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FURTHER STUDIES ON THE ENRICHMENT OF LIME-TREATED CORN WITH WHOLE SOYBEANS

R. BRESSANI, J.E. BRAHAM, L.G. ELIAS and M. RUBIO

ABSTRACT

A series of experiments were performed to study further the effect of supplementing corn with whole soybeans processed in a batch home process and in a continuous industrial operation on chemical composition, presence of antiphysiological substances and protein quality of tortillas. Variables studied with the conventional home cooking process included levels of whole soybeans from 0–20%, cooking times at atmospheric pressure and 96°C from 0–90 min at a constant corn-to-soybean ratio of 85:15 and lime concentration of 0, 1 and 2%. In the industrial process a 90:10 mixture of corn to whole soybeans was used, and processing conditions were those normally employed for corn alone. Although addition of whole soybeans to corn increased total protein and fat content, both the PER and weight data suggest that the optimum amount of soybeans to add to maize falls between 8 and 12%. Levels of soybeans above 12%, did not significantly improve PER any further. Antiphysiological factors were inactivated after 30 min of cooking independent of lime concentration in the home process. These factors were also inactivated in the industrial process as measured chemically and by biological assays. The study demonstrated the feasibility of producing an enriched tortilla flour at the industrial scale, which besides containing a higher protein content and quality, also provides an increased amount of calories to the consumer.

INTRODUCTION

THE BASIC CEREAL of many Latin American countries is corn, consumed in a relatively large variety of forms. In most of the Central American countries and Mexico, the main form of consumption is the well-known tortilla, made either at home or industrially by cooking maize with lime (Bressani et al., 1972; Katz et al., 1974; Bressani, 1972). Even though "tortillas" are flat-round cakes, the size, thickness and appearance preference vary among countries.

In the northern countries of South America corn consumption is in the form of "arepas," made by cooking degermed corn flour with water (Bressani et al., 1972). Besides these two main forms of consumption, corn is used to make drinks called atoles and foods such as "tamales" and the like.

Corn proteins are known to be deficient in the essential amino acids lysine and tryptophan (Bressani and Marenco, 1963). Many efforts have been made to improve their quality by genetic means as in the case of Opaque-2 corn, by adding the deficient amino acids or by protein supplementation (Bressani et al., 1972). This last approach offers various nutritional advantages such as increasing protein content besides improving its quality. Many protein supplements have been tested, and among them soybean protein has received much attention (Bressani and Marenco, 1963; Bressani and Villarreal, 1963; Del Valle and Pérez-Villaseñor, 1974; Franz, 1975; Del Valle et al., 1976; Green et al., 1976, 1977).

Studies carried out in our laboratories with experimental animals have suggested that optimum protein quality is obtained when whole corn flour, either raw or cooked, is supplemented with 4–5g % of soybean protein (Bressani et al., 1974). The improved quality has been confirmed by nitrogen balance studies in children (Bressani et al., 1972). In these studies, nitrogen retention values were similar to those from milk, and significantly above those obtained with common corn flour.

One of the problems in implementing these results is the fact that many population groups still transform maize into tortillas at home, although tortilla flour is industrially produced and available in most corn-consuming countries in Latin America. With the idea of exploring possible ways to implement the above finding in the rural areas, which would also be capable of industrial implementation, various sources of soybean proteins were examined, including whole soybeans (Bressani et al., 1974). With this background information, results from further studies on the uses of whole soybeans as a supplement to corn will be presented.

Authors Bressani, Braham and Elías are with the Div. of Agricultural & Food Sciences, Institute of Nutrition of Central America and Panama (INCAP), Carretera Roosevelt, Zona 11, Guatemala City, Guatemala, C.A. Author Rubio is General Manager of Asesoría de Empresas, S.A. in Monterrey, México.

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MATERIALS & METHODS

Pilot plant studies

Whole corn and whole soybeans produced at INCAP's experimental farm were used in the studies carried out in the pilot plant.

