RAT SERUM PROTEINS AND NUTRITIONAL QUALITY OF FULL-FAT SOY FLOUR: APPLICATION OF RESPONSE SURFACE METHODOLOGY

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SUMMARY

A study was conducted to diagnose nutritional status by determining total serum proteins and electrophoretic patterns of protein reserves. Serum proteins are affected by privation of several amino acids. The purpose of the present research work was to evaluate protein quality of full-fat soy flour (FFSF) obtained from various hydrothermal processes by measuring changes in the serum proteins of Wistar rats. Response surface methodology was used as tool to determine the optimum conditions of hydrothermal process. The mean values of total serum proteins for the experimental group fed treated FFSF, were $\beta_0 = 5.12 \pm 0.13$ g°/o; for the untreated FFSF group, $C_1 = 4.60 \pm 0.28$ g°/o, and for the control group fed casein, $C_2 = 5.63 \pm 0.33$ g°/o. All these values differ at 5°/o of significance ($p \leqslant 0.05$).

Results confirmed that treated full-fat soy flour is nutritionally superior to untreated FFSF, but not to casein.

INTRODUCTION

At present, there is an increasing interest in the use of soybean as an important energetic and protein source. Anti-nutritional factors are normally present which call for the use of proper heat treatment (1). Insufficient heat treatment results in a suboptimal product and excessive treatment reduces the bioavailability of lysine and sulphur amino acids, also affecting the nutritional value of the products (2). Some authors have reported that alkaline processing of soybeans alters the nutrient profiles by reducing the thiamine content (3).

Biological methods used to evaluate protein quality are important,

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particularly when dealing with proteins of similar amino acid composition, but subjected to modifications during processing (4). Moreover, for each species and body function there is an optimal amino acid profile, and profile alterations reduce the efficiency of protein use (5). Serum protein has been related to protein-deficient nutrition. According to Dimopoullos (6), factors that affect protein metabolism may influence total serum proteins or individual protein fractions. Protein depletion has been induced by protein-free diets. As reported by Bressani, Brenes and Braham (7), it can also be induced by the use of protein diets deficient in essential amino acids, which determine protein quality.

Protein reserves are affected by the quantity and quality of protein ingested (8). Determination of serum proteins has been used to find variation of protein reserves. It would be interesting to determine changes in serum proteins for the better diagnosis of nutritional status. Serum proteins are affected by absence of several essential amino acids. Albumin neoformation could be blocked in rats deprived of sulphur and phenolic amino acids, tryptophan and valine (9).

The purpose of the present work, therefore, was to evaluate protein quality of full-fat soy flour obtained from various hydrothermal processes by measuring changes in the serum proteins of rats. Response surface methodology was used as a tool to determine the optimum conditions for hydrothermal process.

MATERIAL AND METHODS

Certified soybean seeds (Paraná variety) were dehulled according to Sastry *et al.* (10), and cotyledons were selected using a mesh sieve of 0.45 - 0.55 cm.

Cotyledons were then processed by the hydrothermal method (11) employing response surface methodology (12) to determine optimum conditions.

Full-Fat Soy Flour Hydrothermal Processing

The important steps of the process were:

1) Soaking of cotyledons in a 1:3 solid/liquid ratio (3 kg cotyledons/3 lt water) for 0, 4 and 8 hours at room temperature.
2) Blanching of cotyledons in tap water containing Na HCO_3 (0, 0.25 and 0.50o/o concentrations) at 96oC, atmospheric pressure for 5, 20 and 35 min in a 1:10 solid/liquid ratio.
3) Dehydration in an air-circulating oven at 50 to 60oC for 20 hours.
4) Grinding in a hammer mill to obtain full-fat soy flour.

Experimental Design

As Table 1 shows, the incomplete 33 factorial design with equidistant levels was used. The plan of work constituted a group of 12 experiments, with three replicates in the central point (for the estimate optimum) totalling 15 experiments at random (Table 1). This procedure was selected

TABLE 1

EXPERIMENTAL DESIGN

Number of treatments	Variables X_1	X_2	X_3
1	−1	−1	0
2	1	−1	0
3	−1	1	0
4	1	1	0
5	−1	0	−1
6	1	0	−1
7	−1	0	1
8	1	0	1
9	0	−1	−1
10	0	1	−1
11	0	−1	1
12	0	1	1
13	0	0	0
14	0	0	0
15	0	0	0

VARIABLES AND LEVELS USED FOR OPTIMIZATION PATTERN

Natural variables	Levels −1	0	+1
X_1	0	4	8
X_2	5	20	35
X_3	0	0.25	0.50

X_1 = Hydration — hours.
X_2 = Blanching — minutes.
X_3 = $NaHCO_3$ - g o/o.

$$\text{Coded variables} = \frac{\text{Variable-Variable at central point}}{\text{Range}}$$

so as to perform an analysis by response surface methology (RSM) to determine the optimum processing conditions.

