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Probiotic Cheese Production Using *Lactobacillus casei* Cells Immobilized on Fruit Pieces

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ABSTRACT

Lactobacillus casei cells were immobilized on fruit (apple and pear) pieces and the immobilized biocatalysts were used separately as adjuncts in probiotic cheese making. In parallel, cheese with free *L. casei* cells and cheese only from renneted milk were prepared. The produced cheeses were ripened at 4 to 6°C and the effect of salting and ripening time on lactose, lactic acid, ethanol concentration, pH, and lactic acid bacteria viable counts were investigated. Fat, protein, and moisture contents were in the range of usual levels of commercial cheeses. Reactivation in whey of *L. casei* cells immobilized on fruit pieces after 7 mo of ripening showed a higher rate of pH decrease and lower final pH value compared with reactivation of samples withdrawn from the remaining mass of the cheese without fruit pieces, from cheese with free *L. casei,* and rennet cheese. Preliminary sensory evaluation revealed the fruity taste of the cheeses containing immobilized *L. casei* cells on fruit pieces. Commercial Feta cheese was characterized by a more sour taste, whereas no significant differences concerning cheese flavor were reported by the panel between cheese containing free *L. casei* and rennet cheese. Salted cheeses scored similar values to commercial Feta cheese, whereas unsalted cheese scores were significantly lower, but still acceptable to the sensory panelists.

Key words: probiotic cheese, *Lactobacillus casei,* cell immobilization, cheese ripening

INTRODUCTION

An upsurge of interest in developing novel foods containing probiotic microorganisms such as *Lactobacillus* and *Bifidobacterium* spp. has taken place in recent years. Such functional foods have great potential for promoting human health by maintaining and improving intestinal microbial balance (Mattila-Sandholm et al., 2002). Probiotic products should be provided with an adequate amount of live bacteria (at least 10^7 cfu/g; Oliveira et al., 2002) to survive the acidic conditions of the upper gastrointestinal tract and proliferate in the intestine to obtain the health-promoting benefits. Methods such as cell immobilization, appropriate selection of acid- and bile-resistant strains, use of oxygen-impermeable containers, and stress adaptation have been proposed for improving the viability of probiotic bacteria (Rao et al., 1989; Ishibashi and Shimamura, 1993; Shah, 2000; Champagne et al., 2005).

Fruits and fruit juices have been used extensively as blended additives during production of a variety of foods, especially in dairy industry. Apple (Kourkoutas et al., 2001, 2002) and pear pieces (Mallios et al., 2004) have been used successfully as immobilization supports of yeast strains for room temperature and low-temperature wine making. The resulting wines were of improved quality with a distinctive aromatic potential. Likewise, apple pieces have been used in *Lactobacillus casei* cell immobilization (Kourkoutas et al., 2005) for probiotic additive fermented milk production. In addition, apple pieces proved to be very effective supports for the survival of apple-immobilized yeast (Kourkoutas et al., 2003) and bacteria (Kourkoutas et al., 2005), as the immobilized biocatalysts were able to reactivate after storage of 120 and 129 d, respectively.

Immobilized lactic acid bacteria (**LAB**) have been used and their effect has been studied during the cheese-making process. Batch and continuous milk prefermentation were performed using entrapped LAB in Ca-alginate beads (Prévost and Diviès, 1987). Dinakar and Mistry (1994) studied the growth and viability of *Bifidobacterium bifidum* added either as a commercially available powder or immobilized on κ-carageenan freeze-dried preparation in Cheddar cheese. Maximum bifidobacteria counts occurred at 18 and 24 wk for the commercial and immobilized preparations, respectively. Also, alginate-immobilized freeze-dried LAB have been used as starters in cheese making (Champagne et al., 1992) and in cheese ripening (Steenson et al., 1987).

Lactobacillus casei is a homofermentative microorganism (Holzapfel and Schilliger, 2002). It is acid toler-

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ant (Fellows, 1997; Kourkoutas et al., 2005) and could thus survive during cheese ripening.

Feta cheese, one of the most significant and popular dairy products in Greece with worldwide acceptance, is a soft, white cheese, usually ripened in brine. Traditionally, Feta cheese is prepared from ewe's milk using only rennet on small family facilities with simple equipment. Today, most Feta cheese is produced from ewes' milk or a mixture of ewes' and goats' milk in organized, commercial cheese dairies, using yogurt culture for lactic acid production. After a short period, rennet is added for completion of precipitation. A variation of Feta cheese is a traditional Greek soft white cheese known as "Katchochiri" (other local names may also exist), which is produced using ewes' or goats' milk, or both, by coagulation using only rennet. Subsequently, treatments such as filtration, salting, and preservation at 4°C are usually carried out. The produced cheese is consumed fresh or after ripening.

The aims of the present study were to investigate 1) the production of a new type of cheese using immobilized *L. casei* cells on fruit pieces as an adjunct culture, 2) the survival of the probiotic culture during cheese ripening, and 3) the implications of the adjunct culture on physicochemical and sensory characteristics of the final product during ripening.

MATERIALS AND METHODS

Cell Immobilization

Lactobacillus casei ATCC 393 (DSMZ, Germany) was used. It was grown on MRS broth (Merck, Darmstadt, Germany). Cell immobilization on apple and pear pieces (~0.5-cm^3 cubes) was carried out as described previously (Kourkoutas et al., 2005). In brief, fruit (apples and pears of Starkin and Conference varieties, respectively) pieces (~500 g) were introduced into 1 L of *L. casei* liquid culture (~10^9cfu/mL), and allowed to ferment overnight at 37°C without agitation. When immobilization was complete (glucose in the liquid culture was <1g/L; concentration of glucose was determined by HPLC using the same method described for lactose determination below), the fermented liquid was decanted, and the supported biocatalysts were washed twice with pasteurized milk. The biocatalysts were then used in cheese production.

Probiotic Cheese Production

Ewes' milk was used for cheese production. It was heated at 65°C for 30 min and then cooled at 37°C. Commercial rennet (0.01%) was added and the whole was left undisturbed for 2 h for curd formation. Subsequently, the curd was cut in squares (~1 cm diameter), left undisturbed for 10 min, and then cloth-filtered. Immobilized *L. casei* on apple and pear pieces were added separately (50 g of immobilized biocatalyst/L of milk used) during cloth filtration, which lasted overnight at room temperature (18 to 22°C) for complete whey removal. Cheese produced from milk containing ~10^9cfu/mL of free *L. casei,* and cheese without *L. casei* cells (called rennet cheese) were produced for comparison. The effect of salt addition on cheese quality characteristics was studied by rubbing 10 g of salt/100 g of cheese on the surface. Ripening of the produced cheeses was monitored at 4 to 6°C for 71 d.

Reactivation of the Supported Biocatalysts

After 7 mo of cheese ripening, apple- and pear-supported biocatalysts (1.4 g) were removed from the salted cheese mass, washed twice with 50 mL of whey, introduced into 100 mL of whey, and tested for their fermentative activity by monitoring pH during lactic acid fermentation. For comparison, the same procedure was followed using equal masses of the remaining salted cheese without fruit pieces, the salted cheese containing free *L. casei,* and salted rennet cheese.

Analyses

Twenty grams of cheese sample was macerated with warm water (40°C) to produce a total volume of 210 mL, and the whole was then filtered (Kirk and Sawyer, 1991). The filtrate was used for lactic acid, lactose, and ethanol determinations.

Lactic acid was determined by HPLC, using a Shimadzu chromatograph (Shimadzu, Kyoto, Japan) with a Shim-pack IC-A1 stainless steel column, an LC-10A pump, a CTO-10A oven at 40°C, and a CDD-6A conductivity detector. A solution of 2.5 m*M* phthalic acid (Merck) and 2.4 m*M* Tris (hydroxymethyl) aminomethane (pH 4.0; Merck) in triple-distilled water was used as mobile phase with a flow rate of 1.5 mL/min. Samples (0.25 mL) were diluted to 25 mL, and 60 μL was injected directly onto the column. Lactic acid concentrations were calculated using standard curves.

Lactose and ethanol were determined by HPLC, using a Shimadzu chromatograph with an SCR-101N stainless steel column, an LC-9A pump, a CTO-10A oven at 60°C, and a RID-6A refractive index detector. Triple-distilled water was used as mobile phase with a flow rate of 0.8mL/min, and 1-butanol (Merck) was used as an internal standard. Then, 0.5 mL of cheese filtrate and 2.5 mL of a 1% (vol/vol) solution of 1-butanol were diluted to 50 mL, so that the final concentration of 1-butanol was 0.05% (vol/vol). Then, 40 μL of the final solution was injected directly onto the column. Lactose

and ethanol concentrations were calculated using standard curves prepared with at least 7 standard solutions by correlating the ratio of lactose or ethanol peak areas divided by 1-butanol peak areas to lactose or ethanol concentrations.

