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RAFFINOSE, STACHYOSE AND SUCROSE CONTENTS OF MUNG BEAN CULTIVARS DIFFERING IN SEED COAT COLOR FROM HYDERABAD-KARNATAKA REGION OF INDIA: EFFECT OF SOAKING AND GERMINATION

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ABSTRACT

Ten samples of mung bean (*Vigna radiata L.*) cultivars including three yellow cultivars representing a broad range of varietal characteristics from Hyderabad-Karnataka region of India were subjected to analysis for their oligosaccharide content. Further, the effect of soaking and germination on the levels of oligosaccharides (raffinose, stachyose and sucrose) contents were investigated. The level of sucrose, raffinose and stachyose in different cultivars of mung beans was found to be in the range from 0.84 to 1.71%, 0.62 to 1.05% and 1.75 to 3.57% respectively. The total level of sucrose, raffinose and stachyose was ranged between 3.48 to 5.37%. The processing methods like soaking and germination reduced oligosaccharide content considerably. Soaking for 12 hr led to the decrease in sucrose by 29 to 70%, raffinose by 19 to 74% and stachyose by 28 to 57% respectively. Compared with soaking, soaking followed germination appeared to be more efficient on the reduction of oligosaccharide content. Germination for 48 hr decreased raffinose and stachyose contents considerably, while sucrose increased by 25-52%.

INTRODUCTION

Legumes are good and relatively cheaper sources of proteins and carbohydrates and dry beans contain up to 60% carbohydrates mainly of starch (Reddy *et al.,* 1984). Among the soluble sugars, oligosaccharides of the raffinose family are found in most legumes and account for 31-76% of the total sugar (Reddy *et al.,* 1984; Champ *et al.,* 1986). The oligosaccharides are important constituents of a wide variety of grain legumes and vary in their distribution among leguminous species and different varieties of same legume (Naivikul and D'Appolonia, 1978; Rao and Belvady, 1978). Oligosaccharides are considered as antinutrients since they are thought to be the major producers of flatulence due to the absence of α-galactosidases in the human intestine, consequently undergoing bacterial fermentation (Reddy *et al.,* 1984; Jood *et al.,* 1986). The flatus forming factor is mainly found in the low molecular weight carbohydrate fractions of legumes, which contain primarily sucrose, raffinose and stachyose (Rackis *et al.,* 1970). The flatulence is the most common symptom associated with pulse consumption which is accompanied by abdominal pain, nausea, cramps, diarrhoea and discomfort (Tanaka *et al.,* 1975; Salunkhe and Kadam, 1989). There are several processing methods like cooking, fermentation, soaking and germination, which could reduce oligosaccharides content of legumes.

Mung bean (*Vigna radiata* L.) is the most important legume due to its high proteins and carbohydarates, and its protein quality is similar to or better than legumes such as chickpea, black gram, peas, pigeonpea etc. (Kataria *et al.,* 1989; Jood *et al.,* 1998). India is the major pulse producing country and among various pulses, mung bean stands third (Chandrasekhar and Ghosh, 2002). Mung bean is the principal crop from which edible bean sprouts, noodles and weaning foods are prepared (Singh, 1988; Singh *et al.,* 1989).

Available literature indicates that information on oligosaccharide content of mung bean cultivars differing in seed coat color from Hyderabad-Karnataka region of India is scarce to the best of our knowledge. In this context, the study was carried out to evaluate the sucrose, raffinose and stachyose content of green and yellow colored mung bean cultivars of H-K region. The effect of soaking and germination on sucrose, raffinose and stachyose content was evaluated and reported.

MATERIALS AND METHODS

The mung bean cultivars, PS-16, Pusabaisaki, GMBLN-2, TAP-7, Vaibhav, Hum-1 and China mung were procured from the Agriculture Research Station, Aland Road, Gulbarga, India. Three yellow colored cultivars were procured from farmers of Hyderabad-Karnataka region and designated as ALM-1, ALM-2, and ALM-3 respectively. The samples were cleaned and stored in the laboratory at 4°C. All chemicals used were of analytical grade.

Processing of mung bean

Soaking

Clean, whole dry seeds were soaked in distilled water (1:10 w/v) for a period of 12 hr at room temperature. The seeds were rinsed and dried at 60ºC in a hot air oven, powdered and analyzed for sucrose, raffinose and stachyose.

