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Quantitative Determination of Flatulence Factors in Legume Seeds and Soy Products

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Abstract

The contents of flatulence factors such as raffinose and stachyose were determined by two-dimensional thin-layer chromatography using a guide-strip technique for some legume seeds and soy products consumed in Korea. Raffinose contents in bean samples were 1.34% in domestic soybean, 1.17% in imported soybean, 0.65% in kidney bean, 0.49% in red bean and 0.49% in mung bean. Stachyose contents were 4.1% in doemstic soybean, 3.6% in imported soybean, 3.0% in kidney bean, 2.9% in red bean and 1.9% in mung bean. The flatulence factors were not detected in soy sauce whereas soy paste contained 46.6 *mg*% of raffinose and none of stachyose. The contents of raffinose and stachyose were 26.7 *mg*% and 53.7 *mg*% in soy curd and 26.5 *mg*% and 41.7 *mg*%, respectively, in soy milk.

Introduction

Legume seeds and several soy products are commercially available for use in foods and the usage of soy products is gradually increasing in Korean diets.[1] Among legumes, soybean is mosty used as the raw material in making soy sauce, soy paste, soy milk, soy curd and in growing bean sprouts. Soy milk is one of the most promising bean products, having advantages in feeding infants who are allergic to cow's milk and adults who have difficulty in lactose digestion.[2] Legume seeds and soy products are also used increasingly as a low-cost, high-quality protein supplement, with or without oil extraction.

These products, however, contain several anti-nutritional factors such as hemagglutinin, trypsin inhibitor, phytic acid and flatulence factors.[3] The flatulence factors are mainly found in the low molecular weight fraction of legumes which consist of primarily raffinose and stachyose.[4] However, α-galactosidase activity which is responsible for the enzymatic hydrolysis of the factors is not present in human intestinal mucosa. After ingestion of legumes, there would be no digestion of galactose-containing oligosaccharides and the flatus produced by fermentation of these factors in human intestine may cause nausea, cramps diarrhea and discomfort.[5]

May attempts have been made to eliminate the oligosaccharides from soybean and its products. There are many methods for analyzing simple sugars and oligosaccharides in bean products by means of paper chromatography,[6] thin-layer chromatography,[7] gas-liquid chromatography[8] and liquid chromatography.[9] Gas-liquid chromatography is one of the most accurate methods, which, however, has a disadvantage of requiring derivatization of the sugars.[10] High-pressure liquid chromatography removes some of the limitations imposed by other methods, but there exist serious problems that other components such as proteins, peptides or amino acids extracted with the solvent will interfere in the chromatogram and also rapidly deteriorate the expensive analytical column.[11] One-dimensional TLC[5,12] and paper chromatography[13] are useful qualitative methods but the resolution is not sufficient for quantitative purposes.

This study was, therefore, attempted to improve the two-dimensional thin-layer chromatography and apply it to estimate the contents of raffinose and stachyose in legume seeds and soy products widely consumed by Korean people.

Materials and Methods

Bean samples

Soybeans (domestic and imported), mung beans, red beans and kidney beans harvested in 1985 were purchased from a market in Shindang-dong, Seoul. Soy milk, soy curd, soy sauce and soy paste were purchased from a grocery market in Shinchon, Seoul. Details of the samples are given in Table 1.

TLC plates and ion-exchangers

To ensure the greatest reproducibility, commercially

Table 1. Details of samples for analysis

Samples	Scientific name or manufacturer
Kideny bean	*Phaseolus vulgaris var. nanus*
Mung bean	*Phaseolus vidissimus*
Red bean	*Phaseolus angularis*
Soybean	*Glycin max*, domestic
Soybean	*Glycin max*, imported
Soy curd	Pulmoowon Food Co., Seoul
Soy milk	Lotte Food Co., Seoul
Soy paste	Sampyo Food Co., Seoul
Soy sauce	Sampyo Food Co., Seoul

available pre-coated silicagel plates (Merck Art. plastic No. 5554, 20 × 20 *cm*) were used. Ion-exchangers used for desalting of samples were Amberlite IR-120B (cation exchanger) and Amberlite IRA-410 (anion exchanger) as purchased from Tokyo Organic Chemical Industry Co.

Determination of flatulence factors in legume seeds[14]

Whole dry beans (10*g*) were ground to pass through 20 mesh sieve and defatted by diethylether in Soxhlet extraction apparatus for 15 hours. These defatted flours were suspended in 100 *ml* of 80% ethanol, refluxed for 2 hours, followed by filtration through a Whatman No. 1 filter paper. The residue was stirred in 100 *ml* of distilled water for 30 minutes and filtered again, then washed with distilled water until the filtrate gave a negative reaction to triphenyltertrazolium chloride reagent. The extracts and washings were combined and concentrated to 100 *ml* in vacuo below 50°C.

