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Improving the Nutritive Value of Cereal Based Foods

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IMPROVING THE NUTRITIVE VALUE OF CEREAL BASED FOODS

Progress Report No. 3

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Improving the Nutritive Value of Cereal Based Foods
Agency for International Development
Contract No. AID/csd-1586
Kansas State University

SUMMARY

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

Accomplishments during 1970 -- Following completion of the survey phase in 1968 and while continuing the experimental screening program begun in 1969, a focusing on the use of three protein sources, i.e., soy flour, chick pea flour, and horse bean flour to fortify the subject food constituted the major portion of the research effort in 1970.

Much emphasis was given during the report period to the nutritional and organoleptic evaluations of chapatis, cous-cous, and Moroccan bread fortified with the high protein flours which the effect of the levels of fortification, process treatment of the protein material, or process treatment of the finished food were studied as variables. This experimental work was designed to pinpoint "best choice" formulations and processes for trial in the field testing phase of the project which is to follow.

Contacts were established in Morocco during the year to test fortified bread in the school lunch program and to test fortified cous-cous by working in the commercial sector with cous-cous manufacturers.

EFFECT OF LEVEL OF AMINO ACID-SUPPLEMENTED PROTEIN ON LYSINE REQUIREMENT

Janet Seymour, D. B. Parrish and C. W. Deyoe

In designing or evaluating diets for animals, and even for man, requirements for essential amino acids are taken frequently from publications such as those of the National Research Council (1963) (1), regardless of total protein content or other characteristics of the diet. Often it is unrealistic or impossible to have diets of high-protein content. In the underdeveloped countries, where protein malnutrition is a severe problem, reason suggests use of minimal satisfactory levels of required nutrients, since even those are difficult to meet. The initial question prompting this study was, is it necessary to use 0.9% lysine, if less than 20% protein is in diets of growing rats.

Although considerable has been written on the subject, the evidence from the literature is not conclusive whether 1) the requirements for essential amino acids may be less than generally accepted, 2) all required amino acids must be in balance in practical diets, and 3) the requirements for essential amino acids increase proportionally to protein content (Almquist, 1970; Jansen, 1962; Young and Zomora, 1968; Rao et al., 1959; Chang and Chao, 1969; Munaver and Harper, 1959; Bressani and Mertz, 1958; Rosenberg, 1959; and Bressani, et al., 1960) (2, 3, 4, 5, 6, 7, 8, 9, and 10).

This report considers the foregoing problems. Specifically it is on a study of the lysine requirement of growing rats fed diets based on wheat in which the other deficient essential amino acids were supplied so that lysine may be assumed to be the limiting factor. The amino acid contents of wheat flour and wheat gluten used in the diets were determined, and the required amino acids, except lysine, were added at 50% of the NRC requirements in the 7%- protein diet, at 80% in the 10%- and 13%- protein diets, and at 100% in the 15%- protein diet. It was necessary to limit the added amino acids to 50% of

requirements in the 7%- protein diet to keep supplemental amino acids from being a significant portion of the protein component.

The diets were composed of 95% extraction Gaines wheat flour, wheat gluten to adjust protein levels, cottonseed oil, and cornstarch to adjust protein and amino acid contents of the diets. Adequate vitamins and minerals were added (Table 1). The freshly prepared flour and mixed diets were kept in a freezer to assure freshness. Weanling male albino rats, 6 per group, were fed the diets individually for 5 weeks. Measurements made were weight gain, protein efficiency ratio (PER) and dry, fat-free tibia weight.

Figure 1 shows results when diets of 10% protein content were supplemented with lysine $^{\bullet}$HCl to make the total content in the diet 0.3 to 0.78%. The weight gains are plotted on a logarithmic scale. From this figure, the lysine requirement is approximately 0.5%. The result for rats fed 0.54% lysine appears to be out of line, but if taken into account in establishing the curve, it would raise the lysine requirement only to about 0.55%. Statistical treatment of the data at the 5% confidence level shows that gains for 0.62, 0.70 and 0.78% lysine do not differ, and that those gains differ from those represented by the other three values on the curve (Table 2).

The PER data and tibia weights are plotted in a similar fashion to weight gains. These curves and statistical treatment of the data lead to the same general conclusion on lysine requirement as found from weight gains. The PER for the 0.46%- lysine diet is generally considered good. Bone minerals and bone ash have been suggested to evaluate response to lysine supplementation. Bone ash was determined on the dry, fat-free tibias. The data plotted as a single horizontal line, at about 63% ash content. This measurement, therefore, was omitted in later studies. Braham _et al._ (1961) (11) found weights of

TABLE 1. COMPOSITION OF DIETS USED, GRAMS PER 100 GRAMS.

Ingredients	Expt. 1	Expt. 2 diets			Expt. 3 diets				
		7%	10%	13%	10%		15%		20%
					+	-	+	-	
Wheat flour	92.6	72.3	90.0	87.0	88.5	88.5	80.3	80.3	72.3
Wheat gluten			1.5	6.46	1.5	1.5	9.75	9.75	17.8
Added amino acids[a]	.311 (5)	.163 (4)	.513 (7)	.055 (2)	.616 (7)	0	.179 (3)	0	0
Cottonseed oil	2.0	2.0	2.0	2.0	3.0	3.0	3.0	3.0	3.0
$CaHPO_4 \cdot 2H_2O$	2.58	2.62	2.58	2.59	2.59	2.59	2.60	2.60	2.62
K_2SO_4	.246	.304	.252	.261	.257	.257	.282	.282	.304
NaCl	.3	.3	.3	.3	.3	.3	.3	.3	.3
MgO	.34[b]	.041	.034	.037	.05	.05	.05	.05	.05
Trace mineral mix[c]	.025	.025	.025	.025	.025	.025	.025	.025	.025
Vitamin premix[d]	.145	.146	.146	.146	.134	.134	.134	.134	.134
Starch[e]	1.45	22.1	2.65	1.12	3.03	3.65	3.43	3.61	3.57
Protein % in diets	10.3	7.1	10.1	13.1	10.1	9.4	15.0	14.8	20.0

[a]Added amino acids, except lysine. In parenthesis is number added.

[b]High addition from calculation error.

[c]Contained in %, Mn 10, Fe 10, Ca 14, Cu 1, Zn 5, I 0.3, and Co 0.1.

[d]Weight of total vitamin mix. Added NRC requirements, except choline chloride 100 mg, menadione .03 mg, vitamin D, 180 IU, and folic acid, biotin, inositol and ascorbic acid.

[e]Starch to adjust for amino acid and protein contents.

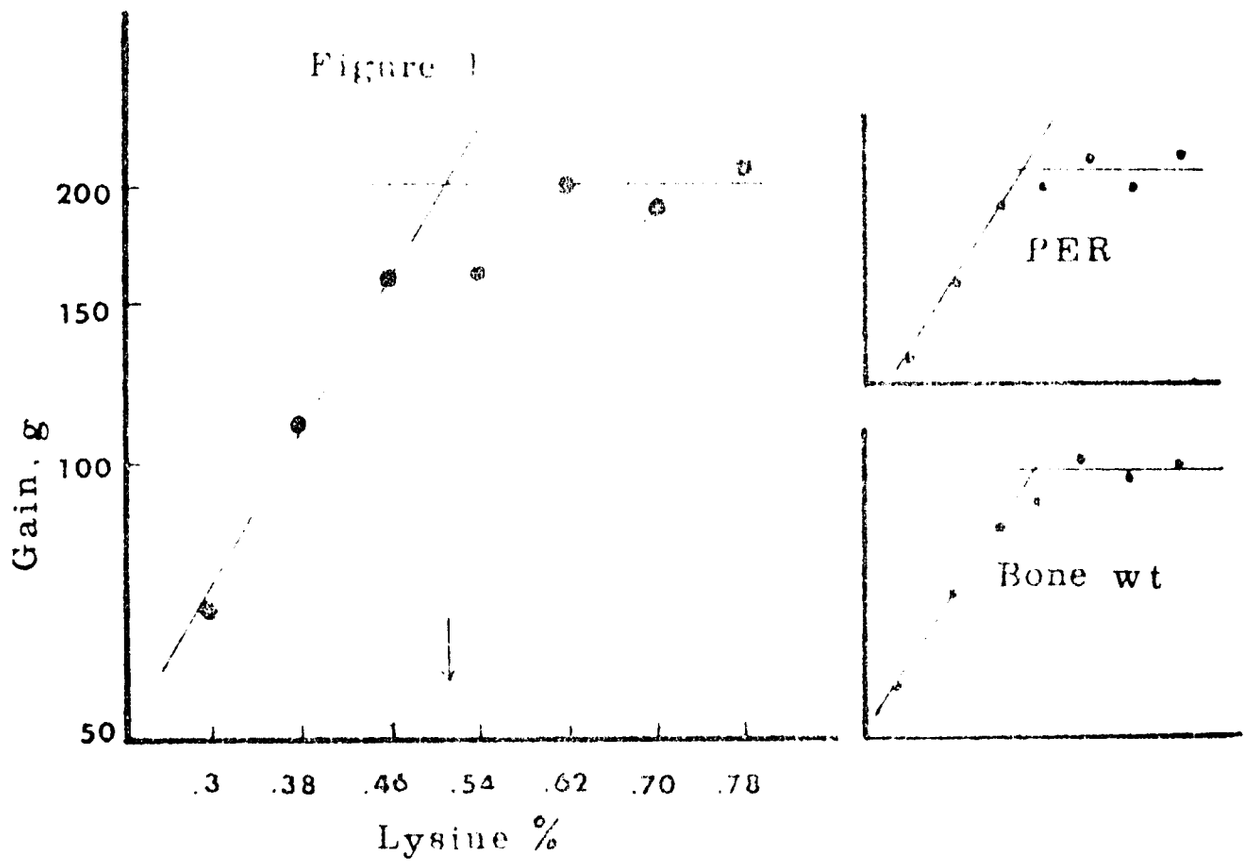
Figure 1
200
150
100
50
Gain, g
.3
.38
.46
.54
.62
.70
.78
Lysine %
PER
Bone wt

TABLE 2. PERFORMANCE OF GROWING RATS FED 10% PROTEIN DIET[a]. Expt. 1.

	% Lysine							LSD, 5%
	.3	.38	.46	.54	.62	.70	.78	
Gain, g	69	110	156	158	197	187	204	26.9
PER	1.74	2.15	2.58	2.71	3.0	2.84	2.91	0.25
Dry, extr. tibia wt, g	.161	.192	.238	.241	.261	.245	.264	0.02

[a]Required amino acids, except lysine, added to 80% NRC requirements for growing rats. Protein plus added amino acids, except lysine, totaled 10.3%.

chicken femurs increased with increasing dietary lysine and that ash contents remained constant.

Figure 2 shows results using 7%-, 10%- and 13%- protein diets containing 4 levels of lysine. In the 7%- protein diets, 0.4% lysine was as satisfactory as higher levels, suggesting that total protein was the growth-limiting factor. The PER values are relatively high, indicating good utilization of the limited amount of protein in the diets.

The graphical presentation of data when 10%- and 13%- protein diets were fed shows that 0.6% lysine gave the best results. Statistically, however, there was no difference among the 0.5%, 0.6% and 0.7% lysine levels, or between the 10%- and 13%- protein diets (Table 3). Plots of the PER and bone weight data, as well as the statistical analysis, lead to essentially the same conclusions as found from weight gains.

Table 4 summarizes data from a study to determine whether more than 10% protein was needed and whether there were advantages to adding required amino acids in addition to lysine. In the diets designated by +, the essential amino acids, except lysine, were added at 80% of NRC requirements in the 10%- protein diets, and at 100% of requirements in the 15%- protein diets. The amino acids were not added to diets designated by -. The star indicates that the only lysine present was that in the flour and gluten of the diets. In the 20%- protein diets, no additions of the amino acids, except lysine, were needed to meet requirements.

In this experiment, rats gained more when fed 15% protein than when fed 10% protein, and the difference in gain on 0.6% and 0.9% lysine was not significant statistically. These data indicate that there was no advantage to adding the required amino acids. The PER values and tibia weight data at each protein level tend to support results from the growth studies.

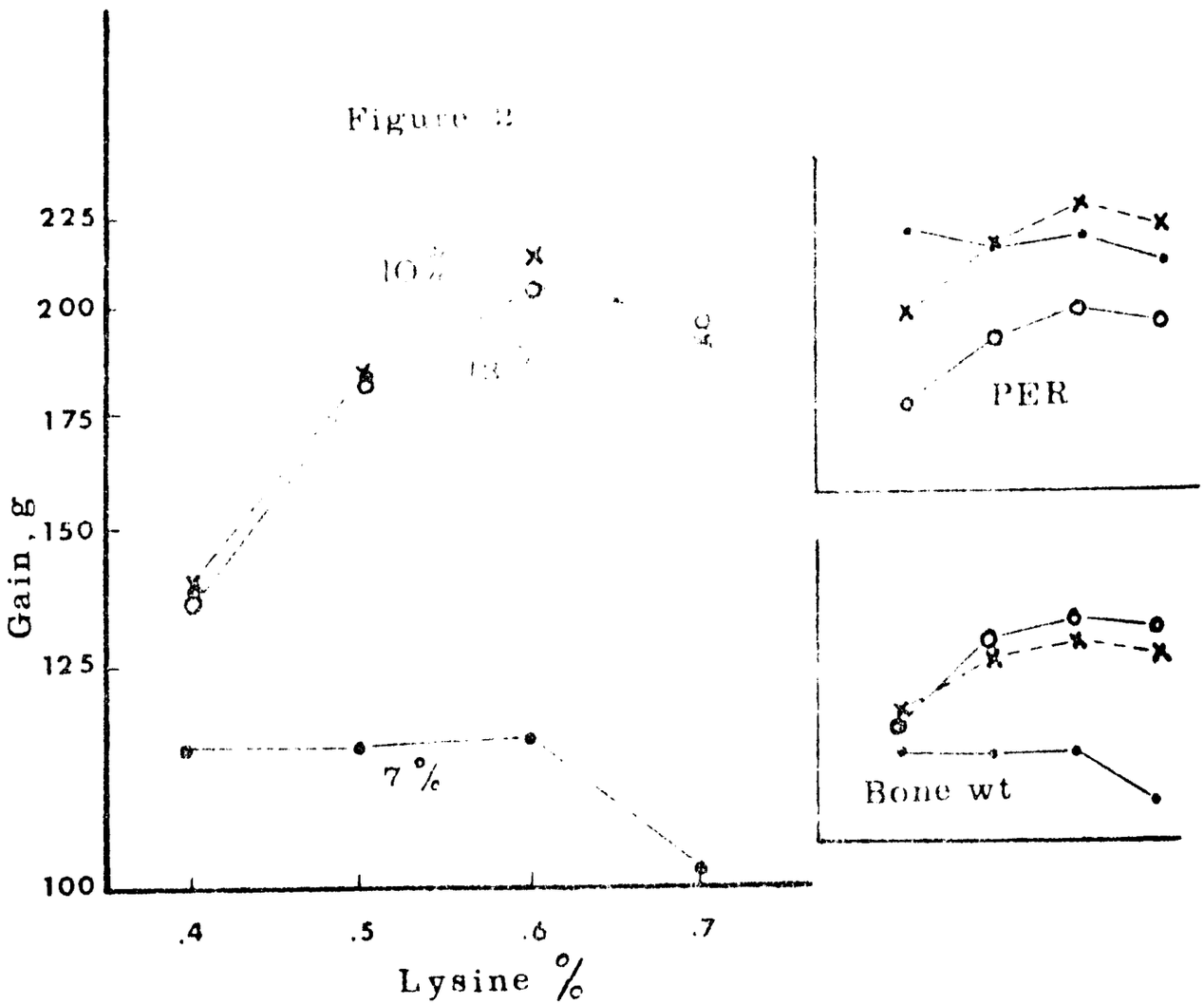
Figure 2
225
200
175
150
125
100
Gain, g
10 %
7 %
.4
.5
.6
.7
Lysine %
PER
Bone wt

TABLE 3. PERFORMANCE OF GROWING RATS FED DIETS CONTAINING DIFFERENT LEVELS OF PROTEIN AND LYSINE[a]. EXPT. 2.

Protein %[b]		% Lysine			
		.4	.5	.6	.7
7	Gain, g	113	114	116	97
	PER	2.95	2.85	2.95	2.74
	Dry, extr. tibia wt, g	.188	.188	.187	.170
10	Gain, g	141	187	217	194
	PER	2.37	2.86	3.17	3.04
	Dry, extr. tibia wt, g	.211	.245	.255	.248
13	Gain, g	139	186	214	199
	PER	1.87	2.23	2.37	2.35
	Dry, extr. tibia wt, g	.205	.253	.270	.265

[a]Required amino acids, except lysine, added to 10% and 13% protein diets to 80% NRC requirements; amino acids added to 7% diet at 50% NRC requirements.

[b]Actual protein levels, including added amino acids, except lysine, were: 7%-7.1%; 10%-10.1%; 13%-13.1%.

TABLE 4. PERFORMANCE OF GROWING RATS FED DIETS CONTAINING DIFFERENT LEVELS PROTEIN AND LYSINE. EXPT. 3.

Protein %[a]		% Lysine						LSD, 5%
		*		0.6		0.9		
		-	+	-	+	-	+	
10	Gain, g	75	73	186	151	177	143	26.4
	PER	1.78	1.90	3.04	2.59	2.91	2.45	0.14
	Dry, extr. tibia wt, g.	.173	.168	.266	.229	.259	.225	0.03
15	Gain, g	102	114	210	224	223	218	26.4
	PER	1.53	1.53	2.28	2.27	2.33	2.33	0.14
	Dry, extr. tibia wt, g	.204	.197	.289	.313	.309	.294	0.03
20	Gain, g	130	-[b]	226	-	250	--	26.4
	PER	1.32		1.77		1.93		0.14
	Dry, extr. tibia wt, g	.247		.325		.337		0.03

*No added lysine. 10% contained 0.28% lysine; 15%, 0.36%; 20%, 0.44%.

-and + indicated whether required amino acids, except lysine added at 80% NRC req. to 10% diet, and 100% to 15% diet.

[a]Actual protein levels including added amino acids, except lysine, were:

10%	15%	20%
- 9.4%	-14.8%	-20%
+10.1%	+15.0%	

[b]No extra amino acids needed to meet NRC req.

Data for the 20%- protein diet indicates that growth was improved, but not significant statistically, when lysine content was raised from 0.6% to 0.9%, and that growth also was improved by increasing the protein content of the diet from 15% to 20%. Although there were no amino acid deficiencies in the 20%- protein diet, except lysine, there may have been some imbalances which might increase the lysine requirement. Leucine was approximately double the NRC requirement, isoleucine was 40% and valine 25% above requirements. This aspect of the study requires further investigation.

Results from this study suggest that the practical lysine requirement for growth of rats fed wheat-based diets supplemented with the other essential amino acids is no more than 0.6%, possibly closer to 0.5%. The need for the other supplemental amino acids might be questioned and needs further study. Rats grew satisfactorily and were in good condition when fed diets containing only 10% protein and adequate lysine.

A projection of these results to growth of man suggests a closer examination of the minimum lysine requirement, especially when it is impractical to use high levels of protein in the diet. As a final note of caution, it should be recognized that these suggestions and conclusions on lysine requirement are based on growth and protein efficiency data only. So little data are available that it is not known whether requirements for lysine, or even total protein, might be higher for mental development, physical endurance, disease resistance and reproduction than for growth.

Further Evaluation of Lysine in Wheat-based Diets. In carrying out Miss Seymour's studies additional questions of interest were raised. One concerns what effect the rather marked imbalance of leucine, isoleucine and valine have on the apparent lysine requirement. The data especially suggested this question in relation to the 15 and 20% protein diets. Two more mature

students, taking an Animal Nutrition Techniques course, have been assigned for their individual animal experiment a small problem each to investigate two aspects of this imbalance question. By using supplemental amino acids the leucine, isoleucine, and valine contents will be brought into a better balanced ratio, and the effect on lysine requirement will be studied. One student will study the 10% protein diet and the other the 20% diet. This work has just commenced since the wheat atta, analyses, and rats are just now available.

Improving the Protein Quality of Millet, Sorghum, and Maize Diets by Supplementation. In 1968, Mrs. S. Pushpamma finished a dissertation on "Protein Quality and Nutritive Value of Three Indian Millets". One of these millets, so called giant millet, is really a sorghum. In this work we studied corn as a standard for comparison.

This work has never been reported at a meeting since Dr. Pushpamma was required to return to India immediately after finishing the work.

A paper based on the results of this work has been submitted for presentation at the Western Hemisphere Nutrition Congress III, August 30 - September 2, 1971. A copy of the abstract of the proposed paper follows.

Improving the Protein Quality of Millet, Sorghum, and Maize Diets by Supplementation

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Department of Biochemistry and Food and Feed Grain Institute, Kansas State University, Manhattan, Kansas 66502

Dietary value of the protein of millets (Bajra and Ragi), sorghum, and maize were studied by amino acid analyses, and by growth and PER of weanling rats fed diets containing the grains, unsupplemented or supplemented with lysine, tryptophan, fish protein concentrate (FPC), non-fat dried milk (DM), brewer's yeast, and isolated soybean protein. Minerals and vitamins were added to all diets. Ragi protein had the highest concentrations of essential amino acids (EAA), except leucine; no amino acids were limiting when compared to the FAO reference pattern. Bajra protein was deficient only in lysine. Sorghum was deficient in lysine, tryptophan and sulfur amino acids. Maize was deficient in lysine, tryptophan, isoleucine and valine. Rankings of both chemical and protein scores were, in decreasing order, Ragi, Bajra, maize, and sorghum. PER correlated better with scores calculated in reference to egg protein than to FAO reference protein. In the unsupplemented diets, Bajra gave the greatest weight gain and PER. PER of Ragi was similar to that of Bajra, but gain was lower due to lower protein content. Supplementing Bajra or Ragi with lysine increased gains and PER equal to or more than corresponding 5 or 10% casein diets. Lysine improved gain and PER of sorghum; tryptophan had a similar effect with maize, but both together caused markedly increased gains with both grains. Protein supplements (added as 3% protein) improved Ragi diets most, but no supplement was uniformly superior with all grains. FPC was most effective with Ragi, DM with maize, and FPC with sorghum. Protein concentrates with small excesses of lysine improved protein quality of Bajra and Ragi considerably because of good distribution of other EAA. Greater use of Bajra and Ragi, with better protein quality than wheat, sorghum, or maize, might help combat general protein malnutrition in many areas.

SOME OBSERVATIONS ON THE LIPIDS AND 70% AQUEOUS ETHANOL-EXTRACTABLE SUBSTANCES FROM CHICK PEA FLOUR

Chick pea (*Cicer arietinum*), also known as Bengal gram and chana, is one of the most important food legumes in India-Pakistan area. The fat content of the seed of chick pea, which is considerably lower than those of soybean and peanuts but higher than those of peas and kidney beans, was reported between 5.0% and 7.1% (Altschul, 1957) (12). When fed to lab animals, a lowering effect on the serum cholesterol level was reported (Mathur *et al.* 1963) (13). The reported fatty acid composition of chick pea fat showed that 82.7 to 85.8% of the total fatty acids were unsaturated, and approximately half of the unsaturated fatty acids was oleic and the other half linoleic. The amount of total saturated fatty acids was reported between 8.9% and 9.5%, over half of it was palmitic acid, with myristic and stearic acids making up the balance (Altschul, 1957) (12).

Systematic study on the extraction of chick pea lipids was made by extracting chick pea flour with petroleum ether, absolute ethanol, water saturated butanol (WSB), and also with petroleum ether after acid hydrolysis. The results are shown in Table 5.

TABLE 5. EXTRACTION OF CHICK PEA LIPIDS BY VARIOUS METHODS.

Extracting Method	Lipids Extracted (% Chick pea flour)	
	As-is moisture basis	Moisture-free basis
Petroleum ether	5.30	5.94
Abs. ethanol extractable/ soluble in ether	4.72	5.29
Petroleum ether extractable after acid hydrolysis	5.60	6.27
WSB	6.14	6.88

Apparently, the water-saturated butanol was the most efficient extracting solvent system among the four tested.

The WSB extracted lipids was further fractionated on silicic acid column (Daftary and Pomeranz, 1965) (14). The amount of non-polar lipids, which were eluted by chloroform, was found to be 72.4% of the total lipids; the polar lipids, which were eluted by methanol, was found to be 24.6%. Qualitative determination of individual components was made by using thin-layer chromatography on silica gel G and solvent systems of different polarity. Positively identified were tri-, di- and mono-glycerides, lecithin, phosphatidyl ethanolamine, phosphotidyl serine and free fatty acids. Triglycerides and lethicin were found to be the predominant components in non-polar and polar lipids, respectively.

Aqueous ethanol was widely used by different workers for the extraction of prolamine, free sugars, free amino acids, polar lipids, saponin, pigments, flavor compounds, etc. In this study, defatted chick pea flour was extracted by 70% aqueous ethanol for five times at a ratio of 10:1 (v/w). The combined extracts were evaporated and adjusted to a definite volume. The total N extracted by the 70% ethanol was found to be 4.85% of the total N in the sample. Brohault and Sandegren (1954) (15) reported that the seeds of dicotyledonous plants in general did not contain the aqueous ethanol extractable protein, i.e., prolamine. Powrie (1961) (16) suggested that the 70% aqueous ethanol extractable nitrogen from kidney beans actually represented the non-protein nitrogenous material in the sample. Disc electrophoresis (Davis, 1964) (17) was used in this study to examine if any proteinaceous material was present in the 70% ethanol extract of chick pea. One faint line, stained by Amido Black, was observed in the gel to which the highest amount of sample was applied. The evidence indicated that very small amounts of protein-like material could

be extracted by 70% aqueous ethanol from the chick pea seed flour, although the most part of the N extracted was non-protein in nature.

Aqueous ethanol has been also widely used in the extraction of low molecular weight carbohydrates from biological materials (Craig, 1949) (18). To examine the free neutral sugars in chick pea extracted by 70% aqueous ethanol, an aliquot of vacuum evaporated extract was passed through a mixed bed Rexyn 300 ion-exchange resin column (size: 3 x 10 cm.), washed, and the eluents was adjusted to volume. The deionized extract was found to have little reducing power by standard AOAC (1962) (19) method, while the results from phenol-sulfuric acid determination (Dubois et al., 1956) (20) indicated the presence of large amounts of carbohydrate in the extract. These observations showed that the most part of the free neutral sugars in chick pea extract were of the non-reducing type. The total 70% ethanol extractable neutral sugar (based on sucrose standard) was found to be 8.36% the weight of defatted chick pea flour (on moisture-free basis).

The individual sugars in the extract were identified by paper chromatography (Hais and Macek, 1963) (21) and thin-layer chromatography on bisulfite impregnated silica gel G (Stahl, 1968) (22). Four major components were found on most of the chromatograms. Three of them were positively identified as sucrose, raffinose and stachyose. The only unidentified major component appeared to be a trisaccharide. Little, if any, detectable amount of monosaccharide was found.

Extraction of the evaporated 70% aqueous ethanol extract with diethyl ether removed the lipids and pigments. After removal of the solvent, an oily liquid was found. Thin-layer chromatography revealed that most of the components in this fraction were the same as those found in total lipids extracted by water saturated butanol, although the relative amount of different components

appeared to be somewhat different. Phospholipids were found in abundance in this fraction. Some new spots were also found on the chromatogram, they are still under study and not yet identified.

The ether insoluble residues of the aqueous ethanol extract contained crystalline as well as amorphous materials. This fraction also had a rather noticeable disagreeable odor, indicating that some flavored substances in the chick pea flour had been extracted and enriched in the process. The present method could be useful in the future study of the flavor aspect of chick pea and its products. The crystalline materials were found to contain a large amount of carbohydrate. Paper chromatography study showed that most of the free sugars were in this fraction. Evidence also indicated that certain glycosides might be also present. Studies on saponins and other glycosides are presently under way.

EFFECT OF PROCESSING ON THE NUTRITIVE VALUE OF CHICK PEAS

Previous literature indicates that cooking, autoclaving, and roasting have been used as different methods of processing in order to improve the nutritive value of some legumes. In the first part of this study, one of these treatments has been elaborated. The chick pea seeds were roasted for 15 minutes at various temperatures and evaluated for protein, amino acid composition and digestibility. The second phase has involved the use of ionizing radiation. The technique employing the use of radiation for breeding purposes has been well exploited, but an important aspect related to radiosensitivity prior to such applications is still at times overlooked by research workers. A proper understanding of this area is the single factor if some measurable response is to be attained. Thus, appropriate initial radiosensitivity estimations lead to awareness that could be made applicable in diverse investigation areas like breeding, food preservation, grain storage, and others.

Stimulation in the first generation following seed irradiation is a view expressed by many workers that may relate to early germination. Stein and Richter (1961) (23), Gunckel and Sparrow (1954) (24), Sax (1963) (25) coupled with better seedling growth in X_1 at low dosages (Mackey, 1956) (26). Similar reports have been made by Skok *et al.* (1965) (27) and Kahan (1969) (28).

Presumably correlated with low dosage X_1 stimulation could be a modification along similar lines of the nutritive status. Such aspects have not been assessed so far. Consequently chick pea seeds were subjected to varied dosages of gamma irradiation after prior LD_{50} estimation so as to evaluate the low dosage stimulatory effect nutritionally.

A. EFFECT OF ROASTING TEMPERATURE

Materials and Methods

Chick pea seeds were heated in a stainless steel pan for 15 minutes at 125°C, 150°C, 175°C, and 200°C in an oven. Fine samples were ground in a mikro-sample mill to pass through a screen size of 0.02 inches. The uniformly milled samples were stored at 4°C in airtight containers. Moisture and protein were determined according to AOAC methods (1965) (29).

The samples were hydrolyzed by the procedure of Waggle et al. (1966) (30) and stored at -20°C until used. Amino acid analyses of the hydrolyzate was conducted following the procedures of Spackman et al. (1958) (31) and Moore et al. (1958) (32) with a Beckman 120 automatic amino acid analyzer.

For enzymatic hydrolysis and digestibility studies, protein digests of the samples were prepared according to the procedure of Villegas et al. (1968) (33) with slight modifications following enzyme inactivation by boiling. After inactivation, the samples were allowed to cool and filtered through a Whatman #4 filter paper. The filtrates were respectively ultrafiltered by the Amicon Ultrafiltration apparatus. A UM-2, 43 m.m. membrane to partition mixtures of solvents above and below 1,000 MW was used. Protein was determined in the ultrafiltrates and the digestibility ratio obtained by

$$\text{Soluble N/Total N} \cdot 100$$

The remainder of the ultrafiltrate was adjusted to pH 2.2 and analyzed for the amino acid composition on the Beckman 120 automatic amino acid analyzer.

Results and Discussion

Enzymatic digestibility decreased significantly with increasing heat treatments from 150°C and higher (Table 6).

TABLE 6. PROTEIN CONTENT, AND DIGESTIBILITY PERCENTAGE OF ROASTED CHICK PEAS

	Treatments					
	Raw	125°C	150°C	175°C	200°C	LSD 0.05
Protein (%)	18.0	18.1	18.6	19.1	19.3	NS
Total N	2.880	2.896	2.976	3.056	3.088	----
Soluble N	0.531	0.467	0.274	0.282	0.213	----
Digestibility (%)	18.43	16.12	9.21	9.21	6.91	3.52

The 125°C roasting treatment has not altered the samples digestibility index, and as evident in Table 7, provides a great majority of slightly higher amino acid values.

Percentages of amino acid requirements satisfied for the standard reference man age 22 (National Research Council, 1963) (34) by samples of the various treatments presented in Table 8 also suggest higher values for all the amino acids in the 125°C roasting treatment.

Corrected data from the amino acid analysis of the ultrafiltrates depicted similar increased trends for 125°C and 150°C. However, the essential total amino acid ratio was higher for 125°C. Recoveries were poor for both and subsequent analyses are presently in progress.

TABLE 7. THE AMINO ACID COMPOSITION OF HEAT TREATED CHICK PEAS AFTER ACID HYDROLYSIS

	Raw	125°C	150°C	175°C	200°C
Lysine	7.134	7.206	7.05[illegible]	6.878	[illegible]
Histidine	2.638	2.516	[illegible].660	2.569	[illegible]
Ammonia	1.447	1.648	[illegible].464	1.625	[illegible]
Arginine	9.185	8.076	8.969	9.165	8.[illegible]7
Aspartic acid	11.867	[illegible]	11.958	11.621	12.142
Threonine	3.789	3.829	3.761	3.741	3.932
Serine	5.138	5.152	5.099	5.074	5.192
Glutamic acid	17.602	17.721	17.418	17.114	17.662
Proline	3.982	4.521	4.297	4.493	4.737
Glycine	4.180	4.266	4.195	4.217	4.335
Alanine	4.036	4.134	4.037	4.355	4.607
Half Cystine	1.810	1.41[illegible]	1.65[illegible]	1.684	5.108
Valine	4.506	4.627	4.633	4.950	5.108
Methionine	1.276	1.336	1.316	1.272	1.163
Isoleucine	4.346	4.372	4.314	4.400	4.484
Leucine	7.880	7.901	7.760	7.828	7.886
Tyrosine	3.346	3.347	3.339	3.178	3.301
Phenylalanine	5.935	5.964	5.919	5.845	5.844

TABLE 8. PERCENTAGES OF AMINO ACID REQUIREMENTS SATISFIED FOR THE STANDARD REFERENCE MAN AGE 22 BY THE RESPECTIVE SAMPLES.

	Treatments				
	Raw	125°C	150°C	175°C	200°C
Lysine	290.00	292.93	288.29	279.59	240.04
Threonine	246.04	248.64	244.22	242.92	255.32
Valine	183.17	188.17	188.33	201.22	207.64
Methionine	37.75	39.82	39.93	37.63	34.41
Isoleucine	202.14	204.28	200.65	204.65	208.56
Leucine	230.18	233.76	229.76	231.60	233.14
Phenylalanine	175.71	177.10	175.12	172.93	172.90

The 125°C roasting treatment, although not differing significantly from the raw chick peas, has provided evidence of having higher amino acid levels, essential : total amino acid ratio, and in better satisfying the requirements for standard reference man (National Research Council, 1963) (34). Higher heat treatments adversely affected the digestibility and manifested these by lower essential : total amino acid ratios.

B. EFFECT OF IONIZING RADIATION

Materials and Methods

Chick pea seeds were treated with gamma irradiation (^{60}Co) dosages of 2.5, 5.0, 7.5, 10.0, 12.5, 15.0, and 20.0 Kiloroentgens for LD_{50} estimation according to Osborne and Lunden's method (1961) (35). Following the LD_{50} determination of 8.5 Kr., seeds were irradiated with dosages of 1, 2, 3, 4, 5, 7.5, and 10 Kr.

Sample preparation, determination of protein, moisture and amino acids were identical to the procedure listed in part (A) of this report. Enzymatic hydrolysis and digestibility studies are in progress.

Results and Discussion

Protein and amino acid compositions of the respective samples are presented in Table 9.

TABLE 9. THE PROTEIN AND AMINO ACID COMPOSITION OF GAMMA RADIATION TREATED SAMPLES AFTER ACID HYDROLYSIS.

		Treatments						
	Raw	1	2	3	4	5	7.5	10
Protein	17.7	17.4	17.6	17.6	17.7	17.7	17.1	17.1
Lysine	7.156	7.213	7.273	7.340	7.411	7.271	7.229	7.230
Histidine	2.640	2.636	2.615	2.766	2.781	2.604	2.675	2.586
Ammonia	1.358	1.407	1.538	1.251	1.383	1.528	1.240	1.463
Arginine	9.220	9.429	8.493	9.253	9.160	8.817	8.684	8.907
Aspartic acid	12.024	12.013	12.241	12.119	11.537	12.187	12.174	12.160
Threonine	3.835	3.850	3.887	3.857	3.893	3.899	3.907	3.945
Serine	5.179	5.126	5.134	5.228	5.243	5.222	5.215	5.289
Glutamic acid	18.089	17.882	18.010	18.063	18.078	17.881	18.057	18.227
Proline	4.146	4.241	4.142	4.186	4.263	4.218	4.517	4.106
Glycine	4.156	4.215	4.226	4.197	4.161	4.287	4.195	4.279
Alanine	3.795	3.804	3.778	3.805	3.741	3.779	3.744	3.809
Valine	4.640	4.544	4.610	4.552	4.497	4.569	4.568	4.439
Isoleucine	4.357	4.386	4.380	4.244	4.260	4.331	4.274	4.261
Leucine	7.837	7.821	7.926	7.846	7.776	7.885	7.851	7.782
Tyrosine	3.152	3.159	3.064	3.081	3.107	3.098	3.148	3.157
Phenylalanine	5.821	5.779	5.908	5.771	5.655	5.822	5.742	5.547

Compared to raw chick peas, slightly higher compositions were apparent for lysine, aspartic, threonine, serine, and glycine in all treatments. Except for valine and glutamic acid, all the amino acids exhibited minutely higher compositions within the range of 1 and 4 Kr.

The greater release of the respective amino acids is presumably due to an irradiation-moisture-oxygen interaction leading to formation of highly oxidative entities like hydroperoxyl radicals and hydrogen peroxide. The

ionizing radiation characteristic of causing further ionizations, and dissipating energy by excitation is very likely to influence processes with enzymatic involvements. The series of reactions leading to the formation of the oxidation entities is outlined by Gordon (1957) (36).

Enzymatic digestibility studies are presently under way. Further studies need to be undertaken after seed moisture manipulations. The enhancement of seed moisture with low gamma dosage application may elucidate the earlier expressed view, as well as provide greater significance.

MICROSCOPIC STUDIES ON RAW AND PROCESSED CHICK PEAS

Some time has been devoted towards establishing a staining procedure for chick pea seeds histological study. Earlier, staining had obscured all cellular components. Using raw chick peas, two techniques have given satisfactory staining and tissue differentiation. 1. Safranin and fast green and 2. Eosin and iodine (protein/starch).

Procedure 1

1. Soak seeds overnight.
2. Fix for 48 hours in FAA (Formalin, acetic acid, Alcohol).
3. Dehydrate in T.B.A. series from 30% to 100%, leaving seeds for two hours in each. Overnight in last series.
4. Infiltration in paraffin. Four changes at 6 to 8 hour intervals.
5. Embedding in paraffin.
6. Sectioning at 15 u with a hand rotary microtome.
7. Float sections onto microslides precoated with a thin film of a gelatin adhesive over which several drops of 3% formaldehyde solution were spread.
8. Warm slide on a plate adjusted to 40°C until slide dries.
9. Staining:
   a. Xylene - ten minutes - two changes.
   b. 70% ethylalcohol - ten minutes.
   c. 95% ethylalcohol - ten minutes.
   d. Safranin - 24 hours.
   e. Absolute alcohol - ten minutes.
   f. Fast green - two seconds.
   g. Absolute alcohol - ten minutes.
   h. Abs. al: xylene 50:50 - ten minutes
   i. Xylene - ten minutes - three changes.
10. Mount in permount.
11. Dry.

Procedure 2

This was similar to that of Jooste and Mackey (1952) (37), except that instead of 0.04% eosin a .1% solution was used. This gives a bright pink stain to the protein. Starch is stained by using 40% iodine, KI solution and diluting the stock solution 150 times. I_2 and KI stain starch a distinct blue.

The starch and protein relationship in temporary mounts is demonstrated by staining the sections with eosin, differentiating in 50% alcohol and staining with iodine.

Weaker protein staining can be enhanced by either a concentrated stain or longer staining period.

This procedure has worked adequately on raw chick peas and may render excellent application to protein/starch relationships and protein orientation in processed samples.

IMPROVING THE NUTRITIONAL STATUS OF CROPS THROUGH MUTATION BREEDING

Induced mutagenesis has been one of the newer principles of augmenting variability in populations that may be put to utilization either directly or through recombination. The various steps involved in mutation breeding procedure are radiosensitivity estimations, M study, selection of macromutants in M_2 and for micromutants in M_3. Irrespective of the selection procedure nutritional evaluation should be undertaken after the M_4, as yield, protein content, and the nutritional value of the protein are the three distinctly different but related factors encompassing the enhanced value of a crop. Continued improvement in the productivity at the expense of protein content and quality has dubious net value from a nutritional point of view as expressed by Johnson et al. (1969) (38) for wheat. Further, improvement of protein quality and quantity at the expense of productivity, or increases in protein content accompanied by undesirable shifts in the ratio would have questionable value, as would also improvement of amino acid balance associated with depressed protein content.

Variants resulting from mutation induction would have an enormous possibility of being influenced in their protein content and quality, considering irradiation influence upon the chromosomal makeup that may ultimately be manifested through altered genetic coding in a varied protein synthetic cycle. Consequently such a mutational manipulation of protein quantity and quality should be positively screened before further advance.

In the present study, four macromutants of white bean (Phaseolus vulgaris L. CV. Blue Lake) designated as SDM-1, SDM-2 (semi-dwarfs), PSHy (pod shape/high yielding), and LfHy (late flowering/high yielding) were evaluated for their nutritional status after the M_4 generation.

Materials and Methods

Fifty gram seed samples of each macromutant and control were ground on a mikro-sample mill to pass through a screen size of 0.02 inches. The uniformly mixed samples were stored at 4°C in airtight containers.

Kjeldahl protein, moisture, sample hydrolysis, amino acid analyses were performed according to established procedures (AOAC, 1965; Waggle *et al.*, 1966; Spackman *et al.*, 1958; and Moore *et al.*, 1958) (29, 30, 31, 32). Cystine and methionine were determined by oxidation (Moore, S., 1963) (39).

Results and Discussion

The protein content (moisture free basis) and amino acid composition of the four macromutants and the control are given in Table 10.

The protein content was nonsignificant. Significant differences are apparent for arginine and threonine.

Percentages of amino acid requirements satisfied for the standard reference man age 22 (National Research Council, 1963) (34) by samples of the various macromutants compared to control are depicted in Table 11.

Protein quality requirements satisfied were higher for LfHy and PSHy for lysine, with the semi-dwarfs being lesser than LfHy and PSHy but similar to control. PSHy was higher than control in satisfying the threonine requirements. LfHy was low. All comparisons were significant at the 5% level.

The nonsignificant ratio of essential:total amino acids ranged from 0.493 to 0.507 for the macromutants compared to 0.496 for the control.

TABLE 10. PROTEIN AND AMINO ACID COMPOSITION OF THE MACROMUTANTS AND CONTROL.

