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Effect of Wild Strains Used as Starter Cultures on Free Fatty Acid Profile of Urfa Cheese

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Key words: Urfa cheese, wild type strains, free fatty acids, lipolysis, sensory analysis

In the present study, the influences of wild-type lactic acid bacteria including *Lactococcus lactis* subsp. *lactis* 1B4, *Lactococcus garvieae* IMAU 50157, *Enterococcus faecium* ATCC 19434, *Enterococcus durans* IMAU 60200 and *Enterococcus faecalis* KLDSO.034 on the composition and free fatty acid contents of Urfa cheeses were evaluated throughout the ripening period. Three different combinations of the strains were employed in the manufacture of cheeses from pasteurised milk. These are: cheese A (strains 1B4+ATCC 19434+IMAU 50157), cheese B (strains 1B4+IMAU 60200+ATCC 1934) and cheese C (strains ATCC 19434+1B4+IMAU 50157+IMAU 60200+KLDSO.0341). The control cheese (cheese D) was produced from raw ewe's milk without starter culture. The basic composition of ripened cheese samples was not significantly affected by wild type strains. C cheese had a higher level of lipolysis than the other cheeses at all stages of ripening ($p<0.05$). Sensory evaluation of the cheese samples revealed that control cheese had significantly higher aroma and flavour scores than the other cheeses.

INTRODUCTION

Urfa cheese which is a brined type cheese native to Southeastern Anatolia region of Turkey, is an artisanal cheese made from either raw milk of Awassi sheep breed or mixture of caprine and ovine milks at appropriate ratios. In traditional Urfa cheese-making practices, the milk is not pasteurised and no starter cultures are used. The milk is coagulated with animal rennet at 30–32°C for about 90 min, cut into small cubes and drained by gravity drainage in special moulds of triangular shape called "parzin". Afterwards, the cheese blocks are dry salted and/or kept in brine (~16 g/100 g NaCl, w/v) for 5–6 months [Özer *et al.*, 2002].

Lipolysis in cheese is a result of action of biolytic enzymes called hydrolases (lipases and esterases) that split the ester linkage between a fatty acid and the glycerol moiety of the triacylglycerol [Lopez *et al*., 2006; McSweeney, 2004]. Among the short-chain free fatty acids propionic, butyric, isobutyric and iso-valeric acids play active role in aroma formation; the medium-chain free fatty acids, such as hexanoic, octanoic and decanoic acids, are visibly arisen from the lipolytic breakdown of milk fat [Randazzo *et al*., 2008].

Owing to increasing consumers' demand towards healthier and safer foods, some modifications in the manufacturing practices of Urfa cheese have been made [Özer *et al*., 2004]. The major modification is the replacement of raw milk by pasteurised milk. The brine concentration has also been reduced from 16–18% (w/v) to 10–12% (w/v). The use of pasteurised milk in cheese manufacturing has made the incorporation of lactic starters including lactococcal strains necessary. This eventually has resulted in lacks of characteristic aroma and flavour attributes of raw milk Urfa cheeses in its industrial counterparts. Therefore new strains that are isolated and identified from traditional Urfa cheeses should be employed in industrial cheese-making practices. At this point, characterisation of traditional cheese flora and preparation of proper strain combinations are of critical importance for obtaining industrial cheese with sensory and physicochemical properties close to its traditional counterpart. Such an attempt was made by Kırmacı [2010] who characterised the predominant microflora of traditional Urfa cheese made from raw sheep's milk. The author demonstrated that the natural lactic flora of Urfa cheese contained the salt-resistant lactococci (mainly *L. lactis*, *L. garvieae*) and enterococci (mainly *E. durans*, *E. faecalis* and *E. faecium*). Although, enterococcal strains are generally recognised as a sign of unsafe production, some of these strains have been used successfully in starter combinations of different European cheeses such as Mozzarella [Parente *et al*., 1989], Feta [Litopoulu-Tzanetaki *et al*., 1993], Venaco [Casalta & Zennaro, 1997] and Cebreiro [Centeno *et al*., 1999]. The present study aimed to determine the FFA profiles of cheeses made from selected starter culture (wild type) combination that was isolated from traditional raw milk Urfa cheese.

MATERIAL AND METHODS

Materials

Raw sheep's milk supplied from Harran University Agricultural Faculty Dairy Plant (Sanliurfa, Turkey) was used

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in the manufacture of Urfa cheese. Calf rennet was used to coagulate milk in liquid form (declared coagulating power of 1:16,000 IMCU, Mayasan A.S., Istanbul, Turkey). Starter strains were selected among the isolates obtained from traditional Urfa cheese produced from raw sheep's milk. The strains used were previously characterised by phenotypic and genotypic 16S rDNA sequence analysis by Kirmaci [2010]. The strains were chosen according to their contributions to the milk microflora and rate of acidification. The strains used with percentage of identity in brackets were: *Lactococcus lactis* subsp *lactis* B14 (99%), *Lactococcus garvieae* IMAU 50157 (96%), *Enterococcus faecium* ATCC 19434 (97%), *Enterococcus durans* IMAU 60200 (96%) and *Enterococcus faecalis* KLDSO.0341 (96%). All chemicals were derived from Sigma-Aldrich Co. (Interlab A.S., Istanbul, Turkey) and Merck (Merck Pharmaceutical and Chemical Trading. Inc., Istanbul, Turkey), and were of analytical grade.

