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Effect of microwave treatment on the antinutritional factors of two accessions of velvet bean, *Mucuna pruriens* (L.) DC. var. *utilis* (Wall. ex Wight) Bak. ex Burck

Kala, B. K. and *Mohan, V. R.

Ethnopharmacology unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin-628008, Tamil Nadu, India

Abstract: The present study was carried out to investigate the role of microwave treatment in eliminating the antinutritional factors such as, total free phenolics, tannin, l-dopa, phytic acid, hydrogen cyanide, total oxalate, trypsin inhibitor activity, oligosaccharides and phytohaemagglutins in the seeds of two accessions of velvet bean, *Mucuna pruriens* var. *utilis*. Microwave treated raw and overnight soaked seeds of both the investigated accessions of *Mucuna* bean showed a time duration dependent increase in the total free phenolics and L-dopa; whereas, level of tannin in both raw and overnight soaked seeds get reduced. The levels of phytic acid, hydrogen cyanide, total oxalate and trypsin inhibitor activity of presently investigated raw and overnight soaked seeds showed time duration dependent decline. Complete elimination of phytohaemagglutinating activity with respect to erythrocyte of 'O' blood group was observed at 8-12 minutes. Oligosaccharides like raffinose, stachyose and verbascose were also significantly ($p< 0.05$) reduced.

Key words: Microwave, antinutritional factors, total free phenolics, accessions, velvet bean

Introduction

Pulses constitute a cheap alternative source of protein and calories, particularly for the people who are unable to afford the high cost of dietary protein from animal sources (Noel and Rosario, 1989). They complement cereals in terms of amino acid balance. On an average, food legumes contain about 20-30 per cent protein on dry weight basis and provide about 350 calories of food energy per 100 g (Pawar and Ingle, 1987). The underexploited legumes / wild tribal pulses, have tremendous potential for commercial exploitation but have remain ignored, than offer a good scope to meet the ever increasing demands for vegetable protein.The velvet bean, *Mucuna pruriens* (L.) DC var. *utilis* (Wall. ex Wight) Bak. ex Burck evaluated in the present study is an under-utilized legume species available predominantly in Asia, Africa and parts of the western hemisphere for food and forage crop. In Southeast Asia the immature pods and leaves of velvet bean are roasted and eaten. In India, the mature seeds are known to be eaten after repeated boiling by a South Indian tribe, Kanikkars (Mohan and Janardhanan, 1995).

Although it has been suggested that the *Mucuna* bean has a good nutritional value, an important constraint for its wider adoption is its content of several anti-nutritional factors (Del Carmen *et al*. 1999; Bressani, 2002). Seeds of *Mucuna* contain several anti-nutritional factors such as L-DOPA, total free phenolics, tannins, haemagglutinin, trypsin and chymotrypsin inhibitors, anti-vitamins, protease inhibitors, phytic acid, flatulence factors, saponins and hydrogen cyanide (Emenalom and Udedibie, 1998; Vadivel and Janardhanan, 2000). The antinutritional factors in the legume seeds adversely affect the protein digestibility (Gupta, 1987). These substances unless destroyed by heat or some other suitable treatment can exert adverse physiological effects when ingested by man and animals (Liener, 1980).

On the contrary, it has been suggested that consumption of low level of certain antinutrients may produce health benefits while avoiding some of the adverse effects associated with their large intake (Thompson, 1988). L-Dopa, a potentially neurotoxic agent used in the treatment of Parkinson's disease, is found in large amounts in *Mucuna* seeds which have been proposed as a medical source of L-Dopa and even in the treatment of Parkinson's disease (Hussain and Manyam, 1997). Epidemiological studies have correlated the consumption of plant produce with high phenolics to reduction of cardio-cerebrovascular diseases and cancer mortality (Hertog *et al*., 1997). The phytic acid of *Mucuna* possesses antioxidant, anticarcinogenic and hypoglycemic activities (Graf and Eaton, 1990; Rickard and Thompson, 1997; Shamsuddin *et al.*, 1997) and are effective at low

*Corresponding author.
Email: *vrmohan_2005@yahoo.com*

concentrations. Liener (1994) reported that the protease inhibitors in *Mucuna* seeds enhance the pancreatic secretary activity.

