.00	19.98[a] ± 1.38	2.45[a] ± 0.01	2.60[a] ± 0.07

[1] Values are average of two replications.
[2] Results are expressed on as is moisture basis.
[3] The result of protein is expressed on 14% moisture basis.
Values followed by the same letter are not significantly different at $p \leq 0.05$ Duncan's Multiple Range Test (Duncan, 1957).

Control bread had the lowest amount of fat and 20% sesame supplemented bread had the highest. The ash content of control was significantly lower than all other variable and the ash content of 20% sesame supplemented bread was significantly higher than the other breads ($p \leq .05$).

Amino Acid Analysis

The total amino acid content of bread expressed as grams of amino acids per 16 grams of total nitrogen and the FAO "reference protein" are given in Table 29. The lysine content of bread increased by increasing the navy bean flour and methionine content increased by increasing sesame flour. Bread with 20% sesame had the lowest lysine and the highest methionine content and bread with 20% navy bean had the highest lysine and the lowest methionine content.

Comparing the essential amino acid composition of control bread and those supplemented with navy bean and sesame flour to the FAO/WHO suggested pattern of amino acid requirements of infant, school children and adult showed that all mixtures contained adequate quantities of essential amino acids to meet the requirements of adults. The lysine, threonine, leucine were lower than the amounts required to support the normal growth of infants and school childrens.

Table 30 shows the percentage loss of essential amino acids after baking. Control bread had the highest percentage loss in lysine and the one with 20% navy bean had the

Table 29. Essential amino acid content[a] of bread substituted with different percentage of navy bean and sesame flour and FAO/WHO suggested pattern of amino acid requirement[b].

Amino Acids	Ratio of wheat : navy bean : sesame flour in bread							Suggested FAO/WHO Patterns		
	100:0:0	90:10:0	90:5:5	90:0:10	80:20:0	80:10:10	80:0:20	Infant	School children	Adult
Lysine	2.27	2.84	2.71	2.25	3.59	3.15	2.20	5.2	7.5	2.2
Histidine	2.10	2.08	2.23	2.00	2.38	2.44	2.74	1.4		
Arginine	3.77	3.73	5.18	5.68	4.19	7.38	7.58			
Aspartic acid	4.25	5.45	5.52	5.62	6.65	6.58	6.28			
Threonine	2.48	2.71	2.80	2.98	3.02	3.25	2.99	4.4	4.4	1.3
Serine	4.59	4.84	4.63	4.70	5.15	4.76	4.98			
Glutamic acid	34.61	31.62	31.59	31.66	28.17	27.54	29.78			
Proline	11.39	12.06	9.75	10.30	10.07	8.71	9.05			
Glycine	3.29	3.10	3.45	3.60	3.35	3.69	3.70			
Alanine	2.95	2.91	3.24	3.34	3.26	3.50	3.51			
Valine	5.56	5.28	4.86	5.10	5.73	5.86	4.31	4.7	4.1	1.8
Cysteine	0.81	2.23	2.08	1.37	1.91	0.65	2.02			
Methionine	1.37	1.27	1.73	1.58	1.17	1.34	1.84			
Total Sulfur amino acids								2.9	3.4	2.4
Isoleucine	4.02	4.03	4.20	3.91	4.39	4.32	3.32	3.5	3.7	1.8
Leucine	7.47	7.40	7.43	7.21	7.87	7.67	6.92	8.0	5.6	2.5
Tyrosine	2.77	2.51	2.47	2.84	2.75	3.03	3.20			
Phenylalanine	5.21	4.99	5.19	4.95	5.50	5.20	4.68			
Total aromatic amino acids								6.3	3.4	2.5
Tryptophan	1.09	0.94	0.94	0.91	0.84	0.94	0.91			

[a] g/100 g Protein

[b] Castro et al. (1976)

Table 30. Percentage loss of amino acids during baking in bread supplemented with navy bean and sesame flour.

Amino Acid	Ratio of wheat : navy bean : sesame flour						
	100:0:0	90:10:0	90:5:5	90:0:10	80:20:0	80:10:10	80:0:20
	%	%	%	%	%	%	%
Lysine	14.66	12.30	7.50	13.40	1.91	5.20+	12.35
Histidine	10.25	14.40	6.69	15.24	2.46	0.00	18.18+
Threonine	14.48	18.37	8.50	0.00	9.03	4.50+	0.33
Cysteine	63.50	10.94+	3.48+	31.84	13.00+	64.00	6.88+
Valine	0.00	1.86	6.36	0.00	9.73+	17.90+	9.07
Methionine	14.9	13.60	3.90	23.30	17.60	30.20	22.30
Isoleucine	5.85	4.04	5.26+	1.03+	6.03+	10.76+	9.78
Leucine	1.45	0.67	3.48+	4.49+	6.50+	12.10+	5.97+
Tyrosine	1.77	13.15	9.52	0.35	6.14	4.80+	9.96+
Phenylalanine	4.20+	0.40	8.35+	7.60+	8.30+	10.87+	5.17+
Tryptophan	22.47+	2.08	3.00	16.50	16.83	16.80	21.55

+ Weight of amino acid increased instead of decreased

lowest. Bread with 10% navy bean and 10% sesame showed a higher lysine content after baking. There is no explanation for this and some other higher values after baking. They are probably due to error in measuring the amino acids. Amino acids in baked bread were determined by analyzer but in flour it was calculated by knowing the amount of amino acids in different flours and yeast. The loss in methionine was also high especially in those with high percentages of sesame. The significant losses of some of the essential amino acids may be explained by the large ratio of crust to crumb in the small experimental loaves.