Total N expressed as CP on dry weight basis was determined using the Kjeldahl procedure and fat in DM content was determined by the Soxhlet process. The former methods as well as moisture content determination were described previously (Kirk and Sawyer, 1991).

Solid-Phase Microextraction, Gas Chromatography/ Mass Spectrometry

Salted cheese samples ripened for 30 d at 4 to 6°C were studied for volatile by-product composition using solid-phase microextraction (**SPME**), gas chromatography/mass spectrometry (**GC/MS**) analysis. Grated samples of cheeses (~7 g) were placed into a 20-mL headspace vial fitted with a Teflon-lined septum sealed with an aluminum crimp seal, through which the SPME syringe needle (bearing a 2-cm fiber coated with 50/30 mm divinylbenzene/carboxen on poly-dimethyl-siloxane bonded to a flexible fused silica core; Supelco, Bellefonte, PA) was introduced. The container was then held at a constant temperature of 80°C for 30 to 35min (Bellesia et al., 2003). The absorbed volatile analytes were then analyzed by GC/MS (Shimadzu GC-17A, MS QP5050, capillary column Supelco CO Wax-10; 60 m, 0.32 mm i.d., 0.25-μm film thickness). Helium was used as carrier gas (linear velocity of 1.5 mL/min). Oven temperature was programmed from 35°C for 3 min, 5°C/min to 110°C, then 10°C/min to 240°C, and held at 240°C for 10 min. The injector was operated in splitless mode. Injector and detector temperatures were 280 and 250°C, respectively. The mass spectrometer was operated in electron impact mode with the electron energy set at 70 eV. Identification was achieved by comparison with standard compounds and data obtained from NIST107, NIST21 (www.nist.gov), and SZTERP (Shimadzu Instruction Manual) libraries.

LAB Viable Cell Counts

Ten-gram portions of duplicate cheese samples were blended with 90 mL of sterilized Ringer solution, and submitted to serial dilutions. Lactic acid bacteria were counted by pour-plating 0.1 mL of each dilution on MRS agar (Merck) after 48 h at 37°C under anaerobic conditions (Anerocult C, Merck).

Preliminary Sensory Evaluation

Cheese samples containing probiotic culture were tested for their sensory characteristics and compared with rennet cheese and with commercial Greek Feta cheese. Samples (~25 g) of cheeses ripened for at least 30 d were presented. Sensory evaluation was conducted by 12 laboratory members previously trained using locally approved protocols. The panel was asked to give scores on a 0-to-10 scale (0 = unacceptable, 10 = exceptional) for attributes grouped into 3 categories: aroma, taste, and flavor. Panelists used water to clean their palates between samples and were unaware of the samples they tasted (samples were labeled with codes for identification). Significance was established at $P < 0.05$. Results were analyzed for statistical significance with ANOVA. Duncan's multiple range test was used to determine significant differences among results [coefficients, ANOVA tables and significance ($P < 0.05$) were computed using Statistica v.5.0; StatSoft, Inc., Tulsa, OK].

Experimental Design and Statistical Analysis

All treatments were carried out in triplicate and the mean values are presented (maximum deviation for all values was ±5% in most cases). Duplicate samples from each treatment were collected at various intervals and analyzed for lactic acid, lactose, volatile by-products, and total LAB. After that, cheeses remained at 4 to 6°C for 7 mo to test survival of LAB.

In the experiments conducted, the effect of probiotic *L. casei* adjunct culture and the effect of salting during cheese ripening were studied. The experiments were designed and analyzed statistically by ANOVA. Duncan's multiple range test was used to determine significant differences among results [coefficients, ANOVA tables and significance ($P < 0.05$) were computed using Statistica v.5.0].

RESULTS AND DISCUSSION

Ewes' milk consists of about 66% moisture, 17% total solids, 5.30% fat, and 6.30% total protein, of which 4.60% is casein, 4.60% lactose, and 0.80% ash (Voudouris and Kontominas, 1990). Nonhomogenized ewes' milk, pasteurized at 65°C for 30 min, was used in the present study due to its sensory characteristics.

Milk coagulation was achieved using rennet only, as is common practice in Greece. It is well known that starter cultures may implement an inhibitory effect on probiotic microorganisms (Gomes et al., 1995, 1998; Daigle et al., 1999). The strategy adopted was the omission of starter cultures and the use of *L. casei* culture as an adjunct probiotic culture instead, because *L. casei* is an acid-resistant microorganism (Fellows, 1997; Kourkoutas et al., 2005) and it survives in the cheese mass during ripening (Broome et al., 1990; Stanton et al., 1998; Antosson et al., 2002).

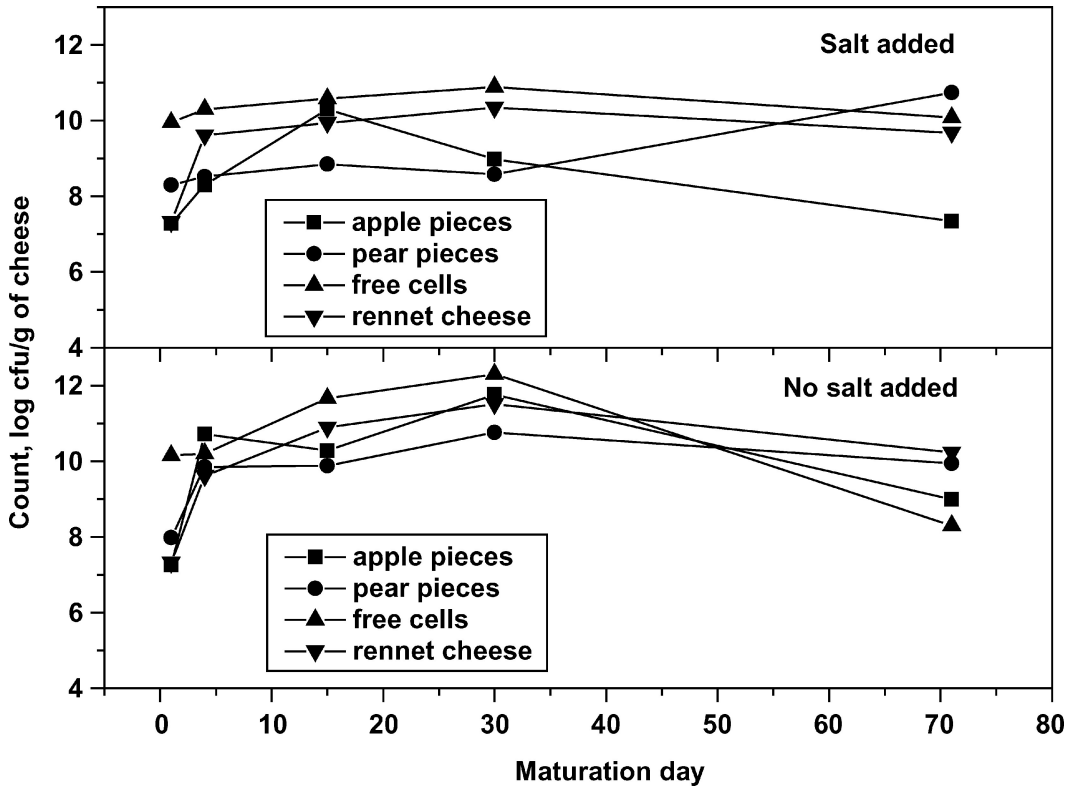

Figure 1. Lactic acid bacteria counts in probiotic white cheese produced by immobilized *Lactobacillus casei* cells on fruit pieces and free *L. casei* cells during ripening for 71 d at 4 to 6°C.

Immobilized *L. casei* cells were added to the curd after cloth filtration and the immobilized biocatalyst was distributed uniformly, as much as possible, in the curd mass. Cheese with free *L. casei* cells and rennet cheese were produced for comparison.

A problem associated with sampling due to uneven distribution of *L. casei* cells in the cheese mass was noticed in cheeses containing immobilized *L. casei* on fruit pieces. However, during cheese production, fruit pieces were mixed with the curd and distributed uniformly, as much as possible. All samples withdrawn at various intervals contained cheese and fruit pieces and effort was made to ensure the withdrawn samples contained an equal amount of fruit pieces and were derived from the same depth of the cheese mass. Therefore, average enumeration of LAB is presented in Figure 1, because LAB cell counts in fruit pieces may have been higher. The same problem was observed during determination of physicochemical parameters. Similar problems concerning uneven distribution exist in commercial products that contain fruit pieces and are acceptable by the consumers (e.g., yogurt with fruit).

