THE EFFECT OF FERMENTATION AND SOYBEAN AND PEANUT SUPPLEMENTATION ON CORN PROTEIN

by

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TABLE OF CONTENTS

INTRODUCTION

Corn (maize) is the largest cereal crop in Rhodesia (1). It supplies 52 to 75% of the calories consumed by rural populations. FAO surveys (2) showed that the caloric value of the food consumed is within 10% of the required 2000 to 2200 calories for an adult. However, there may be a calorie deficit of 30% or more during periods of shortage or for vulnerable groups. A survey by the FAO showed that 4 to 6% of the children and 10% of the pregnant women are seriously malnourished.

Since corn is resistent to crop diseases (1), the main reason for crop failure is lack of sufficient rainfall. In 1967, only 3% of the crop land in Rhodesia was irrigated (3).

Because corn makes up the bulk of the Rhodesian diet, it is an important source of protein even though it is an incomplete protein. Several methods have been used to improve the protein in corn including breeding, fertilization of the land and supplementation. Supplements may change the flavor of corn products and lower their acceptability by the people (4). In Rhodesia, some of the corn is allowed to ferment before it is cooked into a porridge. Sour corn has enough acid to mask the taste of added supplements (5).

Soybeans and peanuts are two possible supplements that could be used with corn. In the past, soybeans have not been grown by Rhodesians but the government is encouraging master farmers and schools to plant soybeans. Teachers and clinic personnel have been given training on how to prepare soybeans in the home. As this knowledge spreads, soybeans will become available to rural people. Peanuts are grown in the gardens of rural people and they are served in a variety of ways. In this study, the effect of soybeans and peanuts on the rate of fermentation of corn was examined. Also, the protein quality of fermented cooked corn supplemented with two levels of soybean and peanut meal was determined.

REVIEW OF LITERATURE

Corn as a Basic Food

Amount of protein. The FAO/WHO recommends 55 grams of protein for African men in the tropics. According to a survey taken by FAO/WHO in 1959, the Rhodesian eats 5 to 10 grams of animal protein per day (2). An adult can eat 0.5 kilogram (dry weight) of corn per day. If corn contains 9% protein, 0.5 Kilogram will supply 45 grams of protein. Thus, the protein supply per capita in Rhodesia just covers the total average requirement. Abbott (6) suggested that supply should be 2% above the requirement to insure distribution. The amount of excess needed depends on spoilage during preparation, availability at proper time and place and income disparity. Moreover, when culture allows productive family members to eat at the expense of the nutritionally vulnerable groups, there may not be sufficient protein for growing children and expentant or nursing mothers.

Table 1 shows that a large percent of the protein comes from grains and that meat, eggs and milk are low when compared to supplies in the United States. Thus, much of the protein is of low quality.

Table 1 - Protein supplies per capita per day in grams (6)

Source	Africa	United States
Grains	32.2	33.4
Starchy roots	7.1	5.2
Nuts	9.0	3.8
Vegetables and fruits	1.7	3.6
Meat	5.8	19.8
Eggs	0.4	3.3
Fish	1.3	2.4
Milk	3.5	18.5
Total	61.0	90.0

Vitamin deficiencies of corn diets. Spector and Metz (7) found corn to be deficient in folate and vitamin B_{12}. Both liver and serum levels of vitamin B_{12} were low when rats were fed a corn diet. Serum folate activity did not reflect depleted folate stores. Supplements of pteroylglutamic acid maintained liver folate levels and vitamin B_{12} supplements prevented depletion of both vitamins.

Bressani et al. (8) reported that the niacin in corn is not completely available for use. Niacin from opaque-2 corn is more available although not higher in amount than in normal corn. Lime treatment causes a loss of niacin but an increase in tryptophan availability and thus an increase in total niacin equivalent of normal corn.

The Need for Improvement of Corn Protein

When a large proportion of protein is from one incomplete protein source, various nutritional problems become acute. A protein is incomplete when it does not provide the right pattern and quantity of essential amino acids. The FAO provisional reference pattern was derived from the amount of individual amino acids required by infants and adults (9). The FAO standards are safe, practical allowances but not so high as to be unattainable by people in developing countries (10).

When chemical analysis of a dietary protein is made, its amino acid pattern can be compared to that of the reference pattern and be given a protein score. The protein score of corn is 42 when compared to the FAO provisional reference pattern. The most limiting amino acids are tryptophan and lysine (8).

Most knowledge dealing with human utilization and metabolism of cereal proteins is based on the assumption that the essential amino acid pattern limits the nutritive value of the grain. Jansen and Howe (11) concluded that since there is a higher percent of non-essential amino acids in corn than essential amino acids, it is unlikely that total nitrogen can be the limiting factor. However, Kies et al. (12) found that "unessential" nitrogen (nitrogen from sources other than the essential amino acids) is the first limiting nitrogenous factor in corn for adults. Nitrogen retention of adults eating a corn diet increased after addition of "non specific" nitrogen (13). The nitrogen source used to supplement the corn diet was glycine-diammonium citrate. The long term effects of "non-essential" nitrogen intake on children has not been reported.

Rhodesian program. In order to provide an enriched corn product for the people, the Rhodesian government obtained the formula for Pro Nutro from the Food Corporation of Durban, South Africa (14). Pro Nutro can be used as a cereal or a soup. It contains soy, corn and peanut flours, non fat milk solids and wheat germ. It contains 22% protein. It is well accepted by the people as an infant food but the whole family cannot afford to use it because it is more economical to use home grown maize than to purchase prepared mixtures.

Better breeding strains. Corn is unknown in its wild state, but it is thought that it was derived from some ancestor of the Mexican grass, teosinte, Euchlaena Mexicana (15). It may be a hybrid between an unknown grass and teosinte. This unknown grass is believed to have been like the earless varieties of pod corn, Zea tunicata.

Corn was probably introduced to Africa after 1502 when Portuguese ships stopped on the African coast enroute to South America and traded seeds for food and water (16). The American Indians evidently passed corn seeds from tribe to tribe. Hybrid lines have been developed from the early American Indian corn.

One strain of corn, Zea mays, is homozygous for the recessive mutant gene, opaque-2 (17). Opaque-2 corn contains twice as much glutelin and half as much zein as other lines tested by Clark et al. (17). Zein is a protein that is lost as a by product of wet-milled corn (18). The change of protein gives a corn that is higher in lysine and tryptophan and has a better balance between leucine and isoleucine than other hybrid lines.

The effectiveness of opaque-2 corn in maintenance of a positive nitrogen balance depends on the caloric intake. For those eating large amounts of corn, opaque-2 corn should be a valuable source of protein (17). Eight young adult men were fed degermed opaque-2 corn for 10 days (19). The digestibility of the corn was near that of egg protein. The biological value and the net protein utilization of degermed opaque-2 corn were 82 and 78, respectively while those values for egg protein were 96 and 92. Whole opaque-2 corn gave lower values for digestibility, biological value and net protein utilization than the degermed opaque-2 corn.

There are other changes brought about by the mutant gene, opaque-2, which are not good. Watson and Yahl (20) found that the kernel size of opaque-2 corn is smaller than normal corn. When cereal protein improvement causes a decrease in productivity, it has limited nutritional value for people depending on cereal grains as a basic food (21). Although only 515 grams of opaque-2 corn would be necessary to supply the essential amino acids as compared to 967 grams of normal maize, the African may still need the larger amount of corn to supply calories necessary for energy. Mossberg (22) pointed out that in countries where high protein cereals are needed for human consumption, people do not have the ability to produce or buy the improved types of grain.

Effects of fertilization. Sauberlich et al. (23) found that corn grown on plots receiving high-nitrogen treatment had 9.3 to 12.0% protein as compared with 6.8 to 8.2% protein in corn grown on plots receiving low-nitrogen treatment. The nitrogen treatment increases the cost of growing corn.

Supplementation. Brock (24) stated that the term supplementation should be used in relation to a meal or a diet. If nutrients are added to bread on a commercial basis, the bread is fortified. When people do not buy ready-made foods, they need to rely on means of supplementation which can be accomplished in the home. Various commercial products have been made that have been fortified with fish flour (25), milk solids, various legumes and lysine (1 and 24). Also, provision of nitrogen in the form of urea can aid the endogenous synthesis of non-essential amino acids (26).

Hansen (1) stated that total protein intake influences the effectiveness of various supplementations. Gallina and Dominguez (26) found that low caloric intake decreases efficiency of high biological value protein. It is even more necessary to have sufficient calories on a low protein diet.

Soybeans. For 50 centuries, people of the Orient have subsisted on products of soybeans (4). The Chinese considered soybeans to be one of the five sacred grains and they were eaten to increase lung power, make the body plump, beautify the complexion and cause a cooling effect on the system (27). Seed was sent to the United States in 1873 by missionaries and they found that soybeans were able to grow in this climate. In the last quarter of a century, soybean production in the United States has grown from insignificant to first place in oil seed production.

Soybeans prefer mellow, fertile clay or sandy loams (27). They are sensitive to changes of soil and climatic conditions. Change in conditions has caused numerous varieties to be developed. The varieties can be divided into yellow, green and black beans but within each group there are different sizes and shapes of beans. When first introduced to a new country, soybeans have few diseases and they are comparatively free of serious insect pests (28). These factors will help soybean production in Africa.