Horn et al. (1958) measured the distruction of amino acids in wheat and some wheat products. His results showed that there was no destruction of amino acids during fermentation of the doughs prior to baking into bread. But during the baking the losses of cystine, lysine and methionine were significant. Stilling et al. (1971) measured the nutritive quality of wheat flour and bread supplemented with either fish protein concentrate or lysine. They found that 6 to 16% of the lysine was lost during the baking. Murata et al. (1979) measured the loss of lysine and threonine during the baking of bread and they concluded that $14 \pm 8\%$ of lysine and $15 \pm 5\%$ of threonine were lost in the whole loaf after baking.

Available Lysine

In order to estimate the availability of lysine in protein supplemented breads a chemical method using 1-fluoro-2, 4 dinitro benzene (FDNB) was used (Peterson and Warthesen, 1979). The results of available lysine expressed as g per 100 g protein are given in Table 31. As it is shown in this table the control bread had the lowest lysine availability. The reason is the low percentage of lysine in wheat flours. Bread with 20% sesame had the lowest availability among the supplemented bread. As the amount of navy bean flour in the bread increased, the availability of lysine increased. These results are in agreement with the results of total lysine (Table 29).

Protein Efficiency Ratio

To determine the nutritional quality of the supplemented bread, an animal study was conducted. The bread was included in diets as the sole source of protein. The diets were fed to the weanling rats, and the growth response of the animals and the efficiency of utilization of the protein were determined. PER is defined as the ratio of the gain in body weight to the the amount of protein ingested by the animal during the test period. The results of food intake, weight gain and PER during the 28 day experimental period are shown in Table 32 and Fig. 4, 5, 6 and 7.

A group of rats was fed a 9% casein protein diet and it was used as control to calculate PER. Food intake,

Table 31. Lysine availability in control bread and bread substituted with navy bean and sesame flours.

	Ratio of wheat : navy bean : sesame flour						
	100:0:0	90:10:0	90:5:5	90:0:10	80:20:0	80:10:10	80:0:20
Available lysine (g/100 g protein)	1.82	3.26	2.36	2.38	3.43	2.74	2.32

Table 32. Growth of rats fed the standard diets containing casein and bread with different percentages of navy bean and sesame flour. Total protein content 9%.

Diet	Food Intake (g)	Protein Intake (g)	Wt. Gain (g)	PER[1]	Adjusted[2] PER	% PER Casein
Casein	371.0^{a} ± 50.5	33.4^{a} ± 4.5	91.4^{a} ± 17.0	2.68^{a} ± 0.23	2.50^{a} ± 0.22	100
100:0:0	253.3^{de} ± 31.7	22.8^{cd} ± 2.9	26.0^{d} ± 9.5	1.12^{d} ± 0.31	1.04^{d} ± 0.28	41.6
90:10:0	315.8^{bc} ± 40.5	28.4^{b} ± 3.6	41.1^{c} ± 9.8	1.43^{c} ± 0.20	1.33^{c} ± 0.18	53.2
90:5:5	311.7^{bc} ± 25.4	28.1^{b} ± 2.3	37.9^{c} ± 4.9	1.35^{c} ± 0.14	1.26^{c} ± 0.13	50.4
90:0:10	246.9^{e} ± 17.5	22.2^{d} ± 1.6	24.1^{d} ± 4.4	1.09^{d} ± 0.18	1.01^{d} ± 0.16	40.4
80:20:0	374.3^{a} ± 48.0	33.7^{a} ± 4.3	64.6^{b} ± 12.7	1.92^{b} ± 0.31	1.79^{b} ± 0.29	71.6
80:10:10	343.1^{ab} ± 25.5	30.9^{ab} ± 2.3	44.3^{c} ± 5.4	1.43^{c} ± 0.11	1.33^{c} ± 0.10	53.2
80:0:20	282.5^{c} ± 25.7	25.4^{c} ± 2.3	24.9^{d} ± 4.8	1.00^{d} ± 0.19	0.90^{d} ± 0.12	36.0

[1] Protein efficiency ratio.
[2] Corrected for casein = 2.5.
Values followed by the same letter are not significantly different at 5% probability level (Duncan, 1957).