Physicochemical parameters for unsalted and surface-salted cheeses during ripening are presented in Table 1. Addition of adjunct culture, salting, and ripening had a significant effect on pH and concentrations of lactose and ethanol ($P < 0.01$), and there was a strong interaction among them ($P < 0.01$). In contrast, lactic acid concentration was only affected by adjunct culture and ripening ($P < 0.01$), but not by salt addition ($P > 0.05$). However, a strong interaction between salting and adjunct culture ($P < 0.01$), between adjunct culture and ripening ($P < 0.01$), and among adjunct culture, salting, and ripening ($P < 0.01$) affecting lactic acid concentration significantly was observed. During ripening, lactose content was significantly decreased and lactic acid was generally increased. Ethanol content was generally at a very low level (0.03 to 0.6 g/100 g of cheese in most cases), whereas pH decreased during ripening in salted cheeses. However, pH was in the range of levels usually observed in commercial products (Table 1). The opposite effect was observed for unsalted cheeses, probably due to invisible spoilage after ripening for more than 30 d (Spencer and Spencer, 1997). The smear microorganisms that develop on the surface of the cheese play a role in the ripening process, both through the action of proteolytic and lipolytic enzymes, and the formation of many alkaline products, mainly

Table 1. Quality characteristics of probiotic white cheese produced by immobilized *Lactobacillus casei* cells on fruit pieces and free *L. casei* cells during ripening for 71 d at 4 to 6°C[1]

		Cheese type							
		L. casei on apple pieces		*L. casei* on pear pieces		*L. casei* free cells		Rennet cheese	
Analysis	Day of ripening	No salt	Salt	No salt	Salt	No salt	Salt	No salt	Salt
Lactose (g/100 g of cheese)	1	1.80 ± 0.1	1.15 ± 0.01	1.90 ± 0.1	2.09 ± 0.1	1.19 ± 0.07	1.57 ± 0.05	2.03 ± 0.1	1.74 ± 0.1
	4	1.38 ± 0.06	1.04 ± 0.001	1.79 ± 0.08	1.27 ± 0.05	1.25 ± 0.05	1.26 ± 0.03	1.73 ± 0.1	1.40 ± 0.05
	15	1.29 ± 0.05	0.92 ± 0.04	0.62 ± 0.03	1.13 ± 0.04	0.59 ± 0.01	0.71 ± 0.02	1.70 ± 0.1	0.89 ± 0.02
	30	0.85 ± 0.02	0.77 ± 0.02	0.47 ± 0.01	0.79 ± 0.01	0.30 ± 0.01	0.70 ± 0.02	0.78 ± 0.03	0.84 ± 0.02
	71	0.71 ± 0.01	0.48 ± 0.01	0.14 ± 0.01	0.21 ± 0.01	Tr	0.34 ± 0.01	0.33 ± 0.01	0.56 ± 0.01
Lactic acid (g/100 g of cheese)	1	0.42 ± 0.01	0.52 ± 0.02	0.48 ± 0.02	0.50 ± 0.05	0.55 ± 0.02	0.39 ± 0.02	0.15 ± 0.002	0.10 ± 0.002
	4	0.62 ± 0.02	0.60 ± 0.03	0.49 ± 0.02	0.60 ± 0.08	0.61 ± 0.03	0.43 ± 0.02	0.22 ± 0.01	0.15 ± 0.002
	15	0.80 ± 0.07	0.63 ± 0.03	0.45 ± 0.02	0.60 ± 0.08	0.64 ± 0.03	0.46 ± 0.02	0.13 ± 0.002	0.13 ± 0.002
	30	0.53 ± 0.02	0.68 ± 0.04	0.45 ± 0.02	0.69 ± 0.03	0.68 ± 0.03	0.48 ± 0.02	0.12 ± 0.002	0.12 ± 0.002
	71	0.54 ± 0.02	0.68 ± 0.04	0.68 ± 0.04	0.94 ± 0.04	0.86 ± 0.04	0.64 ± 0.03	0.86 ± 0.04	0.50 ± 0.01
Ethanol (g/100 g of cheese)	1	0.21 ± 0.01	0.09 ± 0.002	0.22 ± 0.01	0.12 ± 0.005	0.22 ± 0.01	0.09 ± 0.001	0.28 ± 0.01	0.11 ± 0.001
	4	0.29 ± 0.01	0.05 ± 0.001	0.09 ± 0.001	0.20 ± 0.005	0.09 ± 0.001	0.15 ± 0.01	0.28 ± 0.01	0.16 ± 0.01
	15	0.50 ± 0.01	0.05 ± 0.001	0.21 ± 0.01	0.31 ± 0.01	0.14 ± 0.01	0.56 ± 0.01	0.27 ± 0.01	0.12 ± 0.001
	30	0.66 ± 0.02	0.03 ± 0.001	0.18 ± 0.01	0.11 ± 0.005	0.14 ± 0.01	0.51 ± 0.01	0.19 ± 0.01	0.08 ± 0.001
	71	0.09 ± 0.001	1.19 ± 0.01	0.05 ± 0.001	2.12 ± 0.1	0.27 ± 0.01	0.12 ± 0.001	0.34 ± 0.01	0.90 ± 0.05
pH	1	5.3 ± 0.1	5.1 ± 0.1	5.2 ± 0.1	5.1 ± 0.1	5.3 ± 0.1	5.1 ± 0.1	5.9 ± 0.1	5.9 ± 0.1
	4	5.3 ± 0.1	4.9 ± 0.1	5.1 ± 0.1	4.8 ± 0.1	5.2 ± 0.1	5.0 ± 0.1	5.7 ± 0.1	5.4 ± 0.1
	15	5.2 ± 0.1	4.6 ± 0.1	5.0 ± 0.1	4.7 ± 0.1	5.0 ± 0.1	4.9 ± 0.1	5.8 ± 0.1	5.4 ± 0.1
	30	5.4 ± 0.1	4.4 ± 0.1	5.7 ± 0.1	4.3 ± 0.1	5.5 ± 0.1	4.9 ± 0.1	7.0 ± 0.1	5.0 ± 0.1
	71	5.8 ± 0.1	4.6 ± 0.1	6.4 ± 0.2	4.5 ± 0.1	5.9 ± 0.2	4.9 ± 0.1	7.3 ± 0.2	4.8 ± 0.1
Infection	1	–	–	–	–	–	–	–	–
	4	–	–	–	–	–	–	–	–
	15	–	–	–	–	–	–	–	–
	30	–	–	–	–	–	–	+	–
	71	+	–	+	–	+	–	+	–

[1]Tr = Trace; + = cheese spoilage; – = no spoilage.

Table 2. Fat, protein, and moisture content of probiotic white cheese produced by immobilized *Lactobacillus casei* cells on fruit pieces and free *L. casei* cells after ripening for 71 d at 4 to 6°C

	Cheese type								
	L. casei on apple pieces		*L. casei* on pear pieces		*L. casei* free cells		Rennet cheese		Commercial
Analysis	No salt	Salt	No salt	Salt	No salt	Salt	No salt	Salt	Feta
Fat, % in DM	50.0 ± 1.0	64.4 ± 1.4	51.8 ± 0.9	66.7 ± 1.7	64.1 ± 1.4	52.3 ± 1.3	61.0 ± 2.2	55.9 ± 0.9	53.0 ± 1.8
Protein, % in DM	37.9 ± 1.6	33.1 ± 1.9	44.9 ± 1.6	30.1 ± 2.5	31.8 ± 2.4	39.9 ± 1.4	32.1 ± 2.8	37.9 ± 2.4	44.3 ± 1.6
Moisture, %	52.3 ± 1.8	50.1 ± 2.0	54.6 ± 2.1	52.3 ± 2.7	54.5 ± 1.7	42.3 ± 1.9	58.8 ± 1.9	49.6 ± 2.1	57.1 ± 2.2

due to the assimilation of lactate that penetrated the body of the cheese. (Reps, 1993; Smacchi et al., 1999; Addis et al., 2001; Corsetti et al., 2001).

As Stanton et al. (1998) reported, during the early period of cheese ripening, a population of nonpathogenic organisms referred to as nonstarter lactic acid bacteria (**NSLAB**) mainly composed of lactobacilli (*Lb. plantarum, casei,* and *brevis*) and pediococci (*Pediococcus pentosaceus*) survive pasteurization in an attenuated state and proliferate as the cheese is ripened. Number of nonstarter lactic acid bacteria increase rapidly, reaching maximum levels of 10^7 to 10^8 cfu/g in ripened Cheddar cheese within a few days (Stanton et al., 1998). Salting of cheese contributes positively to cheese flavor, and has an impact on microorganisms' growth as salt diffuses in the cheese mass, so that differences concerning salt content in the cheese center and periphery decrease with ripening (Mocquot, 1979; De Leon-Gonzalez et al., 2000). Viability of bifidobacteria and other bacteria is inversely related to the salt concentration (Gomes et al., 1998; Vinderola et al., 2002). In our study, LAB counts were generally increased up to 30 d of cheese ripening, reaching maximum levels of about 10^9 to 10^{12} cfu/g (Figure 1). The increase was higher in unsalted cheeses than in salted cheeses. After 30 d, LAB cell counts were decreased except in the case of salted samples with immobilized *L. casei* cells on fruit pieces, in which some deviations were recorded (Figure 1). The reduction was probably due to high salt in moisture, low pH, and low ripening temperature as recently reported (Stanton et al., 1998). The fact that almost equal initial LAB counts were observed in cheeses containing immobilized *L. casei* and in rennet cheese could be attributed to similar concentration of *L. casei* on the fruit pieces after immobilization to levels of NSLAB in rennet cheese (~10^7 to 10^8 cfu/g). Therefore, increased levels of lactic acid bacteria should not be expected in the cheeses that contained immobilized *L. casei.* Heat treatment at 65°C was applied during raw milk pasteurization in the present study to standardize cheese quality, as is traditionally recommended in Greece, and because it has been claimed that cheeses made from raw, unpasteurized milk possess a better flavor (Adams and Moss, 1997). Consequently, thermophilic LAB might have survived; and thus, levels of 10^7cfu/g of NSLAB in the rennet cheese at the first day of cheese ripening are justified (Adams and Moss, 1997). Similar results have been reported in previous studies describing production of a similar type of cheese using raw milk (Hatzikamari et al., 1999; Nikolaou et al., 2002).