	Protein and Amino Acid Values						
	Control	SDM-1	SDM-2	LfHy	PSHy	LSD 0.05	LSD 0.10
Protein (Moist Free)	28.38	28.51	28.75	27.58	27.55	NS	NS
Amino Acids							
Lysine	6.642	6.502	6.937	7.098	6.793	NS	NS
Histidine	2.763	2.695	2.952	3.110	2.736	NS	NS
Ammonia	1.452	1.356	1.478	1.606	1.295	NS	NS
Arginine	6.602	6.733	7.286	7.210	6.184	NS	.638
Aspartic	12.773	12.984	13.467	12.178	13.244	NS	NS
Threonine	4.492	4.474	4.561	4.005	4.562	.304	.232
Serine	6.248	6.237	6.382	5.942	6.371	NS	NS
Glutamic	17.013	17.248	17.252	17.489	16.612	NS	NS
Proline	4.757	4.768	4.709	4.549	4.298	NS	NS
Glycine	3.979	3.951	4.077	3.859	4.049	NS	NS
Alanine	4.246	4.210	4.321	3.743	4.189	NS	NS
Cystine	0.998	1.004	0.950	1.120	1.068	--	--
Valine	5.730	5.818	5.547	5.399	5.934	NS	NS
Methionine	1.185	1.158	1.196	1.204	1.154	--	--
Isoleucine	4.649	4.583	4.211	4.537	4.738	NS	NS
Leucine	8.527	8.549	8.045	8.160	8.567	NS	NS
Tyrosine	3.261	3.178	3.000	3.439	3.453	NS	NS
Phenylalanine	5.801	5.722	4.646	5.688	5.934	NS	NS
Crude Protein	25.4	25.4	25.7	24.6	24.6	NS	NS

TABLE 11. PERCENT OF REQUIREMENT FOR THE STANDARD REFERENCE MAN, AGE 22, SATISFIED BY THE VARIOUS LINES AND CONTROL.

Amino Acids	Requirement	Percentage of the Amino Acid Requirement Satisfied by Samples					
		Control	SDM-1	SDM-2	LfHy	PSHy	LSD 0.05
Lysine	2.46	275.7	270.4	270.4	297.0	294.0	10.6
Threonine	1.54	297.8	297.2	284.0	268.1	315.5	22.0
Valine	2.46	237.8	242.0	216.6	226.8	256.9	NS
Methionine	3.33	35.1	34.3	35.4	35.6	34.1	--
Isoleucine	2.15	220.8	218.3	188.3	217.8	234.7	NS
Leucine	3.38	257.8	258.9	228.6	248.8	270.1	NS
Phenylalanine	3.38	175.4	173.3	132.4	173.5	187.1	NS

TABLE 12. THE RATIO OF ESSENTIAL TO TOTAL AMINO ACIDS OF THE MACROMUTANTS AND CONTROL.

	Ratio of Values					
	Control	SDM-1	SDM-2	LfHy	PSHy	LSD 0.05
$\frac{\text{Essential amino acids}}{\text{Total amino acids}}$	0.496	0.493	0.482	0.507	0.500	NS

The results obtained after the M_4 study have not indicated the presence of any adverse characteristics thus far assessed in the four macromutants as a consequence of radiation induced variability. Protein content has not differed significantly and neither has the essential:total amino acid ratio, with only the few amino acid variations offering promise.

The further advance of the four macromutants either for subsequent direct utilization (PSHy and LfHy) or recombination (semi-dwarfs) appears promising.

EFFECT OF HEAT PROCESSING ON THE NUTRITIVE VALUE OF CHICK PEAS

Experiment III

In Experiment III (as outlined in Progress Report No. 2, March, 1970, p. 60), the effect of different ways of processing chick peas on the growth of rats was studied. The chick peas were given the following treatments before grinding:

a) No treatment (Diet 2)

b) Popped in damp cloth, hulls removed (Diet 3)

c) Popped in damp cloth, hulls not removed (Diet 4)

d) Soaked in damp cloth overnight, dried without hulls (Diet 5)

e) Soaked in damp cloth overnight, dried with hulls (Diet 6)

f) Soaked in water, drained, dried with hulls (Diet 7)

g) Soaked in water, drained, dried without hulls (Diet 8)

The water used for popping and soaking contained 0.5% sodium chloride and 0.5% sodium bicarbonate.

Diets (10% protein level) were prepared from the chick pea flour by adding vitamins and minerals to meet the NRC requirements, oil, starch, glucose, and cellulose. These diets and a casein control diet (Diet 1) were fed to groups of eight male weanling albino rats for four weeks.

There was little difference in weight gain among the groups. The average gain in weight for the control group was 142.75 g. at the end of four weeks. Average gains for the experimental groups ranged from 147.75 to 158.75 g.

Protein efficiency ratios (PER) were calculated at the end of 28 days (Table 13). Analysis of variance for PER showed that the method of processing

the chick peas did not influence the PER, and the quality of protein supplied by casein was not significantly different from that supplied by chick peas for growth of rats.

Blood samples were drawn from rats at the conclusion of the experiment. The serum was analyzed for total protein (biuret method) and proportions of gamma, beta, $alpha_1$, and $alpha_2$, globulins and albumin (electrophoresis method). Total protein is reported below (Table 13). Calculations of protein fractions are not complete at this time.

TABLE 13. PROTEIN EFFICIENCY RATIOS AND SERUM PROTEIN CONCENTRATIONS FOR RATS.

Diet	PER	Serum Protein g/100 ml
1	2.681	5.82
2	2.611	5.63
3	2.498	5.46
4	2.529	5.50
5	2.683	5.34
6	2.550	5.46
7	2.549	5.40
8	2.468	5.42

The concentration of calcium in the femurs will be studied. Dry bone weight analyses, to be followed by calcium determinations, are now in progress.

EFFECT OF PROTEIN SUPPLEMENTATION (COTTONSEED FLOUR) ON AMERICAN AND INDIA - PAKISTANI - BREAD

Robert J. Robinson, Carlos Sanchez, Mir Khan
and William J. Hoover

Introduction

Cottonseed flour was blended at 8 levels, from 0 to 30% with two wheat flours, low and high in protein content, to give flours of increased protein content.

Farinograph data indicated strengthening only when cottonseed flour constituted up to 8% of a mixture with high-protein flour. Absorption always increased as content of cottonseed flour increased. Extensograph data paralleled those of the farinograph.

Baking by standard pup loaf method showed that above the 15% level cottonseed flour depressed loaf volume and imparted undesirable flavor.

Nan and chapatis (Indian-Pakistani-breads) baked from blends containing 8% cottonseed flour resulted in good products.

The purpose of this study was to determine the effects of cottonseed flour supplementation on American and India-Pakistani breads.

Experimental

Glandless Cottonseed seed flour was obtained from the Cottonseed Products Research Laboratory, Texas A & M University, College Station, Texas 77843. Cottonseed flour was described as greenish-tan, both powdery and particulate in appearance, and intersparsed with black specks. Initially, the odor of the cottonseed flour was characterized by an oily-gassy-rubbery aromatic. Its flavor was marked by the same aromatic and was further described as oxidized, salty, sweet, green vegetation-like, bitter, and astringent (Caul and Archer, 1970) (40).

Two representative hard red winter wheat flours with protein contents of 10.1 and 12.7% (5.75 x N) were tested. A 97% extraction Gaines wheat flour was also used for making Indian-Pakistani breads.

Cottonseed flour samples were blended with each wheat flour at 1, 3, 5, 8, 15, 20, and 30% levels. Each sample of cottonseed-wheat flour blend was analyzed for moisture, protein and ash (AOAC, 1968) (41) (Table 14). Blending cottonseed flour into wheat flour of 10.1 and 12.7% protein content resulted in significantly higher protein content. Analyses of variance (Table 15) of protein content between flours and between cottonseed concentrations shows significant differences between flours and between protein content, i.e., increase in cottonseed concentration showed a significant increase in protein content with the higher protein flour being significantly higher in protein content. Elemental mineral analyses indicated a general improvement in iron content in most blends, especially above 8% level of cottonseed flour in the 12.7% protein wheat flour. Other changes in mineral contents resulting from blending were less significant.

TABLE 14. COTTONSEED AND WHEAT FLOUR BLENDS

	Protein*	Ash	Moisture
Wheat Flour LP	10.1	.36	11.1
Cottonseed Flour	55.7	7.7	6.8
LP 1%	10.6	.44	11.1
LP 3%	11.2	.57	10.9
LP 5%	12.1	.70	11.0
LP 8%	13.4	.93	10.4
LP 15%	16.7	1.4	10.3
LP 20%	19.1	1.8	10.1
LP 30%	23.0	2.4	9.5
Wheat Flour HP	12.7	.39	10.7
HP 1%	12.8	.48	10.8
HP 3%	14.0	.62	10.4
HP 5%	14.8	.78	10.7
HP 8%	16.2	.99	10.9
HP 15%	19.9	1.5	10.9
HP 20%	20.9	1.8	10.1
HP 30%	25.1	2.5	9.6

*% N x 5.7
As is moisture basis.

TABLE 15. AOV - PROTEIN CONTENT OF BLENDS

Variation Due To:	dF	SS	MS	F
Flours	1	44	44	51.33**
Proteins	7	300	42.85	49.99**
Error	7	6	0.8571	
Total	15	350		

**Significant at 1%

Amino acid composition of cottonseed flour, wheat flours and atta was determined with a Beckman Amino Acid Analyzer (Table 16). The overall nutritional qualities of atta protein appeared to be better than those of individual component flours. Methionine appeared to be the limiting amino acid in the cottonseed flour, but lysine was greater than in the wheat flours.

The rheological properties of each sample of wheat flour and blended cottonseed-wheat flour were determined.

Standard farinograph procedures were used (with 300 g. capacity stainless steel bowl) (AACC, 1962) (42). Farinograms of each sample are shown in Figure 3. A summary of the farinograph data is given in Table 17.

Water absorption (57.0-63.2), dough development time, valorimeter values, mixing tolerance index of the samples were not uniform (Table 17). The farinograms for blends at 8 and 15% levels showed good stability.

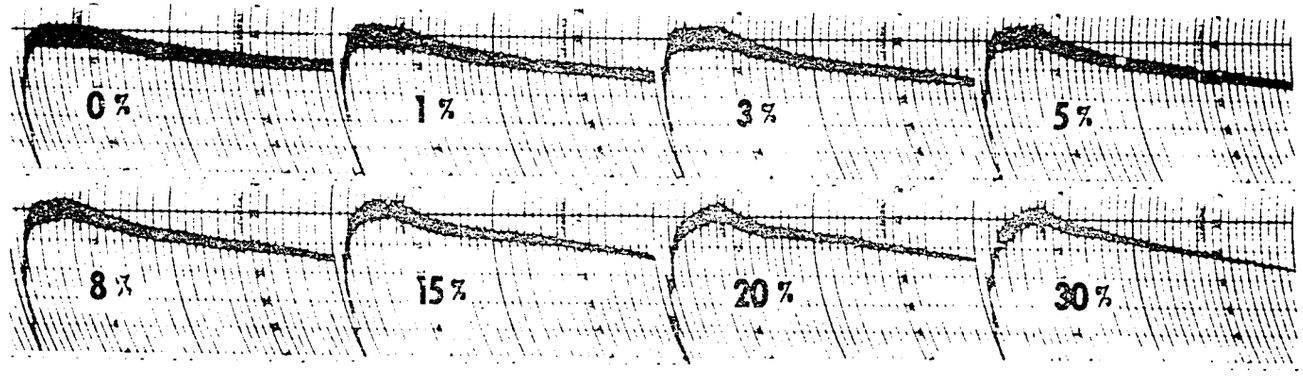
Peak time, absorption and tolerance data were submitted to statistical analyses and the results appear in analysis of variance (Tables 18, 19, and 20). The data indicated significant differences in absorption between flours but not between cottonseed flour concentrations, i.e., absorption increased significantly between flours but not between cottonseed flour concentrations.

TABLE 16. AMINO ACID COMPOSITION OF COTTONSEED FLOUR AND WHEAT FLOURS.

	Cottonseed Flour		W. Flour (10.1)		W. Flour (12.7)		ATTA	
	% Sample	g/16gN	% Sample	g/16gN	% Sample	g/16gN	% Sample	g/16gN
Lysine	2.42	4.49	0.22	2.09	0.29	2.10	0.29	2.92
Histidine	1.51	2.80	0.23	2.18	0.29	2.10	0.22	2.17
Threonine	1.82	3.37	0.27	2.52	0.37	2.69	0.30	2.98
Methionine	0.74	1.37	0.15	1.36	0.21	1.50	0.14	1.41
Valine	2.39	4.24	0.45	4.22	0.57	4.12	0.45	4.49
Isoleucine	1.67	3.09	0.35	3.27	0.48	3.49	0.32	3.21
Leucine	3.25	6.01	0.77	7.15	0.96	6.98	0.64	6.47
Arginine	6.82	12.62	0.39	3.66	0.50	3.60	0.42	4.25
Phenylalanine	2.85	5.27	0.52	4.85	0.71	5.14	0.42	4.27
Protein	55.7		10.1		12.7		9.90	

FARINOGRAMS
COTTONSEED FLOUR AND WHEAT FLOUR BLENDS

LOW PROTEIN HRW



HIGH PROTEIN HRW

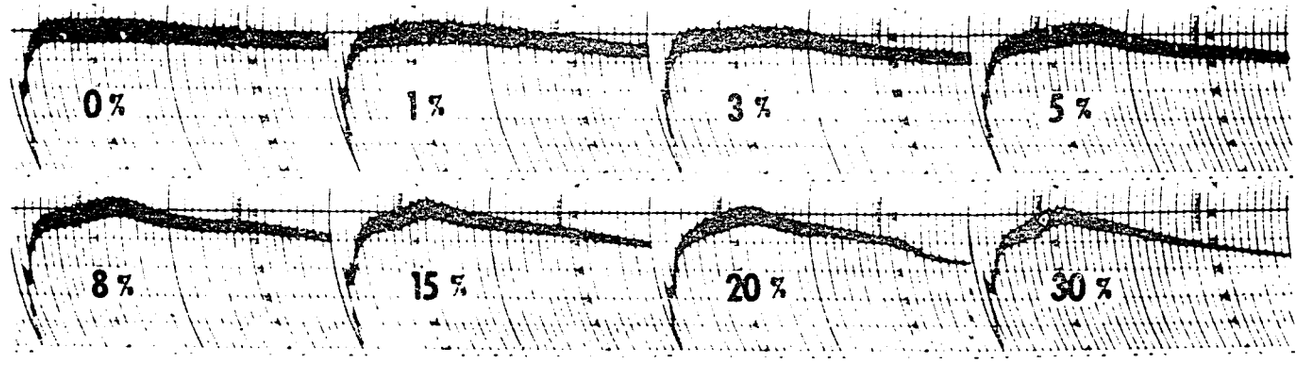


Fig. 3.

TABLE 17. FARINOGRAPH DATA COTTONSEED AND WHEAT FLOUR BLENDS.

	Protein	Absorption	Peak Time	Arrival Time	Departure Time	Tolerance	MTI	Valor-imeter
LP Flour	10.1	57.0	4.0	1.5	7.5	6.0	60	50
LP 1%	10.6	57.0	3.0	1.0	5.5	4.5	80	46
LP 3%	11.2	57.6	4.0	1.0	5.5	4.5	90	50
LP 5%	12.1	59.4	4.0	1.5	6.0	4.5	95	50
LP 8%	13.4	60.7	3.5	1.5	5.5	4.0	90	47
LP 15%	16.7	63.2	3.5	1.5	5.5	4.0	90	48
LP 20%	19.1	66.0	4.0	2.5	5.0	2.5	80	50
LP 30%	23.0	70.0	4.0	2.5	5.5	3.0	90	48
HP Flour	12.7	63.2	7.0	2.0	16.5	14.5	20	71
HP 1%	12.8	63.3	8.0	2.0	14.0	12.0	30	70
HP 3%	14.0	62.9	7.0	2.0	11.0	9.0	40	66
HP 5%	14.8	65.3	7.0	3.5	10.0	6.5	60	66
HP 8%	16.2	66.5	6.5	4.0	9.0	5.0	60	64
HP 15%	19.9	70.0	6.0	4.0	8.5	4.5	60	60
HP 20%	20.9	72.0	6.5	4.5	8.0	3.5	70	57
HP 30%	25.1	76.0	6.0	4.5	8.0	3.5	80	57

TABLE 18. AOV - ABSORPTION (FARINOGRAPH).

Variation Due To:	dF.	SS	MS	F
Flour	1	145.8056	145.8056	15.7346**
Cottonseed Conc.	7	317.1297	45.3071	4.8860N.S.
Error	7	64.91	9.2729	
Total	15	463.6044		

**Significant at 1%
N.S. -Nonsignificant

TABLE 19. AOV - FARINOGRAPH PEAK TIME.

Variation Due To:	dF	SS	MS	F
Flour	1	36	36	9.1626*
Cottonseed Conc.	7	1.25	0.1786	0.4546N.S.
Error	7	2.75	0.3929	
Total	15	40		

*Significant at 5%
N.S. Nonsignificant

TABLE 20. AOV - FARINOGRAPH TOLERANCE.

Variation Due To:	dF	SS	MS	F
Flour	1	40.6407	40.6407	7.5784*
Cottonseed	7	90.3594	12.9085	2.4432 N.S.
Error	7	36.9843	5.2835	
Total	15			

*Significant at 5%
N.S. Nonsignificant

The absorption for the high-protein flour was significantly higher as compared with the low protein flour with increase in cottonseed flour concentration.

Significant differences between flours and a nonsignificant difference between cottonseed flour concentrations were observed in peak time (Table 19), i.e., mixing times were significantly longer with the higher protein flour throughout the cottonseed flour concentrations as compared with the lower protein flour; cottonseed concentration did not effect peak time.

The above table shows significant differences between flours in tolerance and a nonsignificant difference between cottonseed flour concentrations, i.e., the high-protein flour had more tolerance as compared with lower protein flour, cottonseed flour concentration had no effect on tolerance.

Correlation: Correlation coefficients were determined for protein versus absorption, protein versus tolerance, protein versus farinograph peak time and protein versus bakery mixing time.

A strong linear relationship (r=0.9560) between protein content and farinograph absorption was found. This indicates that an increase of cottonseed flour concentration increased absorption as is the case with flour protein.

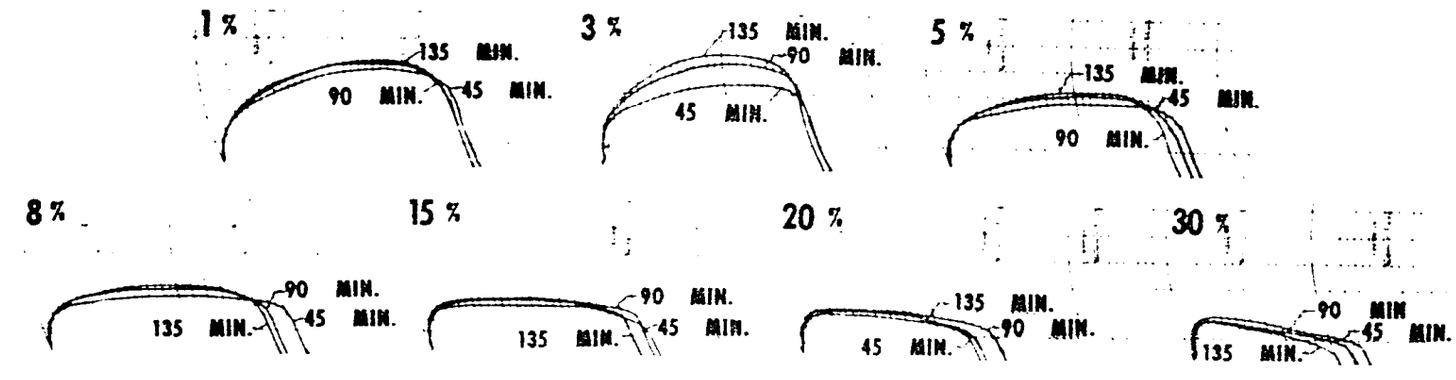
The other factors, i.e., tolerance, peak time and bakery mixing time, had -0.4303, -0.05722, and 0.5322 correlation coefficients respectively. Results indicated low linear relationships between these factors and protein content revealing the lack of gluten character in cottonseed flour protein.

Standard extensograph procedures were used (AACC, 1962) (42). Curves were drawn for duplicate doughs at 45, 90, and 135 minutes (Figure 4). Extensograph characteristics are summarized in Table 21. The extensograph and farinograph characteristics for blends of flour followed similar trends. Area, resistance to extension and extensibility data were submitted to statistical analyses and the results appear in analysis of variance (Tables 22, 23 and 24). The analysis of variance for areas under extensogram was significant for variations due to different flours and different cottonseed flour concentrations. The flour with high-protein content showed a significantly larger area than flour with low protein content. Concentrations of cottonseed flour also affected the areas under the extensogram significantly. The 5% and 8% cottonseed flour concentrations did not show significance in their respective areas under the extensogram. The remaining cottonseed flour concentrations were significantly different in area under the extensogram.

Analyses of Variance (Table 23) on resistance to extension showed significant differences due to flours. The flours with high-protein content exhibited greater resistance to extension than flours with low protein content. Time was nonsignificant in its effect on resistance to extension, but there was a significant difference between cottonseed flour concentrations in

EXTENSIGRAMS
COTTONSEED FLOUR AND WHEAT FLOUR BLENDS

LOW PROTEIN HRW



HIGH PROTEIN HRW

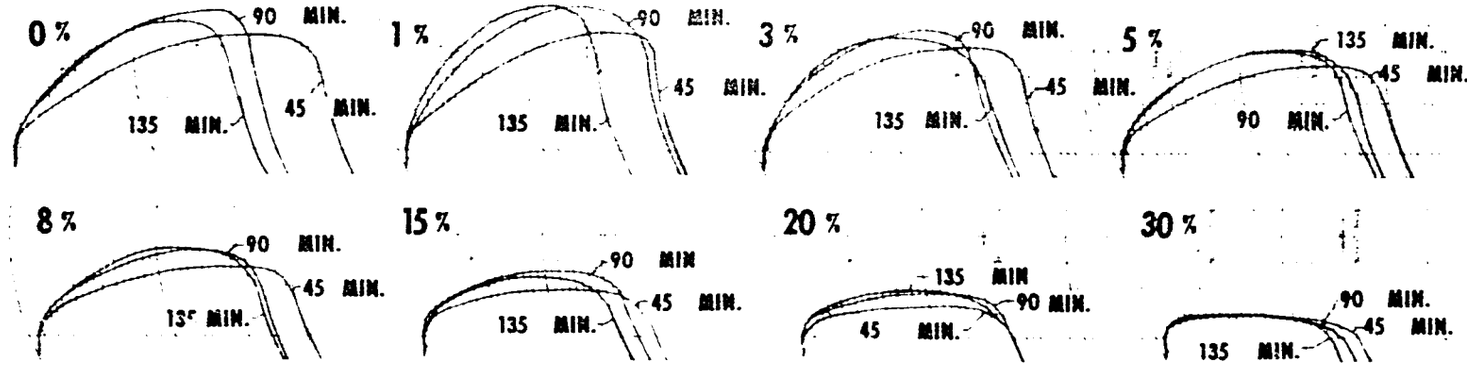


Fig. 4.

TABLE 21. COTTONSEED FLOUR BLENDS EXTENSOGRAPH DATA.

	45 min.				90 min.				135 min.			
	Extensibility	Resistance to Extension	Area In.	Area Cm.	Extensibility	Resistance to Extension	Area In.	Area Cm.	Extensibility	Resistance to Extension	Area In.	Area Cm.
						LP FLOUR						
LP 1%	175	380	13.50	93.8	170	410	14.32	99.5	165	415	14.32	99.5
LP 3%	155	340	10.69	74.3	150	410	13.00	90.4	150	450	14.00	97.3
LP 5%	170	280	10.34	71.9	155	310	10.41	72.3	165	325	10.95	76.1
LP 8%	175	240	8.64	60.1	155	260	8.94	62.1	160	280	9.02	62.7
LP 15%	155	210	6.73	46.8	145	230	7.14	49.6	145	235	6.84	47.5
LP 20%	120	180	4.07	28.3	135	200	5.24	36.4	120	190	4.61	32.0
LP 30%	110	180	3.41	23.7	105	170	3.07	21.3	100	170	2.70	18.8
						HP FLOUR						
HP	230	540	24.93	173.3	190	630	23.45	163.0	170	495	19.84	137.9
HP 1%	190	450	25.80	179.3	175	620	21.79	151.4	150	655	19.83	137.8
HP 3%	200	510	20.20	140.4	170	570	19.16	133.2	175	560	19.33	134.3
HP 5%	200	440	17.30	120.2	170	500	17.61	122.4	180	500	18.52	128.7
HP 8%	195	370	14.53	101.0	170	440	14.75	102.5	170	455	14.95	103.9
HP 15%	165	295	9.82	68.2	155	370	11.54	80.2	140	330	9.49	66.0
HP 20%	150	230	7.12	49.5	150	280	8.58	59.6	150	290	8.49	59.0
HP 30%	140	200	5.55	38.6	130	210	5.25	36.5	120	190	4.78	33.2

TABLE 22. AOV - EXTENSOGRAPH - AREA.

Variation:	dF.	SS	MS	F
Flour	1	14,541.48	14,541.48	116.98**
Time	2	20.92	10.46	0.0083 N.S.
Cottonseed Conc.	6	47,228.17	7,871.30	63.15**
Error	32	3,988.12	124.63	
Total	41	65,778.69		

**Significant at 1%
N.S.-Nonsignificant

resistence to extension. In this case the effect of cottonseed flour can be seen when the concentration is above 5%. Results indicated a difference in the extensograph properties due to different flours. Time or low concentrations of cottonseed flour did not affect the extensograph properties.

TABLE 23. AOV - RESISTANCE TO EXTENSION

Variation:	dF.	SS	MS	F
Flour	1	160,952	160,952	22.81**
Time	2	33,989	16,994	2.408 N.S.
Cottonseed Conc.	6	493,865	82,310	11.663
Error	32	22,585	7,057	
Total	41	711,391		

**Significant at 1%
N.S.-Nonsignificant

Significant differences between flours, times and the cottonseed flour concentration are indicated in Table 24. Flour with low protein content demonstrated significantly lower extensibility than flours with high-protein content.

There was not a significant difference in extensibility when dough was rested for 90 and 135 minutes. But the extensibility at 45 minutes was significantly from 90 and 135 minutes.

There was not a significant difference in extensibility due to 1, 3, 5, 8 and 15% concentration of cottonseed flour. The 20 and 30% cottonseed flour concentrations had a significant effect on extensibility. Results indicated that lower concentrations of cottonseed flour do not contribute to extensibility, but with an increase in cottonseed flour concentration above 15% a significant effect can be seen.

TABLE 24. AOV - EXTENSIBILITY.

Variation	dF.	SS	MS	F
Flour	1	3,172.08	3,172.08	37.32**
Time	2	1,712.55	856.27	10.07**
Cottonseed Conc.	6	16,215.55	2,702.50	31.79**
Error	32	2,719.82	84.99	
Total	41	23,820.00		

**Significant at 1%

Baking Results: Baking experiments were on a laboratory scale. Effects of dough composition were tested by use of a complete formula and a straight dough procedure. The basic dough formula was:

Ingredient	% (Based on Flour)
Flour	100
Sugar	6
Milk	3
Shortening	3
Salt	2
Malt	0.5
Yeast	2
$KBrO_3$	10 ppm
Water	Farinograph

After being mixed to optimum consistency (mixing time correlated with farinograph-dough development), the doughs were fermented at 85°F., punched after 110 minutes, and 50 minutes later punched again, divided, rested 20 minutes, and molded. Proofing was at 90°F. for 45 minutes and baking 25 minutes at 410°F.

The analysis of variance of bakery mixing time between flours and between cottonseed flour concentrations are given in Table 25. The data indicated a significant difference between flours in mixing time but a nonsignificant

effect between mixing times due to cottonseed flour concentrations, i.e., mixing time was significantly longer with flour of high-protein content throughout the cottonseed flour concentrations as compared with flour of low protein content; cottonseed flour concentrations did not affect bakery mixing time.

TABLE 25. AOV - BAKERY MIXING TIME[1].

Variation:	dF.	SS	MS	F
Flour	1	6.602	6.602	6.805*
Cottonseed Conc.	7	4.023	0.5747	0.5924 N.S.
Error	7	6.791	0.9701	
Total	15	17.461		

[1]Swanson 100G Mixer
*Significant at 5%
N.S.-Nonsignificant

Bread Quality: Loaf volumes and weights were determined on freshly baked loaves. After cooling, the loaves were scored with maximum possible values of specific volume 20, crust color 10, symmetry 10, break and shred 10, grain 10, texture 20, crumb color 10, for total 100. Baking data are summarized in Table 26. The total score tended to decrease as concentrations of cottonseed flour increased in both the low and high-protein flours.

A simple analysis of variance was performed on bread quality scores between flours and between cottonseed flour concentrations. Significant differences between flours and cottonseed flour concentrations are shown in Table 27. The visual observations (Figure 5) are in agreement with statistical analysis in that bread quality decreased substantially above 5% level of cottonseed flour concentration. The flour of high-protein content gave significantly higher quality scores as compared with flour of low protein content throughout the cottonseed flour concentrations with overall comparisons being equal at 0, 1, 3 and 5% cottonseed flour concentrations.

TABLE 26. BAKING VALUES AND SCORES.

Sample	Volume (cc)	Specific Vol (cc/g)	Total Score (Points)
LP FLOUR*	655	4.49	73
LP 1%	685	4.69	77
LP 3%	595	3.95	60
LP 5%	613	4.02	63
LP 8%	587	3.90	53
LP 15%	475	3.09	37
LP 20%	435	2.82	27
LP 30%	325	2.07	16
HP FLOUR*	790	5.23	82
HP 1%	745	4.98	83
HP 3%	742	4.93	78
HP 5%	708	4.58	71
HP 8%	687	4.45	65
HP 15%	595	3.78	45
HP 20%	530	3.33	31
HP 30%	350	2.22	18

*Control

TABLE 27. AOV - BREAD QUALITY.

Variation	dF	SS	MS	F
Flour	1	203.0625	203.0625	15.212**
Cottonseed Conc.	7	7568.9375	1081.2760	81.005**
Error	7	93.4375	13.3482	
Total	15	7868.4375		

**Significant at 1%

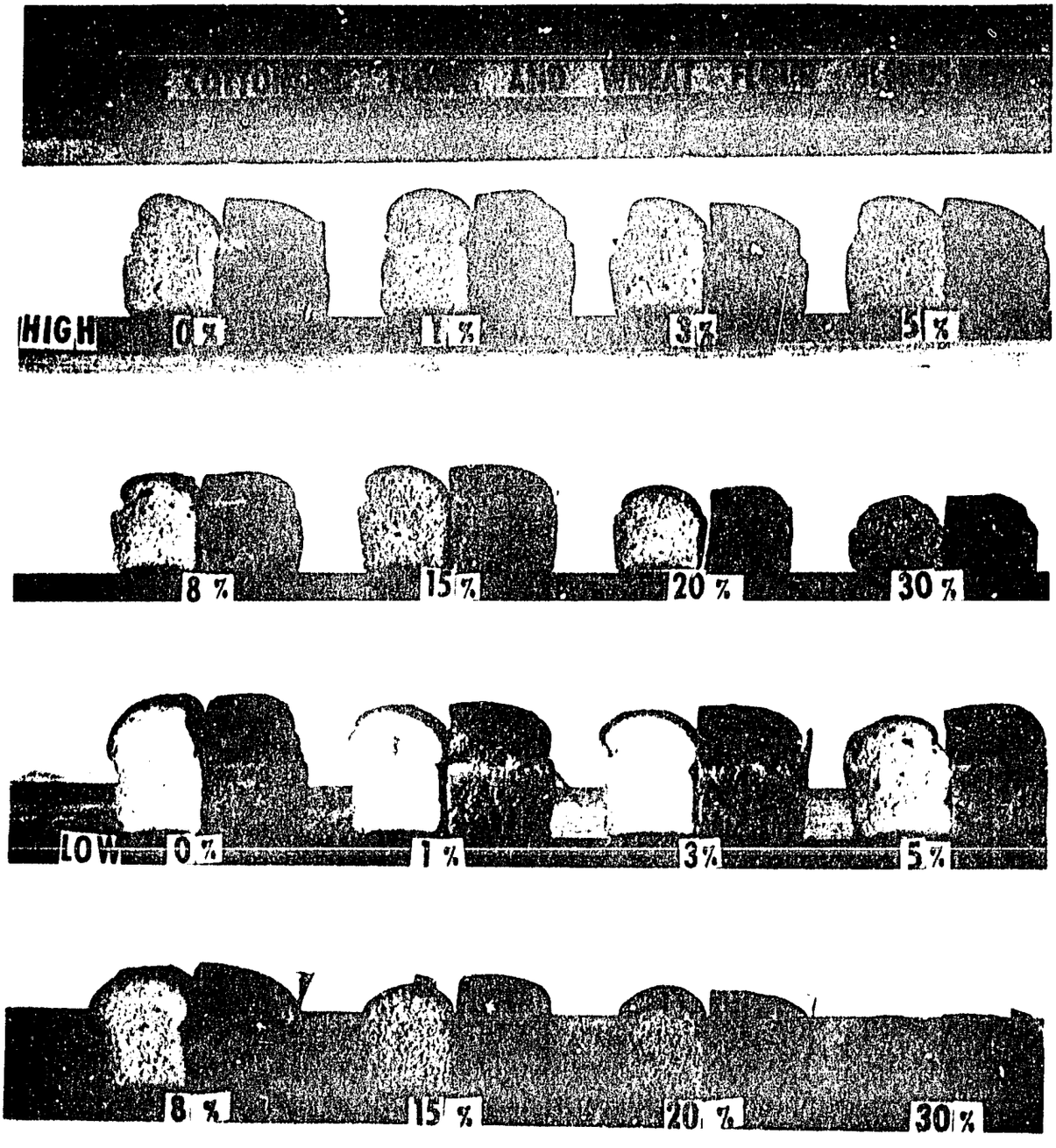


Fig. 5.

Summary

Two flours with different protein content were supplemented with 7 different concentrations of cottonseed flour. Protein and cottonseed flour concentrations produced a significant effect on baking quality. The F values were significant at 1%. Protein content of the flour affected the farinograph and bakery mixing time, but cottonseed flour concentration did not have an effect. Absorption was affected significantly at 1% due to protein content of flours but not between cottonseed flour concentrations. Tolerance was affected significantly due to protein content, but was not affected by cottonseed flour concentration.

Results on the extensograph showed that protein content and high cottonseed flour concentrations affected the extensograph, however, rest periods did not have significant effects.

Correlation between protein and absorption and protein and bakery mixing time were significant at 1 and 5% respectively. Other correlations as between protein and farinograph mixing time and tolerance were negative and non-significant.

SUPPLEMENTATION OF CHAPATIS WITH COTTONSEED FLOUR

Formula for chapatis was:

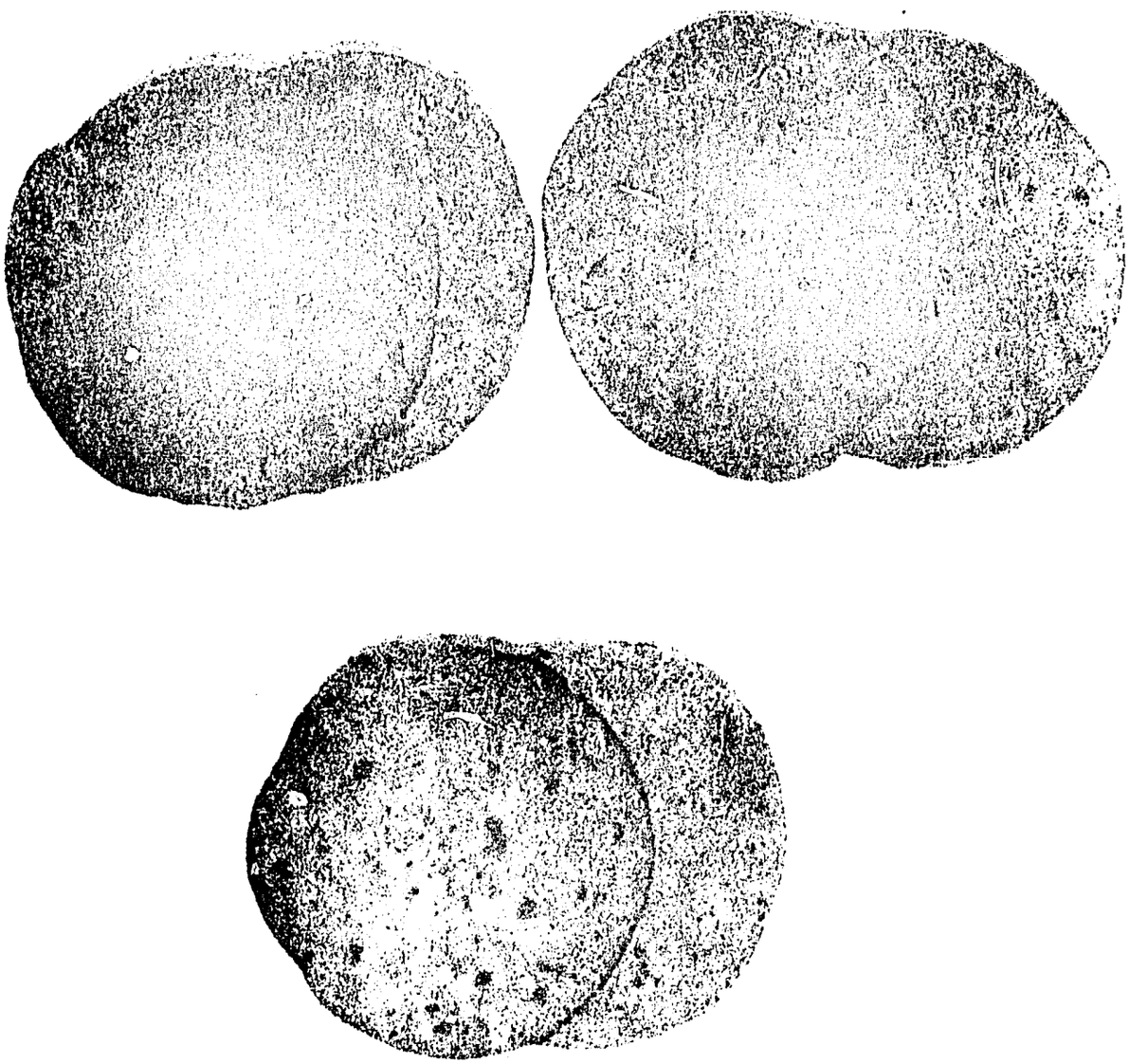
Atta	1362	g.
Salt	28.35	g.
Oil	9.4	g.
Water	878	ml.

The atta, salt, oil and half the water were mixed in a Hobart (N-50) mixing bowl at speed "1" until dough formed. The remaining water was added and mixing continued at speed "2" for one minute. The dough was covered and rested for 30 minutes, then 50 gm. patties, 2½" x ¼", were rolled into 6" circles, and cooked on an electric grill at 400°F on both sides. The chapati was puffed by pressing it with a padded cloth. More water was needed, the greater the concentration of cottonseed flour in the atta.

Cottonseed flour aroma and odor was briefly noted early in the mixing period of the 8 and 15% fortified doughs. Increases in cottonseed flour concentration from 1 to 15% resulted in tacky doughs after mixing, but drier and more inelastic, yet easier to handle doughs after resting. No cottonseed flour aroma and odor was detected during the baking of any fortified chapatis.

A panel scored chapatis for aroma, texture and appearance. Blends with more than 15% cottonseed flour gave objectionable aroma, flavor, texture and appearance. Acceptable chapatis are shown in Figure 6.

ATTA 97% EXTRACTION GAINES WHEAT



CHAPATIS

Fig. 6.

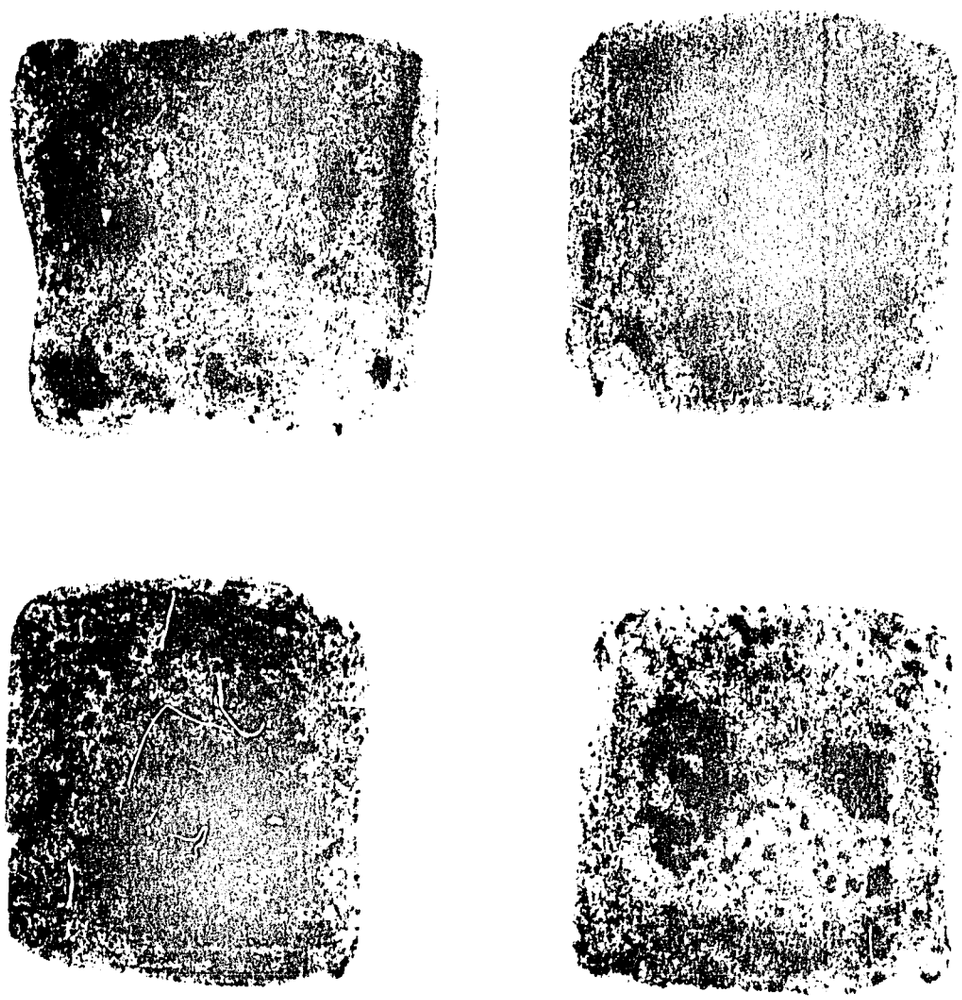
SUPPLEMENTATION OF PARATHAS WITH COTTONSEED FLOUR

Formula and procedure for parathas:

Atta	1362	g.
Salt	56.70	g.
Oil	28.35	g.
Water	890	ml.

The ingredients were mixed according to the procedure for making chapatis. Dough was rolled into a 2½" x ¼" (50 gm.) flat patty then into a 6 to 8" circle. With a pastry brush each circle was painted with 9.5 g. oil and folded into thirds, then folded twice to form a square. The squares were rolled very thin and baked on an electric grill at 400°F. After baking on one side, they were turned, painted with oil, baked, turned again and painted with oil, then removed from the grill and scored subjectively. In this product a much higher level of supplement was acceptable with respect to aroma, flavor, texture and appearance (Figure 7). However, the same reaction occurred with respect to mixing and dough handling.

