EFFECT OF UV TREATMENT ON THE ANTI NUTRITIONAL FACTORS OF TWO ACCESSIONS OF VELVET BEAN, *Mucuna pruriens* (L.) DC var. *utilis* (Wall.ex Wight) Bak. ex Burck

[EFECTO DEL TRATAMIENTO UV SOBRE LOS FACTORES ANTINUTRICIONALES DE DOS VARIEDADES DE FRIJOL TERCIOPELO *Mucuna pruriens* (L.) DC var. *utilis* (Wall.ex Wight) Bak. ex Burck]

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SUMMARY

The effect of UV radiation on the antinutritional factors such as, total free phenolics, tannin, L-dopa, phytic acid, hydrogen cyanide, total oxalate, trypsin inhibitor activity, oligosaccharides and phytohaemagglutinating activity in the seeds of two accessions of velvet bean, *Mucuna pruriens* var. *utilis* collected from Karaiyar and Servalaru, Tirunelveli district, Tamil Nadu, were investigated. UV treatment on overnight soaked seeds showed increase in the level of total free phenolics and tannins. UV treated raw seeds for 20 minutes reduced the level of L-dopa content of *Mucuna pruriens* var. *utilis* white coloured seed coat by 42%, black coloured seed coat by 44% whereas; UV treatment on overnight soaked seeds showed significant (p<0.05) increase in the level of L-dopa. Decrease in the levels of phytic acid**,** hydrogen cyanide and total oxalate in both UV treated raw and overnight soaked seeds as time interval increases. UV treatment (60-90 minutes) completely eliminated the trypsin inhibitor activity in both raw and overnight soaked seeds. Both the studied samples completely eliminated the phytohaemagglutinating activity with respect to the erythrocytes of 'B' and 'O' blood groups at the longest exposure. Oligosaccharides like raffinose, stachyose and verbascose were significantly (p< 0.05) reduced on UV treatment.

Key words: UV radiation; antinutritional factors; L-dopa; accessions; velvet bean.

RESUMEN

Se evaluó el efecto de la radiación UV sobre el contenido de factores antinutricionales como fenoles totales libres, taninos, L-dopa, ácido fítico, HCN, oxalato, inhibidores de tripsina, oligosacáridos y fitohemaglutininas de las semillas de dos variedades de *Mucuna pruriens* var. *utilis* colectada en los distritos de Karaiyar, Servalaru, Tirunelveli, Tamil Nadu, India. El tratamiento UV y el remojo por una noche de las semillas mostró un incremento en los fenoles totales y taninos. El tratamiento UV por 20 min redujó el nivel de L-dopa de la M. pruriens de semilla blanca en 42% y en la semilla negra en un 44%. El tratamiento UV en semillas remojadas por una noche incrementó (P<0.05) el nivel de L-dopa. Se observó un decremento en los niveles de ácido fítico, HCN y oxalatos tanto en las semillas crudas tratadas con UV como en las remojadas por una noche conforme el intervalo de tiempo fue mayor. El tratamiento UV (60-90 min) eliminó completamente los inhibidores de tripsina en semillas crudas y remojadas por una noche. También eliminó la actividad fitohemaglutinante con respecto a los eritrocitos de los grupos B y O con el mayor tiempo de exposición. Oligosacaridos como rafinosa, estaquiosa y verbascosa fueron reducidas por el tratamiento UV (P<0.05).

Palabras clave: Radiación UV; factores antinutricionales; L-dopa; variedades; frijól terciopelo.

INTRODUCTION

Legume pastures have been projected as an economically viable alternative for proteins and calories in developing countries (Famurewa and Raji, 2005). The partial replacement of animal foods with legumes has been shown to improve nutritional status (Guillion and Champ, 1996) due to lower cholesterol level in plant foods. Among the underutilized legumes, seeds of *Mucuna*

constitute source of food for tribals and some ethnic groups of Asia and Africa (Dako and Hill, 1977; Iyayi and Egharevba, 1998). The velvet bean, *Mucuna pruriens* var. *utilis* is one of the under-utilized legumes, consumed by a South Indian hill tribe, the Kanikkars, after repeated boiling (Janardhanan and Lakshmanan, 1985). Recently, Dravidan tribes in the Tirunelveli district have started cultivating it for use as a pulse (Janardhanan *et al*., 2003). Various preparation of this bean is also traditionally consumed in several parts of Srilanka by low-income groups (Ravindran and Ravindran, 1988). In parts of Asia, and Africa, the seeds are roasted and eaten (Haq, 1983). Despite, seeds of *Mucuna* bean is potential source of protein, minerals, dietary fibre and vitamins; they are underutilized because of the presence of antinutritive agents.

