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A New Probiotic Cheese with Antioxidative and Antimicrobial Activity

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ABSTRACT

The aim of our study was to develop an original probiotic cheese based on the Estonian open-texture, smear-ripened, semisoft cheese "Pikantne." Cheese was produced by two methods using cheese starter cultures (Probat 505) in combination with 0.04% of probiotic *Lactobacillus fermentum* strain ME-3 (10^9 cfu/mL) with high antimicrobial activity and antioxidative properties. The probiotic *Lactobacillus* was added into milk simultaneously with starter cultures (cheese A) and into drained curd (cheese B). After addition of probiotic *L. fermentum* ME-3, the cheese composition, flavor, and aroma were comparable to the control cheese (score values = 4.5, 4.2, and 3.7 for control cheese, cheese A, and cheese B, respectively). Cheese A, which had good sensory properties, was chosen for further testing of viability and probiotic properties. The probiotic strain was found to withstand the technological processing of cheese, surviving and sustaining moderate antimicrobial and high antioxidative activity throughout ripening and storage (the ripened cheese contained approximately 5×10^7 cfu/g viable ME-3 cells), although the viability of the ME-3 strain incorporated into the cheese showed a slight decrease between d 24 and 54 after cheese preparation. Semisoft cheese "Pikantne" serves as a suitable carrier of antimicrobial and antioxidative *L. fermentum* ME-3.
(**Key words:** antimicrobial activity, antioxidative activity, lactobacilli, probiotic cheese)

Abbreviation key: LA = linolenic acid, **Mn-SOD** = Mn-superoxide dismutase, **OHEL** = obligately heterofermentative lactobacilli, **TAA** = total antioxidative activity.

INTRODUCTION

Probiotics have been defined as live microbial food supplements that benefit human health (McFarland, 2000; Salminen, 2001). Viable lactic acid bacteria of probiotic foods have several scientifically established and/or clinically proved health effects, such as reduction and prevention of diarrheas of different origin, improvement of the intestinal microbial balance by antimicrobial activity, alleviation of lactose intolerance symptoms, prevention of food allergy, enhancement of immune potency, and antitumorigenic activities (McFarland, 2000; Andersson et al., 2001; Salminen, 2001). Moreover, some studies have shown that certain lactic acid bacteria possess antioxidative activity (Kaizu et al., 1993; Peuhkuri et al., 1996; Kullisaar et al., 2002). They are able to decrease the risk of accumulation of reactive oxygen species in a host organism and could potentially be used in probiotic food supplements to reduce oxidative stress. In a previous study (Kullisaar et al., 2002), it was reported that *Lactobacillus fermentum* strain ME-3 (DSM 14241) has high antimicrobial and antioxidative activity. In healthy volunteers, it has been demonstrated that the consumption of fermented milk containing this *Lactobacillus fermentum* strain exhibited antioxidative and antiatherogenic effects (Kullisaar et al., 2003).

The suitability of different cheeses as carriers for antioxidative *Lactobacillus* strains has not been evaluated. As usual, cheese has certain advantages for a carrier state of probiotic organisms compared with the other, more acidic dairy products. There are different types of probiotic cheeses available on the market worldwide. Bifidobacteria are the most widely used probiotic additives in cheese (Dinakar and Mistry, 1994; Gomes et al., 1995; Daigle et al., 1999). However, there are relatively few scientific reports concerning lactobacilli of human origin as probiotic cheese additives (Gomes et al., 1995; Gardiner et al., 1998; Ross et al., 1999).

Different methods have been described for incorporation of probiotic *Lactobacillus* additives into cheese. Probiotics, as well as other nutritional supplements (antioxidants, vitamins, herbs, etc.), have been added to shredded natural cheese (U.S. Pat. 6090417). Bacteria with probiotic properties could be included with cheese starters (NLAB, 2002) added directly to cheese milk or to the curd before hooping (Gardiner et al., 1998; Ross et al., 1999).

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Probiotic bacteria have many desirable properties, such as safety, bile and acid resistance, adherence to human intestinal cells, colonization of the human gut, and production of antimicrobial substances. Being of human origin is considered to be of great importance (Lee and Salminen, 1995; Saarela et al., 2000). Nevertheless, potential problems may arise when trying to introduce a probiotic strain of human origin to dairy products. A probiotic strain should withstand the manufacturing process without loss of viability or negative effect on the sensory properties of the food product. The strain and the claimed properties should maintain stability in the food product during processing and also during subsequent storage (Lee and Salminen, 1995; Sanders and Huis in't Veld, 1999; Saarela et al., 2000), which could pose a problem for strains of human origin.