Germination

The seeds were first surface sterilized by treating with 0.1% mercuric chloride. The sterilized seeds were rinsed and soaked in distilled water for 12 hr at room temperature. The soaked seeds were placed on moist filter paper bed in Petriplates and incubated at room temperature (37ºC) in the dark. The seeds were moistened with distilled water at regular intervals. The germinated seeds were taken at 12, 24 and 48 hr and dried in an hot air oven at 60ºC to a constant weight and finely powdered. Then analyzed for sucrose, raffinose and stachyose.

Extraction of oligosaccharides

The mung bean seeds were milled to flour and the fraction, which passes through a 400mm sieve was used for the present study. Five gram of flour was extracted with 50mL of 70% aqueous ethanol and kept on orbital shaker at 130 rpm for 13 hr. The contents of the flask were filtered through Whatman No.1 filter paper and the residue was further washed with 25mL of 70% ethanol. The filtrate was evaporated in a rotary vacuum evaporator at 45ºC. The concentrated sugar syrup was dissolved in 5mL of distilled water.

TLC of oligosaccharides

A 100 microliters of the syrup was applied on chromatographic plates (20 cm x 20 cm) coated with microcrystalline cellulose powder. Plates were developed using solvent system n-propanol:ethylacetate:water (6:1:3). The developed plates were sprayed with 1% α-naphthol in ethyl alcohol containing 10% orthophosphoric acid to locate the sugars spots. For quantitative estimation, the area (2 cm x 3 cm) corresponding to each oligosaccharide on chromatogram was scraped off and extracted with 3 mL of distilled water for 1 hr. The extract was filtered through Whatman No.1 filter paper and the oligosaccharides in 1ml of filtrate were estimated by the method of Tanaka *et al.,* (1975).

RESULTS AND DISCUSSION

The levels of sucrose, raffinose and stachyose of mung bean cultivars are presented in the Table 1. The levels of sucrose ranged from 0.84 to 1.71%, raffinose from 0.62 to 1.05% and stachyose from 1.75 to 3.57%. The cultivar Hum-1 found to contain higher levels of sucrose 1.71%, while the cultivar Pusabaisaki contained lower concentration of sucrose 0.84%. The level of raffinose was highest in the cultivar TAP-7 (1.05%) and lowest in cultivar Pusabaisaki (0.62%). Cultivars Vaibhav and PS-16 showed highest (3.57%) and lowest (1.75%) levels of stachyose respectively. The total level of sucrose, raffinose and stachyose in different mung bean cultivars was ranged between 3.48-5.37. The oligosaccharide content of several legume seeds including mung bean has been reported earlier. The relative levels of sucrose, raffinose and stachyose of analysed mung bean samples are within the range presented by others (Naivikul and D'Appolonia, 1978; Tanaka *et al.,* 1975; Trugo *et al.,* 1990). Stachyose was the major oligosaccharide followed by the sucrose and raffinose.

In house hold situations legumes are typically soaked in water at room temperature overnight (12-14 hr). Soaking of the seeds contributed significantly towards reduction of the oligosaccharide content in mung bean cultivars (Table 2). Twelve hours soaking in water led to a decrease of 29 to70% of sucrose, 19 to 74% of raffinose and 28 to 57% of stachyose. The highest and lowest reduction of sucrose was observed in ALM-3 and Hum-1, raffinose in TAP-7 and Pusabaisaki and stachyose in Vaibhav and Chinamung cultivars respectively. Similar results for reduction in sucrose, raffinose and stachyose in the soaked legumes have been reported earlier. Decrease of sucrose by 51%, raffinose by 37% and stachyose by 27% was reported in whole black gram seeds when soaked for 12 hrs in water (Girigowda *et al.,* 2005). Decrease of raffinose by 80% and stachyose by 45% was reported for 16 hr soaked soy bean (Mulimani *et al.,* 1997). Vijayakumari *et al.,* (1996) reported a significant reduction in the levels of stachyose followed by raffinose contents in the seeds of *Mucuna monosperma* DC, during soaking in both distilled water and $NaHCO_3$ solution. Therefore soaking beans in water will reduce most of these sugars and the reduction in oligosaccharides during soaking could be attributed to leaching out in soaking water (Price *et al.,* 1988).

