ARCHIVOS LATINOAMERICANOS DE NUTRICION
Organo Oficial de la Sociedad Latinoamericana de Nutrición

Vol. 45 N° 1, 1995

Effects of dehulling, cooking and storage conditions on protein quality and digestibility of soybeans

L.C. Cabral [1], S.O. Serna-Saldivar [2], A.M. Tinksley [3] and C.W. Weber [3]

SUMMARY. Soybeans were dehulled, stored under two environmental conditions [25°C/75% R.H. (Env. 1) and 38°C/90% R.H. (Env. 2)], optimally cooked and assayed for trypsin inhibitor and protein quality with laboratory rats. Dehulling did not significantly affect protein quality (PER and NPR) and protein digestibility of raw and cooked soybeans. Raw soybeans diets were significantly poorer in protein quality and digestibility when compared with cooked counterparts. PER values of dehulled-cooked soybean diets decreased significantly ($P<0.05$) as seeds were stored for up to 3 months under either environment. There were no significant differences in PER values due to storage during the period from 3 to 6 months. PER values for whole-cooked soybean diets exhibited a significant decline only when stored for 6 months under Env. 2.

RESUMEN. Efecto del descascarado, cocción y almacenamiento sobre la calidad de la proteína y digestibilidad de la soya. Granos de soya fueron descascarados, almacenados bajo dos condiciones ambientales [25°C/75% R.H. (Env. 1) and 38°C/90% R.H. (Env. 2)], cocidos optimamente y evaluados en su actividad de inhibidor de tripsina y de calidad proteica con ratas de laboratorio. El descascarado no afectó la calidad proteica (PER y NPR) ni la digestibilidad de la proteína de granos crudos y cocidos. Las dietas de soya cruda fueron más pobres en calidad proteica y digestibilidad cuando fueron comparadas con dietas de granos cocidos. Los PER's de las dietas de granos de soya descascarados y cocidos disminuyeron significativamente ($P<0.05$) cuando las semillas fueron almacenadas hasta por tres meses en ambos ambientes. No hubo diferencias significativas en valores de PER debido al almacenamiento durante el período de 3 a 6 meses. Los valores de PER para las dietas de granos enteros y cocidos bajaron significativamente solamente cuando fueron almacenados por más de 6 meses bajo el ambiente 2.

INTRODUCTION

Presently, soybeans are one of the best answers for alleviating dietary problems caused by protein/calorie deficiency. World soybean production in 1991 was estimated at 103.1 million metric tons (1), which represents approximately 50 million metric tons of protein. If used directly for human food, this amount would supply approximately 2.4 million people yearly with the recommended daily allowance of 56 g/day/adult. Despite the excellent nutritional qualities of soybeans, their direct use as human food with the exception of some oriental countries, has been quite limited.

Soybean protein has good nutritive value when it is adequately processed in a manner that destroys antinutritional factors but avoids desnutrition of essential amino acids. More importantly, soybean proteins drastically upgrade protein quality of cereals by complementation of essential amino acids. Antunes and Sgarbieri (2) found that concentrations of the limiting amino acids methionine and cystine in pinto beans (*Phaseolus vulgaris*) were reduced considerably by cooking. Decreased levels of the sulfur-containing amino acids resulted in decreased nutritive value. Excessive heat treatment might reduce the available lysine due to inactivation of its epsilon amino group.

Unlike most animal proteins, soybeans have the advantage of being easily stored for a long period of time. The objective of this study was to evaluate the effects of dehulling, cooking and storage on the protein quality of soybeans.

1 Researcher, EMBRAPA-CTAA, Guaratiba, Rio de Janeiro, Brazil.
2 Professor, Department of Food Science and Technology and Graduate Program of Food Engineering, Instituto Tecnológico y de Estudios Superiores de Monterrey, N.L., México.
3 Professors, Department of Nutrition and Food Science, University of Arizona, Tucson, Arizona, USA.

MATERIALS AND METHODS

Samples: Soybeans of the Williams 82 variety grown in Iowa in 1982 were cleaned, dehulled, stored and cooked according to the conditions and methods described in a preceeding experiment (3).

