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Permeate from cheese whey ultrafiltration is a source of milk oligosaccharides

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Abstract

Previously undescribed oligosaccharides in bovine cheese whey permeate were characterized by a combination of nanoelectrospray Fourier Transform Ion Cyclotron Resonance (nESI-FTICR) mass spectrometry and matrix-assisted laser desorption/ionization Fourier transform ion cyclotron resonance (MALDI-FTICR) mass spectrometry. Oligosaccharide composition was elucidated by collision-induced dissociation within the ICR cell. In addition to sialyllactose (the most abundant oligosaccharide in bovine colostrum), we identified 14 other oligosaccharides, half of which have the same composition of human milk oligosaccharides. These oligosaccharides could potentially be used as additives in infant formula and products for the pharmaceutical industry. Because whey permeate is a by-product from the production of whey protein concentrate (WPC) and is readily available, it is an attractive source of oligosaccharides for potential application in human nutrition.

1. Introduction

Interest in human milk oligosaccharides started with the observation that oligosaccharides might be growth factors for the bifidus flora in breast-fed infants (Coppa et al., 2004, 2006). Oligosaccharides are the third most abundant component of human milk (12–14 g L^{-1}), present at an even higher concentration than total protein. Although the oligosaccharide content of cows' milk is about 20-fold lower than that of human milk, several oligosaccharides have been isolated and characterized in bovine colostrum and mature milk (Gopal and Gill, 2000).

The literature reports that oligosaccharides detected in domestic animal milks have some structural features in common with human milk oligosaccharides (Gopal and Gill, 2000; Urashima et al., 2001). Repetitive attachment of galactose and N-acetylhexosamine to lactose through a β-glycosidic linkage characterizes the core of bovine oligosaccharides. The human intestine lacks enzymes able to hydrolyze all β-glycosidic linkages, except the one in lactose. Thus, β-glycosidically-bound galactose is the structural element that protects these molecules

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from digestion during passage through the small intestine (Boehm et al., 2003). Their arrival in the lower intestine may allow them to be fermented by beneficial bacteria and act as prebiotics. Additionally, this fermentation by commensal organisms may produce short-chain fatty acids such as acetic, propionic and butyric acids, which are readily absorbed across the large-bowel wall, providing nutrition for the colonocytes and a source of energy to the body (Modler et al., 1990).

In addition to potential prebiotic activity, oligosaccharides may also act as soluble receptors for different pathogenic microorganisms, thus acting potentially as intestinal mucosa cell protectors. In this sense, they may constitute an additional defense mechanism for newborn infants, whose gastric pH is less acidic than in adults, and whose immune system is not yet mature (Boehm and Stahl, 2007; Gopal and Gill, 2000; Martinez-Ferez et al., 2006).

Both human and bovine milk are known to contain sialyloligosaccharides, especially at the early lactation stage. This class of sugars has gained much scientific focus over the last two decades, as several biological activities have been demonstrated in humans (Rosenberg, 1995). The most common members of the sialic acid family are (Kanamori et al., 1990) N-acetyl-neuraminic acid (NeuAc), and N-glycolyl-neuraminic acid (NeuGc). It has been suggested that these acidic oligosaccharides, in addition to the bifidogenic effect (Idota et al., 1994; Martin-Sosa et al. 2003), have a role in the initial stage of inflammation (sialyl Lewis-X antigen involvement) and may fight influenza virus infection and ulcers caused by *Helicobacter pylori* (Parente et al., 2003; Simon et al., 1997). Other activities include increasing immunity in infants, development of cerebral function and promotion of useful enteric bacterial proliferation (Newburg, 2000; Rivero-Urgëll and Santamaria-Orleans 2001; Schauer, 2000; Wang and Brand-Miller, 2003). In the case of humans, sialyloligosaccharides range from 1 g L^{-1} in colostrums to 90–450 mg L^{-1} in mature milk (Carlson , 1985; Martin-Sosa et al., 2003, 2004), while in bovine-based infant formulas the content of sialyloligosaccharides is as low as 15–35 mg L^{-1} (Martin-Sosa et al., 2003; Wang et al., 2001).

Whey permeate is a by-product obtained when cheese whey is passed through an ultrafiltration membrane to concentrate whey protein. Whey proteins are retained by the membrane, whereas smaller molecules such as lactose and salts pass through the membrane making up the whey permeate. While the protein concentrate can be used in food products, the permeate has so far been of little value. One method of increasing the economic value of whey permeate is to identify bioactive compounds that can be used in the food/health industry. Considering that bovine milk oligosaccharides are mainly made of 3–10 monomers, it is entirely possible that they pass through the membrane and end in the whey permeate. Numerous papers and patents deal with oligosaccharide presence in whey, but they mostly refer to specific galactooligosaccharides (GOS) obtained from lactose by enzymatic reaction (transgalactosylation) and do not refer to the natural milk oligosaccharides produced in the cow lactating mammary gland (Albayrak and Yang, 2002; Goulas and Tzortzis, 2007; Splechtna et al., 2007). A thorough review on galactooligosaccharides has been provided by Tungland and Meyer (2002).