Inactivation of the undesirable components from the dry legume seed is essential for improving their nutritional qualities and effectively utilize them to their full potential as food. To achieve this, several processing methods such as germination, soaking and cooking and dry heat treatment have been used (Siddhuraju and Becker, 2001; Vijayakumari *et al.,* 2007;Vadivel and Pugalenthi, 2008). In the present study an attempt has been made to assess the effect of microwave treatment on the antinutritional factors of two accessions of *Mucuna pruriens* var. *utilis.*

Materials and Methods

Collection of seeds

Two samples of velvet bean, *Mucuna pruriens* (L.) DC var. *utilis* (Wall.ex Wight) Bak. ex Burck (white coloured seedcoat) were collected from Karaiyar and (black coloured seed coat) from Seruvalaru in the Tirunelveli district, Western Ghats, Tamil Nadu. With the help of keys by Wilmot-Dear (1987), the accessions were botanically identified. After thoroughly drying in the sun the pods were thrashed to remove seeds. The seeds, after thorough cleaning and removal of broken seeds, foreign materials and immature seeds were stored in airtight plastic jars at room temperature (25^{o} C).

Treatments

The dry seeds of both the accessions of *Mucuna pruriens* var. *utilis* were exposed for 2, 4, 8, 10 and 12 min in a microwave oven (LG Elecronics, India, Pvt, Ltd, Model No. MC 8087ARH with an output power of 900 W and a frequency of 2450MHz) at 130°C. The above said treatments were also given to seeds of both the accessions of *Mucuna pruriens* var. *utilis* that soaked in distilled water for overnight (Overnight soaked seeds).The overnight soaked seeds are dried at 55° C. All the microwave treated seeds were powdered in a Willey Mill to 60 mesh size. The antinutritional factors such as total free phenolics, tannin, L-dopa, phytic acid, hydrogen cyanide, total oxalate, trypsin inhibitor activity, oligosaccharides and phytohaemagglutinating activity were quantified in both raw (control) and microwave (raw and overnight soaked seed) treated seed samples.

Analyses of antinutritional compounds

The antinutritional compounds, total free phenolics (Bray and Thorne, 1954), tannins (Burns, 1971), the non-protein amino acid, L-DOPA (3, 4-dihydroxyphenylalanine) (Brain, 1976), phytic acid (Wheeler and Ferrel, 1971), hydrogen cyanide (Jackson, 1967), total oxalate (AOAC, 1984) were quantified. Trypsin inhibitor activity was determined by the enzyme assay of Kakade *et al*. (1974) by using benzoil-DL-arginin-*p* nitroanilide (BAPNA) as a substrate. One trypsin inhibitor unit (TIU) has been expressed as an increase of 0.01 absorbance units per 10ml of reaction mixture at 410nm. Trypsin inhibitor activity has been defined in terms of trypsin units inhibited per mg protein.

Extraction TLC separation and estimation of oligosaccharides

Extraction of oligosaccharides was done following the method of Somiari and Balogh (1993). Five g each of both raw and treated seed flours of both the accessions were extracted with 50 mL of 70% (v/v) aqueous ethanol and kept on an orbital shaker at 130 rpm for 13 h and then filtered through Whatman No. 1 filter paper. Residue was further washed with 25 mL of 70% (v/v) ethanol. The filtrates obtained were pooled and vacuum-dried at 45°C. The concentrated sugar syrup was dissolved in 5 mL of double-distilled water. Separation of oligosaccharides was done by TLC. Thirty g of cellulose-G powder were dissolved in 45 mL of double distilled water and shaken well until the slurry was homogeneous. TLC plates were coated with the slurry and air-dried. Spotting of the sugar samples was done by using micropipettes. Five µl aliquots of each sample were spotted thrice separately. The plates were developed by using a solvent system of n- propanol, ethyl acetate and distilled water (6:1:3), and dried (Tanaka *et al.,* 1975). The plates were sprayed with α –naphthol (1%, w/v). Plates were dried in a hot air oven. The separated spots were compared with standard sugar spots. A standard sugar mixture containing raffinose, stachyose and verbascose (Sigma chemical). Separated sugars that appeared were verbascose, stachyose and raffinose. The sugar spots were scrapped and eluted in 2 mL of distilled water kept overnight at room temperature and filtered through Whatman No. 1 filter paper. The filtrates were subjected to quantitative estimation. The eluted individual oligosaccharides were estimated by the method of Tanaka *et al*. (1975). One mL of the eluted and filtered sugar solution was treated with one ml of 0.2 M thiobarbituric acid and one ml of concentrated HCL. The tubes were boiled in a water bath for exactly 6 min. After cooling, the oligosaccharide contents were quantified in a Elico UV-Spectrophotometer model SL 150 at 432 nm. Average values of triplicate estimations were calculated and the content of oligosaccharides was

expressed on dry weight basis.