Lots of 10 kg samples with 0, 4, 8, 12, 16 and 20% whole soybeans replacing equivalent amounts of corn by weight were processed in pilot plant facilities by a standard lime-cooking treatment described previously (Bressani and Scrimshaw, 1958; Bressani et al., 1958). After cooking and washing excess lime, the material was ground wet, dried in a forced draft oven at 60°C overnight, ground in a hammer mill into a fine flour and analyzed for protein by the Kjeldahl method and for fat (AOAC, 1970). These samples were then subjected to biological assay as will be described later.

In a second study, samples of 85% maize and 15% whole soybeans were cooked for 0, 15, 30, 45, 60, 75 and 90 min. During the cooking process samples were withdrawn to determine water uptake in the components and mixture. After cooking, the material was converted into a dry flour as described above. An additional lot was cooked for 60 min, and converted into a dough and then into tortillas. These were air-dried at 60°C in a tray drier, and then ground. All these samples were assayed for protein quality in diets made of 85% of the mixture supplemented with minerals (4%), cod liver oil (1%), maize starch to adjust to 100%, and a B-vitamin mixture. A casein diet was used as control. The samples were analyzed for residual trypsin inhibitor activity by the method of Kakade and Evans, 1966.

In order to obtain additional information on the effects of lime-cooking on the activities of urease and trypsin inhibitors in soybeans, mixtures of maize (85%) and soybeans (15%) were cooked at 96°C in water with 0, 1 and 2% $Ca(OH)_2$. Samples were withdrawn every 15 min up to 90 min and the soybeans were manually separated from the maize, dried at room temperature and assayed for urease (Caskey and Knapp, 1944) and trypsin inhibitors (Kakade and Evans, 1966).

Industrial studies

These were carried out with an industrial enterprise (Asesoria de Empresas S.A.) in Monterrey, México. In this case, 10% whole soybeans was used in a continuous lime-cooking process. Duplicate samples were withdrawn at specific intervals from various places in the process, representing different cooking times. These samples were used to measure the distribution of the two grains during the process, water absorption in the individual grains and mixtures, protein and fat content in the mixture, and urease and trypsin inhibitor activity as well as protein quality as described before. Lots of lime-cooked maize flour, and two lots of the cooked maize/soybean blends were also fed to rats for an 85-day feeding period in order to detect possible adverse effects.

Biological assays

In all animal assays weanling white rats of the Wistar strain from the INCAP colony were used. Eight animals per experimental group were placed in individual all-wire screen cages and fed ad libitum, with water available all the time. Weight gain and food intake were measured every 7 days. The PER assays lasted the conventional 28-day period, while the long-term feeding assay lasted 85 days. In some experiments the animals were sacrificed and the pancreas excised and weighed.

Three types of diets were fed: isocaloric with variable protein content (Diets A); isocaloric and isoproteic (Diets B), and isoproteic with variable energy content (Diets C). A casein control diet containing 11.2% protein and 5% oil was also fed. The PER values were not adjusted to that of the casein diet.

RESULTS

THE MAIZE FLOUR with the various levels of soybeans added before processing were tested biologically using three sets of diets as described previously. The partial composition of the diets are presented in Table 1. As shown in the upper part of the Table, both protein and fat content in the cooked flour increased as whole soybeans percentage in the mixture increased. From the weight gain and protein efficiency ratio data shown in Table 2, it appears that between 8 and 12% whole soybeans produce maximum quality in maize, even though the dietary conditions imposed affected weight gain more than PER. Levels above 12% did not improve further protein quality. This is clearly observed when comparing experiments 1 and 3 with 2.

Information of the rate of water uptake in maize, soybeans and mixture 85:15 during cooking are summarized in Table 3. For maize, water uptake doubled during the first 15 min of cooking and then increased slowly with respect to time; for soybeans, however, the increase during the first 15 min was 5 times as high as the initial value and then increased slowly with respect to time, reaching higher levels as compared to maize. Protein content in the mixture at 10% moisture was 13.2% and fat 7.8%, as compared to 8.9 and 5.2%, respectively, in whole cooked maize.

