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Development of Lactose-hydrolyzed Milk with Low Sweetness Using Nanofiltration

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ABSTRACT : A lactose-hydrolyzed milk with low sweetness was developed using nanofiltration. Raw milk was treated with 0.03% β-galactosidase at 4°C for 24 h to hydrolyze lactose partially. The resultant lactose-hydrolyzed milk containing 0.43% lactose was then concentrated using a nanofiltration membrane to reach concentration factor of 2.13. The concentration factors and coefficients of retention of milk components in nanofiltration were determined. The concentration factor of milk fat was 2.20 which was the highest of the milk components. The coefficient of retention of calcium and riboflavin was 0.96 and 0.76, respectively. However, the coefficient of retention of glucose, galactose, and sodium was 0.21, 0.15, and 0.22, respectively. Raw milk was treated with 0.1% β-galactosidase at 4°C for 40 h to hydrolyze lactose fully and then concentrated to reach a concentration factor of 1.6 by using nanofiltration. The concentrated milk was reconstituted with water. The lactose-hydrolyzed milk had sweetness similar to milk. The compositional ratios of crude protein, calcium, sodium, and riboflavin of lactose-hydrolyzed nanofiltrated milk to those of raw milk were 99%, 97%, 77%, and 80%, respectively. This study showed that nanofiltration of lactose-hydrolyzed milk to remove galactose and glucose did not cause significant loss of calcium. The lactose-hydrolyzed nanofiltrated milk contained 0.06% lactose and had sweetness similar to milk. (**Key Words** : Milk, Nanofiltration, β-Galactosidase, Lactose, Calcium)

INTRODUCTION

Milk is a nutritionally important food which is a rich source for protein of high quality, calcium and riboflavin. The sole carbohydrate in milk, lactose, present at the concentration of 4.8-5.2% is absorbed into the body after its hydrolysis into glucose and galactose by lactase located on mucosal membrane of enterocyte in small intestine. However, most Asians and Negroes including some Caucasians become deficient in digesting lactose due to gradual loss of lactase after weaning (Kim, 1994; Boey, 2001; Jackson and Salvano, 2001). Thus, consumption of more than 20-50 g of lactose in empty stomach by lactase-deficient individuals causes symptoms of lactose intolerance. But 200 ml of milk, equivalent to 10 g of lactose, does not induce the symptoms to most persons. Lactose which is not absorbed in small intestine passes into colon in the person without ability to digest lactose. Lactose increases osmolarity of digesta and thus inhibit absorption of water in colon and is metabolized to increase acidity and to produce gas and toxin by coliform bacteria, which causes soft stool, flatulence, and diarrhea. These unpleasant experiences inhibit further consumption of milk. However, lack in consumption of milk may suppress physical growth in youth and causes osteoporosis in old age due to lack of calcium supply.

Lactose-hydrolyzed milk which is produced by treating milk with microbial β-galactosidase to hydrolyze lactose into glucose and galactose are commercially available for the consumers suffering from lactose intolerance (Kohler et al., 1994). Since the mixture of glucose and galactose is sweeter than lactose and is easily converted into volatile compounds in Maillard reaction during heating in pasteurization and sterilization, lactose-hydrolyzed milk is not popular because of sweetness and off-flavor.

Lactose-hydrolyzed milk with low sweetness was developed (Lange, 2003; Tossavainen and Sahlstein, 2003) and marketed successfully by Valio Ltd. (Mattila-Sandholm and Saarela, 2003). Tossavainen and Sahlstein (2003) separated permeate from milk by using ultrafiltration membrane. The permeate contained lactose and milk salt. Only milk salt was recovered from the permeate by using a series of nanofiltration and reverse osmosis and added to

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the reconstituted milk to maintain flavor. The resulting milk with low content of lactose was subsequently treated with β-galactosidase to produce lactose-hydrolyzed milk with low sweetness.

Membrane filtration is classified into reverse osmosis, nanofiltration, ultrafiltration, and microfiltration based on the molecular weight cut-off of membrane. Nanofiltration membrane retains polyvalent anions such as phosphate and organic compounds whose molecular weights are more than 200-1,000 which is intermediate between conventional reverse osmosis and ultrafiltration membranes (Nyström et al., 1995; Jirage and Martin, 1999). Ultrafiltration membrane retains substances whose molecular weights are more than 1,000-1,000,000 (Renner and El-Salam, 1991; Yoon and Jayaprakasha, 2005).