ATTA 97% EXTRACTION GAINES WHEAT



PARATHA

Fig. 7

SUPPLEMENTATION OF NAN WITH COTTONSEED FLOUR

Formula and procedure for nan:

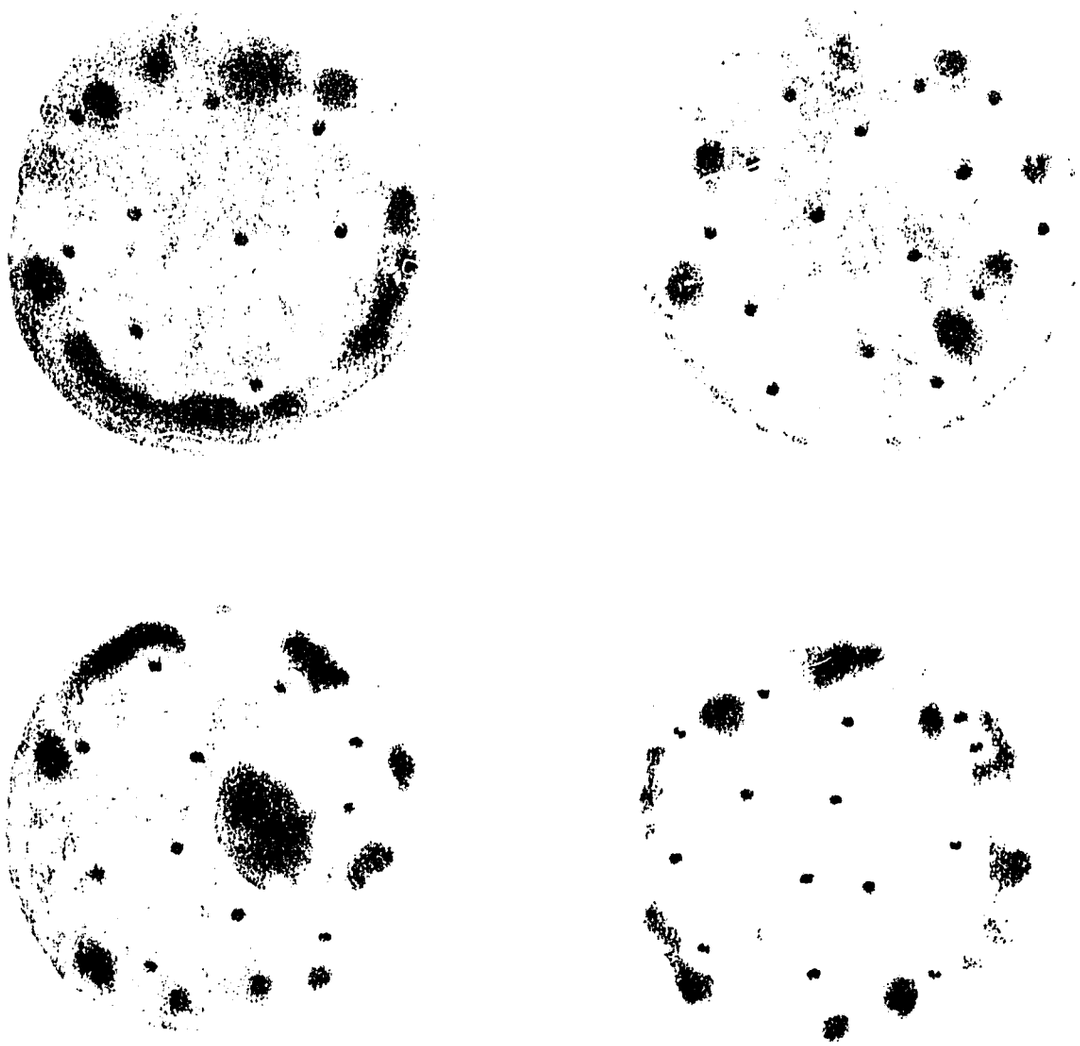
Atta	1362 g.
Compressed yeast	27 g.
Oil	40 g.
Salt	20 g.
Water	895 ml.

A hearth oven was preheated to 500°F with moisture adjustment. Yeast was dispersed in 100 ml. water. All ingredients except half the water were mixed at speed "1" on a Hobart N-50 mixer to dough formation. The remaining water was added and mixing continued at speed "2" for one minute. The dough was proofed for one hour, scaled at 120 g. portions, and rolled on a lightly floured board into 6 to 8" circles ⅛" thick. They were baked in the oven without a rack for 3 minutes on one side and 2 minutes on the other side. Baked nans were wrapped in dry cloth (Figure 8) and later scored by the panel for aroma, flavor, texture and appearance.

The dough mixing and handling properties of the nan followed closely those of the pup loaves. Absorption increased as concentration of the cottonseed flour increased.

Evaluation: Flatulence resulted from the testing of all India-Pakistani-breads made from flours (ATTA) containing cottonseed flour fortification. The 15% fortified products were redolent of cottonseed flour in both aroma and flavor and contained black specks from the cottonseed flour. Lower levels of fortification presented only the greenish and not the oily-gassy-rubbery cottonseed flour aromatic in the aroma and flavor and were sweeter and fuller in aroma and flavor than other fortified products or the control.

ATTA 97% EXTRACTION GAINES WHEAT



NANS

Fig. 8.

This cottonseed flour aroma was noted only by persons familiar with cottonseed aroma, and not by the India-Pakistani student working on the project.

We do need an improved cottonseed flour with more acceptable appearance and digestibility.

EFFECT OF PROTEIN SUPPLEMENTATION (PEANUT FLOUR) ON AMERICAN AND INDIAN-PAKISTANI BREAD

Robert J. Robinson

Summary

Peanut flour was blended at 10 levels, from 0 to 50%, with three wheat flours of low, medium, or high-protein content.

Farinograph data indicated strengthening only when peanut flour constituted up to 30% of a mixture with low protein flour. Absorption always increased as content of peanut flour increased. Extensograph data paralleled those of the farinograph.

Standard pup loaves showed that more than 30% peanut flour depressed loaf volume and imparted undesirable flavor.

Nan, parathas, and chapatis (Indian-Pakistani breads) baked from blends containing 30 to 50% peanut flour were heavy but kept well and improved in flavor on aging.

Materials and Methods

Commercially prepared peanut flour from Gold Kist Peanut Growers, Graceville, Florida, with the proximate analysis shown in Table 28, was used.

Three representative hard red winter wheat flours with protein contents of 10.4, 12.5, and 13.0% (5.75 x N) were tested. A 97% extraction Gaines wheat flour also was used for making India-Pakistani breads.

Peanut flour samples were blended with each wheat flour at 1, 3, 8, 10, 20, 30, 40, and 50%. Each sample of peanut-wheat flour blend was analyzed for moisture, protein, and ash (AOAC, 1968) (41) (Table 29). Blending peanut flour with wheat flour of 10.4, 12.5, or 13.0% protein content significantly raised protein content, and improved iron content of most blends, especially those with at least 10% peanut flour in 13.0% protein wheat flour. Other changes in mineral contents resulting from blending were less significant.

TABLE 28. PROXIMATE ANALYSES OF THE PEANUT FLOUR[1].

Constituent	Percent	Constituent	Percent
Moisture	6.60	Iron (Fe)	0.01
Fat	1.28	Sulphur (S)	0.16
Protein (N x 6.25)	59.81	Chlorine (Cl)	0.04
Fiber	3.92	Copper (Cu)	0.0024
Ash	4.50	Silicon (Si)	0.020
Nitrogen-free extract	23.89	Manganese (Mn)	0.003
Calcium (Ca)	0.063	Zinc (Zn)	0.0014
Phosphorus (P)	0.75	Iodine (I_2)	0.0012
Potassium (K)	1.50	Aluminum (Al)	0.0015
Magnesium (Mg)	0.34	Choline chloride	0.25
	Vitamins		
	Mg/gm		Mgs/lb.
Inositol	520.0	Niacin	113.8
Biotin	1.6	Riboflavin	1.18
Folic acid	2.0	Thiamin	1.23
Pantothenic acid	61.3*	Carotene	0.0
Vitamin B-6	10.6**	Calories	2091/lb.

[1]Manufacturer's analyses.

*As D-calcium pantothenate

**As pyridoxine hydrochloride

TABLE 29. ANALYSES OF PEANUT AND WHEAT FLOUR BLENDS.

	Wheat flour, 10.4% protein*			Wheat flour, 12.5% protein			Wheat flour, 13.0% protein		
Peanut flour %	Moisture %	Protein %	Ash %	Moisture %	Protein %	Ash %	Moisture %	Protein %	Ash %
1	13.0	11.0	0.50	13.5	13.5	0.40	13.1	15.0	0.39
3	13.5	11.6	0.54	13.1	13.9	0.40	12.6	16.2	0.48
5	12.0	12.0	0.58	13.5	14.0	0.48	12.0	16.9	0.56
8	11.7	13.6	0.68	12.9	15.4	0.69	12.1	18.2	0.61
0	14.4	10.7	0.35	12.3	12.5	.43	12.8	14.3	0.44
10	13.0	16.2	0.78	12.5	18.5	0.79	14.3	18.8	0.75
20	12.4	20.7	1.2	11.8	22.6	1.2	11.7	22.8	1.2
30	11.9	24.7	1.5	11.9	27.2	1.6	11.4	22.4	1.5
40	11.7	29.2	2.0	11.3	31.8	2.0	10.9	31.3	2.0
150	10.9	36.6	2.3	11.0	38.2	2.6		36.2	2.4
100	8.6	57.7	4.4						
						ATTA	9.40	9.90	

*Percent Nitrogen x 6.25 Factor.

Amino acid composition of peanut flour, wheat flours, and atta was determined with a Beckman amino acid analyzer (Table 30). The data indicate that the nutritional qualities of wheat flours with 10.4% and 13.0% protein were equal to or lower than those of flour with 12.5% protein content. Nutritional qualities of atta protein appeared to be better than those of individual component flours. Peanut flour contained less methionine but more lysine than wheat flours.

The rheological properties of each sample of wheat flour and blended peanut wheat flour were determined by standard farinograph procedures (with 300 g. capacity stainless steel bowl) (AACC, 1962) (42). Farinograms of each sample are shown in Figures 9 and 10. A summary of the farinograph data is given in Table 31.

Water absorption (61%), dough development time, valorimeter values, and mixing tolerance index of the samples were uniform (Table 31). The farinograms for the blends at the 20, 30 and 40% peanut flour were most stable.

Peak time was not consistent for all samples. With more than 10% peanut flour, peak time lengthened with all samples. From 10 to 40% the mixing tolerance index increased, but it decreased at the 50% level. Hydration time was affected by peanut flour as shown in Figure 10. Valorimeter and stability values of each blend showed no definite trend related to either peanut flour level or wheat variety. Blends with less peanut flour tended to behave as weak flours.

Standard extensograph procedures were used (AACC, 1962) (42). Curves were drawn for duplicate doughs at 45, 90, and 135 minutes (Figures 11 and 12). Extensograph characteristics are summarized in Table 32.

TABLE 30. AMINO ACID COMPOSITION OF ATTA AND OF PEANUT AND WHEAT FLOURS.

	ATTA		Peanut flour		W. flour (10.4)		W. flour (12.5)		W. flour (13.0)	
	Sample %	g/16gN	Sample %	g/16gN	Sample %	g/16gN	Sample %	g/16gN	Sample %	g/16gN
Lysine	0.29	2.92	1.47	2.55	0.22	2.09	0.28	1.73	0.29	2.10
Histidine	0.22	2.17	1.93	3.34	0.23	2.18	0.28	1.75	0.29	2.10
Threonine	0.30	2.98	1.49	2.57	0.27	2.52	0.37	2.33	0.37	2.69
Methionine	0.14	1.41	0.22	0.38	0.15	1.36	0.15	0.96	0.21	1.50
Valine	0.45	4.49	2.29	3.97	0.45	4.22	0.58	3.64	0.57	4.12
Isoleucine	0.32	3.21	1.81	3.13	0.35	3.27	0.48	3.04	0.48	3.49
Leucine	0.64	6.47	3.68	6.37	0.77	7.15	0.96	6.06	0.96	6.98
Arginine	0.42	4.25	7.02	12.17	0.39	3.66	0.54	3.39	0.50	3.60
Phenylalanine	0.42	4.27	2.89	5.02	0.52	4.85	0.64	4.00	0.71	5.14
Protein, %	9.90		57.7		10.7		12.5		14.3	

FARINOGRAMS OF PEANUT AND WHEAT FLOUR BLENDS

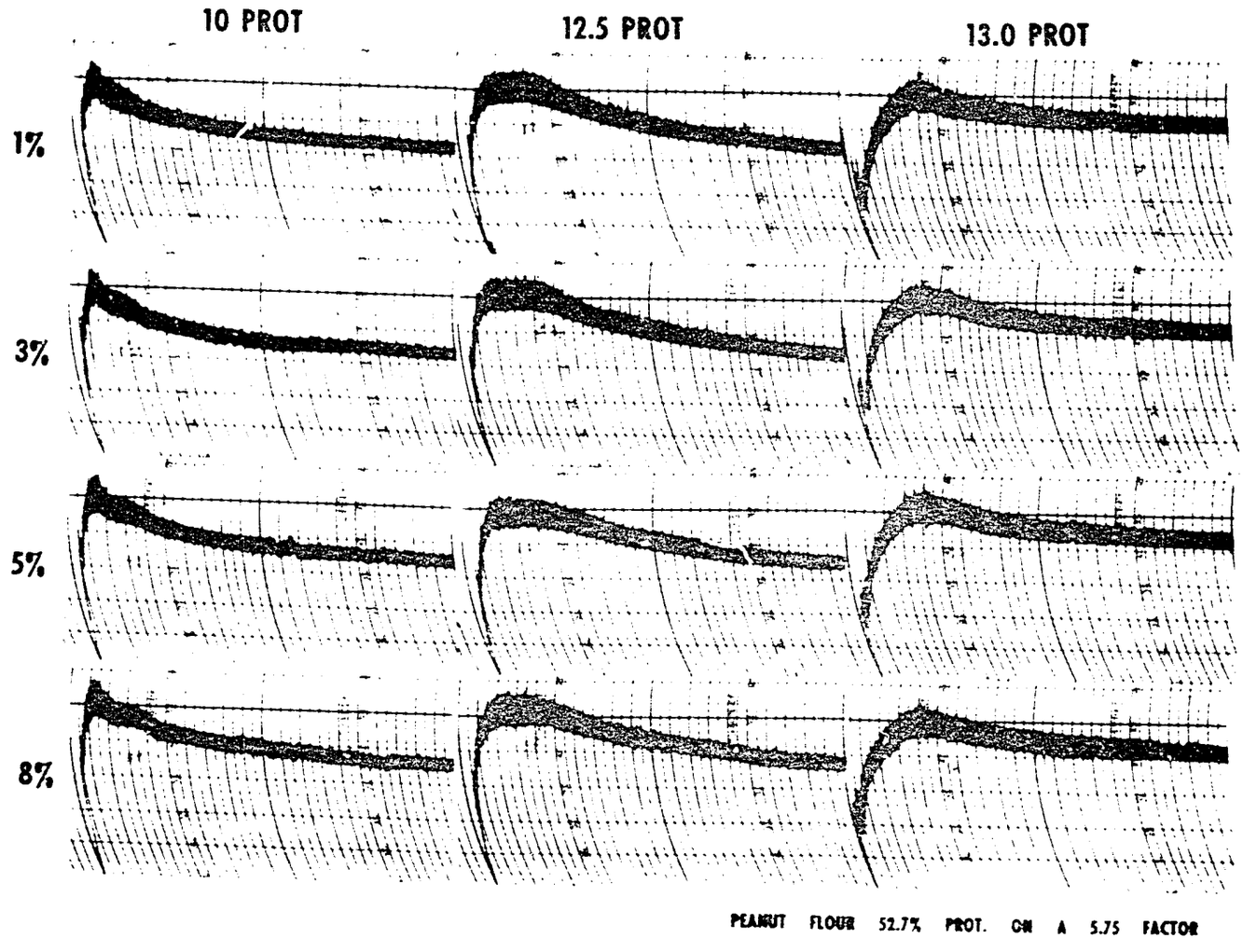


Fig. 9.

FARINOGRAMS OF PEANUT AND WHEAT FLOUR BLENDS

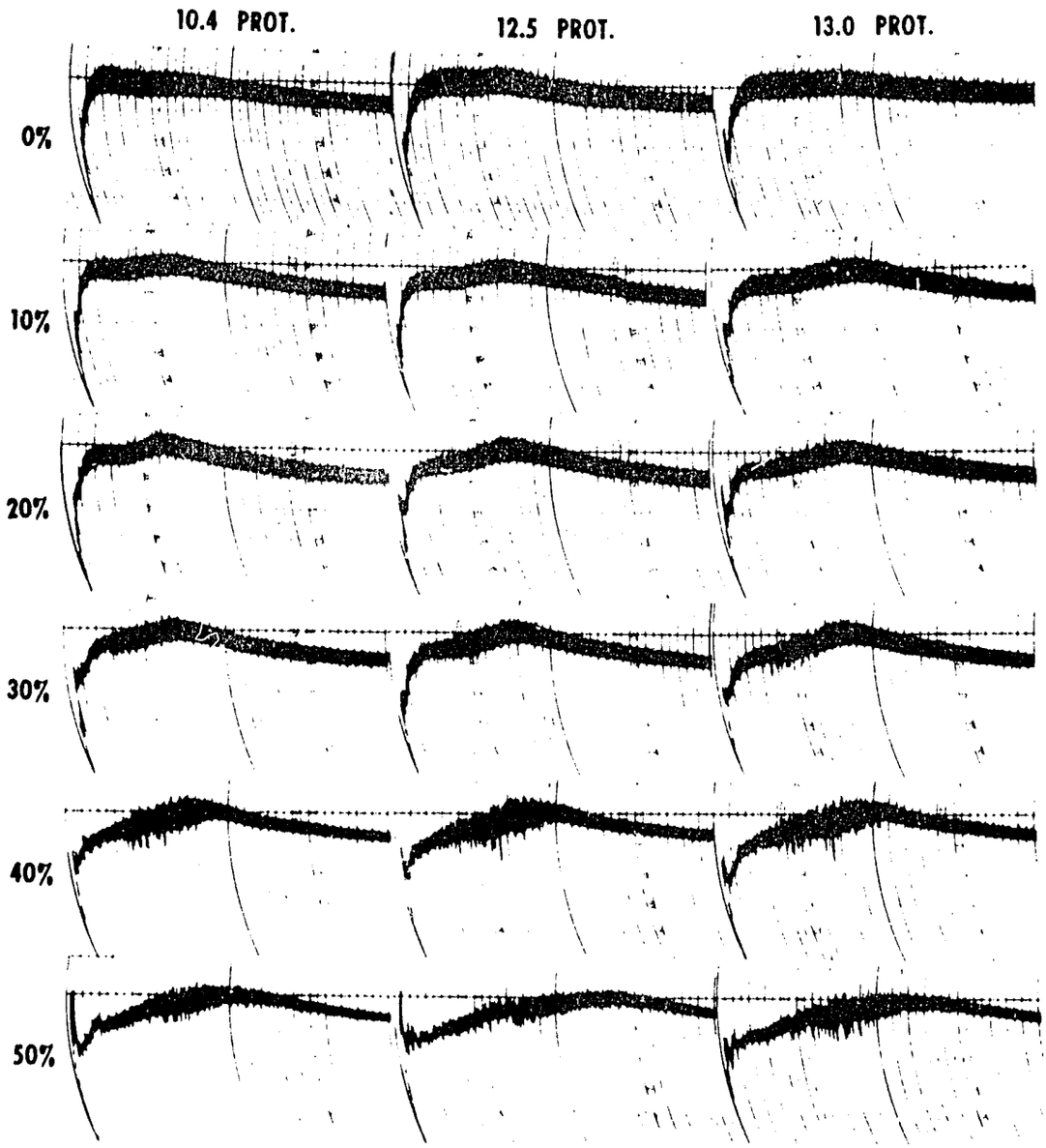


Fig. 10

PEANUT FLOUR 52.7% PROT. ON A 5.75 FACTOR

TABLE 31. FARINOGRAPH CHARACTERISTICS

	% Peanut flour	% Absorp.	Peak time (Min.)	Arrival time (Min.)	Depart time (Min.)	Stability time (min.)	MTI (B.U.)	Valor. Value (B.U.)
Wheat flour (10.4)	1	54.5	1.00	0.75	1.75	1.00	140	12
	3	54.8	1.25	1.00	1.75	0.75	150	13
	5	54.7	1.50	1.00	2.50	1.00	140	15
	8	55.9	1.25	0.75	3.00	1.75	130	14
	0	60.3	2.50	1.00	10.00	9.00	20	31
	10	64.3	6.50	2.00	9.00	7.00	20	62
	20	69.0	6.00	4.00	10.00	6.00	50	58
	30	73.5	7.00	4.00	9.50	5.50	70	61
	40	79.9	8.50	5.00	11.00	6.00	60	68
	50	83.6	10.00	5.50	13.50	8.00	40	73
ATTA	0	64.1	6.25	4.25	11.00	6.75	45	53
Wheat flour (12.5)	1	59.1	3.50	1.25	5.75	4.50	90	39
	3	59.3	3.50	1.00	6.00	5.00	90	39
	5	61.4	3.50	1.50	5.50	4.00	70	39
	8	61.8	3.50	1.25	6.25	5.00	80	39
	0	61.0	6.50	1.50	13.50	12.00	40	59
	10	65.4	7.50	4.00	12.00	8.00	40	63
	20	71.8	8.00	5.00	11.50	6.50	50	66
	30	76.2	8.00	5.00	11.00	6.00	60	66
	40	80.5	9.50	6.00	12.00	6.00	65	72
	50	85.5	13.50	9.00	16.50	7.50	40	83
Wheat flour (13.0)	1	65.0	4.00	2.50	6.50	4.00	60	45
	3	65.3	4.00	2.50	7.00	4.50	65	45
	5	65.8	4.00	2.25	8.50	6.25	60	45
	8	67.3	4.00	3.00	7.00	4.00	50	45
	0	63.5	7.50	1.50	19.00	17.50	30	64
	10	70.3	9.00	6.00	12.00	6.00	60	70
	20	72.3	8.50	5.50	12.00	6.50	50	68
	30	77.0	8.50	6.00	12.00	6.00	60	68
	40	81.5	10.00	6.00	13.50	7.50	60	74
	50	85.0	10.00	7.50	14.50	7.00	40	78

EXTENSOGRAMS OF PEANUT AND WHEAT FLOUR BLENDS

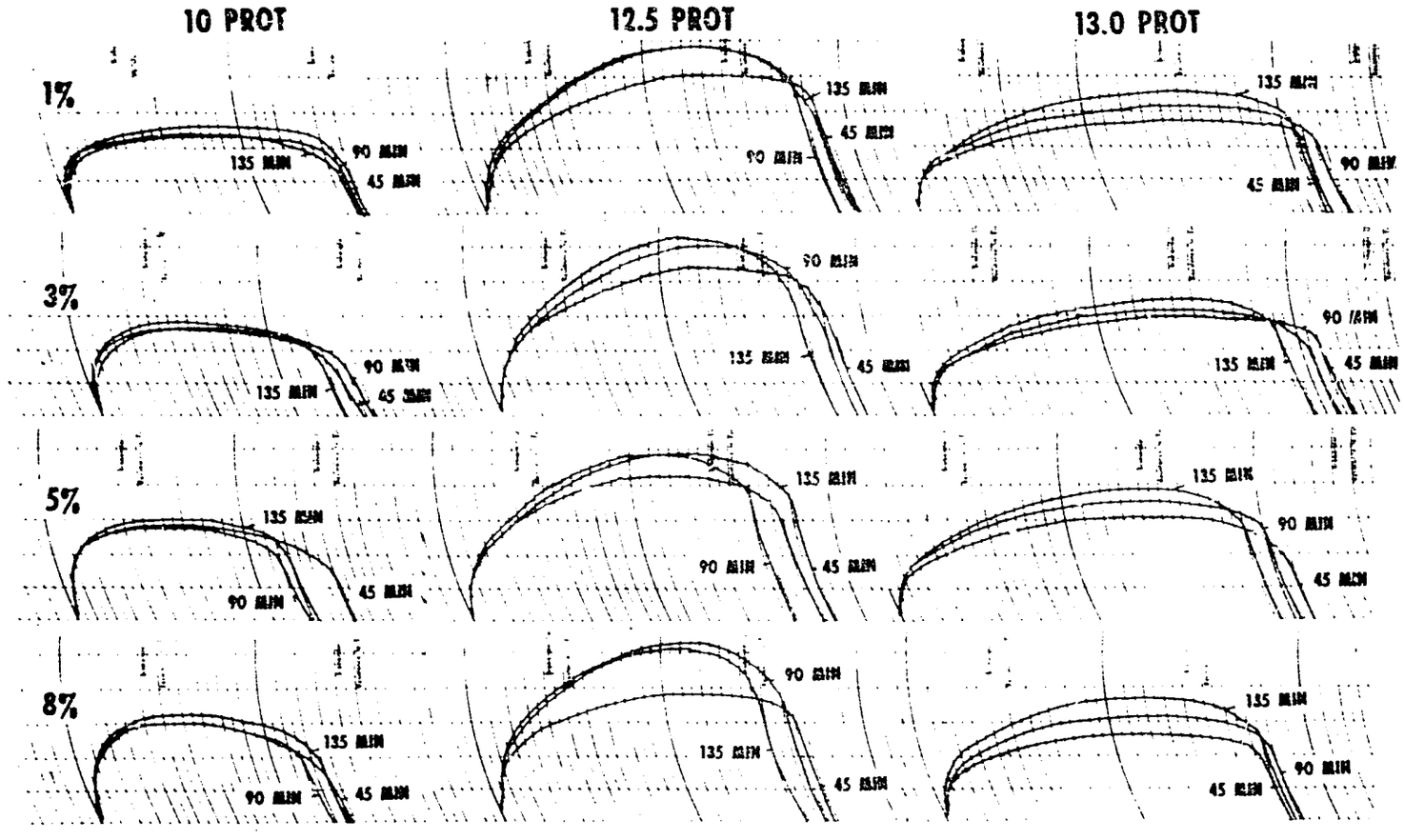
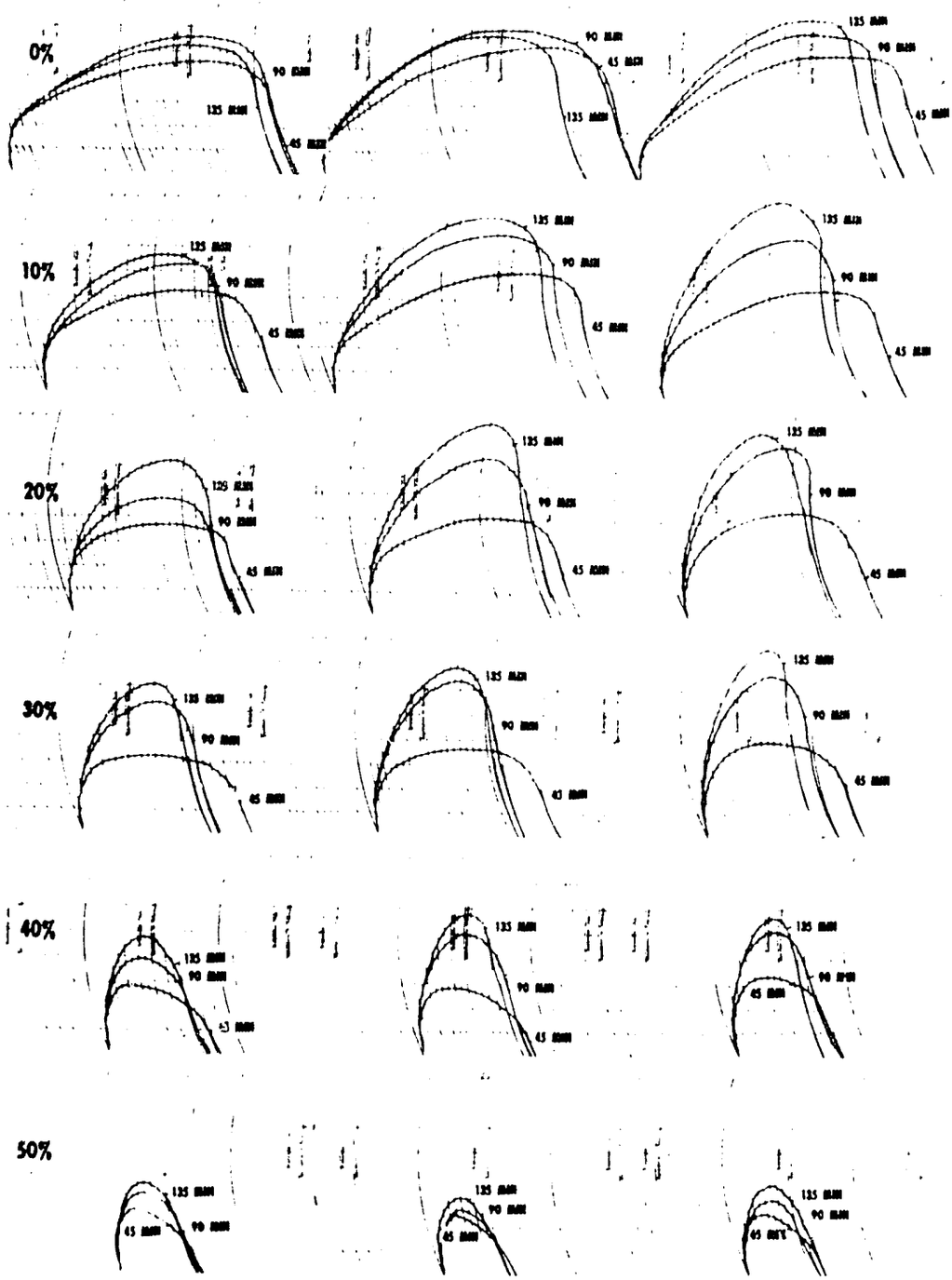


Fig. 11.

PEANUT FLOUR 52.7% PROT. ON A 5.75 FACTOR

EXTENSOGRAMS OF PEANUT AND WHEAT FLOUR BLENDS

10.4 PROT. 12.5 PROT. 13.0 PROT.



PEANUT FLOUR 52.7% PROT. ON A 5.75 FACTOR

Fig. 12

Sample	Extensibility (m.m)			Resistance (B.U.)			Area (CM^2)			Ratio ($\frac{Resistance}{Extensibility}$)		
	45'	90'	135'	45'	90'	135'	45'	90'	135'	45'	90'	135'
Low protein wheat flour (10.4%) blended with peanut flour	(Min.)			(Min.)			(Min.)			(Min.)		
0%	210	205	210	320	335	345	132.1	144.2	148.4	1.52	1.63	1.64
1%	150	148	150	250	220	225	54.4	48.8	46.6	1.67	1.49	1.50
3%	135	140	130	260	260	255	48.5	50.2	50.2	1.93	1.86	1.96
5%	145	125	135	260	250	270	53.0	43.8	50.2	1.41	2.00	2.00
8%	125	115	135	300	300	310	52.6	48.4	56.8	2.40	2.61	2.30
10%	160	155	150	330	390	435	98.2	106.0	112.7	2.06	2.52	2.90
20%	140	130	120	330	430	520	70.6	83.1	94.7	2.36	3.31	4.33
30%	120	105	95	370	485	575	53.0	71.5	73.8	2.67	4.62	6.05
40%	75	70	70	230	355	430	25.8	34.6	38.4	3.07	5.07	6.14
50%	60	60	60	170	240	285	19.1	24.3	27.9	2.83	4.00	4.75
Medium protein wheat flour (12.5%) blended with peanut flour												
0%	240	235	215	290	330	350	170.9	174.3	155.3	1.21	1.40	1.63
1%	190	185	180	290	350	330	101.6	119.6	114.7	1.53	1.89	1.83
3%	185	180	175	310	340	380	108.3	117.0	115.3	1.68	1.89	2.17
5%	170	160	185	320	360	330	96.4	105.0	114.5	1.88	2.25	1.78
8%	165	170	160	310	395	390	87.1	114.1	107.8	1.88	2.32	2.44
10%	205	185	175	310	405	470	132.7	152.4	159.5	1.51	2.19	2.69
20%	150	140	135	350	500	610	90.0	120.1	129.0	2.33	3.57	4.52
30%	135	115	105	345	540	570	69.4	89.5	93.2	2.56	4.70	5.43
40%	85	80	75	295	480	575	34.3	49.7	52.2	3.47	6.00	7.67
50%	55	55	50	130	140	150	18.6	17.5	20.4	2.36	2.55	3.00
High-protein wheat flour (13.0%) blended with peanut flour												
0%	235	190	190	290	340	365	158.8	142.4	148.2	1.23	1.79	1.92
1%	215	215	205	205	235	240	79.5	94.1	89.4	.95	1.09	1.17
3%	215	205	205	220	240	260	84.6	82.4	93.5	1.02	1.17	1.27
5%	205	205	195	230	260	290	82.9	97.8	100.6	1.12	1.27	1.49
8%	175	185	180	215	270	300	61.5	84.5	92.6	1.23	1.46	1.67
10%	185	155	150	315	470	565	110.8	125.6	147.5	1.70	3.03	3.77
20%	145	125	115	390	545	690	87.8	111.6	111.3	2.70	4.36	6.00
30%	120	105	95	370	605	720	61.7	88.4	87.2	3.08	5.76	7.58
40%	80	80	70	330	490	570	36.1	50.0	49.4	4.12	6.13	8.14
50%	65	55	60	200	140	300	19.7	21.9	27.3	3.08	2.55	5.00

The extensograph and farinograph characteristics for blends of flour followed the same trends. Extensibility seemed to decrease as percentage of peanut flour increased. However, there was no apparent difference in resistance. The dough extension decreased and resistance to extension increased as peanut flour in the blend increased (areas under the extensograph curves).

Baking Results. Baking experiments were on a laboratory scale. Effects of dough composition were tested by using a complete formula and a straight dough procedure. The basic dough formula was:

Ingredient	% (Based on flour wt.)
Flour	100
Sugar	6
Milk	3
Shortening	3
Salt	2
Malt	0.5
Yeast	2
$KBrO_3$	10 ppm
Water	Farinograph

After being mixed to optimum consistency (mixing time correlated with farinograph-dough development), the doughs were fermented at 85°F., punched after 110 minutes, punched again 50 minutes later, divided, rested 20 minutes, and molded. Proofing was at 90°F. for 45 minutes and baking was 25 minutes at 410°F.

Loaf volumes and weights were determined on freshly baked loaves. After cooling, the loaves were scored with maximum possible values of specific volume, 20; crust color, 10; symmetry, 10; break and shred, 10; grain, 20;

texture, 20; crumb color, 10, for 100 total. Baking data are summarized in Table 33. Baking qualities and extensograph characteristics correlated for 12.5 and 13.2% protein flours blended but not for blends with wheat flour containing 10.4% protein. Blends with wheat flour of 12.5% or 13.2% protein gave bread with lower total score and specific volume as percentage of peanut flour increased. Peanut flour decreased the total score less when added to wheat flour containing 13.2% protein than when added to that with 10.4% protein.

Increasing peanut flour in the formula (Figures 13 and 14) gave tighter grain and firmer texture. Symmetry of loaves was more consistent as percentages of peanut flour increased to 10%.

TABLE 33. BAKING VALUES AND SCORES.

Sample	Volume (c.c)	Specific vol. (cc/g)	Total sco: (pts.)
A. Low protein wheat flour (10.4%) blended with peanut flour			
0%	708	4.82	73
1%	613	4.35	36
3%	595	4.13	31
5%	528	3.64	22
8%	445	3.03	16
10%	488	3.15	39
20%	388	2.52	25
30%	305	1.99	13
40%	308	1.88	11
50%	273	1.80	11
B. Medium protein wheat flour (12.5%) blended with peanut flour			
0%	815	5.54	80
1%	675	4.59	77
3%	738	5.16	59
5%	720	4.97	53
8%	690	4.63	47
10%	598	3.96	47
20%	438	2.84	29
30%	365	2.28	15
40%	335	2.04	14
50%	268	1.70	11
C. High-protein wheat flour (13.0%) blended with peanut flour			
0%	818	5.53	81
1%	783	5.26	70
3%	790	5.27	65
5%	780	5.17	62
8%	740	4.93	55
10%	535	3.59	35
20%	465	2.96	28
30%	378	2.30	16
40%	308	1.91	13
50%	280	1.73	11

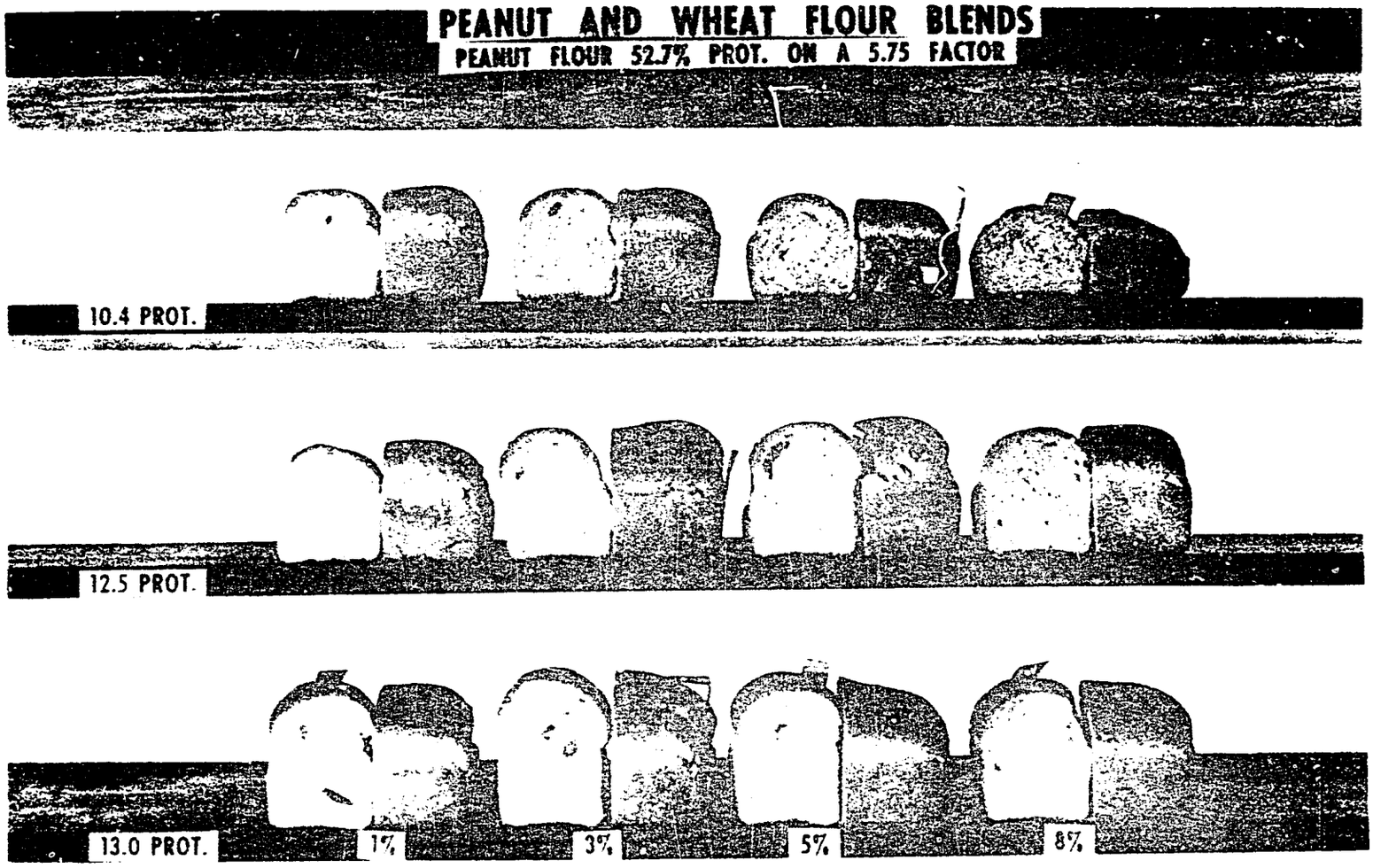
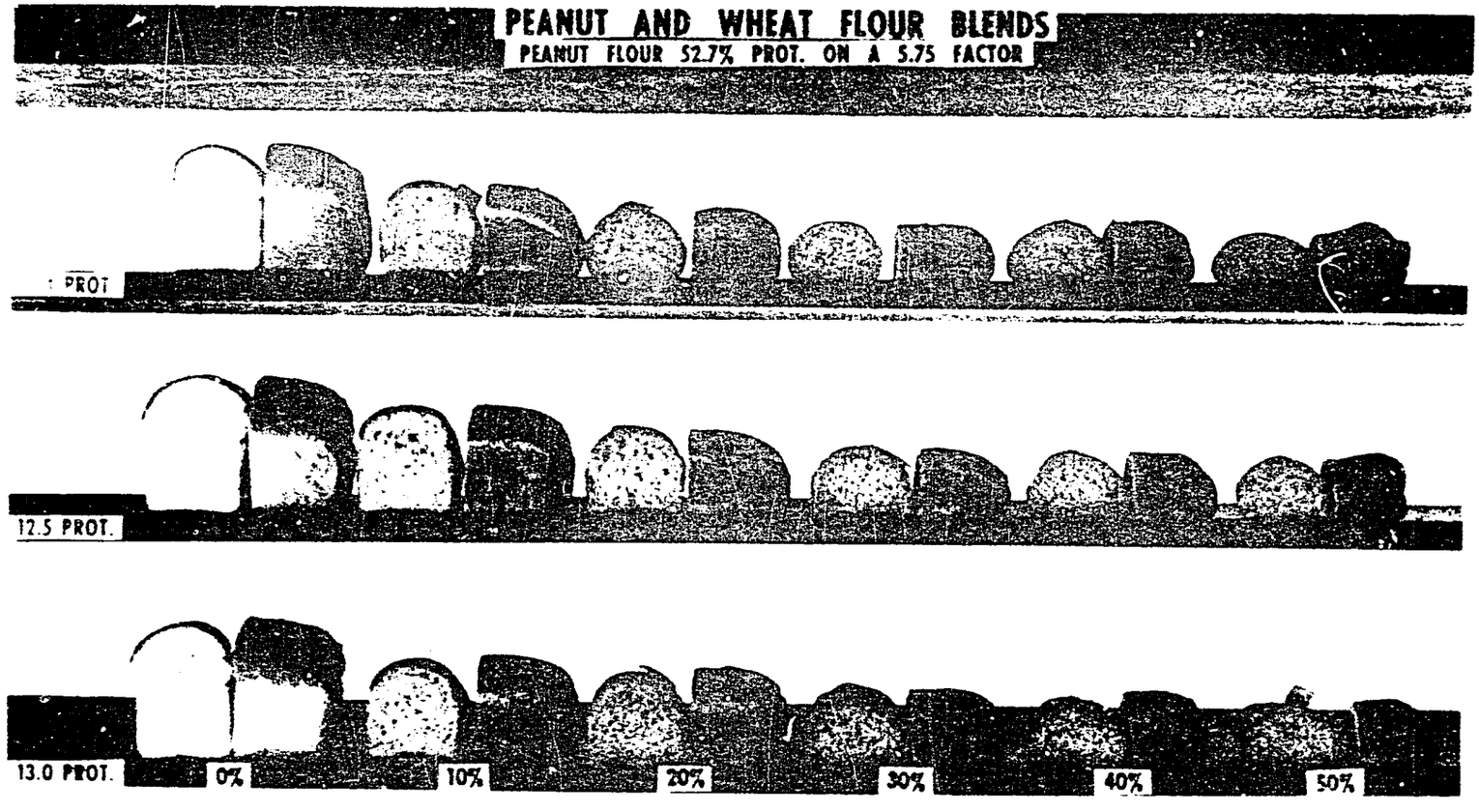

PEANUT AND WHEAT FLOUR BLENDS
PEANUT FLOUR 52.7% PROT. ON A 5.75 FACTOR
10.4 PROT.
12.5 PROT.
13.0 PROT.
1%
3%
5%
8%

Fig. 13.