Salting resulted in slightly higher protein content in cheese containing free *L. casei* cells and in rennet cheese compared with cheeses containing immobilized *L. casei* on fruit pieces (Table 2), probably because the presence of salt may have an inhibitory effect on proteolytic enzymes. In contrast, cell immobilization may have a protective effect on proteolytic activity. Salting resulted in slightly higher fat content in cheeses containing immobilized *L. casei* on fruit pieces, whereas the opposite effect was observed in cheeses containing free *L. casei* cells and in rennet cheeses. The above results could be because the majority of *L. casei* cells were immobilized on fruit pieces and therefore did not contribute to fat lipolysis. Moisture was relatively lower in cheeses produced in the laboratory compared with commercial Feta cheese, as the latter was ripened and stored in brine solution.

Survival of *L. casei* cells was investigated by reactivating separately 1) the immobilized biocatalysts, 2) the remaining part of the cheeses that contained the immobilized biocatalysts, 3) a sample of the cheese that contained free *L. casei,* and 4) a sample of the rennet cheese after ripening for 7 mo. In a final LAB enumeration carried out after 7 mo in salted cheeses, viable cell counts were 10.15, 11.15, 7.69, 7.56, 7.66, and 7.65 cfu/g in apple pieces only, in pear pieces only, in the remaining cheese mass without apple and pear pieces, in the cheese sample containing free *L. casei,* and in rennet cheese, respectively. The above results showed increased LAB cell counts in fruit pieces compared with the remaining cheese mass, which could be attributed to survival of the immobilized *L. casei* cells. This was in agreement with results obtained during reactivation of the immobilized *L. casei* cells, as shown in Figure 2. *Lactobacillus casei* cells immobilized on fruit pieces and

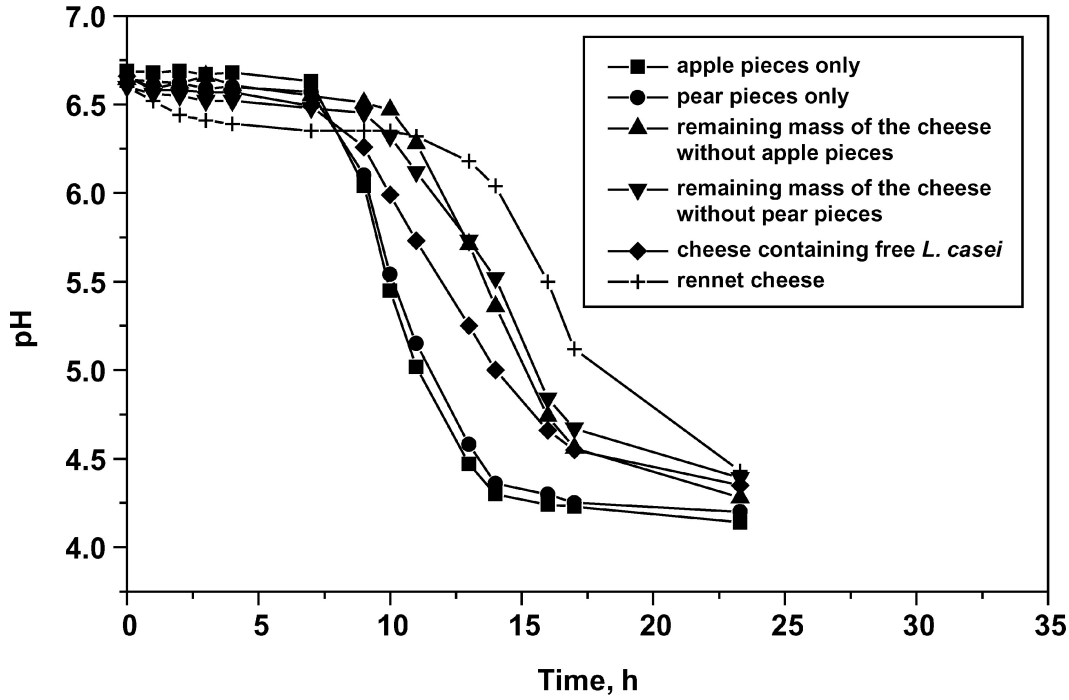

Figure 2. Kinetics of pH during lactic acid fermentation of whey by: a) *Lactobacillus casei* cells immobilized on apple pieces only, withdrawn from salted cheese (■), b) *L. casei* cells immobilized on pear pieces only, withdrawn from salted cheese (●), c) remaining mass of salted cheese produced by *L. casei* cells immobilized on apple pieces (▲), d) remaining mass of salted cheese produced by *L. casei* cells immobilized on pear pieces (▼), e) salted cheese containing free *L. casei* cells (◆), and f) salted rennet cheese (+), after ripening for 7 mo.

especially on apple pieces showed a higher rate of pH decrease and lower final pH value (4.15) compared with other types of cheeses after 7 mo of ripening. The higher pH (4.45) obtained during lactic acid fermentation using pieces of rennet cheese (Figure 1) showed that fruit pieces are an effective support for the acid-tolerant *L. casei* cells during cheese ripening.

SPME and GC-MS Analysis

For evaluation of the aromatic profile, probiotic white cheeses produced by immobilized *L. casei* on apple, pear pieces, and free cells were analyzed with SPME GC/MS and compared with rennet cheese and commercial Feta cheese. Only salted cheeses were analyzed, because an increase in the pH during 30 d of maturation in the unsalted cheeses was observed, accompanied by surface cheese infection and a bad odor. This analysis allowed a qualitative comparison of the aroma-related compounds produced during cheese ripening. The results are summarized in Table 3.

In general, the most important compounds that are usually identified by SPME GC/MS technique in cheeses are esters, organic acids, alcohols, carbonyl compounds, sulfur, and miscellaneous compounds (Barbieri et al., 1994; Engels et al., 1997; Izco and Torre, 2000; Qian and Reineccius, 2002). Nevertheless, this does not imply that some components are less important than others. The flavor of the cheeses seems to depend not on particular key components, but rather on a "critical balance" or a "weighted concentration ratio" of all components present (Izco and Torre, 2000). In regard to the present study, more compounds were detected in cheeses containing immobilized *L. casei* cells on apple and pear pieces than in the other types of cheeses produced. These compounds were probably derived from the fruit pieces introduced in the cheeses. In total, 61 compounds were detected in cheese containing *L. casei* cells immobilized on apple pieces, 71 compounds in cheese containing *L. casei* cells immobilized on pear pieces, 39 compounds in cheese with free *L. casei* cells, 51 compounds in rennet cheese, and 35 compounds in commercial Feta cheese. Presence of *L. casei* cells in cheese with free *L. casei* cells (Bachmann et al., 1997; Bosset et al., 1997; Champagne et al., 2005) and presence of salt (Tzanetakis and Litopoulou-Tzanetaki, 1992; Gerasi et al., 2003) in commercial Feta cheese probably inhibited development and action of some *Lactobacillus* species that produce aroma-related compounds in cheeses. Adjuncts of lactobacilli may suppress the growth of undesirable bacteria in cheese (Martley and Crow, 1993). Vinderola et al. (2002) observed

Table 3. Peak areas ($\times 10^5$) of volatile by-products isolated in probiotic salted white cheeses produced by immobilized *Lactobacillus casei* cells on fruit pieces and free *L. casei* cells after ripening for 30 d at 4 to 6°C using solid-phase microextraction gas chromatography-mass spectrometry[1]

