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Manufacture and characterization of kefir made from cow and buffalo milk, using kefir grain and starter culture

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ABSTRACT

The microbiological and chemical characteristics as well as organic and amino acid profiles of kefir samples made from cow and buffalo milks fermented by kefir grains and starter culture were investigated during storage for 21 d at 4°C. After incubation, lactic, acetic, and citric acid concentrations showed a difference among the samples due to milk type and production methods. Storage time had little effect on the organic acid values of kefir samples. As compared with cow milk kefir, buffalo milk kefir had higher numbers of microorganisms, except lactobacilli, at the end of storage. Whereas pH and titratable acidity exhibited similar changes during storage in all kefir samples, ethanol levels were significantly increased in buffalo milk kefir samples. Glutamic acid was the major amino acid at all sampling times for all samples. Tyrosine, serine, histidine, alanine, methionine, and lysine concentrations were determined to be different in all samples depending on milk type. In general, due to the higher microbial population (especially yeast), kefir made from buffalo milk may be preferred.
Key words: buffalo milk, cow milk, kefir, characterization

INTRODUCTION

Kefir is a fermented dairy product originating from the Caucasus Mountains (Gronnevik et al., 2011). In traditional kefir production, milk is fermented with a starter culture of small, irregularly shaped, and gelatinous yellowish grains (Guzel-Seydim et al., 2000a). Kefir has a slightly sour, acidic taste and is creamy in consistency. It has become a popular drink in many parts of the world, from Japan to eastern and northern Europe (Otles and Cagindi, 2003). Its popularity is mainly based on its nutritive content and health benefits. Kefir has numerous benefits to human health, such as improving lactose digestion and tolerance in adults as well as antimicrobial, antitumoral, antioxidant, antimutagenic, and antiapoptotic effects (Hertzler and Clancy, 2003; Güven et al., 2003; Matsuu et al., 2003; Liu et al., 2005; de Moreno de LeBlanc et al., 2006; Lopitz-Otsoa et al., 2006).

Kefir is generally consumed with meals and alone as a probiotic drink. It is recommended for consumption because of its probiotic bacteria and yeast mixture (Simova et al., 2002). Kefir's probiotic property comes from kefir grains or cultures containing various species of lactobacilli, lactococci, *Leuconostoc* spp., acetic acid bacteria, and yeasts, among others (Wszolek et al., 2001; Witthuhn et al., 2005). The microflora of kefir and kefir grains differs according to their origin and production methods (Temmerman et al., 2004).

The kefir-producing process is divided 2 categories: traditional and industrial methods (Otles and Cagindi, 2003). The main difference between the 2 techniques is the inoculation of kefir grain or culture into milk. Kefir grains are mostly used for traditional kefir production. Due to variable microflora in the kefir grains, sensory properties of kefir samples can show differences depending on the origin of the grains and conditions of storage and handling. For standard production, industrial brands use kefir starter cultures that involve pure kefir microflora strains (Petersson et al., 1985; García Fontán et al., 2006).

Kefir is made from different milks, such as bovine, goat, and ovine (Wszolek et al., 2001). In addition, ewe (Farnworth, 2006), soy (McCue and Shetty, 2005), coconut, and rice milk (Otles and Cagindi, 2003) can be used for producing kefir. Choice of culture and milk type is important for kefir characteristics. In addition, differences between kefir samples produced from various types of milks and cultures have been studied by some researchers. Wszolek et al. (2001) investigated properties of kefir samples produced using 3 types of cultures and bovine, goat, or ovine milk. In another study, the effects on milk type and starter culture on kefir characteristics were reported (Oner et al., 2010).

Buffalo milk differs from other milks not only in terms of its taste, but also in chemical composition

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(Phill, 2005). It has higher calcium and protein and less cholesterol content than cow milk. Moreover, tocopherol level and peroxidase activity of buffalo milk are 2 and 4 times higher, respectively, compared with cow milk (Phill, 2005). Buffalo milk can be considered as a favorable raw material for the production of dairy products based on its fat and protein content. Although some dairy products, such as mozzarella and paneer cheese, buffalo yogurt, and so on, are produced using buffalo milk worldwide, to the best of knowledge no research has been focused on use of buffalo milk as a raw material in kefir production. Therefore, the objective of the current research was to investigate and compare chemical and microbiological characteristics of kefir samples manufactured with cow and buffalo milks using kefir grains or commercial kefir culture.

