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HPLC Determination of Fructo-Oligosaccharides in Dairy Products

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Abstract: Fructo-oligosaccharides is a natural active substance with excellent physiological functions such as improving the gastrointestinal tract, regulating intestinal flora, etc. It is an important nutrient in infant dairy products. Therefore, it is extremely important to accurately detect fructo-oligosaccharides in infant dairy products. Nowadays, the main methods for detection of fructo-oligosaccharides at home and abroad include high performance liquid chromatography, ion exchange chromatography, mass spectrometry, etc. In this article, a method for the determination of fructo-oligosaccharides in modified milk and infant formula milk powder by high performance liquid chromatography (HPLC) was developed. Fructo-oligosaccharides was extracted by water. Protein was precipitated by acetonitrile. The method was performed on NH2 column, the detector was refractive index detector (RID) and the mobile phase was 70% acetonitrile. Fructo-oligosaccharides content of modified milk was in the range of 20-500mg/100g, the recovery was 80.2%-107.1%. The content of Fructo-oligosaccharides in infant formula milk powder was in the range of 100-500mg/100g, the recovery was 71.9%-95.3%. The quantitative detection limit was 20mg/100g. This method was easily to be operated with a high accuracy and the results indicated that it was suitable for the analysis of fructo-oligosaccharides in modified milk and infant formula milk powder.

Keywords: HPLC, Fructo-Oligosaccharides, Modified Milk, Infant Formula Milk Powder

1. Introduction

Fructo-oligosaccharides (FOS), also known as oligofructose or oligosaccharides belongs to sugarcane trisaccharide, is a naturally occurring non-reducing sugar in nature which is mainly composed of 1-Estose (GF2), Nystose (GF3), 1^F-Fructofuranosylnystose (GF4). [1] Oligofructose has the functions of promoting the growth of beneficial bacteria in the intestine, improving digestive system function, preventing diarrhea and constipation, promoting absorption of calcium, preventing dental caries as well as other physiological function. [2-5] Thus, oligofructose is increasingly widely used in a variety of dairy products, infant foods, sweets and beverages, baking and leisure food and health care products. [6, 7] So monitoring the amount of oligofructose added in food is of great significance for examining the efficacy of the product and monitoring the quality of the product.

Currently, the main oligofructose quantification methods include thin layer chromatography, gas chromatography mass spectrometry, ion chromatography and liquid chromatography. [8-20] Among them, Thin layer chromatography has a low sensitivity, gas chromatography mass spectrometry is complicated in pretreatment and expensive, ion chromatography is susceptible to interference from the complex substrate, so these methods are difficult to apply in practical detection. [21, 22] Wheresa HPLC method has the characteristics of high penetration rate and rapid detection, making it the main method for detecting oligofructose. However, at present, the HPLC method for detecting fructo-oligosaccharides in dairy products has the problems such as low resolution, the target peak is easily interfered by lactose and the result is inaccurate, etc. To solve the above problems, the HPLC treatment of fructo-oligosaccharides in dairy products and the conditions of the machine were studied.

2. Materials and Experiments

2.1. Reagent

Acetonitrile was chromatographically pure; 1-Kestose, Nystose, 1F-Fructofuranosylnystose standard products were purchased from Japanese Wako company; modified milk product and infant formula milk powder product of Inner Mongolia Yili Group.

2.2. Equipment

HPLC (1260, with refractive index detector), Agilent, USA; analytical balance (AB265-S), METTLER-DOLEDO company, Switzerland; ultrasonic cleaner (GE013), Kunshan Ultrasonic Instrument Co., Ltd; high speed refrigerated centrifuge (3K15), Sigma, Germany.

2.3. Methods

2.3.1. Chromatographic Conditions

Column: High performance carbohydrate cartridge (Waters, 250mm×4.6 mm, 4μm); flow rate 1mL/min; injection volume 10μL; column temperature 35°C; mobile phase acetonitrile: water = 70:30 (V/V).