Some microorganisms used in cheese making are also a potential source of enzymes with significant transgalactosylase activity. Therefore, there is a possibility of GOS formation in whey during cheese production. However, the structure of GOS produced by enzymatic transgalactosylation on lactose are only made of galactose and glucose units, and do not contain N-acetylhexosamine (N-acetylglucosamine, N-acetylgalactosamine) and sialic acid typical of bovine and human milk oligosaccharides.

Numerous attempts have also been made to concentrate and isolate the milk sialyloligosaccharides naturally present in whey (Pelletier et al., 2007; Sarney et al.,2000;

Sprenger et al., 2007). However, due to the scarcity of commercially available standards for bovine milk oligosaccharides and the technical difficulty of analyzing low-abundant components with analytical techniques such high performance liquoid chromatography (HPLC), which heavily rely on reference compounds, the exact number and type of monosaccharide residues forming those sialyloligosaccharides in whey, with the exception of sialyllactose, is not yet well-known.

The development of desorption/ionization techniques like matrix-assisted laser desorption ionization (MALDI) and electrospray (ESI), coupled with tandem techniques such as collision induced dissociation (CID), has improved carbohydrate research with mass spectrometry. Mass spectrometry provides a sensitive technique to assist in the determination of the structure of carbohydrates, but it is essential that the samples are pure to achieve maximum ionization. Human and bovine milk require several preliminary treatments to eliminate lipids and proteins, which interfere with oligosaccharide determination by mass spectrometry. Such treatments are laborious, time consuming and can potentially cause loss of oligosaccharides. In addition, the fact that oligosaccharides are non-linear polymers creates a high structural diversity that is presently beyond the capabilities of single analytical methods for their characterization. Although the core structure of oligosaccharides comprises only a few molecules, there is a high variety of possible branched structures due to the multiple hydroxyl groups present on monosaccharide and available for glycosylation. Additionally, milk oligosaccharides often have other linkages to fucose (found mainly in human milk oligosaccharides) and sialic acid (found in both human and bovine milk oligosaccharides) at the core molecules. A problem with studying carbohydrate bioactivities is the limited access to well-defined oligosaccharides. Purification of oligosaccharides from natural sources is laborious, and it is difficult to obtain preparations free of contaminants.

Because synthetic oligosaccharides are rare and expensive, and human milk is not available for large-scale oligosaccharide purification, there is an urgent need to find alternative sources from which to obtain sufficient amounts to perform clinical studies and examine their potential for use in human nutrition. Being the product of ultrafiltration, whey permeate requires potentially less pre-treatments as compared with human and bovine milk, as lipids and proteins have already been removed by the membrane and could therefore be straightforwardly analyzed by mass spectrometry.

The aim of the present study was to investigate the presence in cheese whey of oligosaccharides with structures and possibly biological activities similar to those of human milk oligosaccharides. Cheese whey may be a valuable low-cost source of these oligosaccharides, and purification may increase their availability in the food industry.

2. Materials and methods

2.1. Whey origin and chemical characterization

Whey from Gorgonzola cheese was obtained from Santi S.r.l (Novara, Italy). Gorgonzola is a blue veined cheese made from whole cows' milk using *Lactobacillus bulgaricus* and *Streptococcus thermophilus* as starter cultures along with spores of the mold *Penicillium roqueforti*. The literature reports that both *Lb. bulgaricus* and *Str. thermophilus* are able to synthesize the enzyme β-galactosidase (Martini et al., 1991). β-galactosidase belongs to the glycosidase family that shows both hydrolyzation and transglycosylation activities (Rastall and Bucke, 1992). This enzyme may therefore catalyze transglycosylation reactions and synthesize galactooligosaccharides. Whey samples were refrigerated during transportation and stored frozen at −20 °C if not analyzed immediately. Liquid whey was ultrafiltered to obtain whey protein concentrate and whey permeate. Table 1 reports measurement of some Gorgonzola whey composition parameters.

The pH value of each sample was recorded at 20 °C using a digital pH meter (Metrohm 691, Herisau, Switzerland) equipped with a glass electrode inserted directly into the whey samples for the measurement. Dry matter, protein and lipids were determined according to AOAC (1995) procedures.

2.2. Whey Ultrafiltration

A crossflow system designed for rapid laboratory concentration and diafiltration was used for whey ultrafiltration (Ultran®-Miniflex, Whatman, Switzerland). In crossflow ultrafiltration, the solution containing the molecules to be separated flows parallel to the face of the membrane. A pressure difference is applied across the membrane and the permeate passes through the membrane while the concentrated solution (retentate) stays on the high-pressure side of the membrane.

The ultrafiltration membrane was composed of hydrophilic polyethersulfone, with a molecular mass cut off of 10 000 Daltons and a membrane area of 24 cm^2. Pressure transducers were used to measure the pressure at the inlet and outlet. Whey permeate was collected and weighed continuously to measure its flow rate. The feed temperature was controlled by a heat exchanger. The crossflow velocity was changed using a peristaltic pump. Experiments were carried out in total recirculation mode. Temperature was 55 °C and pressure inlet and outlet were 1.0 and 1.8 bar, respectively. Flow rate was 1.5 mL min^{-1}. The retentate containing whey protein concentrate was discarded; the permeate was lyophilized until a white powder containing no residual water was obtained. Whey permeate powder was suspended in deionized water prior to further analyses. Table 1 reports compositional data of the whey protein concentrate and whey permeate so obtained.