Cheese-making protocol

A batch of sheep's milk (100 liters) was divided into four equal parts. Three parts of milk (samples A to C) were pasteurised at 72°C for 2 min (vat system) and cooled to 32°C. The last part was not subjected to heat treatment and converted to cheese without starter culture (sample D, control). Bacterial isolates were enriched in M17 broth at 37°C for 24 h. Then, the isolates were harvested by centrifugation and washed with peptone water. The isolates were then inoculated into sterile skim milk (10 mL) for pre-enrichment for bulk culture and kept at 37°C for 24 h. This culture was used to prepare working culture for cheese making in the same way. The cheeses A, B and C were inoculated with the following starter combinations at a level of 1 g/100 g: *Cheese A*: *E. faecium* ATCC 19434 + *L. lactic* subsp. *lactis* 1B4 + *L. garvieae* IMAU 50157 (with ratio of 1:1:0.5), *Cheese B*: *E. durans* IMAU 60200 + *E. faecium* ATCC 19434 + *L. lactic* subsp. *lactis* 1B4 (with ratio of 1:1:1), and *Cheese C*: *E. faecium* ATCC 19434 + *E. durans* IMAU 60200 + *L. garvieae* IMAU 50157 + *L. lactic* subsp. *lactis* 1B4 + *E. faecalis* KLDSO.0341 (with ratio of 1:1:1:1:1). Food grade calcium chloride was added to the pasteurised milk at a level of 20 g/100 L to restore ionic calcium balance after heat treatment. After inoculation, cheese milk was left at 32°C for 30 min to allow acidity development prior to renneting. The milk was coagulated by means of calf rennet within 90 min. Following coagulation, the curd was cut into cubes (1 cm^3) and put into special cheese cloths of triangular shape, locally called "parzin". The parzins were hung up on a horizontal bar and wheying off was achieved by gravity drainage for about 18 h at room temperature. Each parzin contained approximately 500 g of curd yielding about 100 g of fresh cheese after wheying-off. The cheese blocks were dry salted overnight and then put into pasteurised brine solution (12 g/100 g NaCl, w/v). The cheeses were ripened at 8°C for 180 days.

Chemical analysis

Total solids [ISO, 2004], titratable acidity [Anonymous, 2006] and salt [IDF 1988] were determined. The pH was determined by means of a combined electrode pH-meter (Mettler Toledo GmbH, Giessen, Germany). The fat was determined by the Gerber method [Anonymous, 2006].

Free fatty acid analyses

Fatty acids were extracted as outlined by Garcia-Lopez *et al.* [1994]. A cheese sample (10 g) was grinded and extracted using a mixture of methanol:methylene chloride (1:9). Nonaic acid was used as internal standard. The extract was vacuum extracted (40°C) and methylated with regard to the method of Sukhija & Palmquist [1988]. Fatty acid methyl esters were analysed by GC (Shimadzu GC-17 AAF, V3, 230 V series; Shimadzu Corpora tion, Kyoto, Japan) fitted with flame ionisation detector (FID).. FAME was passed through fused silica capillary column (SP-2380, 100 m - 0.25 mm; Supelco Inc., Bellefonte, PA). Helium was used as a carrier gas at the rate of 2 mL/mon. Injection of 1 μL sample was applied with a split ratio of 1:30 into the injector. Injector and detector temperature were adjusted as 250°C. The initial oven temperature was 40°C for 1.0 min, and then increased to 240°C at the rate of 5°C/min. The final temperature was maintained for 10 min. Fatty acids were identified by contrasting their retention times with a standard fatty acid mixture containing 37 fatty acids (Sigma-Aldrich Chemicals 189–19).

Sensory evaluation

Samples were organoleptically utilised according to the scheme proposed by Bodyfelt *et al*. [1988]. The panel group comprised of ten trained panelist who were familiar with Urfa cheese. A 10 point hedonic scale was used to appraise over perception, aroma and flavour, appearance and colour, saltiness, body and texture scores (1: strongly unacceptable; 10: strongly acceptable).

Statistical analyses

Data obtained were analysed with one-way analysis of variance using SPSS package statistical program (SPSS for Windows release 5.0.1., SPSS Inc., Chicago, IL, USA). Duncan's multiple range test was applied to identify the differences among the cheese groups. The study including cheese-making was done in triplicate.