Most of the soybean protein is packed in the aleurone cells that are in the third layer of the endosperm (28). The outside or palisade layer is responsible for the amount of water taken up by the seed. The second layer is made up of hour glass cells that are thick and readily separated from adjoining layers and from each other. When the outside hull of the soybean is removed, the hour glass cells would be the place of separation so that the third layer made up of aleurone cells would remain with the usable part. Removal of the hull, in a sense, concentrates the protein and makes a food that is easier to digest than food made with the whole soybean (29).

Many different products have been made from soybeans. For use in baked products, there is a choice of three flours: full fat that is 18-20% fat, low fat that is made from press cake, and defatted flour that is obtained by solvent extraction and contains less than 1% fat. Flours with less fat are more costly but of higher nutritional value than full fat soy flour. In bread dough, soy flour serves as a yeast food and thus speeds up fermentation. In the meat industry, soybeans serve as a binder and emulsifier. The Kellogg Company makes a ready-to-eat food which consists of 20-30% solvent extracted soybean and 70-80% yellow corn flakes (4).

One of the inhibiting factors of using soybeans in products is the bean-like flavor that is considered to be bitter by some people. Several methods have been developed which improve the flavor (4). Some of these methods are defatting, debittering, dehulling and roasting. Moisture and heat under various conditions improves flavor and nutritional value because interfering materials such as soyin and trypsin are proteins which are sensitive to heat (29). Steam at 15 pounds for 180 minutes decreased the nutritional value of soybean protein for monogastric animals. Soybeans boiled for 30 minutes caused greater weight gain per gram protein eaten in rats than soybeans cooked with dry heat or steam. Since boiling is the most common method of food preparation in Rhodesia, soybeans could be prepared in the home and they would be a good means of supplementation to be used in a somewhat primitive home situation.

Peanuts. Wild species of peanuts are found abundantly along the Amazon River where they are used to feed cattle (16). Evidence indicates that cultivated forms first appeared in the tropical and subtropical regions of South and North America. Portuguese traders may have taken peanuts to Africa where they spread so fast that soon travelers thought peanuts were indigenous to Africa. Both African and Spanish peanuts are grown in the United States.

Peanuts grow in well-drained soils that are sandy in nature (30). They require warm temperatures for normal development. An increase of temperature during its flowering period increases the yield. Again these are factors which prevail in Rhodesia so peanuts are a good crop for that area.

The components of peanuts vary with environmental factors (30). The skin is high in fiber and ash and it contains appreciable amounts of fat and nitrogenous materials. Removal of the skin would not concentrate the protein as removal of hulls improves soybeans. Milner (31) suggested that the skins, as well as the heart of the seed, should be removed from peanut flour. This would improve the shelf life of the flour but would decrease the nutritional value of the peanut flour.

In order to loosen the skins, the peanuts need a heat treatment. However, long periods of cooking cause a decrease in lysine from 3.4 g/16 g nitrogen to 1.9 to 2.8 g/16 g nitrogen. Maximum temperatures attained in processing peanuts should not exceed 250^{o} F (31). This amount of heat causes formation of free fatty acids. Peanut flour should not contain more than 0.5% of free fatty acids.

Aflatoxin, a product of a mold, causes an economic loss and changes the odor, taste and nutritive value of the peanut (32). Three approaches to eliminating aflatoxin are prevention, removal and inactivation. In order to prevent aflatoxin peanuts should be kept in a dry cool place. Damaged seeds encourage growth of mold that may form aflatoxin. If discolored seeds appear, they should be sorted out from the other seeds. Commercial processing firms could use a solvent mixture that extracts aflatoxin while removing relatively little other material, other than oil. The studies on the effectiveness of heat treatment on inactivating aflatoxin seems controversial. The temperature required to destroy the toxin is above the maximum heat recommended for peanuts and would result in denatured proteins.

Fermented Corn Meal Products

In some areas, corn is fermented and then prepared into an edible food product. The method seems to vary from locality to locality. In all cases, the fermented corn seems to be a desirable food. Perhaps the difficulty in preparation causes it to be used less than regular corn foods.

In Ghana, the fermented corn product is called "kenkey" (33). There they soak whole corn for 1 or 2 days and pour off the soaking water. Then it is ground into a partially bolted meal, mixed with water to form a stiff dough and allowed to ferment for 2 to 3 days. Then two-thirds of it is boiled and mixed with the remaining uncooked fermented corn. The dough is shaped into one pound balls, wrapped in husks and boiled for about two hours. The balls are then ready to eat.

In Nigeria, the people make "ogi" by steeping whole corn in water. The water is poured off and the corn is wetmilled and finally allowed to turn sour (5). The ogi boiled into a porridge is called "agidi". Fermented ogi has a PER and NPU that is inferior to whole corn meal (34). This is probably because of milling and sieving which causes removal of a considerable portion of the hulls, germ and aleurone layer. Fermentation brings about a slight enrichment in thiamin and niacin content of corn meal but not above the amounts found in whole corn.

The sour porridge of Rhodesia is similar to ogi except that the Rhodesian women simply soak ground corn meal overnight and then cook it into a porridge for breakfast. Rhodesian women do not pour off the soaking water.

Factors causing fermentation. Corn fermentation is brought about by natural fermentation. Akinrele and Bassir (34) found four molds present in soaking corn, Cephalosporiun, Fusarium, Aspergillus and Penicillium. The aerobic bacteria present are Corynebacterium michiganese, Leuconostoc Mecenteroids and Lactobacillus plantarum. The Lactobacillus sp. seem to be responsible for the souring process (5). It is a nonmotile, nonsporulating organism that is homofermentative (35). Lactobacillus can be isolated from sour milk. It grows well at pH 5 and in temperatures between 15 and 45° C. There are also yeasts present which are responsible for the odor of the fermented corn. The yeasts that Akinrele and Bassir (34) isolated are Candida Myconderma, Sacchromyces cerevisias and Rhodotorula.

Chemical changes during fermentation. Fermentation is the decomposition of complex organic material into substances of simple composition (35). It can follow several different pathways that produce carbon dioxide and water as final products but various sugars and acids as intermediate compounds. First the carbohydrates of corn meal are broken down to sugars. When simple sugar solutions are fermented by yeast, nitrogenous material is released (36). When the nitrogenous material was analyzed, it was found to be 25% protein and it consisted of small amounts of 10 different amino acids. Alanine was present in the largest amounts and the second highest was lysine. Yeasts vary in their ability to transform glucose to amino acids (37). During the first eight hours of fermentation of bourbon mash that consisted of 75% corn, 13% rye and 12% barley malt, with distillers yeast, flavor components were produced from amino acids. After that time, flavor was produced from carbohydrates at an increasing rate. More flavor originates from amino acids than carbohydrates but some amino acids are formed during fermentation. The percent of protein found in various corn products indicate that there is less protein, on a dry basis, after fermentation.

Akinrele et al. (34) reported that the speed of lactic fermentation of starchy foods is increased by enzyme-rich materials or low molecular weight nitrogen material. They found that the Beta-amylase enzyme of raw soybeans accelerates production of organic acids from the starch and dextrins of corn.

EXPERIMENTAL PROCEDURE

Treatment of Raw Corn, Soybeans and Peanuts

White corn and soybeans were purchased at the Farmers Cooperative Association in Manhattan, Kansas. The corn was ground in the Department of Grain Science and Industry so that it would go through an 8/64 inch sieve. This gave a meal that contained germ, full fat and hull. The hulls of the soybeans were cracked and removed and the remaining part of the soybean was ground to 8/64 inch fineness. Raw Spanish peanuts were purchased from Guy's Foods, Inc., Liberty, Missouri. Peanuts with hulls were put through a Kitchenaid electric grinding attachment three times. The approximate composition of the raw corn, soybeans and peanuts was determined by the methods of the Association of Official Agricultural Chemists (AOAC) (38). Corn was supplemented with soybeans and peanuts in the following combinations:

100% corn (100% C)
90% corn and 10% soybeans (10% S)
80% corn and 20% soybeans (20% S)
90% corn and 10% peanuts (10% P)
80% corn and 20% peanuts (20% P)

The analysis of 17 amino acids and the ammonia content of the three raw products, cooked corn and the fermented mixtures were done on a Spinco (Model 120) Amino Acid Autoanalyzer, which utilizes ion exchange chromatography, in the Department of Grain Science and Industry. Tryptophan was not determined.

Fermentation Study

Five 25 g samples of each of the above combinations were weighed into glass custard cups. Fifty ml of distilled water at 28° C was added to each custard cup at one and one half minute intervals so that 30 minutes were required altogether. The samples were covered and allowed to ferment in an incubator at 28° C. The rate of fermentation was measured by the change in pH (increase in acidity) of samples of the corn, soybean and peanut combinations. The pH of each sample was taken on the expanded scale of a Fisher pH meter (standarized to pH 4.63) every hour from 0 to 24 hours of fermentation time.

To determine the pH, a sample was removed from the incubator and stirred for 30 seconds. The pH meter electrodes were placed in the fermenting mixture and the meter was allowed to stabilize for 45 seconds before taking the pH reading. During the 45 second period the next sample was being stirred. Each sample was placed back in the incubator immediately after the pH reading was taken. The electrodes were cleaned with distilled water between each reading.

On one day, pH readings for the hours 0 and 9 to 24 were taken and on another day, the readings for the hours 0 to 11 were taken. Thus, there were eight replications for the hours 0 and 9 to 11.

