



Effect of Using Attenuated Lactic Starter Cultures on Lipolysis and Proteolysis in Low Fat Kaşar Cheese

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Abstract: In this study, freeze shocked cultures of *Lactobacillus casei* and *Lactobacillus helveticus* were used in the production of low fat Kaşar cheese to accelerate the ripening and improve the flavour and textural characteristics. Physical, chemical and sensory properties of the cheeses with low fat (11 % fat) were evaluated comparatively with that of the low-fat and full-fat controls (28 %) on days 1, 30, 60 and 90 at 5±1°C. According to the results, the use of attenuated cultures has not altered the general composition of the low fat cheeses, however, it has improved the sensory properties significantly and decreased the ripening period by acceleration of the proteolysis, being more evident at the cheese with freeze-shocked *L. helveticus*.

Key Words: Kaşar cheese, low fat, attenuated culture, ripening

Az Yağlı Kaşar Peyniri Üretiminde Yardımcı Kültür Kullanımının Lipoliz ve Proteoliz Üzerine Etkisi

Öz: Bu çalışmada olgunlaştırmayı hızlandırmak, yapı ve tadı iyileştirmek amacıyla az yağlı kaşar peyniri üretiminde dondurularak yardımcı kültür haline getirilmiş *L. casei* and *L. helveticus* mikroorganizmaları kullanılmıştır. Az yağlı (% 11 yağ) ve tam yağlı (% 28 yağ) olarak üretilen ve 90 gün süreyle 5±1°C'de depolanan peynirler; fiziksel, kimyasal ve duyuşsal nitelikler yönünden incelemeye alınmıştır. Araştırma sonuçlarına göre; yardımcı kültür kullanımı az yağlı peynirlerin genel bileşimini değiştirmemiş ancak duyuşsal niteliklerini iyileştirmiş ve *L. helveticus* ' un kullanıldığı örnekte daha belirgin olmak üzere olgunlaşma süresini kısaltmıştır.

Anahtar Kelimeler: Kaşar peyniri, az yağlı, yardımcı kültür, olgunlaştırma

Introduction

Kaşar cheese is one of the greatly consumed traditional cheeses in Turkey. It is mostly produced from cow's milk and also from ewe's or goat's milk or from the combination of the latter two. It is sliceable and semi-firm cheese and falls into the 'pasta filata' group. It is yellowish white or yellow in color, which does not contain pores and is cylindrical or rectangular in shape. Cheeses produced under the names such as Kashkaval, Kashkaval and Kashakovala, Kashkaval Balkan, Kashkaval Preslav, Kasserli in different countries are Kaşar-like cheeses (Gobbetti et al. 2002). According to Turkish Standard-3272, this cheese is classified as "fresh Kaşar cheese" and "old or matured Kaşar cheese" in terms of ripening, and classified as "full fat (45 % m/m), and semi-fat (20 % m/m)" in terms of fat content (Anonymous 1999).

Due to the increasing demand on low fat cheese products, there is also an increase in the production of the low fat or reduced fat cheeses beginning especially

from the 1980s. Fat is not only of nutritional significance in cheese, but also contributes to sensory and functional properties. In the reduced fat cheeses, textural, functional and sensory defects such as rubbery texture, lack of flavor, bitterness, off-flavour and undesirable color are frequently seen (Banks et al. 1989, Drake and Swanson 1995, Mistry 2001).

Accelerated ripening is beneficial for the elimination of flavour and textural defects generally seen in low fat cheeses. It has been reported by Banks et al. (1993) that an improvement can be achieved in the structural properties of a cheese depending on an increased level of proteolysis. One of the methods for acceleration the ripening is the use of attenuated cultures. Attenuated cultures are the strains of microorganisms added to milk for the purpose of increasing the sensorial attributes and improving the textural properties of cheese (El-Soda et al. 2000a). In the preparation of attenuated cultures, physical

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methods such as heating, freezing, freeze drying, treating with lisozyme or an organic solvent are used and by these ways the number and the ability of acid production of the live cells are decreased (El-Soda et al. 2000a, Banks 2004). Among these applications, with the freeze-shocking proteolytic and peptidolytic activities are sufficiently retained for providing the desirable development of body and aroma, since the proteinase, peptidase and esterase enzymes are slightly inactivated instead of 96-98% death rate in live cells (Bartels et al. 1987, Madkor et al. 1998, El-Soda et al. 2000b).