PEANUT AND WHEAT FLOUR BLENDS
PEANUT FLOUR 52.7% PROT. ON A 5.75 FACTOR
PROT
12.5 PROT.
13.0 PROT.
0%
10%
20%
30%
40%
50%

SUPPLEMENTATION OF CHAPATIS WITH PEANUT FLOUR

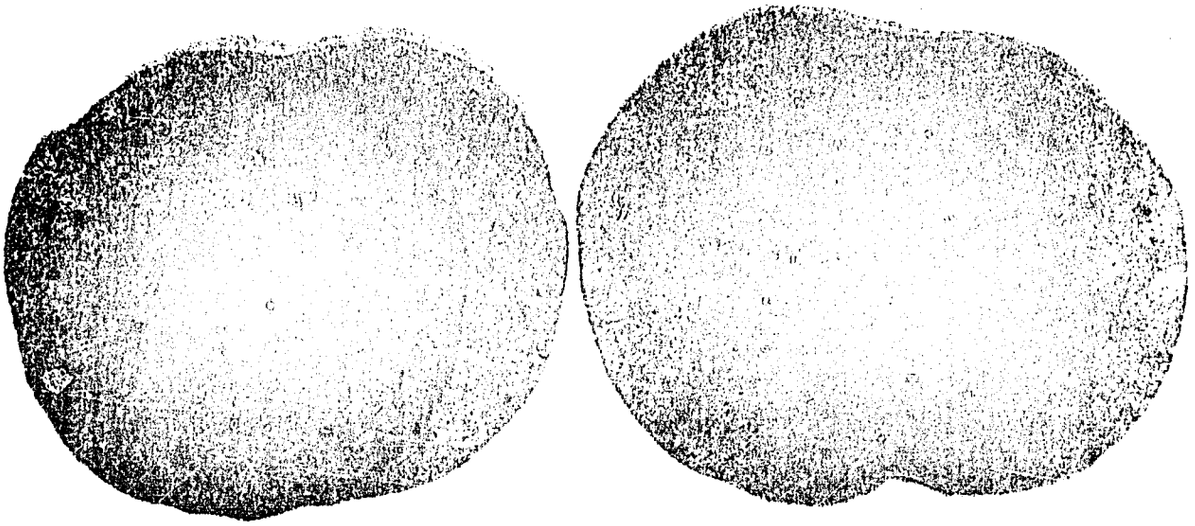
The formula for chapatis was:

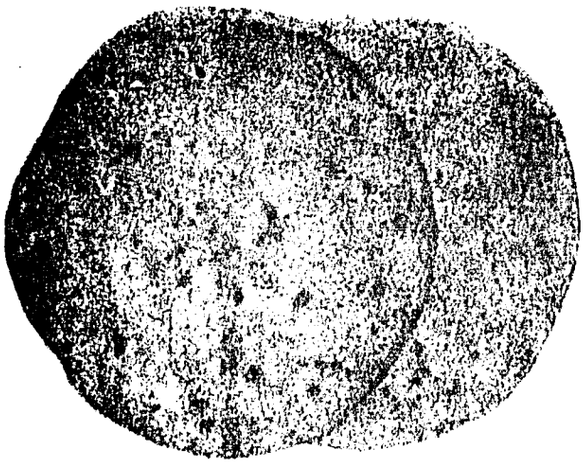
ATTA	1362	g.
Salt	28.35	g.
Oil	9.4	g.
Water	878	ml.

The atta, salt, oil, and half the water were mixed in a Hobart (N-50) mixing bowl at speed "1" until dough formed. The remaining water was added and mixing continued at speed "2" for one minute. The dough was covered and rested for 30 minutes, then 50 g. patties, 2½" x ¼", were rolled into 6" circles, and cooked on both sides on an electric grill at 400°F. The chapati was puffed by pressing its sides with a padded cloth. More water was needed as peanut flour in the atta increased.

An experienced panel scored chapatis for aroma, texture, and appearance. Blends with more than 20% peanut flour were objectionable in aroma, flavor, texture, and appearance. Acceptable chapatis are shown in Figure 15.

ATTA 97% EXTRACTION GAINES WHEAT





CHAPATIS

Fig. 15.

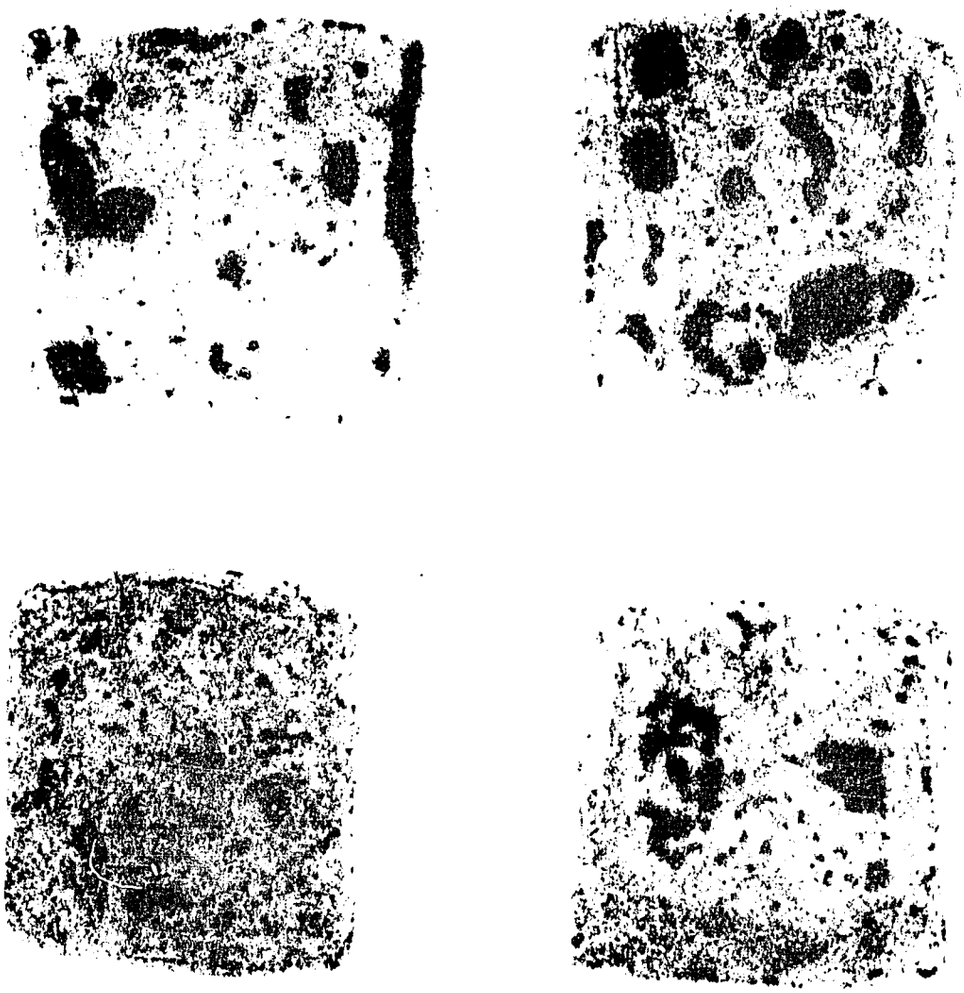
SUPPLEMENTATION OF PARATHAS WITH PEANUT FLOUR

Formula and procedure for parathas:

ATTA	1362	g.
Salt	56.70	g.
Oil	28.35	g.
Water	890	ml.

The ingredients were mixed according to the procedure for chapatis. Dough was rolled into a 2½" x ¼" (50 g.) flat patty then into a 6 to 8" circle. With a pastry brush each circle was painted with 9.5 g. oil and folded into thirds, then folded twice to form a square. The squares were rolled very thin and baked on an electric grill at 400°F. After baking on one side, they were turned, painted with oil, baked, turned again and painted with oil, then removed from the grill and scored subjectively. A much higher percentage of peanut flour was acceptable with parathas than with chapatis, judged by aroma, flavor, texture, and appearance (Figure 16).

ATTA 97% EXTRACTION GAINES WHEAT



PARATHA

SUPPLEMENTATION OF NAN WITH PEANUT FLOUR

Formula and procedure for nan:

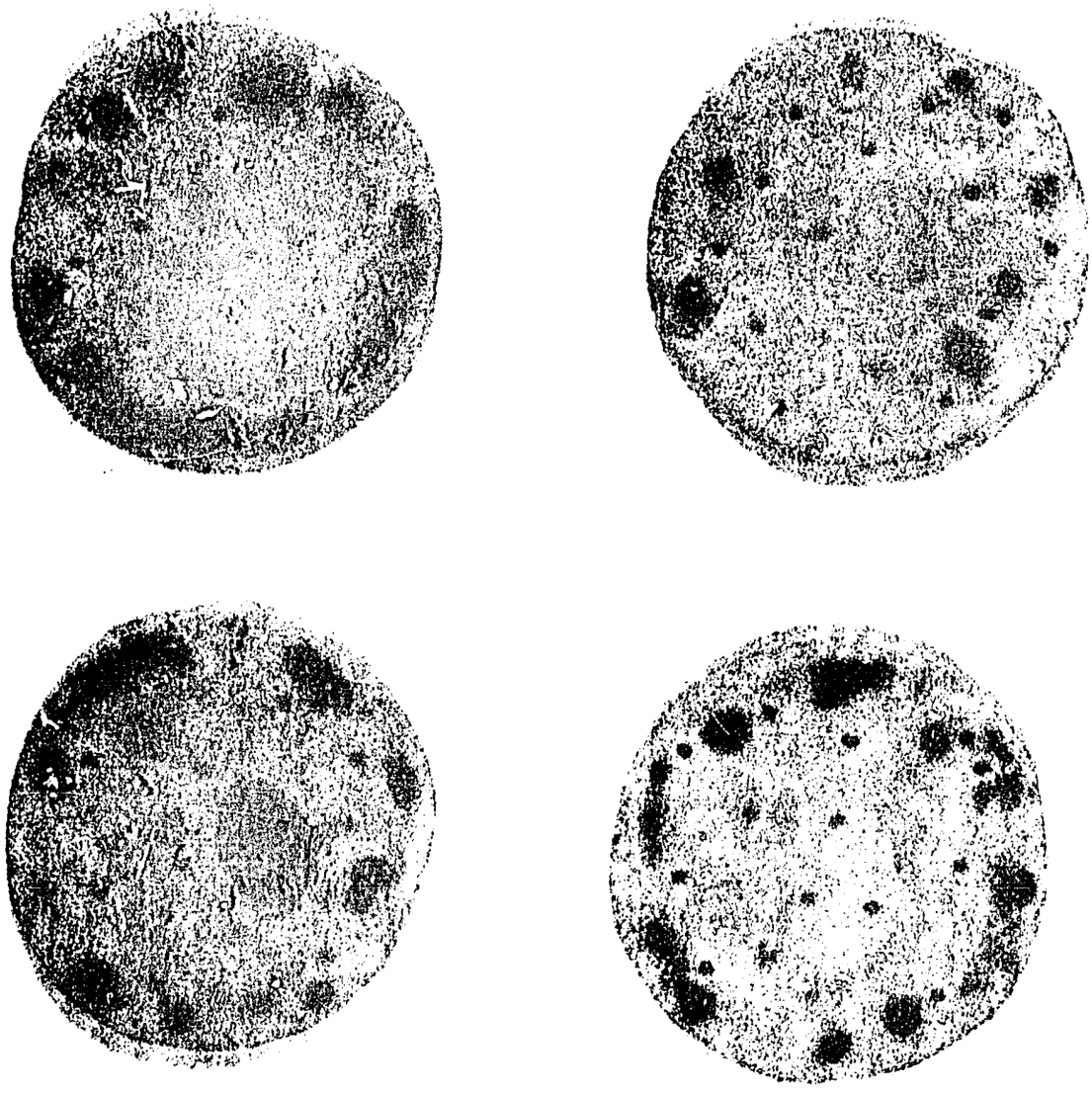
ATTA	1362 g.
Compressed yeast	27 g.
Oil	40 g.
Salt	20 g.
Water	895 ml.

A hearth oven was preheated to 500°F. with moisture adjusted. Yeast was dispersed in 100 ml. water. All ingredients except half the water were mixed at speed "1" on a Hobart N-50 mixer to dough formation. The remaining water was added and mixing continued at speed "2" for one minute. The dough was proofed for one hour, scaled at 120 g. portions, and rolled on a lightly floured board into 6 to 8" circles ½" thick. They were baked without a rack in the oven for 3 minutes on one side and 2 minutes on the other side. Baked nans were wrapped in dry cloth (Figure 17) and later scored by the panel for aroma, flavor, texture, and appearance.

Increased peanut flour affected nans as it had white bread.

As the character of the atta products changed rapidly on cooling, the outside and the inside were tested both when hot and when cool. Products with higher percentages of peanut flour were heavy, had good keeping quality, and improved in flavor and aroma on aging.

ATTA 97% EXTRACTION GAINES WHEAT



NANS

Fig. 17.

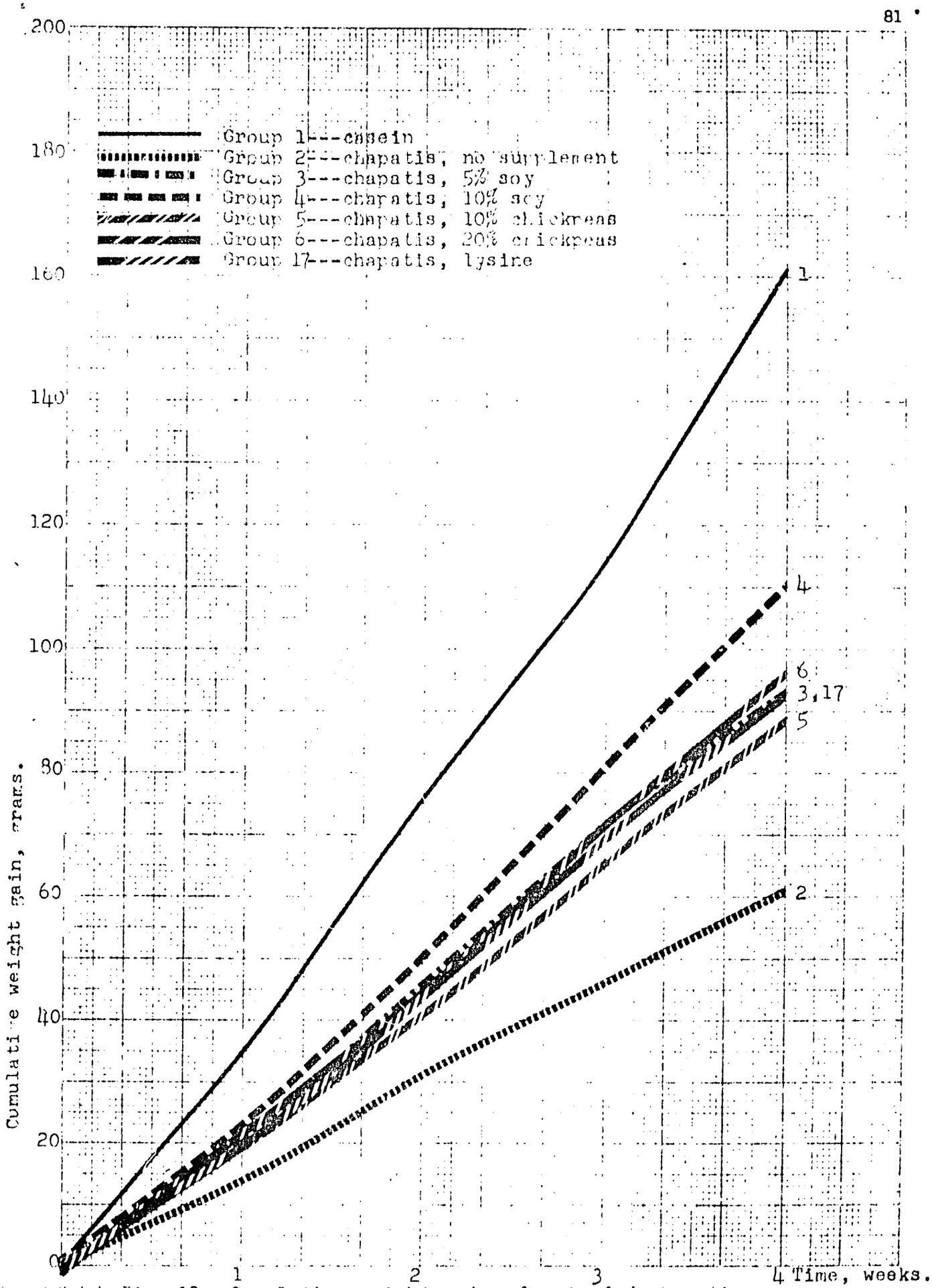
NUTRITIONAL EVALUATION OF SUPPLEMENTED CHAPATIS, PARATHAS AND NAN

Rat Studies I

The effect of supplementing atta products with soy protein concentrate and ground chick peas was studied. Soy was substituted for 5 or 10% and ground chick peas for 10 or 20% of the wheat flour, before the products were prepared. Vitamins and minerals were added after preparation of the product at the same level as in cous cous Rat Study II (see page 107). Both the fat and protein contents were adjusted to 10%. The following rations were prepared.

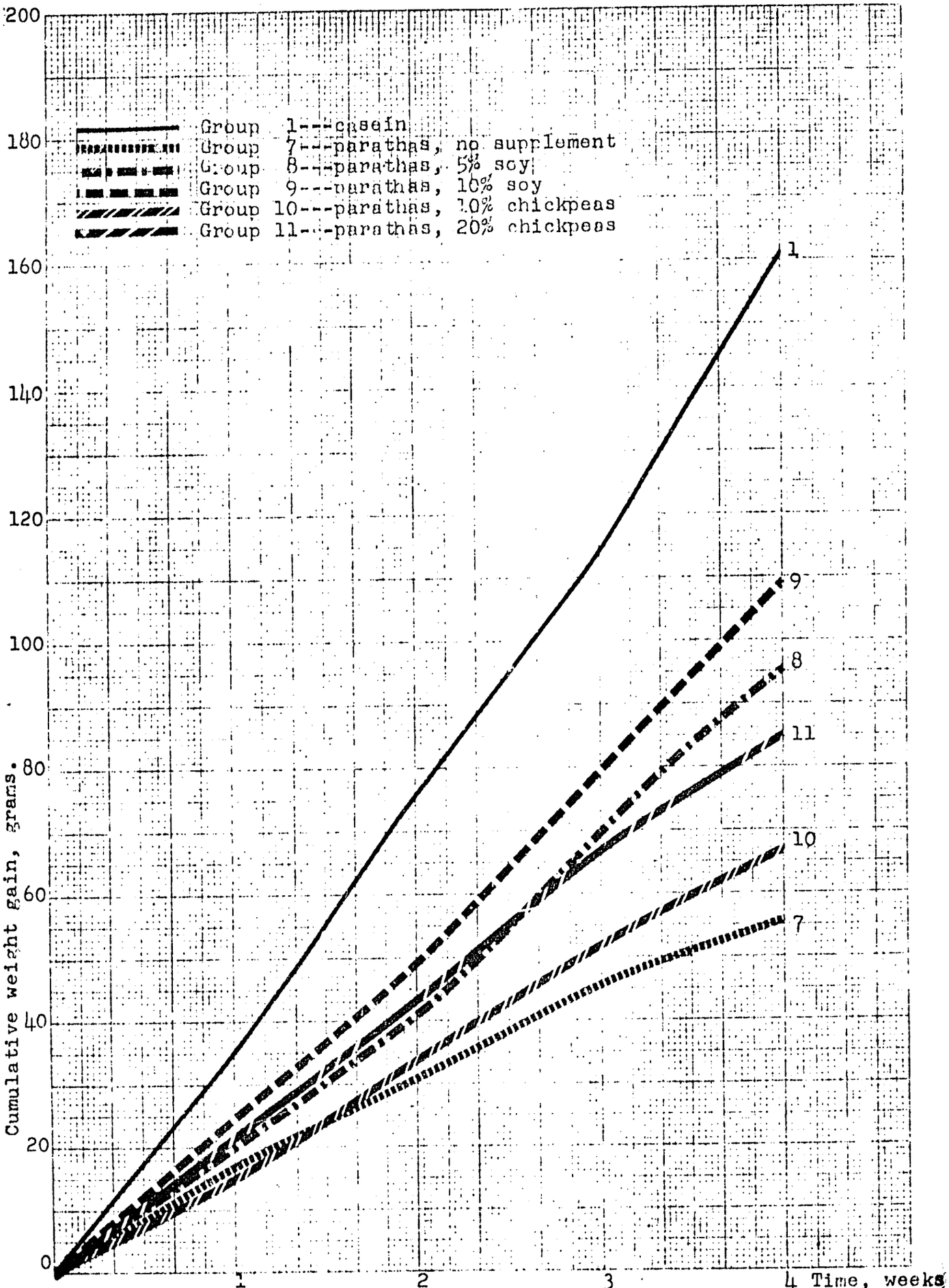
Diet 1 - casein control
Diet 2 - chapatis, no supplement
Diet 3 - chapatis, 5% soy
Diet 4 - chapatis, 10% soy
Diet 5 - chapatis, 10% chick peas
Diet 6 - chapatis, 20% chick peas
Diet 7 - parathas, no supplement
Diet 8 - parathas, 5% soy
Diet 9 - parathas, 10% soy
Diet 10 - parathas, 10% chick peas
Diet 11 - parathas, 20% chick peas
Diet 12 - nan, no supplement
Diet 13 - nan, 5% soy
Diet 14 - nan, 10% soy
Diet 15 - nan, 10% chick peas
Diet 16 - nan, 20% chick peas
Diet 17 - chapatis, 0.19% lysine

Diets were fed to groups of eight male weanling albino rats of the Charles River strain for 28 days. Weekly cumulative weight gains were plotted (Figures 18, 19, 20). PER were calculated at the end of 28 days (Table 34).



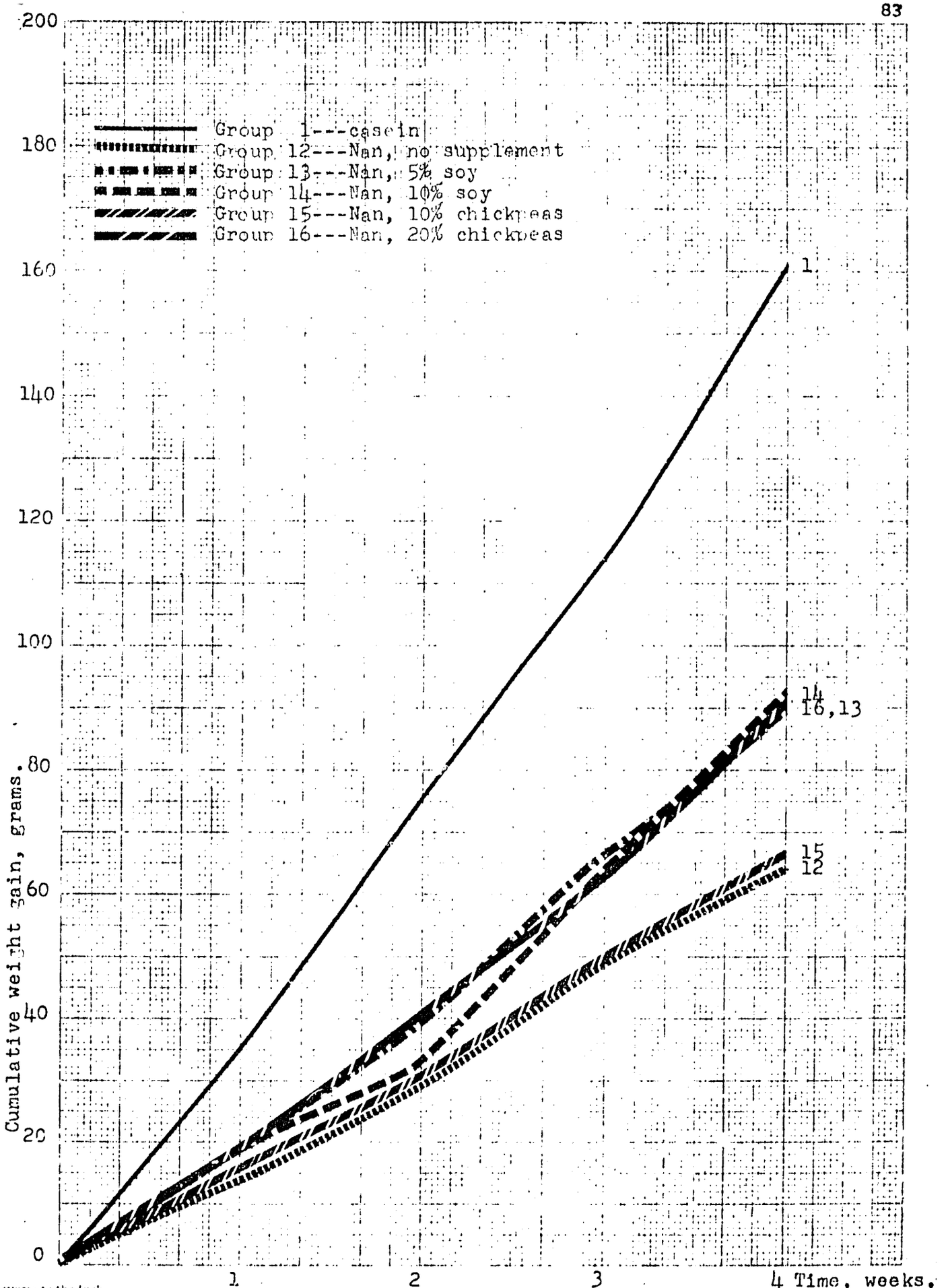
20 squares to the inch

Fig. 18. Cumulative weight gain of rats fed chapatis.



20 Squares to the inch

Fig. 19. Cumulative weight gain of rats fed parathas.



20 Squares to the inch

Fig. 20. Cumulative weight gain of rats fed nan.

TABLE 34. PER FOR DIETS MADE OF ATTA PRODUCTS SUPPLEMENTED WITH SOY OR CHICK PEAS.

Diet	PER	Diet	PER	Diet	PER
1	3.474	1	3.474	1	3.474
	*		*		*
4	2.806	9	2.645	14	2.773
	n.s.		n.s.		*
6	2.754	11	2.491	13	2.408
	*		n.s. *		n.s.
17	2.504	8	2.438	16	2.406
	n.s.		n.s.		*
5	2.479	10	2.208 *	15	2.217
	n.s.		n.s.		n.s.
3	2.462	7	1.989	12	2.164
	*				
2	2.111				
$LSD_{0.05}$=0.222			0.237		0.156

The following generalizations have been made about PER:

1) Casein produced a higher PER than any other protein source studied.

2) Protein quality in chapatis was improved by all supplements added to those products. Protein quality in parathas and nan was improved by all supplements except 10% chick peas.

3) Ten percent soy improved PER more than 5% soy in chapatis and nan, but not in parathas.

4) Twenty percent chick peas improved PER more than 10% chick peas in chapatis, parathas, and nan.

5) Ten percent soy and 20% chick peas were equally effective in chapatis and parathas.

6) [illegible] percent soy and 10% chick peas were equivalent in chapatis and parathas. Lysine did not differ from these supplements in chapatis.

7) Five percent soy improved PER as much as 20% chick peas in parathas and nan.

Some hair loss was noted in a few of the animals, but did not appear to be associated with a particular diet. Dry weight and calcium content of the femurs will be determined.

ORGANOLEPTIC EVALUATION OF CHAPATIS

Three protein supplements have been evaluated for their performance in chapati preparation. A commercially available fish protein concentrate (FPC), a commercially available soy protein concentrate (SPC), and a pilot plant sample of glandless cottonseed flour (CSF) each were used to replace Gaines wheat flour at the 5, 10, and 15% levels by weight. "Standardized" procedures were developed for the preparation and examination of all samples. Flour use properties were described and both flours and chapatis were examined by a flavor profile panel. Differences between the supplemented blends and the unsupplemented control flour were described.

Results and Conclusions. The results appear in Tables 35 and 36. It was concluded that the FPC-supplemented flours had atypical flour use properties and chapati eating qualities. The 15% and possibly the 10% SPC flour appeared to be unacceptable for the same reasons. SPC supplementation at the 5% level did not adversely affect the potential acceptability of the flour blend. The presence of black specks in all CSF-supplemented flours studied contraindicated the use of this particular supplement for chapatis, although other properties of the CSF-supplemented flours were not negative.

Publications

Performance of Protein Supplemented Flours in Chapatis, a Master's thesis by Janet L. Archer, was published in August, 1970. A paper based upon this thesis is being prepared and an abstract of the paper will be included in the program of the May, 1971, meeting of the Institute of Food Technologists.

TABLE 35. PERFORMANCE COMPARISONS BETWEEN EACH TYPE OF SUPPLEMENTED FLOUR BLEND AND THE CONTROL.

	FP[a]			CSF[b]			SPC[c]		
	5%	10%	15%	5%	10%	15%	5%	10%	15%
Flour									
Appearance	-	-	-	-	-	-	?	?	?
Aroma	?	?	-	?	-	-	?	?	-
Flavor	-	-	-	?	-	-	?	-	-
Dough									
Appearance	-	-	-	-	-	-	+	+	+
Aroma-mixing	-	-	-	-	-	-	-	-	-
Aroma-cooking	+	+	?	+	+	?	+	+	+
Mixing properties	-	-	-	-	-	-	-	-	-
Handling properties	-	-	-	?	?	?	?	?	-
Cooking properties	+	-	-	+	+	+	?	[illegible]	-
Chapati									
Appearance	-	-	-	-	-	-	+	-	-
Aroma	-	-	-	-	?	-	?	[illegible]	
Flavor	-	-	-	-	?	-	?		[illegible]
Texture	-	-	-	-	-	-	+	-	-

[a]Food Grade Type I Marine Protein Concentrate, Alpine Marine Protein Industries, Inc.

[b]Glandless cottonseed flour, Texas A & M University.

[c]Fine Grind Swift's Food Protein, Swift Chemical Co.

Key:

- \+ similar to the control
- ? may be slightly different from the control (or small differences occasionally found)
- \- definitely different from the control

TABLE 36. FLAVOR PROFILE COMPARISONS BETWEEN EACH TYPE OF SUPPLEMENTED CHAPATI AND THE CONTROL.

	FPC[a]			CSF[b]			SPC[c]		
	5%	10%	15%	5%	10%	15%	5%	10%	15%
Aroma									
Amplitude	?	-	-	?	?	-	?	?	?
Toasted wheat	-	-	-	-	?	-	+	-	-
Starchy	-	-	-	+	-	-	?	?	?
Raw doughy	-	-	-	-	-	-	-	-	-
FPC character	-	-	-						
CSF character				-	?	-			
SPC character							?	?	-
Flavor									
Amplitude	-	-	-	-	-	-	?	?	?
Sweet	-	-	-	-	+	+	+	+	+
Toasted wheat	-	-	-	-	-	-	+	-	-
Starchy	+	+	+	?	-	?	+	+	?
Raw doughy	+	?	?	?	?	?	?	?	?
FPC character	-	-	-						
CSF character				-	?	-			
SPC character							?	-	-
Aftertaste									
Wheat	?	-	-	-	-	-	+	?	?
Sweet	?	-	-	?	-	-	+	?	-
FPC character	?	-	-						
CSF character				?	?	-			
SPC character								?	?

[a]Food Grade Type I Marine Protein Concentrate, Alpine Marine Protein Industries, Inc.

[b]Glandless cottonseed flour, Texas A & M University

[c]Fine Grind Swift's Food Protein, Swift Chemical Co.

Key:
- + similar to the control
- ? may be slightly different from the control
- - definitely different from the control

EFFECT OF PROCESSING AND PROTEIN SUPPLEMENTATION (HORSE BEAN FLOUR) ON COUS-COUS

V. J. Gainor, R. J. Robinson, A. B. Ward and W. J. Hoover

Cous-cous is a cereal based food common in developing countries of North Africa. This study investigated effects on physical characteristics of processing methods and of supplementing cous-cous with horse bean (Vicia faba) flour. Cous-cous were made from 70% extraction flour, semolina, or 85% extraction flour of durum wheat. The flour was agglomerated with water in 3000 g batches. Agglomerated particles were sieved, separated and sized, then dried steamed 7 min. or autoclaved for 4 min. and dried at 50°C. for 20 hours. Horse bean flour was blended at 3 levels, from 5 to 15%, with three durum flours, to increase protein content of the flours.

Water Absorption Index (WAI) values were greater for the steamed or autoclaved cous-cous than for agglomerated cous-cous. Horse bean flour supplementation decreased the WAI values of steamed cous-cous and increased the WAI values of autoclaved cous-cous.

Water Solubility Index (WSI) values were higher for agglomerated cous-cous than for the steamed or autoclaved samples. (WSI values in general increased as horse bean flour supplementation increased).

The steamed cous-cous had higher amylograph values than did agglomerated or autoclaved cous-cous. Supplementing with horse bean flour tended to decrease the amylograph values.

Literature Review

Agglomeration

Agglomeration occurred when dry, powdery flour base materials were dispersed in a humid atmosphere to wet the surface and form adhesive gluten on the flour particles. The dispersion was then mechanically agitated to

cause random collision and agglomeration, with the agglomerates having a loose bulky density not less than that of the starting material (Gidlow, R., and Teders, R., 1968) (43).

Armstrong, et al., 1968, (44) stated that agglomerates were produced by suspending fine particles in a fluidized bed with heated gas, such as air, an atomizing a fluid-agglomerating agent directly in the fluidized bed, the fluid wetting and agglomerating the particles. Other methods included the spraying of the flour particles with an edible, sticky, cohesive, fluid agglutinant. The particles were moved slightly and mixed with the sticky fluid, forming non-sticky free running agglomerates (Hair and Lawrence, 1968) (45).

The agglomeration of high-protein cereal flour extracted an initial moistening of the flour with subsequent transferral to a mixing zone where it was agglomerated (Galle, 1968) (46). The agglomerates were then dried.

Swanson and Finske, 1968, (47) used a method for the agglomeration of small particles of flour. The moisture contents in the flour particles were lowered by predrying below the equalibrium value at a given temperature in air of suitable relative humidity. The flour particles were then exposed to air at a given temperature and then agglomerated.

In the maccaroni industry, water was sprayed from a pipe with multiple small holes during the agglomeration of semolina (Hummel, 1966) (48). It was not important how the water was added as long as the water was added evenly on the semolina and not on the surrounding metal mixing parts.

Gelatinization

Research has shown that when starch was mixed with water no changes occurred, but as the water was heated, the viscosity of the mixture increased (Meyer, 1968) (49). When the concentration of the starch was at a high enough

level, a paste formed. At times when the starch did not form a thick paste at the high temperature, a gel was formed when the temperature of the suspension was cooled.

Woodruff and Webber, 1933, (50) observed the first changes of heated starch occurring at 55°C., when the largest granules swelled slightly and lost their birefringence. As the temperature was increased, these changes were observed in an increasing number of granules. In 1928 Katz (51) found that gelatinization of starch took place in steps and that for some starches these steps occurred at widely differing temperatures.

Crossland and Favor, 1948, (52) reported that the various stages of swelling occurring during gelatinization of certain starches could be shown by viscosity measurements in the amylograph.

Work done with the modes of swelling of various starches, evaluated over a range of pasting temperatures, showed that the extent of starch solublization during pasting paralleled the swelling of the starch granules (Leach, et al., 1959) (53). Other studies indicated that starches of hard wheats gave higher viscosities than starches of soft wheats (Anker and Geddes, 1944 and Knowles and Harris, 1943) (54 and 55).

The rate of heating was found to have an effect on the degree of swelling in starch (Badenhuizen, 1959) (56). When starch was heated slowly the degree of swelling was reduced, a phenomenon supposed to be due to the additional hydrogen bonds formed during retrogradation.

Anderson, et al., 1969, (57) studies the gelatinization of corn grits by the use of an amylograph test. Five hundred grams of a 9% (dry basis) suspension of the sample (less than 60 mesh) was heated from 29 to 95°C. in 44 minutes. The temperature was then held at 95°C. for 16 minutes, then cooled to 50°C. in 30 minutes. The final cooked paste viscosity served as the criterion of suitability for the product.

Absorption

The absorption of water by grain or products milled from grain products refers to the water being taken into the material (Hlynka and Robinson, 1954) (58). During absorption, several different forces acted to keep the water within the grain. 'Free water' is held in the intergranular and intercellular spaces and within the constituents (such as starch granules) of the material, in which case the absorbing substance acts as a supporting structure. Water is also held to the substance by hydrogen bonds as a result of the interaction between the water molecules and those of the substance. The absorbed water may also combine chemically with the supporting substance.

Anderson, et al., 1969 (57), while working with the gelatinization of corn grits, developed a Water Absorption Index (WAI), and a Water Solubility Index (WSI) from a modification of a method for the measurement of the swell power of starch. A 2.5 gram sample of ground product (less than 60 mesh) was suspended in 30 ml. of water at 30°C. in a 50 ml. tared centrifuge tube. The suspension was stirred intermittently over a 30 minute period, and then centrifuged at 3000 x g for 10 minutes. The supernatant was poured carefully into a tared evaporating dish. The remaining gel was then weighed and the WAI was calculated from its weight. The WSI was calculated from the amount of dried solids recovered by evaporating the supernatants from the WAI test and was expressed as percent of dry solids in the 2.5 gram sample.

The Preparation of Cous-cous

The flour from durum wheat was used as the raw material for the preparation of cous-cous. In this particular study, semolina and 95% extraction flour were used individually as the raw materials.

The raw material was agglomerated with water, then sieved to separate the agglomerated particles. Following the sieving, the particles were either dried, or dried following steaming or autoclaving. The cous-cous particles were steamed prior to consumption.

The steps involved in the preparation of cous-cous are listed below:

1. The raw material was agglomerated in 3000 gram batches.
2. The flour was placed into the mixing bowl of a Hobart (A-200) mixer (with wire whip).
3. The mixer was set to speed number two and turned on.
4. Two hundred seventy-five grams of distilled water was added slowly and continuously over a two-minute period.
5. The mixing was continued for an additional two minutes after the addition of the water.
6. Following the second two-minute period, the mixer was stopped and the sides of the mixing bowl were scraped down.
7. The mixer was then started again at speed number two and 275 grams of distilled water was added over a two-minute period.
8. After the addition of the water, the mixing was continued for two minutes.
9. The mixer was then stopped, and the sides of the bowl were scraped down.
10. The mixer was started again at speed two and 80 grams of water were added over a two-minute period.
11. Following the addition of the water, the mixing was continued for two minutes.
12. The mixer was then turned off and the agglomerated particle was sifted through a nest of sieves consisting of U.S. Standard number eight (2.38 mm), twelve (1.68 mm), and eighteen wire (1.00 mm).

The overs of the number eight wire were considered too large and were discarded. The overs of the number twelve wire were designated as large to medium cous-cous, and the overs of the eighteen wire were considered fine

cous-cous. The throughs of the eighteen wire (pan) were considered too fine and were subject to further agglomeration. Following sieving, the cous-cous particles were processed by one of three different methods.

One method was to dry the cous-cous particles in a forced air oven at 50°C. for 20 hours. The second method was to steam the cous-cous for 7 minutes, then dry in an air oven at 50°C. for 20 hours. The third method used was to autoclave the cous-cous for 4 minutes at 20 psi and 120°C., then dry the cous-cous in the air oven as stated above.

During the addition of water, the agglomerated particles were increased in size, to approximately 2 mm. When too much water was added, the agglomerated particles would stick together and could not be separated through sieving. The amount of water was regulated by the development of the agglomerated particles. When less than the stated amount of water was added to the flour, which had a moisture percentage of approximately 14.5 for semolina and 15.0 for 85% extraction durum flour, the flour would agglomerate slowly and would not attain the desired size. When the amount of water added exceeded the desired amount, the agglomerated particles produced were too large and would slide together. The rate at which the water was added was also important.

If the water was added at a rate faster than stated above, the water would not be evenly distributed among the flour particles, resulting in dry unagglomerated flour particles, and large, moist masses of unseparatable agglomerated particles. Adding the water at a slower rate than stated had no undesirable results with the exception of the longer time needed to prepare the cous-cous. The rate used for the addition of water was the fastest rate that would yield a desirable product.

At the end of preparation and drying the particle size of the cous-cous was determined in the same manner as that of wet cous-cous.

For consumption the procedure was to place the dried cous-cous in cheesecloth and place in the top section of a cous-cous steamer. The material was then steamed for 20 minutes. The cous-cous was then removed and turned and aerated, then was allowed to stand for several minutes. This procedure was then repeated two more times, resulting in a palatable cous-cous product. The above procedure was used for cous-cous which had been agglomerated or agglomerated and steamed prior to drying. When the cous-cous was autoclaved prior to drying, additional steps were added to the procedure. The cous-cous was placed in a strainer and dipped in water for ten seconds, allowed to drain for thirty seconds, then allowed to stand for several minutes prior to steaming. The desired final product was composed of particles which were soft and moist. The cous-cous particles would have a tendency to adhere to each other but would retain their individual identities.

Materials and Methods

Water Absorption and Solubility Tests

Water absorption and water solubility tests were conducted with semolina cous-cous which was agglomerated, agglomerated and steamed, and agglomerated and autoclaved. These samples were also supplemented with horse bean flour at levels of 5, 10, and 15%. The purpose of the tests was to evaluate the effect of method and processing and degree of supplementation with horse bean flour on the water absorption and water solubility characteristics of the cous-cous.

A dried 2.5 gram cous-cous sample, ground to pass through 60 mesh screen, was placed in suspension in a 50 ml. tared centrifuge tube which contained 30 ml. of distilled water. The centrifuge tube was placed in a 30°C. water bath and stirred continuously for 30 minutes at 600 rpm. The suspension was then centrifuged at 3000 Xg for 10 minutes. Following the centrifuging, the supernatant liquid was decanted into a tared evaporating dish and dried for 10.5 hours in a forced air oven at 60°C. The weight of the gel obtained per gram of dry sample was taken as the Water Absorption Index (WAI). The percentage of dry solids remaining, following the evaporation of the supernatant liquid, represented the Water Solubility Index (WSI).