2.3. Oligosaccharide purification: solid phase extraction

As can be seen in Table 1, whey permeate only contains trace amounts of lipids and protein, and therefore does not require delipidization and deproteinization processes usually necessary for milk samples. However, to ensure proper oligosaccharide identification by mass spectrometry, we performed a solid-phase extraction (SPE) to eliminate all salts that escaped the ultrafiltration process and that would interfere with MS analysis.

Five whey permeate samples obtained by ultrafiltration were purified by solid-phase extraction using nonporous graphitized carbon polypropylene cartridges (GCC, 150 mg carbon, 4-mL tube capacity, Alltech, Deerfield, IL, USA). The cartridges were conditioned following the protocol described by Ninonuevo et al. (2006). Three cartridge volumes of deionized water were loaded on each cartridge, followed by three cartridge volumes of an 80:20 deionized water-acetonitrile solution containing 0.05% (v/v) trifluoroacetic acid, followed by three more volumes of deionized water. The whey samples were then loaded onto the cartridges. Subsequently, eight cartridge volumes of deionized water were passed through the cartridge at a flow rate of 1 mL min^{-1} to remove salts and contaminants. The oligosaccharides that had been retained by the solid phase were then eluted stepwise with 2 cartridge volumes of a 90:10 deionized water-acetonitrile solution, 2 cartridge volumes of an 80:20 deionized water-acetonitrile solution, and 2 cartridge volumes of 40% acetonitrile in 0.1% trifluoroacetic acid. Each fraction was dried in a vacuum centrifuge (automatic environmental Speedvac® system AES 2010; Thermo Savant, Holbrook, NY, USA) and 20 μL of deionized water were added prior to mass spectrometry analysis. The graphitized carbon cartridges used in this work were formed by heating carbon to temperatures above 2000 °C, and this type of cartridge has been used extensively to remove polar pesticides and other organic pollutants from water (Crescenzi et al., 1996).

2.4. Qualitative oligosaccharide characterization by mass spectrometry

Oligosaccharide compositions were assigned using the information obtained from tandem mass spectrometry and by using an in-home program, Glycan Finder written in Igor Pro version 5.04B software from WaveMetrics, Inc. (Portland, OR, USA) (Ninonuevo et al., 2006). The algorithm was designed to examine a list of experimentally measured masses and search for all possible monosaccharide combinations matching the experimental mass within a specified tolerance level (mass error). Oligosaccharide compositions were determined based on mass error as low as 10 ppm.

2.4.1. Nanoelectrospray Fourier transform ion cyclotron resonance mass spectrometry—Oligosaccharide characterization was performed on a nanoelectrospray Fourier transform ion cyclotron resonance (FTICR) mass spectrometer (nESI-FTICR MS, IonSpec Corp., Irvine, CA, USA) equipped with a 9.4 Tesla superconducting magnet in both positive and negative ion modes. Cone voltages were maintained at 2000 V to obtain signals. Ions were accumulated in the hexapole and transferred by quadrupole ion guide prior to the ICR cell for detection. Tandem mass spectrometry was performed with sustained off-resonance irradiation-collision induced dissociation (SORI-CID), the simplest and most robust means of achieving multiple collision activation (Laskin and Futrell, 2005). The isolated ion was excited at 1000 Hz, 1000 ms in the ICR cell. Voltage from 3–10 V was used to fragment molecular ions depending on the size of the oligosaccharide.

2.4.2. Matrix-assisted laser desorption/ionization Fourier transform ion cyclotron resonance mass spectrometry—Matrix-assisted laser desorption/ionization Fourier transform ion cyclotron resonance mass spectrometry (MALDI-FTICR MS) was used to improve detection of neutral (not acidic) oligosaccharides. Mass spectra were recorded on a IonSpec Corporation ProMALDI FTICR-MS instrument (Lake Forest, California, USA) equipped with a 7.0 Tesla actively shielded superconducting magnet and an external MALDI source capable of hexapole ion accumulation and fitted with a pulsed Nd:YAG laser (355 nm). External accumulation of ions produced by a variable number of MALDI laser pulses (typically from 10–20) was used to obtain optimum total ion intensity for each sample analyzed. The instrumental conditions for oligosaccharide analysis were as described in detail by Penn et al. (1997). For MALDI, 1 μL of solution containing purified oligosaccharides was spotted on a polished stainless steel target, followed by 0.5 μL 0.01 M NaCl (for positive mode) and 1 μL 0.4 M 2,5-dihydroxybenzoic acid as a matrix. The spots were then allowed to dry under vacuum prior to mass spectrometric analysis.

Internal calibration with oligosaccharides from other food matrices was performed to obtain accurate and precise mass-to-charge information.

3. Results and discussion

Compared with human milk, oligosaccharides are only present in bovine milk in trace amounts; considerably higher concentrations are present in bovine colostrum. As milk production matures post parturition, the concentration of oligosaccharides declines rapidly, making isolation and analysis technically challenging.

3.1. Solid phase extraction

One of the primary requirements for mass spectrometric analysis is elevated sample purity. The solid phase extraction with nonporous graphitized carbon cartridges effectively removed salts and residual contaminants (traces of protein and lipids), thus allowing good identification by mass spectrometry without the need of any further purification.

