Quantitative Determination of Oligosaccharides in Commercial Soybean Lecithin Products by High-Performance Liquid Chromatography

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A method to quantitatively determine the oligosaccharides in a commercial soybean lecithin product by high-performance liquid chromatography (HPLC) was studied. The lecithin product was dispersed in chloroform/methanol/water (10/8/9, v/v) containing an internal standard, and a portion of the water phase was injected onto the anion exchange HPLC column. Oligosaccharides were selectively monitored by a fluorescence detector after a post-column chemical reaction with arginine. This method was highly sensitive ($<0.1 \mu\text{g}$) and also applicable for the determination of saccharide content in soybeans. By this method, we demonstrated that the oligosaccharides in the product were changed into fluorometrically undetectable components by a browning reaction, and that monosaccharides of soybean were not contained in the product.

Color formation due to the browning reaction during heating has limited the utilization of commercial soybean lecithin products in the food industry. This deterioration of the lecithin products was mainly due to the reaction between free amino-groups of phospholipids and aldehydes, especially between phosphatidylethanolamine and oligosaccharides^{1) 2)}. However, there was no critical study about the direct effect of oligosaccharides on the browning reaction in commercial lecithin products. For a better understanding of the browning mechanism in lecithin products and to develop heat stable lecithin products, a quantitative

determination method for oligosaccharides in lecithin products is necessary.

HPLC with an amino column and refractive index detector was used for the quantitative determination of saccharides, but this method was not suitable for the sample which contained a small amount of carbohydrates due to its low sensitivity and selectivity. On the other hand, the fluorometric detection and determination of carbohydrates by HPLC was developed earlier for using ethanolamine³⁾, 2-cyanoacetamide⁴⁾ and arginine⁵⁾. However, ethanolamine was unsuitable for the analysis of soybean oligosaccharides because sucrose was not detectable whereas the reducing monosaccharides gave intense fluorescence³⁾. Moreover, 2-cyanoacetamide was harmful compared to arginine. To establish an adequate determination method for oligosaccharides in lecithin products, we used HPLC with a post-column fluorometric detector using arginine. Furthermore, the oligosaccharide contents of soybeans and heated lecithin samples were measured.

Experimental Procedures

Materials : Commercial soybean lecithin product, AY-Lecithin, prepared by the Honen Corporation (Tokyo, Japan) and U.S. soybeans used for edible oil production were used. All other reagents were obtained from Wako Pure Chemical (Osaka, Japan).

Sample preparation : Heated lecithin samples were prepared from the lecithin product using an aluminum heating block at 180°C. The lecithin sample (0.5 g) was thoroughly mixed with chloroform (10 mL), methanol (18 mL) and water (9 mL) containing 0.1% maltose as an internal standard and centrifuged at $200 \times g$ for 5 min. The upper phase solution was filtered through a $0.45 \mu\text{m}$ disposable filter unit and applied to a HPLC column (10 μL injection).

Analysis : The post-column HPLC method using arginine was achieved according to MIKAMI *et al*⁵⁾; an NR_4 bonded anion exchange column (Shimadzu ISA-07/S2504; 25 cm $\times$ 4 mm i.d.) was used for the resolution of saccharides with borate buffer, pH 8.5–9.0, prepared

by adding KOH pellets to 0.3 M boric acid solution. The flow rate was 0.5 ml/min, and the column temperature was 70°C. The eluent was allowed to react with a mixture of 2% arginine and 3% boric acid solution at 150°C for 10 min, and the fluorescence intensity was measured at λ_{ex} 320 nm and λ_{em} 430 nm using a Shimadzu RF-550 fluorophotometric HPLC monitor. The browning intensity of the heated lecithin samples, which were dissolved in *n*-hexane (1%), was measured at 520 nm using a Shimadzu UV-160 spectrophotometer.

Results and Discussion

The post-column fluorometric detection using the reaction with arginine was mainly developed for the selective and sensitive analysis of monosaccharides. However, the resolution condition for oligosaccharides has not been reported. The preliminary experiment indicated that 0.3 M borate buffer (pH 8.5–9.0) as the effluent and 70°C for column temperature were suitable conditions for the resolution of typical galactosyl oligosaccharides that were found in soybeans (e.g. raffinose or stachyose). While this HPLC analysis was carried out in the water soluble phase, most of the lecithin components were insoluble in water. By pre-treatment using solvent extraction (chloroform/methanol/water), the water phase containing the oligosaccharides could be easily separated from the phospholipids and triglycerides.

