THE EFFECT OF TOASTING ON THE PROTEIN QUALITY
OF WHITE BREAD AND WHITE BREAD
SUPPLEMENTED WITH SOYBEAN

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THESIS

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Supplemented and unsupplemented white breads were baked and toasted at three different toaster settings, light, medium, and dark. Organoleptically, products were highly accepted when toasted at the light and medium temperatures.

Biological tests with rats resulted in a decline in the efficient utilization of the protein with toasted white bread diets, evidenced by poor weight gain, low liver weight, Serum protein, PER (protein efficiency ratio - weight gain/protein intake) and percent digestability.

Increased toasting temperatures reduced the amino acides essential for growth in white bread diets. Supplementation with soybean improved the quality of the bread, possible due to dstruction by heating of the trypsin inhibitor in soybean.

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CHAPTER I

INTRODUCTION

The awareness of the need for consuming foods of high quality protein has prompted many people to evaluate their current nutritional status. Most of the world's population, the tropical and subtropical regions in particular, is dependent on vegetable foods of high carbohydrate content such as cereal grains, starchy roots, and fruits. Any cereal is used directly as human food somewhere in the world. Most carbohydrate foods have become the staple food of the majority of the world's population. To supply these people with an adequate amount of high quality protein foods is an important nutritional concern (1, 4, 9).

In the United States, many people have adopted the vegetarian diets for religious, ecologic, or economic reasons. Some of these diets are satisfactory, but others are not (65). Increasing the nutritive value of such diets would require improving the quality of a staple product. Many researchers have, however, demonstrated that wheat proteins are of poor biological value as compared with that of animal proteins (8). This conclusion was based on the poor growth weight and low protein efficiency ratio [weight gain (gms.)/protein intake (gms.)] values when wheat was the protein source of diets fed growing children and laboratory animals (11). Despite the known fact that the nutritive value of wheat-flour protein is low (18), it is also important to know that the amino acid balance has been improved by the addition of plant proteins in concentrated forms (71, 73) or L-lysine (69).

The conversion of the dough to bread through the process of baking has been shown to cause the destruction of lysine (29, 33) which is the limiting amino acid for growth (36, 75). As a result, baked bread has been demonstrated to have a lower protein quality than bread dough (26, 36). Harden and others (29) and Jansen and others (38) stated that further quality deterioration occurs with prolonged baking time and high temperatures. Thus, in order to improve the nutritional value of bread, a popular food item in the world, but low in some of the essential amino acids, supplementation of nutrients lost during processing is necessary (78). Supplementation of bread makes it convenient to improve the nutritional status of the population (66).

The nutritional quality of wheat flour can be improved by the addition of soy flour, cottonseed flour, and other protein concentrates which will produce bread with high protein quality (44). Rat growth assays have shown that supplementing bread diets with L-lysine (75), soy concentrate (83), or fababeen (35) will markedly improve protein quality scores. In a recent study, Fellers and others (19) found that soybean flour is an excellent nutritional supplement to wheat flour. This supplementation results in an increase in the protein content and provides an improved amino acid balance. Furthermore, soy flour is described as the least expensive source of food protein and easily available in large amounts. Owing to changes in baking properties and eating acceptability by the population, the incorporation of high levels of soy flour into bread has been done with limited success.

Heat treatment of supplemented bread proteins such as over toasting has been found to decrease the nutritional value of such products. Clydesdale (13) explains that the nutritional value of proteins is affected by direct loss of amino acids as well as decreased digestibility and absorbability through interactions with non-nitrogen containing compounds. It was found that heat treatment in the presence of reducing sugar led to Maillard reaction between amino acids and reducing sugars (17).

Heat processing may either improve or impair the utilization of proteins. The heat treatment of soy flour before utilization of the amino acids by animals is done to destroy antigrowth factors which have adverse effects on protein utilization (59). In soybean flour, heat makes the amino acids (for example, methionine) more available by destroying the trypsin inhibitor (12). On the other hand, severe overheating of protein foods impairs their nutritional quality.

It is postulated that the free amino acid groups of arginine and lysine are affected by carbohydrate. The epsilon-amino groups of lysine is highly susceptible to this type of reaction which results in an enzyme resistant inter- and intra-molecular bond. The unavailable amino acids resistant to enzyme cleavage are thus eliminated as waste products, and this effect on animals has resulted in growth depression (25). Studies with infants and laboratory animals have shown that the proteins involved are not utilized by the body, but are excreted (51, 61).

A limited amount of research in this area exists; hence further studies on the effect of toasting on the quality of supplemented bread proteins would make a valuable contribution in the improvement of nutritional status of certain groups.

The objectives of this study were to determine the acceptance of soy white untoasted and toasted bread as compared to untoasted and toasted white bread alone at different temperatures. The biochemical analysis section of the experiment included the effect of diets prepared from untoasted and toasted bread on the protein efficiency ratio (PER), liver weight, liver protein, serum protein, creatinine, and percent digestibility of experimental rats.

Hypothesis

Based on the biochemical analysis data, toasting will reduce the nutritive value of white bread more than it will soy white bread.

There will be a depression in weight gain between rats that ingest the diets made from untoasted and toasted white bread and a more rapid increase in weight gain among those fed untoasted and toasted white bread supplemented with soybean.

There will be a decrease in weight gain with increase in toasting temperature among rats fed toasted bread diets.

The protein quality of white bread alone will be improved by the addition of soybean flour as will be determined by the PER and the effects of diets on liver weight, liver protein, serum protein, creatinine, and percent digestibility.

Review of the Literature

Cereal proteins, when used as a major dietary source, have been inadequate in meeting the needs of the undernourished nations of the world (83). They are limited with respect to the amounts and quality of protein available and are more efficiently utilized in combination with proteins from other sources or when supplemented with the limiting amino acids (32, 52, 53).

Bread is not a food of high quality protein, and the low level of lysine in wheat proteins has been widely discussed (34). Wheat flour is low in lysine, and the baking process can aggravate the lysine deficiency in bread. Previous studies (16, 36, 38, 69) have shown that lysine is the limiting amino acid in bread and that lysine loses its nutritive value during the baking process. The reports of Scrimshaw and Altschul (72) explain the need of wheat flour fortification with lysine, and protein-rich additives such as soy flour for bread making.

Reducing the nutritive losses during processing is one of the ways to improve the nutritive value of bread. Despite bread fortification and supplmentation, the eating pattern can be improved also. Block and others (7), in their study on the effects of baking and toasting on the nutritive value of protein in a cake mix, found that protein efficiency was markedly reduced. Tsen and Reddy (78) reported on the effect of toasting on the nutritive value of bread and pointed out that toasting significantly reduced the nutritive value of bread as demonstrated by rat feeding tests. Their result showed that the food conversion ratio calculated from weight gain and feed intake was increased while the protein efficiency ratio was significantly reduced with toasting and with the degree of toasting (78).

Meanwhile, limited information is available on the effect of toasting on the nutritive value of supplemented bread.

The Nutritive Value of Bread

Wheat is unique among the cereal grains because of the products it produces. For instance, wheat flour is the only flour that will produce good quality bread, cakes, cookies or pasta (34). Cereals are deficient in lysine and some other essential amino acids. Nutritionally they will not provide the quality and quantity of protein needed by young children (79). It is because of this deficiency in proteins and essential amino acids that fortification of bread with amino acids and protein has been recommended (47) in order to improve its nutritional value.

Fleming and Sosulski (21) did a study to evaluate the protein quality of breads supplemented with concentrated plant proteins and lysine, and to determine the possibility of producing a bread which could be rated as an "excellent" source of dietary protein. Their results showed that the addition of lysine had a marked effect on protein ratings due to the increased protein efficiency ratio (PER) values.

Recent studies, using milk, soy, or fish, singly or in combination with lysine monohydrochloride, were undertaken by Jansen (39) to produce high-protein breads. Tsen and Hoover (79) fortified wheat flour with full-fat soy flour in making bread and determined that this fortification can raise protein content, balance essential amino acids, and increase the caloric value of the product.

The nutritive value of bread can be enhanced beyond the level of enrichment additions. Such highly nutritious bread was developed for use in the New York State Mental hospitals when it was discovered that some of the patients ate much more bread and less of other foods than the average person. Because of the participation of the Cornell University faculty members in the development of this bread it was called the "Cornell bread" which contained 6 percent full-fat soy flour and 8 percent nonfat dry milk, based on the weight of the flour (20). These added ingredients increased the protein, vitamin, and mineral content of the bread. Some other breads of improved nutritive value have been developed for household use (57) and for school lunch programs (81)

Improving the Quality of Wheat Proteins

Animal and Human Studies

Dietary proteins are needed for a variety of purposes including replacement of tissue and organ proteins due to continuous metabolic losses, the formation and growth of new tissues during development, pregnancy, lactation and recovery from pathological conditions (84). Young and Scrimshaw explain further that to meet these needs essential amino acids and nonspecific nitrogen sources that comprise the dietary protein are of utmost importance (84).

Early works of Osborne and Mendel (54) demonstrated that the addition of lysine to diets based on wheat proteins improved their nutritional value for the rat. This conclusion was based on the results of the experiment when wheat proteins, especially gluten,were the sole source of proteins in diets fed to rats. The growth of rats was poor, and the protein efficiency ratio (PER) values were low.