Rat Feeding Study

<u>Preparation of corn mixtures</u>. When making food for the diets, 400 g lots of the five combinations of corn, soybeans and peanut meal were mixed with 800 ml distilled water and allowed to ferment for 13 hours. The mixtures were fermented in glass bowls in an incubator at 28° C.

An electronic controlled Trunnion kettle[a] was used to cook the five fermented mixtures and an unfermented corn meal porridge. For each 400 g of meal soaked in 800 ml water, 1200 ml of water was brought to a boil. The fermented mixture was added to the boiling water and cooked for 30 minutes on the medium setting. The unfermented corn meal was cooked in the same amount of water as the fermented corn meal. The cooked porridge was spread one fourth inch or less in thickness on cookie sheets. These pans of porridge were dried for 12 hours at room temperature with fans to circulate the air. The dried chips were

[a]Hotpoint distributed by General Electric Co., Chicago, Ill.

ground in a hammer mill with a medium sieve. Each mixture was analyzed for protein, moisture, fat and ash (38).

Mixing diets. Corn oil, corn starch, salt mixture XIV[a] and vitamins were added to each of these products to make up diets according to the AOAC (38). It was necessary to use a 9% protein level since this was the highest possible level in diets containing 100% corn. For the control diet casein was used as the source of protein.

Animals and their care. Fifty-six weanling male albino rats of the Sprague-Dawley strain were divided into 7 groups so that each group weighed approximately the same. The rats were placed in cages so that each diet was represented by two rats at each of the four rack levels. Food and water were given *ad libitum*. Feed cups were filled each morning. For a four week period, records were kept of food intake and weight gain for each week. Fecal material was collected for the second and third week of the experimental period for determination of digestibility. The nitrogen content of the feces was determined by the macro Kjeldahl method with boric acid modification (41). Protein efficiency ratio (PER) and apparent digestibility coefficient were calculated from the following formulas:

$$\text{Protein Efficiency Ratio} = \frac{\text{g Body Weight Gain}}{\text{g Protein Ingested}}$$

$$\text{Apparent Digestibility Coefficient} = \frac{\text{N Intake} - \text{N in Feces}}{\text{N Intake}} \times 100$$

Statistical Analysis

The degree of acidity developed by the different fermented mixes and the rates of increase in acidity were analyzed statistically. Logistic curves were made to convert the pH - time curves into straight line trends to permit easy

[a]Nutritional Biochemical Corporation, Cleveland, Ohio

study of differences between the rates of development of acidity of the five mixtures.

Analysis of variance were made on food intake, weight gain, PER and digestibility coefficient to determine the differences attributable to protein source in the rat diets. Fisher's least significant difference at the 5% level ($LSD_{0.05}$) was applied to detect which diets were significantly different.

RESULTS AND DISCUSSION

Rate of Fermentation

The rate of fermentation of corn can be determined by rate of change in pH since the process of breaking down the complex molecules in corn causes the formation of various acids. A pH of 7 is neutral and lower values indicate an acid condition.

Table 2 shows the mean pH readings for the five mixtures of corn, soybeans and peanuts at each hour from 0 to 24 while all readings are found in Table 10 in the Appendix. The 0 hour pH readings for the five mixtures were between 6.13 and 6.45. There was little change in pH for the first 8 hours of fermentation. During this time some mixtures decreased in pH and then returned to the beginning pH values. The order of pH values for the mixtures remained the same for the first 10 hours after which the 10% and 20% peanut mixtures had the lowest and third from lowest pH values instead of second and fourth from lowest of pH values. At the end of the 24 hour period, the 100% corn mixture had the lowest pH of all mixtures and the 10% and 20% peanut mixtures were second and third from lowest in pH. The two soybean mixtures were the least acidic for all hours after the tenth hour.

The most rapid change in pH occurred between the tenth and eighteenth hours of fermentation. All of the mixtures passed through a pH of 5.5 between the twelfth and fourteenth hours of fermentation. Aidoo (33) stated that the flavor of kenkey was best liked when it was fermented to pH 5.5, however Aidoo (33) found that kenkey had a pH of 5.2 at 36 hours of fermentation.

Limitations on time and facilities caused the pH readings for the times from 1 to 8 hours, inclusive, after the mixtures were incubated to be taken at a later date than those from 9 to 24 hours, inclusive. However, common readings

Table 2 - Mean pH readings for mixtures of corn, soybeans and peanuts during fermentation for 24 hours

Hours of fermentation	Mixture									
	100% C		10% S		20% S		10% P		20% P	
	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2	Day 1	Day 2
0	6.13	6.07	6.33	6.28	6.45	6.41	6.28	6.16	6.38	6.29
1	6.17		6.35		6.45		6.29		6.39	
2	6.21		6.35		6.44		6.31		6.39	
3	6.26		6.36		6.43		6.32		6.39	
4	6.18		6.31		6.40		6.28		6.36	
5	6.20		6.34		6.42		6.28		6.36	
6	6.20		6.31		6.39		6.27		6.37	
7	6.15		6.26		6.33		6.22		6.31	
8	6.13		6.25		6.31		6.19		6.27	
9	6.12	5.88	6.21	6.07	6.32	6.19	6.18	5.94	6.25	6.12
10	6.06	5.80	6.11	5.98	6.23	6.09	6.09	5.81	6.14	5.95
11	5.98	5.78	6.08	5.94	6.18	6.02	6.01	5.76	6.11	5.87
12		5.71		5.84		5.88		5.66		5.77
13		5.52		5.66		5.70		5.45		5.58
14		5.14		5.32		5.40		5.10		5.23
15		4.78		4.96		5.12		4.76		4.86
16		4.51		4.75		4.89		4.55		4.64
17		4.39		4.64		4.79		4.46		4.54
18		4.26		4.50		4.63		4.31		4.40
19		4.17		4.41		4.53		4.25		4.30
20		4.13		4.38		4.49		4.20		4.30
21		4.09		4.33		4.45		4.17		4.26
22		4.05		4.30		4.41		4.13		4.23
23		4.05		4.26		4.38		4.09		4.18
24		4.00		4.23		4.35		4.06		4.14

were taken at zero, 9, 10 and 11 hours. It was noted that the second set of pH readings (for 0, 1,..., 11 hours) averaged higher at the times in common; namely, 0, 9, 10 and 11 hours. A free-hand curve was used to correct the 0, 1,..., 11 hour pHs down to the level of the 0, 9, 10,..., 24 hour pHs taken previously. This process is shown in Figure 1. Thus, the pH found for 0 hour the second time was reduced by 0.07; that taken at 1 hour was also reduced by 0.07,...; and that pH obtained at 8 hours was reduced by 0.17 of a unit. The average pHs for each time and mix were analyzed for changes with time.

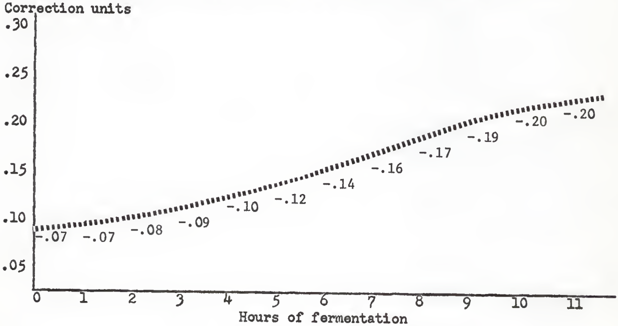


Figure 1 Amounts of adjustment for pH values of the second set of readings.

When the corrected pH readings were averaged and plotted against time in hours, such graphs as those seen in Figure 2 were obtained. Because pH = 7 is neutral and smaller values indicate acidity, the data were converted to measure of acidity by setting $y = 7.00 - pH$.

The graphs then appear as illustrated in Figure 3. These graphs suggest either a cumulative normal change in acidity with time or a logistic increase in acidity with time. The latter point of view is the more logical, and

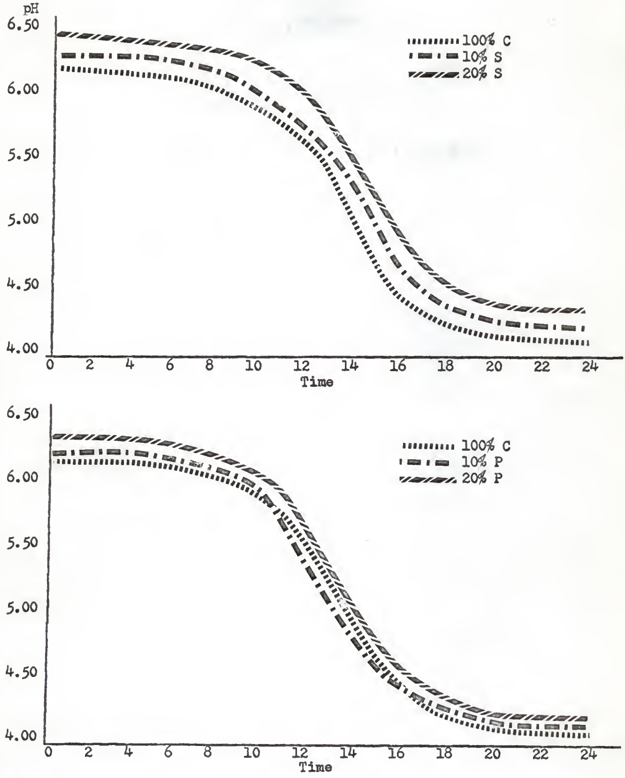
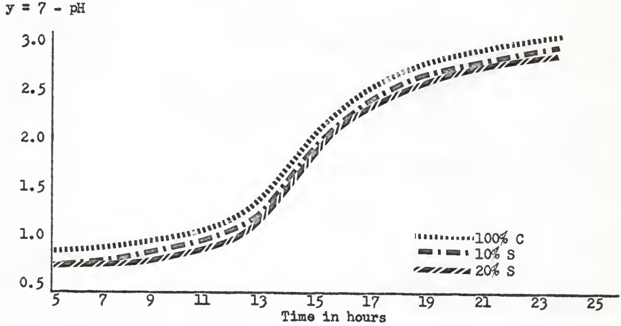


Figure 2 Average corrected pH readings for the fermented mixtures of corn, soybeans and peanuts.

turned out to be the realistic assumption. Attempts to fit cumulative normal curves were unsuccessful, whereas logistic curves do fit well.



