

## Nutrient Composition of 'Nikku muth', a Traditional Soybean Cultivar of Kashmir Valley, with Particular Reference to Content of Lipids and Proteins

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(Received 31 March 1988; revised version received and accepted 11 July 1988)

### ABSTRACT

'Nikku muth', a traditional soybean cultivar of Kashmir Valley, is a nutritive legume, containing 302 g protein, 185 g lipid and 355 g digestible carbohydrates per kilogram of seed. Among the simple proteins, glutelin is the major fraction (91 g/kg) while prolamin constitutes the least (3 g/kg). The oil contains linoleic acid (46.3%) and oleic acid (23.2%) as the major fatty acids whereas myristic (1.5%), palmitic (13.5%), stearic (6.1%) and linolenic (9.4%) acids are in lesser concentrations. The phospholipid fraction which constitutes 10.5% of the oil, also contains linoleic (51.4%) and oleic (21.2%) acids as the major fatty acids. Among the phosphatides, phosphatidyl choline and phosphatidyl ethanolamine constitute 0.83 g and 0.78 g per 100 g of the seed, respectively, while phosphatidyl serine and phosphatidyl inositol constitute 0.09 g and 0.24 g per 100 g of the seed, respectively.

### INTRODUCTION

During a nutrition survey of tribal/hill communities residing in Kashmir Valley, a traditional cultivar of soybean (*Glycine max* L.) locally known as 'Nikku muth', was found to be cultivated in fairly large areas at an altitude of about 1200–1600 m and consumed by local inhabitants after splitting as dal. It is a rich source of proteins and oil in their diet. It is also roasted and ground into a meal and used in food preparations and as cattle feed. In the

local Kashmiri language, 'Nikku' means 'small'. The seeds are comparatively smaller than other soybean varieties.

A literature survey reveals that no nutritional studies have been conducted or data reported earlier on this soybean cultivar. The present study was aimed to analyse, nutritionally, this indigenous cultivar of soybean, with particular emphasis on the content of lipids and proteins.

## MATERIALS AND METHODS

'Nikku muth' (*Glycine max* L.) is cultivated in a fairly large area of Kashmir Valley. The valley is situated at an altitude of about 1200–1800 m. The soil is mostly sandy loam or clayey. For crop cultivation land is thoroughly ploughed, cleaned and brought to a state of good tilth. The seeds are sown at the rate of 7–9 kg per acre in rows and 60–90 cm apart, the depth of sowing being 3–5 cm. Sowing period is from June to July and harvesting in October–November of the year. The climate of Kashmir Valley is temperate. Heavy rainfall occurs in January–March over the entire region. December–February is the coldest part of the year when temperature normally remains between 0 and 5°C. Mean maximum temperature in the warmest months of June–July touches 31°C. Frost is common during December–February. Humidity is high (70–80%) in the morning throughout the year and lowest (30–40%) in the afternoon.

Healthy and mature seeds of 'Nikku muth' were collected from the growing fields around Srinagar in Kashmir Valley. One kilogram of seed was cleaned, dried and mechanically ground to 100–120 mesh size for nutritional analyses.

### Analytical procedure

The procedures carried out for the determination of moisture, ash and protein were based on the standard techniques adopted by the Association of Official Analytical Chemists (AOAC, 1984). The protein value was derived from the nitrogen content, estimated by the Kjeltex system and multiplying by a factor of 6.25. The carbohydrate values were computed by subtracting from 100 the sum of the moisture, protein, fat, fibre and ash contents. Crude fibre was determined by the acid and alkaline digestion method described by AOAC (1984) through the Fibertex system. Phosphorus and iron were estimated by the Fiske & Subba Row (1925) and Andrews & Felt (1941) methods, respectively, while sodium, potassium and calcium were determined flame photometrically. Albumin, globulin, prolamin and glutelin fractions of protein were separated and estimated by solubility principles (Niranjan & Katiyar, 1979).

Lipid was extracted from the powdered seeds by employing petroleum spirit solvent (bp, 60–80°C) in a Soxtec system and purified by the method of Folch *et al.* (1957). For determining the fatty acid composition, methyl esters of fatty acids were obtained following the procedure of Kates (1964) and subjected to gas–liquid chromatographic separation and identification for individual components.

### Isolation and fractionation of phospholipid

Total lipid from the seed powder was extracted by using a chloroform and methanol mixture (2:1, v/v) through a Soxtec system and purified by the Folch *et al.* (1957) method. The crude phospholipid fraction of the lipid was precipitated by using chilled acetone and then separated by centrifugation. Phospholipid was resolved into its components as recommended by Skipski *et al.* (1964). Authentic phosphatides were co-chromatographed simultaneously in each run and located by exposure to iodine vapour (James & Morris, 1964). Different phospholipid constituents (phosphatides) were further identified and confirmed after using specific spray reagents, viz., Dragendroff reagent for choline-containing phospholipid as suggested by Wagner *et al.* (1961), ammoniacal silver nitrate for phosphatidyl inositol and ninhydrin (0.2% in butanol) for phospholipids containing amino groups. Individual spots on TLC plates were eluted in a chloroform–methanol–acetic acid–water (25:10:4:2, v/v) solvent system. Phosphorus in each eluent was estimated by Bartlett's method (1959). Fatty acid composition in the phospholipid was assayed by gas–liquid chromatography as methyl esters following the procedure of Kates (1964).

