J. Dairy Sci. 90:3171–3177
doi:10.3168/jds.2006-810

A Probiotic, *Lactobacillus fermentum* ME-3, Has Antioxidative Capacity in Soft Cheese Spreads with Different Fats

S. Järvenpää,* R. L. Tahvonen,*[1,2] A. C. Ouwehand,† M. Sandell,* E. Järvenpää,* and S. Salminen†
*Department of Biochemistry and Food Chemistry, and
†Degree Programme in Health Biosciences and Functional Foods Forum, University of Turku, FIN-20014 Turku, Finland

ABSTRACT

Our aim was to develop a prototype of a functional spread cheese containing both a specific probiotic and n-3 fatty acids and to analyze the viability of the probiotic and stability of n-3 fatty acids during 4 wk of shelf life. *Lactobacillus fermentum* ME-3 (Lf ME-3) isolated from a healthy Estonian child has been shown to have probiotic and antioxidative properties in several recent studies. In the current study this promising bacterial strain was combined with vegetable oils rich in nutritionally important α-linolenic acid and with unflavored cheese to obtain soft cheese spreads with different fat contents. *Lactobacillus fermentum* ME-3 survived well in all cheeses although the viable count did not increase during 4 wk of storage. The fatty acid composition of cheese triacylglycerols remained stable, whereas the profile of volatile compounds changed: hexanal and pentanal disappeared and the proportion of some alcohols increased. The changes in the profile of volatile compounds show the reductive power of Lf ME-3. A functional spread cheese containing n-3 fatty acids can be prepared with the probiotic *Lactobacillus fermentum* ME-3 strain leading to a reduced need for chemical antioxidants.
Key words: *Lactobacillus fermentum* ME-3, fatty acid, soft cheese, volatile compound

INTRODUCTION

Probiotics are defined as live microbial food and feed ingredients beneficial to health (Salminen et al., 1998). Microorganisms representing many different species and genera have been used as probiotics. Most studies have mainly been focused on lactobacilli and bifidobacteria because of their long history of safe use in foods. One of these strains is *Lactobacillus fermentum* ME-3 (**Lf ME-3**) that was isolated from the intestinal microbiota of a healthy, 1-yr-old Estonian child in 1995 (Sepp et al., 1997). This obligate heterofermentative lactic acid bacterium has been shown to exhibit significant antimicrobial and antioxidative properties in vitro (Kullisaar et al., 2002). It also has a history of safe use in foods in Estonia and other studies support its safety: Lf ME-3 does not contain hemolysins nor does it suppress the growth of common intestinal lactic acid bacteria (Mikelsaar et al., 2002; Songisepp et al., 2005). In laboratory animals Lf ME-3 has been demonstrated to benefit the general health status, to prevent the carrier state of *Salmonella,* and to improve mucosal antioxidative parameters (Mikelsaar et al., 2004; Truusalu et al., 2004). The results of volunteer trials are also promising, as the participants have tolerated well an experimental goat-milk yogurt containing Lf ME-3 without any adverse effects. Moreover, the Lf ME-3 fermented goat's milk expressed antiatherogenic potential; consumption of the yogurt prolonged resistance of the lipoprotein fraction to oxidation, lowered levels of peroxidized lipoproteins and 8-isoprostanes, decreased the glutathione redox ratio, and enhanced the total antioxidative activity (Kullisaar et al., 2003).

α-Linolenic acid (**ALA**, 18:3n-3) is essential for mammals; hence, ALA must be obtained in the diet. It is clear that ALA has actions of its own (Fu and Sinclair, 2000) and acts as a substrate for β-oxidation and carbon recycling (Cunnane et al., 1999). Furthermore, it is cardioprotective (Djoussé et al., 2005, Gebauer et al., 2006). α-Linolenic acid seems to modulate inflammation, which may be one of its benefits regarding cardiovascular disease (Harris, 2005; Basu et al., 2006). As with any polyunsaturated fatty acid (**PUFA**), it is very sensitive to lipid peroxidation (Halliwell and Gutteridge, 1999).

Oxidation of fatty acids in dairy products may have detrimental effects on flavor and storage stability. Usually, antioxidative substances are added to foods containing considerable amounts of different fatty acids. In a recent study, Lf ME-3 cells incorporated into a semisoft cheese expressed good total antioxidative activity (Songisepp et al., 2004). However, it has not been

Received December 4, 2006.
Accepted February 22, 2007.
[1]Corresponding author: raija.tahvonen@utu.fi
[2]Present address: FoodWest Ltd., Vaasantie 1C, FIN-60100 Seinäjoki, Finland.

