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PROGRESS REPORT NO. 5

Improving the Nutritive Value of Cereal Based Foods

Agency for International Development

Contract No. AID/csd - 1586

CONTRACTOR

The Food and Feed Grain Institute
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Submitted March 1, 1973, covering period January - December 1972

IMPROVING THE NUTRITIVE VALUE OF CEREAL BASED FOODS

Progress Report No. 5

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Improving the Nutritive Value of Cereal Based Foods

AID/csd - 1586

Report Summary

A. 1. Improving the nutritive value of cereal based foods.
AID/csd - 1586

2. Dr. William J. Hoover, Principal Investigator

Contractor: Kansas State University
Food and Feed Grain Institute
Shellenberger Hall
Manhattan, Kansas 66506

3. Contract Period (as amended): June 30, 1967 to April 1, 1974.

4. Period covered by Report: January 1, 1972 to January 1, 1973.

5. Total A.I.D. funding of contract to date: $791,097.

6. Total expenditures and obligations through previous contract year -- $175,268.

7. Total expenditures and obligations for current year: $177,788.

8. Estimated expenditures for next contract year: $100,000 (8 months).

B. Narrative Summary of Accomplishments and Utilization.

Development of protein fortified wheat based bread, chapatis, and couscous using soy flour, chickpea flour, and broadbean flour has been accomplished. The products meet the nutritional guidelines established by the project and have proven acceptable to small consumer panels. Field consumer testing evaluations of fortified chapatis have been carried out in Pakistan. Trial marketing potential and operational significance are yet to be determined.

The evaluation of protein resource materials from the standpoint of nutritional value, functional value in foods, and economic implications continue to provide guidance to researchers and industrial concerns world-wide on the use of these high protein materials in various protein fortified food.

Soy fortified wheat flour products based on products demonstrated in this project are now purchased under P.L. 480 Title I and II programs and are finding usage in school lunch and other institutional feeding programs in India, the Philippines, Colombia, and approximately 15 other countries at present.

Soy flour fortification of other cereal based foods such as noodles and cookies have also been accomplished without loss in product acceptability or appreciable increase in cost.

Improving the Nutritive Value of Cereal Based Foods

AID/csd - 1586

ANNUAL RESEARCH REPORT

A. General Background

Although research and product development work on nutritionally improved infant foods or gruels or beverages was under way in the mid-1960's, there was a dearth of information regarding the protein improvement of cereal based foods in the form in which they were traditionally consumed. A basic study was initiated to evaluate the physical, chemical, nutritional and functional properties of all protein resource materials available as potential fortifying agents for cereal based foods. While the implications of this research have worldwide application, the project was given the practical parameters of working, as demonstration foods, with the breadstuffs of North Africa and Pakistan, i.e., bread, couscous, and chapatis.

B. Statement of Project Objectives as Stated in the Contract

The general objective of this research project is to improve the nutritional value of cereal based foods in North Africa and Pakistan by supplementation and process modification without decreasing the food acceptability to the general consuming public in those areas.

C. Continued Relevance of Objectives

Information gathered during the survey phase of the project indicated a lesser emphasis on process modification of wheat milling methods for increased use of wheat millfeeds than had been planned and thus investigations in this area have been limited. The dietary importance of couscous in North Africa was found to be of secondary importance to that of bread. In the latter stages of the research and in the field testing phase, much more emphasis is being given to bread than to couscous.

Increased emphasis is being given to the economic factors which will influence the implementation of protein fortification programs. Expansion of the basic technology to cookies and other traditional cereal based foods was an obvious follow-up shift of emphasis.

D. Accomplishments to Date

1. Specific Objectives for 1972

a. Accomplish acceptability testing programs and marketing potential projections on bread in Morocco and chapatis in Pakistan.

b. Initiate economic evaluations or cost-benefit studies for the use of products in applied nutrition programs.

c. Expand use of high protein bread technology to other LDC's through close cooperation with A.I.D. Project Manager.

d. Expand studies to cookies and other traditional cereal based foods.

e. Evaluate additional protein resource materials, i.e., sunflower flour, triticale, fish protein concentrate, sesame, and rapeseed.

f. Limited studies on processing of protein concentrates from legumes (study economics as soon as possible).

g. Limited studies on nutrient availability (effect of processing and natural vs. synthetic nutrients).