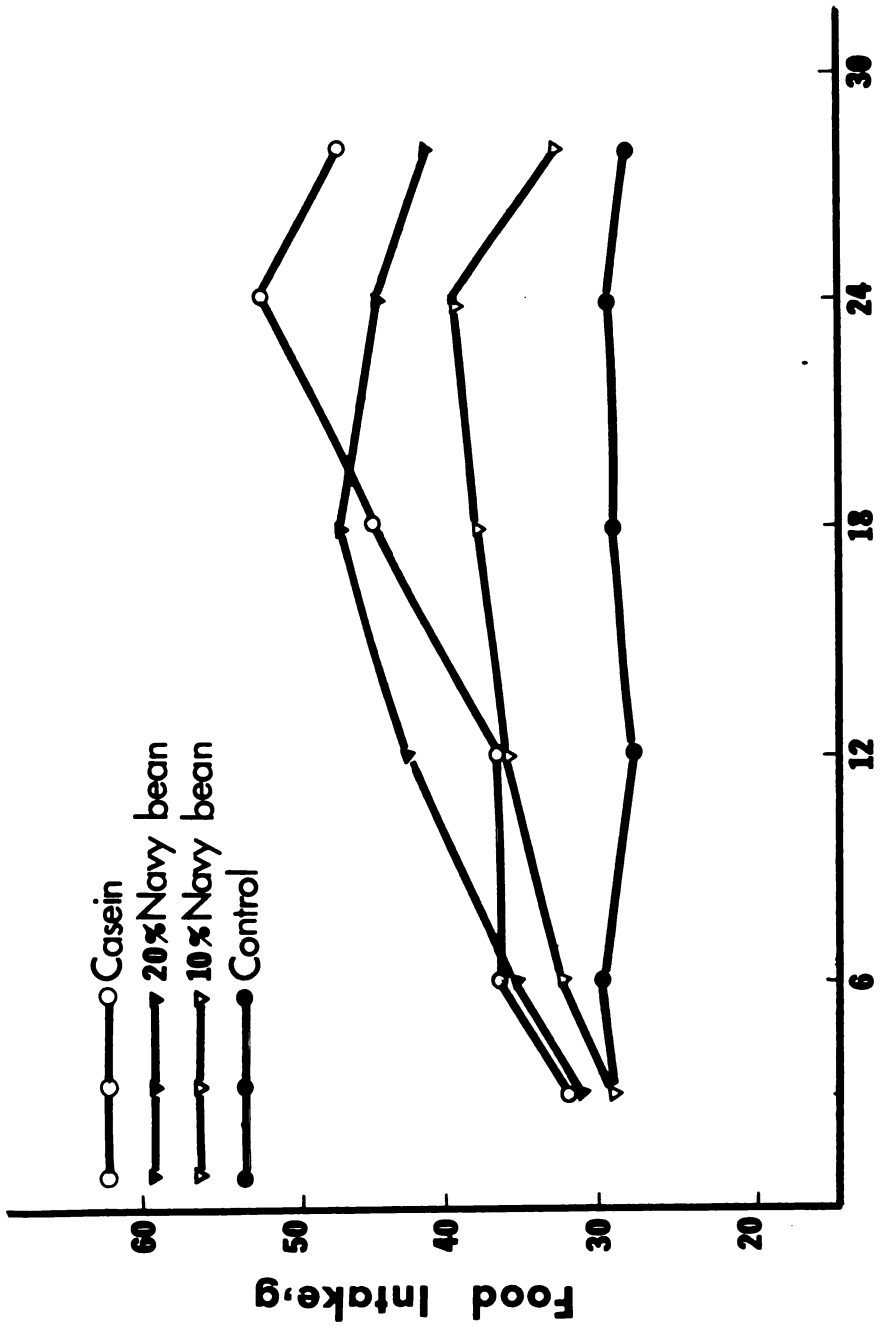


Figure 4. Food intake of weanling rats (av. of 10) fed standard diets containing: casein, control bread and bread substituted with 20% and 10% navy bean.