RESULT AND DISCUSSION

Compositional analyses

The gross compositions of cheese samples are presented in Table 1. A continuous decline in total solids levels of the cheeses throughout ripening period was observed. This may be attributed to the formation of new peptides with high water absorbing capacity as a result of advanced proteolysis [Atasoy *et al*., 2008]. In fact, a decrease in moisture content of the cheeses was expected along with the increase in salt-in-dry matter since salt and water diffusions occur in opposite ways [Luo *et al*., 2013; Papademas, 2006; Madadlou *et al.*, 2007]. Salt penetration was almost complete within the first 30 days of ripening and then the salt-in-moisture levels of the cheeses changed within a very narrow margin. Regarding the salt-in-moisture levels of the cheeses in the same ripening day, no significant variations were noted between the samples ($p>0.05$). The pHs of the experimental cheeses increased until 120 d and then declined gradually towards the end of ripening. The titratable acidity level of the control cheese (sample D) declined continuously throughout ripening

TABLE 1. Gross composition of Urfa cheese made using different starter culture systems.

Storage days	Cheeses	Total solids (g/100 g)	Fat-in-dry matter (g/100 g)	pH	Titrable acidity (g/100 g lactic acid)	Salt-in-moisture (g/100 g)
1	A	54.92^{a1}	26.75^{a1}	4.69^{a1}	0.829^{a2}	3.95^{a1}
	B	55.26^{a1}	26.88^{a1}	4.69^{a1}	0.809^{a2}	4.09^{a1}
	C	53.97^{a1}	26.38^{a1}	4.89^{a1}	1.088^{a3}	4.20^{a1}
	D	54.41^{a1}	26.50^{a1}	4.72^{a1}	0.668^{a1}	3.86^{a1}
30	A	51.69^{b1}	25.13^{b1}	4.88^{b1}	0.674^{ab2}	6.21^{b1}
	B	51.66^{b1}	25.63^{b1}	4.69^{a1}	0.688^{b2}	6.37^{b1}
	C	50.87^{b2}	25.25^{b1}	4.79^{a1}	0.837^{b3}	6.25^{b1}
	D	50.47^{b2}	25.50^{b1}	4.77^{ab1}	0.621^{b1}	6.42^{b1}
60	A	48.73^{c2}	25.25^{b1}	4.91^{b1}	0.679^{ab2}	7.58^{bc1}
	B	49.50^{c2}	25.13^{c1}	4.96^{b1}	0.638^{b12}	6.83^{b1}
	C	48.65^{c1}	25.00^{b1}	4.91^{a1}	0.719^{c2}	7.11^{bc1}
	D	49.05^{c2}	25.13^{b1}	4.74^{a1}	0.567^{c1}	6.99^{bc1}
90	A	48.86^{c1}	24.75^{b2}	5.09^{c12}	0.744^{ab2}	7.70^{bc1}
	B	48.70^{d1}	24.38^{d12}	5.14^{b2}	0.727^{ab2}	7.98^{c1}
	C	48.25^{c1}	24.25^{c12}	5.08^{b1}	0.699^{c2}	7.87^{cde1}
	D	47.33^{d1}	23.38^{c1}	5.05^{b1}	0.509^{d1}	8.00^{bc1}
120	A	48.40^{c1}	24.00^{c1}	5.10^{c2}	0.735^{ab2}	7.95^{c12}
	B	48.35^{d1}	24.13^{d1}	5.13^{b3}	0.638^{b12}	8.34^{c12}
	C	47.49^{d1}	24.00^{cd1}	5.12^{b23}	0.699^{c12}	8.86^{e2}
	D	47.10^{de2}	23.75^{c1}	4.96^{b1}	0.509^{d1}	8.36^{d1}
150	A	46.04^{d1}	23.50^{c1}	5.07^{c1}	0.572^{b12}	8.03^{bc1}
	B	46.51^{e1}	23.50^{e1}	5.07^{b1}	0.672^{b12}	8.52^{bc1}
	C	47.07^{d2}	23.88^{cd2}	5.06^{b1}	0.716^{c2}	8.75^{de1}
	D	45.48^{f1}	23.38^{c1}	4.95^{ab1}	0.457^{e1}	8.77^{cd1}
180	A	46.73^{d1}	23.50^{c1}	4.90^{b1}	0.667^{ab2}	8.00^{bc1}
	B	46.08^{e1}	23.25^{e1}	4.98^{b1}	0.618^{b2}	7.88^{b1}
	C	46.93^{d2}	23.50^{d1}	4.78^{a1}	0.719^{c2}	8.07^{cd1}
	D	46.61^{e1}	23.30^{c1}	4.93^{ab1}	0.426^{e1}	7.85 ^{bc1}

Cheese A: *Enterococcus faecium* ATCC19434+ *Lactococcus lactis* subsp. *lactis* 1B4+ *Lactococcus garvieae* IMAU50157 (1:1:0.5), Cheese B: *Enterococcus durans* IMAU60200+ *Enterococcus faecium* ATCC19434+ *Lactococcus lactis* subsp. lactis 1B4 (1:1:1), Cheese C: *Enterococcus faecium* ATCC19434+ *Enterococcus durans* IMAU60200+ *Enterococcus faecalis* KLDSO.0341+ *Lactococcus garvieae* IMAU50157+ *Lactococcus lactis* subsp. *lactis* 1B4 (0.6:0.4) and Cheese D: control. *Samples showing common superscripts numbers (during storage days) and superscripts letters (between cheeses at the same storage day) do not differ significantly ($p>0.05$).

