Chemical and Nutritional Studies on Hanshi (*Perilla frutescens*), a Traditional Oilseed from Northeast India

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***Perilla frutescens*, an edible oilseed of Northeast India, was evaluated for its nutrient composition and protein quality. It was found to be a rich source of protein (17.0%) and fat (51.7%). The fatty acid profile indicated that perilla oil is rich in polyunsaturated fatty acids, such as linolenic (56.8%) and linoleic (17.6%). The amino acid composition showed that valine was the limiting amino acid of perilla protein. The protein efficiency ratio of the seed protein (2.07) was lower than that of casein (2.99), but comparable to common oilseeds. True digestibility of the seed protein (82.6%) was also lower than that of casein (89.3%).**

KEY WORDS: Fatty acids, oilseed, *Perilla frutescens*, protein quality.

Perilla frutescens, locally called Hanshi, is a member of the Lamiaceae family. It is an annual, aromatic, bushy herb cultivated for its edible seeds by various tribal groups in Northeast India. The plant grows to 150 cm in height and is found in the hills up to an altitude of 3,500 m (1). The seeds are consumed as a delicacy after frying or in combination with vegetables or cereals after cooking. Consumption of this oilseed cuts across age and socioeconomic groups among the tribal populations of Northeast India. To some extent the expelled oil is also used as a cooking medium. Thus, the oilseed contributes significantly towards the nutrient requirements of a cross-section of the region's population.

Literature is scarce and, where available, scanty on the nutrient composition and nutritive value of *P. frutescens*, hence the examination of the chemical and nutritional aspects was of interest.

EXPERIMENTAL PROCEDURES

Mature *P. frutescens* seeds were purchased from the local market of Ukhrul, Manipur, and transported by air to the laboratory for analysis. The seed are greyish in color and resemble mustard seed.

Chemical analysis of the seeds. The raw seeds were pulverized with a mortar and pestle. Aliquots of the pulverized sample of *P. frutescens* seeds were taken for proximate compositional analysis. Protein content (N × 6.25) was estimated by the Kjeldahl method; fat and ash were determined by AOAC methods (2). Total carbohydrates, including fiber, were calculated by difference. Minerals were determined in an atomic absorption spectrophotometer (Varian Techtron model AAS 1000, Varian Associates, Palo Alto, CA) after dry-ashing the sample (3). Phosphorus was estimated by AOAC method (4). The methyl esters of the fat were prepared according to Lowenstein *et al.* (5). The fatty acid composition of the methyl esters was determined by gas-liquid chromatography (GLC) on a Varian 3700 gas chromatograph equipped with flame ionization detector and a 12 ft. × 1/2 in. stainless steel column packed with 10% Silar 10C on Chromosorb W-AW 80/100 mesh. The initial column temperature of 160°C was increased to 226°C at the rate of 3°C/min and maintained at that level for 20 min. The temperature of the injection and detector ports were kept at 230°C. The carrier gas was N_2 at a flow rate of 20 mL/min. Methyl esters were identified and quantitated by comparing the retention times and peak areas of the unknowns with those of the fatty acid methyl esters (FAME) standards. The fatty acid composition was expressed as percent of the total oil.

For amino acid analysis, a defatted perilla sample was hydrolyzed at 110°C for 24 hr with 6N constant-boiling hydrochloric acid in an evacuated sealed ampoule. Excess acid from the hydrolyzate was removed by repeated flash evaporation under reduced pressure. The analysis was carried out by ion exchange chromatography in an automatic amino acid analyzer (Beckman 119-Cs, Beckman Instruments, Fullerton, CA) (6).

Biological evaluation of perilla protein. The pulverized seeds were defatted with n-hexane, and the cake containing 34% protein was used for protein quality evaluation in male Wistar rats. Animals were weaned at 21–23 days and fed a 10% casein diet for 2 days for acclimatization. Weights of animals at the start of the experiment ranged from 45–55 gms. Animals were allocated randomly to three groups of six rats each and housed individually in a battery of cages with facilities for fecal collection. The basal diet contained 83.3% corn starch, 10% groundnut oil, 4% mineral mixture, 1% vitamin mixture, 0.2% choline chloride and 1.5% cellulose. Casein or perilla seed cake was added to the basal diet at the expense of starch to give 10% protein diets (7).