3.2. Qualitative oligosaccharide characterization by mass spectrometry

All five whey permeate samples purified by solid phase extraction produced mass spectra containing the same peak patterns. Different replicates varied only in relative peak intensity in the spectra, thereby confirming the high reproducibility of this method. The application of FTICR-MS for the structural elucidation of oligosaccharides and the improvements in instrumentation that make it possible to study this class of compounds have been previously reported by our group (Park and Lebrilla, 2005). The FTICR-MS coupled to CID provided high mass accuracy, high resolution and the compositional information necessary for their identification without the use of any commercial standard.

The oligosaccharide compositions reported in Table 2–Table 4 were obtained by performing tandem mass spectrometry and by using an in-home program Glycan Finder with a mass accuracy of less than 10 ppm. To illustrate the mechanism of structural elucidation, a simple example of tandem mass spectrometry (n ESI-FTICR SORI-CID) is provided in Fig. 1. In this spectrum, the fragmentation of the ion m/z 632 is observed. Using the exact mass, the predicted oligosaccharide was sialyllactose with the composition 2 Hex+1 NeuAc.

3.2.1. Nanoelectrospray Fourier transform ion cyclotron resonance mass spectrometry—The Mass spectra shown in Fig. 2 and Fig 4 were obtained from the Nanoelectrospray FTICR instrument. The oligosaccharide compositions, along with their mass/charge (m/z) are presented in Table 2 and Table 4. The fraction collected using a 90:10 deionized water-acetonitrile solution, contained eight oligosaccharides—five acidic and three neutral oligosaccharides (Table 2). Because the oligosaccharide corresponding to m/z 632 was in high abundance (Fig. 2), it suppressed the signal of the other oligosaccharides detected by the instrument. It is possible that if that abundant oligosaccharide was removed, more oligosaccharides would be detected. The fraction eluted from the solid phase with 40% acetonitrile solution containing 0.1% trifluoroacetic acid still contained 4 acidic oligosaccharides, of which the m/z 834 and m/z 673 were not present in the other fractions.

3.2.2. Matrix-assisted laser desorption/ionization Fourier transform ion cyclotron resonance mass spectrometry—The Mass spectrum shown in Fig. 3 was obtained from the IonSpec Corporation ProMALDI-FTICR instrument. The oligosaccharide compositions along with their mass/charge (m/z) are presented in Table 3.

The fraction collected using an 80:20 deionized water-acetonitrile solution contained eight oligosaccharides (Table 3), but a closer look reveals that some oligosaccharides (i.e., m/z 892; m/z 656; m/z 818) had the same composition shown in Table 2. The m/z values in Table 3 were increased by about 23 units because of the presence of Na^+ ions that were added to perform the measurement in positive ion-detection mode. The remaining oligosaccharides detected in this fraction (80:20 deionized water-acetonitrile solution), but not in the fraction collected using a 90:10 deionized water-acetonitrile solution, all had a common feature, that is they did not contain any residue of N-acetyl-neuraminic acid.

Comparing the spectra obtained with the two different instruments used for mass spectrometry, we noticed that nESI-FTICR provided greater sensitivity than MALDI-FTICR in the detection of acidic oligosaccharides, particularly in the negative ion-detection mode. On the other hand, MALDI MS used in the positive mode allowed for identification of many neutral oligosaccharides that were not detected using ESI-MS.

Combing the information from the three different fractions and comparing our results with those in the existing literature for bovine milk and colostrum (Gopal and Gill, 2000; Nakamura et al., 2003; Tao et al., 2008; Urashima et al., 2001) it was possible to identify 15 milk oligosaccharides.

The literature reports that the starter bacteria used in Gorgonzola production are able to synthesize the β-galactosidase enzyme and potentially to synthesize galactooligosaccharides. Therefore, we screened GenBank for the presence of genes for the enzymes related to the type of oligosaccharides found in the present work. The oligosaccharides observed in our study contain several residues of N-acetylhexosamine and N-acetyl-neuraminic acid and in one case, N-glycolylneuraminic acid. The genomes of the strains present in the database (*Lb. bulgaricus* ATCC11842, ATCC BAA-365 and *Str. thermophilus* CNRZ1066, LMD9, LMG18311) did not contain any genes annotated for enzymes required for the synthesis of such oligosaccharides. Based on these results, we excluded the possibility that the oligosaccharides measured were of bacterial origin. These oligosaccharides, in fact, can only be created by the complex mechanisms present in the lactating mammary gland.

Of the fifteen oligosaccharides identified combining the three fractions, eight were neutral oligosaccharides and seven were acidic (sialyloligosaccharides).

Published data for sialyloligosaccharides in bovine colostrum and milk differ considerably among various studies, but they all have in common a similar pattern of decrease in the sialic acid content from colostrums to mature milk (Nakamura et al., 2003).

Wang et al. (2001) reported that the sialic acid in human milk oligosaccharides appears to be almost exclusively NeuAc, whereas in bovine milk oligosaccharides, between 35% and 5% (depending on the stage of lactation) of total sialic acid are NeuGc. Out of the 10 sialyloligosaccharides reported by Gopal and Gill (2000), several contained NeuGc and were not found in the present study. Similarly, a recent study published by our group (Tao et al., 2008) confirmed the presence in bovine colostrum of 7 acidic oligosaccharides containing NeuGc, of which only one, with composition 3 Hex +1 NeuGc, was found in whey permeate in the present study.