Repeated experiments of other investigators (22, 23, 31, 68, 80) with laboratory animals, have also demonstrated that the protein value of diets consisting mainly of cereal grains can be greatly improved by supplements of amino acids, especially of lysine, and by supplements of lysine-rich proteins, that the protein quality of diets in which legumes (bean, pulse) are the main protein source can be improved by supplements of sulfur-containing amino acids, and complementary amino acids can be combined to produce a protein mixture of higher nutritional value than any of the individual component proteins.

Block and associates as well as Munaver (48) reported that for growth and maintenance of experimental animals the proteins of wheat are qualitatively poor as compared to that of animal protein. When Rosenburg and Rohdenburg (69) added 0.25 percent of L-lysine monohydrochloride to the white bread fed experimental rats, the PER increased from 1.0 to 1.9. Similarly, Hutchinson and others (36), Bender (5), and

Rosenberg and others (70) revealed that both lysine and threonine are essential for optimum growth in the rat.

According to Jansen (39) lysine fortified white bread was equal in protein value to bread fortified with 3 percent fish protein concentrate (FPC) and also equal to 8 percent toasted soy flour. Also lysine- and threonine-fortified white bread was equal in protein value to bread fortified with 6 percent FPC. Hallab and others (30) reported the supplementation of Arabic bread with L-lysine at a level of 0.3 percent and found that the protein efficiency ratio (PER) increased from 0.40 in the unsupplemented bread to 1.5 in the L-lysine-supplemented bread.

In India, the Central Food Technological Research Institute used blends of wheat, processed full soy-bean and defatted peanut flour with 70:15:15 respectively in several feeding programs (49). Rice and ragi diets were supplemented with this blend. When 20 percent of the diet was replaced with the blend the PER value was satisfactory in animal studies. The PER value increased from 2.0 from feeding a wheat diet to 2.45 with a 10 percent blend supplementation, and 2.7 at the 20 percent blend supplementation (49). Further increase to 2.86 in the PER value was seen when the blends were fortified with limiting amino acids methionine, threonine, and lysine (14). Wheat and Kaffir corn (sorghum vulgare) diets of Indians were improved by adding this vegetable mixture fortified with limiting amino acids (50).

In a nitrogen retention study of young children on the effect of lysine supplementation of wheat flour, Bressani and others (10) showed that feeding protein and calorie at 2 grams and 80 to 100 kilocalories per kilogram of body weight per day revealed that wheat protein can be markedly improved by the addition of lysine alone.

Over twenty years ago, Widdowson and McCance (82) fed 300 undernourished children, aged 4-15 years, diets in which about 75 percent of the total energy was provided by bread and most of the remainder by vegetables. Their subjects' per capita intake of animal protein was about 8 g. per day. Their results showed that the children grew throughout the one year period of the test at a rate that is considered greater than well-nourished English or American children would have done and were judged to be well nourished. The authors at a later test (45) administered to one group of children a pint of milk daily, and to a second group of children an equicaloric amount of biscuits and orange juice. Results showed no differences between the two groups of children considering the diet variables observed during the subsequent 6 months (45). They concluded that with ample food, good sanitation, and appropriate health care, a diet composed largely of wheat products and vegetables was adequate for children aged 4 and/or older.

King and others (41) in a field trial in Haiti fed a supplement of bread or one of bread fortified with lysine to malnourished school children. They reported no benefits from lysine fortification.

In another study, Pereira and others (58) in India, utilized 2-5 year old children who received 54 percent of their calories and 85 percent of their protein from wheat. Their results showed that the group receiving additional lysine grew, on the average, 0-6 cm. more in 4 months than did the group that were not receiving lysine. They also reported that there were no differences between the two groups with regard to weight gain, nitrogen retention, serum protein concentration, or hemoglobin concentration. The protein in the diet contained only 8 percent of the total energy due to the presence of oil and sugar in the diet (58).

Darby (15), in a study of lysine fortification in Iran, reported no benefit from a lysine supplement was observed. Graham and others (27) in Peru reported on the effectiveness of a wheat-noodle supplement for children which was compared to that of the same supplement fortified with fish protein concentrate. The addition of wheat-noodle supplement enhanced nutritional status, but the supplement containing the fish protein concentrate was no more effective (27).

Utilizing The Soybean Protein

Animal and Human Studies

Throughout the world, soybeans are cooked, fermented, or processed by various means before consumption. The raw soybean is not very palatable because of its texture and objectionable flavor (65). Also, in the raw form, nutritive value is low; but when processed with moist heat, like toasting, nutritive value can be improved to nearly that of meat and milk. Precise control of heating and other processing variables ensure adequate destruction of the heat-labile, antinutritional factors to achieve optimum protein quality (65).

The role of the soybean as a traditional food item in diets of populations in Southeast Asia is well recognized (84). The authors further explained that since all age groups are likely to experience an increased use of soy in their diets during the years ahead, and because plant proteins are deficient in one or more of the essential amino acids (such as lysine and tryptophan in hybrid maize, or sulphur amino acids in proteins of legumes), it is apparent that foods based on soy protein to meet the amino acid requirement for humans of all ages be assessed.

In a modified report of Bressani's Study on observations with soybean protein in feeding studies with experimental animals, Young and Scrimshaw (84) reproduced these observations.

1. Raw soybean meal reduces weight and dietary nitrogen utilization.

2. Steam heating improves the nutritional value of raw soybean meal.

3. Growth inhibitors are inactivated by heat.

4. Methionine supplementation markedly improves protein quality of soybean protein.

5. Other types of processes, such as toasting, increase protein quality of soybean.

6. Soybean flour compliments the nutritional value of cereal grains (corn, wheat).

This summary reveals that raw soybean is of low nutritional value in the rat and that its protein quality is improved by heat processing and by supplementation with methionine.

Some researchers have studied extensively the effect of heat on the amino acid composition of different soy products. On the effect of deep fat frying on the amino acid content of tempeh, Stillings and Heckler (74) found that heating at 196°C for seven minutes, the content of most amino acids declined. Especially, lysine and cystine were found to be most susceptible to heat destruction. Similarly, Rios and Barnes (37) reported lysine and cystine losses when autoclaved at 120°C for fifteen minutes and longer during an overheating of soybean protein. When soy milk was heated at 121°C, there was a decrease in the PER value and a decrease in the available lysine. This shows that time and temperature of treatment determines the value of PER obtained. Any roasting of soybeans at 170°C to 185°C for thirty-two minutes showed losses in available tryptophan, total lysine, total cystine, and total histidine (3). Irrespective of the above observations, Osborne and Mendel (55) observed that in order to support the growth of rats the heating of soybean was necessary.

In human studies, many protein-rich additives can be used as protein-fortifiers. Defatted soy flour is a favored fortifier because of its price, availability, protein content, and quality (79). Utilizing full-fay soy flour in wheat flour fortification in making bread can raise protein content, balance essential amino acids, and increase bread's caloric value (77).

Fomon and Siegler (23) recently reviewed the available evidence concerning the growth of infants and children receiving soy as the sole or major source of essential amino acids and total nitrogen. In their studies, commercial soy preparations which were supplemented with DL- or L-methionine were used. On all the studies reviewed, it was found that linear growth and/or body weight gain of infants receiving soy equalled that obtained with cow's milk or human milk when direct comparisons were made.

Fomon and Siegler (23) report that growth responses with infants consuming a methionine supplemented soy protein isolate were found to be essentially the same as those receiving a milk-based formula. The only difference was in weight gain per unit of total energy intake. During the first four months of life, the infants receiving soy formula, gained less than infants given milk-based feeds. Their conclusion was that soy flour and soy isolates are capable of promoting adequate growth of infants when they are the major source of protein in diets provided the diets contain also adequate amount of energy and essential nutrients.

In a recent investigation on nitrogen balance studies in infants and children who had recovered from protein-energy malnutrition, Torun and Viteri (76) administered levels of intake of one of two soy protein isolates and the N balance responses compared with those obtained with milk as the reference protein. Results showed that children receiving graded intakes of a commercially available soy protein isolate (Supro-620, Ralston-Purina Company, St. Louis, Mo.) demonstrated that nitrogen retention increased linearly with increases in nitrogen absorption up to 190 mg/kg/day, after which the nitrogen retention remained constant.

In another comparison, the above authors examined the nitrogen balance responses in children fed two soy protein isolates. The children received test protein intakes less than 190 mg. N/kg/day. They found that the nutritive value of the soy isolates approximates 86-107 percent of milk, depending upon the specific method of comparison. They concluded that the nutritive value of the isolates tested in young children was essentially equal to that of milk protein (76).

The Nutritional Implications of The Maillard Reaction

Browning of foods has always been associated with development of flavors and odors. Some of the changes are desirable because they improve palatability and consumer acceptance of foods. Crust browning of bread has been recognized as a source of bread flavor (60). The most important reactions leading to nonenzymatic browning in foods are those between reducing sugars and free amino acids or free amino groups of proteins. This reaction is called Maillard reaction in honor of the French chemist, L. C. Maillard, who pioneered the research on the basics of browning (60).

This Maillard type of browning that occurs between the Carbonyl group of a reducing sugar and the amino group ($-NH_2$) of a protein or amino acid, takes place in a series of steps not well understood, involving addition, rearrangement, fragmentation into smaller molecules, and finally polymenzation (28).