Fig. 1 shows the typical HPLC pattern of oligosaccharides in commercial soybean lecithin product. In this study, maltose was used as an internal standard, because it was not found in soybeans and was easily separated from other saccharides under this condition (peak 3). Sucrose (peak 1), raffinose (peak 2) and stachyose (peak 4) were completely separated at pH 8.5 within 30 min. The content of total oligosaccharides in the lecithin product was about 4.5%. Although this method was highly sensitive ($<0.1 \mu\text{g}$) for the reducing sugars⁵⁾, monosaccharides were not detected in the product.

This quantitation method was applicable to

Fig. 1 High-performance liquid chromatogram of oligosaccharides in commercial soybean lecithin product on a 25 cm $\times$ 4 mm i.d. NR₄ bonded anion exchange column at pH 8.5

Peak 1, sucrose; peak 2, raffinose; peak 3, maltose (as an internal standard); peak 4, stachyose.

Fig. 2 High-performance liquid chromatogram of saccharides in soybeans on a 25 cm $\times$ 4 mm i.d. NR₄ bonded anion exchange column at pH 9.0

Peak 1, sucrose; peak 2, raffinose; peak 3, maltose (as an internal standard); peak 4, stachyose; peak 5, fructose; peak 6, galactose; peak 7, glucose

Table 1 Comparison of oligosaccharide contents (% by wt) of dry soybean, lecithin product and heated lecithin product

Samples	Sucrose	Raffinose	Stachyose	Fructose	Galactose	Glucose	Total
Dry soybean	5.2	0.5	4.2	1.2	0.7	1.5	13.3
Lecithin product	1.5	0.6	2.4	—	—	—	4.5
Heated lecithin product ^a	0.8	0.3	1.4	—	—	—	2.5

^a at 180°C for 5 min

Fig. 3 Cooperative change in oligosaccharides content (closed circle) and absorbance at 520 nm (open circle) of lecithin product during heating at 180°C

The absorbance of 1% lecithin samples in *n*-hexane was measured.

measure the saccharide content not only in lecithin products but also in soybeans. In this case, the soybean sample should be eluted at higher pH than 8.5 to lengthen the retention time of the monosaccharides. The saccharide content in soybeans was measured at pH 9.0, and fructose, galactose and glucose were detected as shown in Fig. 2. These monosaccharides were not detected in the lecithin product. Since the fluorescence intensities of the reducing sugars are generally higher than those of non-reducing sugars, these monosaccharides show large peak areas. The total saccharide content in soybeans was about 13.3%.

Table 1 shows a comparison of the saccharide contents in soybeans, lecithin product and heated lecithin product. The reason why monosaccharides are not contained in lecithin product is probably as follows: Soybean lecithin

product was produced by degumming from *n*-hexane-extracted crude soybean oil. During the extraction step of soybean oil production, monosaccharides were hardly soluble in the *n*-hexane phase compared with the oligosaccharides, because the hydrophobicity of sugar was increased and accompanied by an increase in the degree of polymerization.

The total oligosaccharide content in heated lecithin product was apparently less than that in the lecithin product, and the decrease in the ratios of the oligosaccharides were nearly equal. Fig. 3 shows the behavior of oligosaccharide content and browning intensity of the lecithin product during heating. Oligosaccharides were changed into fluorometric undetectable components accompanied by an increase in the browning intensity, which indicates that oligosaccharides participate in the browning reaction of lecithin product by heating. Since highly purified phospholipids became slightly brown after heating⁶⁻⁸⁾, the major cause of lecithin product browning was the interaction between saccharides and other components which had free amino groups such as phosphatidylethanolamine. Thus, we consider that elimination of sugar is an effective method to prevent the browning reaction of lecithin products.

Our experimental results indicate that the browning of commercial soybean lecithin products is mainly due to the reaction promoted by oligosaccharides. Therefore, the measurement of oligosaccharide content in lecithin products is essential for the development or improvement of heat stable lecithin products. Since the post-column fluorometric detection using arginine is highly sensitive and selective in comparison with the refractive index detec-

tion, this method is suitable for the quantitative determination of oligosaccharides in lecithin product. In addition, since the sample preparation method is fairly simplified, the saccharide contents in lecithin products or soybeans can be easily and precisely quantified.

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高速液体クロマトグラフィーによる 大豆レシチン製品中のオリゴ糖の定量

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アルギニンを用いたポストカラム蛍光検出高速液体クロマトグラフィーにより、大豆レシチン製品中のオリゴ糖を定量した。簡単な前処理により、迅速かつ精度よく分析できた。レシチン製品の加熱褐変にともないオリゴ糖含量が減少すること、大豆に含まれる単糖類がレシチン製品中には含まれていないことが明らかとなった。