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# Effect of soaking, cooking and autoclaving on phytic acid and oligosaccharide contents of the tribal pulse, *Mucuna monosperma* DC. ex. Wight

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The effects of soaking, cooking and autoclaving on the levels of phytic acid and oligosaccharides, raffinose, stachyose and verbascose, present in two germplasm seed materials of *Mucuna monosperma* collected from Kerala and Tamil Nadu states, were investigated. The loss of phytic acid was found to be higher in distilled water soaking compared to salt water soaking in Kerala germplasm. There was no significant reduction in content of raffinose during soaking in distilled water in Tamil Nadu germplasm. Cooking for 3 h effected significant reduction of phytic acid, raffinose, stachyose and verbascose in both the germplasms studied. The percentage loss of oligosaccharides with autoclaving was higher than with cooking. These results revealed that autoclaving was more effective in eliminating the contents of phytic acid and oligosaccharides, in both the germplasms of the Indian tribal pulse, *Mucuna monosperma*, presently studied.

## INTRODUCTION

Plant foods such as cereals and legumes have consistently been listed as the major potential sources of dietary protein for feeding the world of tomorrow, and research efforts are being directed to this area to identify and evaluate unexploited sources, including tribal pulses (Egbe & Akinyele, 1990; Rajaram & Janardhanan, 1991; Mary Josephine & Janardhanan, 1992; Siddhuraju *et al.*, 1992; Vijayakumari *et al.*, 1993). Since the concentrated protein and other nutrients in tribal pulses are associated with certain antinutrients, the latter have to be eliminated for effective utilization of the pulse nutrients.

Phytic acid, myo-inositol 1,2,3,4,5,6-hexakis (dihydrogen phosphate) widely distributed in mature legume grains, stores most of the grain phosphorus. Phytic acid has antinutritional properties owing to its ability to chelate several minerals and thereby reduces their bioavailability (Nolan & Duffin, 1987).

The oligosaccharides, raffinose, stachyose and verbascose, common in legume seeds, are thought to be the major producers of flatulence. These saccharides are comprised of one, two and three galactose units respectively joined together with sucrose in $\alpha$-D-1-6 linkages. Owing to the lack of $\alpha$-galactosidases in mammalian digestive systems, they pass into the colon where they may produce diarrhoea, flatus gas ($CO_2$, $H_2$ and small amounts of $CH_4$ gases) and their inevitable social discomfort (Wagner *et al.*, 1976; Fleming, 1981).

The boiled seeds of *Mucuna monosperma* are known to be eaten by the tribes of North-Eastern India and Oceanic groups of tribals like Onges, Great Andamanese and Sompens during extreme scarcity of food (Arora & Mehra, 1981). Information regarding the effect of processing on the levels of antinutritional substances in the tribal pulses is not available. Hence in the present study an attempt has been made to assess the effect of soaking, cooking and autoclaving for eliminating phytic acid and oligosaccharides from the seed flour of *M. monosperma*.

## MATERIALS AND METHODS

### Collection of samples

The two germplasm seed materials of *M. monosperma* DC. ex. Wight were collected from Thirunelly Reserve Forest, Wynaad district, Kerala and Siruvani Reserve Forest, Coimbatore district, Tamil Nadu. The samples were coded as Kerala and Tamil Nadu germplasm, respectively.

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**Extraction and estimation of phytic acid**

For the determination of phytic acid content the Wheeler & Ferrel (1971) method was used. Phytic acid was extracted from 3 g seed flour with 50 ml of 3% TCA by shaking at room temperature followed by high-speed centrifugation. The phytic acid in the supernatant was precipitated as ferric phytate by adding excess ferric chloride and centrifuged. The ferric phytate was converted to ferric hydroxide with a few ml of water and 3 ml of 1.5 N NaOH. Then the iron content present in the sample was estimated. The phytate phosphorus was calculated from the iron results assuming a 4:6 iron:phosphorus molecular ratio. The phytic acid was estimated by multiplying the amount of phytate phosphorus by the factor 3.55 based on the empirical formula $C_6P_6O_{24}H_{18}$.