2. Report of Progress in 1972

a. Field Acceptability Testing Programs

Chapatis produced from normal atta and atta fortified with 10% defatted toasted soy flour or 15% chickpea flour were tested for acceptability by 1,018 families at the Pakistan Agricultural University, Lyallpur, Pakistan. The testing procedure was essentially as described in

Appendix I in the Annual Research Report No. 4 for 1971.

The results of the acceptability tests were evaluated using a chi square statistical test and the data were analyzed by use of a computer program. Generally the atta fortified with 10% soy flour was liked as well as regular atta but the 10% chickpea atta was not liked as well. Much of the difficulty for the chapatis made with chickpea flour fortification could be traced to textural changes in the chapatis.

In the testing program, an attempt was also made to determine the extent of nutritional knowledge of these families. Breakdowns were made by income level, but the results were quite confusing and indicate, perhaps, some nutritional biases or knowledge of the person doing the interview. Apparently, however, these people know that something with more protein is good.

Several questions were aimed at the marketing patterns and as to whether these people would pay more for a protein fortified atta or chapati. The results were not clear-cut, however, the question, "Would it be good for chapatis to have more protein?" received more yes answers (82%) than any other question asked. No outstanding point of nutritional intervention in the marketing chain became evident. Perhaps the most likely point of initial entry would be fortification of the atta sold through the government ration shops.

b. Economic evaluations

In November of 1972, Dr. O. J. Scoville, Department of Economics, Kansas State University, prepared a preliminary economic evaluation of protein enrichment of cereal foods in Pakistan. The following paragraphs are taken from the summary

and conclusions of that report.

"A review of available information indicates some inadequacy in quality of the diet of the average Pakistani, particularly with respect to protein, vitamins and minerals, and serious nutritional problems for the most vulnerable sectors of the population, including children, pregnant and lactating mothers, and low-income families.

The Kansas State University Project in Pakistan has been concerned primarily with developing inexpensive means of enriching cereal foods that are important elements in the diets of nearly all Pakistanis and of encouraging the consumption of these foods. Primary attention has been given to the enrichment of atta for use in chapatis and nan. Research emphasis has been on addition of soy flour and chickpea flour to atta.

A high proportion of the food eaten by Pakistanis is from cereals, particularly among low-income families.

According to the Nutrition Survey (1965), ninety percent of Pakistani mothers breast-feed their infants for at least 18 months. Children are introduced to solid cereal food at an early age. The most common solid foods are, in descdending order; flat breads (roti) including chapatis and nan; rice, and biscuits.

Wheat is the predominate cereal produced and eaten in Pakistan. Most wheat is milled into atta (a high-extraction flour) which is used for the flat breads, especially chapatis and nan. These are made from atta, water, and a little salt. Some yeast is added to nan.

About 1 million tons of atta are marketed annually through government-licensed ration shops at a subsidized price. These

shops are mainly in cities. Ration shop atta is said to be variable in quality and to be purchased mostly by low-income families.

The principal indigenous sources of protein supplements in Pakistan are the pulses, especially chickpea (channa). With improved technology, potential sources would also include fish flour, cottonseed flour and peanut flour. Potential imported protein sources include defatted soy flour, non-fat dry milk, fish flour, and the two blends now being used in Pakistan -- wheat-soy blend (WSB) and corn-soy-milk (CSM).

With late 1972 prices, the least expensive sources of protein for addition to atta appear to be chickpea flour, and defatted soy flour. Imported fish flour and non-fat dry milk would be considerably more expensive. Cottonseed flour and peanut flour are not considered because of problems of acceptability.

According to consumer acceptability tests, satisfactory chapatis can be made with atta that has been enriched with up to 10% defatted soy flour or 15% chickpea flour. Atta is reported to have about 10.7% protein content. Addition of soy flour to 10% would increase protein content to 14.8%. The addition of 15% chickpea flour would raise protein content to 12.1%, a level which could be slightly exceeded with only 4% soy flour. With atta at Rs. 19 per maund (82.28 lbs.), the ingredient costs for flour containing 10% soy flour would be Rs. 32.1. Costs for a 15% chickpea-atta flour would be Rs. 24.0 and of 4% soy-atta flour, Rs. 24.2; about 26% more than straight atta. It is estimated that government subsidies on wheat per maund are about equal to the ration shop price of atta. If atta is valued

at its cost to the government, the addition of protein supplements involves a considerably smaller percentage increase in cost.