2.3.2. Standard Solution Preparation

1-Kestose, Nystose, 1F-fructofuranosylnystose standard solution (1mg/mL): weigh 0.025g (accurate to 0.0001g) of these three standard product separately in three 25mL volumetric flasks, dissolve them with water to the constant volume and store the solution in a refrigerator at 4°C.

According to the sensitivity and the content of the FOS of the product, dilute the above standard solution with the mobile phase to a series of mixed standard working solution with suitable concentrations before used.

2.3.3. Sample Processing

Weighed 2.5g of the liquid sample or 1.0g solid sample into a 50mL volumetric flask, accurate to 0.0001g, added 12.5mL of water (ultra-pure water) at 45°C, dissolved and mixed the sample fully, ultrasonic extraction lasted for 10min at room temperature, then the sample was diluted to the constant volume with acetonitrile, continue extracted the sample by ultrasonic extraction for another 10 minutes, filtered the solution through filter paper after 30min standing, the filtrate was filtered through a 0.45μm filter membrane, if the filtered liquid is not clear, centrifugation will be carried out at 10000r/min for 5min at 4°C, the supernatant is filtered through a 0.45μm filter membrane. Performed the filtrate (testing solution) by liquid chromatography.

2.3.4. Results Calculation

$$x = \frac{c \times V \times 100}{m \times 1000} \quad (1)$$

Here: x - the content of GF2 (GF3/GF4) in the sample (g/100g);

c - the concentration of GF2 (GF3/GF4) in the testing solution (mg/mL);

V - the volume of the constant volume (mL);

m - mass of the sample (g).

$$C_{(FOS,g/100g)}=C_{(1\text{-Kestose},g/100g)}+C_{(Nystose,g/100g)}+C_{(1F\text{-Fructofuranosylnystose},g/100g)} \quad (2)$$

3. Results

3.1. Instrument Repeatability, Standard Curve, Linear Range and Detection Limit

Performed the mixed standard solution for six times repeatedly, the relative standard deviations (RSD) of the retention time and the peak area are shown in Table 1 and 2. The RSD of the retention time of GF2, GF3 and GF4 are 0.048%, 0.060%, 0.058%. The RSD of the peak area of GF2, GF3 and GF4 are 1.19%, 2.72%, 0.68%.

Table 1. *Instrument repeatability of the retention time.*

FOS	Retention time/min	RSD/%
GF2	7.006, 7.004, 7.001, 7.005, 7.001, 6.999	0.048
GF3	8.635, 8.634, 8.625, 8.634, 8.628, 8.623	0.060
GF4	10.724, 10.725, 10.718, 10.721, 10.713, 10.706	0.058

Table 2. *Instrument repeatability of the peak area.*

FOS	Peak area/nRIU	RSD/%
GF2	7820, 7752, 8088, 7893, 7687, 8241	1.19
GF3	8038, 7437, 7729, 8087, 7758, 7904	2.72
GF4	7566, 7604, 7444, 7438, 7760, 7896	0.68

The mixed standard working solution of were determined under the above chromatographic conditions. The mass concentration of GF2, GF3 and GF4 were the abscissa (X) and the peak area was the ordinate (Y). Three standard curves were drawn, the linear equations and correlation coefficient were obtained (Table 3). The linear range was 20-500mg/100g. According to the signal-to-noise ratio (RSN)=10, the quantitative detection limit of the three oligofructose was 20mg/100g.

Table 3. *Linear equations and correlation coefficients of the fructo-oligosaccharides.*

FOS	Linear equation	Correlation coefficient
GF2	$y = 88.4\,x - 112.22$	0.9996
GF3	$y = 82.2\,x - 34.96$	0.9990
GF4	$y = 75.94\,x + 106.33$	0.9980