Considering that whey permeate is a by-product obtained from mature milk, it is not surprising that we found six oligosaccharides containing NeuAc and only one oligosaccharide containing NeuGc.

Therefore, whey permeate can be seen as an ideal source of oligosaccharides as it provides acidic oligosaccharides with compositions similar to those present in human milk.

One oligosaccharide found in the present study with the composition 4 Hex + 1 HexNAc has also been found in human milk (Ninonuevo et al., 2006), equine colostrum (Urashima et al., 1991) and Tamar wallaby milk (Bradbury et al., 1983).

Comparing the oligosaccharide compositions identified in the present study with the published data for human milk oligosaccharides (Ninonuevo et al., 2006; Wang et al., 2001), it was possible to identify seven oligosaccharides with the same composition of those present in human milk. These oligosaccharides have the following compositions: 2 Hex+1 NeuAc, 3 Hex + 1 HexNAc, 4 Hex + 1 HexNAc, 4 Hex + 2 HexNAc, 3 Hex+ 2 HexNAc, 2 Hex + 1 HexNAc + 1 NeuAc and 3 Hex + 1 HexNAc + 1 NeuAc (see Table 2–Table 4).

4. Conclusions

The present study builds on previous studies that have assessed the presence in whey, of the acidic oligosaccharide sialyllactose, for which commercial standard exist. In contrast to previous studies, the high resolution and per-species exact mass analysis provided by the instrumentation used in this work, allowed us to identify the exact composition of oligosaccharides for which commercial standards do not exist. This study is the first to determine the compositions of a variety of neutral and sialylated oligosaccharides in

Gorgonzola whey permeate. Seven of the 15 oligosaccharides identified in the present study have the same composition as human milk oligosaccharides. Also, among acidic oligosaccharides, only one contained NeuGc (typically present in bovine colostrum but absent in human milk) while seven contained NeuAc, also found in human milk. These results suggest that whey permeate is a rich source of oligosaccharides with compositions similar to those present in human milk. Future studies are needed to confirm whether all whey oligosaccharides have similar biological activities to human milk oligosaccharides.

Because whey composition depends on the type of technology employed for cheese production, this approach (solid-phase extraction with graphitized carbon cartridges and the mass spectrometry Nanoelectrospray FTICR and MALDI-FTICR) could be used to identify cheese whey sources richest in oligosaccharides. Investigations of oligosaccharide recoveries at pilot scale are needed to determine the true economic efficiencies gained from oligosaccharide production from whey permeate.

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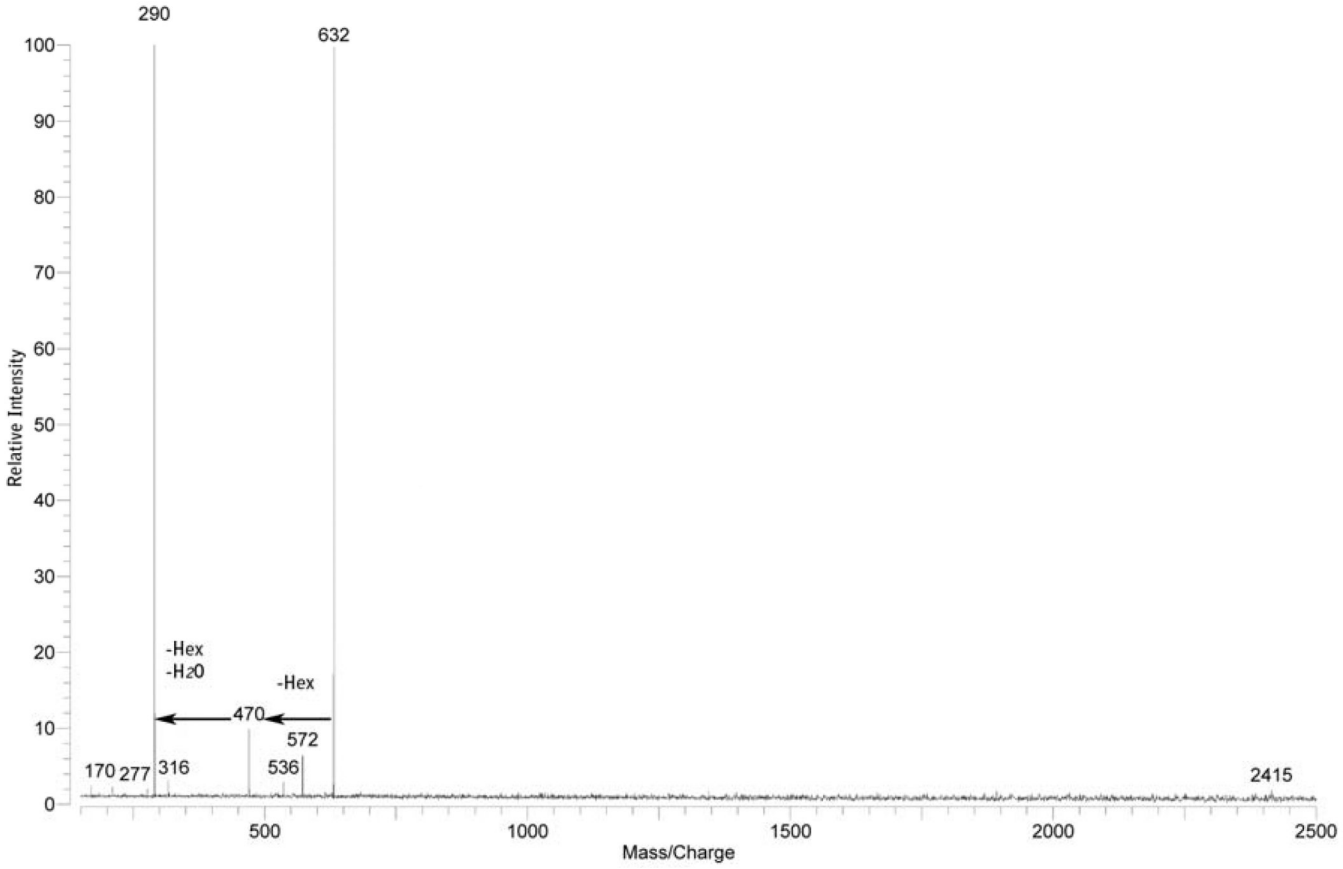

