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There Is Saying That "If Intestines Are Healthy, Skin Gets Healthy."

กาแลคโตซิลแลคโตสในผลิตภัณฑ์กาแลคโตโอลิโกแซคคาไรด์ ช่วยเสริมพรีไบโอติก เสริมสร้างภูมิคุ้มกัน และเสริมสุขภาพผิวหนังของมนุษย์

กาแลคโตโอลิโกแซคคาไรด์ (Galacto-oligosaccharide) หรือ GOS เป็นสายใยของน้ำตาลที่มีความยาวตั้งแต่ 2-5 หน่วย โดยมีน้ำตาล 2 ชนิดเป็นองค์ประกอบหลัก ได้แก่ กาแลคโตส (Galactose) และกลูโคส (Glucose) เชื่อมต่อกันอยู่ด้วยพันธะไกลโคซิดิก (Glycosidic) ชนิดต่างๆ (Nakayama and Amachi, 1999) จัดเป็นสารที่มีคุณสมบัติเชิงพรีไบโอติก โดยร่างกายไม่สามารถย่อยสลายได้ ดังนั้นจะเป็นแหล่งอาหารของแบคทีเรียที่มีประโยชน์ต่อร่างกาย อีกทั้ง GOS มีคุณสมบัติเป็น Bifidus grown factor เนื่องจากมีความสามารถกระตุ้นการเจริญเติบโตของจุลินทรีย์ที่มีประโยชน์ต่อร่างกายในกลุ่มบิฟิโดแบคทีเรียและแล็คโตบาซิลัส ทำให้เกิดการผลิตกรดแลคติกและกรดอะซิติก ส่งผลให้สภาวะในลำไส้เป็นกรด จึงมีผลต่อการยับยั้งการเจริญของจุลินทรีย์ก่อโรค ซึ่งมีประโยชน์ในทางเดินอาหารของมนุษย์ (Gibson and Rastall, 2006)

กาแลคโตซิลแลคโตส คืออะไร กาแลคโตซิลแลคโตส (Galactosyllactose) คือ ไตรแซคคาร์ไรด์ประกอบด้วยแลคโตส และกาแลคโตส โดย 3,4,6 Galactosyllactose เป็นองค์ประกอบสำคัญในน้ำนมแม่ภายหลังจากการคลอดบุตร ซึ่งมีประสิทธิผลในการเพิ่มจำนวนของแบคทีเรียชนิดที่มีประโยชน์ในกลุ่ม Lactic acid และปรับปรุงระบบภูมิคุ้มกัน เพิ่มการดูดซึมของแร่ธาตุ และบำรุงผิวพรรณ รวมถึงป้องกันโรคภูมิแพ้ทางผิวหนังได้อีกด้วย จากการศึกษากาแลคโตโอลิโกแซคคาไรด์ที่อุดมไปด้วยกาแลคโตซิลแลคโตสจะให้ประโยชน์ในเชิงสุขภาพได้หลากหลาย

บำรุงผิวพรรณ งานวิจัยทางวิทยาศาสตร์บางฉบับได้ตีพิมพ์เรื่อง สารพิษที่เกิดขึ้นในทางเดินอาหารประเภท Phenol, p-cresol ผลิตขึ้นจากแบคทีเรียชนิดก่อโรคในกลุ่ม *E. coli, Proteus* sp, *E faecolis* อาจเป็นสาเหตุของการเกิดสิว ริ้วรอย และความแห้งของผิวหนัง (RIizuka-Handbook of diet, nutrition and the skin, 2012)

จากการศึกษายังพบว่าการรับประทาน GOS ที่มีกาแลคโตซิลแลคโตสมีผลช่วยลดการเจริญของแบคทีเรียก่อโรคที่ไม่ใช้ออกซิเจน (Anaerobic) ดังนั้น จะช่วยลดการสร้างสารพิษจากแบคทีเรียกลุ่มดังกล่าว ลดการอักเสบของผิวหนัง ลดการเกิดสิว

Galacto-oligosaccharide or GOS for shot, generally comprise a chain of 2-5 galactose units that arise through consecutive glycosidic linkages, with a terminal glucose unit. (Nakayama and Amachi, 1999). Depending on oligosaccharide composition, GOS products will vary in terms of prebiotic activity, as well as other physiological effects. GOS is effective as a bifidus grown factor stimulates the growth of bifidobacteria and Lactobacillus in the intestine. Most of the bifidobacteria break down lactose in to lactic acid and acetic acid that is excellent in intestinal regulation. (Gibson and Rastall, 2006).

What is Galactosyllactose? Galactosyllactose is trisaccharide composed of lactose and galactose. Especially, 3', 4', 6'-Galactosyllactose is important substance contained in mother's milk immediately after childbirth, and it has various bio-activities, and its effects are proliferation of lactic acid bacteria, immune improvement, enhancement of mineral absorption, and skin (atopy) improvement, etc. Recently, it was found out to be effective in improving many body activities as follow.

Skin and Effects Recent researches have revealed that when we intake protein, phenol substance (Phenol, p-cresol), intestinal toxin (bioactive toxin) generated by intestinal toxic bacteria (*E. coli, Proteus* sp., *E. faecolis*) when the protein is metabolized, is absorbed quickly into intestinal mucosa, and accumulated in the skin by body circulation system, causing various skin troubles such as atopy, acne, skin dryness, and wrinkles caused by dryness, etc. (R Iizuka - Handbook of diet, nutrition and the skin, 2012).

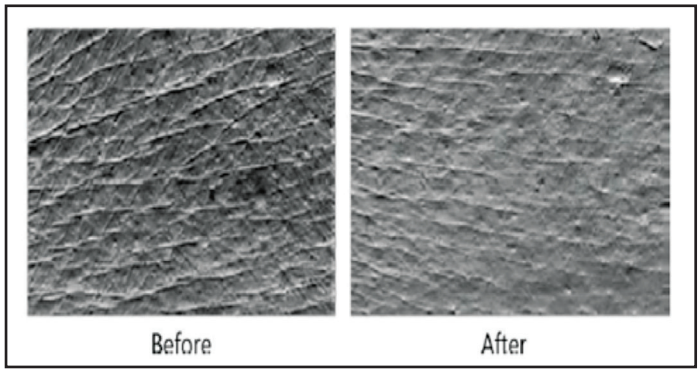
The studies also showed that galactosyllactose-fortified high-purity galacto-oligosaccharide intake reduces anaerobic bacteria so that they cannot generate toxic substances. It improves symptoms of skin troubles and wrinkles among adults. The study on 75 volunteers (30-60 years of age with wrinkles) were orally administrated of 2 grams/day of GOS for 12 weeks resulted that wrinkle area proportion and skin surface after GOS intake were reduced. The skin troubles were improved as well as the moisture amount were increased. (Fig 1)

และริ้วรอยได้อีกด้วย และมีการศึกษาการรับประทาน GOS ในกลุ่มผู้สอบ 75 คน อายุเฉลี่ย 30-60 ปี ที่มีปัญหาริ้วรอยที่ขอบตา ซึ่งให้รับประทาน GOS ที่มีกาแลคโต-ซิลแลคโตส 2 กรัม ต่อวัน เป็นระยะเวลา 12 สัปดาห์ พบว่าริ้วรอยที่ขอบตาจางลง สีผิวมีความสว่างใส อีกทั้งความชุ่มชื้นของผิวหนังมากขึ้น ดังรูปที่ 1

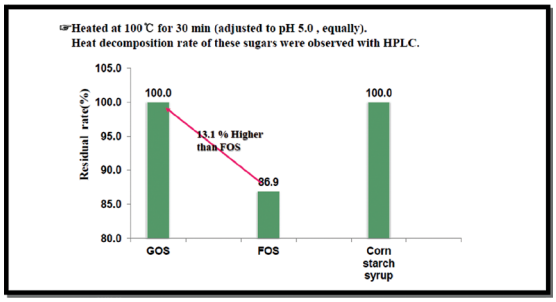
เพิ่มความแข็งแรงของระบบภูมิคุ้มกันโรค การปรับปรุงคุณภาพของจุลินทรีย์ชนิดที่ดีก่อให้เกิดสารต้านจุลชีพ (Antibiotic) เช่น แกรมมาอินเตอร์-เฟอรอน (γ-interferon) การหมักครั้งที่สองของจุลินทรีย์จะก่อให้เกิดความแข็งแรงของระบบภูมิคุ้มกันโรค เช่น เพิ่มจำนวนเซลล์ลิมโฟไซต์ชนิดทีเซลล์ (T cell) และชนิดบีเซลล์ (B cell)

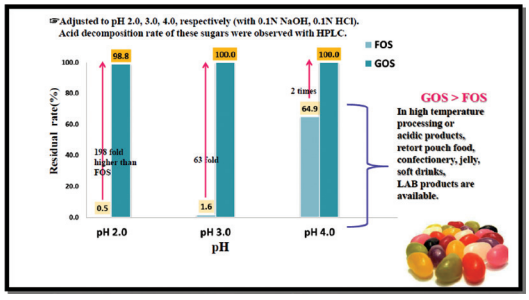
เพิ่มการดูดซึมแร่ธาตุ การปรับปรุงจุลินทรีย์ชนิดที่มีประโยชน์ในลำไส้จะทำให้สภาวะพีเอชในลำไส้เป็นกรดอ่อนๆ ช่วยให้เกิดสภาวะการดูดซึมกลับของแร่ธาตุ เช่น แคลเซียม และแมกนีเซียม และยังปรับปรุงความหนาแน่นของมวลกระดูก

ลดอาการท้องผูก ช่วยลดอาการท้องผูกเพราะกรดที่ผลิตโดยบิฟิโดแบคทีเรียจะช่วยกระตุ้นการบีบตัวของลำไส้และเพิ่มความชุ่มชื้นของอุจจาระ ซึ่งเป็นผลมาจากแรงดันออสโมติก



รูปที่ 1 ความแตกต่างของสภาพผิวและริ้วรอยรอบขอบตาก่อนและหลังจากรับประทาน GOS
Fig 1 Results of wrinkle area proportion and skin surface after GOS intake.