**Determination of oligosaccharides content**

The oligosaccharides were first extracted from samples by treating 5 g of each sample with 25 ml of 80% ethanol at room temperature by repeated shaking. The extraction was repeated twice. The extracts were pooled and concentrated under vacuum at 4°C. The residue was made up to 5 ml with distilled water, and the sugars were separated using a descending paper chromatographic technique using Whatman No. 1 chromatographic paper and the solvent mixture, propanol–ethanol–water, in the ratio of 7:1:2 (v/v) (Tharanathan *et al.*, 1975). A standard sugar mixture containing raffinose, stachyose and verbascose (procured from Sigma Chemical Co., St. Louis, MO, USA) was run simultaneously. After development, *p*-anisidine hydrochloride reagent was sprayed on the papers to reveal the sugar spots (Mukherjee & Srivastava, 1952). For quantitation, the paper (of area 2 × 2 cm) corresponding to each oligosaccharide spot was cut from unsprayed papers and eluted with 3 ml of distilled water. Then the eluted individual sugars were estimated by the phenol–sulphuric acid method described by Dubois *et al.* (1956).

**Treatments**

*Soaking*

Whole seeds were soaked in distilled water and 0.02% sodium bicarbonate solution (pH 8.6) ($NaHCO_3$, 1 g:10 ml) for 3, 6, 9, 12, 15 and 18 h at room temperature. The water was drained off, then the seeds were dried at 55°C, powdered in a Wiley Mill to 60 mesh size and analysed as above for phytic acid and oligosaccharides.

*Cooking*

Separate batches of seeds were cooked in distilled water (100°C) (1 g:10 ml) for 0.5, 1, 1.5, 2, 2.5 and 3 h on a hot plate, the seeds rinsed, dried at 55°C, powdered in a Wiley Mill to 60 mesh size and the contents of phytic acid and oligosaccharides determined as above.

*Autoclaving*

The seed samples were autoclaved at 15 lb pressure (121°C) in distilled water (1 g:10 ml) for 30, 60 and 90 min. Then the seeds were rinsed with distilled water, dried and powdered in a Wiley Mill to 60 mesh size. Phytic acid and oligosaccharides contents were analysed as described above.

*Statistical analysis*

The data were statistically analysed using Duncan's Multiple Range Test (DMRT) by the method of Alder & Roessler (1977).