Fig. 1.

Collision-induced dissociation spectrum of peak 632 m/z. Nano-electrospray FTICR instrument was used in negative ion detection mode. The loss of one hexose followed by the loss of another hexose and water yielded an N-acetyl-neuraminic acid (m/z 290). This composition corresponds to sialyllactose (2 Hex+1 NeuAc).

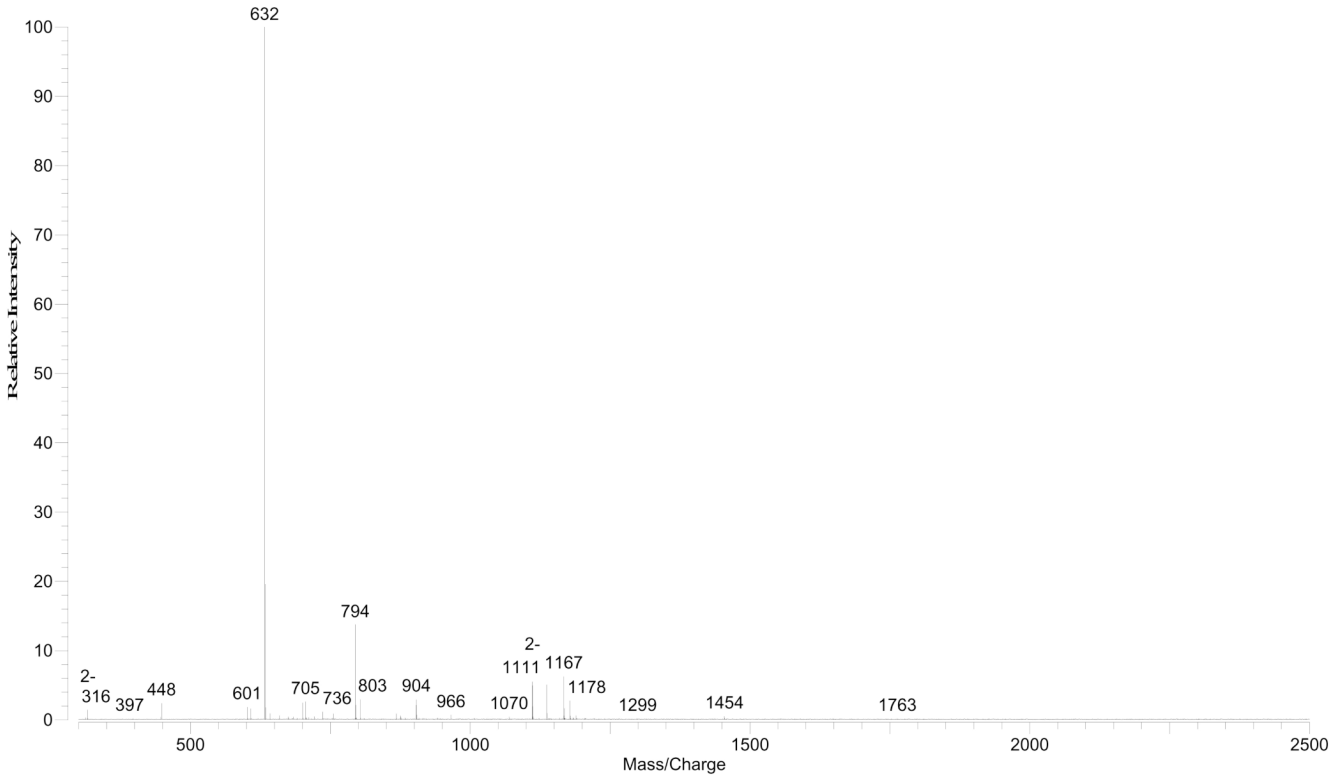

Fig. 2.

Mass spectrum of the whey fraction collected with a 90:10 deionized water-acetonitrile solution from solid-phase medium. The spectrum was recorded using a nESI-FTICR instrument in negative ion-detection mode.

