Effect of Different Commercial Oligosaccharides on the Fermentation Properties in Kefir during Fermentation

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Abstract

Kefir is a traditional fermented milk produced by various lactic acid bacteria (LAB) and yeast, which produce lactic acid, ethanol, carbon dioxide, and other flavor compounds. The purpose of this study was to evaluate the effects of different commercial oligosaccharides, such as maltotriose, fructooligosaccharide (FOS), galactooligosaccharide (GOS), and isomaltooligosaccharide (IMO), on the fermentation properties of kefir. First, we determined the acidification kinetic parameters, such as \( V_{\text{max}} \), \( t_{\text{max}} \), \( t_{\text{pH} 5.0} \), and \( t_{\text{f}} \) of fermented milk supplemented with 4% (w/w) of different oligosaccharides. The probiotic survival and chemical composition (pH, organic acids profile, and ethanol content) of kefir during fermentation were also measured. Compared to control fermentation, all oligosaccharides increased acidification rate and reduced the time to complete fermentation (pH 4.7). The addition of FOS, in particular, resulted in the lowest \( t_{\text{f}} \) and the highest populations of LAB and yeast during fermentation. All oligosaccharides increased ethanol production during fermentation. Further, significant differences were observed in the formation rates of six organic acids during fermentation. This study provided comparative data on the properties of commercial oligosaccharides for kefir manufacturing. Consequently, FOS especially had the potential for adequate and effective oligosaccharides in commercial kefir for the improvement of cost- and time-effectiveness.

Key words: oligosaccharide, kefir, fermentation, fructooligosaccharide

Introduction

Kefir is a traditional fermented milk product originating from the Caucasus mountains. There are two primary ways of making kefir: fermenting by kefir grains or by commercial starter cultures (Thoreux & Schmucker, 2001). Originally, kefir was made by inoculating milk with kefir grain, which is irregularly shaped yellowish-white hard granules (Güzel-Seydim et al., 2000). A kefir grain consists of bacteria and yeast species such as Lactobacillus helveticus, Lactococcus delbrueckii subsp. bulgaricus, Lactococcus lactis subsp. lactis, Leuconostoc mesenteroides subsp. cremoris, and Kluyveromyces marxianus. The biomass of kefir grains slowly increases during fermentation (Gorek & Tramek, 2007). These days, due to economic effectiveness, including the time-saving and hygienic manufacturing process, commercial direct-to-vat cultures are utilized. Microbial populations of kefir produce lactic acid, ethanol, carbon dioxide, and other flavor compounds, such as acetaldehyde, acetoin and diacetyl, which produce the unique kefir flavor. It has been reported that kefir can be considered a probiotic resource as it can improve a variety of health conditions (Rodrigues et al., 2005). The reported scientific benefits of kefir include: antioxidant activity (Liu et al., 2005a; Liu et al., 2005b), antipathogenic activity (Millette et al., 2007), antitumor (Kubo et al., 1992), anticarcinogenic activity (Sarkar, 2007), and anti-inflammatory/immunomodulatory effects (Lee et al., 2007; Thoreux & Schmucker, 2001). Prebiotics are non-digestible food ingredients that beneficially affect the host by selectively stimulating the growth and/or activity of probiotics, thus improving host health (Gibson & Roberfroid, 1995). A prebiotic substrate must not be hydrolyzed or absorbed in the stomach or small intestine; fermentation of the substrate should induce beneficial effects within the host. In terms of diet, resistant starch, non-starch polysaccharides, sugars, and oligosaccharides are representative prebiotics. Oligosaccharides are sugars consisting of approximately 2 to 20 saccharide units, and the following oligomers have been suggested to

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have prebiotic potential: lactulose, fructooligosaccharide (FOS), galactooligosaccharide (GOS), soybean oligosaccharide, isomaltooligosaccharide (IMO), glucooligosaccharide and xylooligosaccharide (Manning and Gibson, 2004).

The aim of this study was to evaluate the effects of various commercial oligosaccharides on the fermentation properties of kefir, including acidification kinetics, the population of lactic acid bacteria and yeast, as well as ethanol and organic acid production during kefir fermentation.

