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Laboratory experiments illustrating evaluation of Raffinose family oligosaccharides of mung bean (*Phaseolus aureus* L.) cultivars

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Abstract

The two different laboratory experiments illustrate the evaluation of raffinose family oligosaccharides for the graduate and post graduate students. The thin layer chromatography (TLC) method is the simple and inexpensive method, while high performance liquid chromatography (HPLC) is the most precise and sensitive technique, useful for the both graduate and post graduate students. Raffinose family oligosaccharides (RFOs) are important constituents of a wide variety of grain legumes and are thought to be the major producers of flatulence. Recent researches showed their beneficial effects also. Mung bean (*Phaseolus aureus* L.) is the principal crop from which edible bean sprouts, noodles and weaning foods are prepared. Two mung bean cultivars analyzed for oligosaccharide content by two different methods; TLC and HPLC, results were compared and discussed. These experiments are mainly intended for the students of plant biochemistry and nutrition.

Key words: TLC; HPLC; Mung bean; RFOs.

Abbreviations: TLC-Thin layer chromatography; HPLC-High performance liquid chromatography; RFOs-Raffinose family oligosaccharides;

Introduction

Plant carbohydrates are synthesized during photosynthesis and serve as chief source of energy for plants and as well as animals. Among plants, legumes are good and relatively cheaper sources of carbohydrates, proteins and minerals. Legumes 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). α-Galactosides, also called as raffinose family oligosaccharides (RFOs), belong to low molecular weight, non-reducing sugars, soluble in water and are widely distributed in the plant kingdom. Raffinose (trimer) is a representative of this group (Fig.1). Apart from raffinose, this group also includes stachyose (tetramer), verbascose (pentamer), ajugose (hexamer), and unnamed so far longer-chain oligosaccharides up to nonasaccharide (Cerning-

Fig.1. Structure of sucrose and raffinose family

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Beroard and Filiatre-Verel, 1980). Oligosaccharides are considered as anti-nutrients 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. These oligosaccharides accumulate in the lower intestine and undergo anaerobic fermentation by bacteria with gas expulsion (H_2, CO_2, and traces of CH_4), causing the flatus effect and sometimes diarrhea and abdominal pain (Reddy and Salunkhe, 1980), and a factor which has tended to render legumes less acceptable. Although oligosaccharides have been considered as an anti-nutrient, but studies showed their beneficial effects also. Oligosaccharides have excellent physiological properties like anti-carcinogenic effect, anti-diabetic, anti-cardiovascular, higher rate of mineral absorption that are beneficial to human health (Masao, 2002).

Mung bean (*Phaseolus aureus* L) is also known as green gram. As a short season growing legume crop, mung bean has been widely cultivated for its edible seeds and pods in the tropical and subtropical regions of the world, especially in Asian countries including India. Mung bean is the principal crop from which edible bean sprouts, noodles and weaning foods are prepared.

The main objective of this experiment paper is to describe an easy and simple experiment to evaluate oligosaccharides by simple TLC and a precise and sensitive HPLC for the students of graduate and post graduate levels. Hence, the study was undertaken to provide a detail insight in to the RFO's taking example of two mung bean cultivars. Separate experiments were carried out to evaluate the oligosaccharide content by TLC and HPLC.

Methods

Plant material

The mung bean cultivar, China mung was procured from the Agriculture Research Station, Aland Road, Gulbarga, India. Another cultivar was procured from farmers of Hyderabad-Karnataka region of India and designated as ALM-1. The samples were cleaned and stored in the laboratory at 4°C. All chemicals used were of analytical grade.

Determination of oligosaccharides by TLC

Extraction of oligosaccharides for TLC

The mung bean seeds were milled to flour and the fraction, which passes through a 0.6mm sieve, was used for the present study. Five gram of flour was extracted with 50ml of 70% aqueous ethanol on orbital shaker at 130 rpm for 13-hours. The contents of the flask were filtered through Whatman No.1 filter paper and the residue was further washed with 25 mL of 70% ethanol. The filtrate was evaporated in a rotary vacuum evaporator at 45°C. The concentrated sugar syrup was dissolved in 5ml of distilled water.

Quantitative-TLC of oligosaccharides

A 100 µL of the syrup was applied as streak on chromatographic plates (20cm x 20cm) coated with microcrystalline cellulose powder. Similarly, 10 µL syrup was applied as spot. The authentic sugars were also spotted. Plates were developed using solvent system n-propanol:ethylacetate:water (6:1:3). The streak portion was covered and the spot potion was sprayed with 1% α-naphthol in ethyl alcohol containing 10% orthophosphoric acid to locate the sugar spots. For quantitative estimation, the area (2cm x 3cm) corresponding to each oligosaccharide of streak portion 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).