A large number of viable organisms are required in order to exert a probiotic effect in the food product. It is postulated that an active probiotic food should contain at least 10^5 cfu/g, and the food should be consumed daily in order to achieve a beneficial effect (Lee and Salminen, 1995). Therefore, it is of great importance to control the stability of probiotic numbers and properties in cheese.

The aim of our study was to develop a tasty original probiotic cheese based on the original Estonian open-texture, smear-ripened, semisoft cheese "Pikantne." The suitability of the probiotic *L. fermentum* ME-3 as a cheese additive was tested and its ability to retain viability and antioxidative and antimicrobial potential in the cheese environment was evaluated.

MATERIALS AND METHODS

Origin and Properties of Microbial Strain

The probiotic *Lactobacillus* strain *L. fermentum* ME-3 was previously isolated from the gastrointestinal tract of a healthy child (Sepp et al., 1997; Mikelsaar et al., 2002). The strain was identified by API CHL 50 System (bioMérieux, Marcy l'Etoile, France) and by internal transcribed spacer PCR using the reference strain *L. fermentum* ATCC 14931 (Annuk et al., 1999). The *L. fermentum* ME-3 has been deposited in the culture collection (Deutsche Sammlung von Mikroorganismen und Zellkulturen, DSM 14241). The patent application has been submitted to the Estonian Patent Agency (Application No. 0356/01PV), as well as to International Bureau of WIPO (Application No. PCT/EE02/00006).

The cells and cell lysate of *L. fermentum* ME-3 have a strong antioxidative potency. The cells produce Mn-superoxide dismutase (**Mn-SOD**) (0.859 ± 0.309 U/mg of protein), contain reduced glutathione (9.95 ± 3.30 μg/mL), and scavenge hydroxyl radicals (~75%). In addition, the cells of ME-3 have high total antioxidative activity (**TAA**) values (29 ± 1.0%) (Kullisaar et al., 2002). *Lactobacillus fermentum* ME-3 has been tested for production of H_2O_2 in a qualitative assay as well as by a quantitative method (Kullisaar et al., 2002). The base value of H_2O_2 production in intact cells was 31 ± 26 μg/mL.

Cheese Manufacture

A probiotic cheese containing *L. fermentum* strain ME-3 has been developed at the Department of Microbiology of Tartu University in cooperation with a small cheese manufacturing plant (Vana-Kuuste Dairy Oy) located in the southern part of Estonia. The probiotic cheese was prepared on the basis of Estonian "Pikantne" cheese.

Freeze-dried cheese inoculant Probat 505 (Wisby, Denmark), containing *Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *cremoris*, *Lactococcus lactis* ssp. *lactis* biovar. *diacetylactis*, and *Leuconostoc mesenteroides* ssp. *mesenteroides* was used as a starter. Cheese was made with 30 L of pasteurized (70 to 76°C, 20 to 25 s) whole milk. Probiotic lactobacillus was incorporated into cheese in two different manners. In the case of cheese A, 0.04% of probiotic strain ME-3 suspension (10^9 cfu/mL) in 0.9% NaCl was added into the pasteurized cow milk, together with 0.5% cheese starter and $CaCl_2$ (25 g per 100 kg) before rennet (12 g/tonne of milk) (Maxiren, Gist-Brocades, The Netherlands) coagulation for 35 min at 35°C. The curd was cut and cooked to 39°C. The procedure lasted approximately 100 min. The acidity of curd was pH 6.4–6.5 at the end of the procedure. After cooking, the curd was placed into the mold to drain off the whey. In the case of cheese B, the same portion of probiotic strain (0.04% of suspension of 10^9 cfu/mL) was added after curd draining. The cheese made without probiotic additive served as a control.

Cheese blocks (3 kg each) were turned from time to time for approximately 210 min in order to allow the curd to mat together. Thereafter, cheese was removed from the mold, salted in 18% brine at 9°C, extracted from the brine bath, allowed to dry for 2 d, and were left to ripen at 12°C for 30 d at a relative air humidity of 85 to 90%. Throughout ripening, cheese blocks were turned around and rubbed with 5 to 7% brine in order to support the uniform formation of smear on the surface of the cheese. After ripening, the cheese blocks were cleaned from the smear, allowed to dry (3 d), covered with paraffin for 2 s at 120 to 150°C, and stored for 30 d at 6°C (Table 1). Three replicates of experimental cheeses A and B were made.

Sensory Evaluation of Cheese

Estonian standard EV ST 616-92 was applied for cheese grading. Evaluation was based on four features,