Quantitative determination of phytohaemagglutinating (Lectin) activity

Lectin activity was determined by the method of Almedia *et al.* (1991). One g of air-dried seed flour was stirred with 10 mL of 0.15N sodium chloride solution for 2h and the pH was adjusted to 4.0. The contents were centrifuged at 10,000 X g for 20 min. and the supernatants were collected separately. The protein content was estimated by Lowry *et al*. (1951) method. Human blood (blood groups A, B and O) was procured from the blood bank of Jothi Clinical Laboratory, Tuticorin. Blood erythrocyte suspensions were prepared by washing the blood samples separately with phosphate-buffered saline and centrifuged for 3 min at low speed (3,000 g for 10 min at room temperature). Supernatants were removed with Pasteur pipettes. The washing procedure was repeated three times. The washed cells were diluted by one drop of cells with 24 drops of phosphate – buffered saline. The determination of lectin was done by the method of Tan *et al*. (1983). Clear supernatant (50 µl) was poured into the depression (pit) on a microtitration plate and serially diluted 1:2 with normal saline. The human blood erythrocyte (A, B and O blood groups) suspensions (25 µl) were added to each of the twenty depressions. The plates were incubated for 3 hours at room temperature. After the incubation period, the titer values were recorded. One Haemagglutinating Unit (HU) is defined as the least amount of haemagglutinin that will produce positive evidence of agglutination of 25µl of a blood group erythrocyte after 3hr incubation at room temperature. The phytohaemagglutinating activity was expressed as haemagglutinating units (HU)/mg protein.

Statistical analysis

The antinutritional factors like total free phenolics, tannins, L-dopa, phytic acids, hydrogen cyanide, total oxalate, trypsin inhibitor activity and oligosaccharides were estimated on triplicate determinations. Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) were used for analysis [SPSS software for windows release 15.0; SPSS Inc., Chicago IL, USA] of any significant difference in anti nutritional composition among the microwave treated seeds of investigated accessions. Significance was accepted at $p < 0.05$.

Results and Discussions

Total free phenolics, tannin and L-dopa

Table - 1 presents the data on effect of microwave on total free phenolics, tannin and L-dopa of seeds of both accessions of *Mucuna pruriens* var. *utilis*. In the current investigation, microwave treated raw and overnight soaked seeds of both the accessions of *Mucuna pruriens* var. *utilis* showed a time duration dependent increase in the total free phenolics; whereas, tannin in both raw and overnight soaked seeds treated with microwave significantly ($p< 0.05$) decreased when compared to control. Randir and Shetty (2004) found that the microwave treatment increase the phenolic content in the germinated seeds of faba bean. They suggested that microwave caused acute heat stress in plant cells which stimulated Pentose Phosphate Pathway (PPP) towards the more production of phenolics and L-dopa. Phenolics have been suggested to exhibit health related functional properties such as, anticarcinogenic, antiviral, antimicrobial, antiinflammatory, hypertensive and antioxidant activity (Shetty, 1997).

L-dopa content of currently investigated raw and overnight soaked seeds of *Mucuna pruriens* var. *utilis* treated with microwave showed a significant ($p< 0.05$) time duration dependent increase. *M. pruriens* var. *utilis* have potential for management of Parkinson's disease since it is rich in pharmacologically active factors L-dopa which is the precursor for dopamine (Manyam, 1995). Recently, there is interest in the over expression of L-dopa from legumes in a high-phenolic antioxidant background to yield low cost and readily available ingredients related to Parkinson's disease management (Randhir *et al.,* 2002).

Phytic acid, hydrogen cyanide and total oxalate

The levels of phytic acid, hydrogen cyanide and total oxalate (Table 3) of presently investigated raw and overnight soaked seeds of *Mucuna pruriens* var. *utilis* showed time duration dependent decline when treated with microwave treatment. Alajaji and El-Adway (2006) reported that microwave cooking treatment decrease the phytic acid content in the seeds of *Cicer arietinum*. Decrease of phytic acid content has been attributed to low inositol and inositol phosphate by the action of free radicals generated during irradiation (De Boland *et al.,* 1975). Therefore, microwave treatment was proved to be effective in lowering phytic acid level.

