Production of a fermented organic rice syrup with higher isomalto-oligosaccharide using *Lactobacillus plantarum*

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Abstract Isomalto-oligosaccharide (IMO) syrup was prepared from organic rice, in which *Lactobacillus plantarum* as a starter was inoculated to raise its purity and also produce a fermented rice beverage. Of the five strains of lactic acid bacteria tested, *L. plantarum* was preferentially selected in terms of a viable cells (7.3×10^8 colony forming unit (CFU/mL) and higher dry cell weight (13 mg/mL). The fermented syrup-based medium did not affect the growth of *L. plantarum*. As expected, the residual sugar content gradually decreased by 1.34% compared with the initial concentration. It was apparent that the residual sugars but not oligosaccharides were removed during the *L. plantarum* fermentation period. The production of lactic acid was the highest (8125.78 mg/kg) among the organic acids produced in the fermented IMO syrup.

Keywords Isomalto-oligosaccharides · Organic rice · Lactic acid bacteria · Prebiotics

Introduction

Prebiotics are by definition a non-digestible food ingredient that benefits the host by promoting increased activity of intestinal microbial flora [1] and can alter the composition of organisms in the gut microbiota. In comparison, probiotics foster the growth and colonization of beneficial

organisms in the intestine [2–4]. The most widely used probiotics are lactic acid bacteria (LAB), including bifidobacteria, lactobacilli and enterococci, but other organisms, such as yeasts and *E. coli*, have been claimed to have probiotic properties [5]. Among the probiotic organisms existing food code in this country, *Lactobacillus* (*L.*) *acidophilus*, *L. casei*, *L. fermentum*, *L. plantarum*, and *L. rhamnosus* in the genus *Lactobacillus* are currently interested.

Recently, the distinction between short-chain, long-chain, and full-spectrum prebiotics has been described. Short-chain prebiotics (e.g., oligo-fructose) contain 2–8 links per saccharide molecule and typically ferment more quickly in the ascending colon, providing nourishment to the bacteria in that area. Longer-chain prebiotics, including inulin, contain 9–64 links and tend to ferment more slowly, nourishing bacteria predominantly in the descending colon. Prebiotics are noteworthy in that they have been applied as additives in infant formulas and a variety of fermented dairy products [6–8].

IMOs, which are gaining popularity in their applications as prebiotics, are a group of branched glycosyl saccharides and belong to malto-oligosaccharides containing one or more α -(1,6)-glucosidic linkages of glucose [9]. In addition, most oligosaccharides, including IMO, are identified as prebiotics in many countries and promote the growth of beneficial gut microbiota to compete with and/or inhibit harmful organisms in the intestinal track of the host [1, 10, 11]. The manufacturing procedures for IMO have been well documented by others; a process using several different enzymes consisting of α -amylase (EC 3.2.1.1), β -amylase (EC 3.2.1.2), pullulanase (EC 3.2.1.41), and transglucosidase or D-glycosyltransferase (EC 2.4.1.24) has been frequently used for the manufacture of IMO-containing syrup [12]. Transglucosidase can both catalyze the

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hydrolysis of α -D-glucooligosaccharides and transfer the glycosyl moiety to 6-OH of glucose, leading to production of isomaltose, panose, and others.

Using prebiotics and probiotics in combination is often described as synbiotics, although the Food and Agricultural Organization (FAO) recommends that the term “synbiotic” be used only if the net health benefit is synergistic. Due to the greater interest in mixtures of probiotics and prebiotics, synbiotics are being studied for development of a commercial product, as it is generally recognized that probiotic bacteria alone do not proliferate in situ without a proper supply of carbon in the intestinal track [13–15].

In this study, organic rice was used to produce the IMO syrup, because it contains less contaminants compared to the non-organic kind. IMO syrup was prepared by treating organic rice with amylolytic enzymes combined with transglucosidase. It was fermented using LAB and measured for bacterial growth, IMO and residual sugar concentrations, and organic acids as a basic study for developing a fermented organic rice beverage.

Materials and methods

LAB strains and chemicals

Once the syrup was prepared, it was inoculated with each of the five starter strains of *L. plantarum* KCCM 12116, *L. casei* KCCM 12452, *L. acidophilus* KCCM 32820, *L. fermentum* KCCM 35269, and *L. rhamnosus* KCCM 3241. Each strain was transferred twice in MRS broth (cat. no. 288130, BD, Franklin Lakes, USA) before inoculation in 10% reconstituted skim milk medium (cat. no. 232100, BD, Franklin Lakes, USA) for main fermentation. Acetonitrile (cat. no. 9017-03) and water (cat. no. 270733) for HPLC analyses, the standard materials such as, oxalic acid (cat. no. 658637), D-tartaric acid (cat. no. 483796), lactic acid (cat. no. L6661), citric acid (cat. no. 251275), fumaric acid (cat. no. 1286708), succinic acid (cat. no. 398055), glucose (cat. no. G5767) and D(+)-maltose monohydrate (cat. no. M5885) were purchased from Sigma (St. Louis, MO, USA).