On the other hand, browning (especially at high temperatures) may destroy some of the nutrients in a food (60). For example, amino acids such as lysine, that have an extra amino group not involved in the peptide linkages of protein, are likely to be involved in the Maillard reaction.

When so involved, they are rendered unavailable during digestion and the nutritive value of protein is lowered (28). Reports by Livingston and others (43), Osner and Johnson (56) and Amaya-F and others (2) confirmed losses in nutritional value of proteins heated in the presence of carbohydrates. In these reports the protein efficiency ratios (PER) were affected by roasting, toasting, baking and drying.

Block and others (8) reported the PER of a cake mix fell from 3.6 to 2.4 after baking for 15 minutes at 200 C and to 0.8 after toasting for one hour at 130°C. The involvement of lysine in the loss of PER was confirmed when lysine was added to the diets of the test animals, which resulted in normal growth response.

Because of lysine supplementation to the diet that restored the PER of the protein, early works on Maillard reaction centered around lysine as the only amino acid affected by the interaction with reducing sugars. In addition to this, Bender (6) pointed out that if multiple assays were made, it was evident that methionine, cystine, tryptophan, and arginine availability in foods would also be affected by heat.

Soybean Trypsin Inhibitors

Raw soybeans contain a number of heat-labile factors that inhibit proteolytic activity of digestive enzymes, stimulate protein synthesis in the pancreas, and enhance pancreatic enzyme secretion. These effects lead to an enlargement of the pancreas and growth inhibition. Various species of animals respond differently (5).

According to Liener and Kakade (42), Mickelson and Yang (46), and Rackis (64), the role of naturally occurring trypsin inhibitors in causing retardation of growth and pancreatic enlargement of experimental animals has been demonstrated in rats and chicks. Recently, Kakade and others (40) indicated that soybean trypsin inhibitor (STI) contributed significantly to the deleterious effects of raw soybeans. Rackis (62), for example, has estimated that the trypsin inhibitors are responsible for approximately 30 to 50 percent of the growth inhibitory effect of raw soybeans and for nearly all of the pancreatic hypertrophic response in rats.

Rackis (63) suggested that the degree of improvement in nutritive value of soybean meal and the simultaneous inactivation of the trypsin inhibitors (TI) and other undesirable components depend upon temperature, duration of heating, and moisture conditions. Rackis and others (65) observed that pancreatic hypertrophy occurred in rats fed raw soy flour containing about 1200 mg. trypsin inhibitor (TI)/100 g. diet. This observation was reversed when the experimental rats were switched to control diets containing 30 percent toasted defatted soy flour.

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CHAPTER II

MATERIALS AND METHODS

Bread Ingredients and Process

The commercial all purpose flour used was bleached wheat flour purchased from a local grocery store and was commercially milled (General Mills, Inc., Minneapolis, Minnesota).

The commercial soybean flour used was a commercial product made from processed soybeans (Central Soya, Chemurgy Division, 1825 North Laramie Avenue, Chicago, Illinois).

A comparison of the composition of commercial all purpose flour and commercial soybean flour is shown in Table I.

TABLE I

COMPOSITION OF ALL PURPOSE FLOUR AND SOYBEAN FLOUR

Sample	Percent Protein	Percent Carbohydrate	Percent Fat
Commercial all purpose flour	11.0	87.0	1.0
Commercial soybean flour (Soyafluff-200W)	53.0	31.0	1.0

Process

Following the standard formula and the straight dough method suggested by Okaka (6), the two different breads (white bread and soy white bread) were baked. To make a loaf of soy flour bread with 20 percent soy flour (53% protein) or soy concentrate (70% protein), the basic breadmaking procedure was used. With the exception of flour, all other ingredients remained constant. Table II shows the basic bread formula.

TABLE II

BASIC BREADMAKING FORMULA

Ingredients	Parts by Weight (g)
White flour	270.0
Soy flour	60.0
Sugar	17.5
Salt	5.25
*Yeast (compressed)	10.5
Hydrogenated Vegetable Shortening	10.5
Yeast food	1.75
Calcium stearoyl-2-lactylak	3.5
Water	220.0**

*If using dry yeast, use less and allow more time to dissolve.

**Volume in milliliters.

The straight dough baking procedure involves five steps.

Step 1. All ingredients in the mixing bowl except yeast, yeast food, sugar and salt were added. Predissolved were yeast, yeast food, salt and sugar in a part of the water used. The mixture was allowed to stand for about ten minutes. Yeast solution was added to the rest of the ingredients in the mixing bowl. The rest of the water was added. The ingredients were mixed for about five minutes at slow speed initially, then at fast speed.

Step 2. The dough was put in an incubator or proof box at about 80-86°F. Relative humidity was high. This was achieved by spreading wet towels on the floor of incubator. Since no incubator was available, the dough was put in a warm oven (oven is always warm without being turned on), as an improvised incubator. It was left for thirty minutes incubation period and then removed to the table.

Step 3. Using a roller board and pin, the dough was flattened to remove as much air as possible. Because of the stickiness of the dough small amount of flour was sprinkled during the rolling process.

Step 4. One pound (454 g.) or (500 g.) of dough was weighed, molded to shape and put in bread pan. The pan with the dough was placed back into the incubator for two hours. Wet towels were placed on the floor of the incubator to give the high relative humidity needed. After two hours, the risen bread was removed and handled carefully.

Step 5. The bread was placed in preheated oven and baked (350 F) for about 25-30 minutes. After 20 minutes the bread was checked to see if it was well browned. The baked product was removed from the loaf pan and allowed to cool.

Slicing and Toasting of Breads

Supplemented and unsupplemented breads were baked and sliced 0.5 inch-thick using an automatic slicer (Handy Manufacturing Co., Chicago, Illinois). A four-slot food toaster (Toastmaster Division, McGraw-Edison Co., Columbia, Missouri), widely used in homes was utilized to toast slices. The toaster heating control had three settings for toasting: light, medium, and dark. This was done to obtain the degrees of toasting that are common on the breakfast table.

Taste Panel Evaluation

Acceptability of untoasted and toasted, supplemented and unsupplemented breads were evaluated by ten home economics students who acted as a panel of judges in evaluating the breads in terms of crust color, crumb color, loaf appearance, slice appearance, flavor and texture. The scores were based on a ten-point Hedonic Scale: Excellent is 9-10; Very good is 7-8; Good is 5-6; Fair is 3-4; Poor is 1-2 (6). Table III shows the taste panel score chart for breads.

TABLE III

TASTE PANEL SCORE CHART FOR BREADS

Attribute	Excellent		Very Good		Good		Fair		Poor	
	10	9	8	7	6	5	4	3	2	1
Crust Color										
Crumb Color										
Loaf Appearance										
Slice Appearance										
Flavor										
Texture										

Diet Preparation

Two different breads (white bread, soy white bread) were baked according to the standard formula and straight dough method. After baking, the loaves were sliced, toasted at light, medium and dark toaster settings, and finally ground. The protein and moisture content of the breads and casein used in preparing the diets are shown in Table IV. Similarly, Table V shows the protein content of the untoasted breads and the toasted breads used for diet preparation.

Prior to diet preparation chemical analysis was done on the ground crumbs. Amino acid composition was done by Dr. Gracy, North Texas State University, Biochemistry Department. See Table VI for chemical analysis of bread samples.

TABLE IV

PROTEIN AND MOISTURE CONTENT OF BAKED BREADS AND CASEIN

Sample	Moisture Percent	Analyzed Protein Percent	Calculated Protein* Percent
White bread	28.6	9.7	10.98
Soy white bread	28.1	18.2	16.40
Casein	3.2	97.4	100.00

*Calculated values from Composition of Foods -- raw processed, prepared, USDA, Agricultural Handbook No. 8 (revised 1963).

TABLE V

PROTEIN CONTENT OF UNTOASTED
AND TOASTED BREADS

Sample	Percent Protein
White untoasted	9.7
White light toasted	9.7
White medium toasted	9.5
White dark toasted	9.5
Soy white untoasted	18.2
Soy white light toasted	18.6
Soy white medium toasted	18.6
Soy white dark toasted	18.5

The casein, soy flour, and ground portions of the breads were incorporated into rat diets formulated to contain eight percent protein from different diets. The compositions of test diets are shown in Table VII. After the diets were made, they were kept refrigerated until used.

TABLE VI

AMINO ACID CONTENT*** OF BREAD SAMPLES

AMINO ACIDS**	WHITE BREAD				WHITE + SOY			
	Untoasted	Light	Medium	Dark	Untoasted	Light	Medium	Dark
Aspartic acid	373.150	576.481	534.214	500.200	1017.407	1017.407	1017.407	796.891
*Threonine	309.291	390.746	325.582	301.264	455.909	455.909	415.300	333.845
Serine	842.425	704.501	689.176	612.774	673.851	689.176	574.351	520.825
Glutamic acid	4168.026	3879.893	3822.319	3841.335	3803.040	3899.172	3322.906	2842.772
Glycine	806.820	640.269	589.171	563.429	742.851	749.190	806.820	569.961
Alanine	732.892	671.680	595.423	534.417	725.266	732.892	481.037	526.792
*Valine	680.997	563.245	510.806	410.806	484.487	412.484	405.930	336.601
*Methionine	153.065	101.955	114.732	114.732	121.121	127.510	101.955	89.177
*Isoleucine	550.259	454.454	406.800	388.681	418.713	418.713	382.724	310.995
*Leucine	937.948	785.801	730.949	688.259	657.730	596.921	718.787	536.112
Tyrosine	332.195	271.660	241.542	211.424	302.077	316.987	316.987	211.424
*Phenylalanine	721.585	281.896	605.037	535.334	535.333	523.763	558.474	419.067
*Histidine	695.080	600.507	560.473	549.450	796.034	796.034	784.721	639.090
*Lysine	593.020	530.534	499.490	405.960	967.538	998.980	998.980	702.470
*Arginine	486.013	402.147	385.549	310.128	670.342	670.342	614.965	486.013

*Essential amino acids for growth in growing rats and in children (except arginine).