Amylograph Tests

Amylograph tests were carried out on semolina cous-cous and 85% extraction cous-cous samples which were processed in different manners and supplemented at varying levels with horse bean flour. The tests were carried out with 500 grams of 9% (dry basis) suspension in water of the sample which had been ground to pass through a 60 mesh screen. The slurry was then heated from 29 to 95°C. in 44 minutes. The temperature was then held at 95°C. for 16 minutes. The slurry was then cooled to 50°C. in 30 minutes.

Results and Discussion

Water Absorption Index and Water Solubility Index

The method of processing cous-cous and the degree of supplementation with horse bean flour had an effect on the Water Absorption Index (WAI). Semolina cous-cous, which was only agglomerated, yielded a WAI value of 0.85. When steaming or autoclaving was added to the processing of cous-cous, the WAI values were 3.29 and 3.47 respectively. When semolina cous-cous, supplemented with horse bean flour at the 5% level, was agglomerated, then steamed,

the WAI value increased to 0.99. When semolina cous-cous, which we supplemented with horse bean flour at the 5% level, was agglomerated, then steamed, the WAI value decreased to 3.21. When the supplemented cous-cous was autoclaved, the WAI increased to 3.62. When semolina cous-cous was supplemented with horse bean flour at the 10% level, the agglomerated samples had a WAI value of 0.96 and the steamed and autoclaved samples had values of 2.86 and 3.76 respectively. With 15% horse bean flour, the value for the agglomerated samples was 0.99, that for the steamed sample was 2.75, and that for the autoclaved samples was 3.91. A summary of these results is shown in Figure 21.

Steaming or autoclaving the cous-cous after agglomeration yielded higher WAI values than when the cous-cous was only agglomerated. The degree of supplementation with horse bean flour had little effect on the value for the agglomerated cous-cous, but had major effects on those for the steamed or for the autoclaved samples. The WAI values, for the autoclaved cous-cous increased with increasing supplementation, cous-cous which was steamed yielded lower WAI values.

Semolina cous-cous, which was agglomerated, yielded a high Water Solubility Index (WSI) of 8.50%. When the semolina cous-cous was agglomerated and then steamed, the WSI value decreased to 6.75%. When agglomerated, then autoclaved, the WSI value was decreased to 3.94%. Supplementation of agglomerated semolina cous-cous with horse bean flour at levels of 5, 10, and 15%, increased the WSI value to 9.[illegible]0, 10.00, and 10.90% respectively. The agglomerated and steamed cous-cous, when supplemented in the same manner, had WSI values of 6.00, 7.14, and 8.91%, respectively. Supplementation at the above three levels with horse bean flour also increased the WSI values for semolina cous-cous, which was agglomerated and autoclaved to 3.20, 4.40, and 5.13% respectively. The results are shown in Figure 22.

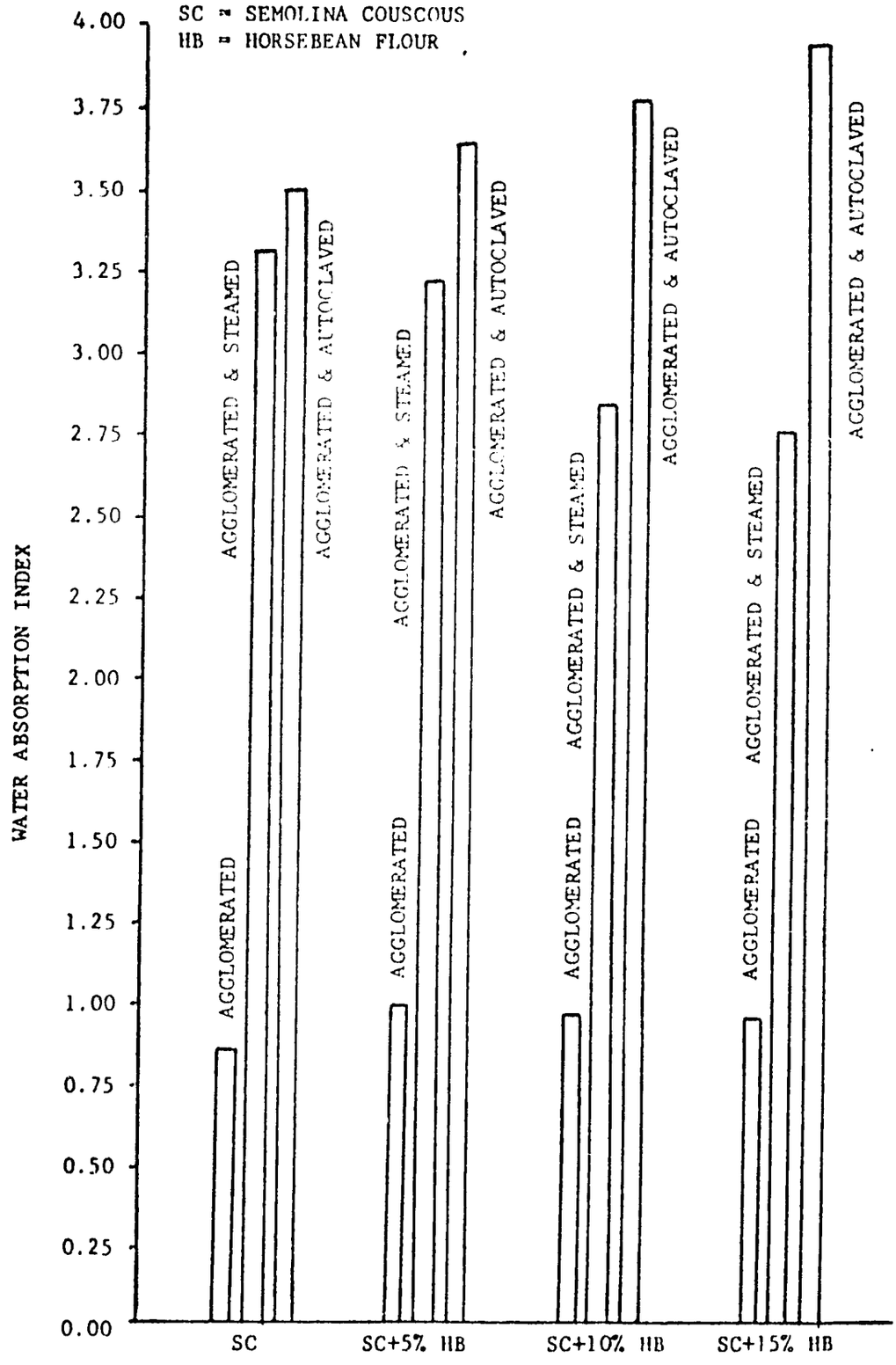


Figure 21. Graph of Water Absorption Index (WAI)

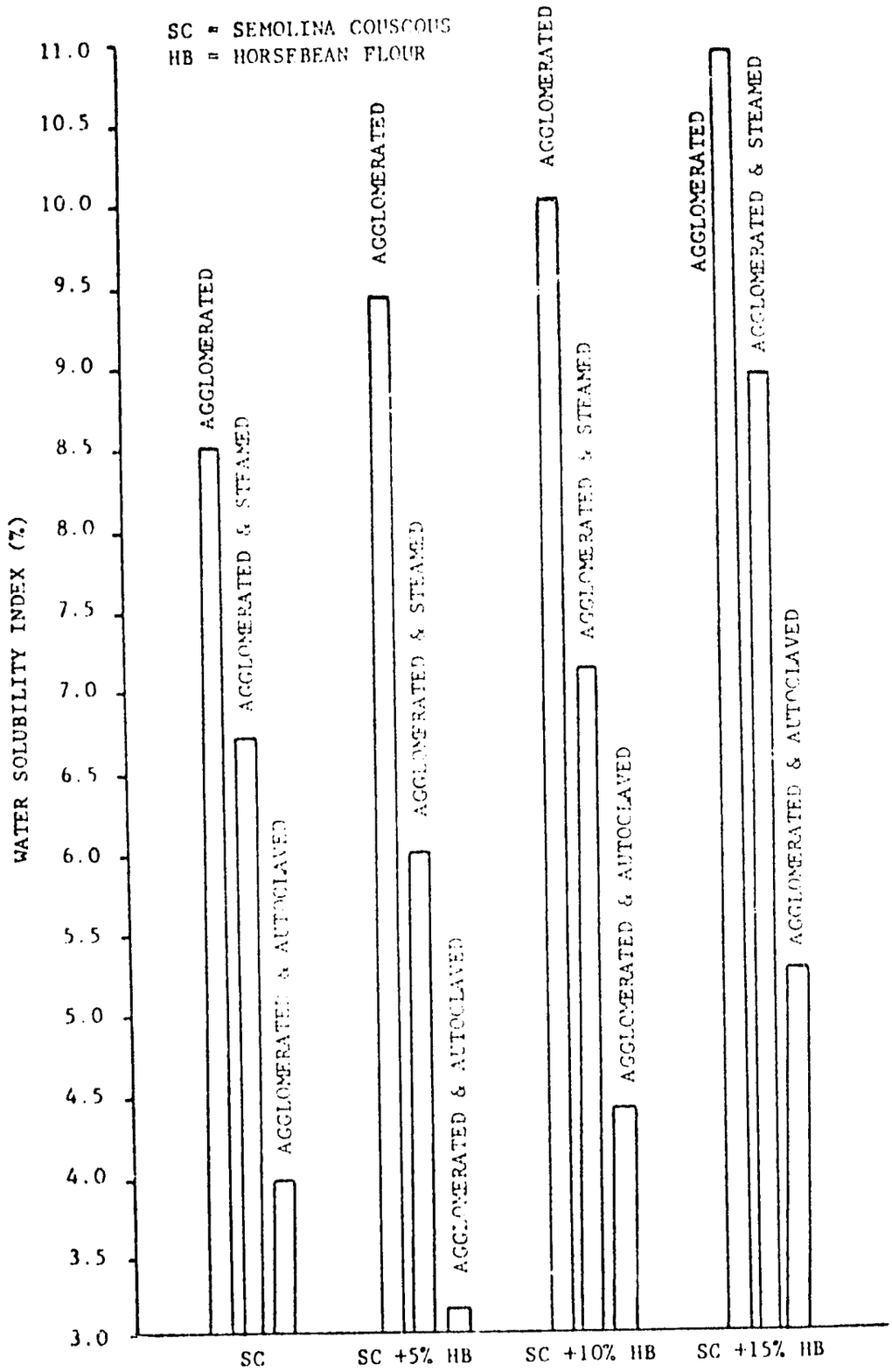


Figure 22. Graph of Water Solubility Index (WSI)

The type of processing of cous-cous had a great effect on the amylograph curve. Semolina cous-cous which was agglomerated had a final amylograph value of 235 Brabender Units (BU). When semolina cous-cous was agglomerated, then steamed for 7 minutes, the amylograph value was increased to 340 BU. The reverse effect was observed when semolina cous-cous was agglomerated, then autoclaved 4 minutes. The amylograph value decreased to 55 BU. The method of processing also had a similar result when 85% extraction cous-cous was tested. Agglomerated 85% extraction cous-cous yielded amylograph values of 375 BU, which were higher than those observed for semolina cous-cous which was agglomerated or agglomerated and steamed. The steamed 85% extraction cous-cous gave a high amylograph value of 460 BU. Autoclaving 85% extraction cous-cous yielded lower values, 38 BU, than the agglomerated or agglomerated and steamed samples of 85% extraction cous-cous. Representation of these results is shown in Figure 23.

Supplementation with horse bean flour had a depressing effect on the amylograph value. The addition of horse bean flour at the 5% level decreased the value from 235 to 155 BU. Further supplementation at the 10 and 15% level decreased the amylograph value to 115 and 100 BU respectively (Figure 24). Supplementation with horse bean flour at 5, 10, and 15% of semolina cous-cous which was agglomerated, then steamed, decreased the value from 340 to 315, 239, and 200 BU respectively. When autoclaved semolina cous-cous was supplemented at the above three levels with horse bean flour, the amylograph value decreased from 59, to 45, 25, and 15 BU. The results are shown in Figures 25 and 26.

Horse bean flour had a similar effect on 85% extraction cous-cous. Supplementation with horse bean flour at the 10% level decreased the amylograph value of agglomerated 85% extraction cous-cous from 375 to 300 BU, and the values of agglomerated and steamed cous-cous from 460 to 360 (Figures 27 and 28).

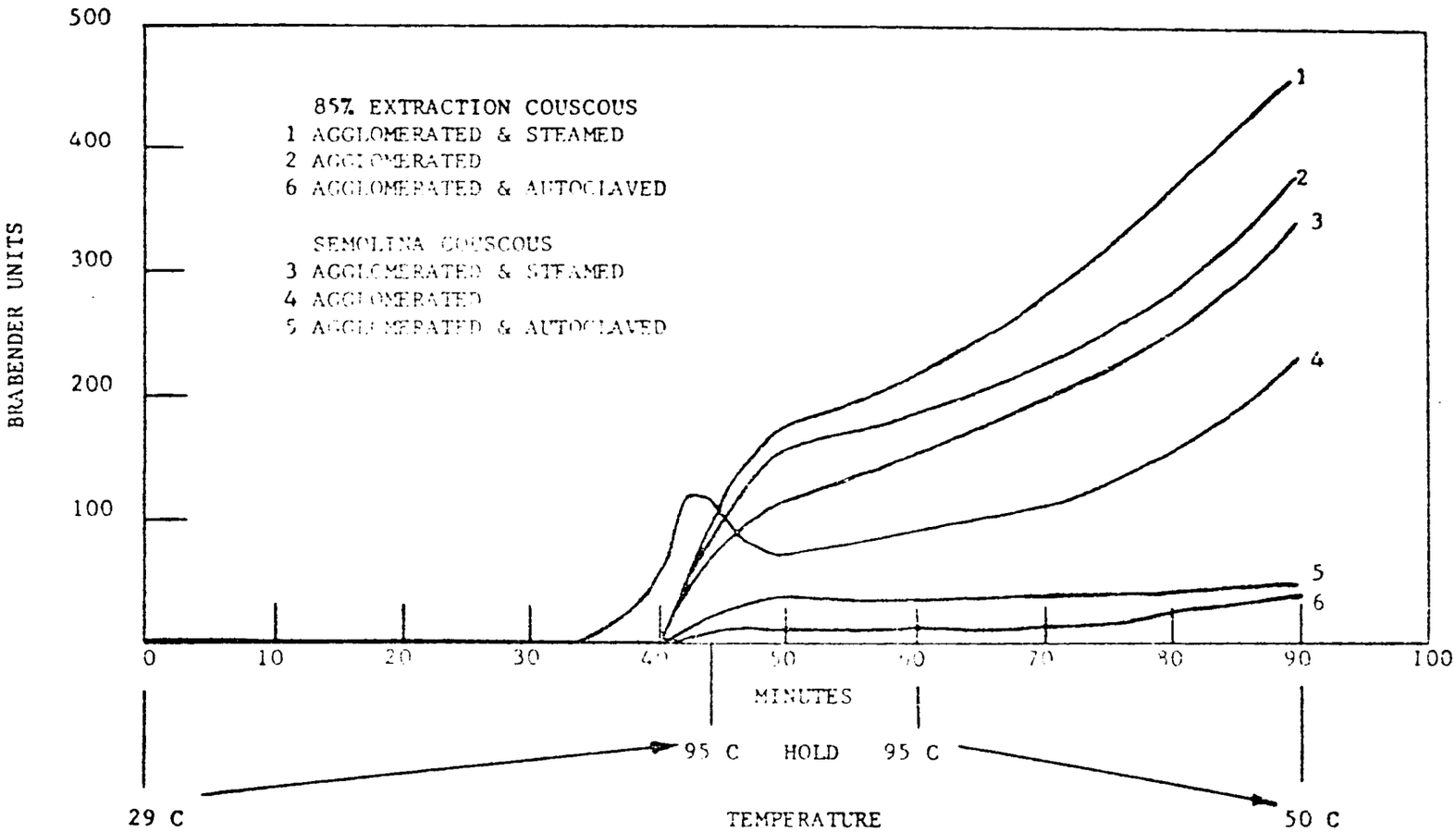


Figure 23. Amylograph curves for Semolina Couscous and 85% Extraction Couscous

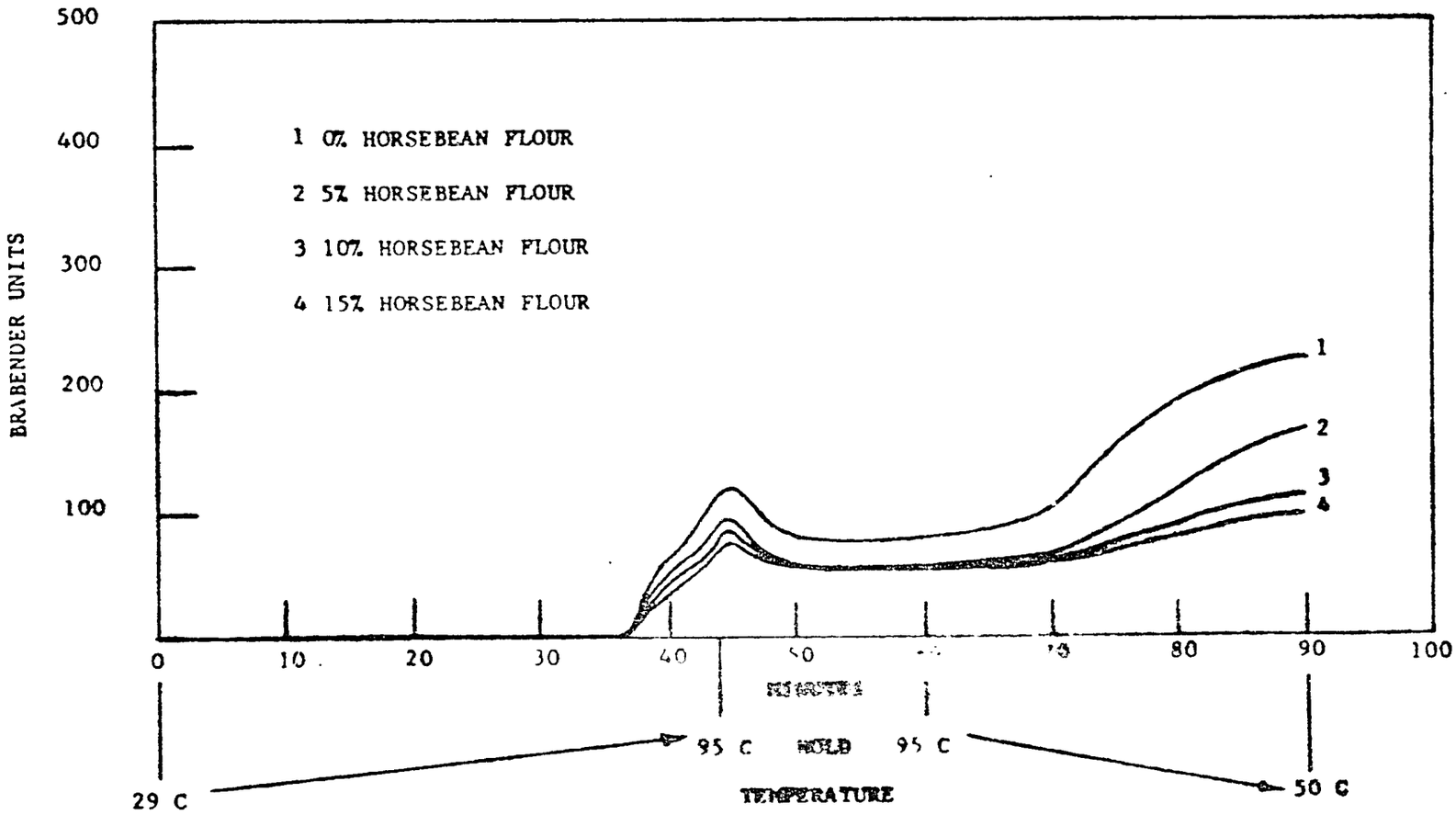


Figure 24. Amylograph curves for Semolina Couscous which was agglomerated and supplemented at three different levels with Horsebean Flour

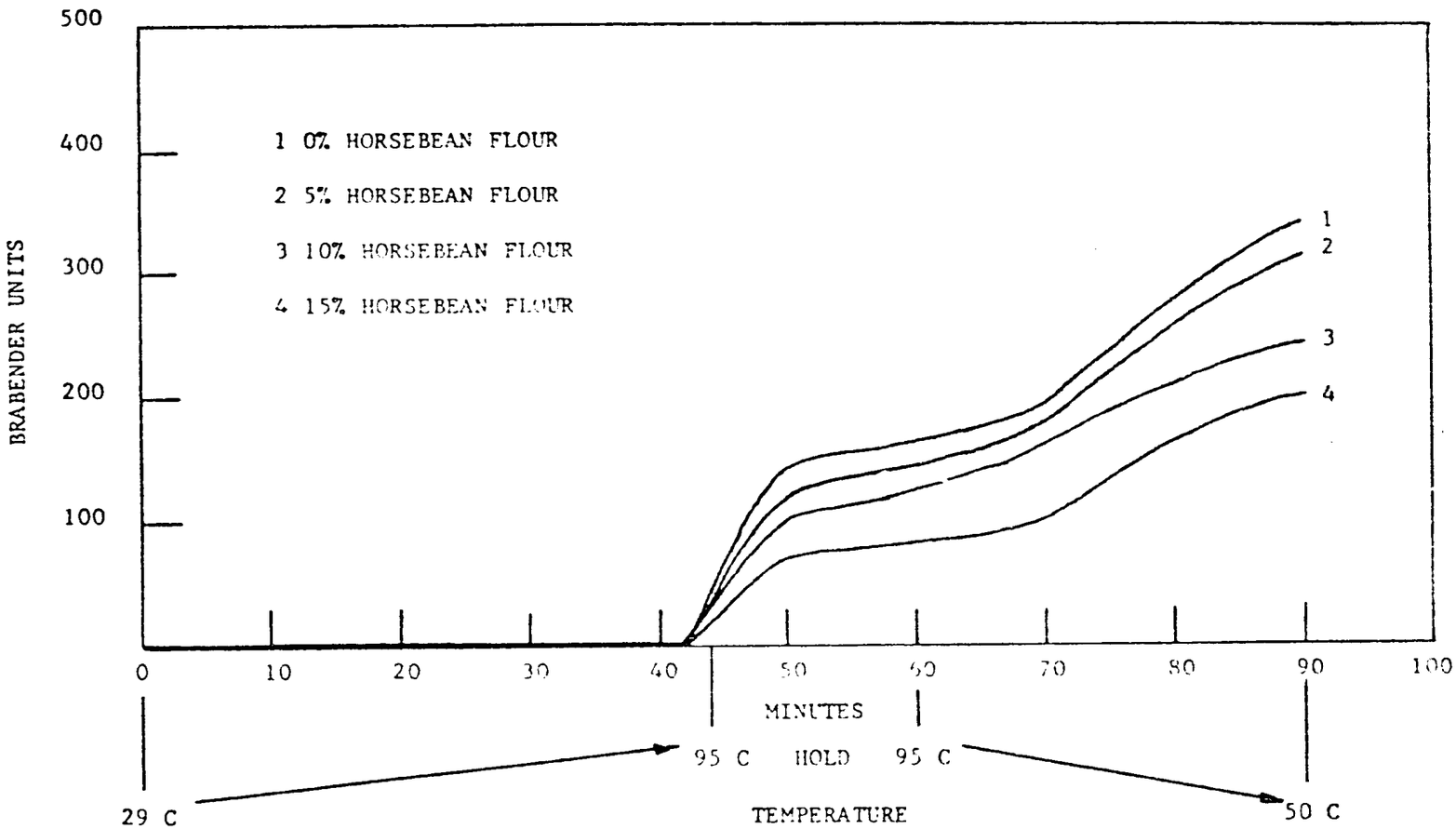


Figure 25. Amylograph curves for Semolina Couscous which was agglomerated and steamed, and supplemented at three different levels with Horsebean Flour.

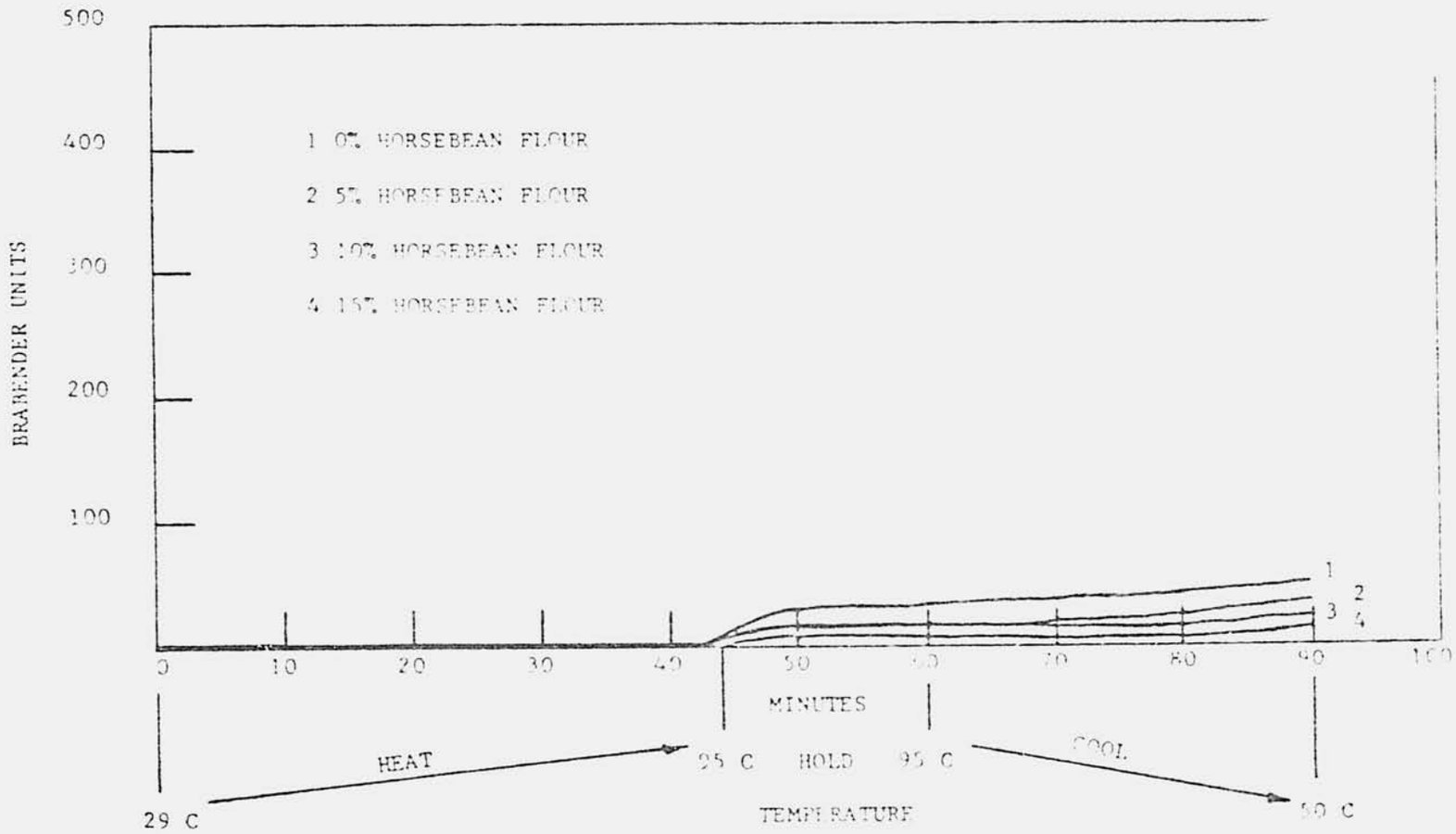


Figure 26. Amylograph curves for Semolina Couscous which was agglomerated and autoclaved, and supplemented at three different levels with Horsebean Flour.

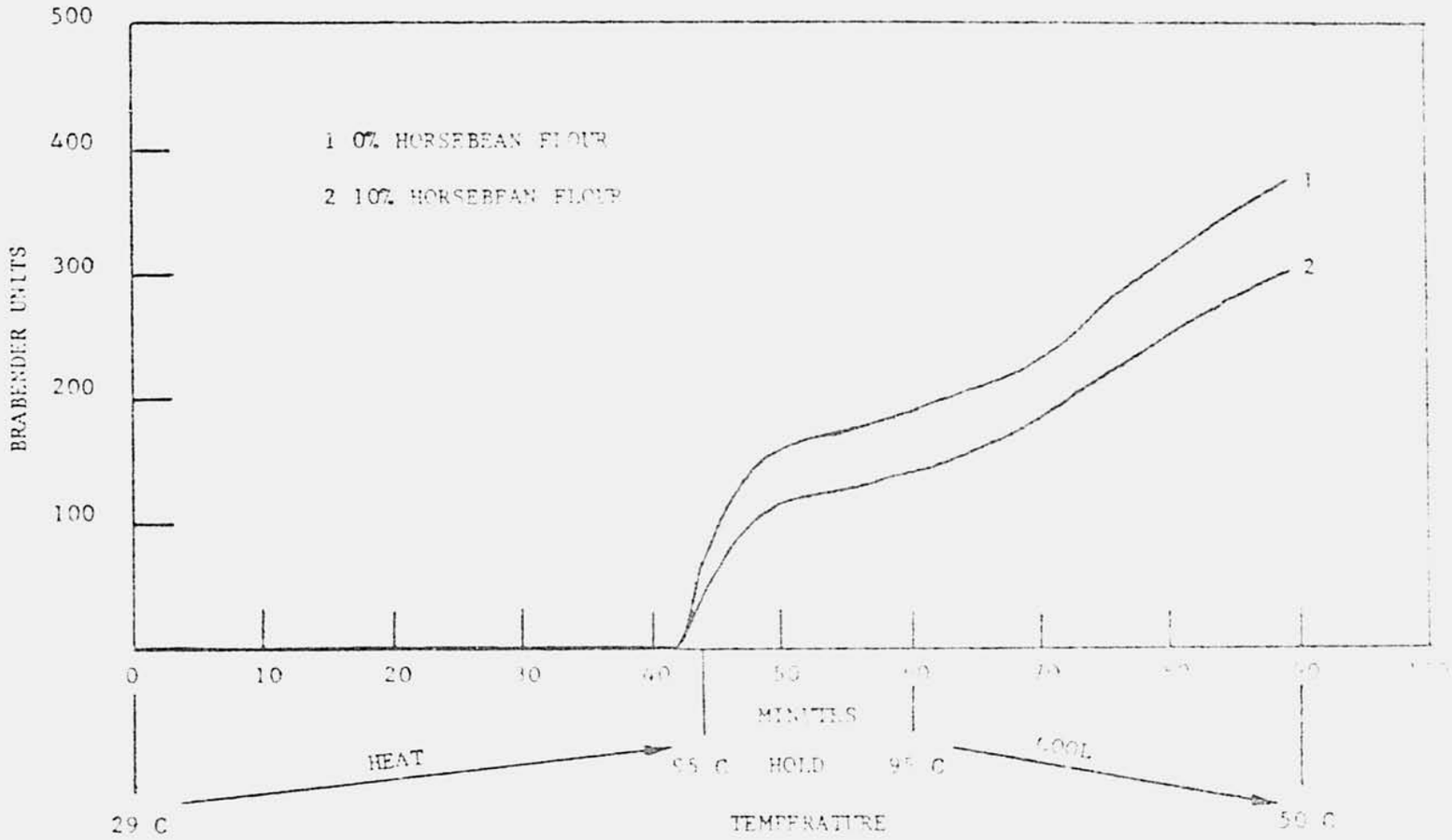


Figure 27. Amylograph curves for [illegible] Extra [illegible] Couscous [illegible] was agglomerated and supplemented with Horsebean Flour.

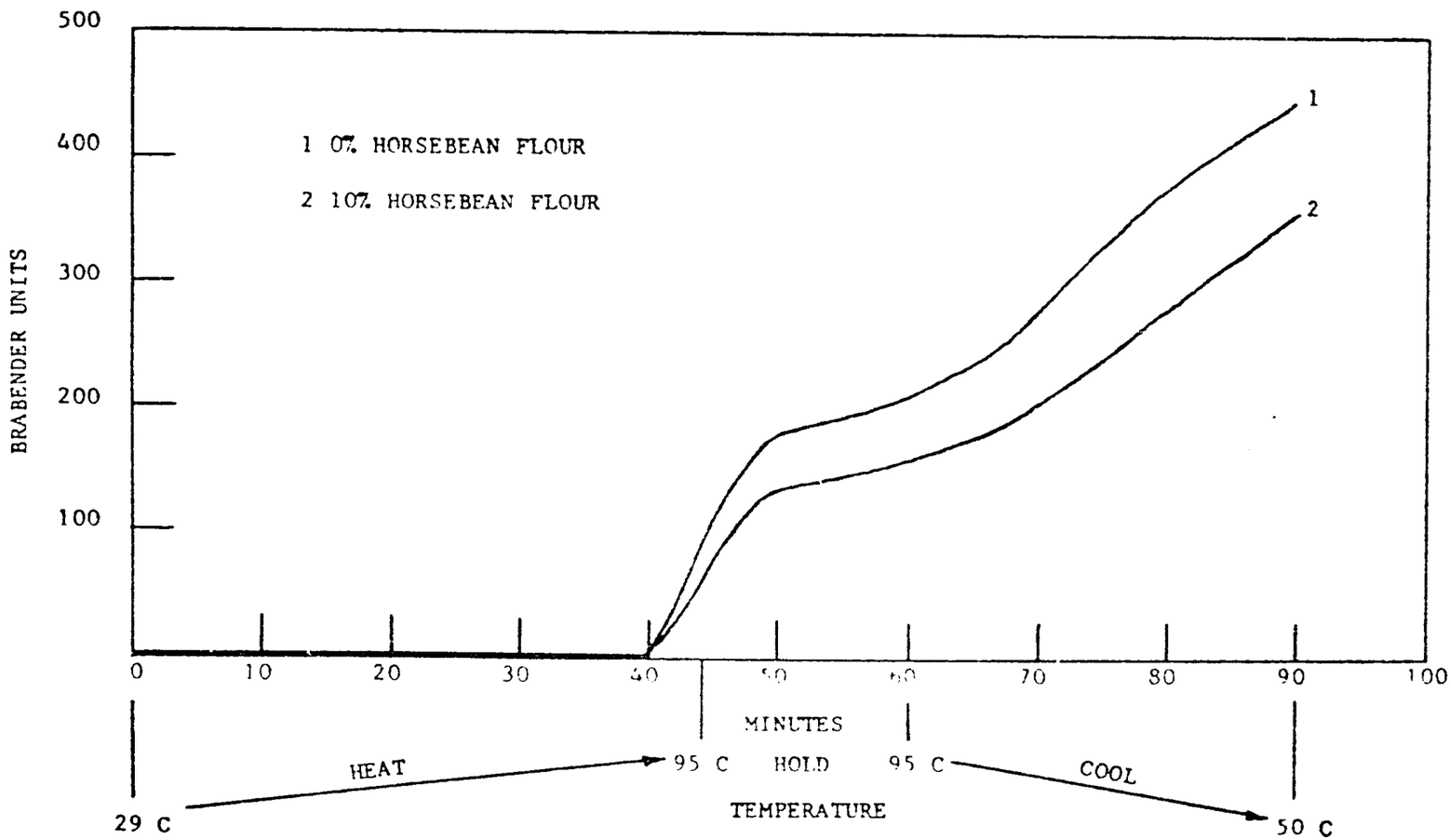


Figure 28. Amylograph curves for 85% Extraction Couscous which was agglomerated and steamed, and supplemented with Horsebean Flour.

Conclusions

The method of processing the cous-cous had a definite effect on the Water Solubility Index. Steaming agglomerated cous-cous lowered the WSI value as compared to agglomerated cous-cous. When the cous-cous was agglomerated and then autoclaved, the WSI value was lowered greatly. In general, the degree of supplementation of cous-cous was directly related to the WSI value. The severity of the treatment tended to decrease the WSI value. Agglomerated cous-cous which was steamed for 7 minutes yielded lower WSI values than cous-cous which was agglomerated. When the cous-cous was autoclaved, the WSI values were greatly lower than those obtained for agglomerated cous-cous.

During this autoclaving process, much of the starch in the cous-cous is gelatinized. Under autoclaving conditions, the structure of the starch granules is broken down. As a result, further swelling of the starch granules during the amylograph test would occur at a very low degree. The steaming process will gelatinize the starch but will not break down the granule structure. The higher amylograph values for steamed cous-cous over those of agglomerated cous-cous could be related to the layer starch granules in the steamed cous-cous. This would be the result of gelatinization of the starch during steaming.

When the percentage of horse bean flour supplementation was increased, the total percentage of cous-cous starch in the slurry decreased. This factor could be responsible for the lower amylograph values and lower increase in viscosity exhibited by the supplemented cous-cous.

NUTRITIONAL EVALUATION OF SUPPLEMENTED COUS-COUS

Rat Study II--Horse Beans and Lysine

Nutritional differences in cous-cous prepared with or without supplementation with horse beans or lysine were studied. Possible causes for the hair loss observed in Study I (Progress Report 2, March, 1970, p. 105) (1969) were investigated.

The following diets were prepared:

Diet 1 - Casein control

Diet 2 - Uncooked 70% extraction flour

Diet 3 - Cooked 70% extraction flour

Diet 4 - Cooked 70% extraction flour with 5% horse bean supplement

Diet 5 - Cooked 70% extraction flour with 10% horse bean supplement

Diet 6 - Cooked 70% extraction flour with 15% horse bean supplement

Diet 7 - Cooked 70% extraction flour with 0.19% lysine supplement

The protein level of this series of diets was 10%. A similar series was prepared in which diammonium citrate was added at the expense of starch to increase the nitrogen content of the diets so that it would be equivalent to 18% protein:

Diet 8 - Casein control

Diet 9 - Uncooked 70% extraction flour

Diet 10 - Cooked 70% extraction flour

Diet 11 - Cooked 70% extraction flour with 5% horse bean supplement

Diet 12 - Cooked 70% extraction flour with 10% horse bean supplement

Diet 13 - Cooked 70% extraction flour with 15% horse bean supplement

Diet 14 - Cooked 70% extraction flour with 0.19% lysine supplement

The lysine contents of Diets 5, 7, 12, and 14 were approximately equal. Vitamins and minerals were added to the cooked cous-cous in amounts necessary to meet NRC requirements. The rations were fed to groups of eight male weanling albino rats of the Charles River strain for 28 days. The animals refused to eat the rations containing diammonium citrate (Diets 8-14) and did not gain normally. Therefore no data will be reported for that portion of the experiment. The animals receiving the first seven diets were continued on the experimental diets for an additional 13 days for further observation of hair loss.

Weekly weight gains for the first seven groups were plotted (Figure 29). For the 28-day period, both weight gain and feed consumption were lower than found for comparable diets in Study I. The increase in weight gain attributed to horse bean supplementation (10% level) was only about half as much in Study II as in Study I. Supplementation with 5% horse beans was almost as effective and supplementation with lysine was more effective than supplementation with 10% horse beans for increasing weight gain. PER were calculated at the end of 28 days (Table 37). Dry weight and calcium content of femurs will be determined.

TABLE 37. PROTEIN EFFICIENCY RATIOS FOR DIETS 1-7.

Diet	PER
1	4.270
	*
6	2.194
	*
5	1.952
	n.s.
7	1.943
	*
4	1.688
	*
2	1.268
	*
3	0.914 $LSD_{0.05} = 0.231$

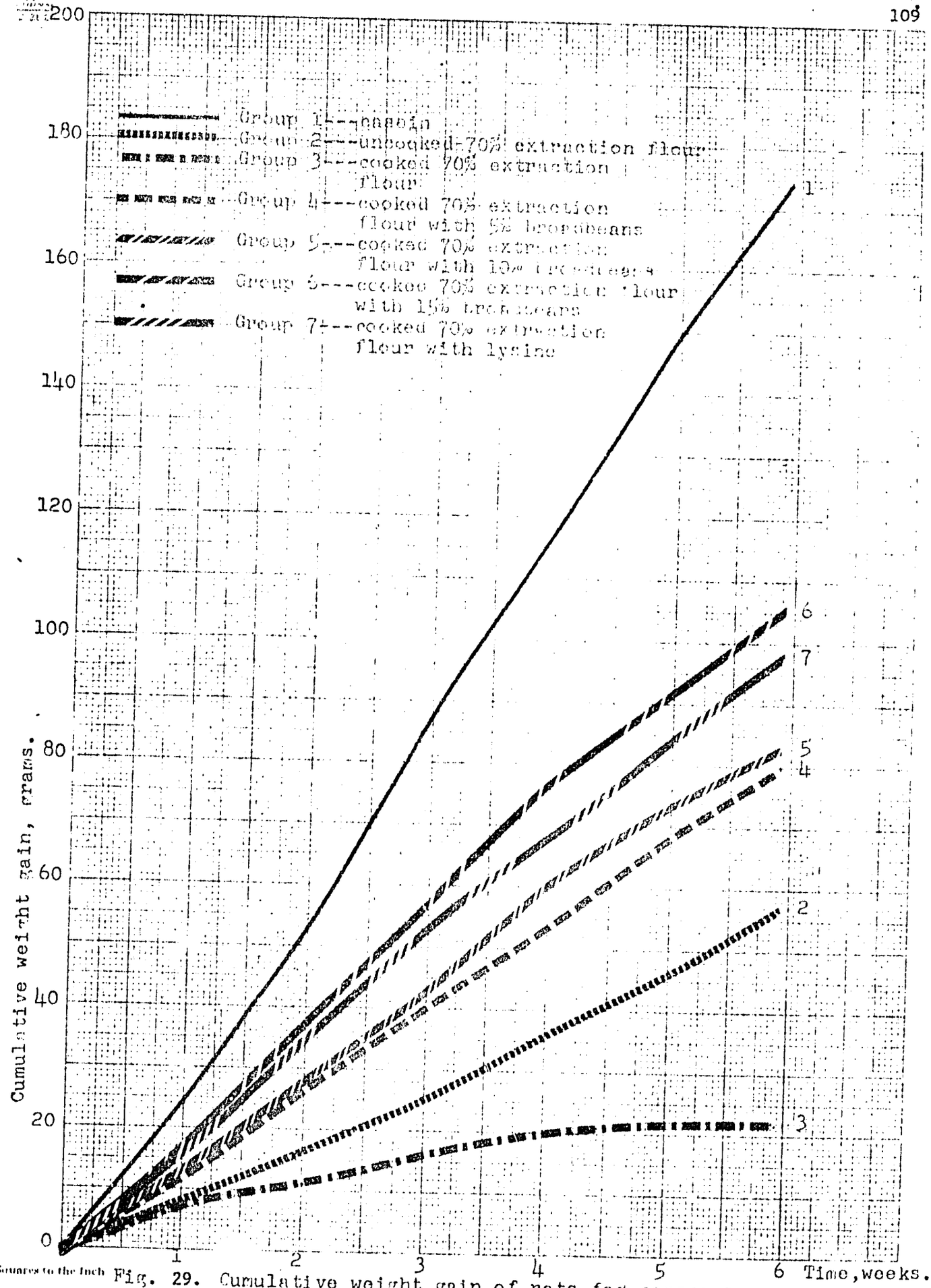


Fig. 29. Cumulative weight gain of rats fed couscous.

The following generalizations were made in regard to PER:

1) There was no difference between supplementation with lysine at the level provided by 10% horse bean supplementation and supplementation with 10% broad beans.