Determination of oligosaccharides by HPLC

Extraction of Oligosaccharides for HPLC

Extraction of oligosaccharides was conducted according to the procedure of Labaneiah and Luh (1981) and Sánchez-Mata *et al.* (1998), with some

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modifications. Ground mung bean flour (0.5 g) was blended in 80% ethanol (40 mL) for 45 min at 57 ± 2°C and added with another 80% ethanol (40 mL) with stirring. The homogenate was centrifuged for 30 min at 1,500*g*; supernatant was collected and filtered through filter paper (Whatman #40). The extract was concentrated using a rotary vacuum evaporator at 50°C by the removal of the ethanol. The concentrate was made up to 10 mL with distilled water. The extract was passed through a Sep-Pak C18 cartridge (Waters, Milford, MA) that was previously washed with methanol (5 mL) and water (5 mL). The eluent (3 mL) was mixed with acetonitrile (7 mL) and filtered through a 0.45-µm nylon membrane filter. Aliquot (90 µL) of filtered eluent was injected to HPLC to quantify oligosaccharide content.

HPLC Analysis

An HPLC system was equipped with an auto-sampler (model 1050, Hewlett Packard, Wilmington, DE), a gradient programmer (model 2360, ISCO, Lincoln, NE), a solvent pump (model 2350, ISCO), and a diode array detector (model 1040A, Hewlett Packard). The mobile phase was a mixture of acetonitrile and water (70:30). The flow rate was 1.0 mL/min. A carbohydrate analysis column (10 µm × 3.9 mm × 30 cm) filled with aminopropylmethylsilyl bonded amorphous silica was used. Commercially available standard raffinose and stachyose were purchased from Sigma Co. (St. Louis, MO). Verbascose was not available commercially. Verbascose was identified by comparing the retention time of verbascose, as reported by Gulewicz *et al.* (2000), with those collected from HPLC analysis of mung beans. The collected fraction of verbascose was evaporated to remove acetonitrile and water (70:30) by blowing a stream of nitrogen gas. The remaining verbascose was then weighed. Raffinose, stachyose, and verbascose were then dissolved in the mixture of acetonitrile and water (70:30) and injected into HPLC to acquire an equation between the concentration and the area in a HPLC chromatogram. The obtained equations were used to estimate oligosaccharide content in mung beans.

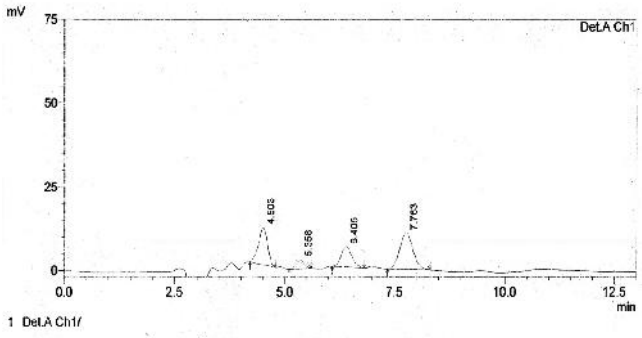

Fig.2a. HPLC analysis chromatogram of mung bean cultivars, ALM- 1 mung bean cultivar. Peaks from left to right represent Sucrose, Raffinose, Stachyose and Verbascose respectively

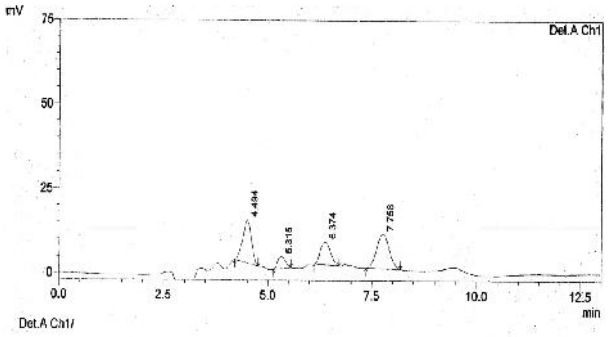

Fig.2b. HPLC analysis chromatogram of mung bean cultivars, China mung bean cultivar. Peaks from left to right represent Sucrose, Raffinose, Stachyose and Verbascose respectively