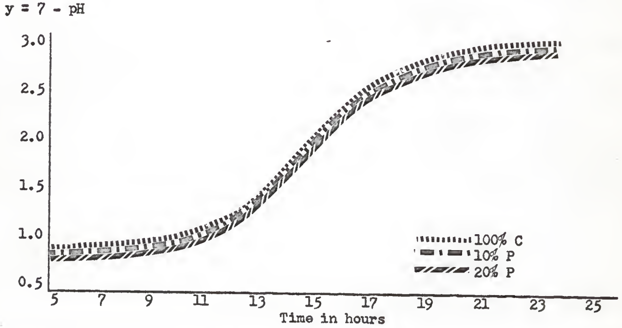


Figure 3 Fermentation curves when pH data is converted to y = 7 - pH.

The logistic curve devised by P. F. Verhulst in 1844 to describe growth of human populations has the mathematical formula

$$y - d = \frac{K}{1 + ce^{rt}}$$

where d and K measure distances to lower and upper asymptotes, respectively, and t = time (39). The c is an extra constant. It can be shown that if $Z = \frac{k-y+d}{y-d}$, then log Z is a linear function of t. This fact enables one to convert the pH-time curves into straight line trends, and thus permits easy study of differences among the rates of development of acidity associated with the five mixtures of meals. The readings for 0, 1, 2, 3 and 4 hours were sufficiently erratic and uninformative that they were omitted from further study. Figures 4 and 5 illustrate how differences between y and z appear graphically.

An IBM 360/50 program was used to analyze the differences in pH produced by the five mixtures of meals during 24 hours of incubation. The differences, if statistically significant, are shown by rejection of the hypothesis that the true regressions of log Z on time are equal. Table 3 shows an analysis of variance which tests that hypothesis plus the hypothesis that the levels of pH are equal among the five mixtures of meals.

Table 3 - Tests for equality of degree of acidity and also equality of rates of increase in acidity

Source of Variation	DF	Mean Square	Significant Level
Degree of Acidity	4	0.0356	$P < .05$
Rates of Change in Acidity	4	0.0636	$P < .005$
Deviation of True Average regression from zero	1	93.6093	$P < .001$
Error	90	0.0139	
Total	99		

It is clear from Table 3 that:

a) There are differences in degree of acidity developed by the different mixtures;

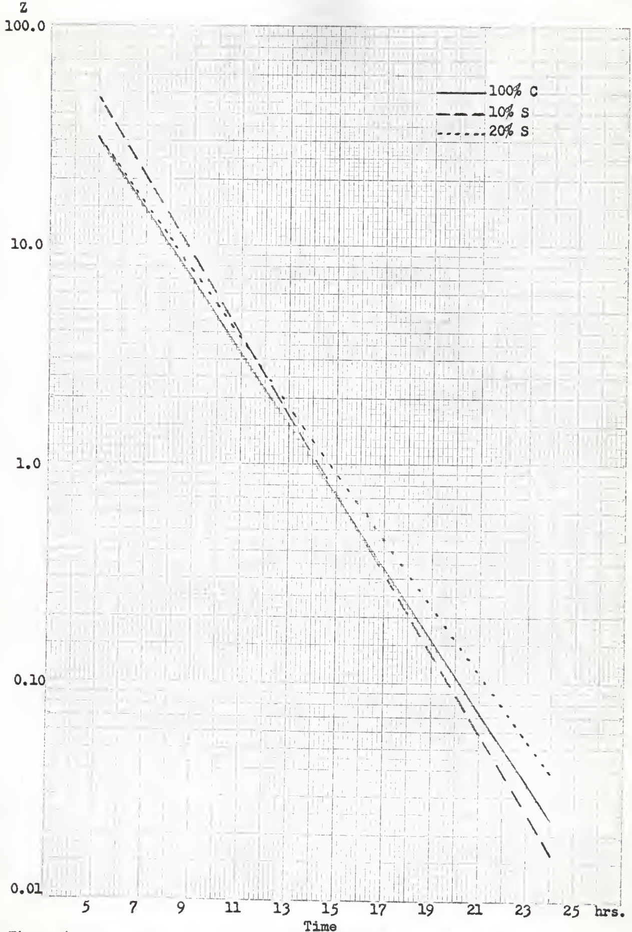


Figure 4 Linear relationship of pH and time for corn and soy mixtures.

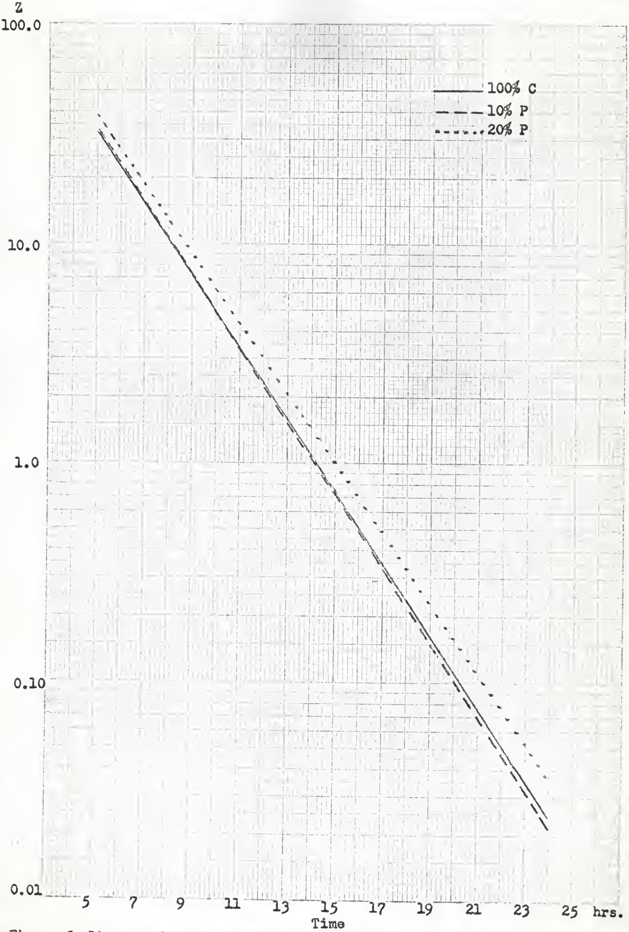


Figure 5 Linear relationship of pH and time for corn and peanut mixtures.

b) There are differences in rates of change in acidity with time under the different mixtures of meals.

Differences in rates of change in acidity of the mixtures is summarized in Table 4. For example, the rate of change in acidity with 10% soybean meal and 90% corn meal was found to be -0.1804, as measured in this study by using logarithms to obtain linear relationships between time and the change in acidity. With 100% corn meal, this rate is -0.1702. The difference between -.1804 and -.1702 is 0.0102 and is essentially at the 10% level of significance. It is too small to be considered significant at the 5% level. Therefore, the numbers -0.1804 and -0.1702 are not over a common solid line but they are over a common dotted line (for lack of 5% significance).

Table 4 - Individual comparisons of rates of change in acidity among mixtures of meals, at the 10% (______) and 5% (_ _ _ _) levels of significance. The rates (regression coefficients) are shown for log Z as a linear function of time

	Mixture of meals				
	10% S	20% P	100% C	10% P	20% S
	(-.1804)	(-.1709)	(-.1702)	(-.1613)	(-.1552)
10% level	______	______			
5% level	_ _ _ _	_ _ _ _	_ _ _ _		
10% level		______	______		
5% level		_ _ _ _	_ _ _ _	_ _ _ _	
10% level			______	______	
10% level					______

Coefficients (such as -0.1804 and -0.1709) above a common solid line <u>are not</u> significantly different at the 10% level. Coefficients above a common dotted line <u>are not</u> significantly different at the 5% level. (e.g., -0.1804 and -0.1702).

Table 4 indicates that:

a) The substitution of 10% soybean meal for corn meal significantly increases the rate of change in acidity with time at the 10% level of significance but not at the 5% level. The substitution of 20% soybean meal has a more striking effect but in the opposite direction; i.e., the rate of decline is decreased. It also is significant at the 5% level instead of the 10% level.

b) The substitution of peanut meal for corn meal in the amounts used does not change its rate of change in acidity significantly away from that produced by 100% corn meal. Increasing the percentage of peanut meal from 10 to 20 significantly increases that rate if one used a 10% level of significance, but not at the 5% level. That is, one is less confident that this change in amount of peanut meal affects the rate of change in acidity than is true with soybean meal. Furthermore, the change is in the opposite direction.