2) Increasing the level of horse bean supplementation increased the PER significantly at each level.

3) Cooking decreased PER.

4) Casein produced higher PER than any other combination of protein sources studied.

Items 2 and 4 also were found in Study I. Item 3 differs from findings of Study I.

At the end of two weeks rats were rated every two to four days for degree of hair loss according to the scale below.

5 - no loss
4 - small amount of hair missing
3 - underside partly bare
2 - underside bare
1 - legs and underside partly bare
0 - legs and underside bare

Group averages are shown in Table 38. In no case did all rats within a group show hair loss--usually three, four, or five of the eight animals would be affected. The hair loss appeared to be the same as observed in Study I; however, in the current study, three of the control rats showed varying degrees of hair loss. After a period of time, hair did start to grow back to partly or completely cover the bare skin. New cases of hair loss continued to appear as late as the fifth and sixth weeks of the study. Two of the animals showing extreme hair loss were examined by a parasitologist; no sign of external parasites was found.

TABLE 38. HAIR LOSS RATINGS.

Diet	Ratings 3 weeks	4 weeks	5 weeks
1	4.57	3.71	3.29
2	4.00	4.50	4.12
3	3.62	3.12	3.00
4	4.12	3.88	3.88
5	4.62	4.25	4.38
6	4.50	4.38	4.25
7	4.62	4.43	4.14

INFLUENCE OF PROCESSING AND PROTEIN SUPPLEMENTATION (HORSE BEAN FLOUR) ON NUTRITIONAL QUALITIES OF COUS-COUS

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B. E. Fryer, and W. J. Hoover

Abstract

Male albino weanling rats were used to determine the ability of cous-cous to support growth and to compare nutritional qualities of cous-cous processed various ways and supplemented with three different levels of protein. Nine blended diets at a 10% protein level were used in Study I (1970). These diets consisted of semolina and 85% extraction flours and cous-cous prepared from these flours. The above ingredients were also supplemented at the 10% level with horse bean (Vicia faba) flour. The diets contained starch, oil, vitamins, and minerals.

Study II (1970) used 14 diets, 7 at 10% protein level and 7 at 18% protein level. Semolina, semolina cous-cous, and autoclaved cous-cous supplemented at 5, 10, and 15% levels with horse bean flour. Two cous-cous diets were supplemented with lysine at a level equivalent to that of 10% horse bean flour. From the studies, which continued 28 days, average weekly weight gain, average weekly feed consumption, feed efficiency, and protein efficiency ratios were calculated. The 10% protein series of Study II (1970) were extended to 42 days.

All diets supported growth and development with significant differences between some. Diets supplemented with horse bean flour gave higher nutritional values than unsupplemented diets.

Effect of Heat Treatment

Heat processing can improve the nutrition value of proteins, wherein the limiting factor was usually lysine (McCollum and Davis, 1950) (59). A new lysine linkage was so slowly digested that part of the lysine entered the blood stream.

The nutritional values of raw and autoclaved wheat germ were compared using the chick as an experimental animal (Attia and Creek, 1965) (60). Raw wheat germ was found to depress growth and fat utilization and also to induce pancreatic hypotrophy. A hemaggutinin factor detected in raw wheat germ was destroyed in the autoclaved sample.

Stevens and McGinnis, 1947, (61) studied the effect of autoclaving lysine in the presence of carbohydrate on utilization by the chick. They found that the decreased nutritive value of over heated soybean oil meal was almost fully corrected by supplementary methionine and lysine. Moran, et al., 1968, (62) found that toasting wheat germ meal significantly improved net protein utilization (NPU), but autoclaving had no effect. Further autoclaving reduced NPU. Toasting or autoclaving for 20 minutes destroyed cysteine. Processing beyond 20 minutes destroyed lysine, cysteine, and arginine.

It was found that steam-pelleting of wheat germ increased the metabolizable energy value for both chicks and roosters, whereas steam pelleting reduced the metabolizable energy values of the middlings and the shorts (Bayly, et al., 1968) (63). The toasting of wheat germ caused an increase in metabolizable energy for roosters but not for chicks. Autoclaving wheat germ caused increase in this value for roosters but decreases for chicks.

Autoclaved wheat germ produced significantly higher weight gain in chicks as compared to diets containing unautoclaved wheat germ (Creek, et al., 1961) (64). Horse bean fed to rats as the sole source of protein at a 12% level of protein was improved by autoclaving through the inactivation of the trypsin inhibitor (Borchers and Ackerson, 1950) (65). It was found that parching increased the biological value of the proteins of some cereals and pulses in rat feeding experiments. In many cases the digestibility coefficient was improved (Acharya, et al., 1942) (66).

By prolonged heating of soybean meal, the liberation of lysine, arginine, and tryptophan by hydrolysis was reduced (Prier and Derse, 1957) (67). The amount of each of the essential amino acids liberated by pancreatic hydrolysis was increased by proper heat treatment and decreased by excessive heat treatment.

Autoclaving casein at 15 psi for 4 minutes had no effect on the amino acid composition as measured by microbiological determination after acid hydrolysis (Hankes, et al.,1948) (68). Autoclaving for 20 hours reduced the cysteine content, but had no effect on the amount of the other amino acids.

Evans, et al., 1948, (69) found that part of the lysine was destroyed when soybean meal was mixed with sucrose and autoclaved for 4 minutes at 121°C. When isolated proteins of soybean were autoclaved, there was little loss of lysine.

Vegetable Protein

Research has shown that for the cereal eating population of the world, the shortage of protein is one of quality rather than quantity (Howe, et al., 1965) (70). Results indicated that under the most adverse conditions, subsistance entirely upon cereal grains, with no intakes of animal protein, the protein consumed when caloric requirements were satisfied would be adequate for both infants and adults if it were of a quality comparable to the animal proteins.

Prier and Derse, 1957, (67) studied the nutritional value of a vegetable protein mixture. They found that animals receiving one gram of protein per day from either casein or the vegetable mixture grew at a slow but equal rate. The protein efficiency ratios were approximately equal. INCAP Vegetable Mixture 9 is an example of a vegetable protein mixture formulated for human feeding (Bressani, et al., 1961) (71). It contains 28% corn, 28% sorghum grain, 38% cottonseed flour, 3% dehydrated leaf meal, and 3% Torula yeast. Baptist and DeMel, 1955, (72) concluded that a suitable mixture of vegetable proteins can support growth in children.

Measurements of the protein efficiency ratio (PER) were carried out on the common legumes used in the Near East and on various legume-containing mixtures (Adolph, et al., 1955) (73). Horse bean (Vicia faba), which was used extensively in Egypt, yielded low PER's. When horse beans were cooked they had a PER of 1.17. When the horse beans were combined with parboiled wheat, a PER of 1.62 was achieved.

Adolph, et al., 1955, (73) studied the method of cooking legume commonly used in the Near East. Most commonly water is added to the legumes which were then cooked till soft, usually about two hours. Horse beans are first soaked in water overnight before cooking.

Everson and Heckert, 1944, (74) tested for biological value of leguminous sources of protein. Heating for 45 minutes at 15 psi improved the nutritional value of many legumes. They found an appreciable difference in the growth promoting qualities of various legumes, and that each source of leguminous protein varies in biological value according to the condition of the legumes and the method of preparation with relation to the application of heat.

Nutritional Studies

Campbell, 1944, (75) determined the protein efficiency ratio (PER) of foods which were fed, at 10% protein level, to rats in a standardized 4 week rat growth study. The determination of the amino acids lysine, methionine, and cysteine was used as a base for the chemical score which correlated well with PER. The PER was found to use the most generally applicable procedure for evaluating protein quality.

Nutritional studies using durum wheat maccaroni supplemented with lysine and milk protein were used to determine the protein efficiency ratio (PER) (Bain, et al., 1964) (76). Tests with rats showed that the mean PER of plain wheat maccaroni made from semolina at a level of 10% protein was 1.11 compared

to 2.48 for enriched maccaroni prepared from a blend of semolina 90, casein 7, and skim-milk solids 3%. Supplementation of both maccaroni products with lysine monohydrochloride (0.25 g./10 g. protein) increased the PER values to 2.11 and 2.63, respectively.

Howe and Dooley, 1963, (77) found that when the food intake of rats was restricted, lysine supplementation of a wheat gluten diet was equally effective in supporting growth. There was no significance as to whether it was incorporated in the diet or given by gauge 6 hours after feeding.

Hegsted and Chang, 1965, (78) studied utilization in growing rats at different levels of intake. Four different proteins, lactobumin, casein, soy protein, and wheat gluten were fed to groups of weanling rats by diets which supplied 7 different levels of each protein for a 3-week period. These workers concluded that the net nitrogen utilization was essentially constant for each of these proteins at all levels of intake up to those which support maximum growth.

Materials and Methods

Nutritional Studies
Study I (1970)

In order to determine nutritional differences in cous-cous prepared from two different flours with and without horse bean flour supplementation, nutrition studies using male albino rats were initiated.

Study I (1970) contained 9 different blended diets which were studied for protein efficiency and feed efficiency. The diets were assayed, and contained added minerals, vitamin and oil. The diets were prepared from cous-cous and from flour samples identical to those used for the preparation of cous-cous.

Diet 1, the control diet, was a blend of casein (ANRC casein), cornstarch, oil, vitamins, and minerals. Diet 2 was a blend of semolina, cornstarch, oil, vitamins and minerals. The third diet was prepared the same way with semolina

cous-cous substitutes for semolina. Diet 4 contained semolina, horse bean flour added at a level of 10% of the semolina, cornstarch, oil, minerals, and vitamins. The fifth diet contained semolina cous-cous and horse bean flour added at a level of 10% of the semolina cous-cous, with the remaining part of the diet the same as above. Diet 6 contained 85% extraction durum flour, while Diet 8 contained the 85% extraction flour with horse bean flour at its 10% level of the durum flour. Diet 7 contained 85% extraction cous-cous, where as Diet 9 contained 85% extraction cous-cous with horse bean flour at the 10% level. The remaining part of the diet was the same as above.

The diets were blended in a V-type liquid-solids blender using a minimum blending time of 5 minutes per blend once all of the ingredients were added.

The individual diets and formulations are contained in Table 39. The blended diets were analyzed for moisture, protein, ash, crude fat, and crude fiber. The proximate analysis is shown in Table 40.

Study Design

The rat study was designed for statistical analysis in a randomsized complete block design. Each of the 9 experimental diets were fed to 8 rats; a total of 72 rats were thus fed for the study. Male albino rats purchased from the Charles River Breeding Laboratories were used in the experiment. At the start of the experiment the rats were 3 weeks old and ranged in weight from 51 to 57 grams.

The individual rats were weighed on the day they were received and then placed in individual wire-bottomed cages. The rats were watered and fed and given two days to adjust to the environment. At the end of 2 days, the rats were weighed and feed consumption recorded. The actual beginning of the experiment started at this time. The rats consumed their feed and demineralized water *ad libitum*. The wasted feed was sifted from feed material, weighed, and the amount recorded. The rats were weighed every 7 days and the amount of feed consumed by each rat was determined on the same day.

TABLE 39. FORMULAS FOR DIETS USED IN RAT FEEDING STUDY, PERCENTAGE, AMOUNTS, AND CALCULATED PERCENTAGE PROTEIN

Diet No.	Ingredient	Percent	Amount/7kg. (gm.)
1	Casein (vitamin free)	11.40	798.6
	Cornstarch	65.40	4580.4
	Oil	7.97	558.0
	Mineral Mix	3.38	236.0
	Vitamin Mix	0.01	0.8
	Choline	0.28	20.0
	Water	11.51	805.8
	Calculated % Protein = 10.8		
2	Semolina	82.30	5761.3
	Cornstarch	6.09	426.5
	Oil	7.93	555.0
	Mineral Mix	3.38	236.4
	Vitamin Mix	0.01	0.8
	Choline	0.28	20.0
	Water	0.00	0.0
	Calculated % Protein = 11.6		
3	Semolina Cous-cous	75.19	5263.2
	Cornstarch	5.28	370.0
	Oil	7.97	558.0
	Mineral Mix	3.38	236.4
	Vitamin Mix	0.01	0.8
	Choline	0.28	20.0
	Water	7.89	551.6
	Calculated % Protein = 11.6		

TABLE 39 (CONTINUED).

Diet No.	Ingredient	Percent	Amount/7kg. (g)
4	Semolina with Horse bean flour	72.72	5090.9
	Cornstarch	14.10	987.4
	Oil	7.92	555.0
	Mineral Mix	3.38	236.4
	Vitamin Mix	0.01	0.8
	Choline	0.28	20.0
	Water	1.56	109.5
	Calculated % Protein = 12.6		
5	Semolina Cous-cous with Horse bean flour	68.26	4778.2
	Cornstarch	11.81	826.5
	Oil	7.97	558.0
	Mineral Mix	3.38	236.4
	Vitamin Mix	0.01	0.8
	Choline	0.28	20.0
	Water	8.29	580.1
	Calculated % Protein = 12.6		
6	85% Extraction durum flour	77.82	5447.5
	Cornstarch	10.47	732.8
	Oil	7.92	555.0
	Mineral Mix	3.38	236.4
	Vitamin Mix	0.01	0.8
	Choline	0.28	20.0
	Water	0.18	12.5
	Calculated % Protein = 11.5		

TABLE 39. (CONTINUED).

Diet No.	Ingredient	Percent	Amount/7kg. (g)
7	85% Extraction Cous-cous	70.92	4964.5
	Cornstarch	9.18	642.4
	Oil	7.97	558.0
	Mineral Mix	3.38	236.4
	Vitamin Mix	0.01	0.8
	Choline	0.28	20.0
	Water	8.26	577.9
	Calculated % Protein = 11.5		
8	85% Extraction durum flour with Horse bean flour	68.58	4801.1
	Cornstarch	17.95	1257.0
	Oil	7.84	549.0
	Mineral Mix	3.38	[illegible]
	Vitamin Mix	[illegible]	0.8
	Choline	0.28	20.0
	Water	1.94	135.7
	Calculated % Protein = 12.2		
9	85% Extraction Cous-cous with Horse bean flour	62.42	4369.5
	Cornstarch	14.12	1198.1
	Oil	7.97	558.0
	Mineral Mix	3.38	236.4
	Vitamin Mix	0.01	0.8
	Choline	0.28	20.0
	Water	8.82	617.2
	Calculated % Protein = 11.8		

TABLE 40. PROXIMATE ANALYSES OF NINE DIFFERENT BLENDED DIETS

Diet No.	Moisture %	Protein %	Ash %	Crude Fat %	Crude Fiber %
1	19.8	10.4	2.3	4.8	2.4
2	12.3	11.6	1.9	5.2	2.3
3	11.7	12.0	2.0	4.7	2.7
4	13.4	12.7	2.4	4.6	2.9
5	13.1	12.6	1.9	4.8	2.7
6	11.9	11.1	2.1	5.1	3.0
7	12.2	11.5	1.8	4.9	2.5
8	13.1	11.9	1.8	4.8	2.8
9	12.0	11.3	2.0	4.8	3.0

Records were maintained for weekly feed intake. The amount of feed needed per rat per week was estimated and weighed at the beginning of each week, and the rat was fed from this supply. At the end of the 7 day period, the unused feed offered to the rat was recombined with the remaining original supply. This feed was weighed and the weight was recorded. The uncorrected feed consumption was determined by subtracting the grams of feed remaining at the end of the week from the grams of feed at the beginning of the period. This amount would represent the amount of feed offered to the rat over the 7 day period. The amount of feed which the rat wasted over the 7 day period was subtracted from the amount of feed offered to the rat. The resulting amount was recorded as weekly feed consumption.

The experiment was continued for 28 days. This time period was considered necessary for the rats to reach maturity. At the end of the experiment, the rats were sacrificed.

The total weight gained and the total feed consumed were calculated for each rat from the weekly data. These data were then used to calculate the average weekly gain and the average feed consumption. Next, the feed efficiency (FE) was calculated by dividing weight gain into feed consumption. The protein efficiency ratio (PER) was then calculated by first determining the amount of protein consumed by the rat, which was done by multiplying total feed consumption by the percentage protein as determined by the proximate analysis. The protein efficiency ratio was then found by dividing the total weight gained by the protein intake. The calculated average weekly gain, average weekly consumption, feed efficiency (FE), and protein efficiency ratio (PER), were analyzed statistically to determine significance.

Study II (1970)

The second nutritional study using male albino rats was made in order to evaluate the diet formulations on a more equal basis, to determine the effect of having sufficient protein for growth and development, and to determine the protein efficiency of diets at 18% protein. Fourteen blended diets were formulated using semolina as the basic source of protein. The duplication of the diets at 18% protein was done to see if an increased level of protein would have an effect on the hair loss observed in Study I (1970). Supplementation with horse bean flour was done at levels of 5, 10, and 15% of the semolina. Seven different blended diets were formulated at 10% protein and then at 18% protein. Three of the diets at each protein-level contained semolina cous-cous supplemented with horse bean flour. Each level also contained a diet composed of semolina cous-cous plus lysine at a level equivalent to horse bean flour supplemented at the 10% level. The remaining diets at each level consisted of a casein control with the other diet containing semolina. Lysine was incorporated in 2 of the diets with semolina cous-cous to see if it would have any effect on hair loss. Diammonium citrate was used toraise the level of nitrogen in the diets to a level of 18% protein.

The diets were blended in the same manner as Study I. The specific diets and formulations with the calculated percentage protein are shown in Table 41. The 14 blended experimental diets were analyzed for moisture, protein, ash, crude fat, and crude fiber. The results of the proximate analysis are given in Table 42.

The design of Study II was the same as Study I. The average weekly weight gain, average weekly feed consumption, the feed efficiency (FE), and the protein efficiency ratio (PER), were analyzed statistically.

TABLE 41. FORMULAS FOR DIETS USED IN RAT FEEDING STUDY: PERCENTAGE, AMOUNT, AND CALCULATED % PROTEIN

Diet No.	Ingredient	Percent	Amount/7 kg.
1	Casein (vitamin free)	11.1	798.0
	Cornstarch	77.0	5386.6
	Mineral premix	3.4	236.4
	Vitamin premix	0.1	4.0
	Choline	0.3	20.0
	Oil	7.6	555.0
	Calculated % Protein	6.9	
2	Semolina	81.3	5690.0
	Cornstarch	7.1	494.6
	Mineral premix	3.4	236.4
	Vitamin premix	0.1	4.0
	Choline	0.3	20.0
	Oil	7.9	555.0
	Calculated % Protein	11.6	
3	Semolina cous-cous	71.9	5036.0
	Cornstarch	16.4	1148.6
	Mineral premix	3.4	236.4
	Vitamin premix	0.1	4.0
	Choline	0.3	20.0
	Oil	7.9	555.0
	Calculated % Protein	10.8	
4	Semolina cous-cous with horse bean flour (5%)	66.8	4678.0
	Cornstarch	21.5	1506.6
	Mineral premix	3.4	236.4
	Vitamin premix	0.1	4.0
	Choline	0.3	20.0
	Oil	7.9	555.0
	Calculated % Protein	10.8	

TABLE 41. (CONTINUED).

Diet No.	Ingredient	Percent	Amount/7 kg.
5	Semolina cous-cous with horse bean flour (10%)	63.3	4432.0
	Cornstarch	25.0	1752.6
	Mineral premix	3.4	236.4
	Vitamin premix	0.1	4.0
	Choline	0.3	20.0
	Oil	7.9	555.0
	Calculated % Protein	10.6	
6	Semolina cous-cous with horse bean flour (15%)	60.2	4212.0
	Cornstarch	28.2	1972.6
	Mineral premix	3.4	236.4
	Vegetable premix	0.1	4.0
	Choline	0.3	20.0
	Oil	7.9	555.0
	Calculated % Protein	10.6	
7	Semolina cous-cous with lysine	70.0	4972.0
	Cornstarch	17.3	1212.6
	Mineral premix	3.4	236.4
	Vitamin premix	0.1	4.0
	Choline	0.3	20.0
	Oil	7.9	555.0
	Calculated % Protein	10.9	
8	Casein (vitamin free)	11.4	798.0
	Cornstarch	66.6	4663.2
	Mineral premix	3.4	236.4
	Vitamin premix	0.1	4.0
	Choline	0.3	20.0
	Oil	7.9	555.0
	Diammonium citrate	10.3	723.4
	Calculated % Protein	15.2	

TABLE 41. (CONTINUED).

Diet No.	Ingredient	Percent	Amount/7 kg.
9	Semolina	78.0	5460.0
	Cornstarch	----	----
	Mineral premix	3.4	236.4
	Vitamin premix	0.1	4.0
	Choline	0.3	20.0
	Oil	7.9	555.0
	Diammonium citrate	10.3	723.4
	Calculated % Protein	18.0	
10	Semolina cous-cous	71.9	5036.0
	Cornstarch	6.1	425.2
	Mineral premix	3.4	236.4
	Vitamin premix	0.1	4.0
	Choline	0.3	20.0
	Oil	7.9	555.0
	Diammonium citrate	10.3	723.4
	Calculated % Protein	16.7	
11	Semolina cous-cous with horse bean flour (5%)	66.8	4678.0
	Cornstarch	11.2	783.2
	Mineral premix	3.4	236.4
	Vitamin permix	0.1	4.0
	Choline	0.3	20.0
	Oil	7.9	555.0
	Diammonium citrate	10.3	723.0
	Calculated % Protein	16.6	

TABLE 41. (CONTINUED).

Diet No.	Ingredient	Percent	Amount/7 kg.
12	Semolina cous-cous with horse bean flour (10%)	63.3	4432.0
	Cornstarch	14.7	1029.2
	Mineral premix	3.4	236.4
	Vitamin premix	0.1	4.0
	Choline	0.3	20.0
	Oil	7.9	555.0
	Diammonium citrate	10.3	723.0
	Calculated % Protein	17.2	
13	Semolina cous-cous with horse bean flour (15%)	60.2	4212.0
	Cornstarch	14.7	1029.2
	Mineral premix	3.4	236.4
	Vitamin premix	0.1	4.0
	Choline	0.3	20.0
	Oil	7.9	555.0
	Diammonium citrate	10.3	723.0
	Calculated % Protein	16.8	
14	Semolina cous-cous with lysine	71.0	4972.0
	Cornstarch	7.0	489.2
	Mineral premix	3.4	236.4
	Vitamin premix	0.1	4.0
	Choline	0.3	20.0
	Oil	10.3	723.0
	Diammonium citrate	10.3	723.0
	Calculated % Protein	17.0	

TABLE 42. PROXIMATE ANALYSIS OF 14 DIFFERENT BLENDED DIETS.

Diet No.	Moisture %	Protein* %	Ash %	Crude Fat %	Crude Fiber %
1	18.9	6.9	2.1	6.0	2.5
2	12.6	11.6	2.4	7.1	2.8
3	12.4	10.8	2.3	5.2	3.7
4	13.3	10.8	2.8	5.6	2.6
5	12.8	10.5	2.6	6.9	3.5
6	13.6	10.5	2.8	7.4	2.4
7	12.4	10.9	2.5	9.7	1.9
8	12.8	13.9	2.0	8.2	1.8
9	12.6	18.0	2.2	13.1	3.1
10	12.6	16.7	2.9	10.3	3.1
11	12.8	16.5	2.7	8.7	2.8
12	13.2	17.0	2.4	5.8	[illegible]
13	13.2	16.6	2.6	8.3	3.0
14	13.3	17.0	2.6	5.9	2.2

*Percent protein = N x 6.25

Results and Discussion

Study I (1970)

The average weekly weight gain (Table 43) was highest for Diet 1, the casein control diet, and lowest for Diet 2, which contained semolina as the source of protein. At the 5% level, there was no significant difference between Diet 2 and Diet 3 which contained semolina cous-cous and Diet 6 which contained 85% extraction flour. Diet 7, which contained 85% extraction cous-cous, was significantly higher in average weekly weight gain than Diet 2, but not significant from Diet 3, 6, and 5 which contained semolina cous-cous with horse bean flour. Diet 5 was not significantly lower than Diet 4 which contained semolina with horse bean flour, but at the 5% level was significantly lower than Diets 1, 8, and 9 which contained casein, 85% extraction flour with horse bean flour, and 85% extraction cous-cous with horse bean flour respectively. There was no significance between Diets 8, 9, and 4. Diet 1 was not significantly higher than Diet 8. At 10.8% protein, the weight gain by rats fed Diet 1 was significantly higher than the gain by rats fed Diets 4 and 5 which has 12.6% protein. Supplementation with horse bean flour caused a significant increase in average weekly weight gain.

TABLE 43. ANALYSIS OF VARIANCE FOR AVERAGE WEEKLY WEIGHT GAIN BY RATS FED NINE DIFFERENT EXPERIMENTAL DIETS.

Source	D.F.	S.S.	M.S.	F-Test	Prob.
Treatments	8	9216.219	1152.027	15.972	0.0000
Error	63	4543.984	72.127		
Total	71	13760.203			

LSD = 8.486

Diet No.	1	8	9	4	5	7	6	3	2

The average weekly consumption of feed (Table 44) by rats was not necessarily the highest by those being fed the diets which gave the greatest average weekly weight gain. Rats which were fed Diet 2 had the lowest consumption, but rats fed Diets 4, 1, 9, 8, and 6 were not significantly different; Diet 4 giving the higher feed consumption and Diet 6 the lowest. There was also no significant difference between the average weekly consumption by rats fed Diets 1, 9, 8, 6, 5, 7, 3, and 2; Diet 8 using the higher and Diet 2 yielding the lowest feed consumption. Rats which were fed Diets 4, 1, 9, 8, and 6 gave significantly higher average weekly consumptions than the rats which were fed Diets 5, 7, 3, and 2.

TABLE 44. ANALYSIS OF VARIANCE FOR AVERAGE WEEKLY FEED CONSUMPTION BY RATS FED NINE DIFFERENT EXPERIMENTAL DIETS.

Sources	D.F.	S.S.	M.S.	F-Test	Prob.
Treatments	8	120078.875	15009.859	1.324	0.000
Errors	63	713861.875	11331.141		
Total	71	833970.750			
LSD = 106.448					
Diet No.	4	1 9 8 6	5	7 3	2

The feed efficiency (FE) was calculated by dividing the weight gained into the feed consumption. A feed efficiency value of 1 would represent a perfect conversion, whereas a high FE value would represent a poorer conversion. The most desirable feed efficiencies were produced by Diets 1, 8, and 9 with no significant differences between them at the 5% level. Diet 1 gave the most efficient conversion. The most undesirable feed efficiencies were given by Diets 6 and 2 with Diet 6 yielding the most undesirable FE value. There was a

significant difference between Diets 2, 3, and 7; 7, 5, and 1. There were no significant differences between the diets which were supplemented with horse bean flour than with the diets being significantly more desirable than the diet with no horse bean flour. These results are shown in Table 45.

TABLE 45. ANALYSIS OF VARIANCE FOR FEED EFFICIENCY OF RATS FED NINE DIFFERENT BLENDED DIETS.

Sources	D.F.	S.S.	M.S.	F-Test	Prob.
Treatments	8	351.882	43.985	36.928	0.0000
Error	63	75.039	1.191		
Total	71	426.921			
LSD = 1.080					
Diet No.	6 2	3	7 4	5	9 8 1

The protein efficiency ratios (PER) were analyzed statistically separate from the other data; the results are shown in Table 46. The effect of horse bean flour supplementation and processing on PER is shown in Table 47. The results from individual, one degree of freedom, comparisons showed that supplementation with horse bean flour generally increased the PER, processing reduced the effect which horse bean flour had on PER by a factor of one-half, 85% extraction flour produced higher PER than semolina, and Diet 1, which contained casein as the source of protein, produced the highest PER.

TABLE 46. PROTEIN EFFICIENCY RATIO (PER) FOR RATS FED NINE DIFFERENT BLENDED DIETS.

Diet	Average PER
1	2.979
8	2.814
9	2.192
4	1.720
5	1.708
7	1.566
6	1.454
3	1.177
2	0.967

TABLE 47. EFFECT OF HORSE BEAN FLOUR AND PROCESSING (COUS-COUS) ON PER.

Treatment	Horse bean flour	No Horse bean flour	Means
Processing	1.950	1.371	1.660
Raw	2.017	1.211	1.614
Means	1.983	1.291	

During the latter stages (third week) of the rat feeding study, hair loss was observed in several of the rats. A mild case of hair loss consisted of a small, bare patch across the belly. As the hair loss increased, the bareness spread over the entire underside as far forward as the throat and also to the thighs and legs. In the more severe cases, the rats would retain a strip of hair along the center back between the two bare thighs. There was a large

variation in hair loss within each dietary groups of rats. The groups of rats fed Diet 1, casein, had no hair loss of any kind; group 2 had no hair loss with the exception of one rat which had extreme hair loss. Group 3 showed all degrees of hair loss, while group 4 had 2 rats showing hair loss, one extreme and one moderate. Group 5 had 3 rats showing hair loss, two of which were extreme. There was extreme hair loss in all 8 rats in group 6, and all degrees of hair loss in group 7. Group 8 and 9 had several with mild hair loss. Due to the randomness in the occurrance and severity of hair loss among the diets, no correlation was found between hair loss and the experimental diets.

Study II (1970)

Diet 1, the casein control at 10% protein, gave the highest average weekly weight gain (Table 48) and was significantly different from all other diets. Diet 10 which contained semolina cous-cous at 18% protein gave the lowest. With the exception of Diet 3, semolina cous-cous, the 10% protein series yielded higher average weekly weight gains than the 18% protein series. Diet 6, semolina cous-cous with 15% horse bean flour, was significantly higher than Diet 5 which contained semolina cous-cous with 10% horse bean flour. Neither of these diets was significantly different from Diet 7 which contained semolina cous-cous with lysine. There was no significant difference among the equivalent diets, Diet 12 to 14, in the 18% protein series. Diet 4, semolina cous-cous with 5% horse bean flour, was not significantly different from Diet 5, but was significantly lower in average weekly weight gain than Diet 7 and higher than Diet 3 which contained semolina cous-cous and Diet 2 which contained semolina. In the 18% protein series, Diet 11, semolina cous-cous with 5% horse bean flour, was significantly higher than Diet 9 and 10 which contained semolina and semolina cous-cous, respectively. Diets 14, 13, and 12 which contained semolina cous-cous

with lysine, semolina cous-cous with 15% horse bean flour, and semolina cous-cous with 10% horse bean flour, were not significantly higher than Diet 11. Supplementation with horse bean flour tended to increase the average weekly weight gain. The use of diammonium citrate to increase the level of nitrogen of the 18% series tended to decrease the average weekly weight gain.

TABLE 48. ANALYSIS OF VARIANCE FOR AVERAGE WEEKLY WEIGHT GAIN FOR RATS FED 14 BLENDED EXPERIMENTAL DIETS.

Sources	D.F.	S.S.	M.S.	F-Test	Prob.
Treatments	13	4994.035	384.156	51.363	0.000
Error	94	703.020	7.479		
Total	107	5697.055			

LSD = 5.471

Diet No.	1	6	7	5	4	2	8	13	12	14	11	3	9	10

Average weekly feed consumption had the highest results with the casein (Table 49) control diet of the 10% protein series and the lowest results with the semolina diet of the 18% protein series. Diet 1 was significantly higher than all other diets with the exception of Diet 6 which was significantly higher than Diet 5, but not higher than Diet 7. There were no significant differences between Diets 7, 5, and 4 or Diets 2, 8, 11, 12, and 13. Diet 4 was significant among Diets 11, 12, 13, and 3, or Diets 12, 13, 3, 10, and 14, or 10, 14, and 9. Horse bean flour supplementation tended to increase the average weekly feed consumption, with the 10% protein series generally higher than the 18% protein series.

TABLE 49. ANALYSIS OF VARIANCE FOR AVERAGE WEEKLY CONSUMPTION OF RATS FED 14 BLENDED EXPERIMENTAL DIETS.

Sources	D.F.	S.S.	M.S.	F-Test	Prob.
Treatments	13	22832.008	1756.308	23.992	0.000
Error	94	6881.184	73.204		
Total	107	29713.191			

LSD = 8.560

Diet No.	1	6	7	5	4	2	8	11	12	13	3	10	14	9

The casein control diet of the 10% protein series gave the most desirable (Table 50) feed efficiency value; while Diet 10, containing semolina cous-cous of the 18% protein series, gave the most undesirable feed efficiency. There were significant differences between Diets 1 and 2, 7 and 14, 4 and 13, 2 and 11, 14 and 3, 13 and 9, and 11 and 10. The feed efficiency values showed more significant differences between the diets of the 10% protein series than the average weekly feed consumption values. Generally the diets of the 10% protein series gave more desirable FE values than most of the 18% protein series.

TABLE 50. ANALYSIS OF VARIANCE FOR FEED EFFICIENCY OF 14 BLENDED EXPERIMENTAL DIETS.

Sources	D.F.	S.S.	M.S.	F-Test	Prob.
Treatment	13	904.500	69.577	13.807	0.000
Error	95	478.746	5.039		
Total	108	1383.246			

LSD = 2.248

Diet No.	10	9	3	11	12	8	13	14	2	4	5	7	6	1

All diets of the 10% series were significant with relation to protein (Table 51) efficiency ratio (PER). Diet 1 gave the highest PER, whereas Diet 10 yielded the lowest. There was no significance between Diets 5 and 7; 8, 3, 14, 13, and 12; 14, 13, 12, and 11; 12, 11, and 9; and 9 and 10. Diets 8 and 3 were significantly higher than Diet 11. Diet 13 was significantly higher than Diet 9 and Diet 11 was higher than 10. Supplementation with horse bean flour tended to improve the PER. Generally the 10% protein series tended to have higher PER's than the diets of the 18% series.

TABLE 51. ANALYSIS OF VARIANCE FOR PROTEIN EFFICIENCY RATIOS OF 14 BLENDED EXPERIMENTAL DIETS.

Sources	D.F.	S.S.	M.S.	F-Test	Prob.
Treatment	13	96.642	7.434	155.816	0.000
Error	95	4.532	0.048		
Total	108	101.174			
LSD = 0.216					
Diet No. 1 6 5 7 4 2 8 3 14 13 12 11 9 10					

Varying degrees of hair loss was observed in all of the 14 diets. The type of hair loss and the distribution of the rats with hair loss among the diets was similar to those observations in Study I (1970). The section of the feeding study involving the rats fed with Diets 1 through 7 was extended 2 weeks to bring the length of the study to 6 weeks.

The extension of the rat feeding study, 2 weeks, tended to increase the significance between feed efficiency values and decrease the significance between protein efficiency ratios.

Hair loss continued to occur at the end of the sixth week of the feeding study. Rats from all of the dietary groups continued to lose hair while some rats from each diet showed signs that new growth of hair was permanent. The distribution of the rats showing hair loss and those showing hair loss and the hair gain was in a manner that could not be related to the individual diets.

The rats fed Diets 8-14, the 18% protein series, showed lower average weekly weight gains, average weekly weight gains, average weekly feed consumption, protein efficiency ratios, and higher feed efficiency values than the rats fed the 10% protein diets 1 to 7. Eighteen percent protein is considered optimum for the growth of rats, but the data from Study II (1970) did not support this theory. The 18% protein diets contained diammonium citrate which may have influenced the acceptability of the diets to the rats.

Conclusion

In Study I (1970), the supplementation of flour and cous-cous diets with horse bean flour improved the diets ability, as a source of protein, to support the growth and development of the rats. By increasing the quantity and quality of the protein through the addition of horse bean flour, the limiting effect of lysine on utilization of the wheat protein is diminished. The 85% extraction flour constitutes a greater percentage of the wheat kernel than semolina. Generally the processing of the flour into cous-cous increased the nutritional value. This could be due to the destruction of enzyme inhibitors. The processing of cous-cous decreased the effect of horse bean flour supplementation as compared to the effect of supplementation of unprocessed flour. The results of heating horse bean flour may be related to the reaction of protein with carbohydrates, leading to the destruction of amino acids and an alteration in the digestibility of the protein by proteolytic enzymes. The reaction of protein in the absence of carbohydrate, which results in a decrease in the availability of some amino acids, may also effect the horse bean flour.

In Study II (1970), diets supplemented at levels of 10 and 15% yielded results which compared more favorably with the casein control than the other diets. In contrast to Study I, the processing of the flour into cous-cous decreased the nutritional value. The nutritional value of the diets increased with increased supplementation with horse bean flour. The diets of the 18% protein series of Study II (1970) which contained diammonium citrate yielded results that were inferior to those of the 10% protein series. The diammonium citrate gave a very bitter and unpleasant taste and aroma to the diets that resulted in their rejection by the rats.

Based on the results of the rat feeding studies, it can be concluded that supplementation of cous-cous with horse bean flour can have positive nutritional qualities. In some instances this processing of the flour into cous-cous decreased the nutritional value, but this was compensated for by the increase in nutritional value resulting from the horse bean flour supplementation.

PROGRESS REPORT ON THE ORGANOLEPTIC PHASE OF AID/csd-1586

During the past year efforts have been continued toward the development of protein-supplemented bread and cous-cous, and chapatis that will be acceptable to the Moroccan and West Pakistani consumers, respectively. At the present time we are still examining and characterizing products in the laboratory, so that it will be possible to select for field-testing those formulas, supplemented flours, or supplemented products which appear to have consumer acceptance potential. With regard to cous-cous, preparations now are being made for initial testing of some of the most promising supplemented samples. In July, 1970, a mission team composed of Drs. Hoover, Deyoe, Robinson, and Caul visited Morocco and established contacts with several cous-cous manufacturers. These manufacturers agreed to produce and package our supplemented cous-cous, if a suitable product could be developed. On January 23, 1971, Dr. Hoover returned to Morocco with three samples of legume protein supplemented cous-cous to give them to the manufacturers for their personal examination. A questionnaire was prepared for (1) recording the manufacturers' reactions to the products and (2) providing us with additional information about the products.

Cous-Cous

Three legume proteins have been investigated as supplements for cous-cous: chick pea flour, horse bean flour, and soy flour. The incorporation of 0.5% Emplex (sodium stearoyl-2-lactylate, Patco Products) and 6 NRA (Type 6 N-Richment-A) added at the rate of 1 oz/100 lb. flour also has been studied. All cous-cous samples were made in the Department of Grain Science and Industry and prepared for organoleptic examination in the laboratories of the Department of Foods and Nutrition. Unless otherwise specified, all samples were cooked according to directions developed two years ago by Kirleis et al., 1969, (79)

and examined for aroma, flavor, texture, and appearance. The results are presented in the following reports.

Preparation of Cous-cous for the Organoleptic Analysis
Reported in Cous-cous Reports #2 and #3

Part I

Three different batches of semolina cous-cous were made from 11/20/70 to 11/22/70. One semolina cous-cous contained chick pea flour at a level of 10% of the semolina. The other two batches of semolina cous-cous contained horse bean flour at levels of 10 and 20% respectively. The three batches of cous-cous also contained Emplex at a level of 0.5% and type 6 N-Richment-A (6NRA) at a level of 0.06%.

Raw Material

The semolina used to make the cous-cous was prepared from a blend of semolina from the Peavey Company (40%) and semolina milled at KSU (60%). The commercial semolina was held in cold storage (40°F) in poly bags until prepared into cous-cous. The KSU semolina was milled on 12/12/69 and was held in fiber drums in cold storages until being made into cous-cous. The horse beans and chick peas were obtained from the Country Store, Kansas City, Mo. The horse bean flour was milled on 12/1/70 and was held in cold storage in poly bags. The chick pea flour was held in cold storage in poly bags although the date of milling was not known. The Emplex was obtained from the C. J. Patterson Company. The 6NRA was received from the Pennwalt Corporation. Both were stored at room temperature until used.

Preparation of Cous-cous

The general procedure for the preparation of the above cous-cous involves the agglomeration of the semolina with water, then sieving to separate the

agglomerated particles. Following the sieving, the particles were air dried after being autoclaved. The steps involved in this procedure are listed below.

1. The raw material was agglomerated 3,000 grams at a time.

2. The flour was placed into the mixing bowl of a Hobart (A-200) mixer (with wire whip).

3. The mixer was set to speed number two and turned on.

4. Distilled water was added slowly and continuously over a two minute period.

5. The mixing was continued for an additional two minutes after the addition of the water.

6. Following the second two minute period, the mixer was stopped and the sides of the mixing bowl were scraped down.

7. The mixer was then started again at speed number two and distilled water was added over a two minute period.

8. After the addition of the water, the mixing was continued for two minutes.

9. The mixer was then stopped, and the sides of the bowl were scraped down.

10. The mixer was started again at speed two and distilled water was added over a two minute period.

11. Following the addition of the water, the mixing was continued for two minutes.

12. The mixer was then turned off and the agglomerated particles were sifted through a nest of sieves consisting of U.S. Standard number eight (2.38 mm), twelve (1.68 mm), and eighteen wire (1.00 mm).

13. The sieved cous-cous was then autoclaved at 20 psi and 120°C. for 4 minutes.

14. The autoclaved cous-cous was then sieved through a U.S Standard number eight wire (2.38 mm).

15. The sieved cous-cous was then placed in an air oven and dried at 50°C. for 20 hours.

16. The dried cous-cous was then sieved for size through the sieves listed in step 12.

17. The dried sieved cous-cous was held at room temperature in poly bags in fiber drums.

Cous-cous Formulation

The amounts of raw materials used in the preparation of cous-cous, and the average amounts of distilled water added per agglomeration are listed below.

Raw Material	Semolina Cous-cous +10% Horse bean fl.	Semolina Cous-cous +20% Horse bean fl.	Semolina Cous-cous +10% Chick pea fl.
Semolina	2684.9 g	2486.6 g	2684.9 g
Horse bean fl.	298.3 g	596.6 g	
Chick pea fl.			298.3 g
Emplex	15.0 g	15.0 g	15.0 g
6NRA	1.8 g	1.8 g	1.8 g
Average amount of water/agglomeration			
1st agglomeration	296 g	395 g	295 g
2nd agglomeration	297 g	210 g	310 g
3rd agglomeration	103 g	105 g	115 g

Part II

Seven different batches of semolina cous-cous were prepared from 12/15/70 to 12/16/70. Two batches contained only semolina as the flour, with one batch enriched with Emplex and 6NRA. Two batches of semolina cous-cous contained soy flour at the 10% level with one enriched with Emplex and 6NRA. The remaining three batches of semolina cous-cous contained horse bean flour at 10 and 20% and chick pea flour at 10%, respectively.

Raw Material

All cous-cous samples were prepared from commercial semolina obtained from the Peavey Company on 12/5/70. The soy flour (Ardex 550) was obtained from Archer-Daniels-Midland. The horse bean and chick pea flours were the same as used in Part I of this report.

Preparation of Cous-cous

The preparation of the cous-cous was the same as stated in Part I.

Cous-cous Formulation

The weight of the raw materials used to prepare the cous-cous and the average amount of water added per agglomeration are shown below.

Two other batches of cous-cous were prepared on 1/15/71 to aid in the organoleptic analyses of the cous-cous prepared in Parts I and II. One was semolina cous-cous with horse bean flour at the 20% level and the other was semolina cous-cous with chick pea flour at the 10% level. Both batches were enriched with Emplex and 6NRA.