Chemical analysis: Protein (N x 6.25) and amino acid compositions of raw, stored and cooked whole and dehulled soybean samples were determined by the micro-Kjeldhal method (4) and ion-exchange chromatography (5), respectively. Amino acids were separated by ion exchange chromatography, and colorimetrically quantified by spectrophotometer after reaction with ninhydrin. Trypsin inhibitor activity was determined according to methodology proposed by Kakade et al (6).

Nutritional study: The nutritional experiments were designed to study the effects of dehulling, storage time and cooking on protein quality and digestibility in laboratory rat tests. Treatments chosen included whole-raw, dehulled raw, and cooked whole and dehulled soybeans stored at 25°C/75% R.H. (Env. 1) and 38°C/90% RH (Env. 2) for 0, 3, and 6 months. A casein-based diet served as the control treatment. A protein-free diet was included to estimate the fecal and urinary metabolic losses of nitrogen.

All diets were formulated following accepted guidelines (4) with constant amounts of AIN vitamins and minerals, cellulose, chlorine chloride and chromic oxide (Table 1). Chromic oxide served as an undigestible marker for estimation of digestibilities. Ground soybean samples were added to balance dietary protein levels to 10% and were supplemented with corn oil to achieve total oil contents of 8%. Starch was added to produce isocaloric diets, and betonite was incorporated as a filler (Table 1).

Groups of six weanling rats of the Sprague-Dawley strain having similar body weight were used per treatment. Animals were individually housed in wire bottom cages for 28 days in an environmentally controlled room (temperature of 23°C; 12 hr schedule of alternating artificial light and darkeness). Consumptions of food and water were *ad libitum*. Rats were weighed weekly, and feed intake was determined twice a week.

Protein efficiency ratio (PER) were calculated as the weight gain/weight protein consumed, and were corrected according to performance of rats fed the control casein diet (PER=2.5). Net protein ratios (NPR) were determined after estimating feed and weight gains on the tenth day of the PER assay. The NPR was calculated as follows (wt. gain on test diet + wt loss on protein free diet/protein consumed from test diet). Apparent protein digestibility was calculated after a representative sample of feces (collected twice a week) was assayed for protein (4) and chromic oxide (7).

Statistical analysis: Analysis of variance and linear regression analysis of the data were performed following Statistical Analysis System procedures (8).

TABLE 1
COMPOSITION OF DIETS USED IN PROTEIN QUALITY EVALUATIONS OF RAW AND COOKED, WHOLE AND DEHULLED SOYBEANS[1]

Storage Conditions								
Time (mo)	Temp. (C)	R.H. (%)	Cooking Time	Protein Source	Starch[2]	Corn oil	Betonite	Others[3]
				Casein4				
—	—	—	—	11.12	67.00	8.00	5.98	7.90
				Protein Free				
—	—	—	—	—	77.00	8.00	7.10	7.90
				Raw Whole Soybean				
—	—	—	—	27.20	60.78	2.56	1.56	7.90
				Cooked Whole Soybeans				
—	—	—	5.9	22.23	63.66	1.97	4.24	7.90
3	25	75	6.3	22.11	63.30	2.05	4.64	7.90
6	25	75	7.1	22.18	63.20	2.01	4.71	7.90
3	38	90	8.0	22.06	63.24	2.03	4.77	7.90
6	38	90	11.0	22.33	63.24	1.96	4.57	7.90
				Raw Dehulled Soybeans				
—	—	—	—	25.24	61.25	2.28	3.33	7.90
				Cooked Dehulled Soybeans				
—	—	—	6.1	21.54	63.60	1.45	5.51	7.90
3	25	75	6.9	21.18	63.81	1.56	5.55	7.90
6	25	75	6.9	21.18	63.81	1.56	5.55	7.90
3	38	90	8.3	21.75	63.56	1.27	5.52	7.90
6	38	90	11.3	21.27	63.64	1.35	5.84	7.90

1 All diets were formulated to contain approximately 10% protein; 8% oil, 3.80 kcal/g and 0.2% BHT (oil weight basis).
2 Cerelose=partially hydrolyzed starch.
3 All diets contained 1.0% AIN (American Institute of Nutrition) 76 vitamin mix, 3.5% AIN mineral mix, 3% cellulose (Celufil a non nutritive bulk), 0.2% Cr_2O_3 and 0.2% choline chloride.
4 Casein= ANRC reference standard (89.2% protein).

