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Effects of different fermentation parameters on quality characteristics of kefir

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ABSTRACT

The main objective of the study was to determine the effects of different fermentation parameters on kefir quality. Kefir samples were produced using kefir grains or natural kefir starter culture, and fermentation was carried out under normal or modified atmosphere (10% CO_2) conditions. The microbiological (lactobacilli, lactococci, *Lactobacillus acidophilus*, *Bifidobacterium* spp., and yeasts), chemical (pH, lactic acid, total solids, protein, ethanol, exopolysaccharide contents), rheological, and sensory properties of kefir samples were investigated during a 21-d storage period. The use of different fermentation parameters or the choice of grain versus natural kefir starter culture did not significantly affect the content of microorganisms. Lactobacilli, lactococci, and yeast contents of kefir samples varied between 9.21 and 9.28, 9.23 and 9.29, and 4.71 and 5.53 log cfu/mL, respectively, on d 1 of storage. Contents of *L. acidophilus* and *Bifidobacterium* spp. were between 5.78 and 6.43 and between 3.19 and 6.14 log cfu/mL, respectively, during 21 d of storage. During the storage period, pH, lactic acid (%), total solids (%), protein (%), acetaldehyde, and ethanol contents of kefir samples ranged from 4.29 to 4.53, from 0.81 to 0.95%, from 7.81 to 8.21%, from 3.09 to 3.48%, from 3.8 to 23.6 mg/L, and from 76.5 to 5,147 mg/L, respectively. The exopolysaccharide contents of the samples decreased during 21 d of cold storage; the samples fermented under modified atmosphere had relatively higher exopolysaccharide contents, indicating higher potential therapeutic properties. The kefir samples exhibited non-Newtonian pseudoplastic flow behavior according to the power law model. According to the sensory results, kefir produced from natural kefir starter culture under CO_2 atmosphere had the highest overall evaluation score at d 1.

Key words: kefir, exopolysaccharide, viscosity, sensory

INTRODUCTION

Kefir is a self-carbonated, refreshing, fermented milk that has unique sensory properties due to a mixture of lactic acid, acetaldehyde, acetoin, ethanol, and other fermentation by-products obtained from a diverse range of microorganisms inherent in kefir grains (Guzel-Seydim et al., 2011). During fermentation, lactic acid bacteria (**LAB**) convert lactose to lactic acid and other flavor compounds, and lactose-fermenting yeasts produce CO_2 and small amounts of ethanol. Kefir has a mildly sour and yeasty flavor with a tangy effervescence depending on the composition of the kefir grains or kefir starter culture (Ertekin and Guzel-Seydim, 2010). Kefir grain is a unique natural starter culture for kefir production. Kefir grains are gelatinous granules, 2 to 15 mm in diameter, consisting of a mixture of microorganisms grouped in a highly organized manner. Kefir grains consist of a blend of LAB, acetic acid bacteria, and yeasts (Wszolek et al., 2006). Kefir can also be produced from natural kefir starter culture, which is obtained from kefir grains. In the preparation of natural kefir culture, kefir grains (2–3%, wt/vol) are fermented in reconstituted milk at 20 to 25°C for about 24 h. The grains are removed and the remaining fermented liquid is aseptically stored at 4°C (Guzel-Seydim et al., 2010). Kefir starter cultures, consisting of a limited variety of pure biotechnologically produced microorganisms, have been used for industrial applications. However, kefir produced with starter culture differs from natural kefir produced from kefir grains because of the loss of characteristic properties of traditional kefir, including the organoleptic qualities and the health benefits, due to the relatively inadequate microflora in custom-made, defined starter cultures.

The microflora of kefir grains is held together in a unique matrix of protein and exopolysaccharide material (**EPS**; Rimada and Abraham, 2001; Frengova et al., 2002). The EPS produced by kefir microorganisms is commonly known as kefiran, which is a water-soluble, branched glucogalactan consisting of equal amounts of D-glucose and D-galactose (Micheli et al., 1999; Mitsue et al., 1999). Kefiran is present in the capsular material of some large, rod-like bacteria, especially lactobacilli. *Lactobacillus kefir*, *Lactobacillus parakefir*, and *Lacto-*

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bacillus kefiranofaciens are the major EPS-producing bacteria present in kefir grain microflora (Cheirsilp et al., 2003a,b; Wang et al., 2008). The EPS produced by kefir microorganisms improves the texture and mouthfeel of the product. In recent years, the use of kefiran in the food industry as a food-grade gum and fortification agent and in the development of novel packaging materials has gained popularity (Kwon et al., 2006; Rimada and Abraham, 2006; Piermaria et al., 2009). Kefiran may have therapeutic immunostimulatory, antimutagenic, antiallergic, and antiulcer activities, and might act as a prebiotic compound (Yoon et al., 1999; WonHo et al., 2003).