Consumption of unprocessed seeds of *Mucuna* is known to cause various ailments in human and the animals. 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). A clinical study confirmed the efficacy of the seeds in the management of Parkinson's disease by virtue of their L-dopa content (Bell *et al*., 1971; Manyam, 1995). The phytic acid of *Mucuna* possesses antioxidant, anticarcinogenic and hypoglycemic activities (Graf and Eaton, 1990; Rickard and Thompson, 1997; Shamsuddin *et al*., 1997) and they are effective at low concentrations. To increase biological benefit and ease of digestion and decrease antinutrient compounds in legumes, traditional procedures, heating or blanching, soaking, roasting are generally used. Most of the methods employed were based on the use of water, chemicals and thermal treatments (Bressani, 2002; Diallo and Berhe, 2003; Gilbert, 2002). The objective of the present study was to investigate the effect of UV 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 seedcoat) from Servalaru in the Tirunelveli district, South Eastern slopes of 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

Dry seeds(raw seeds) of both the accessions of presently investigated *Mucuna pruriens* var. *utilis* were placed in petri dishes and exposed under the UV-B light (15 W Philips lamp, Tempo Instruments and Equipment Pvt. Ltd, Bombay) for 10, 20, 30, 45, 60 and 90 minutes. The above said treatments were also given to seeds of both the investigated accessions of *Mucuna pruriens* var. *utilis* that soaked in distilled water for an overnight (Overnight soaked seeds). The overnight soaked seeds are dried at 55^{0} C. Both the raw and 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 and UV treated seed samples.

Analysis 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 by following the method of Somiari and Balogh (1993). Five grams 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 13hr 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 5mL 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, 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 phyto-haemagglutinating

(Lectin) activity

Lectin activity was determined by the method of Almedia *et al.* (1991). One g of air-dried seed flour was stirred with 10mL of 0.15N sodium chloride solution for 2hours and the pH was adjusted to 4.0. The contents were centrifuged at 10,000 X g for 20min. and the supernatants were collected separately. The protein content was estimated by the 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 3min at low speed (3,000g 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 (50µl) 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 (25µl) 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 heamagglutinin that will produce positive evidence of agglutination of 25µl of a blood group erythrocyte after 3hr incubation at room temperature. The phytoheamagglutinating activity was expressed as heamagglutinating 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 UV 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 UV treatment on total free phenolics, tannin, and L-dopa. In the present study, UV treatment for 30 minutes on the raw seeds showed a significant ($p< 0.05$) reduction in the total free phenolics; whereas UV treated on overnight soaked seeds showed a significant time duration dependent increase in total free phenolics. Similarly, UV treatment on raw seeds for 10 minutes showed maximum reduction in tannin content whereas, UV treated on overnight soaked seeds show a time duration dependent increase in both the accessions of *Mucuna pruriens* var. *utilis*. Shetty *et al*. (2002) found an increase in total free phenolics in germinated faba bean seeds on UV treatment. Winter and Roastas (2008) reported that, UV irradiated soya bean seeds showed increase in

accumulation of phenolics than that of non-irradiated seeds. Bhat *et al*. (2007) reported a dose-dependent increase in phenolic content in seeds of *Mucuna pruriens* irradiated with gamma radiation.

UV treatment for 20 minutes on the raw seeds showed a significant ($p< 0.05$) reduction in the content of L-dopa; whereas UV treated on overnight soaked seeds showed a significant time duration dependent increase in L-dopa of both the accessions of *Mucuna pruriens* var. *utilis*. Ultra Violet light induces the synthesis of phenylalanine–ammonia lyase an enzyme involved in the deamination of phenylalanine, the precursor of several phenolic compounds of plants and L-dopa (Liu and McClure, 1995).

Shetty *et al*. (2002) reported that, UV light has an impact on plant metabolism and development. Among different exposure time, the longest time exposure was effective and it increases the antioxidant activity of plant by enhancing the synthesis of metabolites of neutraceutically important factors such as phenol and L-dopa likely through the Pentose Phosphate Pathway (PPP).

