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Effect of dehulling, cooking and roasting on the protein quality of *Perilla frutescens* seed

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The effect of processing on the protein quality of *Perilla frutescens* was investigated. The amino acid scores for *Perilla* wholeseed and dehulled seed (kernel), as compared to the FAO/WHO/UNU (1985) amino acid requirement for pre-schoolers were 63 and 66, respectively. Net protein ratio (NPR), net protein utilization (NPU) and true digestibility of protein (TDP) of *Perilla* wholeseed as well as kernel in the raw, roasted or cooked forms were evaluated using rat balance assay. Compared to the casein based control diet (NPR 4.8, NPU 79.8, TDP 94.2), all the parameters examined were significantly ($p < 0.05$), lower in the *Perilla* diets (NPR 1.7–3.9, NPU 36.2–65.9, TDP 72.8–87.9). Cooking increased the NPR, NPU and TDP significantly ($p < 0.05$), whereas roasting exerted a negative effect. Dehulling significantly ($p < 0.05$) increased the NPR, NPU and TDP. This study demonstrates that *Perilla* seed is a source of good quality protein that could be used in both human and animal nutrition. © 1998 Elsevier Science Ltd. All rights reserved.

INTRODUCTION

Perilla (*Perilla frutescens* Linn, Britton) belonging to the family Labiatae (Mukherjee, 1940) is a coarse aromatic herb growing 0.5 to 1.5 m tall, occurring in tropical and temperate climates (CSIR, 1966). In India, *Perilla frutescens* is cultivated in the hills of Northeast India, Uttar Pradesh, Himachal Pradesh and Kashmir (Sharma *et al*., 1989). In Northeast India, *Perilla frutescens* is a traditional oilseed grown primarily for its food uses at the household level. *Perilla* seeds are consumed after cooking or roasting along with other foods such as cereals, vegetables, roots or tubers. The main drawback of *Perilla* seed is its thin dark grey brittle hull which imparts a dark colour to the food preparation. Partial dehulling can be achieved by the traditional method which is laborious and often ignored. However, dehulling results in a more appealing light yellow seed with soft texture.

Earlier studies have shown that *Perilla* seed is a potential source of food, rich in fat (51%) and protein (17%) (Longvah and Deosthale, 1991). The use of *Perilla* seeds as food source cuts across age and economic status among the tribal communities in Northeast India. Although *Perilla* seeds represent a less expensive source of dietary protein and fat among the tribals in Northeast India, very little is known about its nutritional potential or the effects of processing on its protein quality. Hence this study was undertaken to evaluate the effects of dehulling, cooking and roasting on the protein quality of *Perilla frutescens* seeds.

MATERIALS AND METHODS

Sample collection, transportation and processing

Perilla seeds were obtained from local cultivators around Kohima town in Nagaland and transported by air to Hyderabad. Upon arrival at the laboratory the samples were winnowed, cleaned of all foreign particles and stored in the cold room at 4°C until further analysis. *Perilla* kernels were obtained by passing the seeds through a Satake rice mill where 97–99% dehulling was achieved. Roasted seeds were prepared by subjecting the whole seed, as well as kernels to dry-roasting in a hot-air oven at 135°C for 30 min. Samples of raw and roasted (whole/dehulled) *Perilla* seeds were defatted with n-hexane for 16–18 h and the subsequent seed meals were used in the diet preparations for protein quality evaluation.

Chemical analysis

Nitrogen contents of the samples were estimated by the Kjeldahl method (AOAC, 1990) and expressed as crude

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protein (N×6.25). For amino acid analysis, defatted samples in triplicates were hydrolysed at 110°C for 22 h with 6 N hydrochloric acid in evacuated sealed ampoules. Excess acid from the hydrolysates was removed by repeated flash evaporation under reduced pressure. To convert methionine and cyst(e)ine to the stable forms, methionine acid and cysteic acid, the samples were treated with performic acid reagent for 16 h and hydrolyzed with 6 N hydrochloric acid for 24 h (Moore, 1963). The analysis was carried out by ion-exchange chromatography in an automatic amino acid analyzer (Beckman 119-C, Beckman Instruments, Fullerton, CA) (Moore *et al*., 1958), Tryptophan was determined chemically by the method of Miller (1967). From the amino acid composition data, amino acid scores (FAO/WHO/UNU, 1985) were calculated.