Composition of Corn, Soybean and Peanut Mixtures

Total protein, moisture, fat and ash content. The amounts of nitrogen, moisture, fat and ash of each of the raw products used in this experiment and the composition of the mixtures after treatment are given in Table 5. The soybeans had about four times as much protein as corn while the peanuts had about three times as much protein as corn. Replacement of corn by soybeans at the 10 and 20% levels caused 30 and 50% increases in protein above the fermented 100% corn. Peanut supplementation at 10 and 20% levels gave increases in protein of 20 and 40% over fermented 100% corn.

Moisture content of the mixtures were nearly the same. Fat content of the peanut mixtures were higher than other mixtures. There was little difference in ash content of the treated mixtures although the ash content of the raw products did vary.

Table 5 - Composition of raw products and corn, soybean and peanut mixtures after cooking and drying

Products and Treatments	Nutrients					
	Moisture	Protein[a]	Fat	Ash	Carbohydrate[b]	Protein Moist-free basis
Raw products	%	%	%	%	%	%
Corn	14.92	8.91	3.08	1.59	71.50	10.47
Soybeans	9.12	35.74	17.83	5.44	31.87	39.33
Peanuts	5.80	28.50	46.11	2.24	17.35	30.25
Cooked mixtures						
Unfermented						
corn	9.76	9.50	2.24	1.26		10.53
Fermented						
100% C	8.30	10.32	2.84	1.58		11.25
10% S	7.91	13.06	3.68	1.98		14.18
20% S	9.07	15.33	4.72	1.05		16.86
10% P	8.02	12.07	5.58	1.50		13.12
20% P	7.13	14.16	8.43	1.64		15.25

[a]N x 6.25
[b]Determined by difference of preceding values

Amino acid composition. Table 6 shows the amino acid analysis of the raw products, cooked corn and the fermented cooked combinations of corn, soybeans and peanuts. The fact that the recovery on the protein basis is near 100 for each chromatogram and the recovery on the nitrogen basis is between 80 and 100 show that these data are reliable.

Most of the amino acids were decreased by cooking unfermented corn except for glutamic acid, alanine, cystine and methionine which increased slightly. The fermentation process caused an increase of 0.1 to 0.3 gram in amino acids of cooked corn except for threonine, methionine, leucine and tyrosine. Increase in lysine and valine may be due to the presence of yeast since it is high in these amino acids. Values for isoleucine and leucine are closer in the fermented corn than in the raw corn. Yeast has a balance of these two amino acids.

A substitution of 10% soybean for corn caused an increase in almost all of the amino acids in the fermented, cooked corn. When 20% soybean was

Table 6 - Comparison of amino acid composition of raw corn, soybeans and peanuts with cooked and fermented, cooked combinations of those products (g. of a. a./100 g. protein on moist free basis)

Amino acids	Raw products			Cooked	Fermented				
	Corn	Soybeans	Peanuts	Corn	100% C	10% S	20% S	10% P	20% P
	g	g	g	g	g	g	g	g	g
Alanine	0.828	2.164	1.239	0.850	0.895	0.892	1.049	0.800	0.887
Ammonia	0.197	0.674	0.500	0.192	0.214	0.246	0.282	0.243	0.221
Arginine	0.543	3.750	3.463	0.490	0.581	0.693	0.869	0.794	0.911
Aspartic acid	0.797	5.766	3.729	0.715	0.833	1.114	1.461	0.953	1.219
Cystine[a]	0.204	0.801	0.363	0.217	0.217	0.244	0.278	0.234	0.238
Glutamic acid	2.126	8.873	6.075	2.152	2.169	2.550	3.023	2.269	2.705
Glycine	0.436	2.093	1.804	0.406	0.487	0.529	0.669	0.541	0.645
Histidine	0.337	1.466	0.672	0.294	0.363	0.361	0.414	0.338	0.343
Isoleucine	0.365	1.712	0.990	0.359	0.410	0.502	0.608	0.423	0.485
Leucine	1.371	3.530	1.983	1.331	1.356	1.520	1.667	1.320	1.423
Lysine	0.361	3.525	1.020	0.308	0.407	0.500	0.674	0.367	0.402
Methionine[b]	0.154	0.493	0.594	0.163	0.139	0.207	0.202	0.199	0.187
Phenylalanine	0.533	2.380	1.649	0.499	0.599	0.658	0.772	0.587	0.703
Proline	1.085	2.479	1.318	0.991	1.104	1.138	1.220	0.984	1.029
Serine	0.577	2.716	1.561	0.505	0.620	0.649	0.805	0.585	0.675
Threonine	0.438	1.939	0.833	0.377	0.384	0.494	0.622	0.415	0.453
Tyrosine	0.497	1.778	1.203	0.423	0.484	0.506	0.591	0.453	0.539
Valine	0.436	1.625	1.247	0.429	0.571	0.629	0.623	0.447	0.640

[a]by oxidation
[b]by oxidation

substituted for corn, increases in valine and methionine were not as great as with 10% soybean substitution. The 20% soybean caused the amount of lysine, aspartic acid and isoleucine to double.

Peanut supplementation resulted in smaller increases in amino acids than the soybean. However, the fermented 20% peanut mixture had slightly higher amounts of arginine and valine than the 20% soybean mixture.

As can be seen in Table 7, the most limiting amino acid in each of the corn mixtures was tryptophan and values for tryptophan are quoted from Orr and Watt (40) since it was not determined. The second most limiting amino acid for corn in this experiment was methionine. When corn was cooked, lysine was destroyed so that it was the second most limiting amino acid for the cooked corn. Fermentation increased lysine content so that methionine was again the second most limiting amino acid in all the fermented mixtures except the 10% peanut mixture. Both mixtures supplemented with peanuts had a low amount of lysine. The other limiting amino acid in corn was isoleucine. Neither fermentation or cooking or supplementation changed the isoleucine content greatly.

Rat Feeding Study

The food consumption, body weight gain, protein efficiency ratio (PER) and digestibility coefficient for each of the animals are presented in the Appendix, Table 11. The mean values for food consumed, weight gain and protein efficiency ratio for the seven diets are in Table 8. The mean apparent degestibility coefficient for each diet is in Table 9. Mean values for each factor were used for discussion. F values and least significant differences at the 5% level for each factor are included in the tables.

Food intake. The fermented corn had a sour flavor and aroma when compared to the unfermented corn but there was no significant difference in

Table 7 - Essential (and related) Amino Acids in the FAO Provisional Pattern of Amino Acids, corn, soybeans, peanuts and products of corn, soybeans and peanuts

Mixture	Iso-leucine	Leu cine	Lysine	Phenyl-alanine	Tyro-sine	Total Sulfur[a]	Methio-nine[b]	Threo-nine	Trypto-phan[c]	Valine
FAO	270	306	270	180	180	270	144	180	90	270
Raw Corn	218 (81%)	818 (267%)	215 (80%)	318 (177%)	298 (166%)	251 (93%)	108 (75%)	261 (145%)	38 (42%)	260 (96%)
Soybean	272	561	560	378	282	226	86	308	86	258
Peanut	204	410	211	341	248	210	130	172	69	258
Cooked Corn	209 (77%)	773 (253%)	183 (68%)	230 (128%)	178 (99%)	250 (93%)	108 (75%)	224 (124%)	--	255 (94%)
Fermented Corn	228 (84%)	753 (246%)	226 (84%)	310 (172%)	269 (149%)	216 (80%)	84 (58%)	213 (197%)	--	317 (117%)
10% S	221 (82%)	670 (219%)	220 (81%)	290 (161%)	223 (124%)	216 (80%)	99 (69%)	218 (121%)	43 (48%)	277 (102%)
20% S	226 (84%)	618 (202%)	250 (92%)	280 (156%)	219 (122%)	191 (71%)	82 (57%)	231 (128%)	48 (53%)	231 (86%)
10% P	202 (75%)	628 (205%)	175 (65%)	280 (156%)	216 (120%)	224 (83%)	103 (72%)	197 (109%)	41 (48%)	213 (79%)
20% P	199 (74%)	584 (191%)	165 (61%)	288 (160%)	221 (123%)	176 (65%)	73 (51%)	186 (103%)	44 (49%)	262 (96%)

[a]Sum of cystine and methionine
[b]Methionine by oxidation
[c]Analysis of tryptophan not done on these samples, values are from Orr and Watt (40)

amounts of feed consumed by rats on fermented and unfermented 100% corn diets. Supplementation of corn with 10% and 20% soybeans resulted in significant increases in food intake. Food intake was very similar for the rats on the 20% soybean diet and those on the casein diet. Rats on the 20% soybean and the casein diets consumed more than rats on the 10% soybean diet but the amount was not significant. The rats on the 10% peanut diet consumed significantly less than rats on the 20% peanut diet.

Weight gain. The pattern of weight gain for the groups of rats was similar to that of food intake. The difference between weight gain of rats fed fermented and unfermented 100% corn diets was not significant. Supplementation with 10% soybeans brought about a significantly greater weight gain than the 100% corn diets. Moreover, weight gain was significantly greater for the 20% soybean diet than for the 10% soybean diet. There were no significant differences for weight gain among the two 100% corn diets and the 20% peanut diet but weight gain for the 10% peanut diet was significantly lower than for all other diets. Weight gain was significantly greater for the casein diet than for any of the corn diets.