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

Variants	Time durarion (in minutes)	Accessions					
		M. pruriens var. *utilis* (white coloured seed coat)			*M. pruriens* var. *utilis* (black coloured see dcoat)		
		Phenol	Tannin	L-dopa	Phenol	Tannin	L-dopa
UV 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}
	10minutes	2.58±0.19^{bc} (-30%)	0.05±0.01^{c} (-64%)	5.23±0.07^{c} (-31%)	2.25±0.09^{c} (-45%)	0.04±0.01^{c} (-78%)	5.58±0.19^{bc} (-30%)
	20'	2.28±0.10^{c} (-38%)	0.07±0.01^{bc} (-50%)	4.35±0.06^{e} (-42%)	1.92±0.11^{d} (-53%)	0.08±0.01^{c} (-56%)	4.58±0.12^{f} (-44%)
	30'	1.77±0.11^{d} (-57%)	0.09±0.01^{b} (-36%)	4.70±0.06^{d} (-38%)	1.56±0.08^{e} (-62%)	0.13±0.01^{b} (-28%)	4.86±0.09^{ef} (-39%)
	45'	2.85±0.08^{b} (-23%)	0.12±0.01^{a} (-14%)	4.88±0.12^{d} (-35%)	3.02±0.09^{b} (-26%)	0.17±0.01^{a} (-6%)	5.16±0.04^{de} (-35%)
	60'	3.79±0.08^{a} (+3%)	0.19±0.01^{d} (+36%)	5.23±0.08^{c} (-31%)	3.88±0.09^{a} (-4%)	0.21±0.02^{ad} (+17%)	5.50±0.08^{cd} (-31%)
	90'	3.92±0.07^{a} (+7%)	0.22±0.01^{e} (+57%)	5.72±0.12^{b} (-24%)	4.68±0.06^{f} (+15%)	0.23±0.02^{d} (+28%)	6.07±0.07^{b} (-23%)
UV 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}
	10minutes	3.71±0.07^{a} (+0.8%)	0.20±0.01^{b} (+43%)	8.02±0.04^{b} (+6%)	4.12±0.08^{ab} (+1%)	0.25±0.02^{ab} (+39%)	8.51±0.15^{b} (+7%)
	20'	3.80±0.08^{ab} (+3%)	0.26±0.01^{c} (+86%)	8.14±0.03^{b} (+8%)	4.21±0.09^{ab} (+4%)	0.31±0.03^{bc} (+72%)	8.71±0.15^{b} (+10%)
	30'	3.98±0.06^{bc} (+8%)	0.29±0.01^{c} (+107%)	8.63±0.05^{c} (+14%)	4.35±0.12^{ab} (+7%)	0.35±0.02^{cd} (+94%)	8.87±0.11^{b} (+12%)
	45'	4.07±0.07^{cd} (+11%)	0.36±0.01^{d} (+157%)	10.7±0.06^{d} (+42%)	4.41±0.11^{b} (+9%)	0.39±0.02^{de} (+117%)	9.66± 0.07^{c} (+21%)
	60'	4.23±0.06^{de} (+15%)	0.43±0.02^{e} (+207%)	10.9±0.03^{d} (+44%)	5.03±0.04^{c} (+24%)	0.43±0.02^{ef} (+139%)	10.36±0.10^{d} (+30%)
	90'	4.43±0.13^{e} (+20%)	0.50±0.03^{f} (+257%)	8.64±0.18^{c} (+14%)	5.49±0.11^{d} (+35%)	0.49±0.03^{f} (+172%)	8.63±0.06^{b} (+9%)

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

Recently, phenolics have been suggested to exhibit health related functional properties such as, anticarcinogenic, antiviral, antimicrobial, antiinflammatory, hypertensive and antioxidant activity (Shetty, 1997). L-dopa enriched *Mucuna* may also have relevance for management of type-2 diabetes. Enhancement of antioxidant type phenolics metabolites is desirable to counter oxidative stress linked to type-2 diabetes and Parkinson's disease. Recently, there is an 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 (Randir *et al*., 2002).

Since , the UV treated *Mucuna* seeds in the present investigation have higher L-dopa and total free phenolics contents in an antioxidant rich background, they could be harvested for the use as a natural source of L-dopa and phenol for the pharmaceutical purposes.

Phytic acid, Hydrogen cyanide and Total oxalate

The levels of phytic acid, hydrogen cyanide and total oxalate (Table 2) of presently investigated raw and overnight soaked seeds of both the accessions of *Mucuna pruriens* var. *utilis* showed time duration dependent decline when treated with UV irradiation. Dry heat treatment has been shown to reduce phytic acid in *Mucuna* species to 47% - 36% (Siddhuraju *et al*., 1996).

