Production of Kefir from Soymilk With or Without Added Glucose, Lactose, or Sucrose
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ABSTRACT: The effects of added glucose, lactose, and sucrose on microbial growth, acid, and ethanol production, and galactosidase activity in soymilk fermented with kefir grains were studied. Immediately after the addition of kefir grains to soymilk, the lactic-acid bacterial counts were higher, but the yeast counts were lower than in milk kefir. After fermentation for 32 h, the concentrations of yeast, lactic acid, and ethanol in soymilk were significantly lower than those in milk kefir. Addition of 1% glucose to soymilk stimulated growth of lactic-acid bacteria and yeast, the production of lactic acid and ethanol, and the $\beta$-galactosidase activity. Nevertheless $\alpha$-galactosidase activity was suppressed by 1% glucose.

Key Words: kefir, soymilk, carbohydrates, growth characteristic, galactosidase activity

Introduction

Kefir is an acidic and mildly alcoholic fermented milk alleged to have originated in the Caucasus mountains. This beverage differs from other milk products in that it is not the result of the metabolic activity of a single species, but of fermentation with a mixed microflora confined to a matrix of discrete kefir grains that are recovered after fermentation (Marshall and Cole 1985). Kefir grains are small, gelatinous, yellowish, and irregularly shaped masses resembling individual miniature florets of a head of cauliflower. Kefir grains contain a complex flora of lactic-acid bacteria, yeasts, and sometimes acetic-acid bacteria. The flora is held together in a slimy polysaccharide matrix named kefiran, which is the result of the microbial metabolism of milk lactose (La Riviere and others 1967; Marshall and others 1984). Yeast and lactic-acid bacteria coexist in a symbiotic association and are responsible for an acid-alcohol fermentation. Various lactic-acid bacteria and yeasts have been identified in kefir grains, including Lactobacillus brevis, L. helveticus, L. kefir, Leucosostoc mesenteroides, Klyveromyces lactis, K. marxianus, and Pichia fermentans (Angulo and others 1993; Lin and others 1999). The microorganisms constituting the kefir grains produce lactic acid, antibiotics, and bactericide, which inhibit the development of degrading and pathogenic microorganisms in kefir milk (Angulo and others 1993). Furthermore, kefiran is reported to possess antitumor activity (Shiomi and others 1982).

Soybeans contain a number of anticarcinogens (Messina and others 1994), and a recent National Cancer Institute workshop recommended that the role of soy foods in cancer prevention be investigated. There is much evidence from studies on experimental animal and human subjects that substituting soy protein for animal protein in the diet reduces the concentration of total and low-density lipoprotein cholesterol in plasma and serum (Carroll and Kurowska 1995). Soymilk, obtained by aqueous extraction of whole soybeans, is well-known and growing in popularity in many areas of the world (Haumann 1984). Soymilk may afford nutrition and health benefits, since it contains no cholesterol or lactose and only small quantities of saturated fatty acids. However, many people find the taste of soymilk undesirable. Soymilk is commonly characterized as having a beany, grassy, or soy flavor, which reportedly can be improved by lactic acid fermentation (Murty and others 1992; Granata and Morr 1996). Kikuchi and others (1998) showed that both soymilk and Bifidobacterium-fermented soymilk have lowering plasma cholesterol effects.

Results and Discussion

Figure 1 shows the changes in lactic-acid bacterial counts during fermentation of soymilk and milk (control) with kefir grains. Immediately after the addition of kefir grains to the milk and soymilk, the concentrations of lactic-acid bacteria were 6 log CFU/mL and 7 log CFU/mL, respectively. This indicates that part of the microflora contained in the kefir grain is transferred to milk and soymilk. Although the initial counts of lactic-acid bacteria in soymilk were higher than those in milk, the microorganisms grew more slowly in soymilk, even with the addition of carbohydrates. After incubation for 32 h, the lactic-acid bacterial counts increased by 2.3 log cycles from 5.9 ± 0.1 to 8.2 ± 0.2 log CFU/mL in milk, but by only 1.6 log cycles from 7.4 ± 0.2 to 9.0 ± 0.1 log CFU/mL in soymilk without added carbohy-

![Fig. 1—Growth curves of lactic acid bacteria in milk and soymilk fermented with kefir grains](image-url)
drate. The major carbohydrates present in soymilk are sucrose, raffinose, and stachyose, whereas in milk it is lactose (Pinthong and others 1980). The fact that lactic-acid bacteria from kefir grains grew well in soymilk, even when no extra carbohydrate was added, means that these organisms can utilize soymilk carbohydrates for growth.