As shown in Table 4, the PER values increased from 1.15 in the raw maize:soybeans 85:15 mixture to values between 2.41 and 2.51 after cooking periods longer than 15 min. Increasing cooking time or the transformation of the

Table 1—Proximate composition of flours and formulation of diets used in the rat experiments

		% Whole soybeans in blend with maize					
		0	4	8	12	14	20
Moisture in flour (%)		7.0	7.6	7.1	7.5	6.4	6.6
Protein (%)		9.7	10.6	11.6	12.5	14.3	14.8
Fat (%)		3.5	4.6	5.0	6.1	7.1	7.4
Diets A[a]	Ingredient[b]						
	Maize + soybeans	90.0	90.0	90.0	90.0	90.0	90.0
	Cottonseed oil	3.6	2.6	2.2	1.2	0.3	—
	Maize starch	1.4	2.4	2.8	3.8	4.7	5.0
	% protein in diet[c]	8.8	9.1	10.0	11.5	12.6	13.0
Diets B[a]	Ingredient						
	Maize + soybeans	90.0	80.0	72.0	65.0	60.0	55.0
	Cottonseed oil	3.6	3.0	3.1	2.7	2.4	2.6
	Maize starch	1.4	12.0	19.9	27.3	32.6	37.4
	% protein in diet[c]	8.8	8.4	8.4	8.5	8.5	8.5
Diets C[a]	Ingredient						
	Maize + soybeans	95.0	95.0	95.0	95.0	95.0	95.0
	% protein in diet	8.5	9.5	10.7	11.9	13.0	13.8
	% fat in diet	4.3	5.4	5.8	6.8	7.7	8.0

[a] Diets A — Constant fat, variable protein; Diets B — Constant fat, constant protein; Diets C — Variable fat; variable protein
[b] All diets were supplemented with 1.0% cod liver oil, 4% mineral mixture and a complete B vitamin mixture.
[c] Diets were adjusted to 6.7% oil content.

Table 2—Weight gain and PER in rats fed different blends of maize and whole soybeans

	% Whole soybeans in blends with maize					
	0	4	8	12	16	20
Diets A[a]						
Avg weight gain, g[b]	31 ± 7.4[c]	57 ± 4.6	80 ± 4.9	110 ± 2.9	114 ± 8.0	139 ± 5.8
PER	1.25 ± 0.27	1.79 ± 0.06	2.11 ± 0.09	2.26 ± 0.06	2.18 ± 0.09	2.41 ± 0.05
Diets B[a]						
Avg weight gain, g[b]	31 ± 7.4	39 ± 3.6	57 ± 3.4	69 ± 2.8	69 ± 4.9	86 ± 5.4
PER	1.25 ± 0.72	1.54 ± 0.11	2.04 ± 0.05	2.29 ± 0.13	2.32 ± 0.13	2.45 ± 0.13
Diets C[a]						
Avg weight gain, g[b]	25 ± 3.9	70 ± 6.9	96 ± 3.6	101 ± 10.2	128 ± 10.3	111 ± 11.3
PER	1.02 ± 0.13	2.13 ± 0.18	2.21 ± 0.08	2.22 ± 0.19	2.30 ± 0.24	2.08 ± 0.27

[a] See Table 1. [Casein diet (10% protein): PER, 2.77[b] ± 0.23. PER values for experimental diets were not adjusted to casein PER.]
[b] Average initial weight, 43g
[c] Standard error

flour into tortilla did not change the quality of the product. It is also of interest to point out the complete destruction of trypsin inhibitor activity (Table 4). These observations were confirmed by pancreas weight of the animals at the end of the 28-day experimental period.

The effects of cooking and lime concentration on urease and trypsin inhibitor activity are shown in Table 5. The raw soybeans showed an average trypsin inhibitor activity of 33 units. The results suggest little, if any, effects of lime concentration in inactivating urease and trypsin although urease activity disappeared faster when lime concentration was 2%. Thus the inactivation is due to temperature and time more than to the other processing conditions.