RESULTS AND DISCUSSION

There were no appreciable differences in protein and individual amino acids contents between whole and dehulled soybean samples treated alike (Table 2). For all soybean treatments, methionine and cysteine were the first limiting amino acids with similar chemical scores. As expected, the casein - base diet had the best chemical score or essential amino acid composition. Among amino acids, only cysteine suffered a substantial decrease as a result of cooking. Reductions in cysteine levels most likely were affected by thermal degradation due to their increased cooking time as a result of storage duration and environmental conditions (3). Storage may not have had a direct effect on total cysteine content, since a substantial decrease occurred even when unstored samples were cooked.

TABLE 2
PROTEIN AND AMINO ACIDS COMPOSITION OF WHOLE DEHULLED SOYBEANS UNDER VARIOUS STORAGE AND PREPARATION CONDITIONS

Storage Conditions					Essential Amino Acid Composition[1]								
Time (mo)	Temp. (C)	R.H. (%)	Cooking Time	Protein (% N x 6.25)	Ile	Leu	Lys	Met	Cys	Phe	Tyr	Thr	Val
					(g/16g N)								
Raw Whole Soybeans													
				46.4	4.0	7.0	5.7	1.1	1.8	4.8	3.6	3.7	4.2
Cooked Whole Soybeans													
0	—	—	5.9	46.4	3.8	7.2	5.6	1.2	1.5	4.7	3.6	3.8	4.0
3	25	75	6.3	46.1	3.8	7.2	5.5	1.3	1.6	4.9	3.7	3.9	4.0
6	25	75	7.1	45.6	3.8	7.4	5.7	1.2	1.3	4.6	3.3	3.9	4.0
3	38	90	8.0	45.9	4.0	7.4	5.5	1.4	1.4	5.0	3.8	3.9	4.1
6	38	90	11.0	45.3	4.0	7.7	5.7	1.2	1.4	5.1	3.8	4.1	4.3
Raw Dehulled Soybeans													
—	—	—	—	42.3	3.4	6.8	5.7	1.1	1.8	4.4	3.1	3.7	3.6
Cooked Dehulled Soybeans													
0	—	—	6.1	47.2	4.0	7.4	5.5	1.1	1.5	4.9	3.5	3.9	4.1
3	25	75	6.9	47.8	3.6	7.0	5.2	1.1	1.4	4.6	3.3	3.7	3.8
6	25	75	7.2	47.5	4.2	7.6	5.6	1.2	1.5	5.3	3.9	3.9	4.3
3	38	90	8.3	46.7	3.7	7.3	5.4	1.1	1.4	5.0	3.8	3.9	3.9
6	38	90	11.3	47.3	4.4	7.7	5.6	1.2	1.3	5.1	3.8	4.0	4.4

1 Essential amino acid profile for 2 years old children (20) in g/100 g protein: ile 2.8, leu 6.6, lys 5.8, met+cys 2.5, phe+tyr 6.3, thr 3.4, trp 1.1 and val 3.5.

The trypsin inhibitor activity of raw and dehulled soybean was very high, and dehulling did not appreciably affected values (Table 3). Storage of whole and dehulled soybeans significantly decreased trypsin inhibitor activity, and the effect was more noticeable in beans stored under Env. 2. Urbaniski et al (9) also found decreased trypsin inhibitor activity in mature soybean varieties subjected to storage.

As expected, cooking of whole and dehulled soybeans destroyed nearly all trypsin inhibitor activity (Table 3). Ferrier (10) ndicated that trypsin inhibitor can be destroyed by boiling seeds for only 5 min. In this study soybean samples were subjected to boiling for a minimum period of 5.9 hr.

The dehulling process did not significantly affect the PER, NPR and protein digestibility for unstored raw or cooked soybean samples (Table 4). Results obtained for PER and NPR are comparable to those normally encountered in the literature (11-15). Apparent digestibilities of casein and soybeans were slightly lower than digestibilities reported by Liener (11-13) and Rackis et al (16), but the relative difference between values for casein and soybean were similar.