The initial counts of yeasts in milk and soymilk without added carbohydrate were $5.8 \pm 0.1 \log \text{CFU/mL}$ and $4.7 \pm 0.2 \log \text{CFU/mL}$, respectively (Fig. 2). In milk, yeast reached the stationary growth phase at 20 h of fermentation, with a concentration of $6.6 \pm 0.1 \log \text{CFU/mL}$. Soymilk kefir with 1% glucose had the highest yeast density ($6.4 \pm 0.1 \log \text{CFU/mL}$) of the soymilks at the end of fermentation, while soymilk without added carbohydrate had the lowest. The yeast count did not differ significantly between milk and 1%-glucose soymilk kefir at the end of fermentation. Yeast is important in kefir fermentation because of the production of ethanol and carbon dioxide, which give the kefir drink its unique taste. Kefir grains usually contain lactose-fermenting yeasts such as *Kluyveromyces lactis*, *Kluyveromyces marxianus*, *Torula kefir*, as well as nonlactose-fermenting yeasts such as *Saccharomyces cerevisiae* and *Pichia fermentans* (Angulo and others 1993; Lin and others 1999). Because the type of fermentable carbohydrates present in soymilk and milk are different, the growth characteristics of microorganisms in kefir grains may change, resulting in dissimilarity of the microflora present in kefir grains. From the above results, we conclude that the addition of 1% glucose greatly enhances growth of both lactic-acid bacteria and yeast in soymilk.

Lactic acid is extremely important for producing high-quality fermented milk, and appropriate concentrations are needed to ensure proper flavor with minimum syneresis during storage (Karleskind and others 1991; Granata and Morr 1996). Previous studies indicated that yogurt culture can not produce adequate lactic acid in soymilk (Lee and others 1990; Karleskind and others 1991). We found that at the end of fermentation, the concentration of lactic acid in milk kefir ($1.6 \pm 0.3\%$) was significantly higher than in soymilk kefir without added carbohydrate ($0.9 \pm 0.1\%$) (Fig. 3). However, adding 1% glucose or lactose to soymilk resulted in lactic-acid concentrations similar to those of milk kefir, showing that the addition of these carbohydrates improves the ability of microorganisms in kefir grains to produce lactic acid in soymilk.

Figure 4 shows the production of ethanol during fermentation of milk or soymilk containing 1% glucose, lactose, sucrose, or without added carbohydrate. At the end of fermentation, soymilk containing 1% glucose or lactose had ethanol concentrations similar to that of milk kefir ($0.25\%$ to $0.26\%$). Soymilk without carbohydrate addition showed the lowest ethanol production ($0.11 \pm 0.01\%$).

Ethanol, together with carbon dioxide, gives kefir its stimulating and effervescent characteristics. The ethanol content in kefir ranges widely in the literature. For example, Marshall and Cole (1985) reported that 0.17% ethanol makes a good kefir, and Duitschaever and others (1987) reported that 0.12% to 0.18% ethanol was produced in sequential fermentation of kefir. Koroleva (1988) indicated that many factors affect the percentage of ethanol in kefir, such as time and temperature of fermentation, starter culture, and containers. Our study showed that the addition of 1% glucose or lactose to soymilk enhances the production
The fermentable carbohydrates in soybeans and soybean products are low-molecular-weight oligosaccharides such as sucrose, raffinose, and stachyose (Mital and Steinkraus 1975). Raffinose and stachyose are made up of three simple sugars: fructose, glucose, and galactose. α-Galactosidase is needed to digest sugars of the galactosido-sucrose series.

Some lactic-acid bacteria possess α-galactosidase and may use galacto-oligosaccharides for growth and acid production (Mital and others 1973). Measuring sugar content of a fermented dairy product is not an accurate index of galactosidase activity because first glucose and then galactose are rapidly metabolized to lactic acid by culture bacteria (Kilara and Shahani 1976). Therefore, the galactosidase activity in kefir must be measured directly to determine the activity of microorganisms in kefir grains.

After 32 h of fermentation, the β-galactosidase activity was significantly higher in milk than in any of the soymilk kefirs, while the α-galactosidase activity was higher in soymilk without carbohydrate added than in milk kefir (Table 1). The addition of 1% glucose or lactose to soymilk increased the β-galactosidase activity of kefir grains but decreased α-galactosidase activity. This result is consistent with that reported by Garro and others (1996), who showed that stachyose and raffinose stimulate the synthesis and activity of α-galactosidase from Lactobacillus fermentum, but that exogenously supplied glucose inhibits the synthesis of the enzyme.