### Operating conditions of GLC

Analysis of methyl esters was done with a Pye Unicam GLC instrument (204 series) equipped with hydrogen flame ionization detector. A glass column, 200 cm × 0.6 cm, packed with 15% Reoplex on 80/100 mesh chromosorb W was used. The carrier gas was nitrogen with a flow rate of 30 ml/min. Column, injector and detector temperatures were maintained at 180°C, 270°C and 300°C, respectively. The results reported are the means of triplicate determinations.

## RESULTS AND DISCUSSION

The raw seeds of the soybean cultivar contain appreciable amounts of protein (302 g/kg) and lipid (185 g/kg). These values are somewhat lower in comparison to some newly developed soybean varieties which contain

400–420 g/kg proteins and 200–220 g/kg lipid. The 'Nikku muth' cultivar also contains fibre (65 g/kg), carbohydrates (355/kg) and minerals (38 g/kg) of which potassium, calcium, sodium, phosphorus and iron are 12.0, 2.0, 0.65, 3.83 and 0.88 g/kg, respectively (Table 1). Crude protein contains glutelin, 91 g/kg; globulin, 41 g/kg and prolamin, 3 g/kg. The residue fraction constitutes the major fraction of nitrogenous matter (Table 1).

Physical characteristics of lipid show an iodine value of 121.8, saponification value, 191.2, and unsaponifiable matter, 2.3% (Table 2) which are more or less similar to that of other cultivated varieties of soybeans (The Wealth of India, 1956). The composition of fatty acids in the lipid is: oleic ( $C_{18:1}$ ), 23.2%; linoleic ( $C_{18:2}$ ), 46.3%; myristic ( $C_{14:0}$ ), 1.5%; palmitic ( $C_{16:0}$ ), 13.5%; stearic ( $C_{18:0}$ ), 6.1% and linolenic ( $C_{18:3}$ ), 9.4%. Total unsaturated fatty acids were found to be 78.9% whereas saturated ones were 21.1% only. One of the main drawbacks of soybean lipid is its unpleasant

TABLE 1

Proximate Analysis and Distribution of Protein Fractions in 'Nikku Muth', a Traditional Soybean Cultivar

<i>Nutrients</i>	<i>'Nikku muth'</i>	<i>Mean representative value in other soybeans</i>
<i>(a) Proximate analysis (g/kg)</i>		
Moisture	55	65
Ash	38	42
Protein	302	400
Petroleum spirit extractives (fat)	185	200
Fibre	65	53
Carbohydrates	355	237
<i>(b) Minerals (g/kg)</i>		
Sodium	0.65	0.71
Potassium	12.00	11.50
Calcium	2.00	2.40
Iron	0.88	0.11
Phosphorus	3.83	4.90
<i>(c) Distribution of protein fractions (g/kg)</i>		
Albumin + globulin + non-protein Nitrogen (water-soluble)	29	
Non-protein nitrogen (NPN)	3	
Globulin (5% NaCl-soluble)	41	
Prolamin (75% alcohol-soluble)	3	
Glutelin (0.25% NaOH-soluble)	91	
Residue	135	

beany flavour which is due to high concentrations of polyunsaturated (linoleic) fatty acids. Some commercially cultivated soya varieties contain up to 63% linoleic acid (The Wealth of India, 1956). In comparison with some other varieties, this cultivar of soybean from Kashmir is more acceptable as it contains only 46.3% linoleic acid.

Phospholipid constitutes 1.94% of total seeds and 10.5% of total lipid and has the same pattern of fatty acids as the total lipid of the raw seeds. This fraction also contains higher percentages of linoleic (51.4%) and oleic (21.2%) acids whereas other components of fatty acids, viz. myristic, palmitic, stearic and linolenic acids, constitute 0.8%, 11.7%, 7.4% and 7.5%, respectively (Table 3). In comparison with other allied legumes, the presence of higher amounts of the total essential fatty acids (linoleic and linolenic) is advantageous as these essential fatty acids cannot be biosynthesized and must come from the diet.