TABLE 3
EFFECTS OF STORAGE AND COOKING ON TRYPSIN INHIBITOR ACTIVITY OF WHOLE AND DEHULLED SOYBEANS

Storage Conditions			Trypsin Inhibitor Activity			
Time (mo.)	Temp. (C)	R.H. (%)	Raw Soybeans		Cooked Soybeans	
			TIU/mg Protein	TIU Inactivation (%)	TIU/mg Protein	TIU Inactivation (%)
Whole Soybeans						
0	—	—	129.1	—	1.4	98.9
3	25	75	103.3	20.0	1.2	99.1
6	25	75	97.6	25.4	1.2	99.1
3	38	90	106.3	18.7	1.1	99.2
6	38	90	89.8	31.4	1.1.	99.2
Dehulled Soybeans						
0	—	—	122.2	—	1.0	99.2
3	25	75	100.7	22.0	0.9	99.3
6	25	75	83.4	28.3	0.7	99.5
3	38	90	92.6	35.4	0.9	99.3
6	38	90	82.3	36.3	1.0	99.2

TABLE 4
EFFECTS OF STORAGE AND COOKING CONDITIONS ON PROTEIN QUALITY OF WHOLE AND DEHULLED SOYBEANS FED TO WEANLING RATS[1]

Storage conditions Time (mo.)	Temp. (C)	R.H. (%)	Cooking Time (hr)	Protein Efficiency Ratio Experimental	Corrected[2] (g gain/g protein fed)	Net Protein Ratio[3]	Apparent Protein Digestibility (%)
				Casein			
—	—	—	—	2.40	2.50 a	4.03 a	86.8
				Raw Whole Soybeans			
—	—	—	—	0.66	0.70 d	2.08 c	64.4
				Cooked Whole Soybeans			
0	—	—	5.9	1.86	1.94 b	3.35 b	79.7
3	25	75	6.3	1.92	2.00 b	3.34 b	82.0
6	25	75	7.1	1.79	1.86 bc	3.08 b	79.5
3	38	90	8.0	1.90	1.98 b	3.36 b	79.2
6	38	90	11.0	1.69	1.76 c	3.11 b	81.2
				Raw Dehulled Soybeans			
—	—	—	—	0.71	0.74 d	2.18 c	66.4
				Cooked Dehulled Soybeans			
0	—	—	6.1	1.89	1.97 b	3.41 b	80.8
3	25	75	6.9	1.66	1.73 c	3.15 b	78.4
6	25	75	7.2	1.60	1.66 c	3.15 b	78.2
3	38	90	8.3	1.65	1.72 c	3.05 b	78.3
6	38	90	11.3	1.59	1.66 c	3.02 b	80.5

1 Means with different letters with in column are significantly different ($P<0.05$).
2 Protein efficiency ratio (PER)= weight gain on test diet/protein intake from test diet.
Adjusted PER = PER (experimental) x [2.5 (standardized value for casein) /2.4 (experimental value for casein)].
3 Net protein ratio (NPR)= (weight gain on test diet + weight loss on protein free diet) /protein intake from test diet.

As expected, the raw soybean diets were significantly poorer in protein quality when compared with cooked counterparts. The significant improvement in protein quality upon cooking may be attributed to the improvement in protein digestibility via inactivation of trypsin inhibitor as well as denaturation of globulins which in nature state are more resistant to enzymatic digestion (14, 17, 18). Kakade et al (17) found that trypsin inhibitor accounts for about 40% of the growth inhibition and pancreatic enlargement of rats fed raw soybeans. The remaining 60% of the growth inhibition and pancreatic hyperthrophy were attributed to the indigestibility of proteins for which trypsin inhibitor was removed by affinity chromatography.

Both duration and condition of storage significantly affected PER values of whole and dehulled soybeans (Tabla 4). PER values of dehulled diets decreased as seeds were stored up to 3 months under either environment. There were no significant changes in PER values due to storage during the period from 3 to 6 months. PER values for whole soybean diets exhibited a significant decrease only when beans were stored for 6 months under 38 C/90% RH. Molina et al (19) and Antunes and Sgarbieri (2) also found a lower protein quality of black beans stored under a variety of conditions. Decreased PER values were attributed to the decreased availability of sulfur containing amino acids from the increased cooking time associated with the stored samples.