Fermentation parameters, such as type of kefir culture (from natural grains or starter cultures), inoculation ratio, temperature, and time, affect the final microbial, chemical, and sensory quality of kefir. Atmospheric oxygen may affect growth and balance (ratio of microorganisms) in kefir microflora. Several methods exist for measuring the viability of LAB, especially *Bifidobacteria* spp. and *Lactobacillus acidophilus* (Scardovi, 1986; Shah, 2000), because of the presence of mainly microaerophilic and anaerobic microflora in kefir grains. The aim of this study was to investigate the effects of different fermentation parameters—kefir grain versus natural kefir starter culture obtained from kefir grains and CO_2 atmosphere during fermentation—on the microbiological, chemical, sensory, and rheological properties of kefir during a 21-d storage period.

MATERIALS AND METHODS

Materials

Kefir grains were obtained from the Department of Food Engineering, Suleyman Demirel University (Isparta, Turkey). Cow milk was supplied from Suleyman Demirel University Ünsüt Dairy Plant. All chemicals were of analytical grade and were from Sigma-Aldrich Co. (St. Louis, MO).

Preparation of Kefir Samples

Kefir grains (**KG**) and natural kefir starter culture (**KS**) were used to ferment milk for kefir production. Natural kefir starter was obtained from kefir grains by straining after first fermentation at 25°C for approximately 22 h, and the fermentation was ended at pH 4.6 (Figure 1). Fermentation time (22 h) between KG and KS was identical. The inoculation rate of culture was decided after preliminary sensory studies. Kefir samples cultured with different inoculation rates (2, 3, and 5%) were evaluated by a sensory panel of 11 experienced panelists; according to their overall evaluation (taste, smell, texture, and appearence), optimal inoculation rates for KG and KS were 2% (wt/vol) and 3% (wt/vol), respectively. The inoculated kefir milk was fermented under normal atmospheric condition (KG and KS) or under 10% CO_2 (**KG-C** and **KS-C**) at 25°C. Fermentation under CO_2 was achieved by using a CO_2 generator (CO-150, New Brunswick Scientific, Enfield, CT). The rate of CO_2 was determined, after preliminary studies, according to LAB growth. The pH was measured using a combined-electrode pH meter (WTW Measurement Systems, Fort Myers, FL). Fermentation was ended at pH 4.6 and the samples were stored at 4°C for 21 d.

Microbiological Analysis

Lactobacilli counts were determined on de Man, Rogosa, and Sharpe (**MRS**) medium (Merck, Darmstadt, Germany) after incubation at 37°C under anaerobic conditions (6% CO_2) for 3 d. Lactic streptococci were enumerated on M17 medium (Merck) at 37°C under anaerobic conditions (6% CO_2) for 2 d. Yeasts were grown on potato dextrose agar (Merck) with addition of 0.14% lactic acid at 25°C for 5 d (Mossel et al., 1995). *Lactobacillus acidophilus* were determined on MRS with addition of 10% (wt/vol) sorbitol (Shah, 2000) and *Bifidobacterium* spp. were grown on MRS with neomycin, nalidixic acid, lithium chloride, and paromomycine sulfate (MRS-NNLP; Shah, 2000) at 37°C under anaerobic conditions (6% CO_2) for 3 d.

Proximate Analysis

Titratable acidity, DM, protein, lactic acid, and fat were determined according to AOAC International (1992) methods. Acetaldehyde and ethanol contents of samples were determined by using a headspace (Turbo Matriks 16, Perkin Elmer, Waltham, MA) gas chromatographic (Auto System XL, Perkin Elmer) method that used the flame-ionization detector, according to Guzel-Seydim et al. (2000).

Purification and Quantification of EPS

The separation and quantification of EPS was carried out according to Zisu and Shah (2003). The proteins in 50 mL of diluted kefir sample were precipitated with 2 mL of 20% (wt/vol) TCA and separated by centrifugation (Sorvall RT7, Kendro Instruments Australia Pty Ltd., Lane Cove, NSW, Australia) at 3,313 × *g* for 30 min at 4°C. The pH of the supernatant was adjusted to 6.8 with 40% (wt/vol) NaOH, and then boiled in a sealed container at 100°C for 30 min to denature the whey proteins. The denatured whey proteins were re-