Improving Constipation GOS has been shown to be effective for improving constipation according to the lactic acid and acetic acid produced from bifidobacteria. Improvement of gut microflora suppresses adherence which resulted from osmotic pressure.

GOS Applications The application of GOS[1] as functional ingredients with 70% galacto-oligosaccharide and galactosyllactose (as contained in mother's milk) is low calorie and its sweetness is 40 % as sweet as sugar. Heated 100 °C for 30 min and adjusted to pH 2-4. The functional ingredients commonly used in food industries for beverages, dairy, coffee and food supplements.

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การประยุกต์ใช้ GOS ผลิตภัณฑ์ GOS[1] อุดมด้วยกาแลคโตโอลิโกแซคคาไรด์ที่มีความเข้มข้นสูงมากกว่าร้อยละ 70 และกาแลคโตซิลแลคโตส (องค์ประกอบใกล้เคียงกับน้ำนมแม่) ให้พลังงานต่ำ และมีความหวานประมาณร้อยละ 40 ของน้ำตาลทราย ทนความร้อนได้สูง 100 องศาเซลเซียส เป็นระยะเวลา 30 นาที ทนสภาวะพีเอชที่เป็นกรดต่ำได้ดี (pH 2-4) จึงสามารถประยุกต์ใช้ได้กับผลิตภัณฑ์เครื่องดื่ม นม นมผง โยเกิร์ต กาแฟ และผลิตภัณฑ์เสริมอาหาร 4CUS

Strengthening the Immune System Improvement of gut microflora generates anti-biotic substance, etc. (γ-interferon), the second metabolic products of those microbes, strengthening the immune system (proliferation of T cell, B cell).

Enhancement of Body Absorption of Minerals Many animal experiments showed that GOS intake enhances body absorption of calcium and magnesium substances, and improves bone density. When GOS is taken, it is not digested in small intestine, but arrives at large intestine. Here, GOS is decomposed by intestinal microorganisms into short chain fatty acid, acidifying intestinal pH, which increases solubility of mineral substances, and absorption rates of them.

ข้อมูลเพิ่มเติม/Additional Information

[1] ผลิตภัณฑ์ Mother's Oligo หรือ Beauty Oligo จาก Neo Cremar ประเทศเกาหลี จัดจำหน่ายโดย บริษัท เพียวเคมีกัลส์ จำกัด สามารถประยุกต์ใช้ในผลิตภัณฑ์อาหารและเครื่องดื่มได้หลากหลาย

[1] Mother's Oligo or Beauty Oligo from Neo Cremar in Korea is distributed by Pure Chemicals Company Limited. Its applications are variety for foods and beverages.

เอกสารอ้างอิง/Reference

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original scientific paper

Evaluation of Prebiotic Effects of High-Purity Galactooligosaccharides *in vitro* and *in vivo*

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Summary

Galactooligosaccharides (GOS) are an important class of dietary prebiotics that exert beneficial effects on intestinal microbiota and gut barrier function. In this study, high-purity GOS (HP-GOS) were investigated *in vitro* and *in vivo* and confirmed as prebiotic ingredients in rat diet. HP-GOS were successfully produced using a two-step process, enzymatic hydrolysis and fermentation by yeast. They were found to serve as a good substrate and carbon source for supporting the growth of probiotic bacteria more effectively than other commercial GOS. Following administration of 1 % (by mass) of HP-GOS to rats, the growth of *Bifidobacterium bifidum* and *B. longum* in the gut increased most rapidly up to 12 h, and thereafter the increase was slow. Therefore, 1 % HP-GOS was found to be acceptable for the growth of probiotic bacteria. Groups of animals that were orally administered HP-GOS and bifidobacteria during the study, and the group administered HP-GOS during the 2nd (days 13–15) and 4th (days 28–30) period of the study had significantly ($p<0.05$) higher numbers of bifidobacteria in faeces than groups receiving a single dose of bifidobacteria. HP-GOS affected the expression of genes encoding glucagon-like peptide-1 (GLP-1) and peptide YY (PYY). There was a significant upregulation of GLP-1 and PYY mRNA with HP-GOS and bifidobacteria intake. We propose that the prebiotic properties of HP-GOS are potentially valuable for the production of functional foods for human consumption.

Key words: high-purity galactooligosaccharides, bifidobacteria, genes encoding GLP-1 and PYY peptides

Introduction

There has been an increasing interest in the regulation of colonic microflora in order to improve the host's health. This has been achieved traditionally by dietary inclusion of live microbes as food supplements known as probiotics. An alternative approach involves the consumption of food ingredients known as prebiotics. Prebiotics may provide advantages to probiotic bacteria in the gastrointestinal tract and additionally exert direct effects on the microflora in the large intestine (*1*).

Galactooligosaccharides (GOS) that consist of 3–10 molecules of galactose and glucose are known to facilitate the growth of desirable intestinal microflora and are considered as potent non-digestible prebiotics (*2*). Commercial GOS that contain complex mixtures of oligosaccharides with different glycosidic linkages and degrees of polymerization are usually synthesized by enzymatic transgalactosylation of lactose by β-galactosidases from various sources such as yeast, fungi or bacteria (*3,4*). In addition, these kinds of products can also contain transgalactosylated oligosaccharides, unreacted lactose, glu-

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cose and galactose, which do not have prebiotic properties but only the caloric value (*5*).

The most efficient process to produce high-purity GOS (HP-GOS) is yeast fermentation of sugars such as glucose, galactose and lactose (*6*). Cardelle-Cobas *et al*. (*7*) first reported the production of non-monosaccharides and HP-GOS by repeated batch fermentation with immobilized yeast cells. A GOS syrup of an increased purity was produced by immobilized β-galactosidase from *Penicillium expansum* F3 and subjected to fermentation by *Saccharomyces cerevisiae* L1 and *Kluyveromyces lactis* L3 (*8*). This was a feasible industrial process to produce high-purity GOS.

Among numerous non-digestible carbohydrate-based prebiotics, convincing scientific evidence for suitability for use as prebiotics exists only for inulin/oligofructose and GOS (*9*). Studies of the prebiotic effects of GOS and FOS in humans have shown that a daily dose of 4–20 g significantly increases the population of lactobacilli and bifidobacteria in the gut (*10*). Other effects, such as hypocholesterolemic effects, prevention of colon cancer, and enhancement of calcium absorption have been described (*11,12*). Numerous studies have reported data on the effects of non-digestible oligosaccharides (NDOs) and dietary fibre content on serum cholesterol and lipid levels; however, only a limited number of reports indicate positive effects of GOS (*13*) or inulin (*14*) on serum cholesterol metabolism in humans. Yamashita *et al*. (*15*) suggested first that dietary inclusion of fructooligosaccharides demonstrated hypocholesterolemic effect in diabetic subjects.

In this paper, the ability of HP-GOS to support the *in vitro* growth of selected strains of probiotic bacteria, namely *Lactobacillus acidophilus, Lactobacillus casei*, *Bifidobacterium bifidum* and *Bifidobacterium longum*, is investigated. In addition, it was determined whether oral administration of HP-GOS affected the growth of bifidobacteria, as well as serum cholesterol and nitrogen levels and the expression of gene encoding glucagon-like peptide-1 (GLP--1) and tyrosine-tyrosine peptide (PYY), which act as significant modulators of appetite *via* their peripheral effects (on the vagus nerve) and/or by influencing directly the arcuate nucleus in the rat.

Materials and Methods

Materials

HP-GOS, containing 75.18 % of galactooligosaccharides (by mass), were obtained from Neo Cremar Co. Ltd, Seoul, Republic of Korea. Commercial GOS, Y-GOS (52.52 % GOS, by mass; Yakult Honsha, Tokyo, Japan), C-GOS (56.25 % GOS, by mass; Doosan Corn Products Korea, Seoul, Republic of Korea), and Q-GOS (41.77 % GOS, by mass; Samyang Genex, Inchon, Republic of Korea) were purchased from a local market in Seoul, Republic of Korea. A commercial β-galactosidase from *Bacillus circulans* was obtained from Bision Corporation (Seongnam, Republic of Korea).