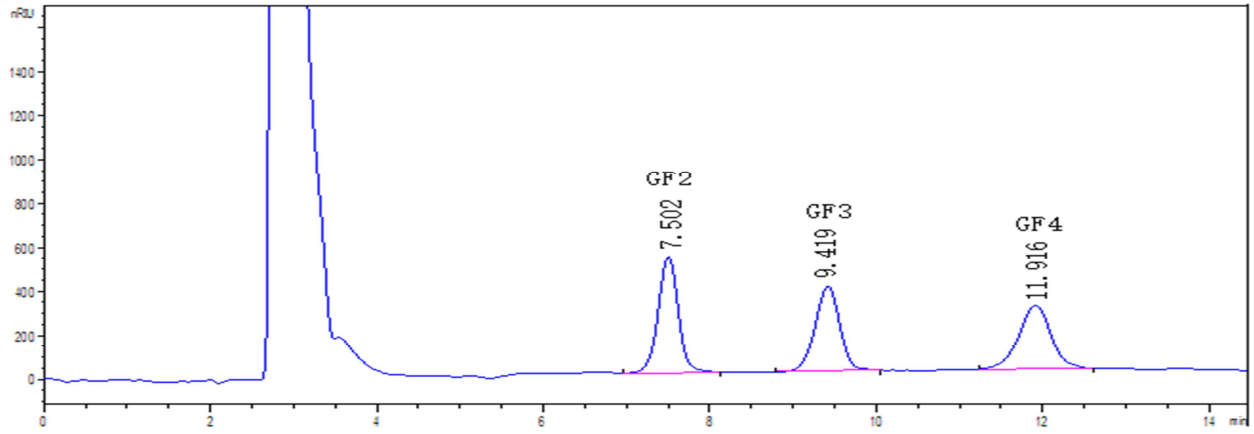

Figure 1. *HPLC chromatogram of the mixed standard solution of fructo-oligosaccharides.*

The chromatograms of the mixed standard solution and samples are shown in Figure 1, 2 and 3. It can be seen from Figure 1 that the retention time of 1-Kestose, Nystose, 1F-Fructofuranosylnystose are 7.502min, 9.419min, and 11.916min, respectively. As can be seen from Figure 2, the retention time of them from the modified milk are 7.560min for GF2, 9.478min for GF3, and GF4 was not detected. As can be seen from Figure 3, the retention time of them from the infant formula milk powder are 7.546min, 9.461min and 11.931min, respectively.