Diets

Diets were formulated according to the procedure of Campbell (1963), consisting of 83.3% corn starch, 10% groundnut oil, 4% mineral mixture, 1% vitamin mixture, 0.2% choline chloride, 1.5% cellulose. The protein (casein or *Perilla* seed meal) replaced cornstarch at 10% protein level in the diets (Table 1).

Animals

Male weanling NIN Wistar rats (21–23 days) weighing 44.5 ± 6.9 g were housed in individual bottom-raised steel cages with filter paper placed below the cages to collect spilled food and faeces. Room temperature was maintained at 22 ± 1°C, relative humidity 65 ± 5% and a 12 h light and 12 h dark cycle. The rats were fed the casein-based control diet for an acclimatization period of 2 days and then randomly distributed into 8 groups of 6 animals each on the basis of equal mean body weights (51.9 ± 6.9 g). Each group of animals was randomly allocated to the different diets as shown in Table 1. Animals were offered water and foods *ad libitum* for 14 days. Records of individual animal body weight changes and daily food intake were maintained. Faeces of individual animals were collected during the last four days (days 11–14) of the study period. Samples of faeces and diets were taken for nitrogen determination (AOAC, 1990). At the end of the 14 days feeding period, animals were sacrificed under ether atmosphere. The whole carcass of each animal was placed individually in conical flasks containing 300 ml of 6 N hydrochloric acid and autoclaved for 3 h at 120°C. The solution was extracted once with ether to remove fat, and diluted to 1 L. Nitrogen contents of the carcass hydrolysates were determined by the macro Kjeldahl method (AOAC, 1990).

From the above data, net protein utilization of carcass (NPU) (Henry, 1965) net protein ratio (NPR), relative net protein ratio (RNPR) (Sarwar *et al*., 1984), apparent digestibility of protein (ADP) and true digestibility of protein (Sarwar and Peace, 1986), were calculated.

Data were analysed using analysis of variance and means were separated using the least significant difference (lsd) test procedures. Pearson correlation coefficients (Ott, 1988) were used to evaluate the relationship between in-vivo biological values (TDR, NPR, NPU).

RESULTS AND DISCUSSION

Protein content and amino acid composition

Protein content of *Perilla* whole seed has been reported to be in the range of 15.7–23.7% (Sharma *et al*., 1989). Our result (Table 2) for *Perilla* whole seed (17.2%) is within the reported range. Dehulling increased the protein content of *Perilla* seed from 17% to 20%. The hull which makes up 18% of the wholeseed had 5% protein. The protein content of *Perilla* seed is comparable to other oilseeds such as sunflower 15.8–23.8% (Earle *et al*., 1968). Defatted *Perilla* wholeseed protein content was 36% and that of *Perilla* kernel meal was 46%. The amino acid compositions of *Perilla* whole seed and kernel proteins are given in Table 3. The essential amino acids of *Perilla* whole seed meal and kernel meal

Table 1. Composition of basal protein-free diet, casein diet and *Perilla* diets fed to rats (g kg^{-1} diet)

Ingredients	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
	Protein-free[a]	Casein	Raw		Cooked		Roasted	
			P. Kernel	P.wholeseed	P.Kernel	P.wholeseed	P.Kernel	P.wholeseed
Groundnut oil	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Salt mixture	40.0	40.0	40.0	40.0	40.0	40.0	40.0	40.0
Vitamin mixture	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Choline chloride	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Cellulose	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Casein	—	123.1	—	—	—	—	—	—
Perilla kernel	—	—	217.4	—	217.4	—	206.0	—
Perilla wholeseed	—	—	—	277.8	—	277.8	—	268.9
Starch	833.0	709.9	615.6	555.2	615.6	555.2	627.0	164.1

[a]Campbell, 1963.