With respect to the samples taken from the industrial plant, Table 6 shows the weight distribution of maize and soybean, a possible problem in the adaptation of the technology since the process was a continuous operation. In theory, the mixture subjected to processing was a 90:10, maize:soybeans mixture. The distribution taken at various times showed a variation of 7.0–26.0% soybeans. It can be concluded that, apparently, even with the variation found, this is not a problem even though some changes will have to be made to insure more consistent results. Analyses of moisture in samples of maize, soybeans and the mixtures as shown in Table 6, indicate a more rapid uptake of moisture by soybeans than by maize, as previously found in the pilot plant studies. Further evidence on the distribution based on chemical analysis is also shown in Table 6. It can be seen that the chemically determined values for protein and fat in the mixture confirm those based on the quantities found individually in maize and soybeans, since there is a 2-point increase in fat from 4 to 6 and a 3.6 point increase in protein content. These data were taken to indicate that the distribution of corn and soybean kernels was as found, that is, slightly higher than originally set, 90:10. The quality improvement is shown in Table 7. The raw mixture had a PER value of 0.46 which increased to 2.04 in the final flour. For comparative purposes the Table includes the PER of the maize flour itself (1.20). The improvement in quality observed is slightly less than the predicted value as established from other studies; however, it may be due to the individual protein quality of the two components.

Analysis of urease and trypsin inhibitor activity indicates again a disappearance of these antiphysiological substances, also corroborated by pancreas analysis, indicated by the IPC values (Elías et al., 1976).

The long-term feeding test results are summarized in Table 8. They show normal development of the animal; no gross pathological signs were observed.

DISCUSSION

PREVIOUS RESULTS reported by various groups (Del Valle and Pérez-Villaseñor, 1974; Franz, 1965; Del Valle et al., 1976; Bressani et al., 1974) on the subject presented in this paper, showed that the addition of soybean protein to maize increases the protein quality and total protein content of the latter. As shown in this and other reports, addition of whole soybeans also provides additional amounts of

Table 3—Water uptake with respect to cooking time in maize, whole soybeans and in 85:15 mixture

Cooking time (min)	Maize	Whole soybeans	Maize:whole soybeans 85:15
0	13.8	10.0	13.4
15	26.1	54.4	31.8
30	31.2	59.3	39.9
45	37.1	59.5	42.0
60	38.3	60.0	43.8
90	42.7	60.6	47.4
Tortilla	39.1	61.4	45.2
% Protein (10% moisture)	8.96	41.21	13.24
% Fat	5.2	22.2	7.8

Table 4—Effect of cooking time on protein quality of maize:whole soybeans blend (85:15) and trypsin inhibitor activity

Cooking time[a] (min)	Avg wt gain, g[b]	PER	TUI/ml[c]
0	30 ± 3.9	1.15 ± 0.11	10.3
15	96 ± 3.8	2.36 ± 0.07	4.4
30	103 ± 5.9	2.46 ± 0.07	0.0
45	90 ± 4.5	2.41 ± 0.08	0.0
60	107 ± 7.6	2.51 ± 0.11	0.0
90	97 ± 4.6	2.43 ± 0.08	0.0
Tortilla	97 ± 6.8	2.34 ± 0.11	0.0
Casein	117 ± 6.6	2.79 ± 0.11	–

[a] Protein in diets: 10.4
[b] Average initial weight, 48g
[c] TUI: Trypsin Units Inhibited

Table 5—Effect of lime concentration on trypsin inhibitor and urease activity of whole soybeans cooked with maize[a]

Cooking time (min)	Lime concentration 0		1%		2%	
	TUI[b]	Urease	TUI	Urease	TUI	Urease
0	29.9	+	36.4	+	38.8	+
15	28.8	+	18.3	+	37.1	+
30	4.8	+	2.8	+	2.8	+
45	4.0	+	0.0	+	2.8	+
60	0.0	–	0.0	–	0.0	–
75	0.0	–	0.0	–	0.0	–
90	0.0	–	0.0	–	0.0	–

[a] Analysis on soybeans only
[b] Trypsin units inhibited

Table 6—Grain distribution, water uptake, protein and fat content of samples withdrawn at various times during industrial processing of maize:soybeans mixture