		Cheese type				
RI[2]	Compound	*L. casei* on apple pieces	*L. casei* on pear pieces	*L. casei* free cells	Rennet cheese	Commercial Feta cheese
Esters						
866	Ethyl acetate	801.7[a]	790.9[a]	nd	357.5[a]	nd
977	Ethyl butyrate	nd	nd	nd	nd	348.7[a]
1067	Propyl butyrate	nd	nd	nd	nd	281.8[a]
1183	Hexyl acetate	nd	68.1[a]	nd	nd	nd
1371	Ethyl octanoate	nd	45.7[a]	nd	28.7[a]	nd
1563	Ethyl decanoate	70.3[a]	nd	nd	91.8[a]	nd
1580	Ethyl dec-4-enoate	52.5[a]	nd	nd	nd	nd
1595	Diethyl butanedioate	135.9[a]	112.2[a]	nd	nd	nd
1642	Propyl decanoate	nd	nd	nd	nd	117.9[a]
1777	Ethyl dodecanoate	nd	21.3[b]	nd	22.4[b]	nd
1838	3,7,11,15-Tetramethyl-hexadecyl acetate	378.4[b]	302.1[b]	201.7[b]	464.2[b]	nd
1864	Heptyl decanoate	nd	nd	nd	nd	198.5[a]
Organic acids						
1452	Acetic acid	nd	nd	nd	nd	3,007.6[a]
1584	2-Ethyl-butanoic acid	nd	nd	nd	946.8[b]	nd
1869	2-Ethyl-hexanoic acid	186.3[b]	nd	nd	nd	nd
1937	Hexanoic acid	nd	nd	nd	nd	2,657.2[a]
2036	*n*-Octanoic acid	574.3[a]	530.1[a]	<10,000[a]	820.0[a]	nd
2138	*n*-Nonanoic acid	372.2[a]	nd	nd	nd	29,356.8[a]
2221	*n*-Decanoic acid	3,891.3[a]	3,316.1[a]	nd	5,991.5[a]	41,657.8[a]
2269	Undecylenic acid	nd	nd	nd	nd	117,238.0[b]
2400<	*n*-Dodecanoic acid	5,559.2[a]	3,853.1[a]	5,139.1[a]	4,670.7[a]	<10,000[a]
2400<	*n*-Tetradecanoic acid	4,987.7[b]	4,615.5[b]	429.7[b]	310.5[b]	<10,000[b]
2400<	Benzoic acid	nd	5,029.1[a]	nd	nd	nd
2400<	*n*-Pentadecanoic acid	nd	nd	nd	nd	<10,000[b]
2400<	*n*-Hexadecanoic acid	4,088.9[a]	3,430.0[a]	1,410.2[a]	2,414.2[a]	<10,000[a]
2400<	*n*-Octadecanoic acid	1,620.1[a]	1,558.1[a]	nd	nd	nd
2400<	9-Octadecenoic acid	nd	nd	nd	nd	<10,000[a]
Alcohols						
905	Ethanol	818.0[a]	2,098.6[a]	1,040.0[a]	2,153.9[a]	4,149.7[a]
985	1-Propanol	nd	nd	nd	nd	1,573.0[a]
1159	[E]-3-Caren-2-ol	nd	nd	138.3[b]	nd	nd
1171	1-Pentanol	32.5[a]	106.3[a]	31.1[a]	84.0[a]	nd
1247	2-Methyl-1-pentanol	nd	<10,000[a]	nd	nd	nd
1265	1-Hexanol	11.2[a]	42.3[a]	nd	44.9[a]	nd
1376	1-Octen-3-ol	nd	22.1[a]	nd	nd	nd
1381	1-Heptanol	nd	10.2[a]	nd	18.7[a]	61.7[a]
1415	2-Ethyl-1-hexanol	529.9[a]	24.2[a]	38.5[a]	154.3[a]	107.7[a]
1431	6-Methyl-5-hepten-2-ol	nd	nd	nd	32.5[a]	nd
1480	1-Octanol	nd	nd	nd	62.6[b]	nd
1545	3-Methyl-2-heptanol	28.4[a]	<10,000[a]	nd	nd	nd
1550	2,3-Butanediol	nd	<10,000[a]	<10,000[a]	918.3[a]	nd
1585	2-Pinen-4-ol	125.4[b]	nd	nd	28.6[b]	nd
1755	2-Methyl-5-[1-methylethenyl]-2-cyclohexen-1-ol	51.6[b]	nd	nd	nd	nd
1780	1-Tridecanol	nd	nd	nd	nd	166.7[b]
1807	Benzyl alcohol	nd	nd	nd	nd	1,235.3[b]
1830	2-Tridecanol	nd	nd	14.1[b]	nd	nd
1911	2,2-Dimethyl-1-decanol	nd	nd	nd	nd	233.7[a]
1916	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	78.9[b]	100.4[b]	90.9[b]	65.4[b]	nd
1964	3-Phenyl-2-butanol	20.3[a]	nd	nd	5.9[a]	nd
1969	2-Methyl-1,4-benzenediol	85.8[b]	nd	nd	nd	nd
1980	2,2,4-Trimethyl-1,3-pentanediol	nd	34.2[b]	nd	nd	nd
2003	4-Methyl-phenol	44.4[a]	43.2[a]	41.8[a]	nd	nd
2012	2-Methyl-phenol	nd	78.0[a]	nd	nd	nd
Carbonyl compounds						
<800	Acetaldehyde	857.7[a]	812.1[a]	1,511.8[a]	695.6[a]	1,092.2[a]
<800	Propanal	1,382.0[a]	622.0[a]	2,020.2[a]	623.2[a]	nd
842	1-Hydroxy-2-propanone	nd	734.3[a]	nd	nd	nd
881	2-Butanone	nd	nd	nd	nd	667.4[a]
886	2-Methyl-butanal	nd	427.2[a]	nd	nd	nd

Continued

Table 3 (Continued). Peak areas ($\times 10^5$) of volatile by-products isolated in probiotic salted white cheeses produced by immobilized *Lactobacillus casei* cells on fruit pieces and free *L. casei* cells after ripening for 30 d at 4 to 6°C using solid-phase microextraction gas chromatography-mass spectrometry[1]

		Cheese type				
RI[2]	Compound	*L. casei* on apple pieces	*L. casei* on pear pieces	*L. casei* free cells	Rennet cheese	Commercial Feta cheese
932	Pentanal	107.7[a]	984.4[a]	nd	265.4[a]	nd
938	3-Methyl-butanal	nd	nd	109.8[a]	nd	nd
990	2-Methyl-2-propenal	27.4[a]	nd	nd	nd	nd
995	2-Butenal	nd	124.3[a]	nd	148.7[a]	nd
1034	2,3-Pentanedione	1.4[a]	50.9[a]	nd	46.2[a]	nd
1040	Hexanal	97.2[a]	261.0[a]	61.2[a]	222.3[a]	nd
1075	[E]-2-Pentenal	26.7[a]	70.4[a]	nd	81.5[a]	nd
1111	Heptanal	293.8[a]	574.9[a]	nd	700.7[a]	nd
1116	3-Methyl-hexanal	nd	nd	70.4[a]	nd	nd
1142	2-Hexenal	nd	142.0[a]	nd	85.0[a]	nd
1205	Octanal	20.9[a]	28.8[a]	nd	31.4[a]	nd
1238	3-Hydroxy-2-butanone	nd	nd	56.6[a]	nd	nd
1242	2-Heptenal	nd	199.2[a]	nd	144.5[a]	nd
1252	6-Methyl-5-hepten-2-one	88.6[a]	19.9[a]	nd	nd	nd
1315	Nonanal	157.7[a]	162.8[a]	nd	166.7[a]	nd
1351	[E],[E]-2,4-Hexadienal	nd	16.2[b]	nd	nd	nd
1358	[E]-2-Octenal	21.3[a]	37.1[a]	nd	48.6[a]	nd
1403	[E],[E]-2,4-Heptadienal	nd	16.4[a]	nd	nd	nd
1426	Decanal	93.4[a]	9.5[a]	nd	nd	nd
1458	Benzaldehyde	51.8[a]	nd	12.7[a]	nd	726.7[a]
1462	3,5-Octadien-2-one	nd	59.1[b]	nd	59.3[b]	nd
1467	[E]-2-Nonenal	333.7[a]	623.1[a]	18.0[a]	584.5[a]	nd
1495	[E],[Z]-2,6-Nonadienal	nd	12.4[a]	nd	16.9[a]	nd
1519	2-Undecanone	35.5[a]	81.6[a]	nd	36.8[a]	nd
1560	[E]-2-Decenal	24.7[a]	<10,000[a]	nd	<10,000[a]	nd
1605	Butyrolactone	nd	nd	nd	nd	2,455.7[a]
1628	2-Pinen-4-one	409.1[b]	nd	nd	98.6[b]	nd
1658	2-Dodecenal	97.1[b]	35.9[b]	nd	nd	nd
1682	[E],[E]-2,4-Decadienal	nd	14.1[b]	nd	nd	nd
1727	3-[3,3-Dimethylbutyl]-cyclohexanone	nd	nd	91.8[b]	50.9[b]	nd
1742	2-Tridecanone	nd	44.0[b]	68.5[b]	nd	nd
1933	6,10,14-Trimethyl-2-pentadecanone	nd	47.6[b]	nd	nd	nd
2160	Delta-undecalactone	384.1[a]	263.1[a]	nd	nd	nd
Miscellaneous compounds						
900	*n*-Nonane	nd	nd	nd	nd	350.7[a]
954	Toluene	16.9[a]	32.4[a]	306.7[a]	248.1[a]	490.3[a]
1000	*n*-Decane	nd	nd	nd	nd	3,427.1[a]
1047	Butyl benzene	16.8[b]	nd	nd	nd	nd
1058	2,4,6-Trimethyl-octane	nd	nd	nd	nd	217.6[b]
1070	3-Methyl-decane	nd	nd	nd	nd	705.6[b]
1081	Beta-myrcene	nd	nd	183.8[b]	nd	nd
1090	2-Ethyl-decane	nd	28.4[b]	nd	8.2[b]	nd
1095	4,5-Dimethylnonane	nd	nd	39.6[b]	nd	nd
1100	Undecane	31.4[a]	nd	nd	nd	1,873.1[a]
1106	Limonene	nd	nd	2,175.0[a]	nd	nd
1128	2-Pentyl-furan	9.0[b]	10.9[b]	nd	nd	nd
1134	1-Methyl-4-[1-methylethyl]-1,4-cyclohexadiene	nd	nd	276.4[b]	nd	nd
1139	1-Decyne	nd	173.7[b]	nd	nd	nd
1200	Dodecane	7.9[a]	nd	nd	nd	nd
1300	Tridecane	83.2[a]	234.3[a]	50.7[a]	159.7[a]	nd
1410	Furfural	18.4[a]	17.3[a]	nd	25.8[a]	nd
1485	2,3,5,8-Tetramethyl-1,5,9-decatriene	nd	15.8[b]	22.2[b]	nd	nd
1490	2,4-Dimethyl-dodecane	21.7[b]	37.3[b]	30.9[b]	nd	nd
1500	Pentadecane	109.9[b]	186.3[b]	38.8[b]	132.0[b]	nd
1590	2-Furanmethanol	71.3[a]	49.1[a]	12.2[a]	43.2[a]	nd
1600	Hexadecane	nd	nd	56.0[a]	nd	nd
1632	Alpha.farnesene	216.0[b]	695.4[b]	nd	nd	nd
1800	Octadecane	nd	54.0[a]	826.8[a]	nd	nd
1884	3,7,11,15-Tetramethyl-2-hexadecene	nd	203.5[b]	231.3[b]	300.6[b]	136.8[b]
1973	2-Methyl-1,3-dioxalane	nd	nd	nd	nd	99.8[b]