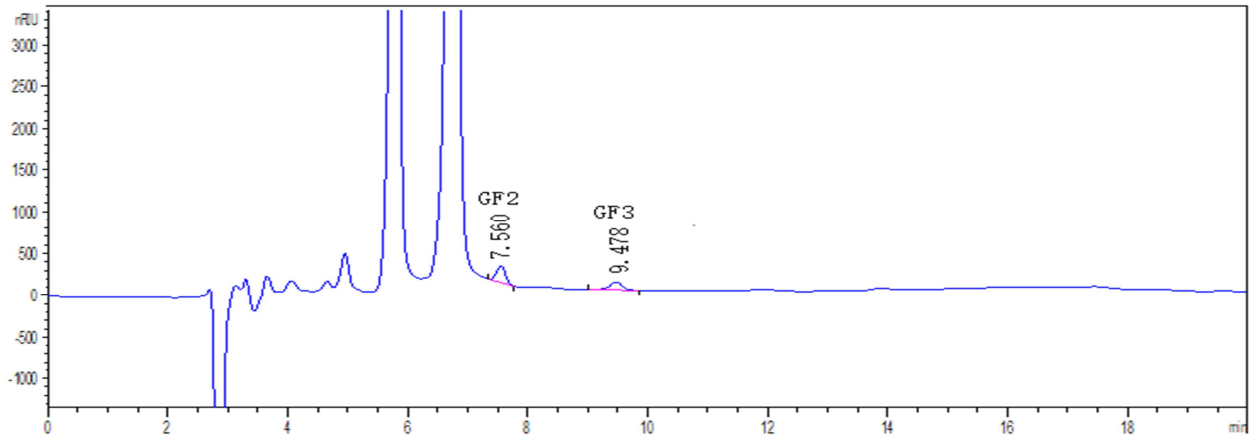

Figure 2. *HPLC chromatograms of modified milk.*

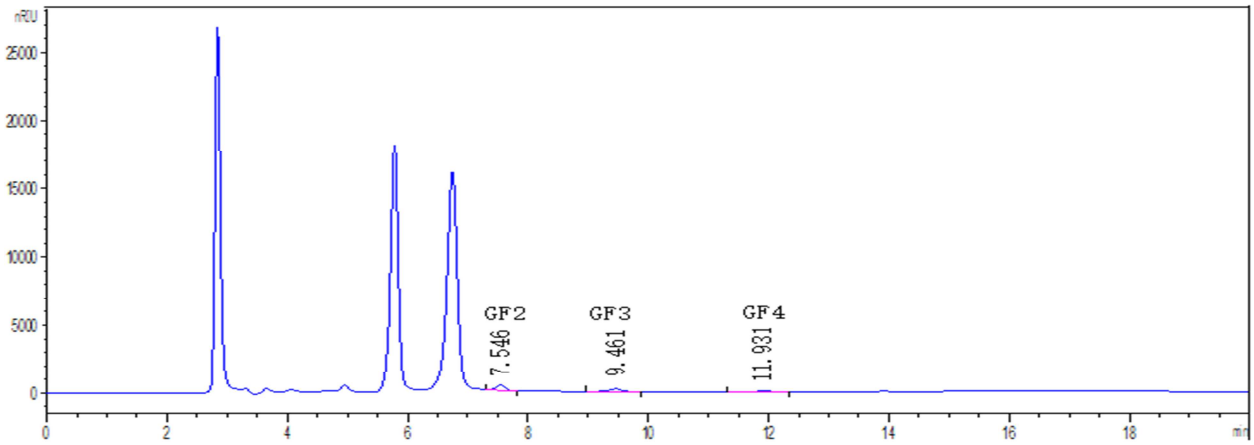

Figure 3. *HPLC chromatograms of infant formula milk powder.*

3.2. Method Recovery and Precision

Different concentrations of GF2, GF3 and GF4 were added to the blank-modulated milk samples, and the recovery experiments were carried out, the determination was performed in parallel six times. As shown in Table 4 and 5, when the added amount were 20, 100, 200, 500mg per 100g for modified milk and 100mg per 100g for infant formula milk powder, the average recovery rate were 83.3%-104.3% for GF2, 80.2%-107.1% for GF3, 81.7%-101.6% for GF4, respectively for modified milk and 71.9% for GF2, 95.3% for GF3, 90.0% for GF4 respectively for infant formula milk powder. Six replicate experiments were performed on samples containing 30mg/100g GF2, GF3 and GF4. The relative standard deviations were 3.19%, 1.67%, and 2.04%, respectively (Table 6).

Table 4. *Recovery of blank sample for modified milk.*

Add amount/mg/100 g	The average recovery rate/%		
	GF2	GF3	GF4
20	83.3	80.2	81.7
100	88.8	89.5	90.4
200	95.6	100.5	97.1
500	104.3	107.1	101.6

Table 5. *Recovery of blank sample for infant formula milk powder.*

FOS	amount/mg/100 g	recovery /%
GF2	100	71.9
GF3	100	95.3
GF4	100	90.0

Table 6. *Precision of the modified milk.*

FOS	Contents/mg/100 g	RSD/%
GF2	25.2, 26.9, 24.7, 25.4, 26.1, 26.4	3.19
GF3	25.3, 24.8, 25.6, 26.0, 25.8, 25.7	1.67
GF4	26.1, 25.7, 25.3, 25.0, 24.8, 26.2	2.04

3.3. Products Results

Determined modified milk products and infant formula milk powder products by this method, the results of 1-Kestose, Nystose, 1F-Fructofuranosylnystose and fructo-oligosaccharides are shown in Table 7 and 8.