**Tryptophan an essential amino acid (destroyed by acid hydrolysis) no data.

***Micrograms/gram bread.

TABLE VII

COMPOSITION OF TEST DIETS

Groups	Casein[a]	Bread	Cerelose[b]	Oil[c]	Vitamin[d] Mixture	Salt[e] Mixture
Casein	8.2	-	75.6	10	2.2	4.0
White untoasted	-	82.5	1.3	10	2.2	4.0
White light toasted	-	82.5	1.3	10	2.2	4.0
White medium toasted	-	84.2	-	10	2.2	4.0
White dark toasted	-	84.2	-	10	2.2	4.0
Soy white untoasted	-	45.9	3.7	10	2.2	4.0
Soy white light toasted	-	43.0	4.08	10	2.2	4.0
Soy white medium toasted	-	43.0	4.08	10	2.2	4.0
Soy white dark toasted	-	43.2	4.06	10	2.2	4.0

[a]"Vitamin free" Casein, Nutritional Biochemical Corporation, Cleveland, Ohio.

[b]Cerelose (Anhydrous dextrose), Nutritional Biochemical Corporation, Cleveland, Ohio.

[c]Crisco, Procter and Gamble Company, Cincinnati, Ohio.

[d]Vitamin Mixture, fed at 22 g/kg of diet supplied the following in g/kg of vitamin mix: Alpha tocopherol, 5; L-ascorbic acid, 45; choline chloride, 75; D-Calcium pantothenate, 3; i-inositol, 5; meandione, 2.25; Niacin, 4.5; P.A.B.A., 5; Pyridoxine HCI,1; Riboflavin,1; Thimin HCI, 1; Vitamin A acetate, 900,000 units; Calciferol CD_2), 100,000 units; Biotin, 20 mg; Folic acid, 90 mg; Vitamin B_{12}, 1.35 mg, Nutritional Biochemical Corporation, Cleveland, Ohio.

[e]Salt mixture Hegsted, Nutritional Biochemical Corporation, Cleveland, Ohio.

Experimental Units

The experimental units of this study were sixty-three weanling rats of the Holtzman Albino strain, twenty-one days old. The rats, whose initial weight averaged 53.5 g. were randomly assigned to nine groups of seven rats per group. Each rat was housed in a screen-bottom cage in an environmentally temperature controlled laboratory. Prior to being fed the test diets, the rats were fed a basal diet containing 10 percent casein for three days.

On beginning the experiment, a test diet (Table VI) and water were given ad libitum. Fresh water was given every other day, feed cups were checked daily and filled as needed. The feed wastage was measured by collecting the spillage every other day. Weights and feed consumed were recorded every other day for the twenty-one days experimental period. Feed wastage, usually minimum, was subtracted from consumption data. The diets were given as shown below:

Group 1: 8 percent Casein (control)

Group 2: Untoasted white bread

Group 3: Light toasted white bread

Group 4: Medium toasted white bread

Group 5: Dark toasted white bread

Group 6: Untoasted soy white bread

Group 7: Light toasted soy white bread

Group 8: Medium toasted soy white bread

Group 9: Dark toasted soy white bread

The composition of the above test diets is shown in Table VI.

Feces were collected in the last three days of the test period. Feces from each group was weighed and stored until analyzed. Nitrogen determinations were done on the samples by micro-Kjeldahl method and the protein content was estimated by the formula %Nx5.7 for white flour, %Nx6.25 for soy flour (4, 8).

Metabolic cages were used in the last part of the experiment where urine samples were collected over a twenty-four hour period. Specimens were preserved with boric acid. Each urine specimen was diluted to sixty milliliters with distilled water and was kept frozen until used for the determination of creatinine excretion.

At the end of the feeding period, the animals were killed by being put to sleep with a mixture of chloroform and ether. An incision was made through the abdomen and thorax. The right pulmonary trunk was severed, allowing bleeding into the pleural cavity where blood was collected with a syringe. About 2-3 mls. of mixed arterial and venous blood was collected from each animal and transferred into tubes where they were spun for fifteen minutes each, the serum separated and collected, and kept frozen until used. The liver was excised from each animal, weighed, and then ground. Fifty milliliters of distilled water was added to each liver before grinding, and an aliquot (0.5 ml.) was used for protein determination.

Biochemical Analysis of Tissues

Protein Efficiency Ratio (PER)

The recognized procedure by the Association of the Official Analytical Chemists (AOAC)(2) was used to determine the PER. This is a method of expressing growth promoting values of protein numerically, developed by Osborne, Mendel and Ferry (7). This method actually correlates growth with an increase in body protein (1). The PER was then calculated by dividing weight gain in grams of the test rats by the total amount of protein consumed by the animal during the test period. The individual PER was determined for each animal over the twenty-one day period, and group means were calculated from individual PER values.

Protein Determination

Protein was precipitated with trichloroacetic acid (TCA) as recommended by Robertson (9). One-tenth milliliter of serum or 0.5 milliliter of liver solution was added to duplicated centrifuge tubes, to which was added 10 percent TCA. The resulting suspension was centrifuged at high speed for about ten minutes. This was followed by withdrawing the supernatant and resuspending the precipitate in a 5 percent TCA and then centrifuged. Both washing and resuspension of the precipitated protein was repeated twice with 5 percent TCA. The final precipitate was resuspended in 0.1N NaOH, mixed thoroughly and kept refrigerated until ready for use.

Liver and Serum Protein

Protein determination proceeded by methods used by Lowry and others (4), and McDonald and others (5). The remaining protein solution from serum or liver (1 ml.) was diluted to 50 milliliters or 60 milliliters with distilled water; putting 0.4 milliliters of this solution in a test tube, enough water was added to bring the final volume to 0.5 milliliters; then 5 milliliters of prepared mixture was added and the contents were mixed thoroughly and incubated at room temperature for 10 minutes. The prepared mixture of Lowry reagent contained 4.9 ml. 2% Na_2CO_3 + 0.05 ml. Sodium potassium tartarate + 0.05 ml. 1% $CuSO_4$ which was prepared daily. After incubation, 0.5 milliliters of phenol reagent (1:1 dilution, phenol with water) was then added and the mixture was mixed thoroughly and allowed to incubate at room temperature for thirty minutes. The absorbance was measured on the Bausch and Lomb Spectronic 20 Colorimeter (Bausch and Lomb, Rochester, New York, 14602) at 700 nm. Reference used to estimate the quantity of protein in each sample was a standard protein serum (5% Bovine albumin, Sigma Chemical Co., St. Louis, Mo. 63178) with a range of 25 to 250 micrograms (μg) protein.

The standard curve was constructed with protein standards of 0.1, 0.2, 0.3, 0.4 and 0.5 milliliters.

Urinary Creatinine

Creatinine determination was by a method used by Folin (3). Each urine sample was diluted to 60 milliliters with distilled water. Five-tenths milliliter of urine was placed into 100 milliliter volumetric flask. Twenty milliliters of one percent picric acid solution was added to each flask; this was followed by the addition of 1.5 milliliters of ten percent NaOH solution. The mixture was mixed gently and allowed to stand for fifteen minutes, then it was diluted to 100 milliliters with distilled water and mixed by inversion.

Portions of the mixture were put into a photometer tube, and the absorbance read on the Bausch and Lomb Spectronic 20 spectrophotometer at 520nm to determine density. Standard creatinine solution containing one milligram per milliliter was used as reference to estimate the creatinine content of each sample. The standard curve was made with creatinine standards of 0.25, 0.5, 0.75 and 1.0 milliliter.

Protein Digestibility

Fecal samples were collected for a period of three days, and food intake was recorded for each animal. The total protein intake was calculated from dietary records. Nitrogen was estimated and protein was determined by Nx6.25 (4, 5).

Digestibility was calculated from the formula:

$$\%\text{Digestibility} = \frac{\text{Protein intake-Protein in feces x 100}}{\text{Protein Intake}} \quad (8)$$

where,

Protein intake = Amount of food eaten x 8%*

Protein in feces = Weight of feces x % Protein in feces

*(diets contained 8% protein).

To calculate % Protein in feces; assume being given the following data:

fecal protein = 5%

fecal weight = 10 gms., Then 10 gm. of feces contain 5 gm. protein. 10 gm. of feces will contain $\frac{5x10}{100}$ (g)

= 0.5 gm. protein.

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CHAPTER III

RESULTS AND DISCUSSION

Chemical Analysis of Toasted and Untoasted Breads

Table V shows the results of the chemical analysis for protein content of different breads. As the figures indicate, light toasted and medium toasted soy white bread contained higher percentages of protein than did untoasted white bread or any of the toasted white breads alone. This is due to the high content of protein in soy flour as compared to all purpose white flour.