After activating the pre-coated silicagel plates in oven at 105°C for 30 *min*, a 20 *μl* aliquot of the concentrated sugar extract was applied on a plate and developed by two-dimensional thin-layer chromatography, using solvent mixtures of n-propanol: ethylacetate:water (6:1:3, *v/v*; 1st run solvent) and n-butanol: acetic acid: water (5:4:1, *v/v*; 2nd run solvent).

The plates were allowed to develop by ascending chromatography to a height of 10 *cm* in a closed glass tank containing 1st run solvent. The plates were dried at 100°C for 10-15 minutes, cooled to room temperature, and then returned to the solvent tank for a second run. The plates were allowed to develop until the solvent was 10 cm past the starting point, and then air-dried in a horizontal position. After the diphenylamine-aniline-phosphoric acid reagent was sprayed to locate sugar spots, the plates were heated in oven at 85°C for 10 minutes. Raffinose and stachyose appeared as violet spots.

The guide-strip technique was used to determine the amounts of raffinose and stachyose as follows. A sugar spot on the chromatogram was scraped off and the sugar was extracted with 2 *ml* of distilled water in a test tube at 70°C for 2 hours with occasional shakings. One *ml* of the eluent was mixed with 1 *ml* of 0.02 *M* thiobarbituric acid and 1 *ml* of concentrated hydrochloric acid. The mixture was heated in a boiling water-bath for exactly 6 minutes, then cooled under running water. The yellow color produced was read at 432 *nm* in spectrophotometer. (Spectronic 21, Bausch & Lomb Co.) The concentration of sugar was calculated from standard curve, ranging from 10-100 *μg* sugar per 20 *μl* aliquot per spot.

Determination of flatulence factors in soy products

A 100 *ml* portion of the boiled sample was poured into 24 *ml* of absolute ethanol and the precipitate was removed by filtration. The filtrate was extracted with a mixture of chloroform and water (5:3) in a separatory funnel. The aqueous phase was concentrated to 25 *ml* volume in vacuo and after removal of contaminating proteins[15], desalted by passing it through two separate ion-exchange resin columns (cation and anion exhangers).

The neutralized eluate was concentrated to 10 *ml* in vacuo and used for thin-layer chromatography. A 20 *μl* aliquot of the purified sugar solution was applied to a pre-coated silicagel plate and the quantitative estimations of stachyose and raffinose were carried out by means of guide-strip technique as described previously.

Results and Discussion

Standard runs for raffinose and stachyose

With the aim of selecting the efficient solvent system to separate the oligosaccharides, several solvents were examined by the thin-layer system of Tanaka *et al.*[14] using tow-dimensional method. The selected solvent systems were proved to be suitable for the fractionation of oligosaccharides, giving compact and nearly circular spots with no tailing. The clear-cut separation of the oligosaccharide spots enabled their quantitative measurements to be made.

A raffinose plate and a stachyose plate after spotting 10-100 *μg* of sugars per spot were developed by two-

dimensional TLC. In each plate, three blank spots were selected and measured by the same methods. Sugar spots from the tested samples were estimated by the guide-strip technique. The concentration of sugars was determined by substracting the mean absorbance of blank spots from that corresponding to the sugar spots. Oligosaccharides tested in the concentration range of 10-100 μg showed a highly singificant linear correlation with the absorbance at 432 *nm* as shown in Fig. 1.

The spectrophotometric determination of the eluates is used since the application of densitometric method is limited to one-dimensional chromatograms. A slight variation in results may occasionally occur, connected with the inevitable variations in spraying intensity, heating time and temperature. Accurate standardization of detection procedures is, therefore, very important for reproducibility, as pointed out by Damonte *et al.*[16]

Content of flatulence factors in legume seeds

For the determination of sugars on TLC, the thiobarbituric acid reaction was chosen because this reaction is not only specific to fructose, but also the interference of glucose, galactose, lipids and proteins is negligible under the conditions of the test.[14] This is in contrast to the phenolsulfuric acid method used by Sugimoto and Van Buren[7], Tanusi *et al.*[6] and Kim *et al.*[9], where their measurements were found to be interfered with the presence of lipids or proteins.

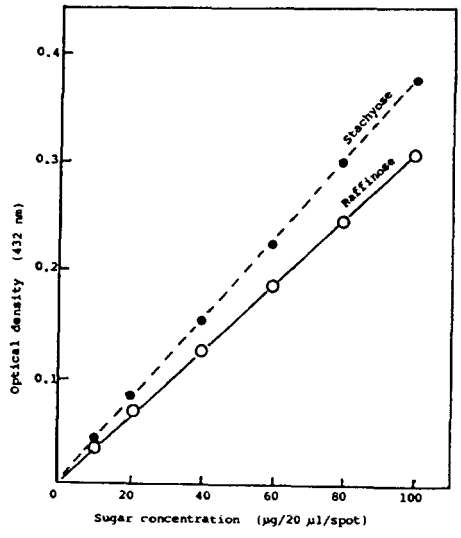

Fig. 1. Standard curves for raffinose and stachyose by thin-layer chromatographic determination

Table 2 shows the contents of different types of oligosaccharides in legume seeds analyzed by the present method and Fig. 2 shows a typical thin-layer chromatogram for the extract of soybean.