period, indicating the catabolism of lactic acid and the production of ammonia by deamination of free amino acids [Grappin & Beuvier, 1997; Prieto *et al*., 2000]. On the other hand, the titratable acidity levels of the cheeses A, B and C fluctuated after 90 days of ripening. This may be attributed to almost complete salt penetration to cheese after 30 days of ripening as increasing salt level in cheese results in decreasing total acidity in brined-type cheeses [Upreti & Metzger, 2007]. The combined effects of starter strains and ripening period on the development of acidity were significant at $p<0.05$. The fat-in-dry matter content of the cheeses decreased significantly ($p<0.05$) during ripening proportionally to decreasing total solids contents of the samples. The effect of starter combinations on the fat-in-dry matter level was insignificant ($p>0.05$). The total nitrogen (TN) contents of the experimental cheeses declined continuously throughout ripening; however, no considerable differences between the cheeses were noted regarding TN concentrations ($p>0.05$). The reduction in the TN levels of the cheeses could be due to hydrolysis of proteins to water-soluble nitrogen (WSN) compounds and their migration into the brine. This may also explain why total solids levels of the cheese declined in the present case during the ripening period.

FFA analysis

Lipolysis in cheese is mainly affected by the type of milk used, stage of lactation, form of milking and milk collection, cooling and agitation in farm tanks. All these factors activate the membrane-bound lipoprotein lipase and thus trigger enzymatic hydrolysis of milk fat [Hassan *et al*., 2013; Lopez *et al*., 2006].

The variations in concentrations of individual short chain FFAs [($\sum C_{4:0}$–$C_{8:0}$)], in the cheese samples during ripening stage are shown in Table 2. Level of lipolysis in the cheeses increased significantly throughout ripening period ($P<0.05$). Butyric acid concentrations of all samples increased during ripening period. This is most probably due to the high specificity of bacterial lipolytic enzymes towards FFA located in the position sn-1,3 of the triglyceride, where short chain free fatty acids SCFFA are predominantly esterified [Avila *et al.*, 2007]. These findings are in good agreement with previous studies for different brined cheeses [Haya-

TABLE 2. Short chain free fatty acids (mg/100 g cheese) of cheese samples at during ripening.