Table 2. *Perilla frutescens* seed protein content ($g\,100\,g^{-1}$)

Sample	Protein Content	
	Before defatting	After defatting
Perilla whole seed	17.2	36.3
Perilla kernel	20.1	46.2
Perilla Hull	5.0	5.2

Values are means of triplicate analyses.

represent 39% and 42% of their total amino acids. These levels are somewhat lower than most animal proteins (Beef 52, egg 55 and cows milk 54). However, they are comparable with other oilseed meals such as cottonseed 39, soybean 42 and sunflower 39 (Bodwell and Hopkins, 1985). Using the FAO/WHO/UNU (1985) pattern of essential amino acid requirement for infants as the reference, the chemical scores for *Perilla* whole seed and kernel were 63 and 66, respectively (Table 4). Lysine was the limiting amino acid in both cases. The amino acid score of *Perilla* whole seeds and kernel were comparable to sunflower (61) and peanut (69), both of which also had lysine as the limiting amino acid (Bodwell and Hopkins, 1985).

Effects of dehulling, cooking and roasting (Table 5)

The final body weights of animals on the casein control diet (112.5 ± 13.5) and the different *Perilla* wholeseed or kernel diets in the cooked, raw or roasted forms varied between 59.3 ± 9.2 g in roasted *Perilla* wholeseed group to 107.9 ± 9.2 g in the cooked kernel group. Body weight gain at the end of the 14 days study period was highest in the casein group (115%), followed by cooked *Perilla* kernel (108%), cooked *Perilla* wholeseed (94%), raw *Perilla* kernel (69%), raw *Perilla* wholeseed (54%), roasted *Perilla* kernel (29%) and roasted *Perilla* wholeseed (14%). Protein intake of animals on the casein diet and raw *Perilla* diets were comparable but lower than cooked *Perilla* diets. However, body weight gain and NPR of animals on the cooked or raw *Perilla* diets were significantly ($p < 0.05$) lower than that of casein. This reflects the inferior protein quality of *Perilla* seeds compared to casein protein. Roasting of *Perilla* seed led to a decreased food intake with a concomitant decreased gain in body weight and NPR. However, cooking of *Perilla* seeds resulted in a significant increase in NPR as compared to the raw *Perilla* seeds. Dehulling resulted in a significant improvement in NPR of animals on the raw, cooked or roasted *Perilla* seed diets, though the essential amino acid contents of *Perilla* wholeseed and kernel were not vastly different. The hull which is mostly composed of cellulose and hemicellulose may have led to the decreased availability and absorption of nutrients in the animals on *Perilla* wholeseed diets (Bressani, 1982). RNPR of animals on the different *Perilla* diets ranged between 35.8 ± 4.9 in the roasted *Perilla* wholeseed to 81.3 ± 3.7 in the cooked *Perilla* kernels. The RNPR values of roasted *Perilla* wholeseed were comparable to lentils (34 ± 2) (Sarwar *et al.*, 1989) whereas roasted kernel and raw *Perilla* wholeseed were comparable to wheat cereal (55 ± 1) (Sarwar *et al.*, 1989). RNPR of raw *Perilla* kernel and cooked *Perilla* wholeseed was comparable to heated skim milk (67 ± 2) (Sarwar *et al.*, 1989). Cooked *Perilla* kernel which had a significantly ($p < 0.05$) higher RNPR than the other *Perilla* diets was comparable to chick peas (80 ± 2) (Sarwar *et al.*, 1989).

Table 3. Amino acid composition of *Perilla* seed (mg g^{-1} protein)

Amino acid	*Perilla* whole seed	*Perilla* Kernel
Valine	33 ± 0.4	39 ± 0.6
Isoleucine	41 ± 1.6	34 ± 0.7
Leucine	62 ± 1.2	74 ± 0.3
Lysine	37 ± 1.3	38 ± 1.0
Tryptophan	13 ± 0.3	15 ± 0.5
Threonine	41 ± 0.7	38 ± 0.3
Tyrosine	35 ± 1.3	39 ± 0.7
Phenylalanine	55 ± 0.3	67 ± 0.6
Cysteine	16 ± 0.9	20 ± 0.5
Methionine	26 ± 0.2	23 ± 0.6
Histidine	31 ± 0.7	27 ± 1.3
Aspartate	97 ± 0.7	84 ± 0.5
Serine	51 ± 1.3	55 ± 0.6
Glutamate	206 ± 1.3	199 ± 1.3
Proline	41 ± 1.5	38 ± 1.1
Glycine	53 ± 1.4	50 ± 1.2
Alanine	45 ± 1.3	53 ± 1.5
Arginine	109 ± 0.3	101 ± 0.4
Total essential amino acids	390	413.2
Total amino acids	992	991.6

Values are mean ± SD of triplicate analyses.