The level of hydrogen cyanide in both the investigated accessions of *Mucuna pruriens* var. *utilis* seems to be negligible when compared with lethal level of hydrogen cyanide (36 mg100 g^{-1}) (Oke, 1969). In the present study, increases in time duration of microwave treatment completely eliminated the hydrogen cyanide and total oxalate content in both the accessions of *Mucuna pruriens* var. *utilis*.

Table 1. Effect of Microwave on Total free phenolics, Tannin and L-dopa of two accessions of *Mucuna pruriens* var. *utilis* (g $100g^{-1}$)

Variants	Time duration (in minutes)	Accessions					
		M. pruriens **var.** *utilis* (white coloured seedcoat)			*M. pruriens* **var.** *utilis* (black coloured seedcoat)		
		Phenol	**Tannin**	**L-dopa**	**Phenol**	**Tannin**	**L-dopa**
Microwave treated raw seeds	Raw	3.68±0.06^{a}	0.14±0.01^{a}	7.55±0.12^{a}	4.06±0.09^{a}	0.18±0.01^{a}	7.93±0.17^{a}
	2minutes	3.78±0.03^{ab} (+3%)	0.12±0.01^{ab} (-14%)	7.84±0.19^{ab} (+4%)	4.24±0.05^{b} (+4%)	0.16±0.01^{ab} (-11%)	8.23±0.08^{b} (+4%)
	4’	3.94±0.02^{bc} (+7%)	0.11±0.02^{bc} (-21%)	8.06±0.18^{bc} (+7%)	4.36±0.03^{b} (+7%)	0.14±0.01^{bc} (-22%)	8.36±0.08^{b} (+5%)
	8’	4.08±0.04^{cd} (+11%)	0.09±0.02^{cd} (-36%)	8.24±0.10^{bc} (+9%)	4.54±0.02^{c} (+12%)	0.12±0.01^{c} (-33%)	8.74±0.06^{c} (+10%)
	10’	4.24±0.06^{d} (+15%)	0.09±0.01^{cd} (-36%)	8.30±0.06^{c} (+10%)	4.66±0.01^{c} (+15%)	0.11±0.02^{c} (-39%)	8.96±0.04^{cd} (+13%)
	12’	4.56±0.15^{e} (+24%)	0.08±0.01^{d} (-43%)	8.77±0.04^{d} (+16%)	4.98±0.01^{d} (+23%)	0.11±0.01^{c} (-39%)	9.10±0.05^{d} (+15%)
Microwave treated overnight soaked seeds	Raw	3.68±0.06^{a}	0.14±0.01^{a}	7.55±0.12^{a}	4.06±0.09^{a}	0.18±0.01^{a}	7.93±0.17^{a}
	2minutes	3.96±0.10^{a} (+8%)	0.11±0.01^{b} (-21%)	8.08±0.08^{b} (+7%)	4.78±0.07^{b} (+18%)	0.14±0.02^{b} (-22%)	8.56±0.18^{b} (+8%)
	4’	4.48±0.09^{b} (+22%)	0.11±0.03^{b} (-21%)	8.56±0.04^{c} (+13%)	5.04±0.08^{c} (+24%)	0.10±0.01^{c} (-44%)	9.16±0.06^{c} (+16%)
	8’	4.78±0.10^{bc} (+30%)	0.09±0.01^{b} (-36%)	9.36±0.17^{d} (+24%)	5.24±0.06^{c} (+29%)	0.08±0.01^{cd} (-55%)	9.78±0.10^{d} (+23%)
	10’	5.04±0.13^{cd} (+37%)	0.05±0.01^{c} (-64%)	9.94±0.16^{c} (+32%)	5.86±0.05^{d} (+44%)	0.06±0.01^{d} (-67)	10.72±0.13^{e} (+35%)
	12’	5.28±0.12^{d} (+43%)	0.03±0.01^{c} (-79%)	10.36±0.02^{f} (+37%)	5.91±0.04^{d} (+46%)	0.03±0.01^{e} (-83%)	11.23±0.15^{f} (+42%)