**Table 1. Contents of phytic acid and oligosaccharides (g $kg^{-1}$DM) in raw and soaked seeds of *Mucuna monosperma**

| Treatment | Kerala germplasm | | | | Tamil Nadu germplasm | | | |
|---|---|---|---|---|---|---|---|---|
| | Phytic acid | Raffinose | Stachyose | Verbascose | Phytic acid | Raffinose | Stachyose | Verbascose |
| Raw seeds | $6.32^{a}$ | $16.2^{a}$ | $12.4^{a}$ | $9.6^{a}$ | $5.03^{a}$ | $13.6^{a}$ | $11.8^{a}$ | $10.7^{a}$ |
| Soaked in distilled water (h) | | | | | | | | |
| 3 | $6.07^{b}$ (4) | $16.2^{a}$ (0) | $12.3^{a}$ (1) | $9.5^{a}$ (1) | $4.63^{b}$ (8) | $13.6^{a}$ (0) | $11.3^{ab}$ (4) | $10.4^{ab}$ (3) |
| 6 | $5.66^{c}$ (10) | $16.1^{a}$ (1) | $11.9^{ab}$ (4) | $9.3^{a}$ (3) | $4.25^{c}$ (15) | $13.4^{a}$ (1) | $10.7^{bc}$ (9) | $10.1^{abc}$ (6) |
| 9 | $5.33^{d}$ (16) | $15.5^{ab}$ (4) | $11.4^{bc}$ (8) | $8.7^{b}$ (9) | $4.00^{d}$ (20) | $13.0^{a}$ (4) | $10.4^{bcd}$ (12) | $9.8^{abc}$ (8) |
| 12 | $4.84^{e}$ (23) | $15.0^{b}$ (7) | $11.2^{c}$ (10) | $8.2^{c}$ (15) | $3.87^{de}$ (23) | $13.0^{a}$ (4) | $9.8^{cd}$ (17) | $9.2^{bcd}$ (14) |
| 15 | $4.51^{f}$ (29) | $15.0^{b}$ (7) | $10.2^{d}$ (18) | $8.2^{c}$ (15) | $3.69^{ef}$ (27) | $12.9^{a}$ (5) | $9.4^{de}$ (20) | $8.9^{cd}$ (17) |
| 18 | $4.11^{g}$ (35) | $14.9^{b}$ (8) | $9.7^{d}$ (22) | $8.0^{c}$ (17) | $3.62^{f}$ (28) | $12.6^{a}$ (7) | $8.4^{e}$ (29) | $8.5^{d}$ (21) |
| Soaked in sodium bicarbonate solution (h) | | | | | | | | |
| 3 | $5.99^{b}$ (5) | $16.0^{ab}$ (1) | $12.2^{a}$ (2) | $9.1^{a}$ (5) | $4.69^{b}$ (7) | $13.4^{ab}$ (1) | $10.8^{ab}$ (8) | $10.1^{ab}$ (6) |
| 6 | $5.64^{c}$ (11) | $15.7^{ab}$ (3) | $11.5^{b}$ (7) | $9.0^{ab}$ (6) | $4.33^{c}$ (14) | $13.0^{ab}$ (4) | $10.0^{bc}$ (15) | $9.6^{abc}$ (10) |
| 9 | $5.43^{d}$ (14) | $15.2^{abc}$ (6) | $10.9^{bc}$ (12) | $8.4^{b}$ (13) | $4.20^{c}$ (16) | $12.7^{abc}$ (7) | $9.9^{bc}$ (16) | $9.2^{bc}$ (14) |
| 12 | $5.24^{e}$ (17) | $14.8^{bc}$ (9) | $10.6^{c}$ (15) | $7.6^{c}$ (21) | $4.02^{d}$ (20) | $12.3^{bc}$ (10) | $9.4^{c}$ (20) | $8.7^{cd}$ (19) |
| 15 | $4.93^{f}$ (22) | $14.4^{c}$ (11) | $9.6^{d}$ (23) | $7.4^{c}$ (23) | $3.82^{e}$ (24) | $12.3^{bc}$ (10) | $9.1^{c}$ (23) | $8.5^{c}$ (21) |
| 18 | $4.74^{g}$ (25) | $14.1^{c}$ (13) | $8.7^{e}$ (30) | $7.0^{c}$ (27) | $3.62^{f}$ (28) | $11.7^{c}$ (14) | $7.6^{d}$ (36) | $7.7^{d}$ (28) |

*All values are averages of three determinations.
Means followed by same letter are not satistically significant according to DMRT ($P < 0.05$).
Values in parentheses indicate the percent loss.

## RESULTS AND DISCUSSION

The data on phytic acid and oligosaccharides, namely raffinose, stachyose and verbascose contents, in raw seeds of two germplasm of *M. monosperma* are presented in Table 1. The two germplasm seed materials of *M. monosperma* are known to contain more phytic acid than the seeds of *Mucuna utilis* (Ravindran & Ravindran, 1988); *Prosopis glandulosa* (Harden & Zolfaghari, 1988); *Vigna unguiculata* (Akinyele, 1989) and *Phaseolus lunatus* (Egbe & Akinyele, 1990). The oligosaccharide contents of the raw seeds estimated in the present study are in agreement with the earlier reports in black gram (Reddy & Salunkhe, 1980) and soybean (East *et al.*, 1972). Raffinose is found to be the major oligosaccharide in both the germplasms of *M. monosperma* as has been reported earlier in *Prosopis glandulosa* and *P. velutina* (Becker & Grosjean, 1980).