The present study is to develop a lactose-hydrolyzed milk with low sweetness by treating milk with β-galactosidase, concentrating lactose-hydrolyzed milk with nanofiltration membrane, and then reconstituting it.

MATERIALS AND METHODS

Milk

Raw milk was purchased from dairy farms. Lactose-hydrolyzed market milk was purchased at groceries. The lactose-hydrolyzed market milk was known to be manufactured under the license granted by Valio Ltd. (Mattila-Sandholm and Saarela, 2003).

Preparation of lactose-hydrolyzed milk

In order to produce milk in which lactose was partially hydrolyzed, 0.03% of commercial β-galactosidase (5,000 lactase activity unit/g, Validase, Valley Research) was added into raw milk, which was then incubated at 4°C for 24 h. In order to produce milk in which lactose was fully hydrolyzed, 0.1% of β-galactosidase was added into raw milk, which was then incubated at 4°C for 40 h. Partial hydrolysis of lactose was performed to determine concentration factors and coefficient of retentions of chemical components including lactose in nanofiltration. Full hydrolysis was done to prepare lactose-free milk.

Nanofiltration of lactose-hydrolyzed milk

The lactose-hydrolyzed milk was heated at 72°C for 5 min to inactivate enzymes in milk, cooled to 45-50°C, and then concentrated using a spiral wound element of nanofiltration membrane (DS2DL, Osmonics) at the pressure of 130-140 psi and at the flow rate of 30 L/min of milk. Lactose-hydrolyzed milk was recirculated, until appropriate concentration factor was obtained. The concentration factor and coefficient of retention were calculated based on the equations as follows,

Concentration factor = feed volume/concentrate volume
= concentration of a component in concentrate
/concentration of a component in feed

Coefficient of retention
= 1-concentration of a component in permeate
/concentration of a component in concentrate

Sensory evaluation

Sensory evaluation panel consisted of ten college students. Friedman analysis was used to compare sweetness of milk samples. The totaled rankings of the samples were tested to determine statistical significance by using Kramer's table (Kim et al., 1993).

Proximate component analysis

Moisture content was determined by adding sea sand into sample, drying partially in water bath, and then drying at 105°C until its weight reached constant level. Solid content was calculated by subtracting moisture content from 100%. Crude protein and milk fat was determined using Kjeldahl method and Gerber method, respectively (Marshall, 1993).

Analysis of calcium and sodium

Atomic absorption spectrophotometer (Perkin-Elmer 5100PC) was used to determine calcium and sodium. Milk samples for the analysis of calcium and sodium were diluted with 0.01% lanthanum oxide and 0.1% cesium chloride, respectively. .

Sugar analysis

After 3.2 ml of ethanol was added to 0.7 g of milk sample, the mixture was centrifuged at 10,000×g for 20 min. 1.0 ml of the supernatant was evaporated under nitrogen gas stream at 40°C. The dried residue was dissolved in 1 ml of distilled water. The dissolved solution was analyzed using a column (Aminex HPX-87P, Bio-Rad) and HPLC instrument (Varian 9012Q). The column was heated at 85°C and deionized distilled water was used as eluent.

Riboflavin analysis

After 1 g of trichloroacetic acid was dissolved in 8.0 ml of milk sample, the solution was centrifuged at 2,000×g for 10 min. The supernatant was collected. 3 ml of 4% trichloroacetic acid was added to the pellet. The mixture was vortexed and centrifuged at 2,000×g for 10 min. The supernatants were pooled and 4% trichloroacetic acid was added to make up to 10 ml for HPLC analysis. HPLC instrument and column were Varian 9012Q and HIQ sil C18 (Kya Tech), respectively. Eluant consisted of 5 mM octanesulfonic acid, 0.5% triethylamine, 2.4% glacial acetic

Table 1. Chemical compositions of raw milk, lactose-hydrolyzed milk, and retentate and permeate obtained after nanofiltration of lactose-hydrolyzed milk at the concentration factor of 2.1