Germination is a process widely used for legumes to increase their palatability and nutritional value. During germination, the seeds undergo marked metabolic changes, and the reserve carbohydrates, including the oligosaccharides of the raffinose family are hydrolyzed (Adjei-Twum *et al.,* 1976). Germination appears to be a potential method to reduce flatulence caused by legumes since during these process α-galactosides will be

Table 1: Sucrose, Raffinose and Stachyose content of mung bean cultivars

S. N.	Cultivar	Oligosaccharides (g/100g)			
		Sucrose	Raffinose	Stachyose	Total
1	ALM-1	1.44 ± 0.03	0.65 ± 0.57	2.25 ± 0.11	4.64 ± 0.23
2	ALM-2	1.17 ± 0.26	0.97 ± 0.17	2.75 ± 0.43	4.89 ± 0.28
3	ALM-3	1.62 ± 0.05	0.69 ± 0.11	2.21 ± 0.04	4.52 ± 0.06
4	GMBLN-2	0.90 ± 0.33	0.81 ± 0.24	2.21 ± 0.15	3.92 ± 0.24
5	PS-16	0.96 ± 0.16	0.81 ± 0.09	1.76 ± 0.23	3.53 ± 0.16
6	Pusabaisaki	0.84 ± 0.10	0.62 ± 0.12	2.03 ± 0.53	3.48 ± 0.25
7	TAP-7	1.67 ± 0.02	1.05 ± 0.07	2.43 ± 0.14	5.15 ± 0.07
8	Hum-1	1.71 ± 0.35	0.93 ± 0.21	2.03 ± 0.02	4.66 ± 0.19
9	Vaibhav	1.02 ± 0.03	0.78 ± 0.03	3.57 ± 0.15	5.37 ± 0.07
10	China mung	1.47 ± 0.23	0.92 ± 0.11	2.66 ± 0.02	5.05 ± 0.12

Range 0.84-1.71; 0.62-1.05; 1.76-3.57; 3.48-5.37; * Values are mean ± standard deviation of three independent determinations.

Table 2: Effect of soaking on the Sucrose, Raffinose and Stachyose content of mung bean cultivars

Cultivar	Oligosaccharides (100g)											
	Dry seeds				12 hr soaking				% of Reduction			
	Sucrose	Raffinose	Stachyose	Total	Sucrose	Raffinose	Stachyose	Total	Suc	Raff	Stac	Total
ALM-1	1.44± 0.03	0.65± 0.57	2.25± 0.11	4.64± 0.23	0.78± 0.09	0.41± 0.31	1.58± 0.02	2.77± 0.14	45	36	29	40
ALM-2	1.17± 0.26	0.97± 0.17	2.75± 0.43	4.89± 0.28	0.83± 0.12	0.27± 0.23	1.94± 015	3.04± 0.16	29	72	29	37
ALM-3	1.62± 0.05	0.69± 0.11	2.21± 0.04	4.52± 0.06	0.48± 0.02	0.30± 0.13	1.35± 0.01	2.13± 0.05	70	56	38	52
GMBLN-2	0.90 ± 0.33	0.81± 0.24	2.21± 0.15	3.92± 0.24	0.39± 0.11	0.23± 0.26	1.23± 0.21	1.85± 0.19	56	71	44	52
PS-16	0.96± 0.16	0.81± 0.09	1.76± 0.23	3.53± 0.16	0.63± 0.12	0.54± 0.02	1.20± 0.37	2.37± 0.17	34	33	31	32
Pusabaisaki	0.84± 0.10	0.62± 0.12	2.03± 0.53	3.48± 0.25	0.53± 0.61	0.50± 0.17	1.23± 0.13	2.26± 0.30	36	19	39	35
TAP-7	1.67± 0.02	1.05± 0.07	2.43± 0.17	5.15± 0.08	0.71± 0.28	0.27± 0.09	1.35± 0.05	2.33± 0.14	57	74	44	53
Hum-1	1.71± 0.35	0.93± 0.21	2.03± 0.02	4.66± 0.19	1.20± 0.12	0.54± 0.43	0.99± 0.11	2.73 ± 0.22	29	41	51	41
Vaibhav	1.02± 0.03	0.78± 0.03	3.57± 0.15	5.37± 0.07	0.69± 0.04	0.56± 0.25	1.53± 0.42	2.78± 0.23	32	28	57	48
China mung	1.47 ± 0.23	0.92± 0.11	2.66± 0.02	5.05± 0.12	1.02± 0.01	0.53± 0.23	1.89± 0.07	3.44± 0.10	30	42	28	31

* Values are mean ± standard deviation of three independent determinations.

