Pak J Sci Ind Res 200144 (6) 356-360

Biological Evaluation of Whole Soymilk Produced by Indigenous Soybean

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(Received 25 November 1999; accepted 9 February 2001)

A study was planned to prepare whole soymilk using two different methods from the indigenous soybean. The extraction of soymilk was carried out from whole soybean and dehulled soybean. The soymilk samples thus prepared were then analyzed and their quality of protein were evaluated by conducting 10 days feeding trial at 10% protein level to weanling albino rats. Skim milk (standard protein), cerelac of wheat (for comparison) and non-protein diets were also involved in feeding trial. Proximate analysis revealed that dehulled soybean milk is more nutritious as compared to whole soybean milk. The data on feeding trial showed Protein Efficiency Ratio (PER) 2.6,1.7; True Digestibility (TD) 90.0,88.0; Net Protein Utilization (NPU) 69.0,58.0 and Biological Value (BV) 77,66 for dehulled soymilk and whole soymilk respectively. Where as for skim milk and cerelac (wheat) PER 2.8,3.1; TD 92,92; NPU 74,66 and BV 81,71 respectively. Based upon the above results it is suggested that soymilk may be prepared on large scale from dehulled soybean and may be used as supplement food for children and population at risk.

Key words: Protein Efficiency Ratio (PER), True Digestibility (TD), Net Protein Utilization (NPU), Biological value (BV).

Introduction

Use of vegetable protein sources in weaning food for infants and children may be recommended due to shortage and higher cost of animal protein sources. Among vegetable sources of protein, soybean is ranked on the top due to its better amino acid profile and availability of vitamins and minerals (Plyer 1988). Soymilk may serve as a good nutritive source for the babies who are malnourished and for those who do not tolerate cow milk and lactose of milk. Evidence suggests that soy protein may be less allergenic than heat-treated cow's milk protein and is probably a better source of nutrition in allergy prone infants (Anon 1983).

Several methods of the soymilk preparation have been tried in developed countries but they are rarely used in Pakistan. The information regarding the digestibility of milk prepared from soybean, particularly indigenous soybean is lacking. The aim of the present investigation was to prepare whole soymilk from indigenous whole soybean and dehulled soybean, and to evaluate the quality of soymilks by chemical and biological methods.

Materials and Methods

The indigenous raw soybean was procured from Ayub Agriculture Research Institute, Faisalabad. It was dried and ground to uniform size and subjected to proximate analysis (AOAC 1990). The experiment was conducted at Animal Nutrition Laboratory, University of Agriculture, Faisalabad.

Preparation of soymilk. Soymilk was prepared from indigenous soybean by the following two processes:

A: One kilograms of the soybean was placed in a 2 liter stainless steel bowl and excess of boiling water was added. The container was allowed to stand for over night. Thereafter, the whole material was blended for 5 min and filtered through cheesecloth under suction. The milk thus obtained was dried in air oven initially at 100°C for one hour and then the temperature was reduced to 60°C to attain a constant weight.

B: Soybean was soaked for 6 h and dehulled with smooth maceration with hands. Whole material was blended for 10 minutes at 80°C to obtain a bland soymilk. Dehulled soymilk was filtered through cheesecloth under suction. It was dried initially at 100°C in air oven for one hour and then the temperature was reduced to 60°C till a constant weight was obtained. This method was described by (Wilkens *et al* 1967).

Biological evaluation. Whole soymilk and dehulled soymilk was evaluated on weanling albino rats following the methods described by Pellet and Young (1980).

Thirty albino rats (mixed sex) of similar age were weaned at the age of 21 days. The rats were fed on stock diet for 7 days before the start of experiment and then divided into 10 groups

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of 3 rats each with a weight difference of ± 5g. Each group of rats was weighed prior to the feeding trial and thereafter daily. The rats were housed in wire screen mesh bottomed cages. A sheet of filter paper was used under each cage for the collection of feces and spilt diet. Water and feed were provided *ad-libitum* to the rats. The temperature of animal room was maintained at 28 ± 2°C. Five diets were prepared as shown in Table 1.