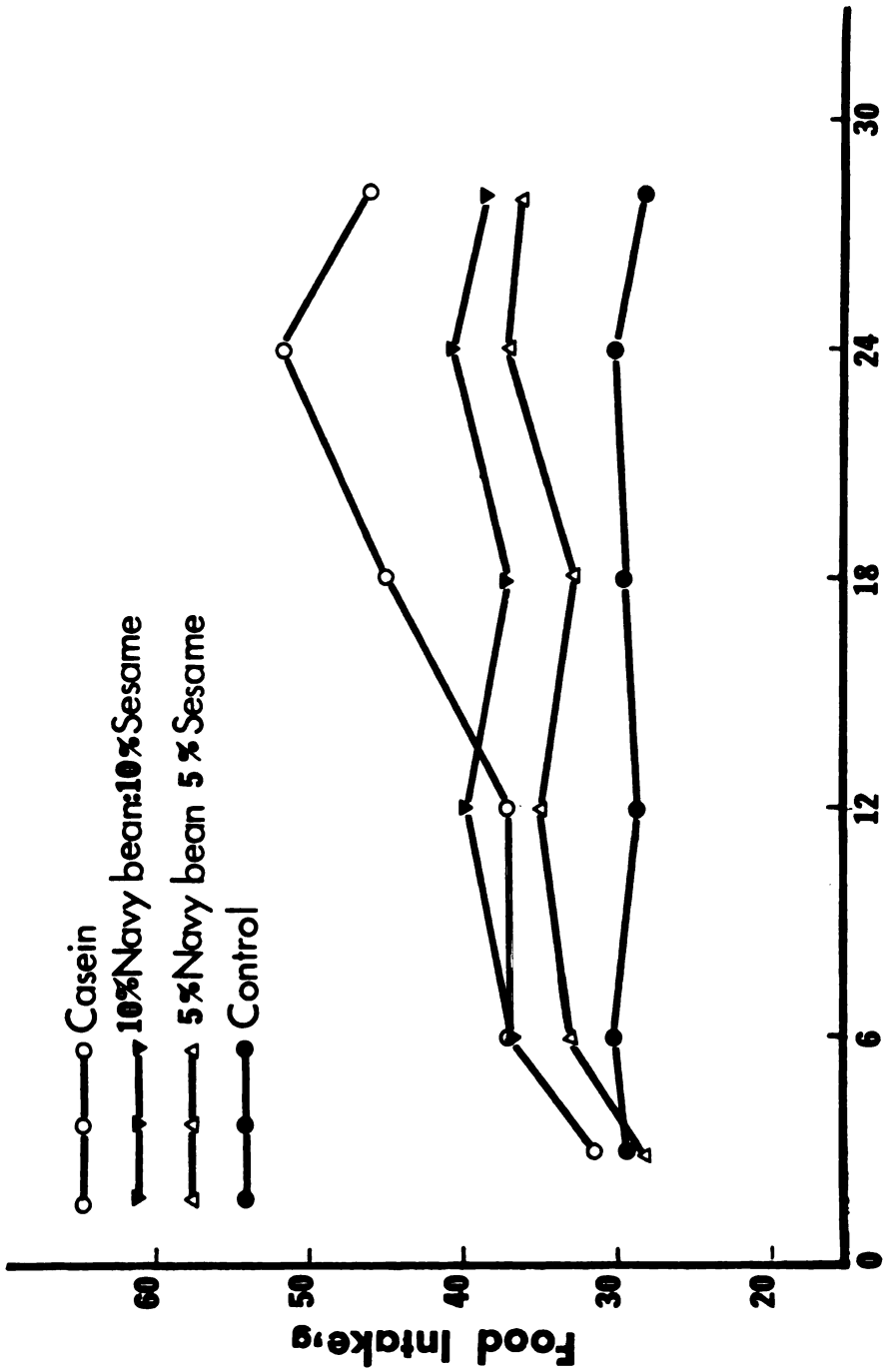


Figure 5. Food intake of weanling rats (av. of 10) fed standard diets containing: casein, control bread and bread substituted with a combination of navy bean and sesame flour.