As reported by Narayanaswamy and others (10, 11), Daniel and others (2), fortification of limiting amino acids in the diet improves the protein quality of the diet. The improvement in protein quality is accounted for by the addition of soy flour with its high protein content to compensate for the lysine deficiency in wheat protein. Soy flour (53 percent protein) contains 1.63 times as much lysine as a white flour of 11.0 percent protein (Table VI).

Comparing the amino acid content of bread samples as shown in Table VI revealed that methionine, arginine, and lysine are the limiting amino acids in the toasted and untoasted white breads while methionine is the first limiting essential amino acid in soy white toasted and untoasted breads. According to Jansen (6) mixtures of soy flour and wheat flour are better balanced in amino acid composition and biological value than either of the two components.

Taste Panel Evaluation

Figure 1 shows untoasted and toasted slices of white bread alone and white bread supplemented with soybean flour. It was found possible to bake acceptable bread containing soy flour.

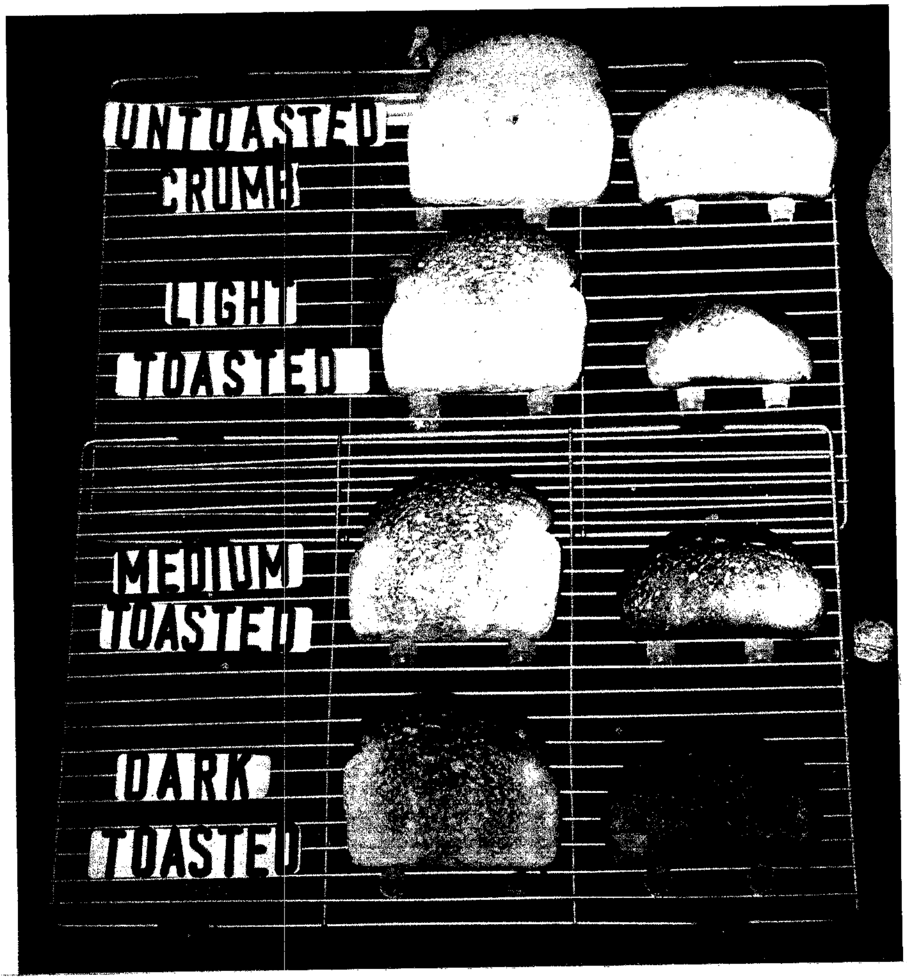

Fig. 1--Typical examples of untoasted crumbs, light, medium, and dark toasted slices. (Left = white breads; Right = Soy white breads).

The crust color of the soy white bread was more brown than the white bread crust. The addition of soy flour contributed to the darker crust color of soy white bread. The crumb showed that soy white bread was darker than that of white bread, its crumb was slightly coarse, and its color can be described as creamy-like, with a roasted nut-like flavor. The supplementation of white bread with soybean enhanced the flavor. There were no differences in loaf volumes of the two breads, but the soy white bread appeared to be a more compact loaf.

The taste panel evaluation scores showed that the products were highly accepted when toasted at light and medium temperatures, but rejected when darkly toasted. Table VIII indicated that there was no significant difference between panelist scores for overall loaf appearance, flavor and texture. There were significant differences in the crust color between the dark toasted soy white bread ($P < 0.05$), and the dark toasted white bread ($P < 0.01$) as compared with the untoasted white bread. Similarly, the crumb color and the slice appearance of the dark toasted white bread were both significantly different from the untoasted white bread.

The panelists' evaluation of the soy white bread showed that the bread can be baked with little or no damage to the loaf volume, crumb, crust, slice appearance, flavor, or texture.

TABLE VIII

TASTE PANEL[a] SCORES[b] FOR BREADS

Groups	Crust Color	Crumb Color
Untoasted white bread	9.66±0.7	9.44±0.88
Light toasted white bread	9.55±0.88	9.33±0.86
Medium toasted white bread	9.0 ±2.00	8.11±2.47
Dark toasted white bread	6.55±2.18**	7.0 ±2.44*
Untoasted soy white bread	8.44±1.13	8.44±1.33
Light toasted soy white bread	8.30±1.05	8.20±1.03
Medium toasted soy white bread	7.55±1.66	7.77±1.20
Dark toasted soy white bread	6.87±1.35	7.37±1.76

[a]Sample size = 10

[b]Mean and standard deviation scores were based on the ten point Hedonic Scale: 9-10 = Excellent, 7-8 = Very good, 5-6 = Good, 3-4 = Fair, 1-2 = Poor.

*Significantly different from untoasted white bread (P 0.05)

**Significantly different from untoasted white bread (P 0.01)

TABLE VIII--continued

Loaf Appearance	Slice Appearance	Flavor	Texture
9.44±1.13	9.22±0.66	8.11±0.92	7.44±2.29
9.37±0.91	9.33±0.86	8.22±1.39	7.25±1.83
9.0 ±2.13	8.66±2.00	8.37±1.59	8.00±1.58
7.85±2.41	8.33±1.80	8.87±1.12	8.11±0.78
8.11±2.84	8.22±1.39	7.71±2.21	7.77±2.53
8.11±1.16	8.10±1.28	8.90±1.10	8.10±2.28
7.37±1.84	7.44±1.23	9.0 ±1.22	7.88±2.26
7.14±1.57	6.87±1.80	9.0 ±1.06	7.25±2.25

Wheat flour fortification with soy flour due to its high protein content, increases the protein content of wheat flour, and improves the amino acid balance of bread. Finney and Matthews (5, () have explained that rejection of breads made from fortified wheat flours were due to breadmaking properties. The use of surfactants such as calcium-stearoyl-2-lactylate, sodium-stearoyl-2-lactylate (SSL) and potassium bromate to improve the baking performance of fortified wheat flour has been recommended (4, 5, 13, 18).

The results of the present study, revealed that the incorporation of one percent calcium-stearoyl-2-lactylate into the breads baked with soybean flour and white flour made them more acceptable. The addition of soy flour to wheat flour will not affect the loaf volume and crumb texture, but the palatability was significantly affected when full fat soy flour was used (20). Using 0.5 percent sodium-stearoyl-2-lactylate, Tsen and Hoover (20) in their study determined that bread supplementation with 24 percent soy flour was acceptable. But prior to the addition of 0.5 percent SSL, it was found that small loaf volumes and poor grain scores resulted when breads were baked with 12 percent to 28 percent soy flour.

Treatments of soy flour (used for supplementation) such as heat-treated and chemically-treated soy flour showed that loaf volume, texture, and odor were affected. The least effect resulted in the chemically treated flours (15).

Quality of breadmaking formula is a basic step towards producing a wheat flour bread supplemented with soy flour that will be acceptable in almost all aspects of bread characteristic properties.

Effect of Diets on Growth of Rats and PER

The growth curves (fig.2) show the response of the animals to the different treatments. The results in Table IX show initial body weight, final body weight, average food intake, average protein intake, and PER values of rats fed different diets.

As the table indicated, the casein group responded with higher values than animals on the bread diets. The casein PER value of 3.70 and the untoasted soy white PER of 1.47 compares within range of the PERs of 3.51 and 1.46 respectively as reported by Rackis and McGee (17).

Table XV shows the differences in average weight gain, food intake, and PERs between treatment groups as were tested for significance by Scheffe's *F* test. The results reveal that among each treatment soy white bread diets were best (with the exception of the control diet - eight percent Casein) and the white bread diets were worst for growth of the animals. The animals fed the soy white bread diets had a higher growth rate as compared to the growth rate of animals fed the white bread alone (fig.3).

