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Short communication: The effects of frozen storage on the survival of probiotic microorganisms found in traditionally and commercially manufactured kefir

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ABSTRACT

Kefir is a fermented milk traditionally made from a unique starter culture, which consists of numerous bacteria and yeast species bound together in an exopolysaccharide matrix produced by certain lactic acid bacteria. Many health benefits are associated with traditionally produced kefir; however, bulging and leaking packaging, caused by secondary yeast fermentation during storage, has limited large-scale manufacture. Commercial kefir products have been designed to reduce these effects by using a pure starter culture consisting of a mixture of bacteria and yeast species that give a flavor similar to traditional kefir, but some health benefits may be lost in commercial production due to reduced microbial diversity and lack of beneficial exopolysaccharides. In this study, traditional and commercial kefir was frozen to study the effects of frozen storage on the viability of probiotic bacteria over time. Traditional kefir was prepared by inoculating 1 L of pasteurized whole goat milk with approximately 30 g of kefir grains. Commercial kefir was prepared by inoculating 1 L of full-fat, pasteurized goat milk with a commercial kefir starter. The milk was allowed to ferment at room temperature (24–28°C) until pH 4.6 was reached. Samples were frozen (−8 to −14°C) immediately following the completion of fermentation and were thawed and plated for lactobacilli, lactococci, and yeasts on d 0, 7, 14, and 30 of frozen storage. Lactobacilli, lactococci, and yeasts were significantly reduced in number during frozen storage; however, the traditionally produced kefir was shown to have significantly higher counts of bacteria and yeast at each sampling. We concluded that frozen storage and the development of frozen kefir products could eliminate most packaging concerns associated with the large-scale manufacture of traditionally produced kefir, resulting in increased production and marketability of this healthful product.

Key words: kefir, kefir grains, probiotics, frozen dairy

Short Communication

In order for a probiotic to benefit human health it must have good technological properties, survive through the upper gastrointestinal tract, and be able to function in the gut environment (Mattila-Sandholm et al., 2002). These properties, as well as many health benefits, have been examined, and kefir has demonstrated a wide array of positive effects such as antitumor and immunostimulating activity in animals (Quiros et al., 2005). With kefir, both prebiotic and probiotic benefits are incurred by the consumer, including competitive exclusion of pathogenic bacteria, increased absorption of nutrients, and immunomodulating effects such as the modification of the balance of immune cells in the intestinal mucosa (Vinderola et al., 2006; Medrano et al., 2008; Maalouf et al., 2011).

During kefir manufacture with grains, lactic acid fermentation slows considerably or stops as the pH declines, but the yeast fermentations continue, allowing for an increase in ethanol production during storage. The secondary alcohol fermentations can lead to substantial changes in flavor as well as bulging or leaking packaging due to the continued production of carbon dioxide gas (Kwak et al., 1996). Commercial kefir production uses a dry starter culture usually containing of up to 12 species isolated from lyophilized kefir grains.

The development of commercial starter cultures has allowed for widespread distribution of kefir and kefir products; however, the demand for traditionally produced kefir is rising, and methods for producing a consistent product with an adequate shelf life are still being developed. This indicates that the consumer preference might be for a product with a flavor akin to traditionally produced kefir. Because taste preferences are met by traditionally produced kefir and because of possible added health benefits of traditional over com-

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mercial kefir, frozen storage and transport could serve as an alternative solution to the problems typically associated with traditionally produced kefir.

Microorganisms present in a cultured dairy product have a high survivability rate; however, numerous reports have observed structural damage to living lactobacilli cells when subjected to freezing and thawing (Breunan et al., 1986; Lopez et al., 1998). Therefore, other considerations, such as the freezing method, must also be taken into account when attempting to provide a product with the highest number of surviving probiotics.

Exopolysaccharides, such as kefiran, might also serve to enhance the survival of probiotic organisms in a frozen dessert by providing a protective coat that may help to ameliorate the harsh conditions associated with freezing and thawing. A study by Monnet et al. (2003) showed a significantly higher cryotolerance during freezing of *Lactobacillus delbrueckii* strains with a mutation causing an excess production of exopolysaccharide.