Table 7. *Results of modified milk products.*

Sample	GF2/ g/100g	GF3/ g/100g	GF4/ g/100g	FOS/ g/100g
S1	0.0498	0.0370	—	0.0868
S2	0.0450	0.0256	—	0.0706
S3	0.0520	0.0312	—	0.0832

Table 8. *Results of infant formula milk powder products.*

Sample	GF2/ g/100g	GF3/ g/100g	GF4/ g/100g	FOS/ g/100g
J1	0.0732	0.1506	0.0434	0.2672
J2	0.0338	0.0636	0.0116	0.1090
J3	0.0750	0.2030	0.0365	0.3145

4. Discussion

4.1. Sample Pretreatment Optimization

The modified milk sample contains a lot of protein, precipitated the protein with lead acetate, methanol and acetonitrile respectively. The results showed that all these three substances can precipitate the protein in the sample, but because the method used a refractive index detector, it has the specificity of reference. Therefore, the sample treated with lead acetate and methanol showed unstable peaks and poor peak shape. When acetonitrile was used as a precipitant and the amount of acetonitrile was adjusted to be close to the flow ratio, the precipitation effect was good, the retention time of the peaks were stable, and the peaks shape were better. Well, acetonitrile was the final choice as a precipitant, the ration was 70% of the constant volume.

4.2. Selection of Chromatographic Conditions

When 65% acetonitrile solution was selected as the mobile phase, the peaks of the kestose and lactose in the sample were partially overlapped, and the resolution was not good enough for detection. When the acetonitrile content was adjusted to 75%, the kestose and the lactose were able to be separated. However the retention time of 1F-fructofuranosylnystose was delayed to 20 min with a broadened peak shape, which was not conducive to accurate quantification. The selection of 70% acetonitrile was able to completely separate the kestose and lactose in the sample, and the retention time of 1F-fructofuranosylnystose was also within 20 min. Moreover, the peaks shape were good, which can be accurately and quantitatively detected. The 70% acetonitrile solution was finally used as the mobile phase.

4.3. Selection of Column Temperature

Performed the testing solution under 25°C, 30°C, 35°C and 40°C column temperature conditions respectively, compared to other spectrums, the spectrum of 35°C column temperature showed the best peak shape, a high resolution as well as most number of plates, thus 35°C column temperature was chosen to be the final column temperature for the method.

5. Conclusion

The method for detecting fructo-oligosaccharides in dairy products was established by using acetonitrile to precipitate protein for the purpose of removing impurities, fructo-oligosaccharides was extracted by water under the condition of ultrasonic extraction, 70% acetonitrile was used as mobile phase, the fructo-oligosaccharides was separated by high performance carbohydrate cartridge and detected by refractive index detector, 1-Estose, Nystose and 1^F-Fructofuranosylnystose were well separated. The quantitative detection limit of the method is 20mg/100g. It has a good linear relationship in the range of 20-500mg/100g. The

accuracy, recovery and precision of the method meet the requirements of qualitative and quantitative detection, and provide reference for the detection of fructo-oligosaccharides in dairy products.

References

[1] B. B. WANG. Health consequences of FOS [C]. Proceedings of the Annual Committee of the Sweeteners Professional Committee 2015, pp. 118-122, 2015.

[2] Z. L. LIU, K. W. LI, J. B. WANG, et al. The physicochemical properties, physiological functions and applications of fructooligosaccharides [J]. China Food Additives, No. 10, pp. 211-215, 2016.

[3] Y. Y. ZHANG, B. ZHANG, A. Y. HAN, et al. Comparison of Three Methods for Determination of Fructooligosaccharides [J]. Food Research And Development, Vol. 36, No. 14, pp. 98-101, July 2015.

[4] X. H. HUANG, G. SONG, L. H. ZHANG, et al. Simultaneous determination of monosaccharides, disaccharides and oligosaccharides in dairy products [J]. China Dairy Industry, Vol. 45, No. 11, pp. 37-39, 2017.

[5] SANGEETHA P T, RAMESH M N, PRAPULLA S G. Recenttrends in the microbial production analysis and application of fructooligosaccharides [J]. Trends in Food Science & Technology, No. 16, pp. 442-457, 2005.

[6] X. J. YU, K. QIU, Z. H. XIONG. A Review of Detection Methods Related to Fructo-oligosaccharides and Galactooligosaccharides in Food [J]. Food Research and Development, Vol. 34, No. 20, pp. 125-129, October 2013.

[7] C. PAN. Determination method of oligofructose content in feed by High performance liquid chromatography-differential refractive [J]. Chinese feed, No. 3, pp. 26-27, 2015.