MATERIALS AND METHODS

Materials

The raw cow (2.6 g/100 g of fat, 11.7 g/100 g of TS, and 6.68 pH) and buffalo milk (4.9 g/100 g of fat, 17.5 g/100 g of TS, and 6.94 pH) were supplemented at the Pilot Dairy Plant (Ondokuz Mayıs University) and a local farm in Samsun, Turkey, respectively. The TS contents of raw cow and buffalo milk were standardized to 11.5 g/100 g by adding drinking water. Kefir grains and direct vat inoculation starter culture were obtained from Pilot Dairy Plant and Danisco Biolacta (Kefir DC1 1,000 L, Danisco Biolacta, Olsztyn, Poland), respectively.

Kefir Manufacture

Raw buffalo and cow milks were pasteurized at 90°C for 15 min after the standardization. The pasteurized milks were cooled down to 25°C using water circulation by a heat exchanger. To obtain kefir made using kefir grains, cooled milks were inoculated with 5% (wt/vol) kefir grains rate and incubated at 24°C for 18 h. At the end of the incubation, kefir grains were separated via a sieve and then the kefir samples were taken into high-density polyethylene bottles.

The procedure for making kefir with kefir starter culture involves different steps. Freeze-dried kefir starter culture was added to raw milks at a level of 0.025 g/L of milk. According to the supplier, the starter culture contains *Lactococcus* spp., *Leuconostoc* spp., *Streptococcus thermophilus*, *Lactobacillus* spp., and kefir yeast. After inoculation, all milk samples were incubated at 24°C for 18 h and kefir samples were taken into high-density polyethylene bottles.

All kefir products were stored for 21 d at 4°C and analyzed at d 1 (after the incubation), 7, 14, and 21 for all analyses. Cow (**C**) and buffalo (**B**) milk kefir made using kefir grains (**KG**) and starter culture (**KS**) were named KG-C, KG-B, KS-C, and KS-B, respectively.

Enumeration of Microorganisms

Kefir samples (10 mL) were dispersed with 90 mL of sterile sodium thiosulfate solution (0.2% wt/vol) and homogenized for 1 min using a stomacher (Smasher, AES Chemunex, Bruz, France). Further dilutions were made using Ringer solution (Merck, Darmstadt, Germany). Lactobacilli counts were determined on de Man Rogosa Sharpe agar (Merck) after incubation at 30°C under anaerobic conditions for 5 d (García Fontán et al., 2006). Lactococci counts were enumerated on M17 Agar (Merck) at 30°C for 3 d (Irigoyen et al., 2005). *Leuconostoc* spp. counts were determined on de Man Rogosa Sharpe Agar (Merck) incorporated with vancomycin hydrochloride (Sigma-Aldrich, St Louis, MO) at 30°C for 3 d (García Fontán et al., 2006) and yeasts were grown on yeast extract glucose chloramphenicol agar (Merck) at 25°C for 3 d (Magra et al., 2012).

Proximate Analyses

The titratable acidity of kefir samples was determined using the AOAC titration method using 0.1 *N* NaOH (AOAC International, 1992). The pH was measured at 15°C using a calibrated pH meter (Eutech Cyberscan pH 2700, Ayer Rajah Crescent, Singapore) by directly submerging the probe into the homogenized kefir sample. Total solid contents of the kefir samples were determined gravimetrically using an oven at 103°C until a constant weight was obtained (approximately 2.5 h).

Ethanol Content

The ethanol content in kefir samples was determined following the method of Erbas (2003) with some modifications. A sample of approximately 2.5 g of kefir was diluted in 20 mL of deionized water and centrifuged at 3,200 × *g* for 30 min at 25°C. The supernatant was collected and filtered using Whatman 42 filter paper (Whatman International, Maidstone, UK) and then kept in the deep freeze until analysis. After thawing, the samples were filtered on a 0.45-μm membrane filter and injected on a gas chromatograph (Shimadzu GC MS–QP2010 Plus, Kyoto, Japan) equipped with ZB-WAX polyethylene glycol colon (30 mm × 25 mm × 0.25 μm, 7HG–G007–17). Operating conditions were