Production of GOS

Batch reactions were performed by incubating β-galactosidase with a 40–45 °Brix lactose solution in a 100-litre incubator shaker at 150 rpm. Lactose (25 kg) was dissolved in distilled water (60 L), and 0.08 % (by mass) β-galactosidase from *Bacillus circulans* was added to synthesize GOS at 55 °C and pH=6.0 for 24 h. All reactions were terminated by incubation at 100 °C for 10 min and the sugar profile was analyzed by high-performance liquid chromatography. To increase the GOS content, 100 mL of 20 % (by mass) GOS syrup produced by enzymatic hydrolysis with β-galactosidase were fermented by 8 % (by mass) of fresh yeast (*Saccharomyces cerevisiae* L1). The fermentation process was carried out in a shaking incubator at 100 rpm and 30 °C for 24 h. After each fermentation cycle, cells were transferred to 20 % (by mass) GOS syrup, which was then filtered and treated with active carbon for decolourization. Ion exchange chromatography using Amberlite® CG-120-II column (Sigma-Aldrich, Buchs, Switzerland) was utilized for further purification. The pooled fractions were evaporated to 45 °Brix and dried with a spray dryer. Lactose and HP-GOS were determined by an HPLC system with Waters® 2414 refractive index detector (RID) (Waters Corporation, Milford, MA, USA) equipped with YMC-Pack Polyamine II column (4 mm×250 mm; YMC Co. Ltd, Kyoto, Japan), column heater (30 °C), and RID detector. Acetonitrile (64 %) was used as mobile phase.

Bacterial strains and growth conditions

Lactobacillus acidophilus DDS-1, *L. casei* KCTC 12452, *Bifidobacterium bifidum* KCTC 3357 and *B. longum* SJ 32 were obtained from the culture collection from the Korea Research Institute of Bioscience and Biotechnology, Daejeon, Republic of Korea. The cultures were grown at 37 °C in modified peptone yeast extract fructose (PYF) medium without carbon source consisting of (in %, by mass): yeast extract 1, peptone 0.5, L-cysteine HCl 0.5 and salt solution 4 (containing (in %, by mass): $CaCl_2$ 0.02, $MgSO_4$ 0.02, K_2HPO_4 0.1, KH_2PO_4 0.1, NaCl 0.2 and Na_2HPO_3 1.0) (*16*). The medium was supplemented with 0.5, 1, 2 or 4 % (by mass) GOS. Microplates were incubated anaerobically at 37 °C in a GasPak™ container (BD (Becton, Dickinson and Company), Franklin Lakes, NJ, USA). The absorbance at 600 nm ($A_{600\ nm}$) was recorded by the microplate reader at 0, 24 and 48 h. At 24 h, after the measurement of $A_{600\ nm}$, the plates were placed in the incubator to restore anaerobic conditions.

Animals and diet

The experimental protocol was reviewed and approved by Institutional Animal Care and Use Committee of Korea University. Four-week-old male Sprague Dawley® (SD) rats were purchased from Daehan Biolink Co. Ltd. (Cheongju, Republic of Korea). The animals were kept in a room at 24 °C and constant atmosphere with 60 % humidity and a 12-hour light/dark cycle. Rats were fed an AIN-93G diet based on the main ingredients of corn starch (40 %) and casein (20 %) (*17*). After an adaptation period, the rats were randomly divided into four groups (N=8): the control group (oral administration of saline), the HP-GOS group (oral administration of HP-GOS), the HP-GOS+BB group (oral administration of HP-GOS and bifidobacteria), and the BB group (oral administration of bifidobacteria). The groups were orally administrated HP-GOS (1.5 mL of the solution of 1 g of HP-GOS and/or 10^9 CFU bifidobacteria) daily for 5 weeks.

Fresh faecal samples were collected weekly (equal mass from four rats per pool) in sterile flasks, and kept at –80 °C for microbiological analysis. To count the total number of bifidobacteria, 3 g of faecal sample were diluted in 25 mL of dilution solution and an aliquot of 0.2 mL was spread on Petri dishes using BL agar. Colonies were incubated anaerobically during 2–3 days at 37 °C under anaerobic conditions and results were measured as the log CFU per gram of faecal sample.

At the end of the study, the rats were euthanized using CO_2 asphyxiation and the liver, kidney and spleen were removed and weighed immediately. The body mass of each rat was measured every week for 5 weeks and the mass of each organ was expressed as 100 g of body mass.

Blood analysis

Blood samples were collected into non-heparinized serum separator tubes. Serum triglycerides (TGs), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) levels were measured by using a FUJI Dri-Chem 3500 system (Fuji Photo Film Co., Osaka, Japan). Concentration of low-density lipoprotein cholesterol (LDL-C), in mg per 100 mL, was calculated according to the method of Friedewald *et al*. (*18*) as follows:

$$\gamma(\text{LDL-C})=\gamma(\text{TC})-\gamma(\text{HDL-C})-(\gamma(\text{TG})/5) \qquad /1/$$

RNA extraction and real-time PCR

Total RNA was obtained from the intestine samples by using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. Reverse transcription was performed by using 1 μg of total RNA with first strand cDNA synthesis kit for real-time polymerase chain reaction (RT-PCR, Invitrogen) with oligo (dT) 15 as a primer (Invitrogen). After cDNA synthesis, real-time PCR was performed using a Power SYBR® Green PCR Master Mix kit (Applied Biosystems, Foster City, CA, USA). Quantitative analysis was carried out using StepOne plus software v. 2.0 (Applied Biosystems). Results were normalized to a validated control gene, β-actin, using the ΔΔCt method (*19*). Using primers to interrogate proglucagon and PYY, RT-PCR was performed by a method reported previously (*20*). The following primers were used for GLP-1 and PYY: proglucagon (NM-012707.2): forward primer: 5′-ATGCGGACGAATACATTTCC-3′, reverse primer: 5′-CTCAGGGCGGTAACTTCAAA-3′; PYY (NM_001034080.1): forward primer: 5′-CAGCGGTATGGGAAAAGAGA-3′, reverse primer: 5′-CATGCAAGTGAAGTCGGTGT-3′; β-actin (NM-031144.3): forward primer: 5′-GCTACAGCTTCACCACCACA-3′, reverse primer: 5′-TGCCGATAGTGATGACCTGA-3′.

Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Sciences, v. 12.0 (SPSS Inc., Chicago, IL, USA). The statistical significance of differences was determined using one-way ANOVA at a significance level of $p<0.05$. All data were significant at 95 % level and reported as the mean value±standard deviation (S.D.).

Results and Discussion

GOS content before and after enzymatic hydrolysis and fermentation

Table 1 summarizes the content of saccharides during different stages of GOS preparation, *i.e*. the enzymatic hydrolysis by β-galactosidase and fermentation by *S. cerevisiae*. After treatment by β-galactosidase, GOS content increased from 0 to 51.0 g per 100 g, and lactose mass fractions decreased from 99.2 to 21.3 g per 100 g (Table 1). Monosaccharide (glucose and galactose) mass fractions also increased from 0.8 to 27.7 g per 100 g after the enzymatic reaction; however, these monosaccharides were completely removed by fermentation with *S. cerevisiae*, whereas lactose levels were slightly increased. In order to increase the purity of GOS, fermentation process was utilized. After fermentation, glucose and galactose mass fractions decreased from 27.7 to 0.0 g per 100 g (Fig. 1 and Table 1). The purity of the GOS preparation increased

Table 1. Saccharide content during galactooligosaccharide (GOS) production

Process	*w*(monosaccharide)	*w*(lactose)	*w*(GOS)
	g/100 g	g/100 g	g/100 g
Raw material	0.8±0.1	99.2±2.6	0.0
After enzyme reaction	27.7±1.8	21.3±1.0	51.0±3.3
After fermentation with *S. cerevisiae* L1	0.0	26.4±1.3	73.6±2.1

Values are expressed as mean±standard deviation

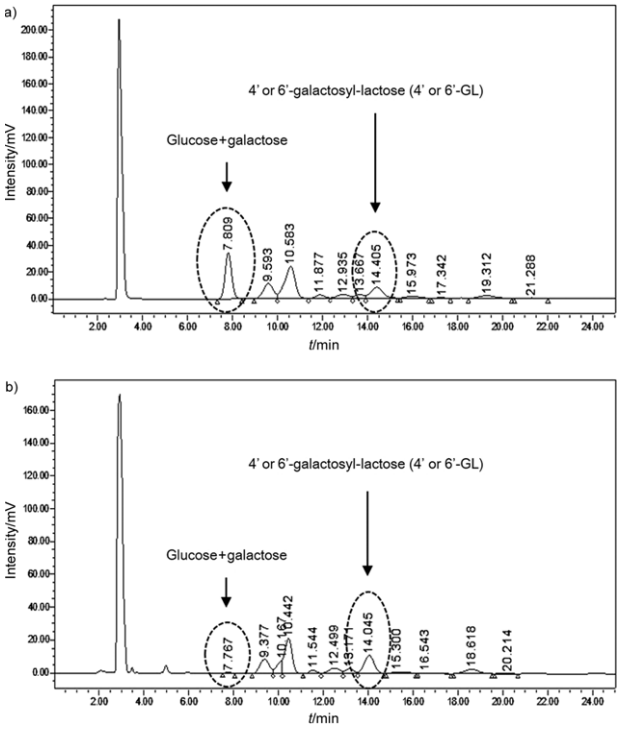

Fig. 1. Carbohydrate profile: a) before and b) after the fermentation with *Saccharomyces cerevisiae* L1

from 51.0 to 73.6 g per 100 g after fermentation with *S. cerevisiae* (Table 1), verifying that HP-GOS were successfully produced using the two-step enzymatic hydrolysis and fermentation by yeast.