If the average Pakistani consumes .84 pound of wheat a day, and if atta comprises 90% of this, the enrichment of atta with 15% chickpea flour would increase food costs by Rs. 0.045 per day (=0.41 U. S. cents). Added costs from 4% soy-flour enrichment would be Rs. 0.054 per day (=0.49 U. S. cents). On an annual basis, cost per person for 15% chickpea enrichment would be $1.50 (U. S.) and for 4% soy flour enrichment $1.79. Applied to the approximately 1 million tons of atta distributed through ration shops, the annual costs would be $9.8 million for the chickpea program, $9.3 million for the soy-flour program.

Of the wheat supply of about 7.5 million tons a year, about 60% does not come to market. Of the marketed portion, about 1 million tons are procured by the government for sale at fixed prices in ration shops. The ration shop atta is ground in roller flour mills.

The most feasible channel for adding high-protein flours and other ingredients to atta would be at the roller mills, which are said to have adequate equipment to permit blending of high-protein flours to wheat atta. The addition of very small quantities of vitamins and minerals would depend upon acquisition of suitable metering and blending equipment. Capital and operating costs for this equipment would be nominal per cwt. of flour.

Addition of protein flours at village mills would be difficult because of the crude nature of the mills and the low technical level of the management.

Addition of protein supplements in the homes would be a possibility, but would require a great deal of consumer education. It is doubtful if needy members of the population could pay the added cost. The traditional within-household pattern of food distribution also might interfere with allocation of the enriched food to the family members most in need of it.

The most efficient strategy for improving the average diets of all low-income persons in the country would be to add a protein supplement at the roller mills to all the atta sold through ration shops.

If it were decided to concentrate a food improvement program on "target" sectors of the population, the most promising channels would appear to be through the Maternal and Child-Health Centers, and possibly through the handicraft centers.

Improvement of protein content through increased supply of existing high-protein foods or creation of new foods involves increased problems of education, preparation and logistics, but there might be advantages in preparing foods with special appeal to the "target" sectors of the population. People in the higher-income sectors might be more willing to pay for the improved quality of nutrition if it were offered as a separate food and not merely as a common food that had been enriched. But, low-income people, to whom the program should be primarily directed, probably could pay little for enriched food. With them, it

might be easier to recoup at least part of the added cost by having the protein enrichment included in a staple food that had low-income or price elasticity.

Further work, looking toward development of nutrition programs for Pakistan and similar countries, should include development of models for the rapid and accurate projection of least cost diets for the population or for specified sectors of it. But, the construction of models, and the further planning of a nutrition strategy call for better information. Specifically, there is need for:

1. Information on food purchases, food preparation, and consumption in low-income families.
2. Study of local marketing and processing channels for wheat and pulses.
3. More knowledge of the functioning and location of ration shops.
4. Better information on the roller mills and the quality of atta produced.
5. Study of the UNICEF and CARE experiences with food distribution.
6. Study of the handicraft centers as potential sites for food programs."

c. Expand use of high protein bread technology to other countries

With the advent of purchase of soy fortified flour under P.L. 480 Title II, breads or buns for school lunch programs are being used in India, Philippines, and Colombia and cookies in Sri Lanka that are based upon the Kansas State University technology. Many other countries including Ecuador, Bolivia,

Nicaragua, Honduras, Guatemala, Jamaica, Peru, and Indonesia have large scale usage trials underway. Commercial evaluations are also underway in Japan, Mexico, Taiwan, and Venezuela.

An effort was initiated to systematically evaluate the commercial potential of soy fortified bread products in countries where A.I.D. has an operating field mission.

d. Expand studies to cookies and other traditional cereal based foods

High Protein Cookies

Fortifying wheat flour with soy flours and soy proteins exerts a marked effect on cookies' width (spread) and thickness (height). As shown in the present study, the spread of cookies reduced, while the thickness increased progressively with increasing fortification-levels of soy flours and proteins.