Protein efficiency ratio. A casein diet is expected to give a PER of 2.50 or higher. The PER of the casein diet in this experiment was 2.818. In Table 8 both the PER values and the PERs corrected to casein (2.50) are given, but the uncorrected values will be used for discussion.

The fermented and unfermented 100% corn diets, with PERs of 1.843 and 1.835 respectively, were not significantly different. Akinrele and Bassir (35) found unfermented ogi, a cooked corn product used in Nigeria, to have a higher PER (0.86) than fermented ogi (0.77). The PER values for both ogi diets were lower than values found in this study because in preparation of ogi the soaking water is discarded. This is not true of the preparation of fermented porridge in Rhodesia.

The PER of the casein diet was significantly higher than those of all other diets. However, the 20% and 10% soybean diets had relatively high PERs of 2.574 and 2.306, respectively. These PER values were significantly different from each other and both were significantly higher than those of all the other diets. There were no significant differences among the PERs for the unfermented 100% corn (1.835), fermented 100% corn (1.843), 10% peanut (1.777) and 20% peanut (1.788) diets.

Table 8 - Mean values, F - values and least significant differences for food consumed, weight gain and protein efficiency ratio

Protein Source	Food Intake	Weight gain	PER	PER corrected to casein 2.50
1. Casein	351.1	97.8	2.818	
2. Unfermented 100% corn	274.4	43.5	1.835	1.628
3. Fermented 100% Corn	279.8	42.2	1.843	1.635
4. 10% soy	333.5	69.0	2.306	2.045
5. 20% soy	355.1	78.0	2.574	2.283
6. 10% peanut	249.2	34.1	1.777	1.576
7. 20% peanut	293.8	47.1	1.788	1.586
F - value	10.320	67.246	28.653	
LSD .05	44.0	8.0	0.072	
	5,1> 7,3,2,6 4> 3,2,6 7> 6	1> 5,4,7,2,3,6 5> 4,7,2,3,6 4> 7,2,3,6 7,2,3> 6	1> 5,4,3,2,7,6 5> 4,3,2,7,6 4> 3,2,7,6	

Digestibility coefficient. The casein diet was the most digestible of the seven diets with an apparent digestibility coefficient of 96.5. Digestibility coefficients of the other diets ranged from 90.0 to 91.8. The unfermented 100% corn diet was more digestible ($P < 0.05$) than the fermented 100% corn diet. Akinrele and Bassir (34) found fermented ogi to be more digestible than unfermented ogi. Since fermentation causes a breakdown of complex compounds, it seems logical that fermented corn would be more digestible than unfermented corn. It is not known why fermented corn in this experiment was not as digestible as unfermented corn.

Supplementation with 10% soy, 20% soy and 20% peanut resulted in digestibility coefficients that were not significantly different from the unfermented 100% corn diet. However, the digestibility coefficient of the 10% peanut diet was significantly lower than all of the diets except the fermented 100% corn diet.

Table 9 - Mean values, F - value and least significant differences for apparent digestibility coefficient

Protein Source	Apparent Digestibility Coefficient
	%
1. Casein	96.5
2. Unfermented 100% corn	91.7
3. Fermented 100% corn	90.0
4. 10% soybean	91.8
5. 20% soybean	91.2
6. 10% peanut	90.0
7. 20% peanut	91.2
F - value	52.112
$LSD_{.05}$	0.9
	1 > 4,2,7,5,3,6 4,2,7,5 > 3,6

Relationship of food intake, weight gain, PER and digestibility. Diets which were consumed in large amounts resulted in good weight gains and high PER values. The only significant difference between the fermented and unfermented 100% corn diets was in apparent digestibility.

The rats on the 20% soybean diet had approximately the same food intake as rats on casein but their weight gain and PER was less than for the casein diet. Perhaps this was because the 20% soybean diet had a lower apparent digestibility. The 10% soybean diet was third from highest in all factors studied. It appears that addition of soybean meal at either 10% or 20% levels improved the nutritional value of fermented corn.

Although rats on the 20% peanut diet consumed more feed and had a greater weight gain than the rats on the 10% peanut diet, PERs for the two diets were almost identical. Addition of 10% or 20% peanut meal did not enhance the nutritional value of fermented corn. In fact, the 10% peanut meal lowered food intake, weight gain, PER and digestibility coefficient. These values were significantly lower for weight gain and digestibility.

SUMMARY

The effect of supplementation with 10% and 20% soybean (10% S, 20% S) and 10% and 20% peanut (10% P, 20% P) on the rate of fermentation of corn meal (100% C) was examined. Also, the protein quality of cooked unfermented corn (unfermented 100% C) was compared to fermented cooked corn (fermented 100% C) and the four fermented mixtures of corn, soybean and peanut meals.

Rate of fermentation over a 24 - hour period at 28° C was measured by change in pH of a 25 gram sample of each of the five mixtures combined with 50 ml of distilled water. For rat diets, 400 gram lots of the five corn mixtures were fermented in 800 ml distilled water for 13 hours which produced a pH of approximately 5.5. The fermented meal was boiled in an additional 1200 ml of water for 30 minutes to form a porridge. It was then dried at room temperatures and ground into a meal. A diet of unfermented 100% corn was prepared in the same manner. The dried corn mixtures served as the source of 9% protein for the rat diets. Oil, corn starch, salt mixture and vitamins were added to complete the diets. Each of the diets were analyzed for content of 17 amino acids and ammonia. Feed consumed, weight gain, PER and apparent digestibility were determined for the six corn diets plus a casein control diet.

The corn mixtures had initial pHs of 6.13 to 6.45 and 24 hour pHs ranging from 4.00 to 4.35. Each of them reached a pH of 5.5 after 12 to 14 hours of fermentation. The substitution of 10% soybean meal for corn meal increased ($P < 0.05$) the rate of change in acidity but substitution of 20% soybean meal decreased ($P < 0.05$) the rate. The substitution of 20% peanuts resulted in a faster change in acidity than substitution of 10% peanut ($P < 0.10$) but neither of the levels of substitutions of peanuts changed the rate of change in acidity

significantly from that produced by 100% corn meal.

Amino acid analysis of raw and unfermented cooked corn indicated that cooking decreased most of the amino acids. Fermentation before cooking resulted in a slight increase in most amino acids as compared to raw corn. The second most limiting amino acid was methionine in all fermented mixtures except the 10% peanut mixture.

Although fermentation resulted in improvement in amino acid content of 100% corn, food consumed, weight gain and PER were not changed and the apparent digestibility was decreased. Substitution of soybean meal at either 10% or 20% levels improved the utilization of fermented corn in that rats consumed more feed, gained more weight and had higher PERs. Substitution of 10% and 20% peanut meal did not enhance the nutritional value of fermented corn.

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REFERENCE LIST

1. Hansen, J. D. L. 1960 The effects of various forms of supplementation on the nutritive value of maize for children. In: Meeting Protein Needs of Infants and Children. National Academy of Sciences - National Research Council, Publication 843, Washington, D. C. p. 89.

2. Report of the FAO/WHO Seminars of problems of food and nutrition in Africa south of the Sahara. Lwiro, Bukavu, Congo 1959 FAO Nutrition Meetings Report Series 25.

3. Statistical Year Book 1970 Statistical Office of the United Nations, Department of Economic and Social Affairs. New York.

4. Burnett, R. S. 1951 Soybean protein food products. In: Soybeans and Soybean Products, Vol. 2, ed., K. S. Markley. Interscience Publishers, Inc. New York. p. 949.

5. Akinrele, I. A., A. Makanju and C. G. Edwards 1969 Effect of soya flour on the lactic fermentation of milled corn. App. Micro. 17:186.

6. Abbott, J. C. 1966 Protein supplies and prospects: the problem. In: World Protein Resources, Advances in Chemistry Series 57. American Chemical Society, Washington, D. C. p. 1.

7. Spector, I. and J. Metz 1966 Folate and vitamin B_{12} metabolism in weanling rats given a maize diet. Amer. J. Clin. Nutr. 19:187.

8. Bressani, R., L. G. Elias and R. A. Gomez-Brenes 1968 Protein quality of opaque-2 corn evaluation on rats. J. Nutr. 97:173.

9. National Research Council 1963 Evaluation of Protein Quality. National Academy of Sciences - National Research Council, Publication 1100.

10. Pike, R. E. and M. L. Brown 1967 Nutrition: an Integrated Approach. John Wiley and Sons, Inc., New York.

11. Jansen, G. R. and E. E. Howe 1964 World problems in protein nutrition. Am. J. Clin. Nutr. 15:262.

12. Kies, C., E. Williams and H. M. Fox 1965 Effect of "non-specific" nitrogen intake on adequacy of cereal proteins for nitrogen retention. J. Nutr. 86:357.

13. Kies, C., H. M. Fox and E. R. Williams 1967 Time, stress, quality and quantity as factors in the nonspecific nitrogen supplementation of corn protein for adult men. J. Nutr. 93:377.

14. Bauernfeind, J. C. 1970 Vitamin fortification and nutrified foods. SOS/70 National Conference of Food Science and Technology, Washington, D. C. p. 217.

15. Collins, G. M. 1912 The origin of maize. J. Washington Acad. of Sci. 2:520.

16. Higgins, B. B. 1951 Origin and early history of the peanut. In: The Peanut: The Unpredictable Legume. National Fertilizer Association p. 18.