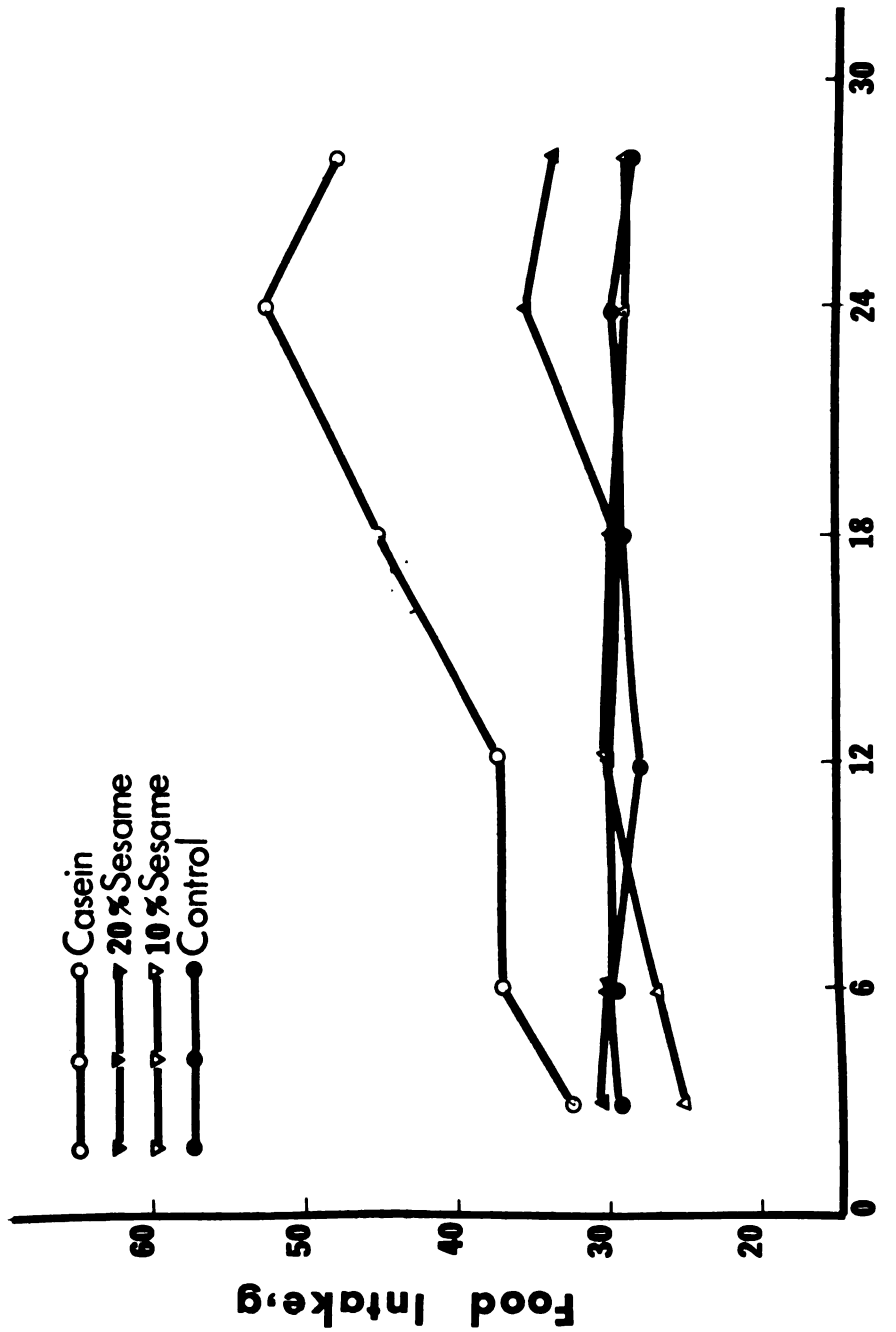


Figure 6. Food intake of weanling rats (av. of 10) fed standard diets containing: casein, control bread and bread substituted with 20% and 10% sesame flour.

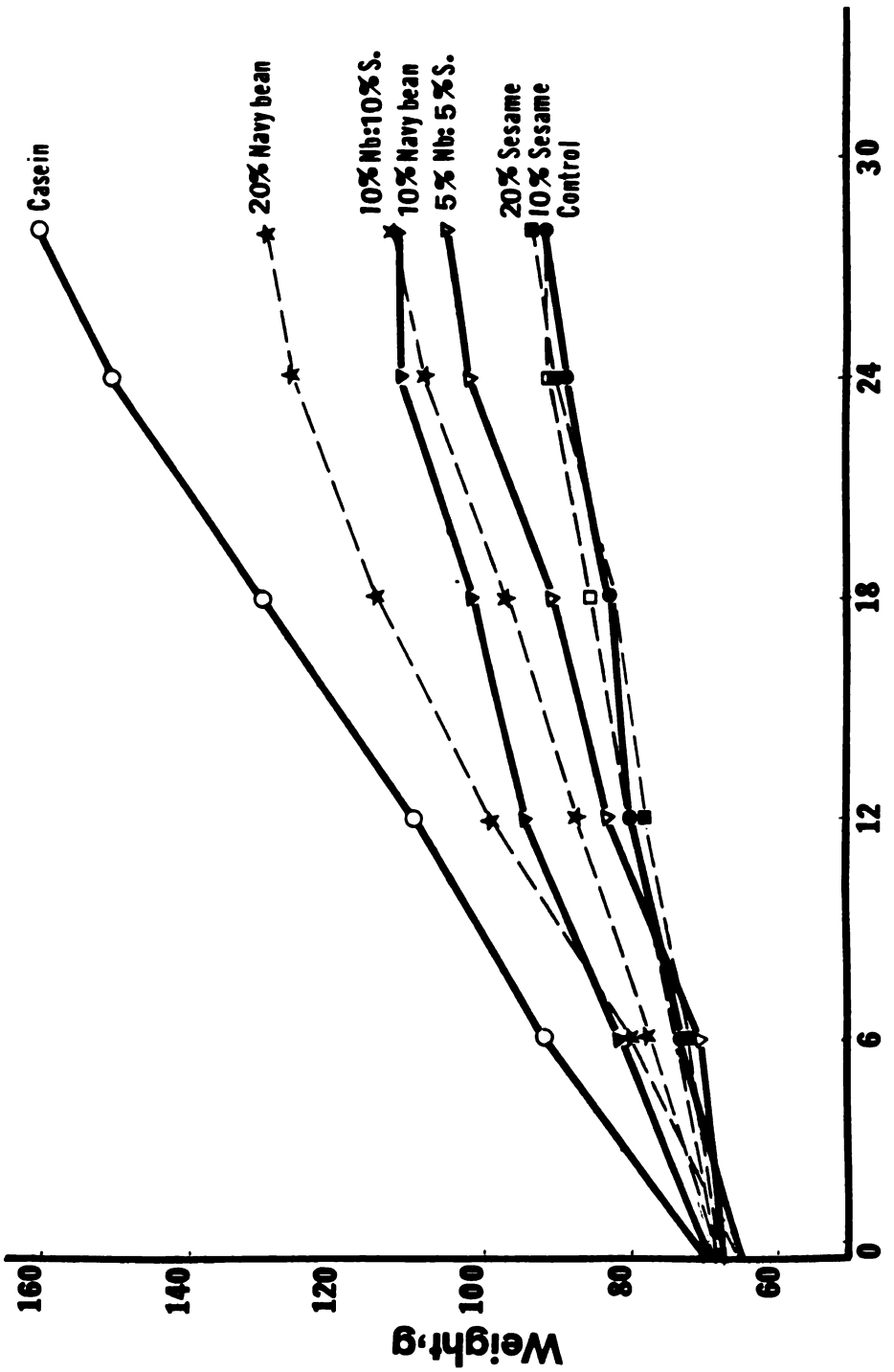


Figure 7. Body weight of weanling rats (av. of 10) fed standard diets containing: casein, control bread and bread substituted with different combinations of navy bean and sesame flour.

weight gain and PER were higher in this group than those groups which were fed with bread. The PER for this group was adjusted to 2.5 as recommended by the National Academy of Science, National Research Council (1963). All the PER values obtained throughout this work were corrected for casein = 2.5.