The protein content of cookies could be readily raised by 60 and 100% with the fortification of 24% soy flour and soy protein isolate. The flavor and texture of such fortified cookies were rated acceptable or nearly acceptable as compared with the control cookies made from wheat flour in the preliminary organoleptic tests.

The cookies' size also varied with the fractions, with different particle sizes, separated from the same soy protein concentrate. Both the finest and coarsest fractions gave small cookies.

The addition of non-fat dry milk, a normal practice for cookie making, reduced the spread ratio of cookies with or without the soy fortification. Preliminary organoleptic tests did not reveal any significant difference in cookies' flavor and texture between cookies with and without non-fat dry milk.

The present results suggest that soy flour and protein are suitable for use as a fortifier to raise the protein content and to balance amino acids in cookies so as to improve the nutritive value of cookies. Soy products can also be used to substitute non-fat dry milk so as to save the production cost and to simplify the cookie-processing.

High-protein noodle and macaroni products

Paste products represent a low cost source of attractive, nutritious food with wide adaptability and popularity. In fact, the use of wheat in the form of pasta products is considerably more widespread in the world today than is its use in breadmaking. Thus, it was felt desirable to increase the nutritional value of these products.

The first project undertaken was to explore whether soy flours can be used as fortifiers for making high-protein noodles. A systematic study was made to examine the effects of soy flour and surfactants on wheat flour suspensions during a heating-holding-cooling cycle with amylograph. Cooking qualities of soy fortified noodles are at present being evaluated. Organoleptic tests will be conducted to examine the eating quality of the finished product.

Soy fortified baby foods

A project has been taken to explore whether soy fortified flour can be used to prepare a low cost baby food (gruel type) for poverty areas in this country and particularly, in developing countries where diets are deficient in both protein and calories. Preliminary organoleptic tests show that the beany flavor of the gruel containing soy fortified flour increases

with the increasing fortifying levels from 12-36%. However, a slight beany flavor at 12% defatted soy flour or 16% full-fat soy flour was noted. This may not be undesirable. Extruded soy products (Brady samples) produce less beany flavor than full-fat or defatted soyflour.

Further work is required to substantiate the above findings and to conduct feeding tests on soy-fortified baby foods, so as to ascertain their nutritional value.

e. Evaluate additional protein resource materials.

<u>High-protein breads from extrusion-cooked soy products</u>

Soy products, particularly, extrusion-cooked by Brady Crop cooker, were suggested by Crowley to have potential value as protein-fortifiers in breads and infant and weaning foods. Baking tests have been conducted to evaluate whether such products could be used as ingredients to produce high-protein bread. The following conclusions are tentatively drawn from the baking study:

1. High-protein breads with an acceptable loaf-volume could be made from wheat flour (a blend of HRW and HRS: Protein 12.4%) and fortified with soy products up to 20% with the addition of SSL. However, most of the loaves showed an open grain.
2. Dehulled soybean products, in general, gave a larger loaf volume and better grain score than whole soybean products.
3. The baking quality of the soy product cooked at lower temperatures was better than that cooked at higher temperatures (W 260 W 288; D 240 D 283).
4. Unless the cooked soy product was ground, the large particles of soy products caused dark brown and yellow

spots in the bread crust and crumb, respectively. The spots were eliminated by grinding the cooked products to pass a 36 mesh.

In view of the promising baking results, additional studies have been in progress to examine the baking performance of soy products (extruded whole and dehulled soybean) processed at a wide range of temperatures (W-235°, 260°, and 300°F; D-223°, 244°, and 300°F).

Feeding tests on the finished breads (D-300°F and W-300°F) have been completed by Dr. Fryer et al.

Corn germ bread

Corn is a major crop in the United States and many Latin American and other countries. Since corn germ contains a rather high protein content (22.4%), it seemed desirable to explore whether high-protein bread could be produced from wheat flour fortified with corn germ flour. In cooperation with Dr. Inglett, NRL, USDA, a project on corn-germ bread was undertaken. Results showed acceptable bread was prepared from wheat flour fortified with 12% corn germ flour (defatted) with the addition of 0.5% SSL, ethoxylated monoglycerides, or sucrose tallowate. Various processing conditions were studied.