Determination of Response Surface

In most RSM problems, the form of relationship between the response and independent variables is unknown. Therefore, a second order quadratic equation (13) was selected in this study because it is the simplest non-linear equation that can be used for optimization with response surface methodology. Responses are total serum proteins and their electrophoretic fractions from rats fed different diets prepared with full-fat soy flour (FFSF) denoted by Y (1 to 6) and the independent variables that affect the responses are: hydration time (X_1), blanching time (X_2) and $NaHCO_3$ concentration (X_3), since these may affect protein quality of FFSF. The general form of the quadratic equation in this study is:

$$Y = \beta_0 + \Sigma_i \beta_i X_i + \Sigma_i \beta_{ii} X_i^2 + \Sigma_i \Sigma_j \beta_{ij} X_i X_j + \epsilon$$

$$i < j$$

Where ϵ is a random error component and β's are the coefficients of the least squares estimators. An analysis of variance (F-test) was done for coefficients of multiple regression equation terms to determine the suitability of the model for response surface analysis, that is, the value of serum proteins as a function of independent variables.

Preparation of Diets and Handling of Animals

Full-fat soy flours were used after determination of proximate analysis by the AOAC methods (Table 2), as the only protein source for preparation of the diets (14). Experimental diets (treated FFSF) were identified with numbers 1 to 15 based on protein source and treatment. Two control diets were used, one with untreated FFSF (C_1), and the other with casein (C_2) as protein source.

Male weanling rats *(Rattus rattus)* of the Wistar strain, 21 days old, obtained from Tecpar[2], were used for the experiments. Animals were divided into 17 groups, identified from 1 to 15, C_1 and C_2, according to protein source of the diets offered, and housed in individual cages (18 cm x 18 cm x 18 cm) provided with screen bottoms. Each group was formed by six animals with a mean body weight of 37 to 40 g, and an individual body weight of 30 to 42 g. A period of three days acclimatization was allowed prior to the initiation of the test. During this period, a standard casein diet was provided for all animals (15), and a 12/12 hr light-dark cycle and a temperature of 24 ± 1°C were maintained. The animals were weighed, and food intake was determined every two days. Spillage food was collected and used to correct the amount of food intake during 50 days of the experimental period, and hematological tests were then performed.

Determination of Serum Proteins

Seventeen blood samples were analyzed per day, in a period of six days, corresponding to 1 sample for each group. Rats from the experimental group were fasted the night before the assays took place. Bleeding was done by severing the jugular vein, and blood was allowed to clot at room temperature for 60 minutes, centrifuged for 10 minutes at 2,500 rpm and centrifugation serum was recovered. Total serum proteins were determined by/Biuret methods (16) using Precinorm U (Boehring)

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TABLE 2

PROXIMATE COMPOSITION OF FULL-FAT SOY FLOURS: TREATED, UNTREATED, AND CASEIN

	Proteins (N x 6.25)	Fat	Ashes	Fiber carbohydrate	Moisture
1	45.18	25.38	4.28	25.15	4.38
2	48.18	16.12	3.61	22.08	4.85
3	47.54	28.90	3.90	19.65	4.66
4	47.75	30.39	3.44	18.42	4.29
5	47.16	26.52	4.00	22.30	5.09
6	48.62	27.66	3.44	20.27	4.08
7	45.68	28.84	4.13	21.34	4.90
8	48.80	27.99	3.71	19.49	4.33
9	48.93	24.46	3.77	22.84	4.46
10	47.64	31.23	3.24	17.88	4.16
11	48.78	25.06	3.83	22.32	5.04
12	47.19	30.48	3.88	18.45	5.66
13	46.62	28.60	3.65	21.13	4.53
14	46.76	29.55	3.62	20.06	4.60
15	46.83	30.04	3.72	19.41	5.02
C1	43.73	22.51	4.89	28.86	8.74
C2	96.70	—	0.71	—	9.48

1 to 15 = Treated full-fat soy flour.
C1 = Untreated full-fat soy flour.
C2 = Casein.

as standard. Electrophoresis was performed by strips of cellulose acetate, veronal buffer 0.4M (diethyl barbiturate sodium 8.24 g/lt). Serum was applied with micropipette using a current of 200 volts for 45 minutes. Staining was done with amido Schwartz. The strips were analyzed by a recording integrating densidometer (Atago Quick).

