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Comparison of antioxidant capacity of cow and ewe milk kefirs

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ABSTRACT

This research aimed to evaluate the effects of using either grain or commercial starter culture on the antioxidative capacity of cow and ewe milk kefirs. The antioxidant capacity of kefir samples during fermentation and 21 d of storage was assessed by using 3 assays: 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) radical cation decolorization; 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) radical scavenging activity assay; and Fe^{+3}-reducing power (ferric reducing antioxidant power assay, FRAP). Vitamin E and β-carotene contents were also quantified. All kefir samples exhibited varying values for DPPH, ABTS, and FRAP assays depending on the starter culture and milk type. Vitamin E and β-carotene contents were similar in all kefir samples during storage. The results of this study suggest that milk type (cow or ewe) and culture type (kefir grains or commercial starter) were the significant parameters for the antioxidative activity of kefir.
Key words: antioxidant capacity, cow, ewe, milk, kefir

INTRODUCTION

Some chronic diseases including cancer, cardiovascular disease, and degenerative disease of aging have been related to the oxidative stress caused by excessive production of reactive oxygen species and oxygen-free radicals (Pizzino et al., 2017). Oxidative stress can also increase oxidation of membrane phospholipids, proteins, and DNA, as well as modification of low-density lipoproteins (López-Pedrera et al., 2016). Aerobic organisms are protected from oxidative stress by various enzymatic and nonenzymatic antioxidant defense systems. When the endogenous defense system cannot prevent the damage of reactive radicals, artificial antioxidants, such as butylated hydroxytoluene, butylated hydroxyanisole, and *tert*-butylhydroquinone, and natural antioxidants are used to impede the detrimental effects of oxidative stress. Because artificial antioxidants have been reported to be toxic, carcinogenic, and unsafe, most consumers prefer natural antioxidants as they believe these are not chemicals and are not subjected to any legislative restrictions of use (Boskou and Elmadfa, 2010; Bartosz, 2013; Landete, 2013).

Milk is a unique food that contains valuable macro- and micronutrients for the growth and development of breastfed infants; it is also a basic source of energy and nutritional components for mammals. It has naturally occurring nonenzymatic antioxidants such as vitamins A, C, and E, carotenoids, uric acid, CLA, and enzymatic antioxidants such as catalase, superoxide dismutase, and glutathione peroxidase. Some proteins in milk (e.g., casein, lactoferrin, α-LA, β-LG) and milk amino acids such as tyrosine, cysteine, tryptophan, and lysine are known to have antioxidant potential. Therefore, the antioxidant defense system of different animal milk and milk products can be considered to have health-beneficial effects against free radical damage (Lindmark-Månsson and Akesson, 2000; Kullisaar et al., 2003; Kitts, 2005; Zulueta et al., 2009).

Kefir has been manufactured and consumed since ancient times for its nutritional, taste, and flavor properties, as well as its potential health benefits. Consumption of kefir is increasing among consumers because of its profound antimicrobial, antibacterial, antitumor, antihypertensive, antioxidative, anticytotoxic, and hypocholesterolemic effects (de Oliveira Leite et al., 2013; Arslan, 2015; Chen et al., 2015; Prado et al., 2015). Kefir is obtained by the fermentation of milk with mixed microflora, including various species of lactic acid bacteria, acetic acid bacteria, and yeasts. The acid-alcoholic fermentation of milk with these microorganisms results in a refreshing and slightly acidic taste. Kefir is produced by either traditional or industrial methods. Traditional kefir production involves addition of kefir grains, whereas industrial kefir has been produced by commercial starter cultures of pure or mixed-strain cultures in powder form. Different animal milks such as cow, ewe, and goat, and plant-based milks obtained from soy, coconut, and rice can be used for production. The type of milk, type and quantity of starter culture,

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time and temperature-related conditions of fermentation, and storage conditions all affect the microbiological, chemical, and sensorial characteristics of kefir (Gao and Li, 2016; Barukčić et al., 2017; Rosa et al., 2017).