Utilization of triticale

Triticale, a synthetic cereal created through a genomic combination of wheat and rye, may become an important new cereal grain for food and feed uses. This attracts interest in exploring the potential uses of triticale through breeding and utilization studies. The International Development Research Center (IDRC) has recently granted almost four million dollars to CIMMYT, The University of Manitoba, and the Agricultural

Research Institute of Ethiopia, to expand their research mainly on the triticale breeding program. As shown in the recent report from CIMMYT, certain triticale lines have been found to be of high fertility and nutritionally superior to wheat. Certain triticale lines will grow on some soil in Latin America which will not sustain wheat.

We, on the other hand, have been directing our research effort to study the utilization of triticale.

Milling Properties of Triticales

Several triticales with different protein contents were milled in a Miag Multomat mill in the Department. Milling properties of triticales were examined. The work was completed.

Breadmaking Quality of Triticale Flours

The farinograph properties and baking performance of three triticale flours of different protein content were studied. The triticale dough developed readily with a low stability toward mixing, indicating deficient gluten quantity and quality. Prolonged fermentation deleteriously affected triticale dough. It could readily rupture the weak dough structure and also reduce reactants for the browning reaction during baking. Accordingly, the finished triticale bread had a broken top and pale crust. By eliminating bulk fermentation and adding 0.5% sodium stearoyl-2-lactylate, 0.25% sucrose tallowate, or 0.25% ethoxylated monoglycerides, acceptable bread was made from triticale flour (13.6% protein) without supplementing wheat flour. However, the low protein (11.1%) triticale flour required wheat flour supplements to produce acceptable bread. Triticale bread staled almost twice as rapidly as wheat bread.

Cookies Prepared from Triticale Flours

In order to produce acceptable triticale cookies, various processing factors such as different triticales, shortening levels, and emulsifiers have been studied.

Organoleptic tests have been undertaken to compare the eating qualities of triticale cookies with those of wheat cookies.

Nutritional and Biochemical Studies on Triticale

Amino acid compositions of triticale and wheat flours were analyzed. Results show that triticale flours contain more lysine and methionine than wheat flour, indicating that the essential amino acid profile is more nutritionally balanced for triticale than wheat.

Our baking studies have indicated that triticale flour possesses higher enzymatic activity than does wheat flour. A study was then made to determine the proteolytic activity of triticale flours, including wheat and rye flours. The results indicated that the proteolytic activity of the triticale flour was greater than that of wheat flour and close to that of rye flour.

The deterioration (weakening) of triticale dough structure during prolonged fermentation, observed in the baking study, is likely due to the high proteolytic activity of triticale.

Sunflower flour

Sunflower seeds (*Helianthus annus*) were obtained from Kansas, North and South Dakota, and from Wisconsin. These seeds were dehulled, flaked and defatted. Proximate analysis of the defatted sunflower seeds showed protein content of 50% and

the amino acid profile revealed lysine at 3.780, methionine at 2.301 and half-cystine at 2.810%. The oil content of these sunflower seeds averaged 40%. Product development from the sunflower seed will include textured protein and protein isolate for blending.

The growing of sunflower seeds is the coming agricultural crop in the United States since farmers are allowed to grow sunflowers on soil bank land for which they obtain seven dollars per acre. The plump black sunflower seeds obtained from Morocco did not grow well in Kansas this year. However, it may be possible to cross this seed with another variety to obtain improved seed suitable for Kansas or other states in the U. S.

Rapeseed

The work with rapeseed as a source of protein for human consumption is continuing. An acceptable clear light tan flour was made from the processed rapeseeds, but the problem of toxin removal during processing still plagues us.

It was found that the toxins were higher in the germ than in the hull. Present rapeseed processing plants do not dehull before processing which results in rapeseed meal high in fiber.

Feeding studies with mice suggest toxins, in addition to the sulphur compounds, may have harmful effect on animal growth and development. It was found that the bleach process (thesis by Earl Weak) would break down allyl and butyl isothiocyanates. The economics of this process has not been checked or compared. The resultant flour from this process has not been checked for acceptability or tested in animal feeding. Chemical tests

indicate toxin breakdown, removal and shift. Product identification, by gas liquid chromatography, of resultants from toxic breakdown are under careful study.

f. Limited studies on processing of protein concentrates from legumes

The processing technology of chickpeas and horsebeans has been successful. These legumes were dehulled, milled, defatted and air classified to obtain five fractions, two of which were in the upper protein ranges 35 to 50%. The extracted process produced isolate with protein contents from 70 to 90%. A blend of the high protein fractions has been successfully converted into textured vegetable protein. The animal feeding trials have not been completed.