Continued

Table 3 (Continued). Peak areas ($\times 10^5$) of volatile by-products isolated in probiotic salted white cheeses produced by immobilized *Lactobacillus casei* cells on fruit pieces and free *L. casei* cells after ripening for 30 d at 4 to 6°C using solid-phase microextraction gas chromatography-mass spectrometry[1]

		Cheese type				
RI[2]	Compound	*L. casei* on apple pieces	*L. casei* on pear pieces	*L. casei* free cells	Rennet cheese	Commercial Feta cheese
2400<	5-Hydroxy-methyl-2-furancarboxaldehyde	4,371.8[a]	3824.0[a]	nd	5462.4[a]	nd
2400<	Squalene	nd	nd	nd	nd	<10,000[b]
Unidentified compounds						
1121	Unknown	nd	nd	12.1[d]	nd	nd
1151	Unknown	nd	73.8[d]	57.0[d]	40.2[d]	nd
1320	Unknown	nd	nd	56.7[d]	nd	nd
1436	Unknown	70.9[d]	87.7[d]	nd	nd	nd
1574	Unknown	153.3[d]	nd	nd	nd	nd
1738	Unknown	114.7[d]	nd	nd	nd	nd
1859	Unknown	165.0[d]	nd	nd	nd	nd
1985	Unknown	nd	nd	nd	nd	34,648.3[d]
2309	Unknown	1,088.1[d]	nd	nd	nd	nd

[1]Peak areas are the mean values of 3 determinations. Identification (superscripts): a = positive identification from mass spectra and retention times that agree with authentic compounds; b = positive identification from mass spectra data only; d = detected; and nd = not detected.

[2]RI = Kovats retention index.

inhibition in growth of LAB in the presence of probiotic bacteria. Furthermore, high salt in moisture may result in a dramatic decline in starter numbers during the early weeks of ripening (Boylston et al., 2004). A comparison between cheese with free *L. casei* cells and rennet cheese showed that the rennet cheese contained more alcohols and aldehydes, which constitute mainly products of proteolysis, and esters than the cheese with free *L. casei* cells.

Most esters encountered in analyzed cheeses are described as having fruity, floral notes. Ethyl acetate was identified in cheese containing immobilized *L. casei* cells on apple and pear pieces and in rennet cheese, whereas ethyl butanoate and propyl butanoate were present only in commercial Feta cheese. Likewise, ethyl octanoate was identified in cheeses containing pear-immobilized biocatalyst and in rennet cheese. The above esters are known for their fruity aroma contribution (Pérès et al., 2001; Qian and Reineccius, 2002) and may derive from milk (Urbach, 1995).

Free fatty acids are important components in cheese flavor and may originate from milk fat lipolysis or from breakdown of amino acids (Urbach, 1993). Acetic and hexanoic acids (identified only in commercial Feta cheese) provide a pungent flavor (Pérès et al., 2001). Octanoic acid (present in all cheeses produced in the laboratory) and decanoic acid (present in all samples tested except in cheese containing free *L. casei* cells; Table 3) are known for their goaty, rancid flavor (Kim Ha and Lindsay, 1992; Molimard and Spinnler, 1996). A number of long-chain fatty acids were also detected such as undecylenic, dodecanoic, tetradecanoic, pentadecanoic, hexadecanoic, octadecenoic, and 9-octadecenoic acids. However, fatty acids with >12 carbon atoms play a minor role in cheese flavor, because they have a high perception threshold (Molimard and Spinnler, 1996).

Alcohols identified included alcohols of the aliphatic series, fusel alcohols, and phenols. Ethanol (identified in all cheese samples) provides an alcohol, mild flavor note, and occurs in fresh milk (Urbach, 1995) and may derive from lactose metabolism, whereas 1-octen-3-ol (detected only in cheese containing immobilized *L. casei* on pear pieces) is well known for its raw mushroom odor (Molimard and Spinnler, 1996). Aliphatic primary alcohols such as 1-pentanol (identified in all cheese samples produced in the laboratory) and 1-hexanol (found in cheese samples containing immobilized *L. casei* cells on apple and pear pieces and in rennet cheese; Table 3) may impart a fruity, nutty note to the flavor of cheese (Engels et al., 1997). 4-Methyl-phenol (*p*-cresol) was detected in cheeses containing immobilized and free *L. casei,* whereas 2-methyl-phenol (*o*-cresol) was detected only in cheese containing immobilized *L. casei* on pear pieces. In general, cresols and phenols may play an important role in cheese produced by sheep milk providing a distinctive sheep-like or sheepyard-like flavor (Kim Ha and Lindsay, 1992).

Carbonyl compounds identified included mainly aldehydes, ketones, and lactones. Straight-chain aldehydes such as acetaldehyde, propanal, 2-butenal, pentanal, hexanal, 2-hexenal, heptanal, 2-heptenal, octanal, 2-octenal, nonanal, decanal, and 2-decenal were detected. The majority of these aldehydes are natural constit-

uents of fresh milk (Urbach, 1995), formed during β-oxidation of unsaturated fatty acids (Collin et al., 1993; Moio et al., 1993; Lee et al., 1996). Hexanal and 2-hexenal provide a green note of immature fruit, whereas octanal, nonanal, and decanal result in an aromatic note resembling orange (Molimard and Spinnler, 1996). Benzaldehyde (identified in cheese produced by immobilized *L. casei* on apple pieces and free cells and in commercial Feta cheese; Table 3) is described as having an aromatic note of bitter almond (Molimard and Spinnler, 1996), and may originate from α-oxidation of phenylacetaldehyde or from β-oxidation of cinnamic acid (Casey and Dobb, 1992).

Methyl ketones are formed in a metabolic pathway connected to the β-oxidation pathway. 2-Butanone (detected only in commercial Feta cheese) provides an acetone note, whereas 2-undecanone (detected in cheeses containing *L. casei* immobilized on apple and pear pieces and in rennet cheese) provides a floral, herbaceous aroma (Molimard and Spinnler, 1996). Likewise, 2-tridecanone with a fruity, green flavor note (Molimard and Spinnler, 1996) was found only in cheese containing pear-supported biocatalyst and free *L. casei* cells. 3,5-Octadien-2-one was detected in cheese containing immobilized *L. casei* on pear pieces and in rennet cheese, whereas 6,10,14-trimethyl-2-pentadecanone was detected only in cheese containing immobilized *L. casei* on pear pieces. 3-Hydroxy-2-butanone (acetoin) was detected only in cheese containing free *L. casei* cells; it may derive from diaketyl reduction, due to the reducing cheese environment.