Food intake and weight gain were increased as the percentage of navy bean flour in the bread increased (Table 32). There was no significant difference ($p \leq .05$) in food intake between groups fed with casein and those which were fed with 20% navy bean and 10% navy bean 10% sesame flour supplemented bread. Groups fed with bread supplemented with 10% sesame showed significantly lower food intake than the groups fed with other supplemented breads. The results of food intake showed that the low quality and imbalanced diet was not accepted by the rats and they ate less in comparison with those which were fed with casein and those with a high percentage of navy bean supplementation. Fleming and Sosulski (1977) reported that lysine caused a significant increase in feed consumption for the control, sunflower, soy and faba bean breads. Weight gain for diets with no additional lysine varied from 24.9 g for wheat flour bread to 114.6 g for casein, but addition of lysine narrowed the range (61.7 to 101.5 g) and, with the exception of casein, increased weight gains of all diets. Kumta et al. (1958) showed that rats refuse to consume imbalanced diets. Bressani et al. (1960) supplemented cereal with limiting

amino acids, which included lysine, tryptophan, methionine, isoleucine, valine and threonine. They fed six children from one year, 5 months to 5 years, 9 months. In one of their experiments, they supplemented the basal diet with all limiting amino acids except lysine. This immediately resulted in vomiting and refusal of the food offered. They concluded that this effect was probably due to the fact that the addition of other amino acids increased the imbalance of the diet with respect to lysine.

Greater nutritional responses were obtained when bread supplemented with navy bean than with sesame flour. The increase in growth rate and PER was in linear relationship with the navy bean content of the bread. The PER of the group fed with 20% navy bean supplemented bread was 1.79 which represented 71.6% of that for casein control diet. This would be expected because navy bean is high in lysine, which is the first limiting amino acid in wheat flour. By increasing the navy bean, the lysine content of the bread increased (Table 29), so better weight gain and PER were obtained. The addition of sesame flour did not have much effect in improving the nutritional quality of bread. There was no significant differnce ($p \leq .05$) in weight gain and PER for the group fed with 10% navy bean and the one which was fed with 10% navy bean, 10% sesame flour supplemented breads. The PER of these two groups was 1.33 which represented 53.2% of that for casein control diet. Bressani et al. (1960) supplemented cereal protein with amino acids and

they concluded that the nutritive value of wheat proteins can be improved markedly by the addition of lysine alone to the wheat diet. Rosenberg (1959) concluded that the biological value of cereal grain is improved by adding the amount necessary to bring the level of the first limiting amino acid to that of the second.

There was no significant difference ($p \leq .05$) in weight gain and PER among control, 10% sesame and 20% sesame supplemented bread. The protein quality of the control bread and those with 10% and 20% sesame flour were 41.6, 40.4 and 36% of that for the casein control group. The relatively low efficiency of the sesame protein supplemented bread in promoting growth is attributed mainly to its low lysine content. After supplementing with sesame flour, the lysine content decreased in comparison with the other amino acids and an imbalanced diet was obtained, which caused low PER in the rats. Some of the rats which were fed with 10% and 20% sesame flour supplemented breads had lost some of their hair at the end of experimental periods. Wilding et al. (1968) measured the nutritive value and dietary properties of soy protein concentrates. They observed that rats fed white breads were significantly smaller than those fed with 15% soy supplemented bread. They also mentioned that at the end of 7 weeks, rats in the group fed white bread were losing a noticeable amount of hair and coat luster.

In using any amino acid pattern for the evaluation of proteins, it is necessary to consider not only the adequacy of the amount of each amino acid per gram of nitrogen when others are present in optimal quentities, but also the effect of excess (Bressani et al., 1963). Rosenberg (1959) evaluated supplementation of foods with amino acid and concluded that, the amino acid supplementation of a deficient protein gives an improved response only when it corrects the most limiting amino acid in such a way as to improve the balance or proportion among the other essential amino acids. Some of the amino acids may have little or no adverse effect on nitrogen utilization when they exceed the quantity per gram of nitrogen recommended in the FAO reference pattern. Others such as methionine, valine, leucine when present in excess may under some conditions produce imbalance (Rosenberg, 1959).

Two important aspects must be considered when protein supplements are used in foods. These aspects are the effect of supplement on total protein and also its effect on the quality of the protein in the product. In this study, addition of sesame flour to bread increased the amount of total protein, but did not increase the quality of the protein supplemented bread. The addition of navy bean not only increased the total protein in bread but also increased the quality of bread.

Based on physical, chemical, organoleptical and nutritional results which were obtained in this experiment,

navy bean flour could be a good supplement for wheat flour for making breads and cookies. Breads and cookies up to and including 20% of navy bean flour substitution were scored as acceptable by most panelists. Cookies with more than 10% sesame were not accepted by the panelists. Certain panel members detected a bitter after-taste in bread with 10% or more sesame which was objectionable.