Acceptable fermented foods have been made from chickpeas and horsebeans. Soy and rice fermented products were used as comparative standards. The protein quantity and quality of these legumes was improved by the fermentation process. Comparative animal studies have not been completed.

g. Limited studies on nutrient availability

Many potential food sources require extensive processing which often may have a deleterious effect on the nutritive value of the protein (Krehl and Barboriak, 1960). Reactions of carbohydrates with amino acids are an example.

Other processes may improve the nutritive value by inactivating digestive enzymes inhibitors. Procedures based on the amino acid composition using acid hydrolysis may not reveal these changes in the nutritive value induced by processing. Therefore, the nutritional quality of a protein in final analysis has been tested with feeding trials. There would be many advantages in

evaluating protein quality by *in vitro* techniques to predict the efficiency of utilization of protein.

Rate of release of essential amino acids using digestive enzymes was estimated by modification of the *in vitro* digestion procedure described by Akeson and Stahman (1964). Bread samples used in rat growth studies were subjected to enzymatic hydrolysis to estimate biological value.

Preliminary trials were conducted to establish optimum conditions and steps for *in vitro* enzymatic hydrolysis of food protein samples to get reproducibility and accuracy in results.

PREPARATION OF SAMPLES

All samples were finely ground and dried over P_2O_4 at room temperature to equalize moisture content. One set of samples were defatted. Both defatted and undefatted samples were used in preliminary trials. Protein content of the samples was determined by the micro-Kjeldahl procedure (AOAC) using a conversion factor of 6.25.

PREPARATION OF PROTEIN DIGEST MIXTURE

Samples containing 100 mg of protein were weighed and incubated for ten minutes in water bath at 37°C prior to addition of enzymes. To this 1.5 mg of pepsin in 15 ml of 0.1N HCl was added and incubated at 37°C for three hours. At the end of this period, 7.5 ml of 0.2N NaOH was added to neutralize the contents. Following neutralization 4 mg of pancreatin in 7.5 ml of pH 8.0 phosphate buffer was added.

This digestion mixture was incubated for an additional 24 hours at 37°C. An enzyme blank was prepared by incubating under the described conditions with protein samples omitted.

Fifty ppm methiolate was added to each digestion mixture to prevent growth of micro-organisms and did not interfere with the digestion. At the end of the digestion period, the samples were centrifuged for 20 minutes at 10,000 rpm at $4^{o}C$. Supernatants were collected in a separate flask.

DEPROTEINIZATION

Eighty milligrams of sulphosalicylic acid was added to each 10 ml of digestion mixture. This mixture was frozen and stored for 24 hours. After 24 hours, frozen samples were centrifuged for 30 minutes at 15,000 rpm at $4^{o}C$. Supernatants were separated and dried using freeze drying equipment. The dry samples were dissolved and diluted to 10 ml with pH 2.2 citrate buffer and stored at -20° till used for amino acid samples.

AMINO ACID ANALYSIS

Amino acid analysis of the samples was conducted using ion exchanger chromotography as described by Moore et al. (1958) with a Beckman Model 120B amino acid autoanalyzer. Basic amino acids were separated on a 10 cm column using pH 5.28 buffer and acidic and neutral amino acids were separated on a 159 cm column using pH 3.25 buffer followed by pH 4.25 buffer after one hour 40 minutes from zero time.

The total amino acid content of the bread samples and of standard protein (whole egg) was determined using acid hydrolyzates.

The amino acid peaks of enzyme digests were identified. The peaks were integrated as described by Akeson and Stahman (1964). The pepsin-pancreatin digest index was used to estimate biological values of test proteins.

RESULTS

The pepsin pancreatin amino acid index values calculated are shown in Table I. The amino acids used in all calculations include lysine, histidine, threonine, cystine plus methionine, valine, isoleucine, leucine, and tyrosin plus phenylalanine. These were the amino acids classified by Rose (23) as essential for growing rats. Tryptophan, partially destroyed during the sulphosalieylic acid procedure, was not determined in the enzyme hydrolyzates. It was also destroyed by acid hydrolysis and was therefore not used in the calculation.