Butyrolactone, which has a pungent, buttery, fetid impact (Molimard and Spinnler, 1996) was only detected in commercial Feta cheese, whereas delta-undecalactone was identified in cheese containing immobilized *L. casei* (Table 3). Generally, hydroxy fatty acids, which are lactone precursors, may be present in milk.

A number of miscellaneous compounds were identified, some groups of which are known to contribute to the complexity of cheese aroma, such as aromatic and nonaromatic hydrocarbons and furanic derivatives. Cheese with free *L. casei* contained more hydrocarbons than did rennet cheese. The presence of free *L. casei* cells probably inhibited the action of enzymes or microorganisms that could convert these compounds to other by-products. Similar results were observed after addition of *L. casei* cells in raw milk during production of Swiss Emmental cheese, in which inhibition of propionic acid fermentation and enterococci was reported (Bachmann et al., 1997). Toluene (identified in all cheese samples) and limonene (identified only in cheese containing free *L. casei* cells) may occur in fresh milk and milk butter, respectively. β-Myrcene (detected only in cheese containing free *L. casei* cells) is a monoterpene found in essential oils. A number of nonaromatic hydrocarbons such as *n*-decane, 2,4,6-trimethyl-octane, 3-methyl-decane, 4,5-dimethyl-nonane, undecane, 1-decyne, dodecane, tridecane, 2,3,5,8-tetramethyl-1,5,9-decatriene, 2,4-dimethyl-dodecane, hexadecane, octadecane, and 3,7,11,15-tetramethyl-2-hexadecene was detected. *n*-Alkanes such as decane, undecane, and dodecane were also reported as volatile flavor compounds of buffalo Mozzarella cheese in a previous study (Moio et al., 1993). However, the hydrocarbons belong to a family of secondary products of lipid antioxidants (Barbieri et al., 1994) that do not have a major contribution to aroma in cheese, although these compounds may serve as precursors for the formation of other aromatic compounds (Arora et al., 1995). Furans such as 2-pentyl-furan, furfural, 2-furanmethanol, and 5-hydroxymethyl-2-furancarboxaldehyde were likely formed by the Maillard reaction (Nursten, 1981). 2-Furanmethanol (present in all cheese samples produced in the laboratory) provides a strong baked aroma (Qian and Reineccius, 2002), whereas 2-pentyl-furan (present in cheese produced with immobilized *L. casei* cells), which may also occur as autoxidation product of linoleic and linolenic acids, has a liquorice aroma (Belitz and Grosh, 1987). Furfural (detected in cheese containing immobilized *L. casei* and in rennet cheese) is known for its cooked, heated milk odor; it has been also reported as volatile constituent of cheeses (Moio et al., 1993).

Regarding peak areas of the identified compounds, the highest maximum peak areas were observed for fatty acids, followed by peak areas of ethanol, aldehydes, ketones, esters, and lastly, hydrocarbons. Peak areas of ethanol were much higher compared with other alcohols in all samples. However, to understand the respective contribution of each aroma-bearing compound to the overall cheese aroma, quantitative studies are necessary to relate the concentration of an individual component with respect to its threshold level, and to observe any synergistic effect at the odor port.

Preliminary Sensory Evaluation

Feta is a very popular cheese in Greece and therefore, the sensory characteristics of the produced cheeses were compared with commercial Feta cheese. Commercial Feta cheese was characterized by a more sour taste. More acids were detected in commercial Feta cheese during GC/MS analysis (Table 3). A fruity taste was predominant in cheeses containing *L. casei* immobilized on fruit pieces, whereas no significant differences concerning cheese flavor were reported by the panel between cheese containing free *L. casei* and rennet cheese. Salted cheeses scored similar values to commercial Feta cheese ($P > 0.05$; data not shown), whereas unsalted

cheeses scores were significantly lower ($P < 0.01$) than those of commercial Feta. However, the unsalted cheeses were accepted by the panel, which is an important consideration for people with high blood pressure.

CONCLUSIONS

As a final consideration, it is concluded that 1) no spoilage was observed in salted cheeses after ripening for 7 mo, 2) fruit pieces proved to be a very effective support for survival of the *L. casei* cells during cheese ripening, and 3) the cheeses produced had a distinctive taste and acceptable sensory characteristics when compared with the very popular Feta cheese.

Cheeses produced using immobilized *L. casei* on fruit pieces could be manufactured with some additions to the traditional cheese-making practice. The production of probiotic cheese in which the probiotic culture would survive and develop during manufacture and throughout its shelf life could lead to a major economic advantage. The fact that immobilized *L. casei* on fruit pieces were reactivated after storage for 7 mo is very promising with regard to survival of probiotic bacteria in an acidic environment such as cheese mass. Probiotic bacteria immobilized on fruit pieces would reach the colon, because the fruit pieces contain cellulose, which is not digested. It is strongly believed that future clinical tests will ensure the beneficial effects of fruit based probiotics.

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REFERENCES

Adams, M. R., and O. M. Moss. 1997. Pages 110 and 269 in Food Microbiology. The Royal Society of Chemistry, Cambridge, UK.

Addis, E., G. H. Fleet, J. M. Cox, D. Kolak, and T. Leung. 2001. The growth, properties and interactions of yeasts and bacteria associated with the maturation of Camembert and blue-veined cheeses. Int. J. Food Microbiol. 69:25–36.

Antosson, M., Y. Ardo, B. F. Nilsson, and G. Molin. 2002. Screening and selection of *Lactobacillus* strains for use as adjunct cultures in production of semi-hard cheese. J. Dairy Res. 69:457–472.

Arora, G., F. Cormier, and B. Lee. 1995. Analysis of odor-active volatiles in Cheddar cheese headspace multidimensional GC/MS/Sniffing. J. Agric. Food Chem. 43:748–752.

Bachmann, H. P., U. Bütikofer, R. Badertscher, M. Dalla Torre, P. Lavanchy, U. Bühler-Moor, B. Nick, J. Jimeno, R. Warmke, W. Grosch, R. Sieber, and J. O. Bosset. 1997. Reifungsverlauf von in folien verpacktem Emmentaler käse mit und ohne zusatz von *Lactobacillus casei* subsp. *casei.* I. Mikrobiologische, chemische, rheologische und sensorische untersuchungen. Lebensm. Wiss. Technol. 30:417–428.

Barbieri, G., L. Bolzoni, M. Careri, A. Mangia, G. Parolari, S. Spagnoli, and R. Virgill. 1994. Study of the volatile fraction of Parmesan cheese. J. Agric. Food Chem. 42:1170–1176.

Belitz, H.-D., and W. Grosh. 1987. Page 168 in Food Chemistry. Springer-Verlag, Berlin, Germany.

Bellesia, F., A. Pinetti, U. M. Pagnoni, R. Rinaldi, C. Zucchi, L. Gaglioti, and G. Palyi. 2003. Volatile components of Grana Parmigiano-Reggiano type hard cheese. Food Chem. 83:53–61.

Bosset, J. O., U. Butikofer, R. Gauch, and R. Sieber. 1997. Reifungsverlauf von in folien emmentaler käse mit ohne zusatz von *Lactobacillus casei* subsp. *casei.* II. Gaschromatographische untersuchung einiger fluchtiger, neutraler verbindungen mit hilfe einer dynamischen dampfraumanalyse. Lebensm. Wiss. Technol. 30:464–470.

Boylston, T. D., C. G. Vinderola, H. B. Ghoddusi, and J. A. Reinheimer. 2004. Incorporation of bifidobacteria into cheeses: Challenges and rewards. Int. Dairy J. 19:315–387.

Broome, M. C., D. A. Krause, and M. W. Hickey. 1990. The use of non-starter lactobacilli in Cheddar cheese manufacture. Aust. J. Dairy Technol. 45:67–73.

Casey, J., and R. Dobb. 1992. Microbial routes to aromatic aldehydes. Enzyme Microb. Technol. 14:739–747.

Champagne, C. P., N. J. Gardner, and D. Roy. 2005. Challenges in the addition of probiotic cultures to foods. Crit. Rev. Food Sci. Nutr. 45:61–84.

Champagne, C. P., N. Morin, R. Couture, C. Gagnon, P. Jelen, and C. Lacroix. 1992. The potential of immobilized cell technology to produce freeze dried, phage-protected cultures of *Lactococcus lactis.* Food Res. Int. 25:419–427.

Collin, S., M. Osman, S. Delcambre, A. I. El-Zayat, and J.-P. Dufour. 1993. Investigation of volatile flavour compounds in fresh and ripened Domiati cheeses. J. Agric. Food Chem. 41:1659–1663.

Corsetti, A., J. Rossi, and M. Gobbetti. 2001. Interactions between yeasts and bacteria in the smear surface-ripened cheeses. Int. J. Food Microbiol. 69:1–10.

Daigle, A., D. Roy, G. Belanger, and J. C. Vuillemard. 1999. Production of probiotic cheese (Cheddar-like cheese) using enriched cream fermented by *Bifidobacterium infantis.* J. Dairy Sci. 82:1081–1091.