Means±SE (N=3) means in the column with unlike superscript differ significantly ($p < 0.05$) Values in the parentheses denotes the loss or gain in percentage

Table 2. Effect of Microwave on Phytic acid, HCN and Total oxalate of two accessions of *Mucuna pruriens* var. *utilis* (mg 100 g^{-1})

Variants	Time duration (in minutes)	Accessions					
		M. pruriens **var.** *utilis* (white coloured seedcoat)			*M. pruriens* **var.** *utilis* (black coloured seedcoat)		
		Phytic acid	**HCN**	**Total oxalate**	**Phytic acid**	**HCN**	**Totalo xalate**
Microwave treated raw seeds	Raw	483.00±0.41^{a}	0.16±0.03^{a}	0.12±0.01^{a}	634.12±0.78^{a}	0.24±0.01^{a}	0.09±0.02^{a}
	2 minutes	464.17±0.19^{b} (-4%)	0.13±0.02^{b} (-19%)	0.10±0.02^{b} (-17%)	610.40±0.34^{b} (-4%)	0.20±0.02^{b} (-17%)	0.07±0.02^{ab} (-22%)
	4’	421.76±0.47^{c} (-13%)	0.09±0.01^{c} (-44%)	0.08±0.02^{b} (-33%)	578.38±0.38^{c} (-9%)	0.18±0.02^{b} (-25%)	0.05±0.01^{bc} (-44%)
	8’	387.44±0.85^{d} (-20%)	0.04±0.01^{d} (-75%)	0.05±0.01^{c} (-58%)	514.13±0.73^{d} (-19%)	0.12±0.01^{c} (-50%)	0.02±0.01^{cd} (-78%)
	10’	354.10±0.65^{e} (-27%)	NIL	0.01±0.00^{d} (-92%)	478.28±0.77^{e} (-25%)	0.04±0.01^{d} (-83%)	NIL
	12’	310.86±0.77^{f} (-36%)	NIL	NIL	414.00±0.46^{f} (-35%)	NIL	NIL
Microwave treated overnight soaked seeds	Raw	483.00±0.41^{a}	0.16±0.03^{a}	0.12±0.01^{a}	634.12±0.78^{a}	0.24±0.01^{a}	0.09±0.02^{a}
	2 minutes	444.00±0.33^{b} (-8%)	0.09±0.02^{b} (-44%)	0.08±0.02^{b} (-33%)	590.10±1.33^{b} (-7%)	0.16±0.02^{b} (-33%)	0.06±0.01^{b} (-33%)
	4’	376.41±0.39^{c} (-22%)	0.05±0.01^{c} (-69%)	0.06±0.01^{b} (-50%)	548.26±1.35^{c} (-14%)	0.12±0.02^{c} (-50%)	0.02±0.01^{c} (-78%)
	8’	352.78±0.29^{d} (-27%)	NIL	0.02±0.01^{c} (-83%)	507.14±0.58^{d} (-20%)	0.06±0.02^{d} (-75%)	NIL
	10’	294.74±0.73^{e} (-39%)	NIL	NIL	424.76±0.64^{e} (-33%)	NIL	NIL
	12’	222.46±0.10^{f} (-54%)	NIL	NIL	351.10±0.71^{f} (-45%)	NIL	NIL

Means±SE (N=3) means in the column with unlike superscript differ significantly ($p < 0.05$) Values in the parentheses denotes the loss or gain in percentage

Table 3. Effect of Microwave on Trypsin inhibitor activity and Phytohaemagglutinating activity of two accessions of *Mucuna pruriens* var. *utilis*