### Soaking

Soaking in distilled water as well as $NaHCO_3$ solution, affects the levels of phytic acid and oligosaccharides in the samples to various extents (Table 1). Kerala and Tamil Nadu germplasm of *M. monosperma* exhibit significant reduction in level of phytate content during soaking in distilled water which confirms the earlier reports in *Vigna unguiculata* (Ologhobo & Fetuga, 1984) and *V. mungo* (Duhan *et al.*, 1989). The percentage loss of phytic acid is higher with distilled water soaking compared to salt water soaking in Kerala germplasm. This agrees with the results of Khan *et al.* (1986) in wheat and Khan *et al.* (1988) in white variety of *Cicer arietinum*, who have reported that the loss of phytic acid is less in the presence of sodium bicarbonate. Nonetheless, in Tamil Nadu germplasm, both distilled water and $NaHCO_3$ solution soaking cause significant reduction in content of phytic acid. Soaking-induced reduction in phytate content in legumes may be attributed to the activity of phytase and diffusion. An increase in the phytase activity, coinciding with a decrease in the level of phytate as a result of soaking in faba bean, has been demonstrated in an earlier study (Eskin & Wiebe, 1983).

In the present study, in Tamil Nadu germplasm, soaking in distilled water does not result in significant reduction in the content of raffinose which agrees with an earlier study in *Dolichos lablab* (Revilleza *et al.*, 1990). During soaking in both distilled water and $NaHCO_3$ solution, significant reduction has been observed in the levels of stachyose followed by verbascose and raffinose contents in both the germplasms studied. In general, oligosaccharide reduction is higher with $NaHCO_3$ solution soaking than with distilled water soaking. Upadhyay & Garcia (1988) have demonstrated that differential solubility of the individual oligosaccharides and their diffusion rates are two factors that could influence the sugar losses during soaking.

### Effect of cooking

When the seeds are cooked for 3 h a reduction of 31 and 39% phytic acid in Kerala and Tamil Nadu germplasm, respectively, is noticed, which is statistically significant. Similar results have been reported earlier in black gram (Kataria *et al.*, 1988; Duhan *et al.*, 1989) and cowpea (Uzogara *et al.*, 1990) with a significant reduction of 29–40% compared to its raw seeds. The statistical data reveal that the loss of phytic acid content is positively correlated with the cooking time. The apparent decrease in phytic acid content of the legume seeds during cooking may be partly attributed to the formation of insoluble complexes between phytate and other components (Kumar *et al.*, 1978).

Cooking caused greater reduction in the level of oligosaccharides than soaking (Tables 1 and 2). Though the

**Table 2. Contents of phytic acid and oligosaccharides (g $kg^{-1}$DM) in raw, cooked and autoclaved seeds of *Mucuna monosperma****

| Treatment | Kerala germplasm | | | | Tamil Nadu germplasm | | | |
|---|---|---|---|---|---|---|---|---|
| | Phytic acid | Raffinose | Stachyose | Verbascose | Phytic acid | Raffinose | Stachyose | Verbascose |
| Raw seeds | $6.32^{a}$ | $16.2^{a}$ | $12.4^{a}$ | $9.6^{a}$ | $5.03^{a}$ | $13.6^{a}$ | $11.8^{a}$ | $10.7^{a}$ |
| Cooked in water (h) | | | | | | | | |
| 0.5 | $5.86^{b}$ (7) | $15.5^{a}$ (4) | $11.6^{ab}$ (6) | $9.3^{ab}$ (3) | $4.79^{b}$ (5) | $13.1^{ab}$ (4) | $10.8^{a}$ (8) | $10.0^{ab}$ (7) |
| 1 | $5.32^{c}$ (16) | $13.6^{b}$ (16) | $10.8^{b}$ (13) | $9.0^{bc}$ (6) | $4.51^{c}$ (10) | $12.3^{bc}$ (10) | $9.6^{b}$ (19) | $9.0^{bc}$ (16) |
| 1.5 | $5.08^{d}$ (20) | $13.3^{bc}$ (18) | $9.4^{c}$ (24) | $8.6^{cd}$ (10) | $4.33^{d}$ (14) | $11.5^{c}$ (15) | $7.9^{c}$ (33) | $8.3^{cd}$ (22) |
| 2 | $4.77^{e}$ (25) | $12.3^{cd}$ (24) | $9.1^{c}$ (27) | $8.23^{dc}$ (14) | $3.97^{e}$ (21) | $10.1^{d}$ (26) | $7.6^{c}$ (36) | $8.1^{cd}$ (24) |
| 2.5 | $4.56^{f}$ (28) | $12.0^{d}$ (26) | $8.7^{c}$ (30) | $7.9^{e}$ (18) | $3.52^{f}$ (30) | $9.5^{d}$ (30) | $7.2^{cd}$ (39) | $7.9^{cd}$ (26) |
| 3 | $4.36^{g}$ (31) | $11.5^{d}$ (29) | $7.3^{d}$ (41) | $7.4^{f}$ (23) | $3.07^{g}$ (39) | $9.2^{d}$ (32) | $6.3^{d}$ (47) | $7.6^{d}$ (29) |
| Autoclaved (min) | | | | | | | | |
| 30 | $5.94^{b}$ (6) | $12.3^{b}$ (24) | $9.4^{b}$ (24) | $7.9^{b}$ (18) | $4.46^{b}$ (11) | $10.0^{b}$ (26) | $7.8^{b}$ (34) | $8.5^{b}$ (21) |
| 60 | $5.64^{c}$ (11) | $10.5^{c}$ (35) | $6.4^{c}$ (48) | $6.7^{c}$ (30) | $4.13^{c}$ (18) | $7.9^{c}$ (42) | $5.5^{c}$ (53) | $7.1^{bc}$ (34) |
| 90 | $4.30^{d}$ (32) | $8.9^{d}$ (45) | $4.0^{d}$ (68) | $4.6^{d}$ (52) | $3.77^{d}$ (25) | $6.7^{c}$ (51) | $3.7^{d}$ (69) | $5.8^{c}$ (46) |