De Leon-Gonzalez, L. P., W. L. Wendorff, B. H. Ingham, J. J. Jaeggi, and K. B. Houck. 2000. Influence of salting procedure on the composition of Muenster-type cheese. J. Dairy Sci. 83:1396–1401.

Dinakar, P., and V. V. Mistry. 1994. Growth and viability of *Bifidobacterium bifidum* in Cheddar cheese. J. Dairy Sci. 77:2854–2864.

Engels, W. J. M., R. Dekker, C. de Jong, R. Neeter, and S. Visser. 1997. A comparative study of volatile compounds in the water-soluble fraction of various types of ripened cheese. Int. Dairy J. 7:255–263.

Fellows, P. 1997. Page 165 in Food Processing Technology. Principles and Practice. Woodhead Publishing Limited, Cambridge, UK.

Gerasi, E., E. Litopoulou-Tzanetaki, and N. Tzanetakis. 2003. Microbiological study of Manura, a hard cheese made from raw ovine milk in Greek island of Sifnos. Int. J. Dairy Technol. 56:117–122.

Gomes, A. M. P., F. X. Malcata, F. A. M. Klaver, and H. L. Grande. 1995. Incorporation and survival of *Bifidobacterium* sp. strain Bo and *Lactobacillus acidophilus* strain Ki in a cheese product. Neth. Milk Dairy J. 49:71–95.

Gomes, A. M. P., M. M. Vieira, and F. X. Malcata. 1998. Survival of probiotic microbial strains in a cheese matrix during ripening: Simulation of rates of salt diffusion and microorganism survival. J. Food Eng. 36:281–301.

Hatzikamari, M., E. Litopoulou-Tzanetaki, and N. Tzanetakis. 1999. Microbiological characteristics of Anevato: A traditional Greek cheese. J. Appl. Microbiol. 87:595–601.

Holzapfel, W. H., and U. Schilliger. 2002. Introduction to pre- and probiotics. Food Res. Int. 35:109–116.

Ishibashi, N., and S. Shimamura. 1993. Bifidobacteria: Research and development in Japan. Food Technol. 47:126–135.

Izco, J. M., and P. Torre. 2000. Characterisation of volatile flavour compounds in Roncal cheese extracted by the purge and trap method and analysed by GC-MS. Food Chem. 70:409–417.

Kim Ha, J., and R. C. Lindsay. 1992. Influence of aw on volatile free fatty acids during storage of cheese bases lipolyzed by kid goat pregastric lipase. Int. Dairy J. 2:179–195.

Kirk, R. S., and R. Sawyer. 1991. Pages, 23, 600–601 in Pearson's Composition and Analysis of Foods. 9th ed. Longman Singapore Publishers Ltd., Singapore.

Kourkoutas, Y., M. Kanellaki, A. A. Koutinas, I. M. Banat, and R. Marchant. 2003. Storage of immobilized yeast cells for use in wine-making at ambient temperatures. J. Agric. Food Chem. 51:654–658.

Kourkoutas, Y., M. Komaitis, A. A. Koutinas, and M. Kanellaki. 2001. Wine production using yeast immobilized on apple pieces at low and room temperatures. J. Agric. Food Chem. 49:1417–1425.

Kourkoutas, Y., A. A. Koutinas, M. Kanellaki, I. M. Banat, and R. Marchant. 2002. Continuous wine fermentation using a psychrophilic yeast immobilized on apple cuts at different temperatures. Food Microbiol. 19:127–134.

Kourkoutas, Y., V. Xolias, M. Kallis, E. Bezirtzoglou, and M. Kanellaki. 2005. *Lactobacillus casei* cell immobilization on fruit pieces for probiotic additive, fermented milk and lactic acid production. Process Biochem. 40:411–416.

Lee, Y. B., I. Laye, Y. D. Kim, and C. V. Morr. 1996. Formation of volatile compounds in whey protein concentrate during elevated temperature storage as a function of water activity. Int. Dairy J. 6:485–496.

Mallios, P., Y. Kourkoutas, M. Iconomopoulou, A. A. Koutinas, C. Psarianos, R. Marchant, and I. M. Banat. 2004. Low-temperature wine-making using yeast immobilized on pear pieces. J. Sci. Food Agric. 84:1615–1623.

Martley, F. G., and V. L. Crow. 1993. Interactions between non starter microorganisms during cheese manufacture and ripening. Int. Dairy J. 3:461–483.

Mattila-Sandholm, T., P. Myllarinen, R. Crittenden, G. Mogensen, R. Fonden, and M. Saarela. 2002. Technological challenges for future probiotic foods. Int. Dairy J. 12:173–182.

Mocquot, G. 1979. Reviews of the progress of dairy science: Swiss-type cheese. J. Dairy Res. 46:133–160.

Moio, L., J. Dekimpe, P. X. Etiévant, and F. Addeo. 1993. Volatile flavor compounds of water buffalo Mozzarella cheese. Ital. J. Food Sci. 5:57–68.

Molimard, P., and H. E. Spinnler. 1996. Review: Compounds involved in the flavor of surface mold-ripened cheeses: Origins and properties. J. Dairy Sci. 79:169–184.

Nikolaou, E., N. Tzanetakis, E. Litopoulou-Tzanetaki, and R. K. Robinson. 2002. Changes in the microbiological and chemical characteristics of an artisanal, low-fat cheese made from raw ovine milk during ripening. Int. J. Dairy Technol. 55:12–17.

Nursten, H. E. 1981. Recent developments in studies of the Maillard reaction. Food Chem. 6:263–277.

Oliveira, M. N., I. Sodini, F. Remeuf, J. P. Tissier, and G. Corrieu. 2002. Manufacture of fermented lactic beverages containing probiotic cultures. J. Food Sci. 67:2336–2341.

Pérès, C., C. Viallon, and J.-L. Beragué. 2001. Solid-phase microextraction-mass spectrometry: A new approach to the rapid characterization of cheeses. Anal. Chem. 73:1030–1036.

Prévost, H., and C. Diviès. 1987. Fresh fermented cheese production with continuous prefermented milk by a mixed culture of mesophilic lactic streptococci entrapped in Ca- alginate. Biotechnol. Lett. 9:789–794.

Qian, M., and G. Reineccius. 2002. Identification of aroma compounds in Parmigiano-Reggiano cheese by gas chromatography/olfactometry. J. Dairy Sci. 85:1362–1369.

Rao, A. V., N. Shiwnarain, and I. Maharaj. 1989. Survival of microencapsulated *Bifidobacterium pseudolongum* in simulated gastric and intestinal juices. Can. Inst. Food Sci. Technol. J. 22:345–349.

Reps, A. 1993. Bacterial surface-ripened cheeses. Pages 137–172 in Cheese: Chemistry, Physics and Microbiology, 2. P. F. Fox, ed. Chapman and Hall, London, UK.

Shah, N. P. 2000. Probiotic bacteria: Selective enumeration and survival in dairy foods. J. Dairy Sci. 83:894–907.

Smacchi, E., P. F. Fox, and M. Gobbetti. 1999. Purification and characterization of two extracellular proteinases from *Arthrobacter nicotianae* 9458. FEMS Microbiol. Lett. 170:327–333.

Spencer, J. F. T., and D. M. Spencer. 1997. Yeasts and the Life of Man: Part I: Helpers and Hinderers. "Traditional" Yeast-Based Industries; Spoilage Yeasts. Pages 226–242 in Yeasts in Natural and Artificial Habitats. J. F. T. Spencer and D. M. Spencer, ed. Springer-Verlag. Berlin, Germany.

Stanton, C., G. Gardiner, P. B. Lynch, J. K. Collins, G. Fitzgerald, and R. P. Ross. 1998. Probiotic cheese. Int. Dairy J. 8:491–496.

Steenson, L. R., T. R. Klaenhammer, and H. E. Swaisgood. 1987. Calcium alginate immobilized cultures of lactic streptococci are protected from bacteriophage. J. Dairy Sci. 70:1121–1127.

Tzanetakis, N., and E. Litopoulou-Tzanetaki. 1992. Changes in numbers and kinds of lactic acid bacteria in Feta and Teleme, two Greek cheeses from ewes' milk. J. Dairy Sci. 75:1389–1393.

Urbach, G. 1995. Contribution of lactic acid bacteria to flavour compound formation in dairy products. Int. Dairy J. 5:877–903.

Urbach, G. 1993. Relations between cheese flavor and chemical composition. Int. Dairy J. 3:389–422.

Vinderola, C. G., G. A. Costa, S. Regenhardt, and J. A. Reinheimer. 2002. Influence of compounds associated with fermented dairy products on the growth of lactic acid starter and probiotic bacteria. Int. Dairy J. 12:579–589.

Voudouris, E. K., and M. G. Kontominas. 1990. Page 220 in Introduction in Food Chemistry. OEDB, Athens, Greece.