Preparation of low-purity IMO syrup from gelatinized organic rice

Organic rice obtained from Chungbuk Organic Rice Business Unit (Cheongju, Korea) was suspended in distilled water at a dry weight concentration of 30% (w/v). To gelatinize, the raw rice was heated at 95 °C for 40 min along with constant agitation. The liquefaction step was proceeded by the injection of 0.02% liquefying enzyme, BAN (Sigma-Aldrich cat. no. A7595, Novozyme,

Copenhagen, Denmark), for 2 h at 65 °C. The saccharification step was done by the injection of 0.05% saccharifying enzyme, FUNGAMYL (Sigma-Aldrich cat. no. A8220, Novozyme, Copenhagen, Denmark), and 0.01% PROMOZYME (Sigma-Aldrich cat. no. E2412, Novozyme, Copenhagen, Denmark) for 48 h at 65 °C. The organic rice IMO used in this study was manufactured by treating organic rice with amylolytic enzymes such as BAN, FUNGAMYL, PROMOZYME and Transglucosidase (Genencor, Palo Alto, CA, USA). The majority of IMO used in this research consists of 3 to 6 monosaccharide units linked together: disaccharides and longer polysaccharides (up to 7 units).

Viable cell counts and dry cell weight

First, 10 mL of IMO syrup (100%) was inoculated with *L. plantarum* in an overnight culture at 2% (v/v) and incubated at 37 °C for 24 h. After tenfold serial dilutions, a 100 μ L aliquot of the appropriately diluted sample was spread on MRS-BCP agar containing bromocresol purple (cat. no. 16447, Sigma-Aldrich, St. Louis, MO, USA) 0.005% (w/v) according to the reference procedure, and yellow colonies were counted after 48 h of incubation at 37 °C. For dry cell weight, the cultures grown in the IMO syrup were precipitated by centrifugation at 5000 $\times$ g for 15 min, washed with physiological saline (0.85% sodium chloride, cat. no. 7548-4400, Daejung Chemicals, Sihung, Korea), and weighed after drying at 60 °C for 2 h.

Determination of free sugars and organic acids

Samples were collected every 12 h for sugar determination, followed by addition of 0.1% (w/v) of commercial active carbon powder (cat. no. CA0446, Samchun Chemical, Pyongtaek, Korea) into the sample being heated in a boiling water bath to eliminate all substances except sugars. After being filtered through a paper filter (Whatman No. 2; cat. no. 1002 110, Whatman, Little Chalfont, UK) and 0.45 μ m PTFE syringe filter (cat. no. 25HP045AN, Advantec, Dublin, CA, USA), in that order, the samples were analyzed. Free sugars in the sample were determined using HPLC (Agilent, Santa Clara, CA, USA) with a Prevail Carbohydrate ES Column (cat. no. 35101, Grace, Columbia, MD, USA) equipped with an RI detector (Agilent), as summarized in Table 1.

In addition, for the analysis of organic acids, 3 g of fermented IMO syrup was diluted in 50 mL of distilled water, followed by the membrane filtration described earlier. An analytical sample was injected into the HPLC-Photo Diode Array Detector (PDA; Shimadzu, Kyoto, Japan) equipped with a C₁₈ column (cat. no. 959990-902, Agilent, Santa Clara, CA, USA). All standard solutions,

Table 1 The operating conditions of HPLC for the analysis of free sugars and organic acids

Items	Condition for free sugars	Conditions for organic acids
Instrument	Agilent 1100 Series HPLC	Shimadzu LC-20AD
Detector	RI, Agilent G 1362A	PDA, Shimadzu SPD-M20A at 210 nm
Column	Prevail™ Carbohydrate ES Coulmn (4.6 × 250 mm)	Agilent C ₁₈ (5 μm, 460 mm × 250 mm)
Column temperature	35 °C	30 °C
Flow rate	1.2 mL/min	0.8 mL/min
Solvent	75% (v/v) acetonitrile	0.05 M KH ₂ PO ₄ (pH 2.8)
Injection volume	20 μL	15 μL

including oxalic acid, D-tartaric acid, lactic acid, citric acid, fumaric acid, and succinic acid, were dissolved in distilled water prior to use. Operating parameters for the organic acid analysis are summarized in Table 1.

Statistical analysis

Each individual experiment was conducted in triplicate, and the data obtained are expressed as mean with standard error. Significance was evaluated at $p < 0.05$ by one-way ANOVA tests in SPSS (Armonk, NY, USA).