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

Variants	Time durarion (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	Total oxalate
UV 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}
	10minutes	450.18±0.30^{b} (-7%)	0.14±0.01^{ab} (-13%)	0.10±0.01^{ab} (-17%)	612.10±0.78^{b} (-3%)	0.22±0.01^{ab} (-8%)	0.08±0.01^{a} (-11%)
	20'	424.00±0.18^{c} (-12%)	0.14±0.01^{ab} (-13%)	0.10±0.01^{ab} (-17%)	592.00±1.00^{c} (-7%)	0.22±0.01^{ab} (-8%)	0.08±0.02^{a} (-11%)
	30'	370.76±0.64^{d} (-23%)	0.11±0.01^{bc} (-31)	0.09±0.01^{bc} (-25%)	542.17±0.43^{d} (-15%)	0.20±0.01^{bc} (-17%)	0.08±0.01^{a} (-11%)
	45'	342.16±0.54^{e} (-29%)	0.10±0.01^{c} (-38%)	0.09±0.02^{bc} (-25%)	510.36±0.94^{e} (-20%)	0.20±0.01^{bc} (-17%)	0.07±0.02^{ab} (-22%)
	60'	320.47±0.81^{f} (-34%)	0.09±0.01^{cd} (-44%)	0.08±0.02^{bc} (-33%)	436.10±0.32^{f} (-31%)	0.18±0.01^{c} (-25%)	0.06±0.01^{ab} (-33%)
	90'	252.07±0.65^{g} (-48%)	0.06±0.02^{d} (-63%)	0.07±0.01^{c} (-42%)	370.62±1.29^{g} (-42%)	0.12±0.01^{d} (-50%)	0.04±0.01^{b} (-55%)
UV 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}
	10minutes	440.33±0.21^{b} (-9%)	0.11±0.01^{b} (-31%)	0.09±0.02^{b} (-25%)	600.36±0.75^{b} (-5%)	0.20±0.01^{b} (-17%)	0.07±0.01^{ab} (-22%)
	20'	414.20±0.45^{c} (-15%)	0.11±0.01^{b} (-31%)	0.09±0.02^{b} (-25%)	540.10±0.72^{c} (-15%)	0.18±0.01^{b} (-25%)	0.07±0.01^{ab} (-22%)
	30'	350.16±0.19^{d} (-28%)	0.10±0.01^{b} (-38%)	0.08±0.01^{b} (-33%)	510.20±0.43^{d} (-20%)	0.18±0.01^{b} (-25%)	0.06±0.01^{bc} (-33%)
	45'	320.26±0.32^{e} (-34%)	0.08±0.01^{bc} (-50%)	0.07±0.01^{bc} (-42%)	450.54±0.31^{e} (-30%)	0.10±0.01^{c} (-58%)	0.06±0.01^{bc} (-33%)
	60'	240.12±0.42^{f} (-50%)	0.06±0.01^{c} (-63%)	0.05±0.01^{c} (-58%)	360.24±0.60^{f} (-43%)	0.09±0.02^{cd} (-63%)	0.04±0.01^{c} (-55%)
	90'	210.46±0.69^{g} (-56%)	0.02±0.01^{d} (-88%)	0.05±0.01^{c} (-58%)	240.16±1.27^{g} (-62%)	0.06±0.01^{d} (-75%)	NIL

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

Highest level of phytic acid found in green gram and black gram cultivars is subsequently decreased due to various treatments; the highest reduction was achieved during pressure cooking treatment (Kakati *et al*., 2010). Decrease of phytic acid content has been attributed to low inosital and inosital phosphate by the action of free radicals generated during irradiation (De Bolland *et al*., 1975). Duodu *et al*. (1999) indicate that phytic acid dehydration by radiation is due to cleavage of phytic acid itself. Therefore, UV treatment was proved to be effective in lowering phytic acid level. It is observed that, the reduction of phytic acid in legume seeds during heat treatment may be partially due to the heat labile nature of phytic acid and the formation of insoluble complexes between phytates and other components (Udensi *et al*., 2007).

The level of hydrogen cyanide in both the accessions of investigated *Mucuna pruriens* var. *utilis* seems to be negligible when compared with lethal level of hydrogen cyanide (36 mg100 g^{-1}) (Oke, 1969). UV irradiation was very effective in reducing the level HCN in both accessions of *Mucuna pruriens* var. *utilis.* Montgomery (1980) reported that , liberated hydrogen cyanide is lost by volitization and cyanide is rapidly converted into thiocyanide compounds.