Materials and Methods

Kefir sample preparation

Kefir starter cultures were purchased from Christian Hansen (Hørsholm, Denmark); XPL-1 and LAF-4. XPL-1 included *Lactococcus lactis* subsp. cremoris, *Lactococcus lactis* subsp. lactis, *Lactococcus lactis* subsp. lactis biovariety diacetylactis, *Leuconostoc mesenteroides*, and *Streptococcus thermophilus* and LAF-4 included *Kluyveromyces marxianus* subsp. *marxianus*. The inoculation concentration of XPL-1 and LAF-4 were 250 U/L and 4 U/L, respectively. After inoculation to pasteurized milk, milk was incubated at 31°C. The desired final pH of the product was pH 4.7. Samples were collected every hour for analysis of kinetic parameters and four times for analysis of bacterial and yeast counts, organic acid and ethanol contents during fermentation.

Oligosaccharides

The following commercial oligosaccharides were supplemented at the concentration of 4% (w/w); IMO (purity >69%, Daesang Co-Op., Korea), FOS (purity>55%, Samyanggenex, Korea), maltotriose (purity>60%, Daesang Co-Op., Korea), GOS (purity>53%, Inggredion, Korea).

Kinetic parameters

The acidification rate (V<sub>max</sub>) was calculated as the time variation of pH (dPH/dt) and expressed as 10<sup>−3</sup> pH units/h. During the fermentation period, the following kinetic parameters were also calculated: (i) t<sub>max</sub> (h), time at which V<sub>max</sub> was reached; (ii) t<sub>pH 5.0</sub> (h), time to reach pH 5.0; and (iii) t<sub>pH 4.7</sub> (h), time to complete the fermentation.

Bacterial and yeast counts

Lactic acid bacteria and yeast counts were carried out in triplicate after 1, 6, 12, 14 h-fermentation. Samples were diluted with sterile saline solution and plated on sterile BCP agar (Eiken chemical Co., Japan) for 3 d at 37°C and on sterile Potato dextrose agar (Difco<sup>TM</sup>, USA) for 5 d at 24°C to determine lactic acid bacteria and yeast counts, respectively.

Ethanol and organic acid content

Samples were analyzed for ethanol production using GC-FID (Agilent, USA) according to the method of Güzel-Seydim et al. (2000). In addition, concentrations of lactic, acetic, citric, succinic, pyruvic, and formic acids in kefir during fermentation were determined using high-performance liquid chromatography (HPLC). Sample extraction was performed according to the method of Kocaoglu-Vurma et al. (2008). A HPLC system (Nanospace I, Shiseido, Japan) equipped with UV-VIS detector monitored at 210 nm, and organic acids were analyzed onto an C18-column (Capcellpak C18 UG120, 4.6×150 mm, 5 µm, Shiseido) and kept at 35°C. The mobile phase was 0.1% H<sub>3</sub>PO<sub>4</sub> in 97.5:2.5 (v/v) water: acetonitrile. Run time was 20 min at 1 mL/min and the injection volume was 7 µL. Peak identities were determined based on retention time of standard compounds and concentrations of individual organic acids were quantified by using a standard curve for each compound relating peak area to the concentration.

Statistical analysis

All analysis was conducted in triplicate, and significant differences expressed as different letters were analyzed using Duncan’s multiple range test. SAS statistical package was used to perform all statistical tests (SAS Inst., 2010). Values of p<0.05 were considered to indicate a significant difference.

Results and Discussion

Kinetic parameters

The results of acidification kinetic parameters by different oligosaccharides, including FOS, GOS, maltotriose and IMO, are compared in Table 1. Values of V<sub>max</sub> ranged from 309.33±5.13 to 469.00±8.72×10<sup>−3</sup> pH units/h. Among the samples, the V<sub>max</sub> of the sample added to FOS was significantly higher than others as 469.00±8.72×10<sup>−3</sup> pH units/h. Other than FOS, V<sub>max</sub> values for other oligosaccharides-supplemented samples were lower than the control. The time to reach V<sub>max</sub> (t<sub>max</sub>) of oligosaccharides-supplemented samples was shorter than the control, except in the case of maltotriose. In particular, the FOS and IMO-supplemented samples reached V<sub>max</sub> more rapidly than the other supplemented groups as 8.33±0.58 h.