Diet A was standard diet containing skim milk, diet B was cerelac diet for comparison while C and D were whole soymilk and dehulled soymilk diets. All these four diets contained 10% protein. Diet E was protein free diet to account for endogenous nitrogen extracted along with the feces.

These diets were assigned randomly to ten groups of rats in such a way that there were two groups of rats on each diet. The diets were fed for 10 days. The spilt feed and fecal material was collected daily from each cage, oven dried weighed and stored separately. At the end of the experiment, the rats were anaesthetized with over dose of chloroform. Their cranial as well as abdominal cavities were opened. Each group of carcass was weighed after oven drying at 105°C to a constant weight. The dried carcass and dried feces were ground and analyzed for nitrogen (Hiller *et al* 1948).

The protein qualities of diets were expressed in terms of following formulae (Pellet and Young 1980).

1. Protein Efficiency Ratio (PER) = Gain in body weight / Protein intake of test diets.
2. Net Protein Utilization (NPU) = B - (B_K-I_K) / I x 100.

Where B= Body nitrogen of the group of rats on test protein diet.

B_k= Body nitrogen of the group of rats on protein free diet.

I_k= Intake of nitrogen by group of rats on protein free diet.

I= Intake of nitrogen by group of rats on test protein diet.

3. True Digestibility (TD) = I - (F_n - F_k) / I x 100.

Where I= Intake of nitrogen by group of rats on test protein diet.

F_n= Fecal nitrogen of group of rats on test protein diet.

F_k= Fecal nitrogen of group of rats on protein free diet.

4. Biological Value (BV) = Net Protein Utilization / True Di gestibility x 100.

Results and Discussion

The nutrient composition of skim milk, wheat cerelac, raw soybean, whole soymilk and dehulled soymilk is shown in Table 2.

Mean values for the biological evaluation of the protein quality of skim milk, cerelac, whole soymilk and dehulled soymilk are given in Table 3 and Fig 1.

The data when subjected to Analysis of Variance (ANOVA) showed highly significant (P<0.01) difference among weight gain of the rats fed on different experimental diets. For further comparison Duncan's Multiple Range Test (DMR Test) was applied and highly significant (P<0.01) better gain in weight was noticed in rats fed on cerelac as compared to those fed on whole soy milk and dehulled soymilk, while a non significant

Table 1
Composition of experimental diets (1000g)

Ingredients	Diets (g)				
	A	B	C	D	E
Skim milk	260	-	-	-	-
Cerelac (wheat)	-	666.60	-	-	-
Whole soymilk	-	-	212.77	-	-
Dehulled soymilk	-	-	-	204.08	-
Corn starch	340	333.40	387.23	395.92	600
Corn oil	100	-	100	100	100
Glucose	150	-	150	150	150
Potato starch	100	-	100	100	100
Mineral mixture	40	-	40	40	40
Vitamin mixture	10	-	10	10	10
Total	1000	1000	1000	1000	1000

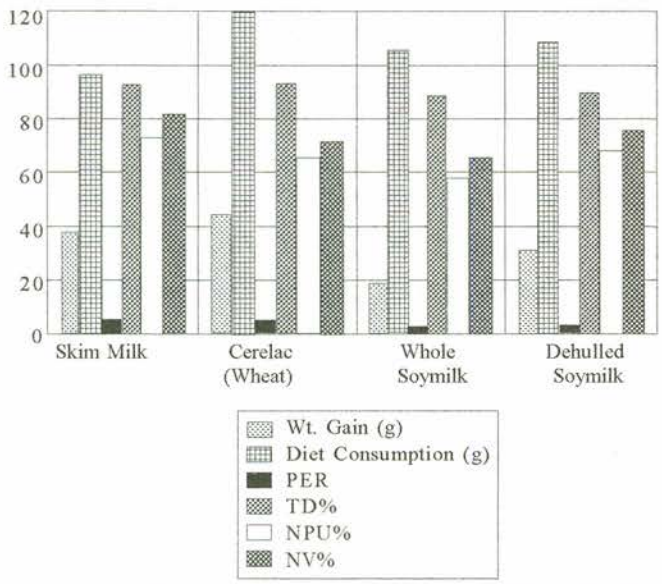

Fig 1. Graphical representation of biological evaluation of diets.