Lactobacilli are bacteria with high activities of aminopeptidase, dipeptitase and protease enzymes, besides, they retain in the cheese matrix, they are not lost with the whey since they are rod shaped microorganisms (Pettersson and Sjöström 1975, Frey et al. 1986a). For this reason, attenuated lactobacilli cells modified by the physical methods like heat-shocked or freeze-shocked have been used to accelerate the ripening in varieties of many cheeses such as Cheddar (El-Soda et al. 1991), Sweedish hard cheese (Ardö and Pettersson 1988), Gouda (Bartels et al. 1987), Ras (Aly 1990, Kebary et al. 1996) and pickled White cheese (Gürsoy et al. 2001, Gürsel et al. 2003).

In this study, *L. casei* and *L. helveticus* microorganisms prepared as attenuated culture by freezing process are used to show their suitability in the manufacture of low fat Kaşar cheese to accelerate the ripening and improve the sensory attributes.

Materials and Methods

Materials: Bulk fresh cow milk was obtained from the herd of Ankara University dairy farm. Commercial culture (TM081, Rhodia/France) containing the mixture of *Streptococcus thermophilus* ve *Lactobacillus delbrueckii ssp. bulgaricus* was used as an acid developer. Culture of *L. casei* (LYO LBC81, Rhodia/France) or *L. helveticus* (LH-B02, Chr. Hansen/Denmark) was used for preparing the attenuated cultures. Liquid calf rennet at the strenght of 1/16.000 was used (Chr.Hansen/Denmark).

Preparation of starter cultures: Lactic starter culture was dissolved in 1 litre of pasteurized milk before being added to cheese milk. Freeze-shocked cultures were prepared as described by Frey et al. (1986b).

Production of Kaşar cheese: Cheese production was carried out in pilot Dairy Plant at Faculty of Agriculture, Ankara University. Milk has

been standardized to fat content of 3.3 % for the full fat cheese (Cheese A) and 1.1% for the low fat cheeses (Cheese B, C, D). For each batch 50 kg of standardized milk were used. Batches of milk, after heat treatment for 1 min at 72°C, have been left for about 1 h for pre-maturation by first adding CaCl₂ (0.02 %) and then adding lactic culture (1 %). Following pre-maturation, one batch of milk was used for the manufacture of low fat control cheese (Cheese B). To the remaining parts of milk *L. casei* (Cheese C) or *L. helveticus* (Cheese D) has been added at the rate of 1 %. Then, standard Kaşar cheese making procedure was followed (Üçüncü 2004). Totally 12 cheese blocks for each cheese (each about 350-400 g) have been left for ripening at 5±1 °C and have been taken for analysis on days 1, 30, 60 and 90. The experiment was carried out with three replicates for each cheese.

Chemical and sensory analysis: In cheese samples, water soluble nitrogen (WSN), nitrogen soluble in 12 % trichloroacetic acid (TCA-SN) and nitrogen soluble in 10 % phosphotungstic acid (PTA-SN) were measured by the Kjeldahl method (Ardö and Polychroniadou 1999). The spectrophotometric method was followed for the analysis of tyrosine (Hull 1947). Total and individual free fatty acids (FFA) were determined by the method of Deeth et al. (1983). Cheese (5 g) was ground with 2.5 g of Na₂SO₄ and then, 5 mL of an internal standart (C₇) and 300 µL H₂SO₄ were added. The mixture was mixed thoroughly for 1 min and hexane (5 mL) was added. Samples were rested for 1 h before the liquid phase was removed and mixed with 2 mL of 6 % solution of formic acid/ether mixture. This mixture was centrifuged at 2000 x g for 10 min. The clear part was transferred into the vials and the vials were stored at -18°C until use. The volume of the cheese samples analyzed was 5 µL. The chromatography system used consisted of an Agilent Model 6890 (Agilent Technologies Inc., USA) instrument fitted with an FID detector. The column used was an Agilent-FFAP capillary 300 x 250 µm x 0.25 µm. The conditions of the determination were as follows: injection temperature, 250°C; split, 1/10; flow rate of the sample, 2mL/min; flow rate of H₂, air and make up gas (N₂), 33 mL/min; 30 mL/min and 30 mL/min, respectively.

Sensory properties were evaluated by 10 expert panel members according to the scoring sheet given in Turkish standard (Anonymous 1999).