In previous studies, milk and milk products (i.e., yogurt, cheese, whey) were shown to have antioxidant properties depending on the presence and activity of natural bioactive components (Taylor and Richardson, 1980; Korpela et al., 1995; Chen et al., 2003; Pulido et al., 2003; Smet et al., 2008; Gupta et al., 2009; Kesenkas et al., 2011; Perna et al., 2013; Shori and Baba, 2013; Chen et al., 2015; Sabokbar et al., 2015; Sah et al., 2015; Yilmaz-Ersan et al., 2015). Although ewe and cow milks are widely used in the manufacture of different fermented milk products, little information is available on the antioxidative characteristics of kefir produced with these milk types. This study had 2 main aims. First, we determined the contents of vitamin E, β-carotene, and total phenolics, as well as total antioxidant capacity of kefir during different fermentation stages. Second, we investigated the varied roles of milk and starter culture type on kefir characteristics.

MATERIALS AND METHODS

Kefir Grains and Inoculum Preparation

Two types of starter cultures were used for manufacturing: traditional kefir grains (obtained from Department of Food Engineering, University of Uludag, Bursa, Turkey) and freeze-dried, direct vat set (**DVS**) culture (obtained from Danisco, Poznan, Poland). The DVS culture contains *Lactococcus lactis*, *Lactococcus cremoris*, *Lactococcus diacetylactis*, *Candida kefir*, and *Saccharomyces unispora.* For reactivation, the kefir grains were transferred 3 times into pasteurized milk, incubated at 25°C for 18 h, separated from the milk, and washed with sterile distilled water. These active grains (5%, wt/vol) were used to inoculate the fermentation medium. Upon receipt, the DVS culture was added 2% (vol/vol) directly to the fermentation medium.

Kefir Production

Raw cow and ewe milks were obtained from the Uludag University Dairy Farm (Bursa, Turkey), heated to 90°C, and kept at that temperature for 10 min. Then, the milks were cooled to the inoculation temperature (25°C) and inoculated with active grains or DVS culture. After inoculation, milks were incubated at 25°C until the pH reached 4.60 to 4.70. From each kefir batch, samples were collected at 4-h intervals during fermentation. At the end of the fermentation, the kefir grains were separated by use of a sterilized plastic sieve (2-mm pore size). After being transferred into 200-mL bottles, the kefir beverages were cooled to 4 to 6°C and stored at 4 ± 1°C until analysis. The samples were analyzed on d 1, 7, 14, and 21 of cold storage. Cow (**C**) and ewe (**E**) milk kefir made using kefir grains (**GR**) and starter culture (**SC**) are designated C-GR, C-SC, E-GR, and E-SC, respectively. Two replications of all batches and 3 replications of the analyses were performed. The pH was monitored using a digital pH meter (Analyzer model 315i/SET, WTW, Weilheim, Germany) throughout fermentation.

Chemicals

2,2-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid) di-ammonium salt (**ABTS**), 2,2-diphenyl-1-picrylhydrazyl (**DPPH**), 2,4,6-tripyridyl-2-triazine (**TPTZ**), Folin–Ciocalteu phenol reagent, potassium persulfate, gallic acid, tocopherol standards (α-tocopherol), HPLC-grade vitamin E and β-carotene were purchased from Sigma-Aldrich (St. Louis, MO); Trolox ([±]-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was obtained from Aldrich Chemical Co. (Steinheim, Germany). All standard solutions were prepared in HPLC-grade methanol (Merck, Darmstadt, Germany).

Extraction of Kefir Samples

Kefir samples (2 g) were blended with 20 mL of aqueous methanolic solution (70:30%, vol/vol) at room temperature in the dark for 4 h with magnetic stirring. The extracts were centrifuged at 1,420 × *g* for 10 min and filtered through qualitative filter paper (Whatman grade 2, 8-μm thickness); the supernatants were used for spectroscopic analysis.

Two grams of kefir was centrifuged and 5 mL of supernatant was saponified with 2 mL of an aqueous KOH (50%, vol/vol) at 65°C for 30 min in a water bath at 40 kHz. After cooling on ice for 15 min, 4 mL (2 × 2) of hexane and ethyl acetate solution (80:20%, vol/vol) was added to extract vitamin E. The organic fractions (3 mL) were evaporated to dryness. Finally, the residue was dissolved in 1 mL of hexane for HPLC-diode array detection (DAD) analysis of vitamin E. The kefir samples were extracted following the same method but without saponification for β-carotene analysis.

Determination of Antioxidant Capacity

ABTS Assay. Total antioxidant capacity of the samples was determined by using $ABTS^{\bullet+}$ radical cation decolorization assay (Sahin et al., 2012). The $ABTS^{\bullet+}$ radical stock solution was produced by re-