Consumer acceptability of acidified dairy foods is typically high in sensory tests conducted on other frozen dairy desserts, such as frozen yogurt (Guinard et al., 1994). The same study also showed that the most-preferred samples of frozen yogurt were the ones with the lowest acidity; these results suggest that an ideal frozen dairy dessert, for most consumers, should combine the sensory properties of ice cream and the nutritional benefits of yogurt (Guinard et al., 1994). However, flavored traditional kefir, which scored high during sensory studies, might be more acceptable to the Western palate than unflavored kefir (Muir et al., 1999). The objective of this study was to quantify viable probiotic bacteria and yeasts in traditionally and commercially produced kefir following various periods of frozen storage.

Two types of kefir were made: one was traditionally produced by inoculation of milk with kefir grains, and the second was made by inoculating milk with a commercial kefir starter. Once fermented, the kefir was divided into 4 aliquots. Three were frozen immediately, and the fourth was left unfrozen and served as a control. The samples were then tested for 3 different types of probiotics: lactobacilli, lactococci, and yeasts. The entire experiment was repeated in triplicate.

Traditional kefir was be prepared by inoculating 1 L of full-fat, pasteurized goat milk (Ryals Goat Dairy, Tylertown, MS) with kefir grains in a liter-sized glass jar. Thirty grams of kefir grains (Cultures for Health, Morrisville, NC) were added to 1 L of milk to give a 3 to 5% ratio of kefir grains to milk as described by Chen et al. (2005). The grains were cultivated, using this method and with the addition of fresh milk weekly, in the Louisiana State University Creamery Building for several months before experimental use.

Commercial kefir was prepared by inoculating 1 L of full-fat, pasteurized goat milk with a commercial kefir starter (Lyo-San, Inc., Lachute, QC, Canada) in a liter-sized glass jar. The milk was allowed to ferment at room temperature (24–28°C) and was agitated by manually shaking every few hours for approximately 24 h to ensure proper mixing of the grains and milk. The kefir fermentation was considered complete when a pH of 4.6 was reached. The grains used to ferment the traditional kefir were recovered by straining the kefir through a fine mesh sieve.

Three 50-g samples of both the traditional and commercial kefirs were collected in separate food-grade plastic containers before storage at -14 ± 6°C, the temperature range that encompasses most household freezers. The samples were frozen immediately following the completion of fermentation (approximately 24 h). The samples were thawed and plated on d 7, 14, and 30 of frozen storage. One additional sample was not frozen and was used as the control for each replication of the experiment; this sample was plated for probiotic microorganisms immediately following fermentation. The frozen samples were allowed to thaw at room temperature for 4 h and were incubated at 37°C for 1 h before plating (Hong and Marshall, 2001).

To quantify the amounts of probiotic bacteria and yeasts in each sample, serial dilutions were made using 0.1% peptone water (Becton, Dickinson and Company, Sparks, MD; Mian et al., 1997). The peptone water was sterilized by autoclaving at 121°C for 15 min, then cooled to approximately 27°C and inoculated with 1% (vol/vol) kefir and further diluted to 10^{-10}. The kefir samples were plated for lactobacilli and lactococci using de Man, Rogosa, and Sharpe (**MRS**) agar (Merck, Darmstadt, Germany) and M17 (Becton, Dickinson and Company) agars (Witthuhn et al., 2005a; Garcia Fontan et al., 2006). To prevent the growth of yeasts on the bacterial plates, 200 mg/L of cycloheximide (Acros, Geel, Belgium) was added to the MRS and M17 agars (Chen et al., 2008). Several dilutions of each sample were plated and each dilution was plated in triplicate. The MRS and M17 plates were incubated anaerobically for 72 and 48 h at 32°C (Irigoyen et al., 2005). Yeasts were grown on yeast extract glucose chloramphenicol agar (Merck) for 5 d at 25°C under aerobic conditions (Gronnevik et al., 2011). Following incubation, growth was determined by counting the number of bacterial and yeast colonies on each plate; colony totals were presented as colony-forming units per milliliter of kefir.

Statistical analysis was performed using the repeated measures ANOVA F-test, with a confidence interval of