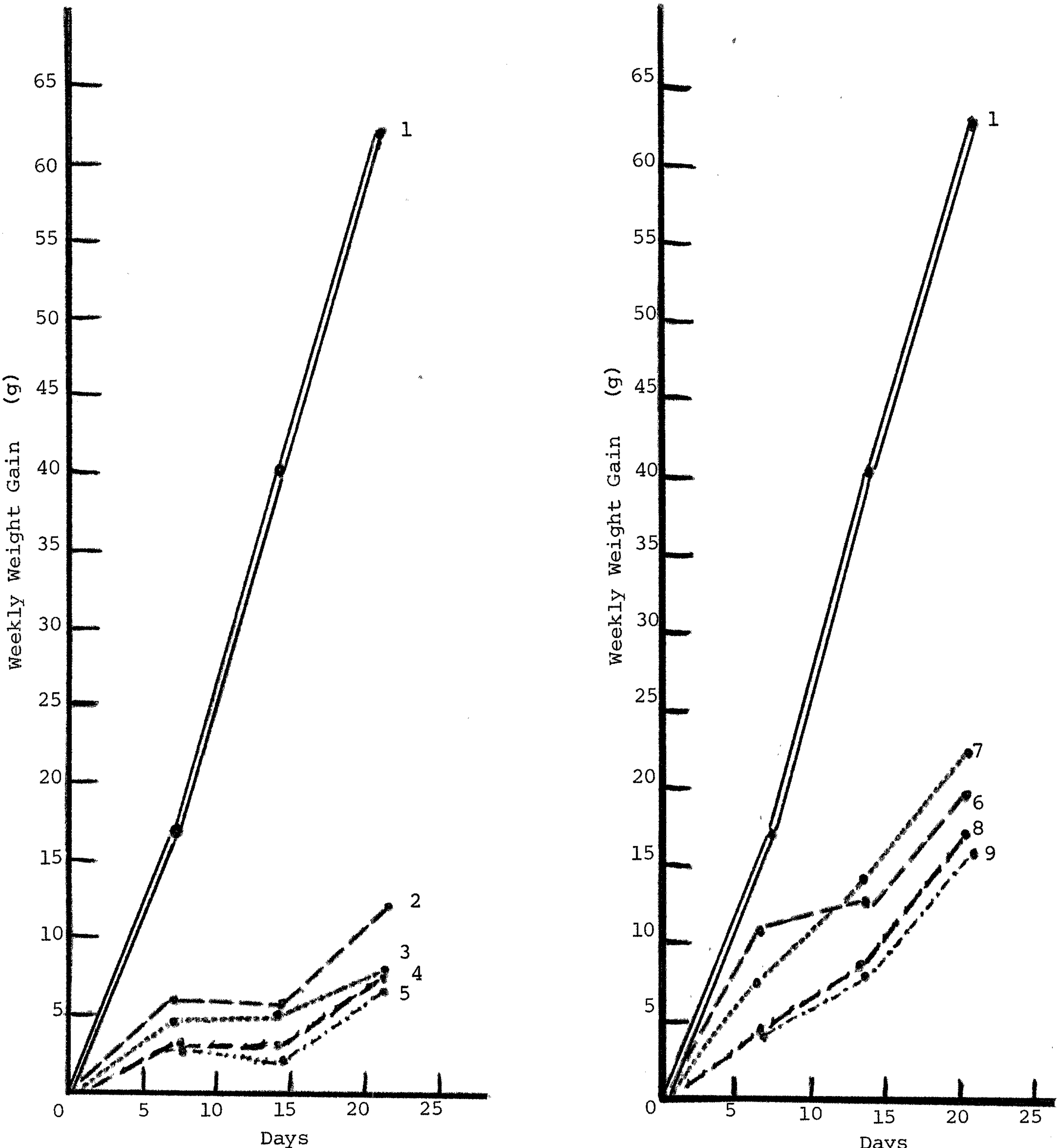

Fig. 2 - Growth Curve of rats fed different diets.

1 = Casein (Control)
2 = Untoasted white bread
3 = Light toasted white bread
4 = Medium toasted white bread
5 = Dark toasted white bread
6 = Untoasted soy white bread
7 = Light toasted soy white bread
8 = Medium toasted soy white bread
9 = Dark toasted soy white bread

TABLE IX

EFFECT ON GROWTH OF FEEDING DIFFERENT DIETS TO WEANLING RATS FOR THREE WEEKS

Groups	Initial Body Weight	Final Body Weight
	(g)	(g)
Casein	60.0±3.86	122.0±15.08
Untoasted white bread	59.2±4.2	69.4± 2.60
Light toasted white bread	62.0±1.63	73.8± 4.70
Medium toasted white bread	60.0±2.51	67.7± 2.0
Dark toasted white bread	59.0±4.16	65.8± 5.70
Untoasted soy white bread	57.6±2.99	78.0± 4.3
Light toasted soy white bread	58.5±4.0	76.0± 5.60
Medium toasted soy white bread	61.0±5.27	71.0± 5.60
Dark toasted soy white bread	63.0±4.04	80.0± 3.86

*PER = Protein efficiency ratio = weight gain (g)/protein intake (g)

**Results are mean ± Standard deviation (SD)

[a]Significantly different from control ($P<0.05$)

[b]Significantly different from untoasted white ($P<0.05$)

[c]Significantly different from untoasted soy white ($P<0.05$)

TABLE IX--continued

Average Weight Gain	Average Food Intake	Average Protein Intake	PER*
(g/rat)	(g/rat/day)	(g/rat)	
62.4 ±12.1^{bc**}	20.07±5.6	16.7 ±3.1	3.7 ±0.48^{bc}
10.2 ± 2.04^{abc}	20.0 ±8.07	16.02±0.86	0.63±0.10^{ac}
11.85± 3.76^{ac}	18.0 ±4.38^{b}	14.7 ±1.79	0.80±0.19^{ac}
7.7 ± 2.49^{ac}	14.7 ±3.03^{a}	11.8 ±1.28	0.65±0.18^{ac}
6.85± 2.85^{ac}	14.1 ±3.74^{a}	11.1 ±1.25	0.62±0.24^{ac}
20.4 ± 1.76^{ab}	16.87±4.22	14.1 ±1.29	1.46±0.26^{ab}
17.42± 3.95^{ab}	14.94±4.31^{a}	11.94±2.10	1.49±0.40^{ab}
22.85± 7.08^{ab}	21.3 ±5.7	17.1 ±1.86	1.36±0.45^{ab}
16.5 ± 2.29^{ab}	19.4 ±5.6^{b}	15.58±2.08	1.06±0.15^{abc}

Fig. 3--Example of Control (right) and animals on experimental diet (left).

Table IX shows the significant differences in weight gain for rats fed toasted and untoasted soy white bread. The animals on the soy white group gained faster with a 32 percent increase in body weight as compared with 15 percent increase in body weight of the rats fed the toasted and untoasted white bread.

Considering the PER values, the untoasted white rose from 0.46 value to 1.46 when supplemented with soy flour. Similarly all the PER values were increased with addition of soy flour, but were decreased with degrees of toasting. Regression equations showing this effect can be seen in Table X.

The regression equations represent the utilization of level of dietary protein from diets of varying types of protein. The slope of the linear regression is the PER. Protein utilization was reduced by toasting white bread medium and dark. Increased utilization was observed by toasting soybean supplemented breads.

It is worth mentioning that during this study it was observed that the untoasted white bread diet group failed to grow within the first week of the experiment. They added few grams during the second week and finally maintained their weight during the rest of the experimental period.

Generally, toasting at dark temperatures decreased the PER values of the unsupplemented breads more than it did the

TABLE X

REGRESSION EQUATION

Groups	Regression Equation
Casein	y*= 13.01 + 2.91x
Untoasted white bread	y = 15.28 + 1.6x
Light toasted white bread	y =-14.89 + 1.82x
Medium toasted white bread	y = -4.32 + 1.03x
Dark toasted white bread	y = 0.238 + 0.60x
Untoasted soy white bread	y = 26.98 +-0.463x
Light toasted soy white bread	y= 10.55 + 0.58x
Medium toasted soy white bread	y = 19.04 + 0.225x
Dark toasted soy white bread	y = 8.34 + 0.522x

*y = a+bx; where y = weight gain; x = protein intake; b = slope or PER; a = intercept.

supplemented breads. The addition of good quality protein will increase the protein quality and quantity of white breads as compared with those of soy white breads.

There were significant differences in food intake as shown in Table IX.

It is expected that the low lysine content of wheat flour can be improved by supplementation with soybean flour. The better rate of growth seen in the animals that were fed bread supplemented with soy flour is attributed to the better quality protein of soybean flour and to the higher lysine content of the soy protein which is the first limiting amino acid in wheat for human nutrition. The study obtained a highly positive correlation ($\underline{r}$ = 0.96) between protein efficiency ratio and weight gain (Table XI).

The significant difference in feed intake and weight gain is presumably due to the variations between the untoasted and toasted breads in respect to their amino acid compositions and availabilities (which regulate the growth of the rat), digestibilities, and palatabilities (21).