Table 1. Experimental design showing content of α-linolenic acid (ALA) in different soft cheeses

Vegetable oil	ALA, g/g of cheese		
Canola oil	0/100[1]		
Canola oil	0.5/100		
Canola oil	2.0/100		
Flaxseed oil	0/100[1]	0/60[1]	0/30[1]
Flaxseed oil	0.5/100	0.5/60 = 0.8/100	0.5/30 = 1.7/100
Flaxseed oil	2.0/100	2.0/60 = 3.3/100	2.0/30 = 6.7/100
Camelina oil	0/100[1]	0/60[1]	0/30[1]
Camelina oil	0.5/100	0.5/60 = 0.8/100	0.5/30 = 1.7/100
Camelina oil	2.0/100	2.0/60 = 3.3/100	2.0/30 = 6.7/100

[1]These products were essentially identical. However, these oil-free blanks were evaluated to determine potential differences between products made on different days and from different curd batches.

assessed whether the antioxidative probiotic strain could prevent PUFA peroxidation and influence the composition of volatile compounds in soft cheeses enriched with different vegetable oils rich in ALA.

The aim of this study was to develop different cow's milk-based soft cheeses containing Lf ME-3 combined with vegetable oils (canola, flaxseed, and camelina) and to assess the survival of Lf ME-3, the changes in composition of fatty acids, and the changes in the profile of volatile compounds in the cheese products during 4 wk of storage.

MATERIALS AND METHODS

Cultures and Cheeses

Lactobacillus fermentum ME-3 (DSM 14241) was supplied by University of Tartu (Tartu, Estonia). A laboratory-scale soft cheese was prepared by dripping whey from a commercial cow's milk-based curd (Vähälaktoosinen maitorahka; Maitokolmio, Toholampi, Finland). The commercial curd was produced by fermenting low-fat, low-lactose milk with a specific starter culture. It contained 0.2% fat, <1% lactose, and potassium sorbate as preservative. Whey dripping was performed in 500-g batches in glass funnels lined with filter paper sachets. Dripping time and temperature was 2.5 h and 5°C, and the curd was covered with aluminum foil to prevent dust contamination. Three or 4 batches were combined and homogenized to yield a base cheese. Next, 1 mL of Lf ME-3 ($\approx 7 \times 10^9$ cfu/mL) in PBS (pH 7.2) and one of the vegetable oils were mixed with the base cheese to obtain 400 g of each product with ALA contents varying from 0 to 6.7 g/100 g (Table 1). The 3 oils used in the study were canola oil (**Can**; Virgino rypsiöljy; Kankaisten öljykasvit Oy, Hämeenlinna, Finland), flaxseed oil (**Fla**; Pellavainen pellavansiemenöljy; Elixi Oil Oy, Somero, Finland), and camelina oil (**Cam**; Camelina öljy; Camelina Oy, Raisio, Finland); all oils were cold pressed.

Treatment designations are shown in Table 1; the numbers following the oil designation (Can, Cam, and Fla) indicate the ALA content in each cheese (g of ALA/g of cheese). The mixing was done first with an ethanol-cleaned spatula and then carefully with a hand mixer (Bamix Mono; ESGE AG, Mettlen, Switzerland) in the same food-grade polypropylene containers in which the cheeses would be stored for the next 4 wk at 5°C. One replicate of each cheese variant was prepared. In addition, different blank cheeses were prepared: oil-free blanks (base cheese and Lf ME-3) and probiotic-free blanks (base cheese mixed with vegetable oil).

Microbiological Analysis

Bacterial analysis of cheeses was performed on the day of production (d 0) and on d 4, 7, 14, 21, and 28. Samples of 1 g were serially diluted (10^{-4} to 10^{-6}) in PBS (pH 7.2), and dilutions were plated on de Man, Rogosa, and Sharpe (**MRS**; Oxoid Ltd., Basingstoke, UK) agar (European bacteriological agar, Laboratorios Conda, Madrid, Spain). The plates were incubated in an anaerobic atmosphere (10% H_2, 10% CO_2, 80% N_2) at 37°C overnight. The counts (cfu/g) of Lf ME-3 were estimated by counting of all visible colonies (white colonies with regular edges). This estimation method was feasible because the diluted samples (10^{-1} to 10^{-4}) of the curd without Lf ME-3 addition did not form colonies during the incubation period on MRS. Each time when the growth of bacteria was tested, additional cheese samples were put in glass vials and stored at –86°C until subsequent analyses.

Extraction of Lipids

The method used in the extraction of lipids was a modification of the Folch procedure (Folch et al., 1957). The extracted samples were from products containing added vegetable oil. Samples were thawed at 4°C before weighing. An amount of cheese corresponding to at least 50 mg of lipids was mixed, as a whole or in several lots, with 18 volumes of 2:1 (vol/vol) chloroform:methanol in stoppered glass tubes. The mixture was vortexed for 2 min. Then, 6 volumes of 0.7% (wt/vol) sodium chloride (aqueous) solution were added and the tubes were inverted 20 times. The mixtures were separated into 2 phases using a DuPont Sorvall TC centrifuge (Sorvall Instruments, Newton, CT) equipped with an H-400 rotor (4 min, $950 \times g$). The lower phase was transferred with a Pasteur pipette to a weighed test tube. The solvents were evaporated under a flow of nitrogen and the tube was weighed again to determine the approximate mass of lipids. The solids were dissolved in hexane containing 0.02% butylated hydroxytoluene (**BHT**, as an