For the production of HP-GOS, selective fermentation for a given microorganism was characterized. During the fermentation, ethanol may be produced depending on the used microorganism, which at toxic volume fractions may compromise the activity of the microorganism. *S. cerevisiae* was used to increase the purity of the mixture of GOS obtained by enzymatic hydrolysis with β-galactosidase from *Bacillus circulans*, with complete removal of the monosaccharides (*21*). The same approach was adopted for the production of GOS mixture from *B. bifidum* biomass (*9*). In a previous research, *Kluyveromyces marxianus* improved the purity of a GOS mixture produced by β-galactosidase from *B. circulans* from 38 to 97 % by selective fermentation of mono- and disaccharides (including lactose) (*6*). With a combined use of *S. cerevisiae* and *K. lactis* the purity of a GOS mixture produced by β-galactosidase from *Penicillium expansum* increased from 29 to 98 % (*8*). In this work, HP-GOS were produced by fermentation with *S. cerevisiae* (Table 1 and Fig. 1), which increased the GOS content from 51.0 to 73.6 g per 100 g after fermentation with *S. cerevisiae*.

Utilization of GOS by intestinal bacteria in vitro

HP-GOS comprise substances with structural differences and have considerably greater prebiotic potential compared to a commercially available GOS mixture. Fig. 2 shows the growth of intestinal bacteria in a medium containing various commercial GOS. In order to confirm that the growth was dependent on GOS utilization, strains were also inoculated into PYF basal medium containing 1 % HP-GOS, Y-GOS, C-GOS or Q-GOS as the carbohydrate source. During the early stages of growth (after 12 h), all strains exhibited a similar growth rate without significant differences. All strains (*L. acidophilus, L. casei, B. longum* and *B. bifidum*) in a medium containing HP-GOS had a higher cell growth rate than the strains grown in the media containing commercial GOS after 12 h of culture. These results suggest that HP-GOS serve as a good substrate and carbon source for supporting the growth of probiotic bacteria.

Growth curves for the given strains grown anaerobically in the medium without GOS or with 0.5, 1, 2 or 4 % (by mass) GOS are shown in Fig. 3. *L. acidophilus* and *L. casei* were able to grow at all the tested GOS mass fractions, both reaching a maximum $A_{600\,nm}$ of 1.15 after 36 h of growth. Cell growth was increased with an increase in HP-GOS mass fraction. Growth of *B. bifidum* and *B. longum* increased rapidly until 12 h and then continued increasing slowly. Therefore, HP-GOS mass fractions above 1 % were found to be acceptable for the growth of probiotic bacteria.

Structural differences of GOS vary notably depending on the conditions and source of enzymes used for their synthesis (HP-GOS and Y-GOS were produced from 4′- or 6′-galactosyl-lactose, and C-GOS and Q-GOS from 4′-galactosyl-lactose) (*4*), which affects the fermentation process as well as their prebiotic properties. Studies of

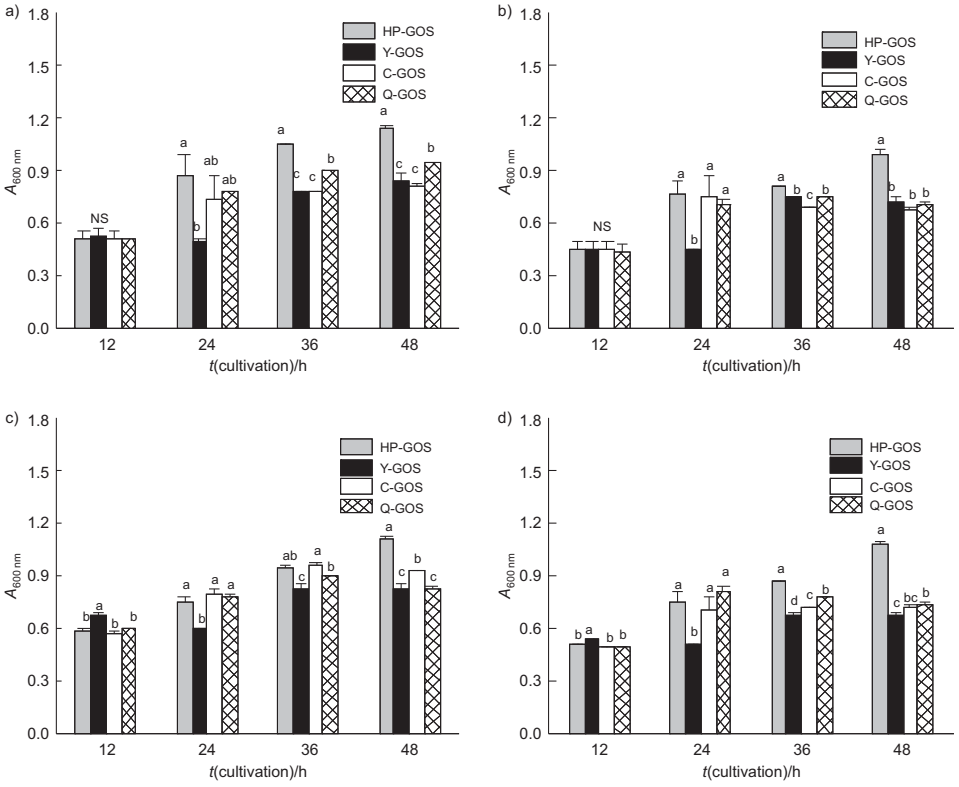

Fig. 2. Growth of: a) *L. acidophilus*, b) *L. casei*, c) *B. bifidum* and d) *B. longum* in modified PYF broth containing 1 % (by mass) of various commercial galactooligosaccharides (GOS). Bars represent the standard error of triplicate measurements. Different letters indicate significant differences at $p<0.05$

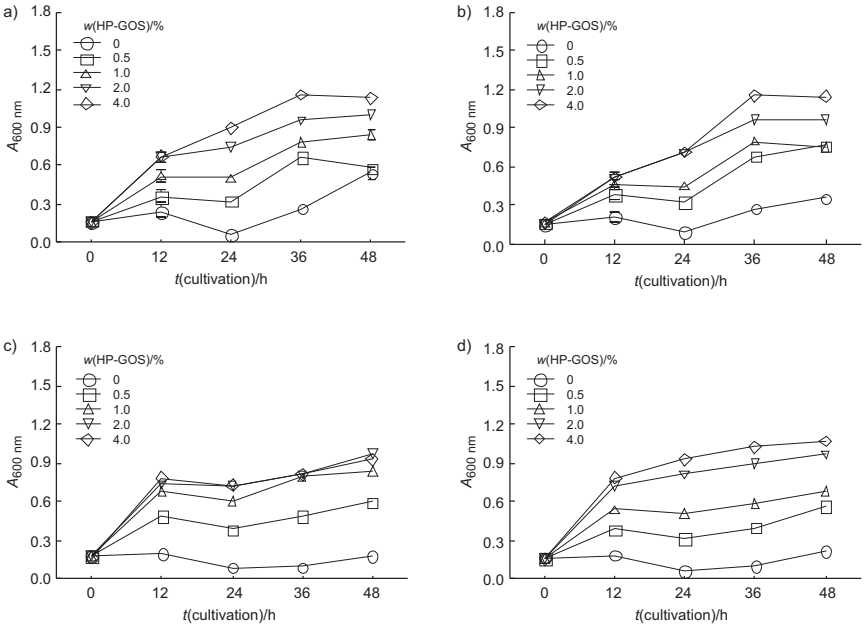

Fig. 3. Growth of: a) *L. acidophilus*, b) *L. casei*, c) *B. bifidum* and d) *B. longum* in modified PYF broth containing various mass fractions of high-purity galactooligosaccharides (HP-GOS). Bars represent the standard error of triplicate measurements

GOS utilization by bacteria have shown that different strains vary in their ability to ferment GOS, with individual strains exhibiting specific substrate preferences (*22, 23*).

We additionally analyzed the utilization of GOS by various probiotic bacteria (Fig. 2). *L. acidophilus*, *L. casei*, *B. bifidum* and *B. longum* had a higher cell growth rate when utilizing HP-GOS in comparison with commercial GOS (Fig. 2), suggesting that 4′- or 6′-galactosyl-lactose may be a more suitable substrate for these strains. The utilization of GOS by a number of probiotic bacteria has been extensively analyzed (*24,25*). It has been shown that 4′-galactosyl-lactose is selectively utilized by all the *Bifidobacterium* strains tested, compared with lactulose and raffinose, whose specificity is less noticeable. Other studies have also shown that some strains of *Lactobacillus*, *Bacteroides* and *Clostridium* ferment GOS, and that transgalactosylated disaccharides may even be better substrates for these bacteria (*26*). Some bacterial species can ferment both 4′- and 6′-galactosyl-lactose, although there are some differences and the growth of bacteria is dose-dependent.