Reduction in PERs was evident with toasting (Table IX). The light, medium, and dark toasted white bread decreased systematically from 0.80 to 0.62. The low PER value from the untoasted crumb is attributed to the feeding patterns of the rats in the group, which led to low weight gains. Tsen and Reddy (21), observed similar decrease in the PER values

TABLE XI

CORRELATION COEFFICIENT OF DEPENDENT AND INDEPENDENT VARIABLES

Independent Variable	Dependent Variables	Correlation Coefficient	Level of Significance
Diet	Negative correlation with weight gain	-0.34	0.003
	Negative correlation with PER	-0.32	0.006
	Positive correlation with creatinine	0.63	0.001
	Positive correlation with creatinine coefficient	0.64	0.001
Weight Gain	Positive correlation with liver weight	0.74	0.001
	Positive correlation with liver protein	0.52	0.001
Food Intake	Positive correlation with weight gain	0.51	0.001
	Positive correlation with PER	0.31	0.007
	Positive correlation with liver weight	0.44	0.001
	Positive correlation with liver protein	0.26	0.02
	Positive correlation with creatinine	0.25	0.02
PER	Negative correlation with diet	-0.32	0.006
	Positive correlation with weight gain	0.96	0.001
	Positive correlation with food intake	0.31	0.007
	Positive correlation with liver weight	0.69	0.001
	Positive correlation with whole liver protein	0.49	0.001
Liver Weight	Positive correlation with weight gain	0.73	0.001
	Positive correlation with food intake	0.44	0.001
	Positive correlation with whole liver protein	0.65	0.001

of toasted slices and established that the decrease in the availability of lysine is induced by the browning reaction upon heating various foods, as reviewed by Reynolds (19). Toasting, they explained, causes browning of bread-slices and consequently makes the lysine in bread less available nutritionally.

Effect of Diets on the Liver Weight, Liver Protein and Serum Protein of Experimental Rats

The data for liver weight, percentage of liver to body weight, liver protein, and serum protein of rats fed different diets are shown in Table XII. There was a positive correlation between liver weight and weight gain ($\underline{r}$ = 0.73), food intake ($\underline{r}$ = 0.44), and whole liver protein ($\underline{r}$ = 0.65) (Table XI) The casein group had the highest liver weights. The liver weights of all the rats fed untoasted and toasted breads were significantly smaller than the livers of rats in casein group ($P < 0.05$).

When the livers were expressed as a percentage of body weight, only the rats fed the dark toasted white bread and the dark toasted soy white bread had a significantly higher liver weight than did the rats in the untoasted white bread group ($P < 0.05$).

The livers of rats on the casein diet significantly contained more protein than the livers of other experimental groups. When liver protein was expressed in milligram per gram liver, the casein group contained significantly more protein than the rats in untoasted white bread groups, light, medium, and dark toasted soy white groups (P 0.05). Also, the casein group, light, medium, and dark toasted of supplemented and unsupplemented groups, as well as the untoasted soy white group, were all significantly higher in their liver protein content than the group of untoasted white bread. The light and dark toasted white bread group, and the dark toasted soy white bread group significantly contained higher liver protein than the untoasted soy white bread group.

When liver protein was expressed in milligram per whole liver, the casein group significantly had a higher liver protein (Table XII) than all the experimental groups with the exception of dark toasted soy white bread group. Significant values within the groups are shown in Table XII. The low liver protein observed in the untoasted white bread group relates to decreased amino acid activating enzymes, and a loss of liver protein occuring with protein depletion (8).

Considering the serum protein, there were no significant differences between the rats on the casein diet and the rats fed untoasted and toasted supplemented and unsupplemented bread diets with the exception of untoasted white bread. However, animals in the dark toasted white bread group had a higher serum protein as compared to the untoasted white bread. Likewise, the rats on the light and medium toasted soy white bread diets contained significantly higher serum protein content than did rats fed untoasted white bread diets ($P<0.05$).

It is evident from the above data that the livers of animals on the casein diet contained slightly more protein than the livers of rats on the supplemented and unsupplemented toasted and untoasted bread diets. Complementing wheat flour with soybean flour increased the protein quality of the bread as measured by the variables in Table XII.

The decreased liver weights of rats in the unsupplemented, untoasted and toasted groups may have resulted from the low protein quality of wheat flour. This is no indication that growth and differences in liver protein content will be altered. The most interesting finding was the high liver protein content of the rats in the casein group. As Brown and others (1) reported on the changes observed in liver composition, it was determined that the rats on the casein diet contained less fat and more protein than the livers of animals on the bread diets. Their findings correlate favorably to the results of this experiment.

Effect of Diets on Creatinine Excretion

The results on the effect of different diets on the urinary excretion and creatinine coefficient are shown in Table XIII. Creatinine excretion of rats fed the casein diet was significantly less than the rats fed light, medium, and dark toasted white and soy white bread diets, as well as the rats fed untoasted soy white bread diets.

TABLE XII

EFFECTS OF DIETS ON LIVER WEIGHT, LIVER PROTEINS, AND SERUM PROTEINS OF EXPERIMENTAL RATS

DIET	Liver Weight	Weight of Liver Per 100g Body Weight
	(g)	(g)
Casein	$3.87 \pm .52^{bc}$	$3.17 \pm .18$
Untoasted white bread	$1.74 \pm .46^{ac}$	$2.51 \pm .56$
Light toasted white bread	$2.68 \pm .44^{ab}$	$3.63 \pm .52$
Medium toasted white bread	$2.24 \pm .37^{a}$	$3.33 \pm .55$
Dark toasted white bread	$2.42 \pm .37^{a}$	$3.65 \pm .73^{b}$
Untoasted soy white bread	$2.72 \pm .45^{ab}$	$3.47 \pm .42$
Light toasted soy white bread	$2.50 \pm .50^{ab}$	$3.30 \pm .70$
Medium toasted soy white bread	$2.78 \pm .58^{ab}$	$3.30 \pm .58$
Dark toasted soy white bread	$2.94 \pm .53^{ab}$	$3.69 \pm .65^{b}$

[a]Significantly different from control ($P<0.05$)

[b]Significantly different from untoasted white ($P<0.05$)

[c]Significantly different from untoasted soy white ($P<0.05$)

TABLE XII--continued

Protein Content of Liver	Total Protein Content of Liver	Protein Content of Serum
(mg/g liver)	(mg/liver)	(mg/ml serum)
188.9±16.3^{b}	733.1 103.6^{bc}	76.5±19.5^{b}
59.7±28.9^{ac}	199.08±54.29^{ac}	43.9±12.32
209.5 ±34.1^{bc}	557.1±102.6^{b}	69.6±13.6
170.0 ±28.4^{b}	377.5± 75.6^{ab}	55.7± 9.28
207.0 ±47.2^{bc}	473.0±123.7^{ab}	75.4±13.15^{b}
156.0 ±24.6^{b}	418.8± 51.8^{ab}	68.7±18.26
143.4 ±20.2^{ab}	353.2± 61.6^{ab}	79.0±21.7^{b}
136.4 ±21.8^{ab}	370.6± 51.3^{ab}	73.3± 8.71^{b}
230.8 ±45.6^{abc}	670.1±131.9^{bc}	65.6±14.5

TABLE XIII

EFFECT OF DIETS ON CREATININE EXCRETION OF RATS

Groups	Initial Body Weight	Final Body Weight
	(g)	(g)
Casein	60.0±3.86	122 ±15.08
Untoasted white bread	59.2±4.20	69.4± 2.60
Light toasted white bread	6.20±1.63	73.8± 4.70
Medium toasted white bread	60.0±2.51	67.7± 2.00
Dark toasted white bread	59.0±4.16	65.8± 5.70
Untoasted soy white bread	57.6±2.99	78.0± 4.30
Light toasted soy white bread	58.6±4.00	76.0± 5.60
Medium toasted soy white bread	61.0±5.27	71.0± 5.60
Dark toasted soy white bread	63.0±4.04	80.0± 3.86

[a]Significantly different from control ($P<0.05$)

[b]Significantly different from white untoasted ($P<0.05$)

[c]Significantly different from soy white untoasted ($P<0.05$)

*Urine, collected on last day of experiment.

**Milligram creatinine excreted in urine per day per kilogram of body weight.

TABLE XIII--continued

Urinary Creatinine (mg/day)	Creatinine Coefficient[2] (mg/kg/day)
1.32±0.71[c]	19.16± 5.28
1.70±0.63[c]	25.0 ± 8.7
2.00±0.25[a]	30.0 ± 8.42[a]
2.40±0.68[a]	35.8 ±10.4[a]
2.14±0.72[a]	32.0 ± 9.90[a]
3.00±1.50[ab]	46.1 ± 9.89[ab]
3.10±2.50[a]	50.5 ±21.96[a]
3.90±1.60[ab]	46.5 ±17.8[a]
4.00±0.98[ab]	49.75±10.85[a]

The creatinine excretion of rats on untoasted, medium toasted and dark toasted soy white bread diets was significantly more than the rats on the untoasted white bread diet. The casein-fed groups and untoasted white bread-fed groups had significantly less excretion than did the rats fed soy white untoasted bread diet.

The creatinine coefficient column of Table XIII showed that the excretion of casein-fed animals was significantly less than those of bread diets (with the exception of un-toasted white bread). The creatinine excretion of rats in the untoasted white bread group was significantly less than those of soy white untoasted bread.

One of the methods used to estimate lean-body mass is the use of urinary creatinine output as the index (12). This method is based on the assumption that the amount of creatinine excreted reflects the size of the muscle mass of the body. A study by Nierkerk (12) explained that the quantity of daily urinary creatinine in humans and in animals is significantly correlated with lean body mass and/or muscle mass.

It has also been shown that obese people and animals excrete less creatinine relative to their body weight than do their thinner counterparts (14). The results of the experiment showed that casein-fed animals have a low level of lean body mass in relation to adipose tissue.