It is known that the level of the oligosaccharides in legume seeds is different among varieties and strains, and also changes during the maturation of the seeds.[17] However, the relative levels of flatulence factors in this study for various legume seeds consumed in Korea are

Table 2. Content of flatulence factors in legume seeds

Seeds	Raffinose (%)*	Stachyose (%)*
Kidney bean	0.65±0.39	3.0±0.32
Mung bean	0.49±0.40	1.9±0.35
Red bean	0.49±0.40	2.9±0.32
Soybean (domestic)	1.34±0.40	4.1±0.32
Soybean (imported)	1.17±0.41	3.6±0.32

* Mean value of triplicate determinations±standard deviation

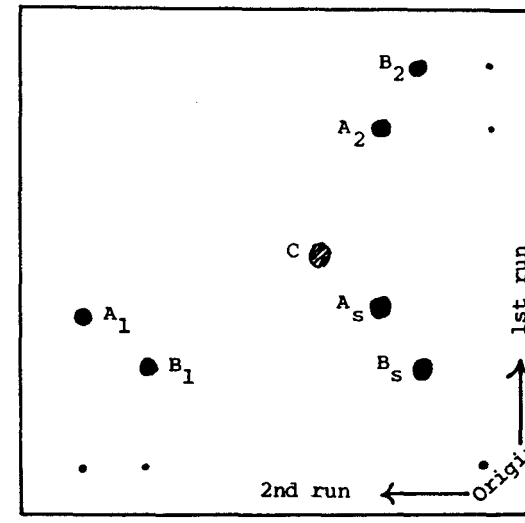

Fig. 2. Thin-layer chromatogram for the extract of soybean

A_1: Raffinose (1st standard solution)
B_1: Stachyose (1st standard solution)
A_2: Raffinose (2nd standard solution)
B_2: Stachyose (2nd standard solution)
A_s: Raffinose from sample
B_s: Stachyose from sample
C: Other sugars from sample

in good agreement with those reported by other workers in foreign countries.[6,14,17-20]

Content of flatulence factors in soy products

This paper demonstrates that the content of oligosaccharides is relatively high not only in soy milk but also in soy curd and soy paste which are most widely used as soy products in Korea, as given in Table 3. However, the oligosaccharides were not detected in soy sauce.

In TLC for soy paste sample, only raffinose spot was observed and stachyose was not. Soy paste results from the fermentation of cooked soybeans by *Aspergillus oryzae*. One advantage of the product appears to be the elimination of some of the oligosaccharides during the cooking and fermentation as pointed out by Horan.[21]

Table 3. Content of flatulence factors in soy products

Sample	Raffinose (*mg*%)*	Stachyose (*mg*%)*
Soy curd	26.7±1.80	53.7±1.44
Soy milk	26.5±0.10	41.7±1.24
Soy paste	46.6±3.60	N.D.
Soy sauce	N.D.**	N.D.

* Mean value of triplicate determinations ± standard deviation

** N.D.: not detectable

Although quantitative determination of oligosaccharides in legume seeds and soy products were made, what levels of oligosaccharides are actually hazardous to human body is not known. Studies in this respect have not been undertaken as yet. Also it is not determined yet to what extent the oligosacharides in legume seeds are changed in the processing of soy products. Further work on this subject, therefore, should be conducted in the future, as a continuation of this study.

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두류 및 대두제품중 가스발생인자의 함량 분석

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두류와 대두 제품중 가스 발생인자로 알려진 raffinose와 stachyose의 함량을 2차 전개법과 guide-strip법을 이용한 얇은막 크로마토그래피에 의하여 정량하였다. 두류의 경우 raffinose 함량은 국산대두 1.34%, 수입대두 1.17%, 강낭콩 0.65%, 붉은팥 0.49%, 녹두 0.49%이었고 stachyose 함량은 국산대두 4.1%, 수입대두 3.6%, 강낭콩 3.0%, 붉은팥 2.9%, 녹두 1.9%이었다. 대두제품의 경우 간장에서는 가스발생인자가 검출되지 않았으나 된장에서는 faffinose 함량은 46.6*mg*%이었고 stachyose 는 검출되지 않았다. 두부의 경우 raffinose는 26.7*mg*, stachyose는 53.7*mg*%이었으며 두유의 경우 raffinose는 26.5*mg*%, stachyose는 41.7*mg*이었다.