As shown in Fig. 3, *B. bifidum* and *B. longum* utilized HP-GOS more rapidly than *L. acidophilus* and *L. casei*. It is considered that the utilization of non-digestible oligosaccharides (NDOs) by bifidobacteria is mediated by the hydrolyzing enzymes produced by these strains. Many *Bifidobacterium* strains produce glycolytic enzymes that hydrolyze a wide range of monosaccharides and various glycosidic bonds, while the activities of the enzymes from other enteric bacteria such as *Lactobacillus*, *Escherichia coli* and *Streptococcus* are less varied and are weaker than those from *Bifidobacterium* (*27*).

Changes in body mass, organ mass and serum parameters

Changes in body mass and organ mass of the rats in control, HP-GOS, HP-GOS+BB and BB groups are shown in Table 2. No significant differences in body mass were found among the three groups. The changes in the masses of the liver and other internal organs of the rats after a 5-week administration of each diet are shown in Table 3.

Table 2. Changes in body mass of rat after treatment with galactooligosaccharides and/or bifidobacteria

Group	*t*/week				
	1	2	3	4	5
	m/g				
Control	44.7±1.0	90.8±2.7	147.7±5.2	201.4±7.4	230.5±6.1
HP-GOS	42.5±2.0	82.0±2.2	129.5±4.7	176.5±6.6	210.6±7.0
BB	43.5±1.5	88.3±3.3	139.6±3.1	193.0±7.9	219.0±8.2
HP-GOS+BB	45.2±1.9	90.3±3.3	147.0±5.1	196.6±5.8	224.3±6.0

Values are expressed as mean±standard deviation. Control=oral administration of saline, HP-GOS=oral administration of high-purity galactooligosaccharides, BB=oral administration of bifidobacteria, HP-GOS+BB=oral administration of HP-GOS and bifidobacteria

Table 3. Changes of organ mass of rat after treatment with galactooligosaccharides and/or bifidobacteria

Organ	*w*/(g/100 g)			
	Control	HP-GOS	BB	HP-GOS+BB
Liver	9.0±0.1	8.4±0.5	8.7±0.4	8.9±0.2
Kidney	2.3±0.1	2.1±0.1	2.3±0.1	2.3±0.2
Spleen	0.7±0.0	0.6±0.0	0.8±0.0	0.7±0.0

Values are expressed as mean±standard deviation. Control=oral administration of saline, HP-GOS=oral administration of high-purity galactooligosaccharides, BB=oral administration of bifidobacteria, HP-GOS+BB=oral administration of HP-GOS and bifidobacteria

No differences were observed in the masses of the liver, spleen and kidney in relation to the body mass in all groups.

Serum glucose, protein, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lipid profiles are shown in Table 4. Groups administered HP-GOS+BB had significantly different AST and ALT levels compared with the control group or the group administered HP--GOS, respectively ($p<0.05$). However, the ALT and AST levels of the group administered HP-GOS+BB were in the normal range for SD rats (17.30–19.77 and 74.4–80.4 U/L, respectively) (*28*). There were no significant differences in the lipid profiles (total cholesterol, HDL cholesterol and triacyglycerol) among the groups.

Enumeration of bifidobacteria in rat faeces

Fig. 4 shows the total bifidobacteria counts in rat faeces, expressed in log CFU per g of faeces, during four periods of the study. The groups that were orally administered HP-GOS and bifidobacteria had significantly ($p<0.05$) higher counts during all four periods, while the group receiving HP-GOS had higher counts in the 2nd and 4th period only in comparison with the control and BB group. In the 2nd and 3rd periods, the same trend was observed in groups administered HP-GOS and HP--GOS+BB. During the entire period, oral administration of HP-GOS+BB resulted in higher bifidobacteria counts than the oral administration of control or single administration of bifidobacteria. There was no significant difference in bifidobacteria counts between the HP-GOS+BB group and the HP-GOS group in the 2nd and 4th periods.

Bifidobacteria in rat faecal samples were counted in the HP-GOS groups with or without bifidobacteria. Our results indicated a positive effect in the synbiotic group (HP-GOS+BB) during the test periods. It is known that most bifidobacteria strains of human intestinal origin can readily use galactooligosaccharides; however, only a few strains from other genera, such as lactobacilli, possess this ability (*24,26*). As far as the effects of probiotic consumption on the bifidobacterial population are concerned, similar results have been observed in children and adults. Benno and Mitsuoka (*29*) reported an increase in the counts of bifidobacteria as well as a remarkable decrease in the counts of clostridia in adult human subjects consuming a daily dose of *B. longum*.

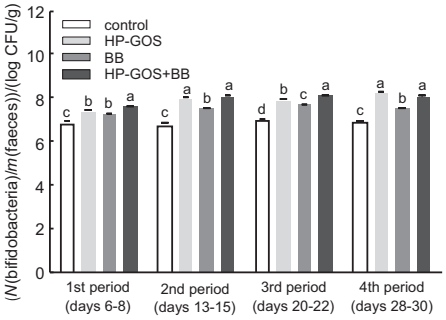

Fig. 4. Number of total bifidobacteria in rat faeces, expressed as mean values of log colony-forming units (CFU) per g of wet faeces, $N=6$. Bars represent the standard error of triplicate measurements. Different letters indicate significant differences at $p<0.05$. HP-GOS=high-purity galactooligosaccharides, BB=bifidobacteria

Expression of genes encoding GLP-1 and PYY in ileum

There was an approx. 1.6-fold increase in PYY mRNA levels in the ileum of rats administered GOS+BB (Fig. 5), which was significantly higher than in the other groups ($p<0.05$). Similarly, GLP-1 mRNA levels in the ileum of rats administered GOS+BB and only bifidobacteria were 1.5- and 1.6-fold higher, respectively, than in rats fed normal diet. There was a significant upregulation of GLP-1 and PYY mRNA with GOS+BB intake.

Endocrine cells in the intestinal mucosa secrete peptides involved in the regulation of food intake and/or pancreatic function; the latter are known as incretins (*30,31*). Endocrine L cells are distributed throughout the intestinal tract, and are predominantly present in the caeco-colon, where fermentation of inulin-type fructans occurs (*32*). GLP-1 and PYY, which are released from intestinal L cells, modulate appetite and thus reduce food intake (*33*). The ability of prebiotic fibre to increase the proglucagon mRNA levels and GLP-1 secretion is well supported (*34,35*). Increased proglucagon expression typically takes place when caecal mass is increased due to the markedly increased bacterial fermentation that occurs with the consumption of prebiotics (*34,35*). It is interesting to note that the fibre-containing prebiotic diet appears to have acute and lasting effects on the ability of L cells to produce and secrete GLP-1 and PYY. In the current study, measurements of GLP-1 and PYY levels were performed

Table 4. Serum levels of glucose, total protein, AST, ALT, and lipid profiles of rats

Group	γ(glucose)	γ(total protein)	AST	ALT	γ(TC)	γ(HDL-C)	γ(LDL-C)	γ(TG)
	mg/dL	g/dL	U/L	U/L	mg/dL	mg/dL	mg/dL	mg/dL
Control	110.7±2.6	18.62±0.07	74.4±2.2^{b}	18.6±0.6^{ab}	79.2±2.5	52.7±2.8	11.4±3.0	75.3±3.0
HP-GOS	111.5±5.4	19.77±0.09	79.6±0.8^{ab}	19.8±0.7^{a}	83.8±2.5	56.2±2.3	14.6±1.6	77.7±1.7
BB	104.0±1.4	18.42±0.09	75.2±1.6^{ab}	18.4±0.9^{ab}	78.7±1.6	54.6±2.0	10.5±3.9	73.5±1.7
HP-GOS+BB	114.5±4.4	17.30±0.08	80.4±1.7^{a}	17.3±0.6^{b}	85.3±0.6	56.0±1.5	16.0±0.8	77.8±1.3

Values are expressed as mean±standard deviation, $N=8$. Mean values with different letters in superscript within the column are significantly different at $p<0.05$ by Duncan's multiple range tests. AST=aspartate aminotransferase, ALT=alanine aminotransferase, TC=total cholesterol, HDL-C=high-density lipoprotein cholesterol, LDL-C=low-density lipoprotein cholesterol, TG=triglyceride. Control=oral administration of saline, HP-GOS=oral administration of high-purity galactooligosaccharides, BB=oral administration of bifidobacteria, HP-GOS+BB=oral administration of HP-GOS and bifidobacteria

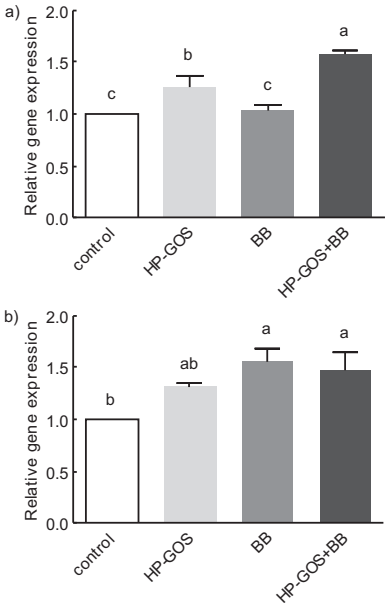

Fig. 5. Expression of genes encoding PYY and GLP-1 in the ileum of mice orally administered saline (control), high-purity galactooligosaccharides (HP-GOS), bifidobacteria (BB), and high-purity GOS+bifidobacteria (HP-GOS+BB) for 4 weeks, N=8. Bars represent mean value±standard error of the mean (SEM). Different letters indicate significant differences at $p<0.05$

at the end of a 5-week period, when GOS as prebiotics were administered as part of the diet; however, we additionally found higher expression of genes encoding GLP-1 and PYY in the HP-GOS group with or without *B. bifidum* at the end of the period in which the high fat-based diet was administered (Fig. 5).