The addition of navy bean not only increased the total protein, lysine and available lysine content of the bread but also increased its quality. Greater growth rate and PER values were obtained for bread supplemented with navy bean flour than with sesame. Addition of sesame flour to bread increased the amount of total protein, but did not increase the quality of supplemented bread.

SUMMARY AND CONCLUSIONS

The objective of this study was to observe the effects of supplementation of wheat flour with navy bean and sesame flours. To achieve this, different combination of navy bean and sesame flour were used to replace 20 and 30% of wheat flour in sugar-snap cookies and 10 and 20% of wheat flour in bread. The chemical composition, baking properties and organoleptic characteristics of bread and cookies as well as the total amino acid content, available lysine and nutritive value of supplemented breads were determined.

In sugar snap cookies the ratio of navy bean to sesame flour was 20:0, 15:5, 10:10, 5:15 and 0:20 for 20% substitution and 30:0, 20:10, 15:15, 10:20 and 0:30 for 30% substitution. Chemical analyses of these cookies showed that protein content increased as the level of navy bean and sesame flour increased. Fat content increased with increasing level of sesame flour substitution except the one with 30% sesame flour. The ash content also increased as the level of sesame flour increased. Raw dough had higher moisture value than baked cookies. The results of moisture also showed that the control cookies lacked water-holding capacity in comparison with high protein cookies, because the control cookie had more water loss

during baking than the protein-supplemented cookies.

Cookie spread and top grain scores were reduced as the percentage of navy bean and sesame flour increased. Those cookies with 20 and 30% sesame flour had the lowest spread ratios and top grain scores among the protein-supplemented cookies. They also were more tough and more force was needed to compress and break them. The force required to break and shear the cookies decreased with increasing level of navy bean substitution, indicating a less crisp and more tender cookie was formed as the level of navy bean flour increased.

Sensory data for the attribute of general acceptability showed that cookies containing up to and including 20% navy bean substitutions were scored as acceptable by most panelists. The panelists did not like the flavor of the cookies when more than 10% sesame was used in the cookies. Cookies containing a combination of 10% navy bean and 10% sesame were also scored as acceptable by the panelists. Comparing the results of cookies substituted with 20% navy bean and sesame flour and those with 30% navy bean and sesame flour showed that cookies with 20% substitution had a better baking properties and organoleptic characteristics than those cookies with 30% substitution level.

Effects of navy bean and sesame flours on physical dough properties were measured by the farinograph. The type of protein supplement had almost no effect on water absorption when 10% level of substitution was used. Water

absorption, arrival time and dough breakdown increased in the dough system by increasing the level of navy bean and sesame flour substitution from 10 to 20%. The bread flour substituted with 20% navy bean had the highest absorption among the flour blends. The dough breakdown was most rapid at the 20% level of replacement as compared to the control and other substituted levels.

The bread baked with navy bean and sesame flour showed an increase in protein content as the percentage of substitution increased from 10% to 20%. Fat and ash content increased by increasing the level of sesame flour. Loaf volume and specific volume of bread decreased as the level of substitution increased from 10 to 20%. There was no difference in volume and specific volume among those breads which were substituted with 10% navy bean and sesame flour. Bread substituted with 10% navy bean, 10% sesame and the one with 20% sesame showed a dramatic reduction in loaf volume and specific volume. Results for compressibility of the bread showed that by increasing the level of sesame flour from 10 to 20% more force were needed to compress the bread and they were firmer in comparison with the control and other protein-supplemented breads.

Taste panel information indicated that the breads containing 10 or 20% navy bean flour were the most satisfactory among the protein substituted bread for tenderness, flavor and acceptability. Some of the panelists commented that the bread with 20% navy bean flour had a

beany taste, but it was not objectionable. At 10% level of incorporation of sesame flour, certain panel members detected a difference in taste and they commented that the bread with 10% or more sesame had a bitter after taste which was objectionable. Based on rheologic, baking and taste panel studies acceptable bread could be produced at the 10% or even up to 20% level of navy bean flour, but in case of sesame flour even a 10% substitution was objectionable.

The total amino acid contents of bread was also determined. The lysine content of bread increased by increasing the navy bean flour and methionine content increased by increasing sesame flour. Bread with 20% sesame had the lowest lysine and the highest methionine content and the bread with 20% navy bean had the highest lysine and the lowest methionine content. The amino acid content of bread was compared before and after baking these result showed that most of the amino acids were lost during baking, but percentage of loss was not consistant among breads substituted with different percentage of navy bean and sesame flour. The availability of lysine was also determined as g per 100 g of protein in control bread and those breads which were supplemented with navy bean and sesame flours. Lysine in the control bread had the lowest availability. As the amount of navy bean flour in the bread increased the availability of lysine was also increased. Bread with 20% sesame had the lowest availability and the

one with 20% navy bean had the highest availability among the protein supplemented breads.