The NPU of animals on roasted *Perilla* diets was significantly ($p < 0.05$) lower than all the other *Perilla* groups and also very much lower than the casein diet. Within the groups of animals on the *Perilla* diets, the cooked *Perilla* kernel group exhibited the highest NPU (65.9 ± 5.7). Like NPR, dehulling of *Perilla* seed resulted in increased NPU ($p < 0.05$); cooking also raised the NPU ($p < 0.05$) while roasting decreased the NPU ($p < 0.05$) of *Perilla* seed protein. NPU of *Perilla* protein varied between 36.2 ± 3.2 in roasted *Perilla* wholeseed to 65.9 ± 5.7 in cooked *Perilla* kernel. The protein quality of *Perilla* seeds in the roasted, raw or cooked forms, as determined by NPU, was comparable to other vegetable proteins such as lentils (38), peanuts (47), lima bean (51) and soybean (66) (Friedman, 1996).

Protein digestibility

In the last 4 days balance period, protein intake, protein output and dietary protein retained were computed and the ratio of protein output/protein intake, ADP and TDP were calculated in rats fed *Perilla* diets and casein control diet (Table 5). Protein intake of animals on the cooked *Perilla* diet was higher than that of animals on

Table 4. Comparison of the essential amino acid (EAA) composition of *Perilla* seed protein and high quality animal proteins with the suggested FAO/WHO/UNU (1985) EAA pattern of requirements for a 2–5 year old child (mg g^{-1} protein)

Essential Amino Acid	EAA[a] FAO/WHO/UNU (1985)	*Perilla frutescens*		Animal products[b]	
		Wholeseed	Kernel	Egg	Cows milk
Histidine	19	31	27	22	27
Isoleucine	28	41	34	54	47
Leucine	66	62	74	86	95
Lysine	58	37	38	70	78
Methionine + Cyst(e)ine	25	42	43	57	33
Phenylalanine + Tyrosine	63	90	106	93	102
Threonine	34	41	38	47	44
Tryptophan	11	13	15	17	14
Valine	35	33	39	66	64
% EAA	—	39	41	51.2	50.4
Amino acid Score	—	63	66	100.0	100.0
Limiting Amino acid	—	Lys	Lys	—	—

[a]Data from FAO/WHO/UNU (1985).
[b]Data from Bodwell (1989).

Table 5. Net protein ratio (NPR), relative net protein ratio (RNPR), net protein utilization (NPU), apparent protein digestibility (ADP) and true protein digestibility (TDP) of casein and *Perilla* seed protein

Group	Gain in body weight (g/2 weeks)	Protein intake (g/2 weeks)	NPR	RNPR	NPU	Ratio protein output/protein intake%	ADP	TDP
Casein	60.2 ± 6.2^a	14.6 ± 1.6^a	4.8 ± 1.6^a	100.0 ± 3.4^a	79.5 ± 7.0^a	7.8 ± 1.8^a	92.2 ± 1.8^a	94.2 ± 2.0^a
Perilla kernel (raw)	35.8 ± 7.0^c	13.8 ± 1.9^b	3.3 ± 0.2^c	68.5 ± 3.0^c	52.0 ± 3.8^c	18.7 ± 1.8^c	81.4 ± 1.8^c	83.6 ± 1.6^c
Perilla wholeseed (raw)	28.1 ± 8.4^d	14.6 ± 2.0^b	2.6 ± 0.3^d	53.3 ± 5.8^d	42.9 ± 5.0^d	23.3 ± 1.8^d	76.7 ± 1.8^b	79.7 ± 1.3^d
Perilla kernel (cooked)	56.0 ± 3.6^a	16.8 ± 0.6^b	3.9 ± 0.2^b	81.3 ± 3.7^b	65.9 ± 5.7^b	14.3 ± 1.9^b	85.7 ± 1.9^b	87.4 ± 1.7^b
Perilla wholeseed (cooked)	48.6 ± 3.3^b	17.2 ± 0.7^b	3.4 ± 0.2^c	70.6 ± 4.3^c	52.5 ± 5.0^c	18.7 ± 1.6^c	81.3 ± 1.6^c	81.0 ± 1.6^c
Perilla kernel (roasted)	17.6 ± 8.9^e	10.1 ± 1.4^c	2.7 ± 0.6^d	55.3 ± 12.8^d	45.3 ± 4.6^d	25.2 ± 2.2^d	74.8 ± 2.2^d	78.3 ± 2.5^d
Perilla wholeseed (roasted)	7.2 ± 8.2^f	8.9 ± 2.2^c	1.7 ± 0.3^e	35.8 ± 4.9^e	36.2 ± 3.2^e	30.9 ± 1.1^e	69.1 ± 1.1^e	72.8 ± 1.3^e