Statistical analysis: Analysis of variance was performed using MINITAB (Ryan et al. 1985), and the results were analyzed as a randomized plot design (Steel and Torrie 1980). Statistically significant differences between means were determined using Tukey's test.

Results and Discussion

Fat contents of Kaşar cheeses are shown in Table 1. Using the attenuated cultures and ripening periods have not caused a significant difference in fat content of cheese ($P>0.05$).

Changes in soluble nitrogenous compounds (WSN, 12 % TCA-soluble N, 10 % PTA-soluble N) and tyrosine values of the Kaşar cheese samples are given in Table 2. The level of soluble nitrogenous compounds and tyrosine values increased gradually in all samples throughout the ripening as reported by Aly (1990), Kebary et al. (1996) and Michaelidou et al. (2003). The increase soluble nitrogenous compounds were at the highest level on day 60, and no significant change has generally been found after that period. When these findings are compared with the other studies performed on Kaşar cheese (Koçak et al. 1996, Güler 2000), it can be said that ripening period could be decreased by using freeze-shocked cultures of *L. casei* or *L. helveticus*, and desired ripening characteristics can be reached within maximum two months. Similar results have been obtained from the studies performed on Ras (Aly 1990, Ezzat and El-Shafei 1991), Cheddar (El-Soda et al. 1991) and Gouda cheeses (Bartels et al. 1987). These results could be attributed to the presence of wide range of proteolytic enzyme systems in lactobacilli. The addition of the freeze-shocked culture of *L. helveticus* was

more effective on the accumulation of soluble nitrogenous compounds in cheese (Aly 1994, Kebary et al. 1996, Madkor et al. 2000).

The percentage concentration of free fatty acids (FFA) in Kaşar cheeses are shown in Table 3. In terms of short (C4-C8) and long (C16, C18, C18:1) chain fatty acids, a significant interaction ($P<0.05$) was found between ripening period and cheese samples. Amounts of butyric, caproic and caprylic acids were higher both in low fat control and the low fat cheese with added adjunct culture than in the full-fat cheese sample. Adding adjunct culture increased the level of butyric, caproic, caprylic acids until 60 days, but then did not cause any change. Concentrations of capric, lauric and myristic acids showed an increase more obviously in the low fat cheese sample with added freeze-shocked *L. helveticus* ($P<0.05$). Palmitic, stearic and oleic acids were the most abundant FFA in all the cheeses throughout the ripening. Especially, oleic acid represented the 55.24 % of total FFA present in the full-fat cheese and 43.44-46.12 % of total FFA in the low-fat cheeses made with or without adjunct culture (Table 3). These results are in accordance with the findings of Güler (2000) and Kondyli et al. (2003). From these results, it seems that the adjunct cultures did not influence the production of FFA significantly. This is attributed to the weak lipolytic activity of the microorganisms in the adjunct cultures (Kondyli 2003).

Table 1. Effects of fat content and storage time on the fat content of Kaşar cheese samples ¹

Main effects	Fat content (%)
Cheese ²	
A	28.042±0.899 a
B	11.708±0.278 b
C	11.833±0.198 b
D	12.125±0.307 b
Ripening time (days)	
1	15.96±2.40
30	16.63±2.23
60	15.69±2.00
90	15.44±2.01

¹ Values are means of 3 replicates ±SEM.

² Cheese: A= full-fat control; B= low-fat control; C= Low-fat cheese with freeze-shocked *L. casei*
D= low-fat cheese with freeze- shocked *L. helveticus*.

a,b; means in the column with different letters were significantly affected by cheese type ($P<0.05$).

Table 2. Effects of fat content and storage time on the nitrogenous compounds of Kaşar cheese samples ¹