Animals in group one were fed the casein diet, in group two the perilla cake diet, and in group three the N-free basal diet. Food and water were offered *ad libitum*. A record of daily food intake and weekly body weight of each animal was maintained throughout the experimental period of 28 days. During the last four days fecal matter was collected from individual rats. Diet and fecal matter were analyzed for nitrogen by the Kjeldahl method. Protein efficiency ratio (PER), net protein ratio (NPR) and true digestibility (TD) were calculated (7).

RESULTS AND DISCUSSION

The proximate and mineral composition of *P. frutescens* is presented in Table 1. The protein and total ash content of perilla seed was comparable to the reported values (8,9). The fat content was found to be more than twice the value reported by Standall *et al.* (8). This difference in fat content may be due to environmental factors. Compared to common oilseeds, the proximate composition of perilla was similar to that of sunflower seed (10). However, total iron in the perilla seed was higher than that of gingelly,

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TABLE 1

Proximate Composition and Inorganic Nutrient Content of *Perilla frutescens* Seeds Compared to Those of Other Oilseeds (all Values are per 100 g Seed)

	Perilla	Groundnut[a]	Linseed[a]	Mustard seed[a]	Niger seed[a]	Safflower[a]	Sunflower[a]
Moisture (g)	7.4	5.5	6.5	8.5	4.2	5.5	5.5
Protein (g)	17.4	18.3	20.3	20.0	23.9	13.5	19.8
Fat (g)	51.7	43.3	37.1	39.7	39.0	25.6	52.1
Ash (g)	3.6	5.2	2.4	4.2	4.9	2.6	3.7
Carbohydrate (g)	20.3	25	28.9	23.8	17.1	17.9	17.9
Energy (Kcal)	615.0	563	530	541	515	346	620
Phosphorus (mg)	710	570	370	700	224	823	670
Magnesium (mg)	275	—	—	—	—	—	—
Calcium (mg)	269	1450	170	490	300	236	280
Iron (mg)	9.0	9.3	2.7	7.9	56.7	4.6	5.6
Manganese (mg)	4.8	1.32	—	2.56	—	1.1	—
Zinc (mg)	4.7	12.2	—	4.8	—	5.2	—
Copper (mg)	.18	2.29	—	0.83	—	1.58	—
Chromium (μg)	20	87	—	63	—	45	—

[a]Values from nutrient composition of Indian foods (10).

TABLE 2

Fatty Acid Composition of *P. frutescens* Seed Oil Compared to that of Other Seed Oils (Values are Percent of Total Oil)

	Perilla	Linseed[a]	Sesame[b]	Mustard[b]	Soya[b]	Safflower[b]
$C_{16:0}$ Palmitic	8.92	7.0	9.7	2.9	9.8	7.8
$C_{18:0}$ Stearic	3.77	4.0	2.4	0.9	2.4	2.1
$C_{10:0}$ Arachidic	—	—	0.9	6.9	0.9	0.8
$C_{16:1}$ Palmitoleic	—	—	—	0.6	—	—
$C_{18:1}$ Oleic	12.92	15.0	28.9	8.9	28.9	17.7
$C_{18:2}$ Linoleic	17.61	18.0	50.7	18.1	50.7	78.5
$C_{18:3}$ Linolenic	56.76	56.0	6.5	14.5	6.5	—
Total saturates	12.69	11.0	13.1	10.7	13.1	10.7
Total unsaturates	87.29	89.0	86.1	88.6	86.1	92.2
Polyunsaturates	74.37	74.0	57.2	32.6	57.2	78.5

[a]Reference 11. [b]Nutrient composition of Indian foods (10).

TABLE 3

Amino Acid Composition (mg/g N) and Quality Score of Essential Amino Acid of *P. frutescens* Seed Protein Compared to That of FAO Whole Egg Protein

	P. frutescens protein				
	Present study	Reported values		FAO[b] whole egg protein	FAO amino acid score of perilla protein
Amino acid		Standall[a]	(FAO)[b]		
Threonine	181	182	225	320	86
Valine	174	113	294	428	62
Cystine	89	83	75	152	89
Methionine	174	86	138	210	126
Isoleucine	234	100	244	393	91
Leucine	374	303	363	551	109
Tyrosine	244	145	225	260	143
Phenylalanine	311	228	313	358	132
Lysine	240	221	238	436	84
Aspartate	556	513	569	601	
Serine	443	343	319	478	
Glutamate	1423	1358	1206	796	
Proline	308	376	206	260	
Glycine	340	238	319	207	
Alanine	296	297	288	370	
Histidine	203	180	175	152	
Arginine	807	143	706	381	
Tryptophan	—	78	75	93	
Essential amino acids	2021	1185	2190	3201	
Total amino acids	6397	5733	5978	6446	

[a]Reference 8. [b]Reference 13.