Variants	Time Duration (in minutes)	Accessions							
		M. pruriens **var.** *utilis* (white coloured seed coat)				*M. pruriens* **var.** *utilis* (black coloured seed coat)			
		TIA (TIU mg^{-1} protein)	**Phytohaemagglutinating activity*** [Hu mg^{-1} protein]			**TIA (TIU mg^{-1} protein)**	**Phytohaemagglutinating activity*** [Hu mg^{-1} protein]		
			A	B	O		A	B	O
Microwave treated raw seeds	Raw	46.40±0.56^{a}	180	66	14	43.70±0.68^{a}	176	74	10
	2 minutes	40.13±0.10^{b} (-14%)	164 (-9%)	51 (-23%)	7 (-50%)	37.24±0.28^{b} (-15%)	161 (-9%)	51 (-37%)	4 (-60%)
	4’	34.78±0.24^{c} (-25%)	151 (-16%)	33 (-50%)	3 (-79%)	30.07±0.20^{c} (-31%)	144 (-18%)	32 (-57%)	2 (-80%)
	8’	28.24±0.14^{d} (-39%)	120 (-33%)	21 (-68%)	NIL	24.12±0.16^{d} (-45%)	121 (-31%)	21 (-72%)	NIL
	10’	19.32±0.09^{e} (-58%)	82 (-54%)	11 (-83%)	NIL	12.78±0.06^{e} (-71%)	96 (-47%)	9 (-88%)	NIL
	12’	6.31±0.02^{f} (-86%)	31 (-83%)	6 (-91%)	NIL	8.30±0.03^{f} (-81%)	42 (-76%)	4 (-95%)	NIL
Microwave treated overnight soaked seeds	Raw	46.40±0.56^{a}	180	66	14	43.70±0.68^{a}	176	74	10
	2 minutes	37.41±0.18^{b} (-19%)	151 (-16%)	42 (-36%)	7 (-50%)	35.20±0.24^{b} (-19%)	132 (-25%)	41 (-45%)	4 (-60%)
	4’	28.30±0.18^{c} (-39%)	102 (-43%)	20 (-70%)	NIL	24.16±0.19^{c} (-45%)	102 (-42%)	29 (-61%)	NIL
	8’	18.76±0.09^{d} (-60%)	60 (-67%)	11 (-83%)	NIL	17.50±0.09^{d} (-60%)	74 (-58%)	11 (-85%)	NIL
	10’	7.33±0.02^{e} (-84%)	21 (-88%)	4 (-94%)	NIL	8.36±0.03^{e} (-81%)	31 (-82%)	6 (-92%)	NIL
	12’	NIL	7 (-96%)	NIL	NIL	NIL	11 (-94%)	NIL	NIL

Means±SE (N=3) means in the column with unlike superscript differ significantly ($p < 0.05$), Values in the parentheses denote the loss or gain in percentage, *values are means of two determinations

Table 4. Effect of Microwave on Oligosaccharides of two accessions *Mucuna pruriens* var. *utilis* (g 100 g^{-1})

Variants	Time duration (in minutes)	Accessions					
		M. pruriens **var.** *utilis* (white coloured seed coat)			*M. pruriens* **var.** *utilis* (black coloured seed coat)		
		Raffinose	**Stachyose**	**Verbascose**	**Raffinose**	**Stachyose**	**Verbascose**
Microwave treated raw seeds	Raw	1.06±0.06^{a}	1.24±0.05^{a}	3.48±0.21^{a}	0.94±0.03^{a}	1.22±0.01^{a}	4.16±0.14^{a}
	2 minutes	0.88±0.02^{b} (-17%)	1.11±0.06^{b} (-10%)	3.10±0.17^{b} (-11%)	0.71±0.02^{b} (-24%)	1.09±0.04^{b} (-11%)	3.91±0.30^{a} (-6%)
	4’	0.64±0.02^{c} (-40%)	0.86±0.04^{c} (-31%)	2.76±015^{b} (-21%)	0.50±0.04^{c} (-47%)	0.82±0.03^{c} (-33%)	3.20±0.28^{b} (-23%)
	8’	0.47±0.01^{d} (-56%)	0.54±0.02^{d} (-56%)	2.31±0.01^{c} (-34%)	0.33±0.04^{d} (-65%)	0.61±0.02^{d} (-50%)	2.74±0.18^{b} (-34%)
	10’	0.23±0.01^{e} (-78%)	0.31±0.01^{e} (-75%)	1.94±0.10^{d} (-44%)	0.18±0.01^{e} (-80%)	0.38±0.02^{e} (-69%)	2.10±0.16^{c} (-50%)
	12’	0.13±0.01^{f} (-88%)	0.17±0.01^{f} (-86%)	1.54±0.06^{e} (-56%)	NIL	0.16±0.01^{f} (-87%)	1.31±0.06^{d} (-69%)
Microwave treated overnight soaked seeds	Raw	1.06±0.06^{a}	1.24±0.05^{a}	3.48±0.21^{a}	0.94±0.03^{a}	1.22±0.01^{a}	4.16±0.14^{a}
	2’ minutes	0.76±0.12^{b} (-28%)	0.94±0.10^{b} (-24%)	3.01±0.27^{b} (-14%)	0.60±0.02^{b} (-36%)	0.94±0.04^{b} (-23%)	3.50±0.27^{b} (-16%)
	4’	0.52±0.10^{c} (-51%)	0.66±0.06^{c} (-47%)	2.44±0.13^{c} (-30%)	0.41±0.01^{c} (-56%)	0.71±0.02^{c} (-41%)	3.01±0.18^{c} (-28%)
	8’	0.36±0.02^{cd} (-66%)	0.30±0.04^{d} (-76%)	1.94±0.07^{d} (-44%)	0.21±0.01^{d} (-78%)	0.31±0.01^{d} (-75%)	2.34±0.12^{d} (-44%)
	10’	0.18±0.04^{de} (-83%)	0.14±0.01^{e} (-89%)	1.21±0.06^{e} (-65%)	0.09±0.01^{e} (-90%)	0.11±0.01^{e} (-91%)	1.66±0.10^{e} (-60%)
	12’	NIL	NIL	0.94±0.04^{e} (-73%)	NIL	NIL	1.11±0.11^{f} (-73%)