Results

The levels of sucrose, raffinose, stachyose and verbascose of mung bean cultivars are determined by TLC and HPLC are presented in the Table 1. The TLC analysis revealed the presence of RFOs and stachyose was the major oligosaccharide followed by the sucrose and raffinose. Verbascose was not detected. While the results of HPLC analysis revealed the presence of verbascose in both the cultivars. The total level of oligosaccharides in two different mung bean cultivars was ranged between 4.6 -5.42g/100g. By TLC method it was not possible to detect verbascose in mung bean samples. However, by HPLC, the most sensitive method than TLC, it was found to be present in both the cultivars. The sucrose, being the low molecular weight sugar, hence eluted first, and then followed by raffinose, stachyose and lastly high molecular weight

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Table 1. Sucrose, Raffinose, Stachyose and Verbascose content of mung bean cultivars by TLC and HPLC methods

Cultivar	Method	Oligosaccharides (g/100g)				Total
		Sucrose	Raffinose	Stachyose	Verbascose	
ALM-1	TLC	1.44±0.03	0.65±0.57	2.25±0.11	ND	4.64±0.23
	HPLC	1.71±0.13	0.43±0.05	1.28±0.07	1.98±0.12	5.42±0.11
China mung	TLC	1.47± 0.23	0.92±0.11	2.66±0.02	ND	5.05±0.12
	HPLC	1.64± 0.02	0.49±0.21	1.17±0.19	1.69±0.03	5.00±0.07

* Values are mean ± standard deviation of three independent determinations
ND- Not detected

varbascose (Fig. 2a & 2b). Ajugose which is higher oligosaccharide was not detected in both the mung bean cultivars possibly due to low concentration or absence.

Discussion

These results clearly indicate that HPLC method is accurate, precise and sensitive to evaluate RFOs, but it is quiet expensive. The comparison of results obtained by TLC and HPLC methods for oligosaccharides was carried out. The values of total oligosaccharide content from TLC and HPLC methods almost lies in the same range i.e. 4.64-5.05 and 5.00-5.42g/100g respectively. The levels of sucrose and raffinose were in the almost same range. But the stachyose content analysed by TLC was found to be slightly higher than the values obtained by HPLC method. Verbascose was detected by HPLC and was not detected by TLC due to very low concentrations. On the other hand TLC experiments are easy to handle, inexpensive.

Conclusions

It is concluded that the thin layer chromatography is the simple and inexpensive method, while HPLC analysis is the most precise and sensitive technique for the evaluation of RFOs. These experiments are mainly intended for the students of plant biochemistry and nutrition and can be assigned as a project for further investigations. The students can be assigned for initial screening of RFOs as a project by making group of three or four students for further investigations, such as;

1. To analyse RFOs from mung bean extract by changing different solvent systems for development of TLC plates.
2. To analyse RFOs from different legumes such as red gram, black gram, bengal gram, soybeans etc.
3. To further analyse RFOs from germinating seeds and the effect of different chemicals such as salts, pH values, temperature, osmoregulators, hormones, sugaras, etc. on RFOs during germination of mung bean seeds and report the difference found in the level of RFOs.

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References

1. Cerning-Beroard J and Filiatre-Verel A (1980) Characterisation and distribution of soluble and insoluble carbohydrates in lupin seeds. *Z Lebensm Unters Forsch*, *171,* 281-285.
2. Gulewicz P, Ciesiolka D, Frias J, Vidal-Valverde C, Frejnagel S, Trojanowska K and Gulewicz K (2000) Simple method of isolation and purification of α-galactosides from legumes. *J Agric Food Chem. 48,* 3120-3123.
3. Labaneiah MEO and Luh BS (1981) Changes of starch, crude fiber, and oligosaccharides in geminating dry beans. *Cereal Chem*. 58, 135-138.
4. Masao H (2002) Noval physiological functions of oligosaccharides. *Pure Appl Chem. 74,* 1271-1279.
5. Reddy NR and Salunkhe DK (1980) Changes in oligosaccharides during germination and cooking of black gram and a fermentation of black gram/rice blend. *Cereal Chem*. *57,* 356-360.

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6. Reddy NR, Pierson MD, Sathe SK and Salunkhe DK (1984) Chemical, nutritional and physiological aspects of dry bean carbohydrates-A Review. *Food Chem*, *13,* 25-68.

7. Sánchez-Mata MC, Peñuela-Teruel MJ, Cámara-Hurtado M, Díez-Marqués C and Torija-Isasa ME (1998) Determination of mono, di and oligosaccharides in legumes by high-performance liquid chromatography using an amino-bonded silica column. *J Agric Food Chem*, 46*,* 3648-3652.

8. Tanaka M, Thanankul D, Lee TC and Chechester CO (1975) A simplified method for the quantitative determination of sucrose, raffinose and stachyose in legume seeds. *J Food Sci*, 40*,* 1087-1088.