Chemical components	Raw milk	Lactose-hydrolyzed milk*	Retentate	Permeate
Solid (%)	13.0	12.9	23.4	4.4
Crude protein (%)	3.34	3.37	6.70	0.14
Milk fat (%)	4.3	4.3	9.2	0
Crude ash (%)	0.71	0.71	1.33	0.31
Lactose (%)	4.83	0.43	0.73	0.23
Glucose (%)	0	2.26	2.73	2.17
Galactose (%)	0	1.76	1.95	1.66
Ca (mg %)	117.1	112.7	166.3	6.07
Na (mg %)	36.9	38.8	41.7	32.4
Riboflavin (mg %)	0.08	0.07	0.12	0.03

* Lactose was hydrolyzed partially.

acid. 15% methanol (Albalá-Hurtado et al., 1997).

RESULTS AND DISCUSSION

Changes of milk composition in nanofiltration

Lactose-hydrolyzed milk was produced by adding 18 ml of β-galactosidase to 60 L of raw milk and incubating at 4°C for 24 h. The lactose-hydrolyzed milk was concentrated to reach the concentration factor of 2.1 using the spiral membrane element for nanofiltration. The chemical compositions of raw milk, lactose-hydrolyzed milk, concentrate, and permeate were analyzed (Table 1). The concentrations of lactose in raw milk and lactose-hydrolyzed milk were 4.83% and 0.43%, respectively, which showed that 91% of lactose was hydrolyzed. But there were little changes in other chemical compositions during the enzyme treatment.

The concentrations of most chemical components in the concentrate were higher than those of permeate after nanofiltration. The permeate was a clear greenish yellow solution. The differences in the concentrations of glucose, galactose, and sodium between the concentrate and the permeate were relatively small, indicating low retention in nanofiltration. The major chemical components in the permeate were glucose, galactose, ash, and lactose.

The concentration factors and coefficients of retention of the chemical components in lactose-hydrolyzed milk (Table 2) were calculated using data in Table 1. The concentration factor of milk fat was 2.20 and similar with the concentration factor of lactose-hydrolyzed milk, which indicated that milk fat did not penetrate the nanofiltration membrane. Since the void volumes of pump and filtration element used in nanofiltration of lactose-hydrolyzed milk were not known, it was often difficult to measure feed volume and concentrate volume which should be measured to calculate concentration factor of lactose-hydrolyzed milk.

Table 2. Concentration factors and coefficient of retentions of chemical components in nanofiltration of lactose-hydrolyzed milk at the concentration factor of 2.1

Chemical components	Concentration factor	Coefficient of retention
Solid	1.81	0.81
Crude protein	1.99	0.98
Milk fat	2.20	1.00
Crude ash	1.87	0.77
Lactose	1.70	0.68
Glucose	1.21	0.21
Galactose	1.11	0.15
Ca	1.48	0.96
Na	1.07	0.22
Riboflavin	1.72	0.76

The concentration factor of milk fat could be used to estimate concentration factor of the lactose-hydrolyzed milk.

Milk fat forms large spherical globules which range from about 1 μm to 12 μm in diameter and are surrounded by a phospholipid-rich layer, milk fat globule membrane. Thus, it may not pass through the nanofiltration membrane the molecular weight cut-off of which is 150-300.

The coefficient of retention of crude protein was 0.98. Non-protein nitrogen of low molecular weight as well as true protein are present in milk. The non-protein nitrogen content of milk represents 5-6% of the total N in milk. The single largest contributor to the non-protein nitrogen in milk is urea (DePeters and Ferguson, 1992). The nanofiltration membrane may not retain urea the molecular weight of which is 60.06.

The coefficients of retention of riboflavin, lactose, glucose, and lactose were 0.76, 0.68, 0.21, and 0.15, respectively. The disaccharide, lactose, was partially retained by the membrane, but the monosaccharides, glucose and galactose, seemed to pass through the membrane. Because the molecular weight of riboflavin was similar with that of lactose, riboflavin also seemed to be partially retained.

In the preliminary experiments the retention characteristics of the nanofiltration membranes, CK, DK, DL, HL, and GE, manufactured by Osmonics were determined to select an appropriate nanofiltration membrane. The estimated coefficient of retention of lactose, glucose, riboflavin in nanofiltration of lactose-hydrolyzed milk ranged 0.75-0.95, 0.26-0.89, and 0.00-1.00, respectively (results not shown). The nanofiltration membrane DL was selected based on this result.