Samples	Storage days	Butyric acid ($C_{4:0}$)	Caproic acid ($C_{6:0}$)	Caprylic acid ($C_{8:0}$)	Total FFA ($\sum C_{4:0}$–$C_{8:0}$)
A	1	1.41 ± 0.05^{a1}	1.31 ± 0.13^{a1}	0.93 ± 0.25^{b1}	3.65 ± 0.33^{a1}
	30	1.93 ± 0.10^{b2}	1.83 ± 0.04^{a2}	1.39 ± 0.13^{b2}	5.15 ± 0.27^{b2}
	60	2.87 ± 0.03^{a3}	2.54 ± 0.18^{a3}	2.33 ± 0.09^{b3}	7.74 ± 0.30^{ab3}
	90	4.12 ± 0.03^{b4}	3.21 ± 0.01^{a4}	3.46 ± 0.19^{b4}	10.79 ± 0.23^{b4}
	120	4.53 ± 0.05^{a45}	5.23 ± 0.22^{a5}	4.67 ± 0.22^{b5}	14.43 ± 0.49^{ab5}
	150	5.07 ± 0.18^{a5}	5.65 ± 0.13^{a56}	5.08 ± 0.23^{b56}	15.80 ± 0.54^{ab6}
	180	5.99 ± 0.33^{ab6}	6.25 ± 0.31^{a6}	5.60 ± 0.28^{c6}	17.84 ± 0.92^{a7}
B	1	1.42 ± 0.14^{a1}	1.34 ± 0.11^{b1}	0.98 ± 0.07^{a1}	3.74 ± 0.32^{ab1}
	30	1.77 ± 0.13^{a12}	1.89 ± 0.12^{a2}	1.43 ± 0.05^{a2}	5.09 ± 0.30^{b2}
	60	3.05 ± 0.14^{b2}	2.81 ± 0.23^{b3}	2.47 ± 0.32^{ab3}	8.33 ± 0.69^{b3}
	90	3.45 ± 0.34^{a2}	3.42 ± 0.21^{ab4}	3.49 ± 0.39^{a4}	10.36 ± 0.94^{a4}
	120	4.79 ± 0.34^{b23}	5.27 ± 0.45^{ab5}	4.77 ± 0.31^{a5}	14.83 ± 1.10^{b5}
	150	5.30 ± 0.32^{b4}	5.98 ± 0.57^{b6}	5.26 ± 0.66^{a56}	16.54 ± 1.55^{bc6}
	180	6.26 ± 0.47^{b5}	6.31 ± 0.52^{a7}	5.73 ± 0.49^{bc6}	18.30 ± 1.48^{ab7}
C	1	1.56 ± 0.12^{b1}	1.42 ± 0.03^{b1}	1.08 ± 0.06^{a1}	4.06 ± 0.21^{b1}
	30	1.89 ± 0.09^{b12}	1.97 ± 0.21^{a12}	1.62 ± 0.12^{a2}	5.48 ± 0.42^{c2}
	60	3.18 ± 0.38^{b2}	3.06 ± 0.23^{b3}	2.64 ± 0.25^{a3}	8.88 ± 0.86^{c3}
	90	4.21 ± 0.29^{b3}	3.89 ± 0.35^{b4}	3.62 ± 0.15^{a4}	11.72 ± 0.79^{c4}
	120	5.22 ± 0.35^{c4}	5.31 ± 0.48^{b5}	5.11 ± 0.58^{a5}	15.64 ± 1.41^{c5}
	150	5.41 ± 0.22^{b45}	6.12 ± 0.31^{b6}	5.46 ± 0.41^{ab56}	16.99 ± 0.94^{c6}
	180	6.47 ± 0.32^{b5}	6.50 ± 0.41^{b6}	5.92 ± 0.39^{6}	18.89 ± 1.12^{b7}
D	1	1.36 ± 0.14^{a1}	1.24 ± 0.02^{a1}	0.85 ± 0.11^{a1}	3.45 ± 0.27^{a1}
	30	1.71 ± 0.25^{a12}	1.89 ± 0.22^{a2}	1.31 ± 0.07^{a2}	4.45 ± 0.44^{a2}
	60	2.77 ± 0.12^{a2}	2.41 ± 0.12^{a3}	2.23 ± 0.27^{a3}	7.41 ± 0.51^{a3}
	90	3.79 ± 0.12^{ab3}	3.30 ± 0.25^{a4}	3.24 ± 0.34^{a4}	10.33 ± 0.61^{a4}
	120	4.62 ± 0.36^{a4}	5.11 ± 0.32^{a5}	4.28 ± 0.32^{a5}	13.91 ± 0.86^{a5}
	150	4.95 ± 0.25^{a45}	5.45 ± 0.19^{a5}	4.86 ± 0.30^{a56}	15.26 ± 0.74^{a6}
	180	5.43 ± 0.25^{a5}	6.16 ± 0.21^{a6}	5.37 ± 0.19^{a6}	17.76 ± 0.65^{a7}

Cheese type description as in Table 1. *Samples showing common superscripts numbers (during storage days) and superscripts letters (between cheeses at the same storage day) do not differ significantly ($p>0.05$).

loglu *et al.* 2013; Atasoy & Turkoglu, 2009; Bohdziewicz, 2006, Perotti *et al*., 2005; Georgala *et al*., 2005; Buffa *et al*., 2001]. At the beginning and end of ripening, the C cheeses had the highest butyric acid content than the other cheeses. The concentrations of caproic acid and caprylic acid showed similar trend to butyric acid in all samples. The total concentration of SCFFA [($\sum C_{4:0}$–$C_{8:0}$)] increased significantly ($p<0.01$) in all cheese samples during ripening period (Table 2). The increase in FFAs in cheese during ripening has been reported previously in matured cheese [Delgado *et al*., 2009; Mallatou *et al*., 2003]. At the end of storage, the volatile FFA content of the C cheese was significantly ($p<0.01$) higher compared to the other cheeses.

The addition of wild type bacterial strains to the pasteurised milk affected the volatile FFAs levels significantly during ripening period ($p<0.01$). In contrast, Atasoy & Turkoglu, [2009] showed that Urfa cheese made from raw milk had higher total FFAs levels than those made from pasteurised milk. Short chain fatty acids (C4 to C8) were at much higher concentrations in the C cheeses. Low and high levels of short chain fatty acids indicate low and high lipolytic action of the natural microflora of the corresponding cheeses [Collins *et al*., 2003]. Generally, the levels of butyric, caproic and caprylic acids were low in all experimental cheeses, indicating low lypolitic activity of lipases/esterases from the wild type strains. As a result, rancid flavour was not noticed in the cheese samples during ripening.

The concentrations of capric (C10:0) and lauric (C12:0) acids in the cheese C were significantly higher than the cheeses B, C and D (control) at all stages of ripening ($p<0.001$). Among medium-chain FFA, lauric acid had the lowest concentration in all cheese samples (Table 3). However, myristic acid was the predominant medium-chain FFA at the beginning of ripening period. After 120 days of ripening, lauric and myristic acid concentrations were close to each other in all cheese groups. Similar results were reported by Atasoy &Türkoğlu [2008, 2009]. Despite the quantitative importance of medium and long chain FFA, they are not the main factor for the cheese flavour [Freitas & Malcata, 1998; Attaie & Richert, 1996].