It is acknowledged that an optimum balance in microbial populations in the digestive tract is associated with good nutrition and health, and that this may be achieved by the consumption of probiotics. In particular, HP-GOS exhibit a greater prebiotic activity than other commercial GOS.

Conclusion

High-purity galactooligosaccharides (HP-GOS) were successfully produced using a two-step process, enzymatic hydrolysis and fermentation by yeast. They were found to serve as a good substrate and carbon source for supporting the growth of enteric bacteria, compared with other commercial GOS. The beneficial effect of regular intake of HP-GOS is attributed to the intestinal survival of probiotic *Lactobacillus* and *Bifidobacterium* strains. The health benefits associated with the consumption of HP-GOS in humans include improvement of intestinal tract health, increase in the expression of genes encoding GLP-1 and PYY, decrease in the risk of certain cancers, blood pressure control, and reduction of serum cholesterol levels. On the basis of our findings, we propose that the prebiotic properties of HP-GOS are valuable in the production of potential health-enhancing foods and supplements, and that HP-GOS may be used as a functional food ingredient for human consumption.

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Disclosures

None of the authors of this study has any financial interest or conflict with industries or parties.

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Original Article

Dietary galacto-oligosaccharides improve skin health: a randomized double blind clinical trial

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Background and Objectives: To study the effects of galacto-oligosaccharides (GOS) on the skin, we investigated skin-related parameters in healthy adults who received GOS for 12 weeks. **Methods and Study Design:** This double-blind, randomized, placebo-controlled study included subjects divided into two groups (control and GOS) by stratified block randomization. The GOS group received 1.0 g of GOS twice a day, whereas the control group received only vehicle. **Results:** The results showed that the increase in corneometer values from baseline to week 12 was significantly greater in the GOS group than in the control group (6.91 vs 2.88 arbitrary units, $p<0.05$). The transepidermal water loss (TEWL) in the GOS group was reduced significantly after 12 weeks of GOS treatment (20.1 g/h/m^2 at baseline vs 17.5 g/h/m^2 at week 12, $p<0.05$). The differences in total and percentage of wrinkle areas between the two groups were statistically significant after 12 weeks of GOS treatment ($p<0.05$). **Conclusion:** Our findings support that oral treatment with GOS is beneficial to the skin and present the possibility of new nutritional strategies for skin care.

Key Words: galacto-oligosaccharide, prebiotics, probiotics, skin hydration, wrinkle

INTRODUCTION

Oligosaccharides show interesting properties, and some are already recognized and included in foods as ingredients.[1] They are an important factor that promotes the growth of intestinal flora dominated by bifidobacteria and lactobacilli.[2] On the basis of the analysis of human milk oligosaccharides, a mixture of 90% short-chain galacto-oligosaccharides (GOS) and 10% long-chain fructooligosaccharides (FOS) have been developed.[3] Studies in preterm and term infants have shown that food supplementation with GOS and FOS produces an intestinal flora similar to that found in breastfed infants.[4] FOS are well known for their contribution to digestive health. GOS have also emerged, with strong clinical evidence, as beneficial to both digestive and immune health.[5] As a stable, soluble ingredient, GOS are an ideal choice for formulating foods and beverages for digestive and immune health. Owing to its similarity to the human milk oligosaccharides, GOS have attracted worldwide attention from researchers.[5,6]

Because GOS are hydrolyzed only by a specific group of colonic bacteria, they are classified as prebiotics.[1] Prebiotics are typically nondigestible fibre compounds that pass undigested through the upper part of the gastrointestinal tract and stimulate the growth or activity of advantageous bacteria that colonize the large bowel by acting as a substrate for them.[1,7] Prebiotics can provide benefits not only for the gut but also for the skin. The intestine is the body's main immune organ, and the mucosal immune system of the gut is linked to the immune system of the skin through migration of immune cells. Prebiotics may also influence the bioavailability of nutrients and thereby affect the condition of the skin.[8,9]

The skin and digestive tract are the largest organs of the human body. They are also two of the oldest structures developed in the evolutionary process to provide the organism with essential information about the outside world, largely by delivering nutrients from the outside world. The digestive tract has a similar and parallel role to that of the skin in providing nutrients to the body. Nutrients, whether delivered through the digestive tract or skin, can be seen as a source of information that literally transforms the body.[10] Cosmetics have long been applied to prevent skin aging. However, while the benefits of cosmetics to skin are promising, they are limited to the topical site of application.[11] On the other hand, the con-

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sumption of foods that contain prebiotics has been found to improve the condition of the skin.[12] Functional foods targeting "beauty from within" are already on the market, and some have limited scientific evidence suggesting their efficacy.[13]

In this study, we investigated whether intake of GOS had beneficial effects on human skin and may represent a novel approach for skin care. To this end, we investigated skin-related parameters in healthy adults who received GOS for 12 weeks.

MATERIALS AND METHODS

Preparation of GOS

The GOS used in this study were provided by Neo Cremar Co. (Seoul, South Korea). Briefly, batch reactions were performed by incubating β-galactosidase with a 40°Bx to 45°Bx lactose solution in a 100-L incubator shaker at 150 rpm. Lactose (25 kg) was dissolved in distilled water (60 L), and 0.08% β-galactosidase from *Bacillus circulans* was added to synthesize GOS at 55°C and pH 6.0 for 24 h. All reactions were terminated by incubation at 100°C for 10 min. Then, 100 mL of 20% GOS syrup produced by the β-galactosidase was fermented by using 9% weight fresh yeast (*Saccharomyces cerevisiae* L1) in an incubator shaker at 100 rpm and 30°C for 24 h. The resulting solution was then filtered and treated with active carbon for decolorization. Ion exchange chromatography (Amberlite CG-120-II1 × 8, Fluka, Buchs, Switzerland) was applied for further purification. The pooled fractions were evaporated to 45°Bx and dried with a spray dryer.

Subjects

Subjects were recruited through advertisements in a local newspaper. Individuals who responded to the advertisements were interviewed in order to ensure they met the experiment criteria. Eighty-four healthy Korean volunteers, aged 30–69 years, with fine wrinkles at the outer corner of the eyes, called lateral canthal lines, were chosen for this study. Before enrolling in the study, all the participants were informed of the risks, benefits, and possible complications of the treatment, and each participant provided investigators with a written informed consent. The exclusion criteria were Fitzpatrick skin types I or IV, allergies, photosensitivity, tanning sunburns, infections, pregnancy, and breastfeeding. Also excluded were subjects who had undergone wrinkle removal or peeling procedures within the previous 6 months.

Study protocol

The study was conducted in accordance with Good Clinical Practice guidelines, the Declaration of Helsinki, and local laws and regulations. The protocol was approved by Korea University and patients gave written informed consent (KU-IRB-14-119-A-2-[R-P-1]). The double-blind, randomized, placebo-controlled study included subjects who were divided into two (control and GOS) groups by stratified block randomization. For 12 weeks, the subjects in the GOS group were asked to take GOS (1 g in a capsule) twice a day. The total daily GOS dose was 2 g. This dosage was selected based on preliminary studies.[14,15] The control group received only the vehicle (100% dextrin), which was the same size and color as the GOS capsule. The subjects were asked not to change their diet or lifestyle during the study.

Skin assessments

The corneometer value was measured by using Corneometer CM 825 (Courage and Khazaka Electronic GmbH, Cologne, Germany). Corneometer CM 825 uses the high dielectric constant of water for analyzing the water-related changes in the electrical capacitance of the skin. It displays hydration measurements in system-specific arbitrary units (AU).[16] Transepidermal water loss (TEWL) was measured by using TEWAmeter TM 300 (Courage and Khazaka Electronic GmbH). The TEWAmeter TM 300 measurements were based on diffusion in an open chamber and measured as $g/m^2/h$.[17] Extent of wrinkling was measured by using a replica method. Briefly, after an adhesive paper (diameter, 11 mm) was attached, translucent silicon was mixed in a small plastic cup containing two components, a basic substance and a catalyst (Courage and Khazaka Electronic GmbH). A layer of the silicone mixture was spread over the restricted area of the adhesive paper and left to dry for 5 min. When the silicone mixture had dried sufficiently, the specimen was stored in a tracing paper envelope until analysis. The skin replica was analyzed by using Skin-Visiometer SV 600 (Courage and Khazaka Electronic GmbH). The total wrinkle area (mm^2), percentage of wrinkle area (%), average wrinkle depth (μm), and number of wrinkles were measured.