Trypsin inhibitor activity and Phyto-haemagglutinating activity

Table 3 presents the data on effect of UV on trypsin inhibitor activity and phytohaemagglutinating activity. The level of trypsin inhibitor activity in the presently investigated raw and overnight soaked seeds of *Mucuna pruriens* var. *utilis* showed a time duration dependent decline and complete elimination was observed at longest exposure (60-90 minutes). Mulimani and Paramjyothi (1993) reported that, soaked seeds of red gram treated with UV for 90 minutes, completely destroyed the trypsin inhibitor activity.

In the present study, UV treatment on raw and overnight soaked seeds showed a time duration dependent decrease in phytohaemagglutinating activity. UV treatment for 60-90 minutes completely eliminated the phytohaemagglutinating activity with respect to erythrocytes of 'B' and 'O' blood groups. Autoclaving for a shorter period (5-30minutes) completely destroyed the lectin activity (DeMuelenaere, 1964; Kakade and Evans, 1966; Kortt and Caldwell, 1985 and Tan *et al*., 1983). ManciniFilho *et al*. (1979) reported that ionizing radiation with dose of 50 kGy destroyed 50% of activity.

Oligosaccharides

Table 4 presents the data on effect of UV on oligosaccharides. In the present investigation, raw and overnight soaked seeds of both the accessions of *Mucuna pruriens* var. *utilis*, oligosaccharides like raffinose, stachyose and verbascose were significantly ($p< 0.05$) reduced on UV treatment. The treatment, autoclaving significantly reduced the content of raffinose, stachyose and verbascose compared to other cooking method (Vijayakumari *et al*., 1995). This reduction in the content of oligosaccharides as a result of cooking has been probably due to heat, hydrolysis of complex oligosaccharides to simple disaccharides or monosaccharides to form other compounds (Onigbinde and Akinyele, 1983).

CONCLUSION

Similar to other unconventional methods such as gamma radiation, electron beam irradiation, microwave heating and ionizing radiation, UV irradiation is also proved to be very effective processing method in reducing/eliminating the antinutritional factors such as phytic acid, hydrogen cyanide, total oxalate, trypsin inhibitors, oligosaccharides and phytohaemagglutinating activity. To eliminate/enhance phenolics and L-dopa from the nutritional/pharmaceutical point of view, the UV treatment is desirable and comparable with earlier reports. It may be possible to set specific time duration to retain optimum level of total free phenolics, and L-dopa in *Mucuna* seeds for desired nutritional or pharmaceutical purposes. This study provides the basis for further research on improving the health promoting value of legume in general and *Mucuna* specifically.

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

Variants	Time duration (minutes)	Accessions: *M. pruriens* var. *utilis* (white coloured seedcoat) TIA (TIU mg^{-1} protein)	Phyto-haemagglutinating activity* [Hu mg^{-1} protein] A	B	O	*M. pruriens* var. *utilis* (black coloured seedcoat) TIA (TIU mg^{-1} protein)	Phyto-haemagglutinating activity* [Hu mg^{-1} protein] A	B	O
UV treated raw seeds	Raw	46.40±0.56[a]	180	66	14	43.70±0.68[a]	176	74	10
	10minutes	32.10±0.19[b] (-31%)	166 (-8%)	52 (-21%)	9 (-36%)	31.20±0.24[b] (-29%)	152 (-14%)	61 (-18%)	6 (-40%)
	20'	26.40±0.12[c] (-43%)	148 (-18%)	41 (-38%)	9 (-36%)	28.10±0.15[c] (-36%)	131 (-26%)	48 (-35%)	4 (-60%)
	30'	18.33±0.08[d] (-60%)	122 (-32%)	34 (-48%)	5 (-64%)	14.34±0.08[d] (-67%)	104 (-41%)	30 (-59%)	2 (-80%)
	45'	7.41±0.01[e] (-84%)	92 (-49%)	20 (-70%)	4 (-71%)	7.21±0.06[e] (-84%)	84 (-52%)	24 (-68%)	NIL
	60'	2.36±0.04[f] (-95%)	61 (-66%)	9 (-86%)	NIL	NIL	62 (-65%)	11 (-85%)	NIL
	90'	NIL	32 (-82%)	NIL	NIL	NIL	28 (-84%)	4 (-95%)	NIL
UV treated overnight soaked seeds	Raw	46.40±0.56[a]	180	66	14	43.70±0.68[a]	176	74	10
	10minutes	30.30±0.17[b] (-35%)	151 (-16%)	52 (-21%)	9 (-36%)	29.10±0.19[d] (-33%)	143 (-19%)	50 (-32%)	4 (-60%)
	20'	24.10±0.15[c] (-48%)	132 (-27%)	41 (-38%)	7 (-50%)	22.42±0.06[c] (-49%)	114 (-35%)	32 (-57%)	2 (-80%)
	30'	10.41±0.17[d] (-78%)	108 (-40%)	32 (-52%)	2 (-86%)	10.31±0.13[d] (-76%)	94 (47%)	16 (-78%)	NIL
	45'	4.36±0.04[e] (-91%)	82 (-54%)	16 (-76%)	NIL	2.36±0.01[e] (-95%)	63 (-64%)	8 (-89%)	NIL
	60'	NIL	40 (-77%)	4 (-94%)	NIL	NIL	31 (-82%)	2 (-97%)	NIL
	90'	NIL	18 (-90%)	NIL	NIL	NIL	14 (-92%)	NIL	NIL