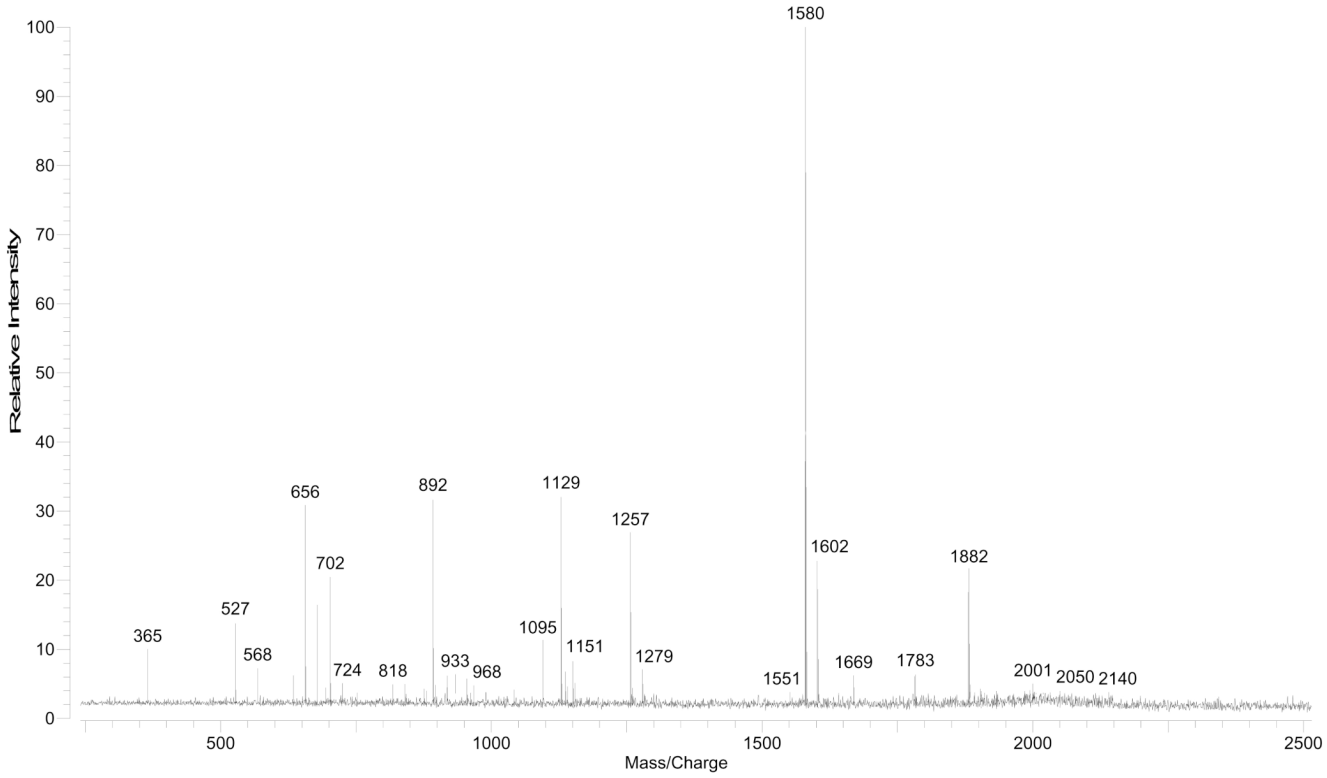

Fig. 3.

Mass spectrum of the whey fraction collected using an 80:20 deionized water-acetonitrile solution from solid-phase medium. The spectrum was recorded using a MALDI-FTICR instrument in positive ion-detection mode.