The coefficients of retention of calcium and sodium in the lactose-hydrolyzed milk were 0.96 and 0.22, respectively. All the minerals in milk are distributed between a soluble phase and colloidal phase (Varnam and Sutherland, 1994). While monovalent ion, such as sodium, exists largely or totally, in the soluble phase, as much as

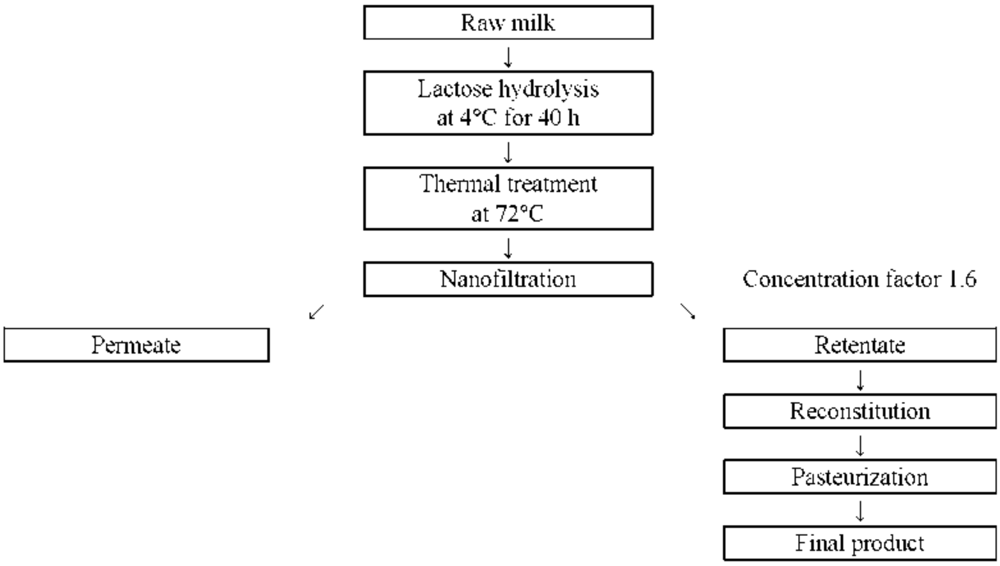

Figure 1. Process for producing lactose-hydrolyzed milk using nanofiltration.

Table 3. Chemical compositions[a] of raw milk, market milk, lactose-hydrolyzed market milk, and lactose-hydrolyzed nanofiltrated milk

Chemical components	Raw milk	Market milk	Lactose-hydrolyzed market milk	Lactose-hydrolyzed nanofiltrated milk[b]
Crude protein (%)	3.14±0.03	3.13±0.02	3.36±0.02	3.10±0.04
Milk fat (%)	3.5±0.1	3.5±0.0	3.5±0.1	3.5±0.1
Lactose (%)	5.00±0.03	4.95±0.08	0	0.06±0.01
Glucose (%)	0	0	1.51±0.02	1.45±0.05
Galactose (%)	0	0	1.41±0.03	1.29±0.08
Ca (mg %)	101.1±2.4	98.5±1.3	92.9±2.5	97.5±0.9
Na (mg %)	46.4±0.7	41.9±2.1	33.8±0.9	35.5±0.2
Riboflavin (mg %)	0.10±0.0	0.11±0.01	0.08±0.0	0.08±0.0

[a] Determined in duplicate. mean±standard deviation

[b] Lactose was hydrolyzed fully.

66% of the calcium may be in the colloidal phase. because calcium ions strongly associate with phosphate and citrate to form colloidal calcium phosphate citrate and also bind to caseins to form colloids. The calcium in colloidal phase may not pass through the nanofiltration membrane.

Production of lactose-hydrolyzed milk with low sweetness

90 ml of β-galactosidase was added to 90 l of raw milk, which was then incubated at 4°C for 40 h in order to hydrolyze lactose completely. The lactose-hydrolyzed milk was heated to 73°C for 5 min, cooled to 45°C, and then concentrated using the spiral wound element until the concentration factor reached 1.6. The concentrated lactose-hydrolyzed milk was reconstituted by adding deionized tap water and subsequently heated at 65°C for 30 min. cooled to 4°C, and stored in refrigerator (Figure 1).