Excellent resolution of the amino acids was obtained with the pepsin-pancreatin digest with the exception of cystine which was not observed in most samples tested. Cystine when present appeared in the same place as cystine of acid hydrolysates. It was also noted that threonine did not separate completely from serine in most of the digests. Integration of amino acids peak was done as described by Akeson and Stahman (1964).

The pepsin-pancreatin digest index was calculated in essentially the same manner as the "pepsin digest residue amino index" described by Sheffner et al. The amino acid concentrations were expressed as grams of amino acid per 100 grams of protein.

The eight amino acids essential to human nutrition plus cystine and tyrosine were used. The concentration of these amino acids in whole egg and experimental bread are determined in the complete hydrolyzed proteins and in pepsin-pancreatin digests. The ratio of the percentage of each amino acid in the enzyme digest of bread to the percent of that amino acid in the enzyme digest of whole egg gives the "egg ratio." A similar calculation is

made for the residue fraction.

The geometric mean of the adjusted egg ratios was then computed logarithmically by averaging logarithms of the egg ratio, and obtaining anti-logarithms. Egg ratios less than one were considered as one in order to avoid negative logarithms. In computing egg ratios, percentage concentrations of amino acids in excess of those present in standard protein were disregarded. The geometric mean of the fractions are each multiplied by a factor to correct further the degree of proteolysis of the test protein relative to that of standard egg protein. This gives a corrected geometric mean.

The percentage of total essential amino acids released from test breads by enzymatic digestion are shown in Table I.

Pepsin-pancreatin amino acids index values given in Table I show higher index values for bread supplemented with soy flour protein than white flour bread, bread containing 14% NFDMS and Blend A flour bread. Similarly higher estimated biological values, 67.152, 67.60, 66.261, were obtained for breads containing 10.5% soy flour, 10.5% soy flour + 0.5% SSL and 12% soy flour + 0.5% SSL, respectively, compared to 56.51, 56.26, and 65.5% for breads made of white flour, Blend A flour and white flour + 0.3% lysine.

Data of the pepsin-pancreatin digest amino acid index and PER values calculated for rat growth and the biological value of bread proteins are also given in Table I. Table I also includes total lysine content of tested bread samples as analyzed by acid hydrolyzates. Also given is available lysine as determined by reacting the C-amino group with TNBS reagent.

TABLE I

	Pepsin-Pancreatin[a] Digest Index	Estimated[b] Biological Value	PER[c] Rat Growth	Total[d] Lysine Content %	Lysine[e] Released by Enzymatic Digestion %	Available[f] Lysine by TNBS %
Regular white bread	51.99	56.51	1.428	1.715		75.3
10.5% soy flour	61.78	67.152	2.328	3.070		84.1
10.5% soy flour + 0.5% SSL	62.2	67.60	2.171	3.152		81.4
12% soy flour + 0.5% SSL	60.96	66.261	2.185	3.228		80.3
14% NFDMS	56.12	61.00	1.867	3.053		72.2
White flour + 0.3% Lysine	60.2	65.50	2.461	3.296		86.7
Blend A flour + 0.5% SSL	51.76	56.26	1.25	2.019		76.90

[a]Pepsin-pancreatin digest index

[b]Estimated biological valine; P-P digest index/digestibility

[c]PER; calculated by rat growth study uncorrected to Std. Casein

[d]Total lysine; analysis of acid hydrolysis

[e]Lysine: g/100gms essential amino acid released by enzymatic hydrolysis

[f]Available lysine; determined by Kokade and Linear 1968, procedure using TNBS acid determining -TNBS lysine

It will be seen that breads having higher pepsin-pancreatin amino acids and estimated biological values also show higher PER values calculated by weight gain during the four-week rat growth study.

Data also show soy flour breads not only containing higher levels of the essential amino acid lysine, but also higher levels of this amino acid released by enzymatic action which would be available to animals. The chemical assay of available lysine results also agree with these results.

Therefore, the estimated biological values obtained by dividing the pepsin-pancreatin digest index by digestibility are closely correlated with biological values.

At this time no further work is planned in this area.