In total phospholipid, phosphatidyl choline, 0.83 g and phosphatidyl ethanolamine, 0.78 g/100 g seeds are the major fractions while phosphatidyl serine 0.09 g and phosphatidyl inositol 0.24 g/100 g seeds are the smaller fractions (Table 3). The lecithin (total phosphatides) content of sundried 'Nikku muth' seeds is 1.94% which is comparable with that of other varieties

TABLE 2

Physico-Chemical Characteristics and Fatty Acid Composition of 'Nikku Muth' Seeds Lipid

	'Nikku muth'	Mean representative value in other soybeans
<i>(a) Characteristics of lipid</i>		
Lipid content (g/kg seed)	185	200
Iodine value	121.8	129.5
Saponification value	191.2	193.4
Unsaponifiable matter (g/100 g lipid)	2.3	24
Refractive index $n_d^{30^\circ}$	1.474	1.473
<i>(b) Component fatty acids (g per 100 g total fatty acids)</i>		
C <sub>14:0</sub>	1.5	—
C <sub>16:0</sub>	13.5	—
C <sub>18:0</sub>	6.1	—
C <sub>18:1</sub>	23.2	20.5–50.0
C <sub>18:2</sub>	46.3	45.7–63.1
C <sub>18:3</sub>	9.4	8.5–12.1
Total unsaturated fatty acids	78.9	
Total saturated fatty acids	21.1	
Polyunsaturated fatty acids (essential fatty acids)	55.7	

Values are means of triplicate determinations.

**TABLE 3**  
Phosphatides and Fatty Acid Composition in Phospholipid Fraction of Lipid

	‘Nikku muth’	Representative values in other soybeans
<i>(a) Composition of phosphatides (per 100 g seed)</i>		
Phosphatidyl choline	0.83	0.44–0.55
Phosphatidyl ethanolamine	0.78	0.49–0.59
Phosphatidyl serine	0.09	—
Phosphatidyl inositol	0.24	0.68–0.78
<i>(b) Component fatty acids (g per 100 g total fatty acids obtained from phospholipid fraction)</i>		
C <sub>14:0</sub>	0.8	
C <sub>16:0</sub>	11.7	
C <sub>18:0</sub>	7.4	
C <sub>18:1</sub>	21.2	
C <sub>18:2</sub>	51.4	
C <sub>18:3</sub>	7.5	
Total unsaturated fatty acids	80.1	
Total saturated fatty acids	19.9	

Values are means of triplicate determinations.

of soybeans (Peterson & Johnson, 1978; The Wealth of India, 1956). Hence, this cultivar has scope for commercial exploitation for the commercial production of industrial food grade lecithin, which is used as a viscosity reducer, emulsifier and wetting agent in the food, pharmaceutical and cosmetic industries. These findings are relevant to the socio-economic development of the local hill communities.

### CONCLUSION

‘Nikku muth’ (*Glycine max* L.), a traditional soybean cultivar of Kashmir Valley, is a nutritive food rich in protein (302 g/kg) and lipid (185 g/kg) which is a rich source of essential fatty acids (55.7%). In addition to fulfilling dietary requirements, ‘Nikku muth’ may be exploited as an alternate source of commercial grade lecithin.

### ACKNOWLEDGEMENT

Grateful thanks are due to the Department of Environment, Government of India, for providing financial assistance under the ‘All India Coordinated

Research Project on Ethnobiology' and to Shri B. M. Kapoor, Chairman, Technical Cell, for going through the manuscript and suggesting suitable changes.

## REFERENCES

- Andrews, J. S. & Felt, C. (1941). The iron content of cereals. *Cer. Chem.*, **18**, 819.
- AOAC (1984). *Official Methods of Analysis of the Association of Official Analytical Chemists*. (14th edn), Washington, DC.
- Bartlett, G. R. (1959). Phosphorus assay in column chromatography. *J. Biol. Chem.*, **234**, 446-68.
- Fiske, C. H. & Subba Row, Y. (1925). Colorimetric estimation of phosphorus. *J. Biol. Chem.*, **66**, 375-400.
- Folch, J., Lees, M. & Stanley, G. H. (1957). A single method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.*, **226**, 497-509.
- James, A. T. & Morris, L. J. (1964). *New Biochemical Separations*. D. Van Nostrand Co., London, p. 327.
- Kates, M. (1964). Simplified procedures for hydrolysis or methanolysis of lipids. *J. Lipid Res.*, **5**, 132-5.
- Niranjan, G. S. & Katiyar, S. K. (1979). Chemical analysis of some wild leguminous seeds. *J. Indian Chem. Soc.*, **56**, 722-5.
- Peterson, M. S. & Johnson, A. H. (1978). *Encyclopaedia of Food Science*, Vol. 3, The Avi Publishing Co., Westport, CT, p. 461.
- Skipski, V. P., Peterson, R. F. & Barclay, M. (1964). Quantitative analysis of phospholipids by thin layer chromatography. *Biochem. J.*, **90**, 374-8.
- The Wealth of India (1956). *A Dictionary of Indian Raw Materials*, Vol. IV, Council of Scientific & Industrial Research, New Delhi, p. 149.
- Wagner, H., Horhammer, L. & Wolff, P. (1961). Serum lipids and glycoproteins in atherosclerosis and diabetes. *Biochem. J.*, **334**, 175-84.