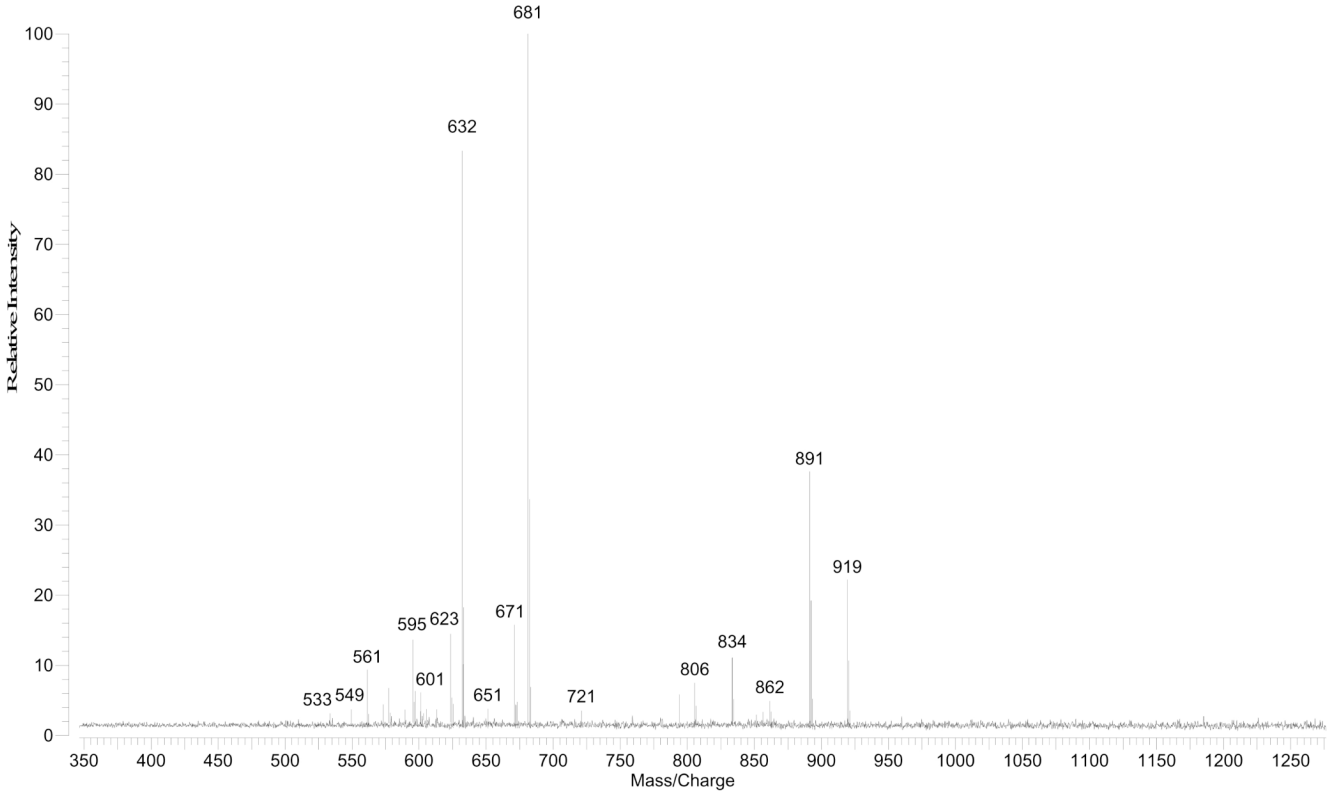

Fig. 4.

Mass spectrum of the whey fraction collected using a 60:40 deionized water-acetonitrile solution containing 0.1% trifluoroacetic acid from solid-phase medium. The spectrum was recorded using a nanoESI-FTICR instrument in negative ion-detection mode.

Table 1

Composition of whey, whey protein concentrate and whey permeate. Values are expressed as a percentage on a wet basis and are the average of three determinations. Protein was calculated by multiplying nitrogen content by 6.38.

Sample	pH	Dry matter %	Lipid %	Protein %
Whey	6.57 ± 0.05	6.31 ± 0.04	0.20 ± 0.02	0.60 ± 0.01
Whey protein concentrate	5.96 ± 0.03	18.73 ± 0.02	0.40 ± 0.01	6.37 ± 0.03
Whey permeate	6.50 ± 0.02	4.87 ± 0.02	0.10 ± 0.04	0.17 ± 0.05

Table 2

Oligosaccharides in the fraction eluted from the solid phase with a 90:10 deionized water-acetonitrile solution and their constituent monosaccharides[a]. Data were obtained using a nESI-FTICR instrument in negative ion-detection mode and tandem mass spectrometry. Oligosaccharide compositions were assigned using an in-home Glycan Finder program.

m/z $[M-H]^-$	Hex	HexNAc	NeuAc	NeuGc	relative intensity
632.10	2		1		100
794.03	3		1		13.72
964.90	1	1	2		0.72
1158.90	4	1	1		0.3
706.02	3	1			0.3
809.96	3			1	0.24
867.99	4	1			0.18
1038.85	2	2	1		0.18

[a]Hex, hexose; HexNAc, N-acetylhexosamine; NeuAc, N-acetyl-neuraminic acid (sialic acid); NeuGc, N-glycolylneuraminic acid.

Table 3

Oligosaccharides in the fraction eluted from the solid phase with an 80:20 deionized water-acetonitrile solution and their constituent monosaccharides[a]. Data were obtained using a MALDI-FTICR instrument in positive ion-detection mode and tandem mass spectrometry. Oligosaccharide compositions were assigned using an in-home Glycan Finder program.

m/z $[M+Na]^+$	Hex	HexNAc	NeuAc	Relative intensity
892.29	4	1		31.79
656.20	2		1	30.79
527.16	3			13.90
1095.36	4	2		11.19
365.11	1	1		9.97
568.18	2	1		7.26
933.32	3	2		6.35
818.25	3		1	4.93

[a] Hex, hexose; HexNAc, N-acetylhexosamine; NeuAc, N-acetyl-neuraminic acid (sialic acid).

Table 4

Oligosaccharides in the fraction eluted from the solid phase with a 60:40 deionized water-acetonitrile solution containing 0.1% trifluoroacetic acid and their constituent monosaccharides[a]. Data obtained using an nESI-FTICR instrument in negative ion-detection mode and tandem mass spectrometry. Oligosaccharide compositions were assigned using an in-home Glycan Finder program.

M/z $[M-H]^-$	Hex	HexNAc	NeuAc	Relative intensity
632.12	2		1	83.89
794.12	3		1	5.83
834.54	2	1	1	5.22
673.16	1	1	1	4.75

[a]Hex, hexose; HexNAc, N-acetylhexosamine; NeuAc, N-acetyl-neuraminic acid (sialic acid).