[8] HAYASHI S, YOSHIYAMA T, FUJI N, et al. Production of a novel syrup containing neofructooligosaccharides by the cells of Penicilliumcitrinum [J]. Biotechnology Letter, No. 22, pp. 1465-1469, 2000.

[9] PARK J, OH T, YUN J W. Purification and characterization of a novel transfructosylating enzyme from Bacillus macerans EG-6 [J]. Process Biochemistry, No. 37, pp. 471-476, 2001.

[10] VACCARI G, LODI G, TAMBURINI E, et al. Detection of oligosaccharides in sugar products using planar chromatography [J]. Food Chemistry, No. 74, pp. 99-110, 2000.

[11] Y. A. WEI, H. L. ZHENG, S. H. WU, et al. Separation and Analysis of Fmctooligosaccharides by High—Performance Anion Exchange Chromatography [J]. Food Science, Val. 36, No. 14, pp. 151-155, 2015.

[12] L. J. GENG, J. R. HUANG, F. FENG, et al. Determination of fructooligosaccharides in milk powder using high performance liquid chromatography coupled with pulsed amperometric detection [J]. Chinese Journal of Chromatography, Vol. 32, No. 12, pp. 1380-1384, December 2014.

[13] J. M. ZHANG, Y. F. LIU, H. C. TANG, et al. Determination of Fructooligosaccharide in Foods by High Performance Ion Exchange Chromatography Method [J]. Food Research and Development, Vol. 33, No. 1, pp. 135-138, January 2012.

[14] Y. B. XU, Z. J. ZHENG, Y. XU, et al. Simultaneous Determination of Monosaccharides, Disaccharides, and Fructooligosaccharides in Inulin Hydrolysates by High Performance Anion Exchange Chromatography [J]. Food Science, Vol. 37, No. 02, pp. 77-81, 2016.

[15] Y. F. LIU, D. LI, H. C. TANG, et al. Determination of Fructooligosaccharides in Milk and Milk Products by High Performance [J]. Journal of Dairy Science and Technology, Vol. 35, No. 01, pp. 40-42, 2012.

[16] Y. LIU, T. DING, S. L. XU, et al. Determination of Fructo-oligosaccharide in milk powder by high performance liquid chromatography-quadrupole/electrostatic field orbitrap high resolution mass spectrometry [J]. Chinese Journal of Chromatography, Vol. 33, No. 10, pp. 1040-1045, October 2015.

[17] L. ZHANG, J. H. ZHANG, J. H. ZHANG, et al. Determination of fructo oligosaccharide and galactooligosaccharides in milk powder by high performance liquid chromatography coupled with evaporative light scattering detector [J]. Journal of Food Safety and Quality, Vol. 8, No. 12, pp. 4729-4735, December 2017.

[18] L. Z. XU, X. Y. LI, J. F. HUANG, et al. Simultaneous determination of monosaccharides, disaccharides and fructo-oligosaccharides in milk powder by ultra-filtration clean up coupled with high performance liquid chromatography [J]. Journal of Food Safety and Quality, Vol. 6, No. 2, pp. 633-638, February 2015.

[19] S. F. ZHANG, X. L. BI, L. YING, et al. Determination of the total amount of Fructooligosaccharides in infant formula milk powder by high performance liquid chromatography with evaporative light scattering detector [J]. China Dairy Industry, Vol. 46, No. 3, pp. 45-48, 2018.

[20] Y. Y. ZHANG, B. ZHANG, L. G. YU, et al. Determination of Fructooligosaccharides in Food by Evaporative Light Scattering Detection [J]. Food Research and Development, Vol. 36, No. 23, pp. 131-134, December 2015.

[21] Z. P. ZHENG, Y. F. GAO, Y. H. PANG, et al. Determination of FOS in Milk Powder by HPLC-MS/MS [J]. Sugarcane and Canesugar, No. 4, pp. 59-63, August 2017.

[22] X. Y. LI, J. F. HUANG, Y. P. XIAN, et al. Recent Advances in Analytical Techniques for Fructooligosaccharides in Foods [J]. Journal of Dairy Science and Technology, Vol. 37, No. 3, pp. 31-33. 2014.