Results of this study indicated that soybean protein quality is mainly dictated by its digestibility, which is affected by the presence of trypsin inhibitors and nature of globulins. Storage significantly decreased the amount of trypsin inhibitor in raw soybean samples. However, protein qualities of stored products were slightly lower due to the amount and availability of sulfur containing amino acids.

REFERENCES

1. FAO. Production Yearbook. Vol 45. Rome, Italy. 1991.
2. Antunes PL. & Sgarbieri V.C. Influence of time and conditions of storage on technological and nutritional properties of a dry bean (*Phaseolus vulgaris L*) variety Rosinha Gz. J. Food Sci. 44: 1703. 1979.
3. Cabral L.C.; S.O. Serna-Saldivar & A.M. Tinsley. Effects of dehulling and storage conditions on cooking requirements and chemical composition of soybeans. Arch. Latinoamer. Nutr. 45: 42-45.1995
4. AOAC. «Official Methods of Analysis», 13th Edition. Assoc. Off. Anal Chem. Washington, DC. 1980.
5. Spackman D.H.; W.H. Stein & S. Moore. Automatic recording apparatus for use in the chromatography of amino acids. Analytical Chem. 30:1190. 1958.
6. Kakade M.L.; J.J. Rackis. J.E. McGhee & G. Puski. Determination of trypsin inhibitor activity of soy products: A collaborative analysis of an improved procedure. Cereal Chem 51:376. 1974.
7. Kimura F. & V.L. Miller. Improved determination of chromic oxide in cow feed and feces. J. Agric. Food Chem 5:216. 1957.
8. Freund R.J. & R.C. Littell. SAS System for Regression. SAS Institute, INC. CARY, NC. 1986.
9. Urbaniski G.E.; L.S. Wei & A.I. Nelson. Effect of freeze damage on soybean quality and storage stability. J. Food Sci. 45:208. 1980.
10. Ferrier L.K. 1975. Simple processing of whole soybeans pp 178-188 In: Soybean production, protection and utilization. D.K. Whigham (ed). Addis Ababa, Ethiopia. Proceedings. College of Agriculture, Univ. of Illinois at Urbana-Champaign INSTOY Series N° 6 pp 178-188. 1974.
11. Liener I.E. Legume toxins in relation to protein digestibility. A review. J. Food Sci. 41: 1076. 1976.
12. Liener I.E. Nutritional aspects of soy protein products. J.Am Oil Chem Soc. 54:454A. 1977.
13. Liener I.E. Nutritional value of food protein products. pp 203-277 In: Soybeans: Chemistry and Technology A.K. Smith & S.J. Circle (ed). The AVI Publishing Co., Inc. Westport, CN. 1978.
14. Liener I.E. Factors affecting the nutritional quality of soya products. J. Am Oil Chem Soc. 58:406. 1981.
15. Sgarbieri V.C.; R.S. Garuti; M.A.C. Morales & L. Hartman. Nutritional and sensory evaluation of mixtures of soybean (*Glyxine max L.*) and common beans (*Phaseolus vulgaris L.*) for direct use as human food. J. Food Sci. 43:208. 1978.
16. Rackins J.J., McGhee J.E. & Honig D.H. Processing soybeans into foods: selected aspects of nutrition and flavor. J. Am Oil Chem Soc. 52:249A. 1975.
17. Kakade M.L., Hoffa D.E. & Liener I.E. Contribution of trypsin inhibitors to the deleterious effect of unheated soybeans fed to rats. J. Nutr. 103:1772. 1973.
18. Walker A.F. & Kochhar N. Effect of processing including domestic cooking on nutritional quality of legumes. Proc. Nutr. Soc. 41:41. 1982.
19. Molina M.R. de la Fuente G. & Bressani R. Interrelationships between storage, soaking time, cooking time, nutritive value and other characteristics of the black bean (*Phaseolus vulgaris*). J. Food Sci. 40:587.
20. FAO/WHO/UNU. In: Protein and Energy Requirements. Tech. Rep. Ser. 724, World Health Organization, Geneva. 1985.

Recibido: 11-01-1994
Aceptado: 04-08-1994.