*All values are average of three determinations.
Means followed by same letter are not statistically significant according to DMRT ($P < 0.05$).
Values in parentheses indicate the percent loss.

level of reduction is not commensurate with the duration of cooking, the longest cooking time results in the greatest reduction in the level of oligosaccharides in both the germplasms of *M. monosperma*.

Kerala and Tamil Nadu germplasm seed materials of *M. monosperma*, when subjected to cooking for 3 h, show significant reductions in contents of raffinose (29 and 32%) and stachyose (41 and 47%), respectively. Somiari & Balogh (1993) reported mean decreases of 44 and 28.6% in the raffinose and stachyose contents of three different cultivars of cowpea after 50 min cooking. The significant reduction of verbascose content in the present study is in agreement with an earlier report in black gram (Reddy & Salunkhe, 1980). On the other hand, Udayasekhara Rao & Belavady (1978) have reported an increase in level of raffinose after cooking.

Decrease in contents of raffinose, stachyose and verbascose due to cooking might be attributed to heat hydrolysis of the oligosaccharides to simple disaccharides and monosaccharides or to the formation of other compounds (Onigbinde & Akinyele, 1983). On the other hand, Price *et al.* (1988) have reported that cooking alone will not be sufficient to bring about any significant reduction in the flatulence-inducing activity of cowpeas.

**Effect of autoclaving**

The loss of phytic acid due to autoclaving is 32 and 25% in Kerala and Tamil Nadu germplasms, respectively. Compared to cooking, autoclaving causes less loss of phytic acid content in Tamil Nadu germplasm of *M. monosperma*. Ologhobo & Fetuga (1984) and Uzogara *et al.* (1990) also have observed that autoclaving causes less loss of phytic acid content compared to cooking in *Vigna unguiculata*.

*M. monosperma*, Kerala and Tamil Nadu germplasm, exhibits significant loss of raffinose (45 and 51%), stachyose (68 and 69%) and verbascose (52 and 46%), respectively, when compared to the raw seeds during autoclaving. In general, more significant reduction of all the three oligosaccharides has been observed during autoclaving than during cooking. Phytic acid and oligosaccharides present in significant amounts in *M. monosperma*, like other food legumes, are markedly reduced when subjected to domestic processing including ordinary cooking processes. Autoclaving is the most effective method of getting rid of the antinutrients presently investigated, thereby enhancing chances for increased and versatile utilization of these protein-rich tribal pulses as a food and protein supplement.

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