No skin-care products were applied to the measured sites for at least 2 h before the measurements. A small area of each location was wiped with ethanol 1 h before the parameters were measured in a room at a temperature of 20-25°C and relative humidity of 30-40%. The crow's foot area was measured three times, and the mean value was recorded and analyzed. The Corneometer and TEWL measurements were repeated for each subject every fourth week (four times), and wrinkling was measured for each subject twice, at baseline and week 12. After week 12, adverse effects, including erythema, edema, bruising, and altered pigmentation, were assessed by questioning subjects and observing skin responses.

Statistical analysis

All statistical analyses were performed by using the Statistical Package for Social Sciences version 12.0 (SPSS Inc., Chicago, IL, USA). The differences between the two groups (control vs GOS group) were statistically evaluated by performing a *t* test. A repeated-measures analysis of variance followed by Bonferroni-adjusted pairwise comparisons was used to assess the differences in the change from baseline to each week within groups. All data were two-sided at the 5% significance level and were reported as means ± standard error of the mean (SEM).

RESULTS

The baseline characteristics are listed in Table 1. Eighty-four individuals were selected for participation in this study. Of these individuals, five were withdrawn from the trial as follows: four (two from the control group and two from the GOS group) failed to complete the study, and

Table 1. Baseline skin characteristics of healthy adults

Variable	GOS group (n=39)	Control group (n=40)
Gender, n (%)		
Women	36 (92.3)	37 (92.5)
Men	3 (7.7)	3 (7.5)
Age (years)		
Mean	51.1±1.31	50.4±1.33
Range (min-max)	34-68	32-66
Menopausal status, n (%)	14 (35.9)	15 (37.5)
Blood pressure (mmHg)		
Systolic blood pressure	125±9.46	129±5.67
Diastolic blood pressure	91.1±6.25	87.3±5.51
Pulse (beats/min)	70.2±6.78	71.2±6.68
Blood glucose (mg/dL)	138±20.7	130±12.2
Body mass index (kg/m^2)	22.9±3.02	23.5±2.13
Energy intake (kcal/day)	2011±416	1879±358
Carbohydrate (%)	69.1±4.02	68.7±2.33
Protein (%)	17.8±2.15	17.2±1.72
Fat (%)	13.1±2.28	14.1±2.12
Metabolic disease, n (%)	0 (0)	0 (0)
Skin diseases, n (%)	0 (0)	0 (0)
Drug use, n (%)	0 (0)	0 (0)
Corneometer value (AU)	70.5±1.76	73.0±1.45
TEWL (g/h/m^2)	20.1±1.09	17.8±1.12
Total wrinkle area (mm^2)	14.1±1.08	15.0±0.99
Percentage of wrinkle area (%)	53.0±4.06	56.3±3.70
Wrinkle depth (cm)	5.54±0.57	5.09±0.77
Number of wrinkles	304±32.4	244±35.6

AU: arbitrary units; GOS: galacto-oligosaccharide; TEWL: transepidermal water loss.
All data were reported as means±standard error of the mean.

one (from GOS group) was noncompliant. As a result, 79 participants met the study requirements (male: 6 female: 73, age 30–39 years: 3, 40–49 years: 37, 50–59 years: 25, 60–69 years: 14). None of the participants withdrew from the study because of GOS treatment-related adverse effects. No adverse effects were experienced by the subjects, and none of the withdrawals were considered to be due to the study products. The control (age: 50.4 years) and GOS groups (age: 51.1 years) ultimately consisted of 40 and 39 participants, respectively. The control and GOS groups had mean Corneometer values of 73.0 and 70.5 AU and TEWL of 17.8 and 20.1 g/h/m^2, respectively. Furthermore, the control and GOS groups had similar results regarding the following wrinkle parameters: total wrinkle area, percentage of wrinkle area, wrinkle depth, and number of wrinkles. The initial values of all the variables did not significantly differ between the two groups. The subjects were healthy adults who did not have metabolic diseases, use any pharmaceutical drugs, or consume alcohol.

Figure 1 and 2 shows the changes in Corneometer values and TEWL, respectively. The increase in Corneometer value from the baseline to week 12 was significantly greater in the GOS group than in the control group (6.91 vs 2.88 AU, respectively; $p<0.05$). The Corneometer values in the control group were not significantly different between baseline and week 12, whereas GOS treatment significantly influenced the Corneometer values at 4 weeks (70.5 AU at baseline vs 73.0 AU at week 4, $p<0.05$).

The initial values of TEWL did not significantly differ between the two groups (Table 1). At week 4, the reduction in TEWL from the baseline in the GOS group was significantly greater than that in the control group (−1.93 vs. −0.52 g/h/m^2, respectively; $p<0.05$). TEWL did not significantly differ between the baseline and week 12 in the control group but was significantly reduced in the GOS group after 12 weeks of GOS treatment (20.1 g/h/m^2 at baseline vs 17.4 g/h/m^2 at week 12, $p<0.05$).

Figure 3 presents the changes in wrinkle formation after 12 weeks of GOS treatment. The differences in total and percentage of wrinkle area between the control and

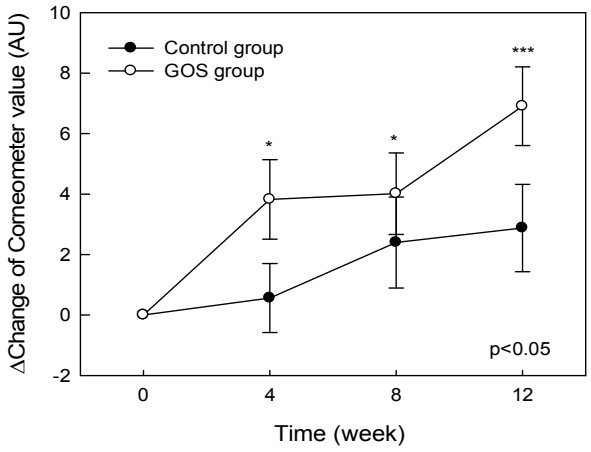

Figure 1. Effects of galacto-oligosaccharides (GOS) on the Corneometer value in the healthy adults. The asterisks indicate significant differences ($^*p<0.05$, $^{***}p<0.001$) between the baseline and the indicated week by a repeated-measures analysis of variance followed by Bonferroni-adjusted pairwise comparisons within groups. The *p*-value shown on the graph indicates a significant difference between changes in the two groups at the indicated week by *t* test. All data were two-sided at the 5% significance level and are reported as means±standard error of the mean.

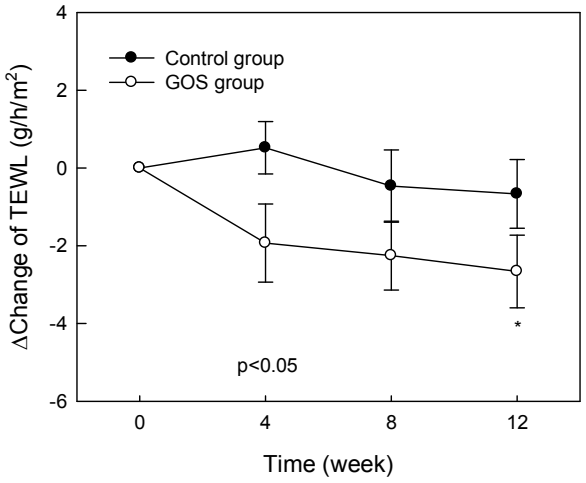

Figure 2. Effects of galacto-oligosaccharides (GOS) on transepidermal water loss (TEWL) in healthy adults. Asterisks indicate a significant difference ($^{*}p<0.05$) between the baseline and the indicated week by a repeated-measures analysis of variance followed by Bonferroni-adjusted pairwise comparisons within groups. The p value shown on the graph indicates a significant difference between changes in the two groups at the indicated week by t test. All data were two-sided at the 5% significance level and are reported as means±standard error of the mean.

GOS groups were significant after 12 weeks of GOS treatment ($p<0.05$). The GOS group showed a reduction in total wrinkle area and percentage of wrinkle area after 12 weeks of GOS treatment (total wrinkle area: −3.25 mm^2; percentage of wrinkle area: −12.2%), whereas the control group showed a slight increase in these parameters (total wrinkle area: 1.07 mm^2, percentage of wrinkle area: 4.02%). Furthermore, the wrinkle depths and number of wrinkles in the GOS group were also lower than those in the control group, although these differences were not significant. As shown in Figure 4, the replica photographs of a subject who received GOS for 12 weeks showed that wrinkles in the crow's feet region were markedly improved compared with the baseline.