TABLE 4

Food Intake, Protein Efficiency Ratio (PER) and Net Protein Ratio (NPR) in Groups of Rats Fed Casein and Perilla Protein (Mean ± SE of Six Animals in Each Group)

Diet group	Protein (%)	Food intake (g/4 weeks)	Body weight gain (g/4 weeks)	PER	NPR
Casein	10	268 ± 8.8	77.7 ± 3.2[a]	2.99 ± 0.08	3.67 ± 0.04
P. frutescens	10	255 ± 11.9	49.0 ± 2.2[a]	2.07 ± 0.09[a]	2.87 ± 0.11[a]
Protein-free	—	103 ± 6.2	18.8 ± 3.1	—	—

[a]Significantly different at 0.1% level.

TABLE 5

Nitrogen Intake, Fecal Nitrogen, Nitrogen Absorbed and True Protein Digestibility in Groups of Rats Fed Diets Based on Casein and Perilla Protein (Mean ± SE of Six Animals in Each Group)

Diet group	Nitrogen intake (g/4 days)	Fecal nitrogen (g/4 days)	Nitrogen absorbed (g/4 days)	True nitrogen digestibility (%)
Casein	0.781 ± 0.36	0.098 ± 0.04	0.677 ± 0.17	89.3 ± 0.7
P. frutescens	0.690 ± 0.46[a]	0.134 ± 0.14[b]	0.555 ± 0.36[c]	82.6 ± 2.1[c]
Protein-free	0.030 ± 0.03	0.014 ± 0.02	0.014	—

[a]Significantly different at 5% level.
[b]Significantly different at 1% level.
[c]Significantly different at 0.1% level.

mustard, safflower and sunflower. Similarly, it was relatively rich in manganese content.

Perilla seed oil was found to be highly unsaturated, exceptionally rich in linoleic acid (18:2) and linolenic acid (18:3), similar to linseed oil (Table 2). Other vegetable oils known to have appreciable amounts of linolenic acid are linseed, mustard and soybean. The perilla oil, like linseed, was 6–8 times higher in its linolenic acid content compared to mustard and soybean. The linoleic acid contents of perilla, linseed and mustard were almost the same. The ratio of linolenic and linoleic was 3.0 in perilla and linseed oils and 0.8 in mustard seed oil. The significance of these observations in relation to the nutrition of tribal populations that consume perilla seed oil needs investigation.

The amino acid composition of perilla seed protein is given in Table 3. Based on essential amino acid score, valine was found to be the limiting amino acid. Standall *et al.* (8) have reported isoleucine as the limiting amino acid of perilla seed protein. Compared to whole egg as a reference protein, the total essential amino acid content of perilla protein was much lower.

Feeding trials in weanling rats have shown that the food intake of animals fed perilla protein diet was not significantly different from those fed casein protein (Table 4). However, the gain in body weight was much smaller, 49 g in the perilla-fed group against 78 g in the controls. The quality of perilla protein (2.07) in terms of PER value was significantly ($P<0.001$) lower than that of casein (2.99). However, perilla protein was comparable with another oilseed protein, *e.g.*, sesame, in its quality (12). The digestibility of perilla protein was also significantly ($P<0.001$) lower than that of casein (Table 5). Consequently, the protein available for absorption was much lower. Low total essential amino acid content of the protein undoubtedly contributes to the poor protein quality of perilla seed protein.

From the results of the present investigations it appears that *Perilla frutescens* seed is a good source of protein, comparable in quality to some other oilseed proteins. It was found to be a rich source of oil containing essential polyunsaturated fatty acids. This oil may be used to nutritional advantage by blending it with saturated oils and fats to provide essential fatty acids.

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