RESULTS AND DISCUSSION

Serum protein fractions from all groups are presented in Table 3. Our results are similar to those obtained by Pestana (17) except for Υglobulins, which were lower than those found by the author.

Total serum protein and serum albumin may be related to protein quality of the diet. As stated by Leveille and Sauberlich (18), a decrease in total serum protein may be attributed to a drop in albumin serum concentration.

Serum protein fractions of groups 1 to 15 were analyzed by multiple regression equations. The analysis of regression and analysis of variance for the model are shown in Tables 4 and 5, and as we may observe, R^2 represents the per cent of variation explained by the model. Values of

TABLE 3

SERUM PROTEINS OF RAT GROUPS FED WITH DIFFERENT DIETS

Group No.	Total serum proteins g o/o Y_1	Albumin g^o/o Y_2	Globulins g^o/o α_1 Y_3	α_2 Y_4	β Y_5	Υ Y_6
1	5.24	2.85	0.72	0.64	0.99	0.17
2	5.50	2.87	0.62	0.68	1.32	0.28
3	5.08	2.59	0.72	0.56	0.98	0.28
4	5.30	2.86	0.64	0.67	0.97	0.15
5	5.12	2.83	0.76	0.55	0.94	0.18
6	5.32	2.83	0.73	0.64	1.03	0.22
7	5.12	2.64	0.78	0.64	0.94	0.16
8	5.24	2.65	0.70	0.69	1.05	0.21
9	5.22	2.55	0.78	0.67	1.07	0.21
10	5.04	2.58	0.80	0.65	0.97	0.14
11	5.16	2.70	0.71	0.67	0.92	0.15
12	5.02	2.44	0.86	0.68	1.03	0.16
13	5.07	2.61	0.75	0.57	1.01	0.20
14	5.27	2.75	0.80	0.69	0.91	0.14
15	5.03	2.64	0.65	0.61	0.94	0.19
β_0	5.12	2.66	0.73	0.62	0.95	0.17
C_1	4.60	2.44	0.51	0.46	1.06	0.13
C_2	5.63	3.13	0.72	0.63	1.01	0.27

β_0 = Animal's serum from experimental group fed treated FFSF; mean values of central point trials.
C_1 = Animal's serum from control group fed untreated FFSF.
C_2 = Animal's serum from control group fed casein.

R^2 indicate that the present model could be used. Since the multiple regression analysis is not significant at 5o/o level ($p \leqslant 0,05$), the model does not establish differences among treatments. Consequently, the mean in central point (β_0) may be used).

Applying Duncan test to compare serum total proteins and then albumin of β_0, C_1 and C_2 (Table 3), one can appreciate that these values differ at the 5o/o level of significance ($p \leqslant 0.05$). Values in decreasing order are C_2' β_0 and C_1, and the α and Υ globulins seem to be unaffected by protein ingestion. The difference in the results of β_0, C_1 and C_2 is not significant at the level 5o/o ($p \leqslant 0.05$). As stated by Allison and Fitzpatrick, the α globulins are considered proteins of specific biological importance since they are preserved (8). Consequently, α β and Υ globulin levels may not provide good estimates of protein nutritional quality.

The means of body weight and the amount of protein ingested during the experiment were analyzed by multiple regression in groups 1 to 15. The results revealed that there were no significant differences at the 5o/o significance level ($p < 0.05$). The Duncan Test was used to compare mean body weight of groups β_0 (146.16 g), C_1 (90.11 g) and C_2 (191.37 g). All of these values proved to be significantly different; $p \leqslant 0.05$. The

TABLE 4

MULTIPLE REGRESSION EQUATION AND β CALCULATED PARAMETERS

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3$$

Y	Total serum proteins	Albumin	α_1-Globulin	α_2-Globulin	β-Globulin	γ-Globulin
B_0	5.1233	2.6633	0.7333	0.6233	0.9466	0.1753
B_1	0.1000	0.0375	-0.03625	0.036250	0.0337	0.0071
B_2	-0.0850	-0.0625	0.02375	-0.0125	-0.0113	-0.0113
B_3	-0.0200	-0.0451	-0.0025	0.02125	-0.0100	-0.0099
B_{11}	0.1233	0.1495	-0.0516	-0.011667	0.0254	0.0364
B_{22}	0.0333	-0.0205	-0.0066	0.025833	0.0304	0.0097
B_{33}	-0.0466	-0.0755	0.0608	0.018333	0.0179	-0.0211
B_{12}	-0.0100	0.0625	0.0050	0.0175	-0.0225	-0.0586
B_{13}	-0.0200	0.0025	-0.01250	0.0100	0.0049	-0.0008
B_{23}	0.0100	-0.0725	0.03250	0.0075	0.0549	0.0210
R^2	0.8550	0.8406	0.7516	0.6872	0.7487	0.8605