DISCUSSION

Purba et al investigated the association between actinic skin damage and dietary intake.[18] This study addressed whether food and nutrient intakes were correlated with skin wrinkling in a sun-exposed site. The results suggested that subjects with a lower intake of milk/milk-products, butter, margarine and sugar products had less skin wrinkling in a sun-exposed site. This study illustrated that skin wrinkling in a sun-exposed site in older people of various ethnic backgrounds may be influenced by the types of foods consumed.

Currently, the use of prebiotics as functional food ingredients to manipulate the composition of gut microbiota in order to improve health has sparked great interest.[19] For the live microbiota (probiotics), which are intended to colonize the large intestine and confer physiological health benefits to the host, specific substrates (prebiotics), which confer a health benefit to the host associated with modulating the microbiota, may be used.[20] Prebiotics can improve the survival of a probiotic organism because its specific substrate is readily available for its fermentation and results in advantages to the host that the live microorganisms and prebiotic offer.[20,21]

Probiotics are known for their potential to modify host immune responses, providing an additional possible mechanism aimed at skin health. The mechanistic basis of skin effects induced by probiotics is thought to be represented by changes in systemic immune responses. In particular, the modulation of specific T-cell subsets such as stimulation of T-helper type 1 cells in the gut mucosa,

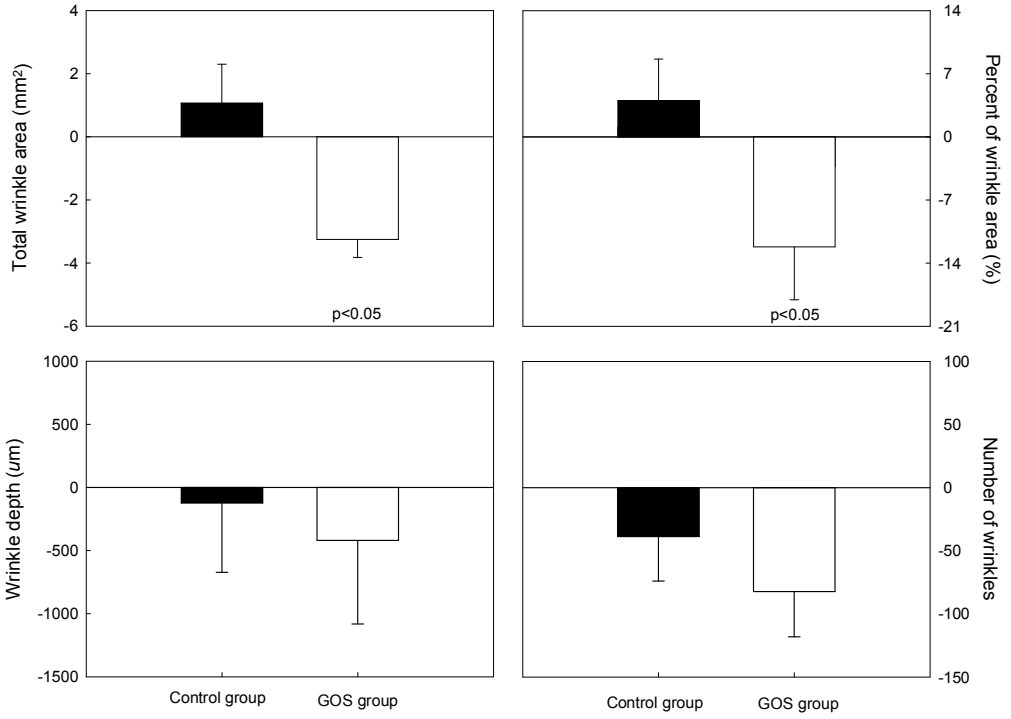

Figure 3. Effects of galacto-oligosaccharides (GOS) on wrinkle formation in healthy adults. The p values indicate a significant difference between changes in the two groups at each week by t test. All data were two-sided at the 5% significance level and are reported as means±standard error of the mean.

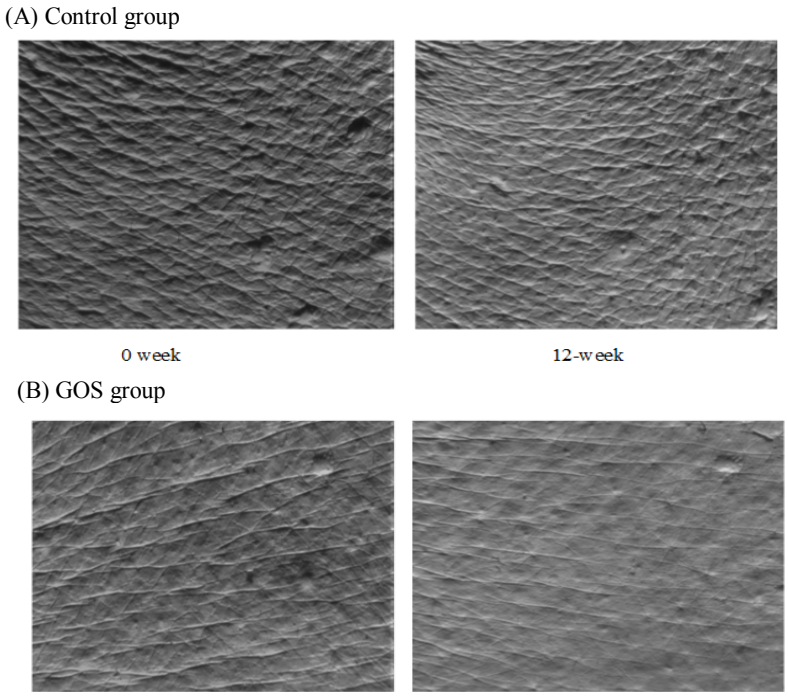

Figure 4. Replica photography of crow's feet in healthy adults who received galacto-oligosaccharides (GOS) for 12 weeks.

which may subsequently influence immune responses in other tissues, may play a role.[22,23] Probiotics protect the skin immune system against ultraviolet B radiation-induced immunosuppressive effects in hairless mice.[22] Similar effects have been described in humans, and it has been proposed that the consumption of probiotics may represent a novel approach to protect the skin immune system.[24,25] The significant improvement on the course of atopic dermatitis has been reported in infants given probiotic-supplemented elimination diets.[26,27] Another target for probiotics may be skin barrier function. A double-blind, randomized clinical study has shown that a 24-week skin intervention with a fermented dairy product in female volunteers significantly reduced TEWL and improved stratum corneum barrier function compared with a placebo product.[28]

GOS as a prebiotic also effectively blocked atopic dermatitis-like skin lesions in a human-like model of atopic dermatitis, NC/Ng a mice, by at least partly inducing production of interleukin 10 and suppressing the production of cytokines such as interleukin 17, which are involved in skin inflammation.[29] In our preliminary tests,[12] we found that GOS administration in hairless mice suppressed the increase in TEWL and concomitant decrease in skin hydration, which reflects barrier function perturbation after ultraviolet B irradiation. Furthermore, GOS administration also resulted in increased *CD44* gene expression, which was associated with maintenance of hyaluronic acid homeostasis, compared with no treatment. The effects of GOS plus a mixture of four probiotics in preventing allergic diseases were reported in pregnant women and their infants.[30] It has been speculated that not only diseased but also healthy skin may benefit from oral treatment with prebiotics.

In this study, oral treatment with GOS in healthy adults improved skin hydration (Corneometer value and TEWL), which is critical for maintaining healthy skin and an important component of basic skin care (Figure 1 and 2). Our results also indicated that GOS improved total and percentage of wrinkle area in healthy adults compared with non-treated subjects after 12 weeks of treatment (Figure 3 and 4).

In our preliminary tests,[31] several probiotics (*Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium longum*, and *Bifidobacterium bifidum*) in GOS showed higher cell growth than the other GOS after 12 h of culture except *B. longum* culture at 36 h. These results suggest that GOS was a good substrate and carbon source for supporting the growth of probiotics. The intestinal flora is part of a complex ecosystem, and many of its constituent bacteria remain unidentified.[32] However, strong evidence suggests that the intestinal flora influences the postnatal development of the immune system. Stimulation of the entire intestinal flora by prebiotics might be a more effective method of altering immune development than by adding bacterial species to the intestinal ecosystem. In contrast to probiotics that introduce exogenous bacteria into the colonic microbiota, prebiotics aim to stimulate the growth of one or a limited number of the potentially health-promoting indigenous microorganisms, thus modulating the composition of the natural ecosystem.[32,33]

Therefore, GOS as a prebiotic might more effectively promote skin health than single or complex probiotics because of the increase of health-promoting indigenous microorganisms. In conclusion, our findings support that oral treatment with GOS was beneficial to the skin, and present the possibility of new nutritional strategies for skin care. However, further research is necessary to fully understand the effects of GOS on skin health and to understand in detail how ingested prebiotics might influence the skin through intestine-mediated changes.

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AUTHOR DISCLOSURES

None of the authors have any conflicts of interest associated with this study. None of the authors had a financial interest or personal affiliation that compromised the scientific integrity of this work.

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