Raw Material	Semolina Cous-cous	Semolina Cous-cous + Emplex + 6NRA	Semolina Cous-cous +10% soy fl.	Semolina Cous-cous +10% soy fl. + Emplex + 6NRA	Semolina Cous-cous +10% horse bean fl.	Semolina Cous-cous +20% horse bean fl.	Semolina Cous-cous +10% chick pea fl.
Semolina	3000.0 g	2983.2 g	2700.0 g	2684.9 g	2700.0 g	2400.0 g	2700.0 g
Horse bean fl.					300.0 g	600.0 g	
Chick pea fl.							300.0 g
Soy fl.			300.0 g	298.3 g			
Emplex		15.0 g		15.0 g			
6NRA		1.8 g		1.8 g			
Average amount of water/agglomeration							
1st agglomeration	260 g	300 g	[illegible] g	[illegible] g	200 g	350 g	300 g
2nd agglomeration	260 g	300 g	300 g	[illegible] g	200 g	150 g	300 g
3rd agglomeration	[illegible] g	100 g	100 g	[illegible] g	150 g	50 g	100 g

Cous-cous Report # 2. Initial laboratory examination has been completed for the following cous-cous samples:

Sample	Date of Preparation
1. Semolina (72% extraction)	4/12/70
2. Semolina with 10% chick pea flour and Emplex-6NRA	11/22/70
3. Semolina with 10% horse bean flour and Emplex-6NRA	11/20/70
4. Semolina with 20% horse bean flour and Emplex-6NR*	11/20/70

*It is understood that 6NR is 6NRA without vitamin A (note: the supplemented cous-cous had not been sieved to remove large clumps and fines, although sieving to size is the usual procedure).

The purpose of this series of organoleptic examinations was to see whether Emplex would suppress the beany character typical of some legume protein supplements. Each supplemented cous-cous sample was cooked and examined only once.

Results and Discussion

The results are presented in Table 52. Although it was not possible to assess the effect of Emplex separately from 6NRA, none of the cooked supplemented cous-cous possessed supplement character in aroma or flavor. However, none were typical of the semolina cous-cous (control). Furthermore, all supplemented cous-cous evolved "off" aromatics during preparation and were atypical in appearance. Texture of the horse bean supplemented cous-cous during preparation was gummy and unlike the control, as was the texture of all cooked supplemented cous-cous. It remains to be seen whether the atypical aroma and flavor properties of the supplemented cous-cous will be masked when the cous-cous are prepared under homeuse conditions (e.g. steamed over and served with one of the traditional Tajines). But the "off" aromatics evolved during preparation and the atypical appearance of these supplemented samples may well alert consumers negatively. The gummy clump

TABLE 52. COUS-COUS SERIES #2.

	Semolina	Semolina + 10% chickpea flour + Emplex-6NRA	Semolina + 10% horse-bean flour + Emplex-6NRA	Semolina + 20% horsebean flour + Emplex-6NR
Aroma of uncooked cous-cous	oxidized, little Semolina identity	not legumy, oxidized, cereal character	cereal identity, not beany	cereal identity, not beany
Aroma in preparation room during steaming	OK	"off"--burnt, starch	"off"--sourish, starchy, beany	"off"--sourich, starchy, beany
Cooked cous-cous:				
aroma	starchy, little Semolina identity	little Semolina identity, "suspect" but not "off"	some off notes, not beany, less flour identity but more amplitude than Semolina	some off notes, not beany, less flour identity but more amplitude than Se
flavor	same as aroma	same as aroma	same as aroma	same as aroma
color	OK	tan	darker than the Semolina	darker than the Semolina
texture	OK	gummy, chewy	OK except for a few gummy particles	OK except for a few gummy particles

formed during preparation of the horse bean supplemented cous-cous also might alert the consumer, but these textural abnormalities probably occurred because the supplemented cous-cous had not been sieved to size.

Cous-cous Report # 3. Initial laboratory examination has been completed for the following cous-cous samples:

Sample	Date of Preparation
1. Semolina	12/15/70
2. Semolina with Emplex-6NRA	12/15/70
3. Semolina with 10% soy flour	12/15/70
4. Semolina with 10% soy flour and Emplex-6NRA	12/15/70
5. Semolina with 10% horse bean flour	12/15/70
6. Semolina with 20% horse bean flour	12/15/70
7. Semolina with 10% chick pea flour	12/15/70

Each supplemented cous-cous sample was cooked and examined only once, with the exception of the sample supplemented with 10% horse bean flour. It was not examined, since no "offness" was detected in the 20% supplemented sample.

Results and Discussion

The results of the organoleptic work on this series of cous-cous are presented in Table 53. No gummy clumps formed during the preparation of any cous-cous in this series. Some "off" aromatics were noted during the preparation of all of the suplemented cous-cous and all differed from the control in color. The 10% chick pea supplemented sample was less tender than the control. None of the cooked cous-cous in this series appeared definitely "off" in either aroma or flavor, although a slight supplement character sometimes was detected in the flavor of the 10% chick pea and 10% soy supplemented cous-cous.

Based upon examination of the two pairs of cous-cous samples that differed only in the presence or absence of Emplex-6NRA, some information was gained about the organoleptic effects of Emplex-6NRA although information is insufficient to

TABLE 53. COUS-COUS SERIES #3.

	New Semolina	New Semolina + Emplex-6NRA	10% Soy	10% Soy + Emplex-6NRA	20% Horsebean	10% Chickpea
aroma of flour	OK	---	caramel, amine-like, legumy?	---	not beany, "atypical"	legumy?
aroma of uncooked cous-cous	OK	leathery, rubbery, no grain identity	dill-pickles-like, molasses, beany	molasses-like, rubbery	OK	legumy
*aroma in preparation room during steaming	OK		sweetish, caramel; barely off		legumy	
cooked cous-cous:						
aroma	OK	sauerkraut-like	less blended than control	sauerkraut-like	different from control but not off	OK
flavor	OK	OK	less blended than control beany? bitter?	OK	different from control but not off	legumy?
color	OK	OK	red-gold	red-gold	gray to tan	darker than control
texture	OK	OK	OK	OK	OK	chewy resilient

*brackets indicate those cous-cous that were prepared at the same time in the same room but in separate cookers

advise either for or against its addition. Emplex-6NRA when added to the 10% soy-supplemented cous-cous seemed to produce a more blended flavor, masking the supplement character which sometimes had been observed in the sample without Emplex-6NRA. Additionally, the presence of Emplex-6NRA coincided with the presence of sauerkraut-like aromatics in the aroma of the cooked cous-cous. The latter finding prompted re-examination of the Emplex-6NRA supplemented cous-cous in series #2, since sauerkraut-like aromatics had not been reported in association with all Emplex-6NRA additions in that series. Sauerkraut-like aromatics actually had been noted in the aromas of the 10 and 20% horse bean-supplemented cous-cous with Emplex-6NRA (but not the 10% chick pea-supplemented cous-cous with Emplex-6NRA) and were reported as a part of some "off" notes observed.

Re-examination confirmed the finding of sauerkraut-like aromatics in the aroma of the 10% horse bean-supplemented cous-cous with Emplex-6NRA and not in the aroma of the 10% chick pea sample, but failed to confirm previously observed sauerkraut aromatics in the 20% horse bean-supplemented cous-cous. As before, all cous-cous were described as somewhat "off" in both aroma and flavor, although only the 10% chick pea-supplemented cous-cous with Emplex-6NRA was legumy in both aroma and flavor.

Examination of Cous-cous as a Dessert. Since up to the present, we have been able only to surmise whether or not "off" aromas and flavors might be detected under home-use conditions, 4 of the Emplex-6NRA-containing cous-cous of series #2 and 3 were prepared and served with butter, sugar, cinnamon, and milk for organoleptic examination. The samples prepared were:

Series #2--10% chick pea with Emplex-6NRA
--20% horse bean with Emplex-6NRA
Series #3--10% soy with Emplex-6NRA
--Semolina with Emplex-6NRA

A recipe obtained from members of the Moroccan delegation who visited KSU on 4/1/69 was used as a guide. Cous-cous were prepared in the usual manner. After the final steaming period, butter was worked into the cous-cous, which were then piled on a platter and sprinkled with powdered sugar and cinnamon. Warmed milk was added at the base of the cous-cous and was allowed to be absorbed. Amounts of butter, sugar, cinnamon, and milk were not measured for this initial trial.

For this "consumer-type" assessment of the cous-cous, the participation of an additional panel member was obtained. This person was not trained in flavor analysis nor was she familiar with cous-cous. At the time of the panel session, she was informed only of the order in which the samples were to be examined for aroma, flavor, appearance, and texture, and that the first sample was the control product (semolina cous-cous with Emplex-6NRA).

Results and Discussion

The results are presented in Table 54. All three panelists reached a consensus about the samples. The soy-supplemented cous-cous was similar to the control. No "offness" was found in aroma or flavor, and the soy-supplemented cous-cous had more amplitude of flavor than the control. The color of the soy-supplemented cous-cous was red-gold in comparison to the light yellow control, and the texture was similar to the control.

The horse bean-supplemented cous-cous differed from the control in color and texture. It was gray to tan in color and fine to mushy in texture (note--this cous-cous had not been sieved to size). The aroma was not "off" but bitterness was detected in the flavor. It was suggested that perhaps the bitterness could be overcome with more cinnamon and sugar.

The chick pea-supplemented cous-cous was definitely "off" in both aroma and flavor. It was observed that the addition of sugar improved the flavor except for the bitterness which remained. This cous-cous was darker in color than the control and less tender.

TABLE 54. COUS-COUS AS A DESSERT.

	New Semolina + Emplex-6NRA	10% Soy + Emplex-6NRA	20% Horse bean + Emplex-6NRA	10% Chick pea + Emplex-6NRA
aroma	OK	OK	OK	legumy
flavor	OK	OK	bitter	legumy, bitter
color	OK	red-gold	gray to tan	darker than control
texture	OK	OK	mushy	less tender than control

This examination of cous-cous as a dessert revealed that (1) sauerkraut-like aromatics if present were masked and (2) supplement character of all but the chick pea-containing cous-cous was masked (with the exception of bitter in the horse bean-supplemented cous-cous).

Boiled Cous-cous Procedure. The following "quick cooking" method was used successfully to prepare cous-cous samples made in the Department of Grain Science and Industry at KSU. Only one further modification was required--whereas the Dromadaire cous-cous required only 2 min. boiling time, all of the KSU products required 3 min. for proper water absorption.

1. Pour ½ cup cous-cous gradually into 1 cup boiling water.
2. Continue to boil for 2 minutes stirring occasionally or until water is almost absorbed.
3. Remove from heat, cover tightly, let stand for about 10-15 minutes.
4. Fluff with a fork and serve as is.

Modified instructions from Dromadaire Cous-cous,
A. Bonnefoy et ses Fils, Asnieres, France--omits additions of oil and salt.

Cous-cous samples to be taken to Morocco on 1/23/71 were previewed using the boiling method of preparation. Results of the organoleptic examinations are presented in Table 55. The results appear to indicate that fewer volatiles escape from the cous-cous during boiling than during steaming.

TABLE 55. BOILED COUS-COUS.

	Dromadaire	Semolina + Emplex-6NRA	10% Soy	10% Chickpea + Emplex-6NRA	10% Horsebean + Emplex-6NRA
color	grayed	OK	red-gold	dark, not yellow	OK
texture	mushy, chewy after standing	OK	more resilient than plain Semolina	slightly chewy?	OK
aroma	not macaroni-like, oxidized?	starchy, acidic + ?, rye bread-like	sweet, caramel more amplitude than plain Semolina, toasted cereal	starchy	not macaroni-like, more total aroma than Dromadaire painty?
flavor	raw starchy, oxidized? less sweet than 10% horsebean	starchy, not wheaty, greenish when cold	more flavor than plain Semolina, starchy? caramel? sweetish, greenish, legumy, beany	off, much total flavor, legumy, bitter, sweet, starchy, green, fatty, tongue bite	starchy, sweetish, bitter, painty?

Cous-cous Report # 4. The following three pairs of cous-cous samples were examined to gain more information about the organoleptic effects of Emplex-6NRA additions:

1. Semolina cous-cous & Semolina cous-cous with Emplex*-6NRA**
2. 20% horse bean cous-cous & 20% horse bean cous-cous with Emplex*-6NRA**
3. 10% chick pea cous-cous & 10% chick pea cous-cous with Emplex*-6NRA**

*0.5%
**1 oz/100 lb. flour

All the above samples were prepared with "new" Semolina in the Department of Grain Science and Industry (12/15/70-1/16/71) and cooked according to the procedure developed by Kirleis et al., (1969) (79).

Results

The results of the organoleptic examinations are presented in Table 56.

Discussion

No off aromatics were noted during the preparation of the Semolina cous-cous. Semolina cous-cous without Emplex-6NRA was starchy and quite bland in aroma and flavor. Semolina cous-cous with Emplex-6NRA was less starchy, sourish, and perhaps more ample in aroma. Emplex-6NRA additions had a similar effect on the flavor but did not boost amplitude. The addition of Emplex-6NRA had no effect on texture or appearance.

Horse bean aromatics evolved during the preparation of the horse bean supplemented cous-cous samples, but were not noticed per se in the aroma or flavor of the cooked samples either with or without Emplex-6NRA. Both samples did, however, have some supplement character and a bitter aftertaste. Sauerkraut-like aromatics and sourish taste were detected in the aroma and flavor, respectively, of the sample containing Emplex-6NRA. Supplement character may have been diminished by Emplex-6NRA additions, and the color of the Emplex-6NRA-containing cous-cous may have been lighter. Emplex-6NRA additions had no discernible effect on the texture of the cous-cous.

TABLE 56. COUS-COUS SERIES #4.

	Semolina	Semolina + E-6NRA	20% HB	20% HB + E-6NRA	10% CP	10% CP + E-6NRA
Cooking room aroma*	[OK]		[horse bean aromatics]		[chick pea aromatics]	
Cous-cous: Aroma:	OK	less starchy, more ample than plain Semolina, sourish	sweetish, molasses-like	less ample than 20% HB; sauerkraut-like aromatics	no chick pea aromatics	no chick pea aromatics; less total aroma than 10% CP; sauerkraut-like aromatics
Flavor:	OK	less starchy than plain Semolina; sourish	not beany some supplement character, bitter aftertaste	not beany may be less supplement character than 20% HB; bitter aftertaste; sourish	chick pea aromatics	chick pea aromatics; bitter; less amplitude than 10% CP
Texture	OK	OK	OK	OK	chewy?	chewy?
Appearance	OK	OK	dark tan	light tan	OK	OK

*brackets indicate samples prepared simultaneously in the same room but in separate cookers

Chick pea aromatics were noted during the preparation of the chick pea supplemented cous-cous, and in the flavor though not in the aroma of the cooked cous-cous--both with and without Emplex-6NRA. The cous-cous containing Emplex-6NRA had less total aroma than the cous-cous without Emplex-6NRA, and sauerkraut-like aromatics were associated with the presence of Emplex-6NRA. The 10% chick pea supplemented cous-cous with Emplex-6NRA had less amplitude of flavor than the cous-cous without Emplex-6NRA and was bitter. Emplex-6NRA additions had no effect on texture or appearance of the cous-cous.

Conclusions

It appears that the organoleptic effects of Emplex-6NRA additions were small in unaged cous-cous made from relatively "fresh" Semolina. The additive combination neither improved nor degraded, although its presence was marked by sourish, sauerkraut-like notes. It remains to be seen how these samples will age.

SOME OBSERVATIONS OF THE MILLING AND BAKING QUALITY OF MOROCCAN WHEAT

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Abstract

Seven wheat samples were obtained from various locations in Morocco. These samples were Norteno, Penjamo, Inia, Siete Cerros, Tobari, BT 908 and BD 2777. Tobari gave exceptional milling yield. No milling problems were encountered except with BD 2777 which was exceptionally hard and required additional reduction to give a reasonable flour yield.

Tobari, Siete Cerros and Inia were found suitable for production of white pan bread. Norteno, Inia, Siete Cerros, Tobari and BT 908 were suitable for blending. Penjamo and BD 2777 were found suitable for cous-cous production with BD 2777 having the greater suitability.

Introduction

Evaluation of milling and baking characteristics is one of the important aspects in a wheat breeding program. Good milling and baking characteristics are part of the criterion for cultivar selection.

The breeding materials developed in Morocco consisted of seven cultivars of wheat and were evaluated for milling and baking characteristics in the Department of Grain Science and Industry, Kansas State University. Bison, winter wheat, was used as control in the laboratory. The seven cultivars consisted of partly the Mexican germ plasm and partly from the lines developed in Morocco by Dr. Edwards and Dr. Acosta. The purpose of the study was the evaluation of the milling and baking characteristics of seven cultivars of Moroccan wheat. The samples were received through the United States Agency for International Development (USAID) in Rabat, Morocco.

Experimental

Characterization and Milling of Wheat Samples.

The samples were characterized according to the following methods:

Wheat Test Weight - Test weight is the weight per Winchester bushel with the weight expressed to the nearest tenth of a pound. Determination was made as described in Circular No. 921 issued by the United States Department of Agriculture (1915; second approval date 1961).

Weight per Hectoliter

lbs/U.S. bu x 454g/lb x 2.8379 bu/Hectoliter x 1Kg ÷ 1000g = Kg/Hectoliter

(e.g.)

60.4 lbs/U.S. bu x 454g/lb x 2.8379 bu/Hectoliter x 1Kg ÷ 1000g = 77.8 Kg/Hectoliter

1000 Kernel Weight - The weight in grams of 1000 kernels of wheat was determined with an electronic seed counter, using a 40g sample from which all foreign material and broken kernels have been removed.

Density - The density is determined with a Beckman Air Pycnometer, using a 14.4 gram sample.

$$\text{Density} = \frac{14.4 \text{ grams}}{\text{Volume (cc.)}}$$

Pearling Value - 20g of wheat with all foreign material and broken kernels removed is retained for one minute in a Strong Scott Laboratory Barley Pearler equipped with a No. 30 grit stone and a 10 mesh screen made of wire .041 inches in diameter (Tyler Code "Fijor"). Pearling value is percent of original sample remaining over a 20 mesh wire after pearling.

Wheat Size Test - Two hundred grams of wheat are placed on the top sieve of a stack of 3 Tyler standard sieves (numbers 7, 9, 12). The stack of sieves is placed in a Ro-Tap sifter and sifted for 60 seconds. The percentage remaining on each sieve is then determined, multiplied by factors of 78, 73, and 67 respectively, and summed to obtain a single number denoting the theoretical flour yield.

Protein - AACC Cereal Laboratory Methods, 46-10. The total nitrogen content is multiplied by 5.7 and the results are expressed as percent protein on a 14% moisture basis.

Ash - AACC Cereal Laboratory Methods, 08-01. The percent remaining after ignition is expressed as percent ash on a 14% moisture basis.

Moisture, ash and protein tests were determined according to Cereal Laboratory Methods (1962) (81). The samples were cleaned and tempered to 16% moisture for 18 hours and then milled on a Buhler Automatic Experimental Mill Model MLU-202 is set, using a representative control wheat to give a 68-70% total product extraction at a .36-.40% ash.

The mill was warmed up for one hour and a control sample (Bison) was run. The feed rate was set at 120 grams per minute before each sample was milled. After each sample was run, the mill was brushed down and allowed to clean for ten minutes. Flour streams, bran, and shorts were weighed and extraction calculated as grams flour/grams total product. The milling flow is shown in Figure 30.

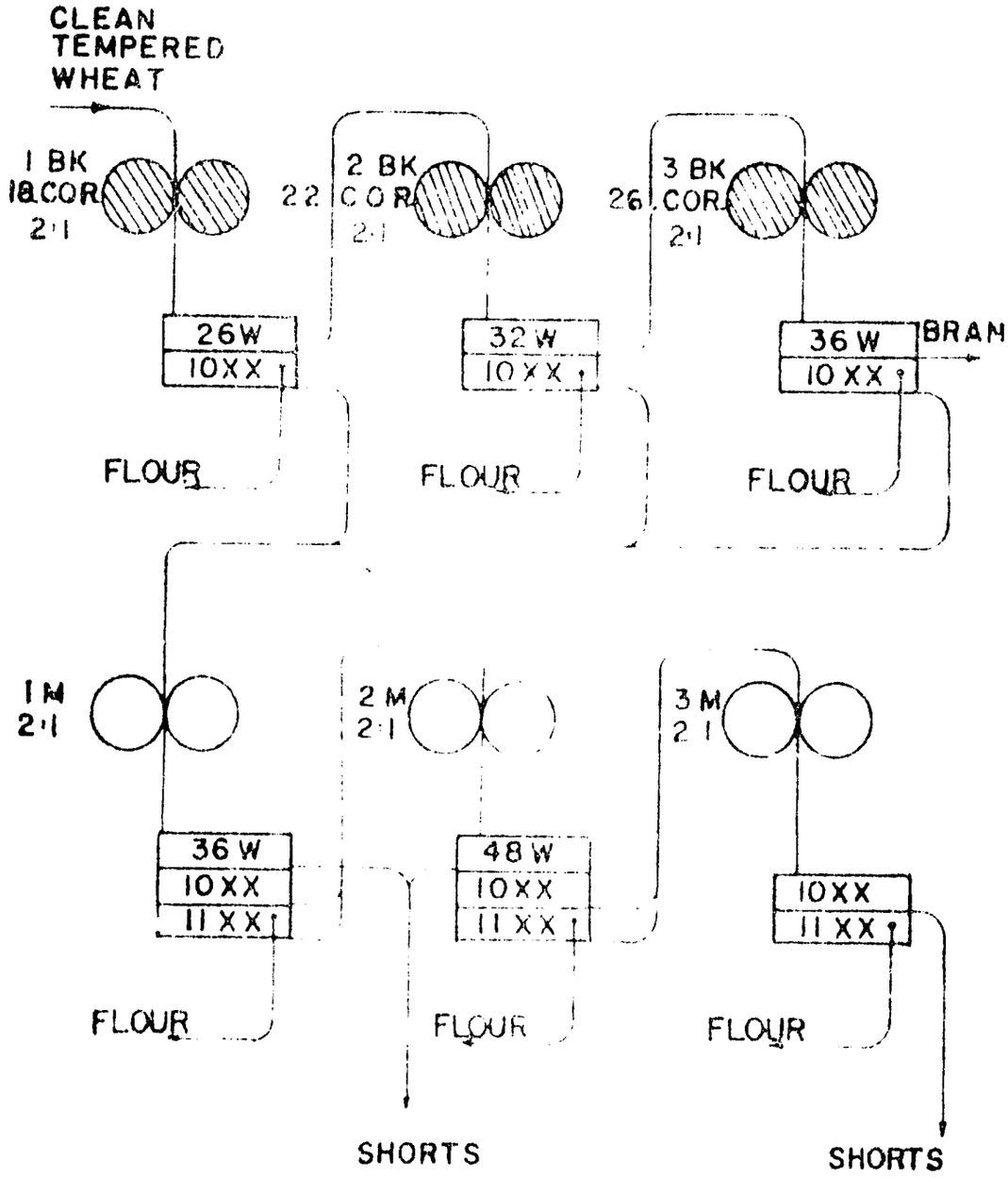
Since cultivars Penjamo and BD 2777 did not show well for straight grade milling and baking, semolina processing was applied and the flow is shown in Figure 31.

Cous-cous was made with the resultant semolina according to the method of Gainor *et al.*, (1971) (82).

Evaluation of the Rheological Properties of the Flours.

To determine flour color, Agtron determination was made by the slurry method using 20 grams of flour and 25 ml. water. Zero was set using disc No. 63 and 100 was set using disc No. 85. These determinations were made using the Model M-4 green Agtron for measuring 546 MCN wavelength light.

Farinograms were made by mixing 50 g. flour (14% moisture basis) in a small bowl with sufficient distilled water to give a maximum dough consistency centered around the 500 B.U. line. For extensigrams, doughs were prepared in the large farinograph bowl from 300 g. flour and solutions in amounts calculated to obtain a maximum dough consistency around the 500 B.U. line. The doughs were



FLOW SHEET FOR BUHLER EXPERIMENTAL MILL

Figure 30

KANSAS STATE UNIVERSITY *Manhattan, Kansas 66502*

CURRICULUMS IN
MILLING TECHNOLOGY
FEED TECHNOLOGY
BAKERY MANAGEMENT

DEPARTMENT OF GRAIN SCIENCE AND INDUSTRY
MILLING INDUSTRIES BUILDING
TELEPHONE: 532-6561

DURUM FLOW AID F-32

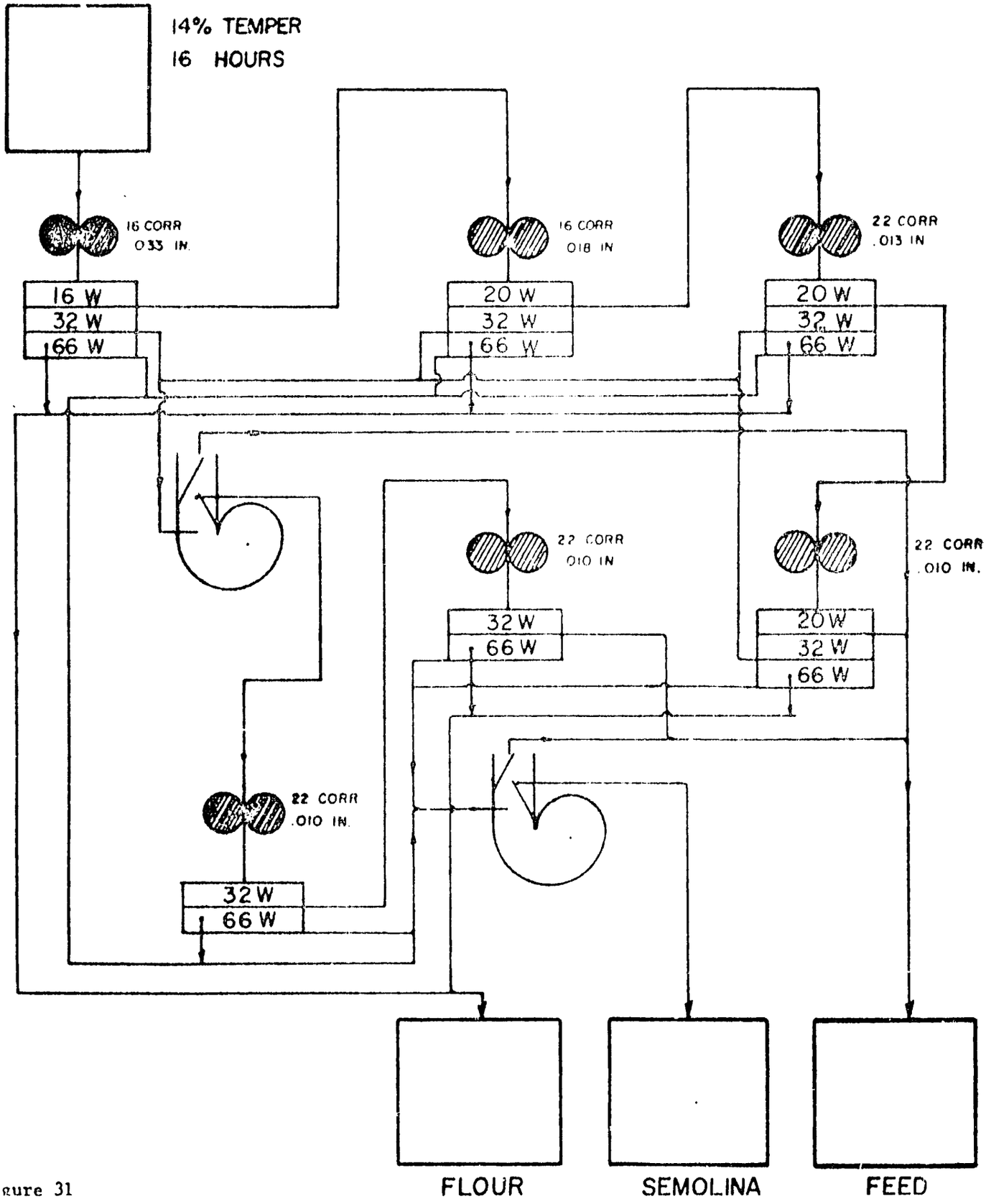


Figure 31

mixed for 1 min., rested 5 min. and remixed. Two 150 g. portions of developed doughs were scaled, rounded 20 times, moulded into cylinders and placed in the extensograph cabinets maintained at 30°C. Curves were drawn for duplicate dough at 45, 90 and 135 min.

Bread Baking Results

Baking quality of cultivars were evaluated by the pup loaf straight dough method with the following formula:

Formula:

Flour	100%	200g	
Water	Variable	--	Farinograph obsn.
Yeast	2	4g	
Salt	2	4g	
Sugar	6	12g	
NFDM	3	6g	
Malt	0.1	0.2g	
$OBrO_3^-$	(15 ppm)	15 ppm	

1. Mix: optimum by feel: Doughs were mixed in a Swanson 200g. mixer to optimum.

2. Dough wt: Divide dough into two equal pieces, round, and ferment.

3. Punching schedule (machine punched):
   a) First punch 1 hr. 50 min.
   b) Second punch at 2 hr. 40 min.
   c) Mould and pan at 3 hrs.

4. Method of moulding:
   a) By hand as described in AACC 7th Edition, 1962.

5. Baking:
   a) Temperature, 425°F
   b) Time, 25 min.

6. Scoring:
   a) Measure volumes immediately after baking.
   b) Score internal and external characteristics the following day.

The following day, the loaves were cut and scored. The maximum scores were: loaf score 20, crust color 10, symmetry 10, break and shred 10, grain 20, crumb color 10, texture 20, and total score 100.

Results and Discussion

The breeding materials developed in Morocco consisted of seven cultivars, namely Norteno, Penjamo, Inia, Siete Cerros, Tobari, BT 908 and BD 2777. The results of the physical wheat tests are shown in Table 57. General observations indicated all samples were of good quality and sound grain with very few broken kernels as shown in Table 57. All test weights and theoretical yields were within the acceptable limits. Tobari gave exceptional milling yield. No milling problems were encountered except with BD 2777 which was exceptionally hard and required additional reduction to give a reasonable flour yield. All physical wheat tests on BD 2777 indicated that this wheat belongs to the Durum groups.

When semolina milling was applied to Penjamo and BD 2777 the yields were feed 18.4%, semolina 71.4% and flour 10.2%. Consequently, the semolina yield was good and BD 2777 was simplest to mill. Semolina from both wheats made good cous-cous, but the semolina from BD 2777 produce cous-cous with better color and more acceptable texture.

Farinograms and extensigrams of the seven flours are shown in Figures 32 and 33, respectively.

A summary of the flour test data is given in Table 58 and of farinograph data in Table 59. Extensograph results are summarized in Table 60.

Peak time and stability were used for scoring mix time and mixing tolerance, respectively. Bread mean scores were correlated with overall farinograph and extensograph data, (Table 61).

Loaf volumes were taken immediately after baking (Figure 34) and loaves were scored the following day (Table 61).

TABLE 39.

MOROCCAN PHYSICAL WHEAT TESTS

SAMPLE NO.	BISON 70-001	MONTANA 009-70	PENJAMO 010-70	RIA 011-70	SIETE CERROS 012-70	TOBAN 013-70	BT-903 014-70	RD-2777 015-70
TEST WEIGHT lbs/U.S. bushel	61.8	63.1	62.4	63.3	62.1	63.4	59.9	61.5
HECTOLITER kg/hectoliter	79.6	81.3	80.4	81.6	80.0	81.7	77.2	79.2
1000 KERNEL WEIGHT (grams)	35.49	40.83	38.99	39.88	34.54	37.91	38.28	40.16
14% MOISTURE BASIS	35.78	43.26	41.16	42.38	36.79	40.29	40.77	42.87
WHEAT SIZE OVER 7W (%)	81.5	87.5	86.5	85.5	77.5	83.0	80.5	77.0
OVER 9W (%)	18.0	11.5	12.5	14.3	21.5	16.5	19.0	22.5
OVER 12W (%)	00.5	1.0	1.0	-	1.0	0.5	0.5	0.5
THEORETICAL YIELD (%)	77.04	77.32	77.27	77.28	76.82	77.13	76.89	76.83
PEARLING VALUE	66.20	61.95	55.90	66.55	61.95	67.35	49.10	61.95
DENSITY grams/CC	1.39	1.423	1.425	1.423	1.443	1.421	1.473	1.438
*PROTEIN**	14.3	11.7	11.4	11.9	11.2	11.9	12.8	14.3
ASH	1.5	1.3	1.5	1.5		1.3	1.3	1.7

*14.0 MOISTURE BASIS
**(N×5.7)

FARINOGRAMS OF MOROCCAN WHEAT FLOURS AND AMERICAN STANDARD

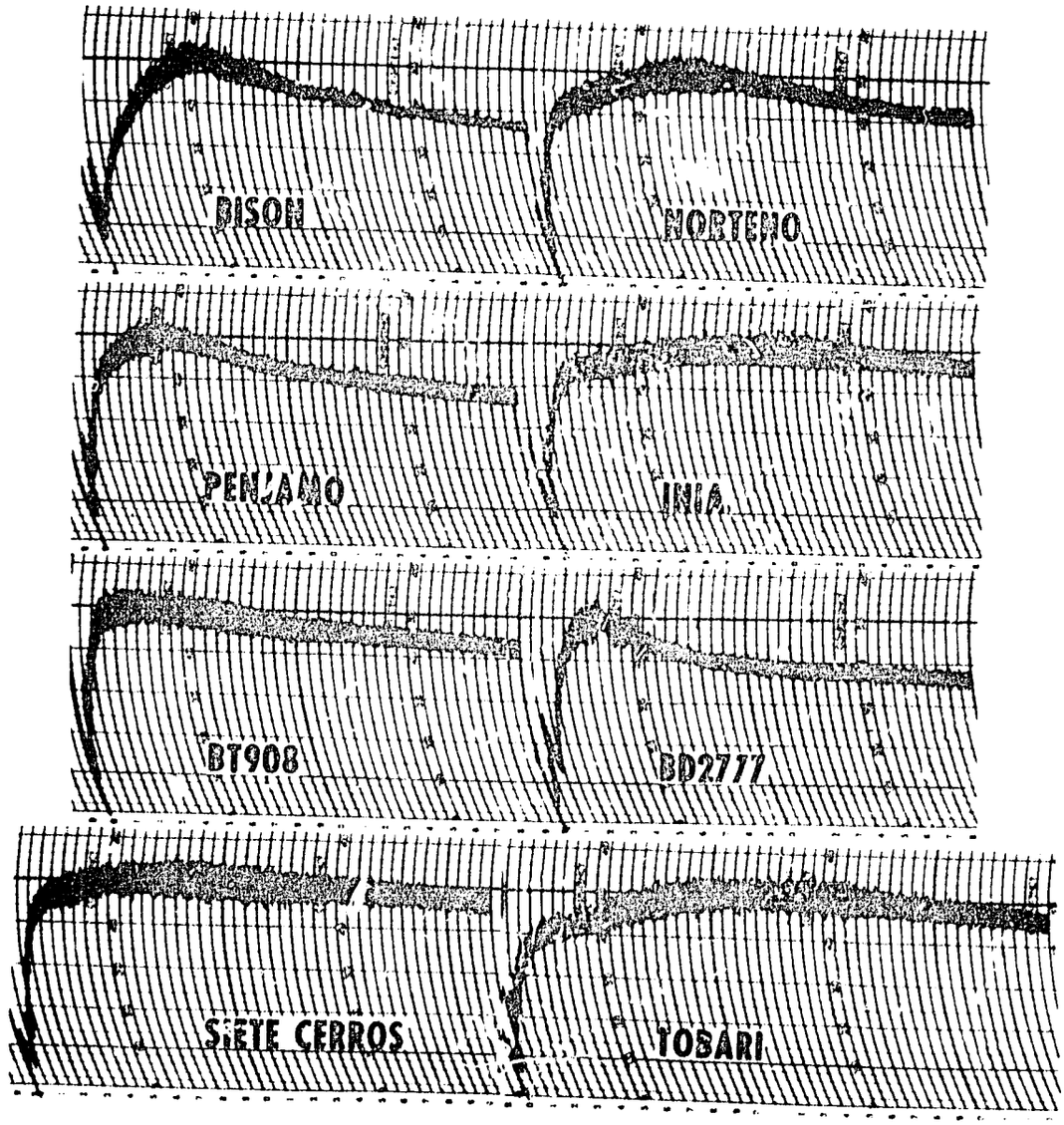


Fig. 32.

EXTENSOGRAMS OF MOROCCAN WHEAT FLOURS AND AMERICAN STANDARD

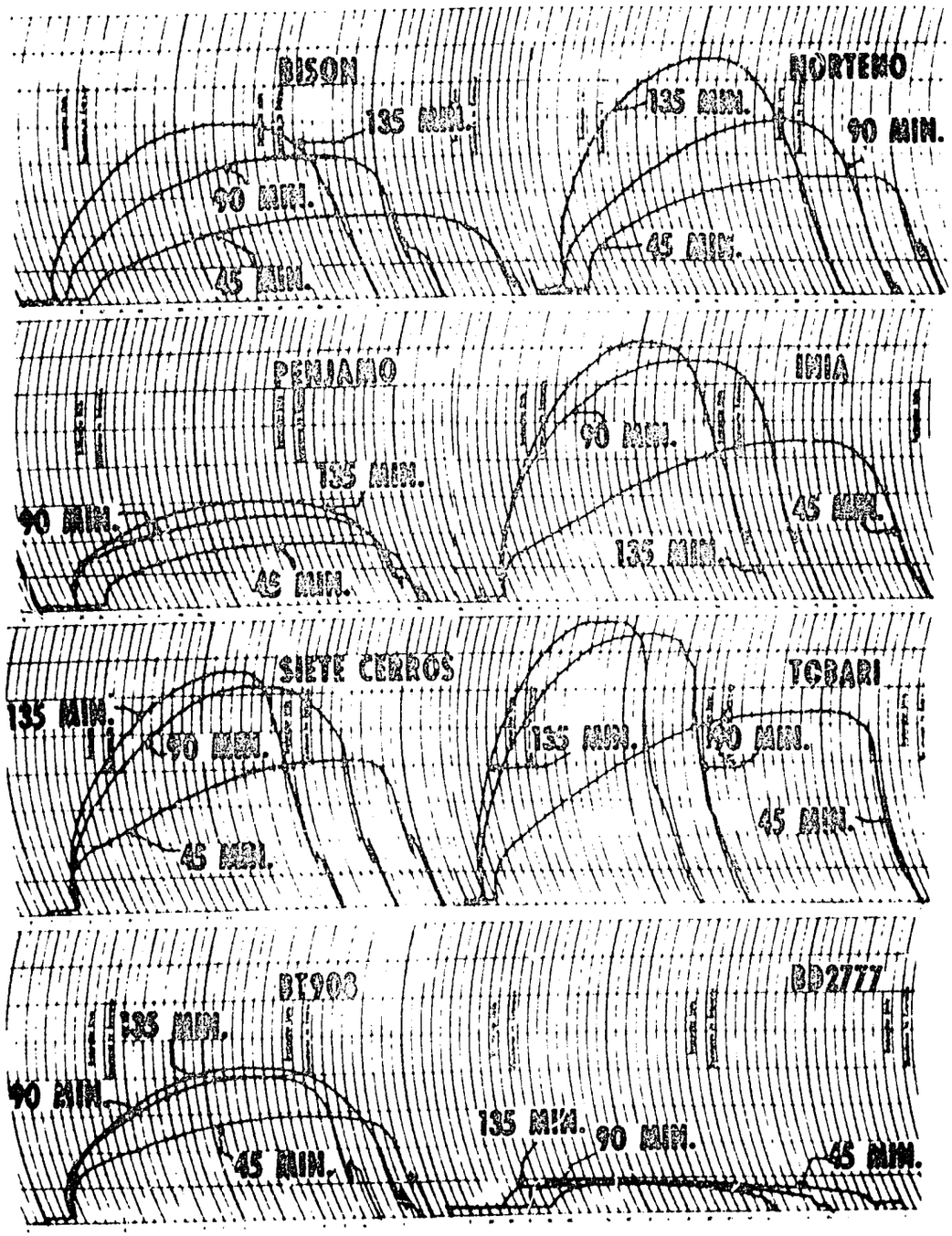


Fig. 33.

FLOUR TESTS

	BISON 70-001	NORTENO 009-70	PENJAMO 010-70	INIA 011-70	SIETE CERROS 012-70	TOBARI 013-70	31-908 014-70	BD-2777 015-70
PROTEIN%	13.0	10.5	9.8	10.6	9.8	10.5	10.7	13.6
ASH%	0.49	0.42	0.39	0.39	0.43	0.4	0.39	0.92
COLOR (AGTRON)	68.0	64.0	75.0	71.0	63.0	69.0	67.0	25.0
MOISTURE	10.8	10.3	10.8	10.4	10.3	10.3	9.6	8.9
ABSORPTION%	69.0	68.6	68.3	69.7	72.3	69.3	65.3	82.0
ARRIVAL TIME (min.)	4.0	4.5	2.75	5.0	3.0	6.5	1.25	2.5
PEAK TIME (min.)	5.25	7.25	4.0	10.75	8.0	13.5	3.0	3.5
STABILITY	3.0	6.0	3.50	10.25	16.0	1[illegible].5	7.0	1.75
M.T.I. B.U.	90.0	55.0	75.0	25.0	25.0	20.0	40.0	110.0
VAL. METER B.U.	46.0	57.0	40.0	73.0	62.0	81.0	30.0	34.0
LOAF VOL. CM^3	888.0	798.0	725.0	805.0	745.0	825.0	723.0	628.0
MEAN SCORE	69.0	66.0	59.5	83.0	70.5	85.5	61.5	39.5
LOAF VOL.% FLOUR PROTEIN	68.3	76.0	74.0	75.9	76.0	78.3	67.6	46.2
SUITABILITY FOR WHITE PAN BREAD	-	-	-	+	+	+	-	-
BLENDING PURPOSES	+	+	-	+	+	+	+	-
FOR COUS-COUS			+					+

MOROCCAN WHEAT FLOUR

FARINOGRAPH DATA

SAMPLE	PROTEIN	ABSORP. (%)	ARRIVAL TIME (min.)	PEAK (min.)	STABILITY (min.)	M.T.I. (B.U.)	VALORIMETER VALUE (B.U.)
70-001 CONTROL	13.0	69.0	4.00	5.25	3.00	90	46
009-70 NORTENO	10.5	68.6	4.50	7.25	6.00	55	57
010-70 PENJAMO	9.8	68.3	2.75	4.00	3.50	75	40
011-70 INIA	10.6	69.7	5.00	10.75	10.25	25	73
012-70 SIETECERROS	9.8	72.3	3.00	8.00	16.00	25	62
013-70 TOBARI	10.5	69.3	6.50	13.50	13.50	20	81
014-70 BT-908	10.7	65.3	1.25	3.00	7.00	40	30
015-70 BD-2777	13.6	82.0	2.50	3.50	1.75	110	34

TABLE 60.