17. Clark, H. E., P. E. Allen, S. M. Meyers, S. E. Tuckett and Y. Yamamura 1966 Nitrogen balances of adults consuming opaque-2 maize protein. Amer. J. Clin. Nutr. 20:825.

18. Boundy, J. A., J. E. Turner, J. S. Wall and R. J. Dimler 1966 Influence of commercial processing on composition and properties of corn zein. Cereal Chem. 44:281.

19. Young, V. R., I. Ozalp, B. V. Cholakos and N. S. Scrimshaw 1971 Protein value of Colombia opaque-2 corn for young adult men. J. Nutr. 101:1475.

20. Watson, S. A. and K. R. Yahl 1967 Comparison of the wet-milling properties of opaque-2 high lysine corn and normal corn. Cereal Chem. 44:488.

21. Johnson, V. A., P. J. Mattern and J. W. Schmidt 1970 The breeding of wheat and maize with improved nutritional value. Pro. Nutr. Soc. 29:20.

22. Mossberg, R. 1970 Practical problems concerning the marketing of cereals with improved protein value. Pro. Nutr. Soc. 29:39.

23. Sauberlick, H. E., W. Chang and W. D. Salmon 1953 The amino acid and protein content of corn as related to variety and nitrogen fertilization. J. Nutr. 51:241.

24. Brock, J. F. Protein malnutrition, requirement and supplementation 1961 In: Meeting Protein Needs of Infants and Children. National Academy of Sciences - National Research Council, Washington, D. C. p. 103.

25. Smith, M. M. and M. A. Kenney 1967 Effect of Fish-corn mixtures on growth antibody formation in rats. J. Amer. Dietet. A. 54:500.

26. Gallina, D. L. and J. M. Dominguez 1971 Human utilization of urea nitrogen in low calorie diets. J. Nutr. 101:1029.

27. Morse, W. J. 1950 History of soybean production. In: Soybeans and Soybean Products. Vol. 1, ed., K. S. Markley. Interscience Publishers, Inc., New York. p. 4.

28. Williams, L. F. 1950 Structure and genetic characteristics of the soybean. Vol. 1, ed., K. S. Markley. Interscience Publishers, Inc., New York. p. 113.

29. Altschul, A. M. 1960 Present status of proteins from oilseeds. In: Meeting Protein Needs of Infants and Children. National Academy of Sciences - National Research Council, Washington, D. C. p. 517.

30. Harris, H. C. and R. W. Bledsoe 1951 Physiology and mineral nutrition. In: The Peanut: The Unpredictable Legume. National Fertilizer Association. p. 52.

31. Milner, M. 1966 General outlook for seed protein concentrates. In: World Protein Resources, Advances in Chemistry Series 57. American Chemical Society, Washington, D. C. p. 52.

32. Goldblatt, L. A. 1966 Some approaches to the elimination of aflatoxin from protein concentrates. In: World Protein Resources, Advances in Chemistry Series 57. American Chemical Society, Washington, D. C. p. 216.

33. Aidoo, S. E. 1967 Studies on kenkey - a food product made from corn in Ghana. Master's Thesis - Kansas State University.

34. Akinrele, I. A. and O. Bassir 1967 The nutritive value of "ogi", a Nigerian infant food. J. Trop. Med. Hygiene 70:279.

35. Brock, T. D. 1970 Biology of Microorganisms. Prentice-Hall, Inc., Englewood Cliffs, New Jersey. pp. 101 and 538.

36. Clapperton, J. F. 1970 Materials formed by yeast during fermentation. J. of Int. Brew. 77:36.

37. Reazin, G. H., H. Scales and A. Andreasen 1970 Mechanism of congener formation in alcoholic grain fermentations. J. Agr. Food Chem. 18:585.

38. Association of Official Agricultural Chemists 1965 Methods of Analysis. Washington, D. C.

39. Pearle, R. 1940 Medical Biometry and Statistics, ed., W. B. Saunders. Phila., Pa. p. 459.

40. Orr, M. L. and B. K. Watt 1957 Amino acid content of foods. Home Economics Research Report No. 4. U.S.D.A., Washington, D. C.

APPENDIX

Table 10 - The pH readings during 24 hours of fermentation time

Mixture 100% C

Hours of fermentation	Mixture #1 Day #1	Mixture #1 Day #2	Mixture #2 Day #1	Mixture #2 Day #2	Mixture #3 Day #1	Mixture #3 Day #2	Mixture #4 Day #1	Mixture #4 Day #2	Mixture #5 Day #1	Mixture #5 Day #2
0	6.08	6.09	6.12	6.14	6.06	6.14	6.05	6.15	6.07	6.17
1		6.15		6.14		6.17		6.23		6.19
2		6.19		6.22		6.22		6.28		6.22
3		6.26		6.28		6.27		6.21		6.25
4		6.16		6.18		6.18		6.25		6.21
5		6.14		6.19		6.21		6.22		6.24
6		6.19		6.20		6.20		6.17		6.19
7		6.14		6.15		6.15		6.13		6.15
8		6.10		6.19		6.15		6.09		6.12
9	5.91	6.13	5.93	6.19	5.89	6.13	5.84	6.02	5.86	6.09
10	5.84	6.09	5.83	6.09	5.81	6.08	5.78	5.94	5.76	6.04
11	5.80	6.01	5.80	5.99	5.80	5.97	5.74		5.77	5.99
12	5.72		5.73		5.74		5.76		5.68	
13	5.53		5.51		5.54		5.55		5.49	
14	5.18		5.12		5.18		5.15		5.09	
15	4.86		4.75		4.79		4.80		4.72	
16	4.56		4.49		4.53		4.52		4.46	
17	4.46		4.36		4.37		4.40		4.37	
18	4.32		4.25		4.24		4.28		4.23	
19	4.21		4.15		4.15		4.19		4.15	
20	4.13		4.12		4.12		4.16		4.12	
21	4.12		4.09		4.09		4.10		4.06	
22	4.07		4.05		4.05		4.07		4.04	
23	4.07		4.05		4.03		4.06		4.02	
24	4.01		4.00		4.00		4.02		3.99	

10% S

Hours of fermentation	Mixture #1 Day #1	Mixture #1 Day #2	Mixture #2 Day #1	Mixture #2 Day #2	Mixture #3 Day #1	Mixture #3 Day #2	Mixture #4 Day #1	Mixture #4 Day #2	Mixture #5 Day #1	Mixture #5 Day #2
0	6.29	6.34	6.29	6.34	6.30	6.33	6.27	6.32	6.26	6.35
1		6.36		6.36		6.35		6.34		6.37
2		6.36		6.34		6.34		6.35		6.37
3		6.37		6.35		6.37		6.35		6.36
4		6.31		6.31		6.32		6.30		6.32
5		6.38		6.34		6.32		6.35		6.33
6		6.31		6.31		6.31		6.31		6.32
7		6.27		6.26		6.26		6.26		6.26
8		6.25		6.26		6.24		6.27		6.26
9	6.19	6.22	6.07	6.20	6.08	6.20	5.99	6.24	6.03	6.19
10	6.05	6.15	5.95	6.12	5.98	6.10	5.96	6.17	5.97	6.11
11	5.99	6.11	5.98	6.02	5.93	6.08	5.91	6.12	5.89	6.08
12	5.92		5.91		5.77		5.84		5.79	
13	5.76		5.81		5.49		5.63		5.61	
14	5.43		5.50		5.11		5.31		5.27	
15	5.08		5.07		4.88		4.88		4.93	
16	4.83		4.79		4.73		4.70		4.72	

Table 10 (cont.)

Hours of fermentation	Mixture 10% S (cont.) Mixture #1 Day #1	Mixture #1 Day #2	Mixture #2 Day #1	Mixture #2 Day #2	Mixture #3 Day #1	Mixture #3 Day #2	Mixture #4 Day #1	Mixture #4 Day #2	Mixture #5 Day #1	Mixture #5 Day #2
17	4.69		4.68		4.66		4.59		4.61	
18	4.53		4.50		4.55		4.46		4.48	
19	4.44		4.39		4.46		4.38		4.39	
20	4.41		4.35		4.45		4.34		4.35	
21	4.35		4.30		4.41		4.30		4.32	
22	4.32		4.26		4.38		4.27		4.29	
23	4.28		4.21		4.34		4.23		4.24	
24	4.25		4.18		4.29		4.20		4.22	
					20% S					
0	6.43	6.44	6.43	6.45	6.42	6.45	6.41	6.45	6.38	6.45
1		6.47		6.45		6.45		6.45		6.46
2		6.44		6.44		6.45		6.45		6.44
3		6.44		6.43		6.43		6.43		6.42
4		6.41		6.40		6.40		6.40		6.40
5		6.44		6.43		6.42		6.42		6.42
6		6.38		6.41		6.42		6.39		6.38
7		6.31		6.35		6.36		6.34		6.33
8		6.34		6.28		6.31		6.29		6.33
9	6.20	6.34	6.21	6.33	6.18	6.32	6.20	6.32	6.19	6.30
10	6.14	6.27	6.09	6.24	6.09	6.26	6.08	6.21	6.09	6.18
11	5.90	6.22	6.05	6.19	6.00	6.13	6.08	6.16	6.07	6.13
12	5.68		5.98		5.79		5.98		5.98	
13	5.50		5.86		5.48		5.78		5.91	
14	5.23		5.65		5.10		5.39		5.65	
15	5.02		5.34		4.94		5.04		5.20	
16	4.85		5.04		4.77		4.84		4.99	
17	4.78		4.89		4.73		4.77		4.82	
18	4.63		4.69		4.60		4.61		4.64	
19	4.57		4.57		4.51		4.49		4.51	
20	4.51		4.52		4.50		4.47		4.45	
21	4.49		4.48		4.45		4.43		4.42	
22	4.45		4.44		4.42		4.39		4.38	
23	4.42		4.38		4.38		4.39		4.33	
24	4.40		4.36		4.34		4.33		4.30	
					10% P					
0	6.16	6.29	6.17	6.31	6.14	6.27	6.18	6.27	6.16	6.26
1		6.31		6.27		6.29		6.30		6.31
2		6.32		6.33		6.31		6.31		6.30
3		6.31		6.36		6.32		6.32		6.32
4		6.29		6.32		6.32		6.22		6.27
5		6.28		6.28		6.29		6.26		6.30
6		6.32		6.29		6.25		6.25		6.25

Table 10 (cont.)