Cooking time (min)	% distribution (d.w.)[a] Maize	Soybeans	Water uptake % moisture Maize	Soybeans	Mixture	In mixture[b] Protein, %	Fat, %
0	87.2	12.8	12.6	8.8	12.2	12.1	6.2
15	74.0	26.0	29.4	42.0	32.6	–	–
30	92.3	7.7	35.0	51.0	35.8	12.3	5.6
45	90.0	10.0	38.1	57.0	40.0	–	–
60	93.0	7.0	38.3	53.0	40.0	11.4	5.5

[a] d.w.: dry weight. Average distribution: 87.3% maize, 12.7% soybeans.
[b] Air-dried weight basis

Table 7—Protein quality, urease and trypsin inhibitor activity of maize:soybeans samples withdrawn at different times during industrial processing

Product	Avg wt gain, g[a]	PER[b]	Urease	TUI/ml[c]	ICP[d]
Raw mixture	10 ± 2.1	0.46 ± 0.10	+	6.2	0.328
15 min cooked	41 ± 4.1	1.52 ± 0.08	+	4.4	1.009
30 min cooked	48 ± 3.7	1.65 ± 0.11	–	2.0	1.692
Final product (flour)	61 ± 2.7	2.04 ± 0.06	–	0.8	2.029
Cooked maize (flour)	32 ± 3.1	1.20 ± 0.08	–	–	1.601
Casein	99 ± 5.3	2.82 ± 0.12	–	–	2.714

[a] Average initial weight: 50g
[b] Average protein in diets: 8.5%
[c] Trypsin units inhibited
[d] Pancreas growth index (Average wt gain/pancreas wt X 100

Table 8—Long term rat feeding test with lime cooked maize and maize:whole soybeans

Lime-cooked product	Avg wt gain, g[a]	Food efficiency[b]	Pancreas wt/100g rat	ICP[c]
Maize flour	102 ± 4.8	9.7	0.155	4.330
Maize: soybean flour	126 ± 7.6	8.7	0.199	3.589
Maize: soybean flour	169 ± 5.9	7.3	0.171	4.516
Casein	170 ± 9.3	7.4	0.227	3.410

[a] 85 days — average initial weight: 50g
[b] g food consumed/g weight gain; (See Table 6)
[c] Protein in diets: 4.8%

energy as fat (Bressani et al., 1974). The fat content increases on the average from a value of 3.7% on tortilla flour to 5.1% on whole soybeans-supplemented flour, thus increasing the energy density of the food. It is common to find in Guatemalan rural areas (Bressani et al., 1972) an intake of 14–22 tortillas/person/day, which is equivalent to an intake of 12.9–20.3g fat which increases to 17.8–28.0g when the corn is supplemented with 12–15% whole soybeans, thus increasing the total energy as well as total protein intake of the individual, with a more efficient utilization. Children would benefit less because of a lower tortillas intake; however, a greater nutritional benefit would result if such a food was offered as a gruel or drink.

The alkaline cooking process can be carried out at home as well as by using the industrial process and as the results showed, there is a complete inactivation of the antiphysiological factors present in soybeans. Of particular concern was the possibility that with a continuous process as the industrial processing is, the two grains would separate. On the average, raw corn kernels weigh 0.325 g/grain while raw whole soybeans weigh 0.152 g/grain. Furthermore, corn kernel density is around 0.730 g/ml, while that of soybeans is 0.680 g/ml. Due to the higher rate of water uptake by soybeans as compared to corn, however, a more or less constant distribution is maintained, as the material proceeds along the cooking, washing, drying and grinding processes. It is also important to indicate that cooking time used in the preparation of tortillas is also adequate to cook soybeans.

From the results of the studies, it may be concluded that enrichment of corn with not less than 8% whole soybeans increases protein content and protein quality as well as energy content of tortillas. Furthermore, both processes inactivate the antiphysiological factors present in the whole soybeans added to corn. This, as well as the increases in nutrient content and quality, are of great nutritive significance to corn-consuming populations in Latin America.

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