Results and discussion

For the purpose of making fermented organic rice beverage, IMO syrup was prepared by treating organic rice with amylolytic enzymes combined with transglucosidase as a primary preparation. Then, a single *Lactobacillus* strain among the probiotic lactic acid bacteria claimed under the Korean Food Law was chosen to ferment and to use up the glucose and maltose. Growth behavior of lactic acid bacteria, changes of IMO and residual sugar concentrations, and organic acids were determined during the fermentation. As expected, the IMO purity increased while the residual sugar decreased when the prepared IMO syrup was fermented with a *L. plantarum*, which might be beneficial as a gut microbiota. The high concentration of lactic acid in the fermented IMO syrup could contribute to a yogurt-like flavor [16] and to extend shelf life of the fermented IMO beverage.

Viable counts and dry cell weight

In order to monitor the growth behavior in the IMOs syrup, five strains of lab-stock lactic acid bacteria (*L. plantarum* KCCM 12116, *L. casei* KCCM 12452, *L. acidophilus* KCCM 32820, *L. fermentum* KCCM 35269, *L. rhamnosus* KCCM 32411) were inoculated into the medium, and counts of the viable cells in the samples were performed at various

time points during the fermentation process. As shown in Figs. 1 and 2, the highest cell number of 7.2×10^8 CFU/mL was obtained for *L. plantarum*. Likewise, the dry cell weight of *L. plantarum* was 13 mg/mL more than that of the other strains used, indicating that *L. plantarum* is highly desirable for fermenting IMO syrup preparations. *L. plantarum* is commonly found in a wide range of fermented foods and in the human gastrointestinal tract. This wide range may be due to its ability to uptake different sugars and peptides and to adapt to various environmental conditions [17].

Effect of IMO syrup concentration on bacterial growth

In order to assess how the IMO syrup concentration affects bacterial growth, varying concentrations of the IMO-based medium were prepared, from the starting concentration of 25–100% (non-diluted). As shown in Fig. 3, bacterial count of *L. plantarum* gradually increased with the amount of IMO syrup added. The concentrations of 25, 50, and 100% correspond to 1.5×10^8 CFU/mL, 4.2×10^8 CFU/mL, and 7.0×10^8 CFU/mL in viable cell count, respectively. It was thought that no inhibitory substances against *L. plantarum* were present in the IMO syrup, as bacterial

Fig. 1 Viable cell counts of IMO syrup fermented with one of five strains of lactic acid bacteria

Fig. 2 Dry cell weight of IMO syrup fermented with one of five species of lactic acid bacteria

Fig. 3 Viable cell counts of *L. plantarum* KCCM12116 in IMO syrup that varies in sugar content

growth was not affected by increasing IMO syrup concentration. The bacterial count was stably maintained at a level greater than 10^8 CFU/mL in the IMO-based syrup for 7 days after fermentation.

Changes in the concentrations of residual sugars

In order to increase the purity of the IMO syrup, LAB has been exploited to remove the simple residual sugars, such as glucose and maltose, during fermentation, as has been demonstrated based on higher IMO concentration after fermentation [18]. In the present study, the IMO syrup was fermented for up to 24 h with *L. plantarum*, which was chosen based on its growth behavior, and concentrations of the residual sugars (glucose and maltose) were monitored during fermentation (Fig. 4). Glucose level in the IMO syrup increased slightly from 6.15 to 6.56% as the incubation time went on due to digestion of maltose. However, maltose level tends to decrease rapidly from 3.09 to 1.49% at 12 h and to 1.34% at 24 h of incubation. This significant

Fig. 4 Changes in residual sugar concentration in IMO syrup during fermentation with *L. plantarum* KCCM12116

reduction of residual sugars after 24 h of fermentation with *L. plantarum* indicated that the purity of IMO was elevated in the fermented syrup from organic rice.

Changes in organic acid concentrations

For the IMO syrup fermented with *L. plantarum*, individual organic acid concentrations were determined as listed in Table 2. As expected, lactic acid was quantified at the highest amount of 8125.78 mg/kg, followed by 107.83 mg/kg for D-tartaric acid, 5.27 mg/kg for oxalic acid, and trace amounts for the other acids. Hong and Ko [19] have claimed that fermented milk with rice powder showed higher LAB counts than the fermented milk alone. Similarly, addition of waxy-white rice gave more viable counts than that of brown rice. In this experiment, greater lactic acid production is a desirable characteristic in terms of a typical yogurt-like flavor development, overall

Table 2 Concentrations of organic acids in the fermented IMO syrup produced by single-step method

Organic acids	Content (mg/kg)
Oxalic acid	5.27 ± 0.57 ^a
D-Tartaric acid	107.83 ± 1.27 ^b
Lactic acid	8125.78 ± 3.47 ^b
Citric acid	N.D.
Fumaric acid	N.D.
Succinic acid	N.D.

N.D. not detected

^{a,b} Means in the same row with different letters are significantly different ($p < 0.05$)

acceptability, and extended shelf life of functional fermented rice products [20]. This fermented organic rice requires further research in order to develop commercial products.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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