3. Research Design

The basic design of the research project is as appropriate at this time as when the project was initiated. Emphasis has shifted generally only as stated in Section C. Research findings and relevant economic and political factors have dictated the emphasis given to the specific objectives for 1973.

E. Dissemination and Utilization of Research Results

Interest in the results of this project have been made to quickly disseminate project findings through traditional technical and trade publications, participation in technical meetings, conferences and workshops, correspondence and material exchange, and a large number of visitors and trainees.

Technical Meetings at Which Papers were Presented in 1972

American Association of Cereal Chemists, Regional Meeting, Minneapolis, Minnesota

Cookie and Cracker Meeting, Columbus, Georgia

Composite Flour Symposium, Bogota, Colombia

American Association of Cereal Chemists, National Meeting, Miami, Florida

American Soybean Association, Columbus, Ohio

International Nutrition Conference, Mexico City, Mexico

Protein Conference, Southern Regional Research Lab, New Orleans, Louisiana

Participated in Workshops

Fortified bread workshop with Kansas School Lunch Services and the Kansas Wheat Commission, Hutchinson, Kansas

Workshops and seminars on fortified bread, Mexico City, Mexico

Workshops and seminars on fortified bread, Japan

Workshops and seminars on fortified bread, Taiwan

Correspondence and Material Exchange

A large volume of correspondence with workers in the field from all areas of the world has been established and continues. Samples of all products have been furnished upon request and samples of high protein materials have been tested when submitted to Kansas State University.

Visitors

Many workers from overseas have visited our laboratories and reviewed and discussed results.

Publications

High-protein soy bread. Special report -- Japanese Institute of Baking (1972) pamphlet.

High-protein wheat foods. Taiwan Wheat Products Promotion Council (1972) booklet.

Development of high-protein resources. Food Industries 4:3-5, 1972.

Recommendations on improving nutrition of staple food in Taiwan. Food Industries 4:6-8, 1972.

Productos de panification con alto valor nutrition a base de soya. Soyanoticals Avg., 1972, Mexico.

Proceso para mejorar el valor protenico del bolillo. Mexican National Council of Science & Technology. 1972 monograph.

The use of emulsifiers for producing protein fortified bread. PAG 11 (3) 42-50, 1972.

Protein malnutrition, a serious problem for people whose diets consist mainly of cereal or starchy products, has aroused interest in fortifying commonly-consumed foods with protein. Because bread is a staple in many countries, it is an ideal food to fortify with protein. Of all protein-rich additives now available, soy flour is the most attractive in price and quality. Soy flour (defatted) is a valuable additive for bread, not only because of soy flour's high protein, but also for its 3.2 per cent to 3.8 per cent lysine. For example, adding 12 parts soy flour to 100 parts wheat flour could increase total protein content about 30 per cent, depending on the protein content of the wheat flour used. Lysine is the first limiting amino acid in wheat flour. The lysine content of such fortified bread would be about twice that of white (wheat) bread. Thus, the soy-fortified bread can greatly improve human diets nutritionally.

F. Statement of Expenditures and Obligations and Contractor Resources

AID/csd - 1586

	Firm Budget 4-1-72 to 3-31-73	Actual Expenditures 4-1-72 to 3-31-73
Salaries	$110,330	$107,094
Equipment & Books	8,000	5,556
Supplies	2,773	5,234
Travel & Transportation	5,632	5,931
Other	1,736	3,583
Overhead	49,317	47,871
	$177,788	$175,269

No significant problems or changes in project management were encountered during the year due to budget restrictions.

G. Work Plan and Budget Forecast for Coming Year

Objectives for 1973

1. Expand use of high protein bread technology to other LDC's through close cooperation with A.I.D. Project Manager.
2. Select target countries for initiation of programs leading to implementation of commercial fortified bread products.
3. Conduct economic evaluations or cost-benefit studies of fortified cereal-based foods in selected countries.
4. Continue application of fortification technology to cookies and other traditional cereal based foods.
5. Continue evaluation of additional protein resource materials and protein concentrates from legumes.

Budget for Period 4-1-73 to 11-30-73

Salaries	$55,299
Consultants	1,000
Equipment & Books	8,000
Supplies	3,000
Travel & Transportation	6,112
Other Direct Costs	1,870
Overhead	24,719
	$100,000