The total long chain FFA contents of the cheeses were higher than those of the medium chain FFAs (Table 4). At the beginning of ripening, palmitic acid (C16:0) concentration of the cheese C was significantly ($p<0.001$) higher than the other cheeses and this remained unchanged at the later stages of the ripening. Among the FFA, the palmitic (C16)

TABLE 3. Middle chain free fatty acids (mg/100 g cheese) of cheese samples at during ripening.

Samples	Storage days	Capric acid ($C_{10:0}$)	Lauric acid ($C_{12:0}$)	Myristic acids ($C_{14:0}$)	Total FFA ($\Sigma C_{10:0}$–$C_{14:0}$)
A	1	4.36±0.18^{b1}	2.22±0.24^{a1}	9.67±0.31^{a1}	16.25±0.73^{a1}
	30	4.90±0.19^{a2}	3.04±0.22^{a2}	10.30±0.44^{a2}	18.54±0.85^{a23}
	60	7.24±0.22^{b3}	4.93±0.38^{ab3}	12.13±0.51^{a3}	24.30±1.11^{b34}
	90	10.62±0.13^{a34}	5.80±0.19^{a4}	14.29±0.29^{b4}	30.71±0.61^{b4}
	120	14.35±0.31^{a5}	7.86±0.36^{a5}	15.49±0.38^{b5}	37.70±1.05^{a5}
	150	16.41±0.33a^{b6}	8.12±0.27^{a6}	17.20±0.19^{b6}	41.73±0.79^{a6}
	180	17.67±0.21^{a7}	9.20±0.44^{a7}	18.76±0.72^{a7}	45.73±1.37^{b7}
B	1	4.40±0.33^{b1}	2.28±0.22^{a1}	10.00±0.38^{b1}	16.78 ±0.93^{b1}
	30	5.25±0.19^{b12}	3.08±0.29^{a2}	10.43±0.21^{a1}	19.56±0.69^{b2}
	60	6.92±0.25^{ab3}	5.19±0.13^{b3}	12.23±0.26^{b2}	24.34±0.64^{b3}
	90	10.58±0.09^{a}	5.89±0.32^{a4}	13.74±0.19^{a3}	30.21±0.60^{a4}
	120	14.44±0.34^{a5}	7.77±0.19^{a5}	15.39±0.43^{b4}	37.70±0.96^{a5}
	150	16.96±0.41^{b6}	8.58±0.36^{b6}	17.26±0.61^{b5}	42.80±1.38^{b6}
	180	18.11±0.39^{b7}	9.44±0.26^{b7}	18.56±0.57^{a6}	46.11±1.22^{b7}
C	1	4.72±0.18^{c1}	2.58±0.22^{b1}	10.69±0.19^{c1}	17.99±0.59^{c}
	30	5.33±0.24^{b2}	3.35±0.32^{b2}	11.30±0.22^{b2}	19.78±0.78^{b}
	60	7.41±0.31^{b3}	5.57±0.41^{c3}	12.54±0.43^{b3}	25.52±1.15^{c3}
	90	11.57±0.64 ^{b4}	6.53±0.21^{b4}	14.95±0.35^{c4}	33.05±1.20^{c4}
	120	15.64±0.24 ^{b5}	7.89±0.61^{a5}	16.08±0.61^{c5}	39.31±1.46^{b5}
	150	17.89±0.36^{c6}	8.95±0.32^{c6}	17.81±0.34^{c6}	44 44.65±1.02^{c6}
	180	18.90±0.21^{c7}	9.96±0.42^{c7}	19.66±0.45^{b7}	48.52±1.08^{c6}
D	1	4.08±0.21^{a1}	2.10±0.29^{a1}	9.83±0.22^{a1}	15.91±0.72^{a1}
	30	4.72±0.29^{a2}	3.31±0.18^{b2}	10.23±0.18^{a1}	18.16±0.65^{a2}
	60	6.65±0.32^{a3}	4.78±0.41^{ab3}	11.90±0.25^{a2}	23.23±0.98^{a3}
	90	10.44±0.33^{a4}	5.64±0.22^{a4}	13.72±0.33^{a3}	29.60±0.88^{ac4}
	120	14.55±0.41^{a5}	7.66±0.47^{a5}	15.01±0.41^{a4}	37.67±1.29^{a5}
	150	16.06±0.23^{a6}	8.34±0.55^{b6}	16.74±0.55^{a5}	41.14±1.33^{a6}
	180	17.44±0.52^{a7}	9.12±0.51^{a7}	18.51±0.38^{a6}	44.97±1.41^{a6}

Cheese type description as in Table 1. *Samples showing common superscripts numbers (during storage days) and superscripts letters (between cheeses at the same storage day) do not differ significantly ($p>0.05$).

and oleic (C18:1) acids were the principal fatty acids in all cheeses at all ripening stages. The highest volatile FFAs contents were determined in C cheeses. These results are consistent with other long-time maturated cheeses [Delgado *et al.,* 2011; Talpur *et al*., 2008].