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It can also be deduced from the experiment that the decrease in creatinine excretion observed in untoasted white bread fed animals was due to muscle wasting or continuing losses of lean body mass. This observation is similar to that explained by Oser (14), that urinary creatinine decreased to below normal levels when the musculature has become atrophic.

Digestibility of Protein by Rats

Table XIV shows the results of the digestibility of protein by rats. This part of the experiment was based on the data collected within the last three days of the experimental period. The digestibility values show that animals in the casein group had the highest value. This value was significantly higher than all the other experimental values obtained. With the exception of the casein group, all other experimental values obtained showed that the untoasted white bread had a significantly higher digestibility value. When digestibility was compared within the groups, it was found that toasting affected the digestibility of the proteins in the animals fed the white bread diets.

There is incomplete hydrolysis of the protein by digestive enzymes; as a result the unutilized products are excreted as waste. Growth impairment which has been demonstrated in the experimental animals is one of the most

TABLE XIV

DIGESTIBILITY OF PROTEIN BY RATS

Groups	Average Fecal Weight	Average Moisture Content
	(g)	(percent)
Casein	3.0634	2.78
Untoasted white bread	2.6260	2.79
Light toasted white bread	3.6517	3.63
Medium toasted white bread	2.5977	3.64
Dark toasted white bread	3.3174	3.31
Untoasted soy white bread	2.7939	3.50
Light toasted soy white bread	3.3135	2.90
Medium toasted soy white bread	3.7186	2.44
Dark toasted soy white bread	3.8120	2.91

[a]Significantly different from the control ($P<0.05$)

[b]Significantly different from the untoasted white ($P<0.05$)

[c]Significantly different from the untoasted soy white bread ($P<0.05$)

$$\text{*Protein digestibility} = \frac{\text{Protein Intake} - \text{Protein in feces}}{\text{Protein Intake}} \times 100$$

TABLE XIV--continued

Protein in Feces	Protein Intake	Percent Protein Digestibility*
0.0912	2.0060	95.4± 2.8[c]
0.1223	1.4760	91.7± .54[ac]
0.1808	1.5020	87.9± 2.75[abc]
0.2800	1.2740	78.0± 4.1[ab]
0.2262	1.1280	79.9± 1.13[abc]
0.2385	1.3440	82.2± 2.6[ab]
0.2390	1.4640	83.6± 3.8[ab]
0.4184	1.9880	78.9± 4.3[ab]
0.3601	1.9080	81.1±11.3[ab]

pronounced symptoms which results. The results of this experiment explain the high digestibility value obtained from the casein diet-fed rats.

The low percent digestibility of proteins by rats in the unsupplemented, toasted groups are due to the effect of toasting. Eggum and Christensen (3) reported that when different proteins were ingested with carbohydrate, the behavior of the meals was largely determined by the properties of the protein. As has been stated before, toasting causes the browning of bread slices and consequently, makes the bread's lysine less available nutritionally.

Considering the soy white bread group, the inconsistency in the protein digestibility values has been attributed to the effect of trypsin inhibitor, a substance that inhibits the function of the enzyme trypsin needed for protein digestion (16). Lewis and Taylor (7), reported that soy flour can support positive nitrogen balance but not as efficiently as autoclaved flour. With adequate heat treatment, soy flour will be more beneficial nutritionally.

According to Rackis (16), raw full-fat and defatted soy flours inhibited growth, depressed metabolizable energy and fat absorption, reduced protein digestibility, caused pancreatic hypertrophy, stimulated hyper- and hyposecretion of pancreatic enzymes, and reduced amino acid, vitamin, and mineral availability. He explained the interrelationship between these effects could cause a decrease in an animal's ability to utilize the essential nutrients rather than fail to utilize these nutrients due to the presence of toxic substances. There was no abnormal findings in his study with the exception of pancreatic hypertrophy.

TABLE XV

ANALYSIS OF VARIANCE TABLE FOR WEIGHT GAIN FOOD INTAKE, PER, LIVER WEIGHT, LIVER PROTEIN, SERUM PROTEIN, AND CREATININE EXCRETION

Source	Sum of Squares	Degrees of Freedom	Variance Estimate	F Ratio	Level of Significance
Weight Gain					
Between	16616.1270	8.	2077.0159	66.3550	P<0.001
Within	1690.2857	54.	31.3016		
Total	18306.4127	62.			
Food Intake					
Between	47984.2857	8.	5998.0357	3.7291	P<0.001
Within	86855.7143	54	1608.4392		
Total	134840.0000	62			
PER					
Between	54.5569	8.	6.8196	65.3830	P<0.001
Within	5.6423	54	0.1043		
Total	60.1892	62			
Liver Weight					
Between	18.6947	8.	2.3368	5.8246	P<0.001
Within	21.6649	54.	0.4012		
Total	40.3596	62			

TABLE XV--continued

Liver Weight/100g/BW[a]	7.3215	8.	0.9152	1.4409	NS[b]
Between	34.2981	54.	0.6351		
Within	41.6195	62			
Total					
Liver Protein gm/l gm					
Between	147639.9111	8.	18454.9889	16.1770	P<0.001
Within	61603.9538	54.	1140.8140		
Total	209243.8649				
Serum Protein					
Between	7188.8571	8.	898.6071	1.9464	NS
Within	24930.8571	54.	461.6825		
Total	32119.7143	62.			
Creatinine					
Between	54.5315	8.	6.8164	4.5271	P<0.001
Within	81.3070	54.	1.5057		
Total	135.8385	62.			
Creatinine Coefficient					
Between	8431.5040	8.	1053.9380	4.7975	P<0.001
Within	11863.0717	54.	219.6865		
Total	20294.5757				

[a]BW = Body weight

[b]NS = Not significant

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CHAPTER IV

CONCLUSION

The nutritive value of bread was significantly reduced by toasting. This conclusion was based on the data collected when the effect of toasting on the protein quality of white bread and white bread supplemented with soybean flour was studied. White bread was supplemented with eighteen percent soy flour. The finished product showed a net increase in the available protein content as compared to unsupplemented bread. Supplementation of breads with soybean flour on the other hand, decreased the volume of the loaves, produced creamy, gray-like crumb colors which were darker, coarser, and more compact. Supplementation of breads with soybean enhanced the flavor.

Scoring for the organoleptic properties on a ten-point Hedonic scale for crumb, crust, loaf appearance, slice appearance, flavor, and texture, the ten panelists showed that the products were highly acceptable when toasted at light and medium temperatures. The dark toasted was rejected. Statistically, there were no significant differences in the overall panelist acceptance of loaf appearance, flavor and texture.

Biological studies using rats were carried out to determine the effect of toasting on the protein quality of the baked products. The protein quality of the diets was measured by the protein efficiency ratio (PER). Nine diets containing casein, untoasted white, white bread toasted at light, medium, and dark temperatures; untoasted soy white bread, and soy white toasted at light, medium and dark temperatures, were fed to rats as the only source of protein at the eight percent protein level for twenty-one days. Calculations included gains in body weight, food intake, protein efficiency ratio (PER) and the determination of the liver weight, liver and serum protein, creatinine excretion and percent digestibility.

The significant reduction in PERs was evident with toasting especially when toasted darker. Growth depression occurred within the rats in the unsupplemented white bread group as compared with the rats in control and in the soy supplemented white bread group. The regression equation in this study represents the utilization of the level of dietary protein from diets of varying types of protein. The slope of the linear regression is the PER. Protein utilization was reduced by toasting white bread at medium and dark temperatures (Untoasted white: $Y = -15.28 + 1.60\ x$; medium toasted white: $Y = -4.32 + 1.03\ x$; dark toasted white: $Y = 0.24 + 0.60\ x$). Increased utilization was observed by toasting soybean supplemented breads, but these values were not significantly different (Untoasted soybean: $Y = 36.98 - 0.46\ x$;

toasted soybean: $Y = 19.04 + 0.225\ x$; dark toasted soybean: $Y = 8.34 + 0.522\ x$). Liver protein, Serum protein in supplemented-bread-fed rats was higher than those of unsupplemented-bread-fed rats. There was a positive correlation between whole liver protein and PER ($\underline{r} = 0.49$), and between liver weight and whole liver protein ($\underline{r} = 0.65$) and between weight gain and PER ($\underline{r} = 0.96$).

With regard to the amount of creatinine excreted in the urine, it was found that obese and malnourished animals excreted less creatinine than did the animals with more muscles and less adipose tissue. The casein fed rats had more adipose tissues and secreted less creatinine, while the decrease in the excretion of creatinine within the unsupplemented white-bread-fed rats was due to muscle degradation.

Apparent digestibility of protein from different diets was examined. The rats in the casein group had the highest protein digestibility values, as compared with rats on the experimental diets. The decrease in the protein digested, which resulted from toasting of white bread, reflected poor utilization of the protein. Heat treatment however did not impair the utilization of proteins from soy supplemented diets. Regression equations derived from protein intake versus weight gain enhanced the utilization of the soybean supplemented bread. This effect was possible due to further destruction of the trypsin inhibitor which is not completely destroyed during the processing of soybeans for flour.

Generally, the nutritive value of white bread was reduced by toasting, especially when toasted at the medium and dark temperatures. It is, therefore, recommended that white breads be toasted at very light temperatures.

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