Table 3: Effect of germination on the Sucrose, Raffinose and Stachyose content of mung bean cultivars

Cultivar	Oligosaccharides (100g)											
	12 hrs Germination				24 hrs Germination				48 hrs Germination			
	Sucrose	Raffinose	Stachyose	Total	Sucrose	Raffinose	Stachyose	Total	Sucrose	Raffinose	Stachyose	Total
ALM-1	2.10± 0.02	0.48± 0.12	0.72± 0.09	3.30± 0.07	1.32± 0.45	0.18± 0.23	0.62± 0.11	2.12± 0.26	2.58± 0.14	0.11± 0.27	0.26± 0.02	2.94± 0.14
ALM-2	2.13± 0.15	0.49± 0.09	1.23± 0.56	3.86± 0.26	1.58± 0.03	0.19± 0.07	0.95± 0.33	2.72± 0.14	2.46± 0.11	0.11± 0.19	0.81± 0.10	3.38± 0.13
ALM-3	1.53± 0.12	0.41± 0.26	1.00 ± 0.02	2.94± 0.13	0.84± 0.08	0.15± 0.13	0.56± 0.17	1.55± 0.12	2.16± 0.01	0.08± 0.15	0.53± 0.04	2.76± 0.06
Vaibhav	1.83± 0.03	0.65± 0.11	0.86± 0.45	3.33± 0.19	1.46± 0.36	0.27± 0.01	0.74± 0.07	3.47± 0.14	2.10± 0.61	0.08± 0.18	0.41± 0.11	2.58± 0.30
Hum-1	1.53± 0.13	0.49± 0.49	0.68± 0.23	2.70± 0.28	1.20 ± 0.05	0.15± 0.34	0.63± 0.13	1.98± 0.17	2.65± 0.02	0.12± 0.09	0.54± 0.17	2.31± 0.09

degraded producing available sugars (King and Puwastien, 1987). Germination also resulted in a significant reduction of raffinose family sugars in mung bean cultivars (Table 3). As the period of germination was prolonged, *i.e.* from 0-12, 12-24, and 24-48 hr, significant and successive reduction in α-galactosides was observed. A loss of 18-49% of raffinose, 54-76% of stachyose occurred during 12hr germination which was enhanced further with rise in the period of germination. After 48 hr of sprouting, decrease in raffinose by 84-90% and stachyose by 70-89% was observed. Highest loss of raffinose and stachyose were observed in Vaibhav, while less decreases were observed in ALM-1 and ALM-2 respectively after 48 hr germination. When compared to soaking, germination for 48 hr seemed to be having the most pronounced effect on decreasing the raffinose and stachyose content of the mung bean seeds. Diminishing effect of germination of legume seeds on raffinose and stachyose have been reported by several authors. Trugo *et al.,* (1990) reported a considerable reduction in α-galactosides (64% for raffinose and 79% for stachyose) after the third day of germination. Complete disappearance of stachyose and raffinose in cowpea, pea, black gram, pigeon pea and soyabean after 48 hr germination was reported (Sampath *et al.,* 2008). Reddy and Salunkhe, 1980 reported complete disappearance of raffinose and stachyose in black gram after 48 hr germination which may be due to the hydrolysis of oligosaccharides by α-galactosidase enzyme. Breakdown of raffinose family oligosaccharides by active α-galactosidases in pea seeds during germination and post-germination events was reported (Blochl *et al.,* 2008). In our study we observed that 48 hr germination produced a considersble reduction in raffinose and stachyose while, sucrose increased by 25-52%. Similar observations were made by several authors. The reduction of á-galactosides was followed by an increase of about 40% of sucrose after the third day of germination in *Phaseolus vulgaris* (Trugo *et al.,* 1990). Reddy and Salunkhe (1980) reported an increase in sucrose content in black gram during the first 24 hr of germination and which remained fairly constant thereafter. Increase in sucrose levels during early days of germination was observed in *Schizolobium parahyba* (Magalhaes *et al.,* 2009). We have observed an initial increase in sucrose content upto 12 hr of germination, followed by decrease after 24 hr

and again increase in levels after 48 hr of germination. The increase in sucrose content during germination was a consequence of the seed metabolism which needs available sugars as energy sources (King and Puwastien, 1987). The oligosaccharides sucrose, raffinose and stachyose are present predominantly in mung bean, and are thought to be the major producers of flatulence when these foods are consumed. Soaking and germination is an integral part of traditional methods of processing mung bean seeds in India, thus offers the dual advantage of saving energy costs by shortening cooking time as well as reducing certain flatus producing oligosaccharides. Such treatments could enhance the utilization of mung bean and serve as potential food source especially for infants, pre-school children and pregnant and lactating women and old age people, without flatulence obstacles compared to raw seeds.

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