The lactose-hydrolyzed nanofiltrated milk was subjected to sensory evaluation in which market milk and lactose-hydrolyzed market milk were compared as references. The milk samples were ranked based on the order of sweetness by ten inexperienced college students. The sums of rankings of lactose-hydrolyzed market milk. lactose-hydrolyzed nanofiltrated milk, and market milk were 18. 22. and 29, respectively. But the differences were not statistically significant at the level of 0.05 according to Kramer's table.

The chemical compositions of raw milk, market milk. lactose-hydrolyzed market milk, and lactose-hydrolyzed nanofiltrated milk were determined (Table 3). The ratio of crude protein. calcium. sodium. and riboflavin in lactose-hydrolyzed nanofiltrated milk to those in raw milk were 0.99. 0.96. 0.77, and 0.80, respectively, which showed that the losses of crude protein and calcium were very low. However, the loss of riboflavin was higher than expected from its coefficient of retention in Table 2.

The lactose-hydrolyzed market milk available commercially was known to be produced using ultrafiltration to remove lactose from milk before hydrolysis of lactose. Milk salt is recovered from the permeate of ultrafiltration using nanofiltration and reverse osmosis and added back to the milk (Tossavain and Sahlstein. 2003).

Though the protein content of lactose-hydrolyzed market milk was higher than the other milks, the calcium and sodium content were lower than the other milks. The lactose-hydrolyzed nanofiltrated milk produced in this study had calcium content higher than the lactose-hydrolyzed market milk. The manufacturing process of the lactose-hydrolyzed milk in this study was relatively simple (Figure 1) and losses of nutrients, such as protein and calcium, were minimal.

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REFERCENCES

Albalá-Hurtado, S., M. T. Veciana-Nogués, M. Izquierdo-Pulido and A. Mariné-Font. 1997. Determination of water-soluble vitamins in infant milk by high-performance liquid chromatography. J. Chroma. 778:247-253.

Boey, C. C. 2001. Lactase deficiency among Malaysian children with recurrent abdominal pain. J. Paediatr. Child Health. 37:157-160.

DePeters, E. J. and J. D. Ferguson. 1992. Nonprotein nitrogen and protein destribution in the milk of cows. J. Dairy Sci. 75:3192-3209.

Jackson, K. A. and D. A. Savaiano. 2001. Lactose maldigestion, calcium intake, and osteoporosis in African-, Asian-, and Hispanic-Americans. J. Am. Coll. Nutr. 20:198S-207S.

Jirage, K. B. and C. R. Martin. 1999. New developments in membrane-based separations. Trends Biotechnol. 17:197-200.

Kim, J. W. 1994. Studies on the lactose intolerance of Korean. Korean J. Dairy Sci. 16:105-114.

Kim, K.-O., S.-S. Kim, N.-K. Sung and Y.-C. Lee. 1993. Practice and application of organoleptic test. Shikwang, Korea.

Kohler, J. J., A. B. Erickson and J. L. Meyer. 1994. Method and apparatus for producing reduced lactose milk. U.S. patent, 5,357,852.

Lange, M. 2003. Process for making a lactose-free milk and milk so processed. U.S. patent 20030031754.

Marshall, R. T. 1993. Standard methods for the examination of dairy products. APHA, Washington.

Mattila-Sandholm, T. and M. Saarela. 2003. Functional dairy products, Woodhead Publishing Ltd. pp. 9-10.

Nyström, M., L. Kaipia and S. M. Luque. 1995. Fouling and retention of nanofiltration membranes. J. Membrane Sci. 98:249-262.

Renner, E. and M. H. Abd El-Salam. 1991. Application of ultrafiltration in the dairy industry, Elsevier Applied Science.

Tossavainen, O. and J. Sahlstein. 2003. Process for producing a lactose-free milk product. WO 03/094623.

Varnam, A. H. and J. P. Sutherland. 1994. Milk and milk products -technology, chemistry, and microbiology. Chapman and Hall, London.

Yoon, Y. C. and J. M. Jayaprakasha. 2005. Production of functional whey protein concentrate by monitoring the process of ultrafilteration. Asian-Aust. J. Anim. Sci. 18:433-438.