R^2 = Per cent of variation explained by the model.

same procedure was used to compare the mean amount of protein ingested in groups β_0 (47.4 g), C_1 (21.67 g) and C_2 (49.61 g). The test did not show a significant difference between the groups β_0 and C_2, although the remaining values were different at a significance level of 5o/o. The weight increase obtained in group β_0 and C_2 were different when the same amount of protein was ingested.

The results obtained confirm that FFSF treated by hydrothermal method is nutritionally better than untreated FFSF, but not so in the case of casein, as the protein source in animal diets. This statement is confirmed by other authors in the literature (19). Aside from this fact, these conclusions agree with Buassi *et al.* (20), who determined the protein nutritional quality of these flours by chemical (methionine and cystine) and biological (net protein ratio) methods.

Measurement of serum proteins, as stated by Bressani, Gómez-Brenes and Braham (7), is a less sentive procedure than nitrogen balance in the evaluation of protein quality. In the work herein reported, the same results could be drawn from chemical, nutritional and hematological indicators.

The interactions of processing variables of FFSF, at the levels used in our experiment, did not reveal any modification in protein quality which could alter the hematological indices of serum proteins.

Since de response surface methodology could not detect differences in the serum proteins analyzed from various treatments, the use of 20 minutes blanching, without prior hydration or addition of sodium bicarbonate, is suggested.

TABLE 5

EFFECT OF EXPERIMENTAL DIETS ON TOTAL SERUM PROTEINS AND ELECTROPHORETIC PATTERNS

Analysis of variance							
		Y_1 Total serum protein g o/o	Y_2 Albumin g o/o	Y_3 α_1-Globulin g o/o	Y_4 α_2-Globulin g o/o	Y_5 β-Globulin g o/o	Y_6 γ-Globulin g o/o
Source of variation	Degrees of freedom	Q.M (F)	Q.M (F)	Q.M (F)	Q.M (F)	Q.M (F)	Q.M (F)
Regression	9	0.0238(1.44)	0.0233(2.33)	0.0051(0.87)	0.0024(0.64)	0.0033	0.0033
Deviation	3	0.0011(0.07)	0.01 (1.63)	0.0011(0.20)	0.0008(0.21)	0.00(0.90)	0.00(0.47)
Experimental error	2	0.0165	0.01	0.0058	0.0037	0.00	0.00

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RESUMEN

PROTEINAS SERICAS EN RATAS, Y CALIDAD NUTRICIONAL DE LA HARINA INTEGRAL DE SOYA: APLICACION DE LA METODOLOGIA DE ANALISIS DE SUPERFICIE DE RESPUESTA

Se llevó a cabo un estudio para diagnosticar el estado nutricional mediante la determinación de proteínas séricas totales y patrones electroforéticos de las reservas proteínicas. La privación de varios aminoácidos afecta a las proteínas séricas.

El propósito de este trabajo de investigación fue evaluar la calidad de la proteína de harina integral de soya sin desgrasar (FFSF) obtenida de varios procesos hidrotérmicos, a través de la medición de cambios en las proteínas séricas de ratas Wistar. Se utilizó la metodología de análisis de superficie de respuesta como instrumento para determinar las condiciones óptimas del proceso hidrotérmico. Los valores medios de las proteínas séricas totales del grupo experimental alimentado con FFSF tratada, fueron: $\beta_0 = 5.12 \pm 0.13$ g°/o; para el grupo que recibió FFSF sin tratar, $C_1 = 4.60 \pm 0.28$ g°/o, y para el grupo control alimentado con caseína, $C_2 = 5.63 \pm 0.33$ g°/o. Todos los valores difieren al nivel de 5°/o de significancia ($P \leqslant 0.05$).

Los resultados confirmaron que la harina integral de soya sin desgrasar, tratada, es nutricionalmente superior a la FFSF sin tratar, pero no lo es con respecto a la caseína.

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