FLOURS OF MOROCCAN WHEAT
EXTENSOGRAPH DATA

SAMPLE NO.	REST PERIOD (min)	RESISTANCE TO EXTENSION (B.U.)		EXTENSIBILITY (mm)		RATIO (R.E./E)		ENERGY	AREA cm^2
70-001 (CONTROL) BISON	45	220	210	190	150	1.38	1.40	55.60	66.31
	90	270	245	160	200	1.42	2.47	91.20	97.33
	135	380	400	164	162	2.32	1.23	107.72	106.75
009-70 NORTENO	45	250	220	180	178	1.39	1.24	64.56	79.85
	90	320	320	168	167	1.90	1.90	97.78	101.97
	135	560	420	140	142	4.00	2.96	97.01	117.45
010-70 PENJAMO	45	140	150	185	160	0.76	0.94	36.06	42.76
	90	200	210	168	172	1.19	1.22	57.92	54.25
	135	250	245	163	170	1.53	1.44	66.05	62.95
011-70 INIA	45	240	240	220	213	1.09	1.13	117.52	124.81
	90	450	500	145	160	3.10	3.13	139.38	111.07
	135	580	570	140	137	4.14	4.16	120.74	124.03
012-70 SIETE CERROS	45	300	260	182	184	1.65	1.41	98.36	109.84
	90	580	460	148	160	3.92	2.88	131.45	131.84
	135	540	520	133	147	4.06	3.54	111.26	126.68
013-70 TOBARI	45	300	300	116	215	2.59	1.40	144.03	141.19
	90	550	600	148	133	3.72	4.51	127.32	142.61
	135	830	700	130	110	6.38	6.36	109.20	147.58
014-70 BT-908	45	220	220	172	162	1.28	1.36	66.05	67.73
	90	340	310	145	153	2.34	2.03	79.40	83.27
	135	320	320	145	175	2.21	1.83	94.23	77.92
015-70* BD-2777	45	90	210	175	148	0.51	1.42	34.31	14.96
	90	80	70	152	193	0.53	0.65	17.16	13.16
	135	90	60	135	93	0.67	0.36	8.26	12.45

*DOUGH WAS STICKY AND HARD TO HANDLE

TABLE 61.

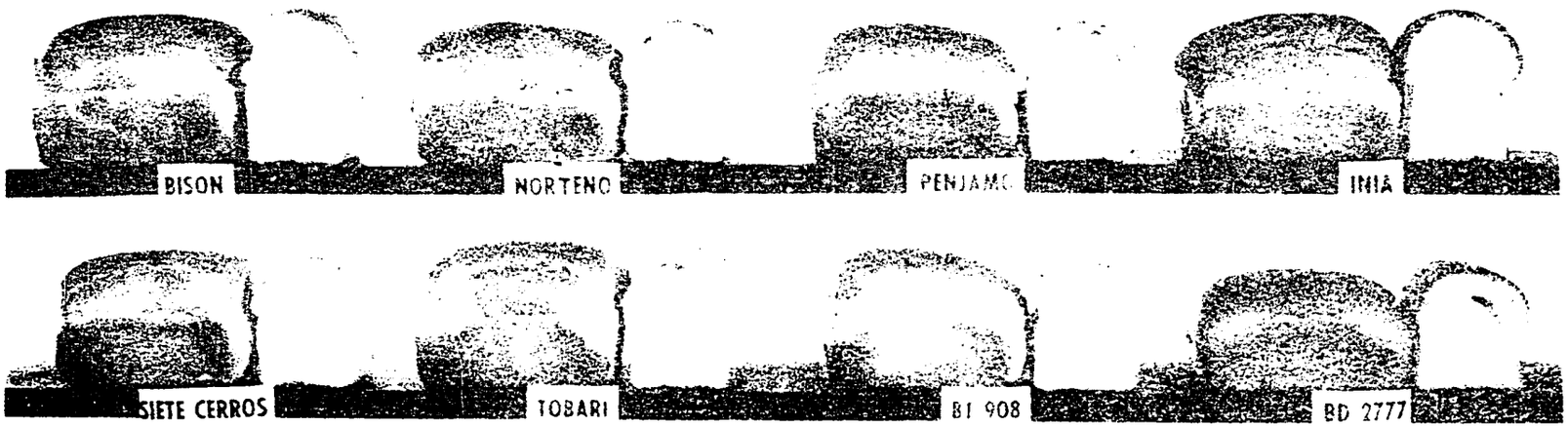
BAKING EVALUATION OF MOROCCAN WHEAT

CHARACTER -ISTICS	BISON 70-001	NORTENO 009-70	PENJAMO 010-70	INIA 011-70	SIETE CERROS 012-70	TOBARI 013-70	BT-908 014-70	BD-2777 015-70
(A) ABSORPTION	6	6	6	6	6	6	5	8
(B) MIX TIME	4	4	3	7	6	10	2	3
(C) MIX.TOL.	2	4	3	8	10	10	5	1
(D) DOUGH HANDLING	9	8	7	9	6	9	8	5
(E) LOAF VOL.	9	8	7	8	7	9	7	4
(F) SYMMETRY	9	8	7	9	6	9	8	5
(G) BREAK & SHRED	7	8	6	10	7	9	8	5
(H) GRAIN	9	9	9	9	9	9	9	4
(I) TEXTURE	8	7	7	9	7	6	5	4
(J) INHERENT QUALITY	6	5	5	8	6	8	6	4
MEAN SCORE	69	66	59.5	83	70.5	85.5	61.5	39.5
*PAN BREAD	-	-	-	+	+	+	-	-
*BLENDING PURPOSES	+	+	-	+	+	+	+	-
SPECIFIC VOL.	5.85	5.17	4.75	5.14	4.77	5.48	4.77	3.65
ACTUAL LOAF VOL. cc	888	798	725	805	745	825	723	628
*MIXING TIME min.	2.5	2.75	2	3	4	4	2	1.75

*SWANSON 200gm BOWL MIXER

A/2+B+C+D/2+2E+F/2+G/2+H+I+2J=MEAN SCORE

MOROCCAN WHEAT SAMPLES AND AMERICAN STANDARD



Flours were evaluated for suitability for white pan bread and blending purposes. The results showed that Norteno and BT 908 were recommended for blending but not for baking. These flours showed high scores for internal characteristics but low scores on mixing tolerance and mix time which are not desirable for baking (Table 58).

Penjamo and BD 2777 were shown to be unsuitable for baking or blending. Both flours showed very low scores in mix time and mixing tolerance which are critical for blending, and relatively low scores for internal loaf characteristics. BD 2777 was characterized by very poor handling properties and a yellow crumb color.

The three remaining flours, Siete Cerros, Inia and Tobari are shown to be recommended for baking and blending with the latter having relatively high mean scores.

Proximate analysis is shown in Table 58. Siete Cerros is shown to have low protein content revealing its apparent high-protein quality. BD 2777 has the highest protein content but a relatively low protein quality with respect to baking and blending. Norteno and BT 908 have approximately the same protein content and quality. Penjamo is shown to have the same protein content as Siete Cerros but of low quality with regard to baking and blending.

MOROCCAN BREAD

Objectives: To produce an improved Moroccan loaf of bread with and without protein supplementation.

Formulation and Specifications:

Bread Formula

Ingredients	grams	% (on flour basis)
flour	1500	100
yeast	15	1
salt	30	2
water	variable	variable

Control Flour (PL480) Specifications:

Ash	0.45
Moisture	12.2
Protein	10.5 - 11.5

Baking Procedure:

The procedure for the production of Moroccan bread was standardized to reduce the number of variables and to eliminate the possibility of errors. Standard mixing, fermentation, moulding, proofing, and baking methods and times were employed.

The bread produced was considered of excellent quality with a very acceptable color and flavor.

Mixing:

The dry inbredients were incorporated for one minute using an Artofex mixer. The water and the compressed yeast solution were then added to the dry ingredients. The mixing time was determined to be optimum for the control flour (PL480) at 4 minutes. The optimum mixing times for the control flour with the addition of 12% of each soy, horse bean, and chick pea flours were respectively 6, 4½, and 5 minutes. The water used was at a temperature of 80°F., while the average dough temperature after optimum mixing was 82°F.

Absorption:

The following percent water absorptions were employed for all the standard mixes including the ones containing 0.5% SSL.

Control Flour (PL480)		= 61%
" "	+ 12% soy	= 71%
" "	+ 12% horse bean	= 63%
" "	+ 12% chick pea	= 64%

Fermentation:

After mixing, the dough was placed into metal fermentation pans and then into a fermentation cabinet at a controlled temperature of 86°F. and 86% relative humidity for 1½ hours.

Moulding:

The dough was punched, scaled to 465 grams, and then rounded and sheeted on a dough sheeter to approximately 9" in diameter and between ¼ to 1/3" thickness.

Proof:

Two moulded doughs were placed on each wooden board on top of wax paper to prevent sticking. The doughs were then placed back into the fermentation cabinet and proofed for 40 minutes at the controlled conditions of 86°F. and 86% R.H.

Baking:

After proofing, the dough was given a 5 minute rest at room temperature. Each dough was then cut with a sharp knife approximately ½" in depth across the diameter of the dough before transferring to baking sheets. The wax paper was retained under each dough to prevent sticking to the baking sheets. The baking sheets containing the doughs were immediately placed into a gas reel type oven preheated to 400°F. and baked for 25 minutes. After baking the bread was cooled to room temperature and the weight and volume measurements recorded for each loaf (Table 62).

TABLE 62. MOROCCAN BREAD WEIGHT AND LOAF VOLUMES

	Average Weight (grams)	Average Volume (cubic centimeters)
Control PL480	417	1325
12% Soy	419	1175
12% Horse bean	412	1225
12% Chick Pea		1250
Control + 0.5% SSL	409	1300
12% Soy + 0.5% SSL	419	1325
12% Horse bean + 0.5% SSL	410	1275
12% Chick pea + 0.5% SSL	410	1300

Results:

Visually it was determined that the control flour (PL480) produced a loaf of bread with the lightest crust color followed by the CP, soy, and with the HB having the darkest crust color respectively.

By comparing the results from flour supplementations at 12% level, it was concluded that the addition of soy gave both the best grain and texture while the addition of HB was the least desirable.

The control HB and CP bread had a higher volume than the control soy bread.

With the addition of 0.5% SSL the 12% soy bread gave the overall best response followed by CP and HB breads respectively.

In all cases the addition of 0.5% SSL gave lighter crumb color to the breads and increased volume.

In general the soy flour produced a bread with a slightly yellow crumb while the HB flour gave a gray cast and with the CP somewhere in between these two.

The control loaves and 12% soy loaves were taken to Morocco for examination and acceptability studies.

ORGANOLEPTIC EVALUATION

MOROCCAN-TYPE BREAD

Starting in January 1970, an 8-week organoleptic project was carried out as part of the bread orientation work. Five-loaf batches of the typical round bread were baked from 80% extraction Gaines 68 wheat, milled 6/6/69 and 1/20/70 in the Department of Grain Science and Industry. Protein supplementation was effected by increments of horse bean flour, milled 7/28/69 and 12/30/69, and of chick pea flour, milled 1/13/70. Both flours were 80% extraction.

Organoleptic testing was performed by a trained flavor panel of six persons. Samples of breads to be examined were retained, after cutting, in Ziploc(R) plastic bags, in order to avoid drying and loss of aroma.

For aroma examination, quarter-loaf sections were presented to each panelist. The panelists split the quarter loaf and sniffed the freshly exposed surfaces of the crumb. Top crust aroma was sniffed after the crust was slit by thumb nail. Intact bottom crust was sniffed. For flavor examination, freshly cut 1-inch slices taken from the mid-portion of the loaf were cut in half, and two halves were bagged for each panelist.

The panel members recorded separate aroma and flavor observations for crumb, top crust and bottom crust. They were asked to indicate first the total intensity of aroma (or total intensity of flavor) before recording the individually detectable character notes of the three bread parts. The character notes with their respective intensities were recorded in the order perceived. Demineralized distilled water was used to rinse mouth in between samples. Control bread was used to build up the descriptive vocabulary for the aroma and flavor properties of the Moroccan bread, prior to the tasting of the bread --

with protein supplements. The following supplements were used for this organoleptic work: horse bean flour at 5, 7.5, 10, and 20%; and chick pea flour at 5, 7.5, 10, 12.5, 15 and 20%.

Results and Discussion

Table 63 summarizes the findings in comparison to the control bread.

The prominent aroma and flavor characteristics of the control bread were toasted, wheaty, yeasty, burnt, chewy crumb, and tough, chewy bottom crust. Yeasty appeared to be more evident in the bland aroma and to a lesser extent in the flavor of crumb. Stronger toastedness of crust seemed to cover the yeasty note.

When corn meal or bran was used as antistick agent, their presence was detected in the aroma of bottom crust.

Musty aroma was prominent in the bread baked from flour stored at room temperature as compared to the fresh milled flour stored at 4°C.

The prominent horse bean characteristics were described as musty, bitter, beany, legumy and like black walnuts. At the 5% horse bean level, a slight bitterness was detected in the top and bottom crusts; at 7.5%, legumy, musty aromatics were detected in the crumb aroma, but at low intensities. At neither level did these characteristics create an impression of horse bean identity.

Increasing horse bean flour from 7.5 to 10% caused the mustiness to be prominent, and the degree of detection by the panel members was much higher. At the 10% concentration of horse bean flour, bitterness became a major flavor component, detected in all portions of the bread, with a higher intensity in the crust than in the crumb. Greater Mailliard reaction with increasing levels of horse bean flour causing significant darkening of crust appears to be the probable explanation of the observed bitterness.

TABLE 63. COMPARISONS BETWEEN SUPPLEMENTED MOROCCAN-TYPE BREADS AND THE CONTROL.

	Horsebean Flour				Chickpea Flour					
	5%	7.5%	10%	20%	5%	7.5%	10%	12.5%	15%	20%
Appearance										
Volume	+	?	-	-	?	?	-	-	-	-
Top crust color	+	?	-	-	?	-	-	-	-	-
Texture										
Crumb	+	?	-	-	?	?	-	-	-	-
Bottom crust	+	?	-	-	?	?	-	-	-	-
Aroma										
Crumb	+	?	?	-	?	-	-	-	-	-
Top crust	+	+	+	?	?	?	?	-	-	-
Bottom crust	+	+	+	?	+	+	+	+	+	+
Flavor										
Over-all	+	+	?	-	?	?	-	-	-	-
Crumb	+	+	-	-	?	-	-	-	-	-
Top crust	+	?	-	-	?	-	+	-	-	-
Bottom crust	+	?	-	?	?	?	?	-	-	-

Key
+ : similar
? : slightly different from control
- : definitely different from control

A broader spectrum of horse bean characteristics became more evident at 20% level. In addition to the mustiness of 10% level, beany, legumy and black walnut-like aromatics plus the bitterness of horse bean flour were the prominent flavor notes in different portions of the bread. When the legume and/or black walnut aromatics were not detected, bitterness may be the clue to presence of horse bean.

Bread made with fresh-milled horse bean flour assumed more of a yeasty (i.e., yeast plus its by-products) character and less of horse bean-beany character. It was also observed that the horse bean flour exposed to air seemed to lose much of its aromatics.

While the higher levels of horse bean flour were detectable to the panel of experienced tasters, the importance of the flavor changes may or may not be as important to consumers as the effects of horse bean on crust color, volume, gumminess of crumb texture and toughness of crust. The general observations of the panel were that crust color darkened with increasing amounts of horse bean. At 10%, the bread had a golden amber top crust but with more than 10%, the crust became a dark, reddish, brown color.

Volume decreased considerably with each increment of horse bean above 7.5%. A possible explanation of this is that the horse bean flour diluted the gluten, causing the dough to become less elastic, and thus decreased the gas retaining property of the dough. Toughness of crust increased and crumb became gummy and dense as the amount of horse bean was increased. Gumminess of crumb can probably be attributed to large quantities of pentosans contributed by the horse bean flour.

Breads made with 5, 7.5, and 10% chick pea flour decreased in volume and increased in darkness of color. The crumb aroma at these levels was described

as yeasty and musty. This was considered to be a major aroma component. Other minor components such as tongue bite, bitter, pea-like were observed in the aroma or flavor in all portions of the bread. The "unidentified" flavor notes could be described as being an off-aroma or -flavor not found in the unsupplemented bread nor identifiable as chick pea.

At the 12.5% chick pea flour level, the bread had dominant bitter, chick pea and salty characteristics. While the chick pea aroma was detectable at the 12.5% level as a major component of the crumb and top crust, chick pea identified in the crumb flavor became dominant at the 15% level of chick pea.

At the 20% chick pea level, the chick pea aroma and flavor became increasingly obvious in all positions of the bread. The flavor evaluation clearly demonstrated that the bread lost much of its wheaty aroma and flavor characteristics as the amount of chick pea flour was increased in the bread.

Comparison of salted and unsalted Moroccan-type bread at the 12.5% chick pea flour level, suggested that chick pea flavor note was less dominant in the salted bread. It appeared that salt suppressed the chick pea flavor but increased the toasted aroma and flavor. It may be that by increasing toasted flavor, salt caused suppression of the chick pea flavor.

In general, addition of chick pea flour to Moroccan-type bread caused development of musty, yeasty, chick pea, bitter and tongue bite flavor characteristics in the bread. While these characteristics were detected by persons trained in flavor, it is not known whether these factors would be detected by a consumer group. The loss of wheaty characteristics in the bread might be a drawback to a consumer group. The loss of volume, increased crust color and toughness of the crust and gumminess of crumb observed with increasing amounts of chick pea flour (as with the addition of horse bean flour), might be objectionable to the Moroccan consumer.

NUTRITIONAL EVALUATION OF SUPPLEMENTED MOROCCAN BREAD

Rat Study I

The effect of supplementing Moroccan bread with ground chick peas or ground horse beans was studied. These were substituted for 10 or 20% of the wheat flour before the bread was prepared. Vitamins and minerals were added after preparation of the bread at the same level as in cous-cous Rat Study II (page 123). Both the fat and protein contents were adjusted to 10%. The following rations were prepared:

Diet 18 - casein control
Diet 19 - Moroccan bread, no supplement
Diet 20 - Moroccan bread, 10% chick peas
Diet 21 - Moroccan bread, 20% chick peas
Diet 22 - Moroccan bread, 10% horse beans
Diet 23 - Moroccan bread, 20% horse beans
Diet 24 - Moroccan bread, 0.19% lysine

Diets were fed to groups of eight male weanling albino rats of the Charles River strain for 28 days. Weekly cumulative weight gains were plotted (Figure 35). PER were calculated at the end of 28 days (Table 64).

TABLE 64. PER FOR DIETS MADE OF MOROCCAN BREAD SUPPLEMENTED WITH CHICK PEAS OR HORSE BEANS

Diet	PER
18	2.863
	*
23	2.249
	n.s.
24	2.089
	n.s.
21	1.965
	n.s.
20	1.825
	n.s.
22	1.790
	*
19	1.495

$LSD_{0.05} = 0.222$

The following generalizations were made regarding PER:

1) Casein produced a higher PER than any other protein source studied.

2) Protein quality was improved by all supplements added to the bread.

3) In Moroccan bread 20% horse beans improved PER more than 20% chick peas. Lysine did not differ from either of those supplements but improved PER more than 10% horse beans and 10% chick peas.

4) Ten percent chick peas did not improve PER in Moroccan bread more than 10% chick peas, but 20% horse beans was more effective than 10% horse beans.

Only four animals (on three different diets) showed any sign of hair loss.

Dry weight and calcium content of the femurs will be determined.

NUTRITIONAL EVALUATION OF PROTEIN FORTIFIED BREAD

The development of highly nutritious cereal based foods is of great interest by scientists as well as by industry. The most conventional way of consuming cereal is in the form of bread. Thus, any improvement in bread is an assurance of consumable material and great utilization. Use of soy flour to increase protein quantity and quality is attempted in several ways. Several products, based on soy protein, are commercialized. The literature on the nutritive value of soybean proteins has been reviewed by Altschul (1958) (83). According to Wolf and Smith (1961) (84) food utilization of soybeans in the United States is still so new that many problems must be solved before main patterns of use are established. Much of the research on the nutritional value of soybean protein has been conducted on defatted soybean meal for animal feeding. The results may not be applicable to several important soy products prepared for human use.

The present work was conducted to evaluate the nutritive value of bread in which 12% soy flour was added to improve the protein quality of bread. The diets were prepared from bread prepared and baked as it would be to correlate the results directly to common practices. The additives SSL (Sodium Stearoyl-2-Lactylate) and ST (Sucrose Tallowate) were incorporated in raw baking formulas of bread. These additives had been found by Tsen and Hoover (1971) (85) and by Pomeranz et al., (1969) (86) to be highly beneficial to high-protein bread quality. The diets as tested were designed to indicate whether SSL or ST had significant effects as measured by growth and appetite.

Two studies were designed to evaluate nutritive value of enriched bread in which soy flour was used as a source of protein enrichment.

The experimental diets were compared with diets made of 12% soy flour without treatments. Diets made up with casein were included as a control diet. SSL was used in Study I. In Study II, ST was incorporated in the baking formula. In an additional study, regular bread with standard enrichment of vitamins and minerals was used for comparison.

Study I

Bread samples were baked in the experimental baking laboratory in the Department of Grain Science, KSU. Six bread samples were classified as follows:

1. Bread made with 12% soy flour.
2. Bread made with 12% soy flour + 0.5% SSL
3. Bread made with 12% soy flour + 2.5% SSL
4. Bread made with Blend A flour.
5. Bread made with Blend A flour + 0.5% SSL
6. Regular white bread.

The bread loaves were sliced and dried in heated air ovens at 50°C. for 12 hours and ground to a uniform particle size. Crude protein and moisture were determined on these samples by AOAC Methods (Table 65).

TABLE 65. PROXIMATE ANALYSES OF BREAD SAMPLES

Type of Bread	Moisture %	Protein %	Ash %
12% soy flour bread	5.0	17.8	---
12% soy flour + 0.5% SSL bread	4.6	17.5	---
12% soy flour + 2.5% SSL bread	4.4	17.5	---
Blend A flour bread	5.0	15.1	3.4
Blend A flour + 0.5% SSL bread	4.5	15.1	3.4
Control bread	4.7	13.6	---

The growth study using 22-day-old weanling male Charles River strain albino rats (weight range 46-52 grams) was conducted using a completely randomized experimental design, with eight rats per diet treatment. The rats were housed in individual wire bottom cages in an environmentally controlled laboratory. The diets were prepared from ground bread and a vitamin and mineral premix designed to support optimum rat growth. Two percent fat was added as vegetable oil to each diet to reduce dustiness and increase palatability. In the six bread diets, the only protein source was bread. Since no starch was added to equalize protein content, the protein content of the diets differed. Diet 7 was a control made using casein, starch and the same levels of vitamins, minerals and vegetable oil used in the bread diets. Table 66 shows the composition of the seven diets. Proximate analysis of the diets are given in Table 67.

During the study, individual weights were recorded weekly. Food consumed was computed weekly for each group of rats. The test diets and water were given ad libitum. Feed wastage was measured and used in calculations of food consumption data.

At the end of the third week, a palatability test was conducted using six rats to determine if differences in feed preference might explain difference in growth and feed consumption of rats fed Diets 1 and 3. Diets 1 and 3 were supplied in two separate feeding cups to each individual rat. The position of each cup was changed daily for one week. At the end of one week, the amount of diet consumed by each rat was determined.

Study II

In this study, bread samples were baked following the same procedures used previously. Bread samples are classified as follows:

1. Control bread.
2. Bread made using 12% soy flour + 0.5% ST.
3. Bread made using 12% soy flour + 2.5% ST.
4. Bread made using 12% soy flour.

TABLE 66. COMPOSITION OF EXPERIMENTAL DIETS.

Diets	1	2	3	4	5	6	7
Ingredients							
Bread (ground)	92.5	92.5	92.5	92.5	92.5	92.5	-----
Vitamin premix[1]	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Mineral premix[2]	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Vegetable oil	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Starch + cerelose[3]	----	----	----	----	----	----	81.23
Casein[4]	----	----	----	----	----	----	11.27

[1]Vitamin premix: Vitamin A (250,000 IU/gm) 800 mg; Vitamin D (200,000 IU/gm) 100 mg; Menadione sodium bisulfite (100%) 1 mg; Vitamin E (25%) 600 mg; Riboflavin (100%) 2.50 mg; Thiamin HCl (100%) 1.25 mg; Pyridoxine HCl (100%) 1.25 mg; Calcium pantothenate (100%) 8.00 mg; Niacin (100%) 15.00 mg; Vitamin B_{12} (0.1%) 5.00 mg; Choline chloride (100%) 750 mg; and Carrier (starch) (Food grade) 5200 mg.

[2]Mineral premix: Dicalcium phosphate 27.04 g; Salt 3.00 g; Trace mineral mix* 0.50 g; Potassium chloride 3.05 g; and Magnesium sulfate 1.06 g.

[3]Starch: Cerelose 4:1.

[4]Casein: Vitamin free.

*CCC trace mineral mix contains (%): Mn, 10; Fe, 10; Ca, 14; Cu, 1; Zn, 5; I, 0.3; and Co, 0.1. Calcium Carbonate Company, Quincy, Illinois.

TABLE 67. PROXIMATE ANALYSIS OF DIET.

Diets	Moisture %	Protein* %	Ether Extract %	Ash %	Fiber %
12% soy flour bread	5.9	16.7	1.6	5.2	1.7
12% soy flour + 0.5% SSL bread	5.5	16.2	1.9	5.0	1.6
12% soy flour + 2.5% SSL bread	5.5	16.4	2.2	5.3	1.9
Blend A flour bread	6.2	14.0	1.9	5.5	1.7
Blend A + 0.5% SSL bread	5.8	14.0	1.9	5.5	1.6
Casein control	5.9	12.7	1.8	4.5	1.1
Regular bread control	13.0	11.0	1.7	3.0	1.2

*Protein % Kjeldahl Nitrogen x 6.25.

The bread loaves were sliced and dried in a heated air oven at 50°C. for 12 hours and ground to uniform particle size.

The growth study with 21-day-old weanling Charles River strain albino rats (weight range 50-59 grams) was conducted using a completely randomized experimental design, eight replications per diet, as in Study I. The only source of protein in the diets was bread, no starch was added; thus, protein content of diets differed. Diets were prepared using the same vitamin and mineral premixes as in Study I and 3% vegetable oil was added (Table 68). The diets were analyzed for crude protein, for moisture, ash, and crude fat by AOAC Methods (Table 69).

Diets and water were supplied ad libitum, weight gain and feed consumption were recorded weekly.

A separate experiment (#3) was conducted in which enriched flour was used to bake breads and no additional vitamin or mineral premix was supplemented. Breads were classified as follows:

1. Regular white bread, made of enriched flour.
2. Regular white bread + 3% dry milk solids.
3. 12% soy flour bread + enriched flour + 0.5% SSL.

The growth study was conducted at the same time as the previous experiment. Feed composition and proximate analyses are shown in Table 70 and 71.

Amino acid composition of the diets were determined following acid hydrolysis using the procedure of Spackman and Moore et al., (1958) (32) with a Spinco Amino Acid Auto-Analyzer. Amino acids found are given in Table 72.

The available lysine content of the bread samples was determined using the method of Kakade and Liener (1969) (87).

TABLE 68. COMPOSITION OF DIETS.

Diets	1	2	3	4	5	6	7
Ingredients							
Bread (ground)	91.5	91.5	91.5	91.5	91.5	91.5	91.5
Vitamin Premix[1]	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Mineral Premix[2]	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Vegetable Oil	3.0	3.0	3.0	3.0	3.0	3.0	3.0

[1]Vitamin premix: Vitamin A (250.000 IU/gm) 800 mg; Vitamin D (200,000 IU/gm) 100 mg; Menadione sodium bisulfite (100%) 1 mg; Vitamin E (25%) 600 mg; Riboflavin (100%) 2.50 mg; Thiamin HCl (100%) 1.25%; Pyridoxine HCl (100%) 1.25 mg; Calcium pantothenate (100%) 8.00 mg; Niacin (100%) 15.00 mg; Vitamin B_{12} (0.1%) 5.00 mg; Choline chloride (100%) 750 mg; and Carrier (starch) (Food grade) 5200 mg.

[2]Mineral premix: Dicalcium phosphate 27.04 g; Salt 3.00 g; Trace mineral mix* 0.50 g; Potassium chloride 3.05 g; and Magnesium sulfate 1.06 g.

*CCC trace mineral mix contains (%): Mn, 10; Fe, 10; Ca, 14; Cu, 1; Zn, 5; I, 0.3; and Co, 0.1. Calcium Carbonate Company, Quincy, Illinois.

TABLE 69. PROXIMATE ANALYSIS OF DIETS

Diets	Moisture %	Protein* %	Ether Extract %	Ash %
Regular white bread diet	7.2	13.5	2.9	4.6
12% soy flour with 0.5% ST bread	6.8	17.2	3.0	5.1
12% soy flour with 2.5% ST bread	7.0	16.8	3.2	5.2
12% soy flour bread	7.3	17.2	3.0	5.3

*Protein - Kjeldahl nitrogen x 6.25

TABLE 70. COMPOSITION OF DIETS.

Diet	1	2	3
Ingredient			
Bread ground	97.0	97.0	97.0
Vegetable oil	3.0	3.0	3.0

TABLE 71. PROXIMATE ANALYSIS OF DIET
STUDY 2, SET 2

Diets	Moisture %	Protein* %	Ether Extract %	Ash %
Control enriched regular flour bread	9.2	13.9	3.0	5.3
12% soy flour with 0.5% SSL bread	11.1	17.3	3.0	2.8
Enriched flour white bread with 3% dry milk solids	4.5	15.2	2.7	2.5

*Protein = Kjeldahl nitrogen x 6.25.

TABLE 72. AMINO ACID COMPOSITION AND PERCENTAGES.

Amino Acids	Gram/100g Protein	% of NRC Requirements Met For Growing Rats	% of NRC Requirements Met For Standard Ref: Man Age 22
		Enriched Flour Bread Diet	
Lysine	1.775	39.4	72.1
Histidine	1.934	129.0	----
Arginine	3.476	347.6	----
Threonine	2.782	111.3	180.6
Half Cystine	3.308	----	----
Valine	4.409	126.0	179.2
Methionine	1.278	42.6	37.8
Isoleucine	3.604	144.2	167.6
Leucine	6.986	174.6	206.7
Tyrosine	3.280	----	----
Phenylalanine	5.092	113.1	150.6
		12% Soy Bread + .5% SSL	
Lysine	2.521	56.0	102.5
Histidine	1.657	110.5	----
Arginine	3.073	307.3	----
Threonine	2.971	118.9	192.9
Half Cystine	1.855	----	----
Valine	4.315	123.3	175.4
Methionine	1.245	41.5	36.8
Isoleucine	3.707	148.3	172.4
Leucine	7.112	177.8	210.4
Tyrosine	3.126	----	----
Phenylalanine	4.805	106.8	142.2
		Enriched Bread + Dry Milk Solids	
Lysine	1.944	43.2	79.0
Histidine	1.916	127.8	----
Arginine	3.147	314.7	----
Threonine	2.695	107.8	175.0
Half Cystine	2.402	----	----
Valine	4.247	121.3	172.6
Methionine	1.442	48.1	42.7
Isoleucine	3.516	140.6	163.5
Leucine	6.888	172.2	203.8
Tyrosine	2.940	----	----
Phenylalanine	4.636	103.0	137.1

Results Experiment I

The weight gains during the four-week test period (Table 73) show no significant differences ($P < 0.01$) between the rats fed Diets 1, 2, 3, and 7. These were the diets containing 12% soy flour bread and the casein control. Gain data of rats fed Diets 4, 5, and 6 (bread made with our soy flour) were statistically similar but significantly different ($P = 0.01$) from Diets 1, 2, 3, and 7. Rats fed diets containing bread having 12% soy flour + 0.5% and 2.5% SSL gained less than rats fed diets containing 12% soy flour bread. This difference, however, was not significant statistically.

Rats fed diets containing 12% soy flour had the highest feed consumption (Table 73). The palatability test indicated that rats voluntarily consumed more of Diet 1 made with 12% soy flour bread. Thus, rats on Diet 1 voluntarily consumed more diet and gained most when compared to diets made with breads containing SSL.

Protein utilization data (Table 73) indicated diets were significantly different ($P = 0.01$). Best protein utilization was by rats fed the casein diet (2.41 grams of gain per gram of protein). Rats fed diets containing 12% soy flour bread were significantly more efficient in protein utilization than rats fed diets made up of Blend A or the control bread. Protein utilization was 1.84, 1.61, and 1.84 for Diets 1, 2, and 3 made using 12% soy flour, 12% soy flour + 0.5% SSL and 12% soy flour + 2.5% SSL, respectively. Protein utilization was significantly lower ($P = .01$) for rats fed diets containing bread made of Blend A flour and without SSL (1.11 and 1.25 respectively) and the control diet made of white regular bread (1.15).

The amino acid composition of diets made with 12% soy flour and regular white bread (Table 72) also indicate the nutritive value of the protein enriched bread. The 11 essential amino acids required for growing rats were in higher concentrations in soy bread than in regular white bread.

TABLE 73. SUMMARY TABLE OF WEIGHT GAIN, FEED CONSUMPTION, PROTEIN UTILIZATION, AND FEED CONVERSION, STUDY 1

Diet	Average Weight Gain (g)	Average Feed Consumption (g)	Protein Utilization* Gain in Grams/ Grams Protein Consumed	Feed Conversion Grams Feed/ Grams Weight Gain
12% soy flour	122.6[b]	409.3[a]	1.84[b]	3.35
12% soy flour with 0.5% SSL	106.0[bc]	399.1[a]	1.61[b]	3.76
12% soy flour with 2.5% SSL	113.7[bc]	374.7[a]	1.84[b]	3.29
Blend A bread	41.0[a]	262.2[b]	1.11[c]	6.39
Blend A bread with 0.5% SSL	51.3[a]	289.3[b]	1.25[c]	5.63
Regular white bread (control)	37.0[a]	249.3[b]	1.15[c]	6.73
Casein control diet	100.2[c]	381.0[c]	2.41[a]	5.01

[a,b,c]Significant at 1% level.

* Diets were not adjusted to isonitrogenous basis, calculated as is protein content of diet.

Some of the rats fed diets made of Blend A (Diets 4 and 5) were supplemented with 0.3% lysine at the end of the third week of the experiment to determine if a lysine deficiency might explain the poorer gain in those rats compared to rats receiving 12% soy bread. Marked increases in weight gain were observed in those rats fed the lysine supplemented diets.

Study II

The weight data (Table 74) for five weekly periods shows there were no significant differences among the rats fed Diets 2, 3, and 4; i.e. diets made of 12% soy flour bread with and without addition of ST in baking formula (P = 0.01). The rats fed Diet 4, 12% soy flour bread with ST, gained more (average 157 grams) when compared to the averages of 134.2 and 132.0 grams gained by rats fed Diets 2 and 3. These differences were not statistically significant, however. There was significant differences (P = 0.01) in weight gain between Diet 1 (regular white bread control diet) and Diets 2, 3, and 4 (P = 0.01).

Feed consumption data (Table 74) revealed significant differences between diet intakes (P = 0.01). Rats consumed more 12% soy flour bread than regular white bread.

Rats fed diets prepared using 12% soy flour bread were significantly more efficient in protein utilization (grams gain per grams protein consumed) than rats fed the control diet (P = 0.01). Feed conversion data (Table 74) shows that Diets 2, 3, and 4 were better utilized than the control diet.

Another experiment was included as Study III to evaluate the nutritive value of normal enrichment bread (vitamins and minerals) when containing 12% soy flour. This enriched bread was compared with a commercial formula for regular white bread using vitamin and mineral enriched flour, and bread with

TABLE 74. SUMMARY OF WEIGHT GAIN, FEED CONSUMPTION, PROTEIN UTILIZATION, AND FEED CONVERSION 0-5 WEEKS, STUDY 2

Liet	Average Weight Gain	Average Feed Consumption	Protein Utilization Weight in g/ Protein in g. consumed	Feed Conversion g Feed Consumed/ g Weight Gain
Control Diet	32.00[b]	328.00[b]	1.073[b]	6.88
12% Soy Flour + Bread with 0.5% ST	102.37[a]	534.62[a]	1.461[a]	3.978
12% Soy Flour + Bread with 2.5% ST	100.30[a]	543.12[a]	1.434[a]	4.114
12% Soy Flour Bread	120.50[a]	571.62[a]	1.590[a]	3.651

[a,b]Significant at 1% level.

3.0% dry milk solids (DMS). No additional vitamin or mineral premix was added in preparation of diets. Composition of diets and proximate analysis is given in Table 70 and 71, respectively.

Rat growth data (Table 75) for four week periods show a significant difference between the rats fed Diet 2 made of 12% soy flour and Diet 1 (control) and 3 (P = 0.01). Feed consumption was higher for Diet 2 than 1 and 3. Protein utilization results indicate rats fed Diet 2 were more efficient in protein utilization. Diet 2 was significantly better than Diets 1 and 3 (P = 0.05).

TABLE 75. SUMMARY OF AVERAGE WEIGHT, FEED CONSUMPTION, PROTEIN UTILIZATION, AND FEED CONVERSION 0-4 WEEKS, STUDY 3.

Diets	Weight Gain	Feed Consumed	Protein Utilization Weight in g/ Protein in g. consumed	Feed Conversion g Feed Consumed/ g Weight gain
Enriched Flour White Bread (control)	13.62[a]	191.37[a]	0.516[a]	14.045
12% Soy Flour + Enriched Flour + 0.5% SSL Bread	101.75[b]	384.62[b]	1.562[b]	3.480
Enriched Flour + 3% DMS Bread	17.62[a]	171.00[a]	0.655[a]	9.702

*No vitamin and mineral premixes were supplemented.

a, a & b Statistically significant at 1% level.

Two groups of rats on Diets 1 and 2 were continued on the experimental diets for six weeks to observe effects over a longer period. Growth trends were similar. The rats on Diet 2 consumed more diet and grew more when compared to Diet 1.

At the end of three weeks a palatability test was again conducted using eight rats to determine if differences in feed preparation affected growth and feed consumption. Diets 3 and Diet 4 (12% soy flour with and without ST) and Diet 2 from the second part of the study (12% soy flour + 0.5% SSL bread) were compared for one week. Eight rats were individually supplied two diets in separate cups. The consumption data show that rats voluntarily preferred Diet 4 over Diet 3. The comparison of Diet 4 and Diet 2 indicated a preference for Diet 2. In Diet 4, vitamin and mineral premixes were added while Diet 2 contained no vitamin and mineral premix. The palatability study also revealed the addition of SSL in the baking formula had no deleterious effect on the taste preference of rats.

The amino acid composition of bread used in Study 2 is given in Table 72. Seventeen amino acids were determined. The amino acid composition shows higher concentrations (grams of acid per 100 g. of Kj protein) of all amino acids in supplemented bread when compared to the control diet. Table 72 shows the NRC requirements met in percentage by the concentration of the essential amino acids for growing rats in bread samples. These results showed the concentration of essential amino acids and percentages met by the enriched bread was higher than compared to control bread. This explains the higher weight gain and protein utilization of rats fed 12% soy flour bread.

Lysine is the most limiting essential amino acid in cereal based foods. Availability of lysine is an essential factor in determining the nutritive value of the product.

Available lysine of the samples are shown in Table 76. The results also indicate that lysine content of enriched 12% soy flour bread was more available than control regular white bread, enriched flour white bread and bread with 3% DMS. This also explains the higher nutritive value of enriched 12% soy flour bread.

TABLE 76. AVAILABLE LYSINE IN BREAD SAMPLES

Samples	Lysine Content in Sample g Lysine/ 100 g Sample	Available Lysine in Sample g Lysine/ 100 g Sample	Total Available Lysine in Sample
12% Soy Flour Bread	0.460	93.47	0.430
12% Soy Flour Bread + 0.5% ST	0.400	97.50	0.390
12% Soy Flour Bread + 2.5% ST	---	---	0.360
Control Bread (White Regular Bread)	0.265	86.7	0.230
Enriched Flour Bread	0.272	88.2	0.240
12% Soy Flour + Enrich Flour + 0.5% SSL	0.491	81.4	0.400
Enriched Flour + Dry Milk Solids Bread	0.295	64.4	0.190

Rats on Diets 1 and 2 made of enriched flour bread and 12% soy flour bread + 0.5% SSL were kept for two more additional weeks (i.e. up to six weeks) on the same experimental diet to see the trend of weight gain and feed consumption if changed during prolonged feed intake. The result (Table 75) shows that the trend of weight gain and feed consumption was still higher and rats on Diet 2 consumed more feed and gained the most weight.

At the end of the fourth week, eight rats fed Diets 1 and 3 were supplemented with 0.3% lysine for one week. The average weight gain of four rats fed Diet 1 and 3 supplement with lysine were 13.5 g. and 15.0 g. whereas control rats fed the same diet gained only 6.7 and 3.5 grams respectively. The results also indicate that these diets were deficient in lysine content. Amino acid composition of these diets (Table 72) also revealed that the lysine content was low in these diets. The data of nutritional studies, amino acid composition and available lysine show that 12% soy flour bread is more nutritious, contained higher amounts of lysine and the lysine content of the bread was available as determined biologically and estimated chemically.

Summary

The nutritional value of enriched bread made of 12% soy flour and white flour in which SSL and ST were incorporated was determined. Three different rat growth studies were conducted. Growth, protein utilization, and feed conversion were studied and compared with casein control and diets made of regular white bread. In one of the studies diets were prepared with ground bread and 3% vegetable oil without the addition of mineral and vitamin premix as to evaluate bread as it is consumed. The nutritive value of the bread samples measured by growth and protein utilization significantly ($P < .01$) increased when 12% soy flour was added. The compounds SSL and ST which were incorporated in the baking formula to improve the physical properties of the bread.

Biological measures of bread protein quality has been compared with values from chemical methods. Amino acid make up of the enriched bread was higher when compared to regular white bread and regular bread with 3% DMS. Percentage of essential amino acids supplied by enriched bread were higher. Most of the lysine content present in the 12% soy flour bread appeared to be available to animals when expressed as available lysine.

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TECHNICAL PAPERS RESULTING FROM PROJECT

Fryer, B. A., S. A. Vesecky, C. W. Deyoe, and W. J. Hoover.
Effect of supplementation of high extraction wheat flour with various amino acids, vitamins and minerals on growth and PER of rats. In preparation.

Vesecky, S. A., B. A. Fryer, C. W. Deyoe, and W. J. Hoover.
Effect of cooking on protein quality of chapatis supplemented with amino acids or broad beans. In preparation.

Vesecky, S. A., B. A. Fryer, C. W. Deyoe, R. J. Robinson, and W. J. Hoover.
Effect on protein quality of supplementation of chapatis, parathas and nan with soy and chick pea flours. In preparation.

Bass, Janet L. and Jean F. Caul.
Lab evaluation of three protein sources for use in chapati flours. In preparation.

Shamsuddin, M., C. W. Deyoe, D. B. Parrish, W. J. Hoover, and C. C. Tsen.
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Deyoe, C. W., F. K. Shoup, G. D. Miller, J. Bathurst, D. Liang, P. E. Sanford, and L. S. Murphy.
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Influence of processing and protein supplementation (horse bean flour) on nutritional qualities of cous-cous. In preparation.

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Farrell, E. P.
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