difference with skim milk. Skim milk had non- significant difference with cerelac and dehulled soymilk, while highly significant difference with whole soymilk.

Weight gain of rats fed on dehulled soymilk was less than skim milk but comparable, may indicating deficiency of certain essential amino- acids. Similar results were found in rats on shoyu (a fermented soybean sauce), (Khader 1983). Weight gain in dehulled soymilk diet was less than cerelac (wheat based). It may be due to that cerelac is a commercial baby food and it may contain wheat, skim milk and flavors. In case of whole soymilk the weight gain was lower than other three diets, indicating that it may contain more trypsin inhibitors due to hulls. It was reported that higher trypsin inhibitors impairs the quality of soybean (Ali 1995).

Non-significant difference was observed among the consumption of four diets. It means that acceptability of four diets were the same. Present study showed that acceptability of soymilk was somewhat higher than skim milk. Similar results were found that kittens ate 3 different levels of soy protein and avoided casein diet (Cook *et al* 1985). It was also found that yogurt containing 50% soymilk along with 50% cow's milk were highly acceptable (Kinik and Akbulut 1996). Diet consumption of cerelac was slightly higher than soymilk. It was probably due to the reason that cerelac is a commercial baby food and its organoleptic characteristics have been improved by addition of some flavors and skim milk. It is, therefore, suggested that if soymilk would be prepared commercially, addition of some flavors, is a must to increase the organoleptic characteristics. It was concluded that acceptability of soymilk was increased by addition of 0.1% salt, 0.5% sugar and 0.25-ml l^{-1} vanilla essence (Singh *et al* 1996).

Table 2
Proximate composition (%age) of skim milk, cerelac (wheat), raw soybean, whole soymilk and dehulled soymilk

% Composition	Skim milk	Cerelac (wheat)	Raw soybean	Whole soymilk	Dehulled soymilk
Moisture	4.00	2.50	7.00	5.35	5.40
Crude protein	37.00	15.50	34.00	47.00	49.00
Ether extract	1.00	9.00	22.50	24.00	27.75
Crude fiber	0	1.40	4.25	0	0
Ash	9.00	2.70	5.50	7.25	6.50
Nitrogen free extract	49.00	68.90	26.75	16.40	11.35

N- Factor for Soybean Products, 5.71; For Skim Milk, 6.38; For Cerelac (wheat), 6.25.

Table 3
Biological evaluation of diets (containing 10% protein)

Analysis	A	B	C	D
	Skim milk	Cerelac (wheat)	Whole soymilk	Dehulled soymilk
Weight gain (grams)	37.17	44.75	18.92	31.17
Diet consumption (grams)	95.73	118.68	105.20	109.01
PER	2.8	3.1	1.7	2.6
TD%	92	92	88	90
NPU%	74	66	58	69
BV%	81	71	66	77

Analysis of data and Duncan's Multiple Range Test revealed significant ($p<0.01$) difference in PER values for experimental diets. The difference among skim milk, cerelac and dehulled soymilk was non-significant and whole soymilk was significantly different from skim milk, cerelac and dehulled soymilk. The differences between the PER values of two soymilks were due to the presence of hull and trypsin inhibitor in whole soymilk. The time for blending was also different. PER for dehulled soybean was 2.30 as compared to whole raw soybean for which PER was 1.34, (Arshad *et al* 1985). The PER value of dehulled soymilk was 2.6 which was comparable with PER of skim milk (2.8). Heat treatment may have improved the protein quality of dehulled soymilk because it was given 10 min blending at 80°C. Same results were found by Liener (1976), Lowgren and Hambraeus (1988) and Byongki and Pask (1995). PER of soymilk diets were lower than cerelac. It may be due to the reason that cerelac is a commercial baby food and its organoleptic characteristics have been increased by the addition of minerals, vitamins, flavors and skim milk.