Generally, the total FFAs contents found in the cheeses were significantly higher in the cheese C. This may be related to higher lipolytic activity of enterococcal strains in the cheese C. Compared to many cheese varieties, the total FFAs content of Urfa cheese was markedly lower. Storing in high salt brine (>12%, w/v) may be the main reason of low lipoysis in Urfa cheese. The inhibitory effect of NaCl on FFAs was emphasised by other researchers [Katsiari *et al*., 2000; Pavia *et al*., 2000].

Sensory evaluations

The cheese samples were evaluated organoleptically based on appearance and colour, aroma and flavour, body and texture, saltiness and overall perception. The results for 180 day old cheeses are presented in Table 5. With respect to the appearance and colour, no differences were found between cheese samples. Control cheese (D) received significantly lower body and texture scores at 180 d ($p<0.05$). This could be due to the lower titration acid value than other cheeses at the end of ripening, which has a significant impact on the development of the textural properties of cheese. However, the body and texture scores of the other cheeses showed similarities at 180 d. Conversely, the cheese D had significantly higher aroma and flavour scores than the other cheeses ($p<0.01$). All cheeses had similar saltiness scores at 180 d. The cheese C had the lowest overall perception scores at 180 d. The cheese C was criticized for the slight bitterness at day 180. All the samples were found to be consumable by the panelists with less pronounced in the sample C. Atasoy & Turkoglu, [2009] used termophillic and mesophillic cultures in the production of Ufa cheese. The cheeses made by thermophillic culture showed similar sensory evaluations with cheeses made from raw milk, on the other hand panelists gave lower flavour scores to cheeses made by the mesophilic culture.

CONCLUSION

Based on the results obtained from the present study, the use of various combinations of *L. lactis* subsp. *lactis* B14, *L. garvieae* IMAU 50157, *E. faecalis* KLDSO.0341,

TABLE 4. Long chain free fatty acids (mg/100 g cheese) of cheese samples at during ripening.