Means±SE (N=3) means in the column with unlike superscript differ significantly ($p < 0.05$), values in the parentheses denote the loss or gain in percentage

Microwave causes an acute heat stress which completely destroys the hydrogen cyanide and total oxalate. Montogomery (1980) reported that liberated hydrogen cyanide is lost by volatilization and cyanide is rapidly converted in to thiocyanide compounds.

Trypsin inhibitor activity and phytohaemagglutinating activity

From the data presented in the Table 3, it is observed that the level of trypsin inhibitor activity in the presently investigated raw and overnight soaked seeds of both the accessions of *Mucuna pruriens* var. *utilis* showed time duration dependent decline when treated with microwave. Dielectric heating (Borchers *et al*., 1972; Simovie *et al*., 1972), infra-red cooking (Faber and Zimmerman, 1973) and microwave radiation (Wing and Alexander, 1971) were also reported to inactivate the inhibitor, causing improvement in the nutritional quality of soybean. Hernandez-Infante *et al*. (1998) reported that microwave cooking destroyed trypsin inhibitors to a degree similar to that observed in six legumes cooked using the conventional method. Alajaji and El-Adway (2006) reported that microwave cooking treatment decrease the trypsin inhibitor activity (80.50%) in the seeds of *Cicer arietinum*.

In the present study microwave treatment on raw and overnight soaked seeds showed maximum reduction in phytohaemagglutinating activity. Khalil and Mansour (1995) reported that, boiling and autoclaving of faba bean completely eliminated the phytohaemagglutinating activity. Hernandez-Infante *et al*. (1998) reported that microwave cooking of common bean failed to destroy the phytohaemagglutinating activity.

Oligosaccharides

Table 4 presents the data on the effect of Microwave on oligosaccharides. In the present investigation, raw and overnight soaked seeds of different accessions of *Mucuna pruriens* var. *utilis*, oligosaccharides like raffinose, stachyose and verbascose were significantly ($p< 0.05$) reduced on microwave treatment. Alajaji and El-Adway (2006) reported that microwave treatment reduced the raffinose, stachyose and verbascose in *Cicer arietinum*. Rao and Vakil (1983) reported that irradiation of green gram at 2.5 kGy reduced the level of oligosaccharides by 20% including a 50% reduction in stachyose and raffinose; the two most gas forming sugars. Kidney bean, irradiated at 3 kGy and germinated for 48h, lost nearly 57, 70, and 32% of their raffinose, stachyose, verbascose contents respectively. The decrease in oligosaccharide content was accompanied by an increase in the concentration of total soluble sugars (Ghazy, 1990).

Conclusion

Currently- employed conventional processing methods are relatively ineffective for the reduction or removal of antinutritional factors. In this context, microwave treatment is possible alternative and additional processing technique for reducing both heat stable and heat labile antinutrients. In particular, microwave levels (i.e. increasing treatment time duration) seem to be effective in inactivating antinutrients such as phytic acid, hydrogen cyanide, total oxalate, trypsin inhibitor activity, oligosaccharides and phytohaemagglutinating activity. And it may be possible to set the microwave to specific time duration to retain optimum levels of L-dopa in *Mucuna* seeds for desired nutritional or pharmaceutical purposes.

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