The digestibility of all the diets showed non-significant difference among four diets indicating that all four diets are highly digestible because of low fiber contents. The proximate analysis (Table 2) of both soymilks showed no fiber in it. This suggested that soymilk prepared from two different methods did not affect the digestibility of protein in soymilk. Protein digestibility was not affected by heating (Liener 1976) same results were shown by Lowgren and Hambraeus (1988).

For Net Protein Utilization (NPU), the ANOVA and DMR Test showed significantly higher difference between cerelac and whole soymilk, dehulled soymilk and whole soymilk, dehulled soymilk and cerelac. It was resulted that protein utilization of four diets was different. The utilization of dehulled soymilk was significantly lower than skim milk indicating that it may be deficient in sulphur- containing amino acids as compared to skim milk. Sarwar (1991) and Young (1991) also studied this deficiency. But NPU value of dehulled soymilk was significantly higher than cerelac (wheat). It may be due to that legumes have better essential amino acids profile than cereal (Pyler 1988). It was found that legumes were higher in thiamin, nicotinic acid, tryptophan and minerals along with lysine as compared to cereal (Doughty and Walker 1982). The difference of dehulled soymilk from whole soymilk was also significant, this was due to that whole soymilk was given only 5 min blending (room temperature) as compared to 10 min blending on constant temperature (80°C) to dehulled soymilk. It may be due to some toxins like trypsin inhibitor along with Kunitz and Bowman Birk inhibitors were left in whole soymilk. The two inhibitors Kunitz and Bowman Birk were found to be inactivated at 137°C (Rouhana *et al* 1996). Additional heat treatment was suggested to destroy trypsin inhibitors of soybean (Byongki and Park 1995). The loses of vitamins and minerals, due to over night soaking of whole soymilk, were high as compared to dehulled soymilk (soaked for only 6 h), also noted in the study of Albrecht *et al* (1966) and Borhan and Snyder (1979).

The ANOVA and DMR Test, for biological values, showed highly significant difference among four diets, indicating retained protein out of absorbed protein was almost different from each other. The reason, for BV of dehulled soymilk was less than skim milk, is that it may be deficient in methionine and cystein as compared to skim milk, and thus retained nitrogen out of absorbed was less in case of dehulled soymilk but it was higher from cerelac, indicating that retention of nitrogen in cereal based foods was less as compared to legumes, although methionine and cystein were present in good amount, but it was deficient in lysine which is also an essential amino acid and it is higher in legumes as compared to cereals (Doughty and Walker 1982).

BV of whole soymilk was significantly less than all diets. It may be due to over night soaking that reduced the solid materials, oligo-saccharides and B complex vitamins. 3-4% losses of these materials were noted at room temperature in soybean (Albrecht *et al* 1966), while 10.4% loss at 45°C, due to over night soaking (Borhan and Snyder 1979).

The difference in BV of two soymilks may be due to the amount of inhibitors especially of trypsin inhibitor present in whole soymilk, which impairs its quality as compared to dehulled soymilk. It was reported that higher trypsin inhibitor impairs the quality of protein (Ali 1995). The quality of dehulled soymilk was improved due to blending at maintained temperature of 80°C, which reduced the inhibitors present in soy protein (Lowgren and Hambraeus 1988; Byongki and Park 1995).

Acknowledgement

The author has deep sense of obligation to Dr. Kausar Almas, Dr. Nighat Bhatty, Dr. Abu Saeed Hashmi and Dr. Abrar Hussain Gillani for their keen interest, skillful guidance and encouragement for the completion of this degree program.

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