Values are mean ± SD of 6 animals in each group.
Values with different superscripts are significantly ($p < 0.05$) different.

the casein or raw *Perilla* diets but not significantly different. However, protein intake of animals on the roasted *Perilla* diets was significantly lower than the other groups. The average values of protein retained were 4.8 ± 0.7 g for casein diet, 5.0 ± 0.3–5.1 ± 0.6 g for cooked *Perilla* diets, 3.6 ± 0.7–3.9 ± 0.6 g for raw *Perilla* diets and 2.0 ± 0.2–2.3 ± 0.4 g for roasted *Perilla* diets. Compared to the casein group, high protein output/protein intake ratios were observed in rats fed the *Perilla* diets. Rats fed dehulled seed diets had lower protein output/protein intake ratios than rats fed wholeseed diets. The roasted *Perilla* wholeseed had the lowest digestibility (72.8 ± 1.3%) and the highest faecal protein output/protein intake ratio (30.9 ± 1.1). A negative correlation coefficient ($r = -0.996$, $p < 0.001$) was observed between the ratio of faecal protein output/protein intake and TDP. Similar observations were made in rats fed red kidney bean diets (Wu Wu *et al.*, 1995). Rats consuming wholeseed diets had higher ratios of faecal protein output/protein intake than their kernel counterparts. TDP values of casein were significantly higher than *Perilla* diets. Dehulling resulted in increased TDP in the cooked (5%), raw (6%), or roasted (7%) *Perilla* diets. Among the different *Perilla* diets, the cooked kernel diet exhibited the highest TDP (87.4 ± 1.7), significantly higher than the other groups. Roasting of *Perilla* seeds produces a distinct flavour which may be the reason for the reduced food intake and hence lowered performance of animals on roasted *Perilla* diets. In contrast, cooking increased the NPU/NPR of *Perilla* protein by inactivation of certain residual antinutritional factors. This is consistent with the suggestion that proteolytic enzyme inhibitors and toxic factors are inactivated by heat-processing (Uerbersax *et al.*, 1991). ADP was 4.7–5.4% lower than TDP in the roasted *Perilla* diets, 2.7–3.1% lower in raw *Perilla* diets and 2.0–2.1% lower in cooked *Perilla* diets. TDP of *Perilla* protein was comparable to other vegetable proteins such as Pinto beans (79 ± 1.5), kidney beans (81 ± 1.2), lentils (84 ± 1.4) and chick pea (89 ± 0.4) (Sarwar, 1987).

Protein quality estimation by TDP was compared with NPR and NPU. There was a positive correlation between TDP and NPR/RNPR ($r = +0.935$, $p < 0.001$), between TDP and NPU ($r = +0.912$, $p < 0.001$), and between NPR and NPU ($r = +0.889$, $p < 0.001$). The excellent amino acid profile of *Perilla* seed protein is confirmed by NPR, RNPR, NPU and TDP. The data furnished by this study demonstrate that *Perilla* seed is an untapped high protein source of reasonably good quality protein as compared to other oil-seed protein that could be used in both human and animal nutrition.

CONCLUSIONS

The potential of *Perilla* seed protein can be increased by dehulling the seed, followed by cooking. Thus, consumption of *Perilla* seed, in combination with cereal-based diets, as is normally done by the tribal communities in north east India, could improve the dietary well-being of these people. It is therefore necessary to preserve and promote a wider cultivation of such under-exploited food plants.

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