Samples	Storage days	Palmitic acid ($C_{16:0}$)	Stearic acid ($C_{18:0}$)	Oleic acid ($C_{18:1}$)	Linoleic acid ($C_{18:2}$)	Total FFA ($\Sigma C_{16:0}$–$C_{18:2}$)	Total FFA ($\Sigma C_{4:0}$–$C_{18:2}$)
	1	7.85±0.65^{a1}	19.99±0.18^{b1}	16.01±0.32^{a1}	1.22±0.12^{b1}	44.57±1.27^{a1}	64.47±2.19^{a1}
	30	12.52±0.54^{a2}	27.50±0.43^{b2}	20.29±0.18^{a2}	1.82±0.09^{a2}	62.01±1.24^{b2}	85.70±3.36^{b2}
	60	16.88±0.28^{a3}	37.55±0.33^{b3}	26.34±0.76^{a3}	2.83±0.31^{a3}	83.40±1.68^{a3}	115.44±3.09^{ab3}
A	90	23.67±0.19^{b4}	48.03±1.21^{b4}	32.78±0.99^{b4}	3.33±0.11^{a4}	107.81±1.50^{ab4}	149.31±2.34^{b4}
	120	29.20±0.81^{a5}	58.35±0.64^{a5}	38.81±0.43^{b5}	4.74±0.38^{a5}	131.10±2.26^{a5}	183.23±3.80^{a5}
	150	35.01±0.54^{a6}	64.35±0.86^{a6}	42.36±1.15^{a6}	5.20±0.19^{a6}	146.92±1.74^{a6}	204.45±3.07^{d6}
	180	38.37±0.42^{a7}	69.22±0.92^{ab7}	46.99±1.32^{a7}	6.42±0.61^{a7}	161.34±2.27a^{b7}	224.91±4.16^{a7}
	1	20.17±0.29^{b1}	8.20±0.09^{ab1}	16.14±0.31^{a1}	1.02±0.12^{a1}	45.53±0.81^{b1}	66.05±1.93^{b1}
	30	27.99±0.17^{b2}	12.31±0.14^{a2}	20.34±0.17^{a2}	1.88±0.07^{a2}	65.52±0.55^{c2}	90.17±1.34^{c2}
	60	37.10±0.44^{a3}	17.71±0.32^{b3}	26.63±1.03^{a3}	2.79±0.07^{a3}	84.23±1.86^{b3}	116.90±2.79^{b3}
B	90	49.84±0.62^{c4}	23.78±0.11^{b4}	32.17±0.31^{a4}	3.29±0.21^{a4}	109.08±1.25^{b4}	149.65±1.99^{b4}
	120	59.31±0.39^{b5}	30.14±0.41^{b5}	38.15±0.67^{a5}	4.69±0.05^{a5}	132.39±1.52^{b5}	184.92±2.88^{b5}
	150	64.95±0.21^{ab6}	35.74±0.62^{b6}	42.45±0.89^{a6}	5.84±0.13^{b6}	148.98±1.85^{b6}	208.32±3.35^{b6}
	180	69.42±0.53^{b7}	39.75±0.33^{b7}	46.80±1.14^{a7}	6.39±0.41^{a7}	162.34±2.38^{b7}	226.75±4.08^{a7}
	1	20.63±0.55^{c1}	8.55±0.32^{b1}	17.01±0.65^{b1}	1.28±0.09^{b1}	47.47±1.61^{c1}	69.52±2.41^{c1}
	30	28.50±0.43^{c2}	13.91±0.27^{b2}	21.14±0.31^{b2}	2.23±0.06^{b2}	65.78±1.07^{c2}	91.04±2.07^{d2}
	60	38.08±0.39^{c23}	18.72±0.44^{c3}	27.44±0.98^{b3}	3.03±0.12^{b3}	87.27±1.93^{c3}	121.67±3.44^{c3}
C	90	50.50±0.71^{d4}	24.38±0.49^{c4}	33.90±1.01^{c4}	3.87±0.26^{b4}	112.65±2.47^{c4}	157.42±3.86^{c4}
	120	60.38±0.64^{c5}	31.61±0.17^{c5}	40.36±0.43^{c5}	4.93±0.11^{b5}	137.28±1.35^{c5}	192.23±3.22^{c5}
	150	65.62±1.32^{b6}	36.53±0.87^{c6}	44.91±0.21^{c6}	5.99±0.08^{b6}	153.05±2.48^{c6}	214.69±3.84^{c6}
	180	70.87±0.42^{c7}	40.64±0.21^{c7}	48.43±0.86^{b7}	7.02±0.41^{b7}	166.96±1.89^{c7}	234.37±3.09^{b7}
	1	19.18±0.75^{a1}	7.96±0.07^{a1}	16.12±0.51^{a1}	1.14±0.03^{ab1}	44.40±1.36^{a1}	63.76±2.35^{a1}
	30	26.24±0.64^{a2}	12.45±0.31^{a2}	20.26±1.05^{c2}	1.78±0.10^{a12}	60.73±2.10^{a2}	83.34±2.89^{a2}
	60	36.90±0.38^{a3}	17.20±0.43^{ab3}	26.54±0.75^{a3}	2.86±0.07^{a3}	83.60±1.63^{a3}	114.24±3.06^{a3}
D	90	47.36±1.12^{a4}	22.83±0.18^{a4}	32.13±0.21^{a4}	3.44±0.25^{a4}	105.76±1.76^{a4}	145.69±2.95^{a4}
	120	58.23±0.98^{a5}	29.86±0.56^{b5}	38.31±0.38^{5}	4.76±0.32^{a5}	131.16±2.24^{a5}	182.74±4.09^{a5}
	150	64.52±1.38^{a6}	34.89±0.87^{a6}	42.97±1.05^{b6}	5.12±0.19^{a6}	147.30±3.49^{a6}	203.70±5.16^{c6}
	180	69.13±0.87^{a7}	38.07±0.21^{a7}	46.77±0.49^{a7}	6.37±0.41^{a7}	159.74±1.98^{a7}	221.67±3.64^{a7}

Cheese type description as in Table 1. *Samples showing common superscripts numbers (during storage days) and superscripts letters (between cheeses at the same storage day) do not differ significantly (p>0.05).

TABLE 5. Sensory evaluation of 180 day-old cheese samples.

Cheese samples	Appearance and colour	Aroma and flavour	Body and texture	Saltiness	Overall perception
A	4.54±0.21^{a}	8.18±0.58^{b}	4.22±0.24^{b}	3.32±0.11^{a}	20.26±0.88^{a}
B	4.75±0.36^{b}	8.32±0.84^{b}	4.13±0.19^{b}	3.42±0.08^{b}	20.62±0.81^{b}
C	4.35±0.15^{a}	7.24±0.54^{a}	4.63±0.42^{c}	3.28±0.10^{a}	19.50±0.69^{a}
D	4.80±0.49^{b}	9.06±0.66^{c}	3.35±0.33^{a}	3.60±0.18^{a}	20.81±0.95^{b}

Cheese type description as in Table 1. Samples showing common superscripts letters (between cheeses at the same storage day) do not differ significantly (p>0.05).

E. faecium ATCC 19434 and *E. durans* IMAU 60200 offers certain advantages compared to raw ewe's milk cheeses in regard to the development of lipolysis. Especially, the starter combination including all above strains in equal proportions (cheese C) was found to be more efficient for faster development of lipolysis in Urfa cheese made from pasteurised ewe's milk. Whereas panelists gave the lowest overall perception degree to the C cheeses. Surely, there are a number of parameters that need to be properly addressed before offering a starter or starter combination to cheese industry. Although the lipolytic performances of the strains employed in the present case were superior to that of raw milk microflora, the safety of enterococcal strains for cheese applications has to be assured.

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