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

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

Variants	Time duration (minutes)	Accessions					
		M. pruriens var. *utilis* (white coloured seedcoat)			*M. pruriens* var. *utilis* (black coloured seedcoat)		
		Raffinose	Stachyose	Verbscose	Raffinose	Stachyose	Verbascose
UV 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}
	10minutes	0.92±0.02^{b} (-13%)	1.12±0.04^{b} (-10%)	3.40±0.08^{a} (-2%)	0.89±0.01^{a} (-5%)	1.16±0.02^{a} (-5%)	4.10±0.08^{ab} (-1%)
	20'	0.90±0.01^{bc} (-15%)	0.98±0.02^{c} (-21%)	3.26±0.10^{a} (-6%)	0.82±0.02^{b} (-13%)	1.01±0.03^{b} (-17%)	3.86±0.08^{b} (-7%)
	30'	0.84±0.04^{c} (-21%)	0.98±0.02^{c} (-21%)	3.11±0.09^{b} (-11%)	0.71±0.02^{c} (-24%)	0.96±0.06^{b} (-21%)	3.56±0.12^{c} (-14%)
	45'	0.66±0.02^{d} (-38%)	0.81±0.06^{d} (-35%)	2.84±0.07^{c} (-16%)	0.62±0.03^{d} (-34%)	0.72±0.07^{c} (-41%)	3.04±0.05^{d} (-26%)
	60'	0.62±0.01^{de} (-42%)	0.66±0.01^{e} (-47%)	2.66±0.05^{c} (-24%)	0.54±0.01^{e} (-43%)	0.66±0.05^{c} (-46%)	2.86±0.06^{d} (-31%)
	90'	0.56±0.01^{e} (-47%)	0.62±0.06^{e} (-50%)	2.21±0.04^{d} (-36%)	0.54±0.03^{e} (-43%)	0.61±0.04^{c} (-50%)	2.51±0.09^{e} (-40%)
UV 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}
	10minutes	0.94±0.02^{b} (-11%)	1.03±0.02^{b} (-17%)	3.10±0.16^{b} (-11%)	0.76±0.02^{b} (-19%)	1.02±0.02^{b} (-16%)	3.99±0.06^{a} (-4%)
	20'	0.82±0.01^{c} (-23%)	0.94±0.02^{c} (-24%)	2.92±0.18^{b} (-16%)	0.75±0.01^{b} (-20%)	0.94±0.03^{c} (-23%)	3.44±0.15^{b} (-17%)
	30'	0.76±0.01^{d} (-28%)	0.86±0.01^{d} (-31%)	2.44±0.06^{c} (-30%)	0.61±0.02^{c} (-35%)	0.86±0.01^{d} (-30%)	3.24±0.10^{b} (-22%)
	45'	0.61±0.01^{e} (-42%)	0.66±0.01^{e} (-47%)	2.10±0.04^{d} (-40%)	0.58±0.01^{c} (-38%)	0.74±0.01^{e} (-39%)	2.76±0.13^{c} (-34%)
	60'	0.61±0.02^{e} (-42%)	0.61±0.01^{f} (-51%)	1.72±0.03^{e} (-51%)	0.44±0.01^{d} (-53%)	0.62±0.02^{f} (-49%)	2.50±0.14^{c} (-40%)
	90'	0.44±0.01^{f} (-58%)	0.57±0.01^{f} (-59%)	1.24±0.03^{f} (-64%)	0.39±0.01^{e} (-59%)	0.40±0.01^{g} (-67%)	1.96±0.05^{d} (-53%)

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

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