To determine the nutritional quality of the supplemented bread, an animal study was conducted. The bread was included in diets as the sole source of protein. Protein quality of the diets were evaluated by Protein Efficiency Ratio (PER). Greater nutritional responses were obtained when bread was supplemented with navy bean than with sesame flour. The increase in growth rate and PER were in linear relationship with the navy bean content of the bread. When PERs for the 20% navy bean supplemented and 20% sesame supplemented diets were compared to 100 for that of casein, they were 71.6 and 36.0 respectively. For the 100:0:0, 90:10:0, 90:5:5, 90:0:10 and 80:10:10 mixture of wheat:navy bean:sesame flour, the PER's were 41.6, 53.2, 50.4, 40.4 and 53.2% respectively.

The increase in PER value with increasing navy bean flour would be expected, because navy bean is high in lysine, which is the first limiting amino acid in wheat flour. The addition of sesame flour did not have much effect on improving the nutritional quality of bread. The reason is because of low lysine content of sesame flour. So after supplementing with sesame flour the lysine content in comparison to the other amino acids decreased and an imbalanced diet were obtained which caused low PER in the rats fed with these diets.

PROPOSAL FOR FUTURE RESEARCH

Effort to improve the quality and quantity of proteins in bread and other baked products is of growing interest all over the world and especially in the developing countries. Formulating new products to meet specific nutritional needs using combinations of cereal grains, legumes and other protein concentrates has been the objective of many Agency for International Development projects. Many of these projects have ended in failure because of the lack of commitment by government or industry, lack of acceptability of the products and lack of education concerning the purpose of the products.

The prospects for commercial production and wide spread consumption of a new product in different countries will depend in the first place on local acceptance. The new product should have a good taste and characteristics, and also a good price to be accepted by the people. If the new food is a kind of local bread, to explore consumers acceptability of that bread pilot bakeries will need to be established to prepare and market the bread and at the same time a nutrition advisory group is necessary to educate the people about their nutritional problems.

The technology of producing different protein concentrates is still beyond the economical capacity of these countries. Edible legume seeds will be more highly utilized especially if 1) quick cooking bean processing technology is more generally adopted on a commercial basis, and 2) more acceptable, nutritious and digestible foods are developed from legume seeds. The area of processing of sesame seed for the production of an acceptable flour still needs research to overcome the strong bitter taste that exists in this seed.

Based on textural, organoleptical and nutritional characteristics of the supplemented bread navy bean flour was a good substitute for wheat flour at a 20% level. Nevertheless, more research is necessary to determine the maximum amount of navy bean which can be used. More feeding studies with navy bean supplemented products are necessary, especially in human subjects in order to get reliable evaluation of supplemented products. In case of sesame no improvement was observed in protein quality and also the texture and taste of the supplemented bread was not accptable.

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APPENDIX

SUGAR-SNAP COOKIE SCORE CARD

Name _____ Date _____ Sample # _____

	Points	Score
A. SURFACE APPEARANCE		
1. Shape		
a) Uniform sphere; evenly rounded	7	
b) Less circular shape; inconsistent	4	_____
c) Sunken center; outer ridge	1	
2. Surface Color		
a) Excellent	7	
b) Fair	4	_____
c) Poor	1	
3. Surface Characteristics		
a) Excellent	7	
b) Fair	4	_____
c) Poor	1	
B. INTERIOR APPEARANCE		
1. Distribution of Cells		
a) Uniform; visible sheeting, thin crust	7	
b) Heterogenous distribution, moderately thick crust	4	_____
c) Cake-like; no discernible cells, thick crust	1	
DESCRIBE WHAT YOU SEE: _____		
2. Shape & Size of Cells		
a) Excellent	7	
b) Fair	4	_____
c) Poor	1	
CIRCLE ONE OF THE FOLLOWING:		
Geometrical shape: spherical, oval, elongated collapsed		
Size: small medium large		
3. Interior Color		
a) Excellent	7	
b) Fair	4	_____
c) Poor	1	
C. EATING CHARACTERISTICS		
1. Texture		
a) Crisp; requires considerable force to bite through	7	
b) Slightly chewy; requires moderate force to bite through	4	_____
c) Chewy; requires little force to bite through	1	
2. Mouthfeel		
a) Acceptable	7	
b) Moderately acceptable	4	_____
c) Unacceptable	1	
CIRCLE ONE OF THE FOLLOWING: crunchy, gritty adhesive, harsh, gummy, smooth, powdery, Other:_____		
3. Flavor		
a) Acceptable	7	
b) Moderately acceptable	4	_____
c) Unacceptable (pronounced off-flavors present)	1	
If the cookie is scored 3 or below for flavor, circle one of the following: chalky, greasy, metallic, rancid, Other:_____		

COMMENTS:

Figure 8. Score card used for sensory evaluation of sugar-snap cookie.

BREAD SCORE SHEET

Sample Number: Name:

Date:

Instructions:

Please place an "X" along the vertical line indicating your judgment.

Crust Color

Pale — Dark

Crust Character

Thick Rubbery — Soft Tender

Grain Texture

Coarse Uneven cells — Uniform cells

Tenderness

Tough — Tender

Flavor

Bad — Excellent

Overall Acceptability

Unacceptable — Acceptability

Comments:

Figure 9. Score card used for sensory evaluation of white bread