The viscosity of soymilk kefir was lower than that of milk kefir (Fig. 5), and the kefir grains subcultured in soymilk were much smaller than those subcultured in milk (results not shown). At least 24% of the dry matter of the kefir grain consists of kefiran, which is thought to be produced by the lactobacilli in the grain. Growth conditions (such as temperature, medium composition, and incubation time) and carbon source have a considerable influence on lactic-acid bacterial exopolysaccharide yield and composition (Cerning and others 1992). Thus, the smaller size of the kefir grains in soymilk in this study may suggest that lactic-acid bacteria produce less kefiran when grown in soymilk.

### Materials and Methods

#### Kefir grains

Kefir grains were collected from households in northern Taiwan. In the laboratory, they were propagated at 20 °C for 20 h with twice-or thrice-weekly transfers in sterilized cow milk or soymilk (50 g/L) and kept at 4 °C or −80 °C for short- and long-term storage, respectively.

#### Preparation of soymilk

Soymilk was prepared by a modification of the procedure of Mital and others (1974). One kg dry, mature, whole soybeans was soaked in 3 L distilled water at 25 °C for 24 h. The soak water was decanted, and the beans were washed and ground in 6 L boiling distilled water in a blender (Waring Division, Dynamics Corporation, New Hartford, Conn., U.S.A.). The resulting suspension was filtered through 3 layers of cheesecloth, autoclaved 15 min at 121 °C, and stored at 4 °C until used.

#### Kefir manufacture

Sterilized reconstituted skim milk (10% w/v), soymilk, and soymilk containing 1% glucose, lactose, or sucrose were each inoculated with 5% kefir grains. All inoculated milk and soymilk samples were incubated at 20 °C for 32 h. During the fermentation, samples were taken every 4 h for cell counting and analysis of other properties.

#### Enumeration of culture microorganisms

Lactic-acid bacteria were enumerated on spread plates of MRS agar (Merck, Darmstadt, Germany) containing 200 mg/L cycloheximide after anaerobic incubation at 30 °C for 5 d. Yeasts were enumerated on potato dextrose agar (Difco, Detroit, Mich., U.S.A.) containing 100 mg/L chlorotetracycline after aerobic incubation at 25 °C for 5 d. The results are expressed as logarithmic colony forming units (log CFU)/mL kefir.

#### Determination of lactic-acid production

To measure the concentrations of lactic acid, 1 g of each sample was mixed with 9 mL deionized distilled water, and 0.1 N NaOH was used as the titrant. Phenolphthalein was used as the indicator. Titratable acidity was expressed as percent lactic acid in the kefir.

#### Determination of ethanol

The concentrations of ethanol in samples were determined...
by enzymatic methods described by Bernt and Gutmann (1974).

Lactase assays

β-Galactosidase activity was measured by determining the rate of hydrolysis of o-nitrophenol–β-galactopyranoside (Sigma Chemical Co., St. Louis, Mo., U.S.A.), and α-galactosidase activity was measured by determining the rate of hydrolysis of o-nitrophenol–α-galactopyranoside (Sigma Chemical Co., St. Louis, Mo., U.S.A.). Hydrolysis of these substrates results in the release of o-nitrophenol, a highly chromogenic compound that can be measured spectrophotometrically (Citti and others 1965). A 1-mL sample was added to 50 mL 0.1 M phosphate buffer (pH 7.0) containing 0.001 M MgSO₄ and 0.005 M β-mercaptoethanol. Then 1 mL of the diluted sample was withdrawn, and 2 drops of chloroform and 1 drop of 0.1% sodium dodecyl sulfate were added to it. The mixture was vortexed for 10 s and incubated at 37°C for 5 min.

Next, 1 mL of 15 mM o-nitrophenol–β-galactopyranoside or 10 mM o-nitrophenol–α-galactopyranoside solution in 0.02 M phosphate buffer (pH 7.0) was added to the assay mixture, and the mixture was vortexed for 10 s and incubated at 37°C for 10 min. The reaction was terminated by adding 1 mL 0.5 M Na₂CO₃. The sample was then centrifuged at 16,000 × g for 15 min to remove cell debris. The release of o-nitrophenol was determined at 420 nm. A standard curve was used to determine the amount of o-nitrophenol that was liberated. One unit of enzyme activity released 1 μM of o-nitrophenol/ min.

Determination of viscosity

Viscosity was determined with a Brookfield Viscometer (Stoughton, Mass., U.S.A.) equipped with a helipath stand and a LV-3 spindle.

Statistical analysis

Data were analyzed using the general linear model procedure of the SAS software package (SAS Institute 1996). Duncan multiple range test (Montgomery 1991) was used to detect differences between treatment means. All experiments were replicated three times.

References


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