Hours of fermentation	Mixture 10% P (cont.) Mixture #1 Day #1	Mixture #1 Day #2	Mixture #2 Day #1	Mixture #2 Day #2	Mixture #3 Day #1	Mixture #3 Day #2	Mixture #4 Day #1	Mixture #4 Day #2	Mixture #5 Day #1	Mixture #5 Day #2
7		6.23		6.25		6.22		6.22		6.22
8		6.14		6.20		6.21		6.21		6.21
9	5.98	6.16	5.99	6.20	5.94	6.20	5.91	6.19	5.90	6.18
10	5.84	6.05	5.86	6.12	5.73	6.10	5.84	6.09	5.82	6.07
11	5.82	5.98	5.80	6.04	5.62	6.02	5.80	6.00	5.79	6.00
12	5.73		5.78		5.39		5.74		5.68	
13	5.58		5.64		5.09		5.53		5.45	
14	5.27		5.31		4.77		5.10		5.16	
15	4.90		4.88		4.56		4.73		4.75	
16	4.62		4.59		4.45		4.54		4.56	
17	4.49		4.50		4.39		4.48		4.46	
18	4.32		4.31		4.29		4.34		4.33	
19	4.24		4.23		4.23		4.28		4.27	
20	4.19		4.18		4.21		4.24		4.22	
21	4.14		4.14		4.18		4.20		4.19	
22	4.11		4.08		4.15		4.17		4.17	
23	4.07		4.05		4.10		4.12		4.09	
24	4.03		4.02		4.06		4.11		4.10	
					20% P					
0	6.29	6.39	6.30	6.38	6.29	6.38	6.29	6.40	6.30	6.39
1		6.39		6.38		6.39		6.39		6.43
2		6.40		6.39		6.40		6.40		6.40
3		6.40		6.39		6.39		6.39		6.40
4		6.37		6.38		6.39		6.39		6.30
5		6.33		6.36		6.37		6.40		6.36
6		6.35		6.37		6.37		6.39		6.39
7		6.30		6.30		6.31		6.32		6.32
8		6.28		6.28		6.26		6.28		6.26
9	6.09	6.26	6.13	6.25	6.11	6.27	6.16	6.25	6.12	6.23
10	5.96	6.14	5.97	6.15	5.94	6.19	6.03	6.16	5.86	6.14
11	5.93	6.10	5.90	6.06	5.90	6.14	5.98	6.14	5.68	6.13
12	5.84		5.82		5.85		5.92		5.43	
13	5.67		5.62		5.70		5.79		5.12	
14	5.29		5.20		5.38		5.50		4.81	
15	4.88		4.85		4.95		5.06		4.60	
16	4.65		4.66		4.68		4.71		4.52	
17	4.51		4.59		4.56		4.59		4.47	
18	4.38		4.49		4.41		4.41		4.34	
19	4.30		4.44		4.32		4.30		4.29	
20	4.27		4.42		4.28		4.26		4.28	
21	4.22		4.29		4.25		4.21		4.24	
22	4.21		4.34		4.18		4.17		4.20	
23	4.16		4.30		4.17		4.14		4.15	
24	4.12		4.22		4.10		4.10		4.15	

Table 11 - Food consumed, body weight gain, PER and digestibility for each of the rats

Diet and rat number	Food Intake	Body weight gain	PER	Digestibility coefficient
				%
Casein				
11	366	91	2.517	97.0
12	317	92	2.937	95.3
13	352	100	2.875	96.2
14	383	104	2.748	96.5
15	325	75	2.336	96.8
16	338	103	3.084	97.1
17	381	110	2.922	97.1
18	347	107	3.121	96.1
Unfermented 100% corn				
21	295	46	1.836	91.6
22	285	45	1.836	92.2
23	220	28	1.480	90.7
24	297	55	2.153	91.3
25	259	44	1.975	91.6
26	264	44	1.938	93.2
27	337	50	1.725	92.0
28	238	36	1.759	90.8
Fermented 100% corn				
31	253	43	2.058	89.5
32	305	45	1.786	89.9
33	295	31	1.272	91.8
34	277	43	1.879	89.1
35	326	52	1.931	91.6
36	231	41	2.149	88.1
37	298	42	1.706	90.0
38	253	41	1.962	90.2
10% Soy				
41	355	71	2.227	92.6
42	314	69	2.447	90.9
43	313	68	2.419	91.9
44	294	51	1.932	93.0
45	368	71	2.148	92.0
46	379	79	2.321	91.6
47	283	65	2.558	91.5
48	362	78	2.399	91.1

Table 11 (cont.)

Diet and rat number	Food Intake	Body weight gain	PER	Digestibility coefficient
				%
20% Soy				
51	333	75	2.643	91.2
52	329	64	2.283	90.5
53	394	87	2.592	91.5
54	362	81	2.622	90.6
55	369	83	2.640	92.6
56	322	69	2.515	90.9
57	363	84	2.716	91.0
58	369	81	2.576	91.0
10% Peanut				
61	245	37	1.954	88.7
62	218	31	1.840	90.3
63	255	42	2.131	89.7
64	276	39	1.828	91.0
65	220	32	1.882	89.2
66	233	19	1.055	90.5
67	229	35	1.977	89.9
68	318	38	1.546	90.4
20% Peanut				
71	276	44	1.779	90.5
72	302	50	1.848	91.6
73	344	58	1.882	90.3
74	274	44	1.792	91.9
75	259	42	1.810	90.6
76	295	47	1.778	90.7
77	311	50	1.794	93.1
78	289	42	1.622	90.9

THE EFFECT OF FERMENTATION AND SOYBEAN AND PEANUT SUPPLEMENTATION ON CORN PROTEIN

by

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B. S., Kansas State University, 1964

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The effect of supplementation with 10% and 20% soybean (10% S, 20% S) and 10% and 20% peanut (10% P, 20% P) on the rate of fermentation of corn meal (100% C) was examined. Also the protein quality of cooked unfermented corn (unfermented 100% C) was compared to fermented cooked corn (fermented 100% C) and the four fermented mixtures of corn, soybean and peanut meals.

Rate of fermentation over a 24-hour period at 28°C was measured by change in pH of a 25 gram sample of each of the five mixtures combined with 50 ml of distilled water. For rat diets, 400 gram lots of the five corn mixtures were fermented in 800 ml distilled water for 13 hours which produced a pH of approximately 5.5. The fermented meal was boiled in an additional 1200 ml of water for 30 minutes to form a porridge. It was then dried at room temperatures and ground into a meal. A diet of unfermented 100% corn was prepared in the same manner. The dried corn mixtures served as the source of 9% protein for the rat diets. Oil, corn starch, salt mixture and vitamins were added to complete the diets. Each of the diets were analyzed for content of 17 amino acids and ammonia. Feed consumed, weight gain, PER and apparent digestibility was determined for the six corn diets plus a casein control diet.

The corn mixtures had initial pHs of 6.13 to 6.45 and 24 hour pHs ranging from 4.00 to 4.35. Each of them reached a pH of 5.5 after 12 to 14 hours of fermentation. The substitution of 10% soybean meal for cornmeal increased ($P < 0.05$) the rate of change in acidity but substitution of 20% soybean meal decreased ($P < 0.05$) the rate. The substitution of 20% peanuts resulted in a faster change in acidity than substitution of 10% ($P < 0.10$) but neither of the levels of substitutions of peanuts changed the rate of change in acidity significantly from that produced by 100% cornmeal.

Amino acid analyses of raw and unfermented cooked corn indicated that cooking decreased most of the amino acids. Fermentation before cooking resulted in a slight increase in most amino acids as compared to raw corn. The most limiting amino acid in the corn mixtures was tryptophan. The second most limiting amino acid was methionine in all fermented mixtures except the 10% peanut mixture.

Although fermentation resulted in improvement in amino acid content of 100% corn, food consumed, weight gain and PER were not changed but the apparent digestibility was decreased. Substitution of soybean meal at either 10% or 20% levels improved the utilization of fermented corn in that rats consumed more feed, gained more weight and had higher PERs. Substitution of 10% and 20% peanut meal did not enhance the nutritional value of fermented corn. In fact rats on the 10% peanut diet consumed less feed, had less weight gain and had lower PERs than rats on fermented 100% corn.