Compounds	Ripening (days)	Cheese ²			
		A	B	C	D
WSN (%)	1	0.287±0.042 bC*	0.513±0.037 aC*	0.513±0.037 aC*	0.593±0.001 aC*
	30	0.500±0.100 bB*	0.920±0.030 aB*	0.887±0.001 aB*	0.913±0.099 aB*
	60	0.600±0.055 cAB*	1.173±0.042 abA*	1.067±0.068 bA*	1.253±0.093 aA*
	90	0.693±0.068 cA*	1.187±0.034 bA*	1.203±0.015 bA*	1.373±0.050 aA*
TCA-SN (%)	1	0.127±0.009 aC**	0.207±0.013 aC**	0.233±0.017 aD**	0.230±0.006 aD**
	30	0.220±0.012 bBC**	0.440±0.025 aB**	0.423±0.012 aC**	0.427±0.017 aC**
	60	0.287±0.017 bB**	0.557±0.003 aA**	0.540±0.031 aB**	0.610±0.035 aB**
	90	0.437±0.057 cA**	0.580±0.042 bA**	0.643±0.007 bA**	0.773±0.024 aA**
PTA-SN (%)	1	0.093±0.007 aC**	0.100±0.006 aC**	0.097±0.007 aC**	0.110±0.012 aD**
	30	0.197±0.003 bAB**	0.210±0.006 abB**	0.210±0.006 abB**	0.247±0.012 aC**
	60	0.193±0.003 cB**	0.267±0.007 abA**	0.263±0.003 bA**	0.313±0.003 aB**
	90	0.243±0.009 cA**	0.270±0.020 bcA**	0.293±0.012 bA**	0.383±0.027 aA**
Tyrosine value (mg/g)	1	0.593±0.012 aA**	0.897±0.095 aB**	0.950±0.095 aB**	0.913±0.023 aB**
	30	1.053±0.113 aA**	1.687±0.162 aB**	1.683±0.072a B**	2.063±0.286 aB**
	60	1.420±0.096 bA**	3.497±0.723 aA**	3.957±0.173 aA**	4.210±0.020 aA**
	90	1.520±0.114 cA**	3.253±0.632 bA**	5.057±0.740 aA**	4.377±0.102 abA**

¹ Values are means of 3 replicates ±SEM.

² Cheese: A= full-fat control; B= low-fat control; C= Low-fat cheese with freeze-shocked *L. casei* D= low-fat cheese with freeze-shocked *L. helveticus*.

a-b; means in the same row with different letters were significantly affected by cheese type

A-C; means in the same column with different capital letters were significantly affected by ripening period

*; P<0.05, **; P<0.01

Results of sensory evaluation are given in Table 4 and 5. The full-fat cheese sample had the highest scores from body, texture and flavour assessments. It is thought that FFA concentration is effective on this result. It is stated by Molimard and Spinnler (1996) and Collins et al. (2003) that the low and medium chain fatty acids contribute more to the flavor formation than

the long chain ones. Among the low-fat cheeses, that one with freeze-shocked *L. casei* has been preferred more in terms of texture and flavor. The reasons of this results the use of LBC-81 which was suggested as the aroma developer culture by the supplier company (Katsiari et al. 2002).

Table 3. Effects of fat content and storage time on the free fatty acids of total FFAs Kaşar cheese samples ¹

Free fatty acids	Ripening (days)	Cheese				Mean
		A	B	C	D	
Butyric (C _{4:0})	1	0.06±0.00 7f	0.36±0.044 bcd	0.34±0.032 bcd	0.28±0.009 de	0.26±0.038
	30	0.09±0.006 f	0.44±0.034 abcd	0.54±0.073 abcd	0.50±0.059 ab	0.39±0.058
	60	0.13±0.003 ef	0.48±0.013 abc	0.37±0.022 bcd	0.43±0.029 abcd	0.35±0.041
	90	0.13±0.002 ef	0.37±0.015 bcd	0.37±0.011 bcd	0.32±0.027 cd	0.30±0.030
	Mean	0.10±0.009	0.41±0.019	0.40±0.030	0.38±0.030	
Caproic (C _{6:0})	1	0.05±0.006 g	0.34±0.030 a	0.29±0.029 abc	0.27±0.005 abcde	0.24±0.035
	30	0.04±0.004 g	0.25±0.002 abcde	0.30±0.050 ab	0.28±0.044 abcd	0.22±0.034
	60	0.06±0.001 g	0.20±0.005 bcde	0.19±0.007 cde	0.22±0.003 bcde	0.17±0.018
	90	0.07±0.002 fg	0.18±0.010 de	0.19±0.007 cde	0.17±0.010 ef	0.15±0.015
	Mean	0.05±0.003	0.24±0.020	0.24±0.020	0.23±0.016	
Caprylic (C _{8:0})	1	0.04±0.002 e	0.13±0.005 a	0.11±0.009 ab	0.11±0.002 ab	0.10±0.010
	30	0.04±0.000 e	0.08±0.006 bcd	0.09±0.008 bc	0.09±0.012 bc	0.08±0.007
	60	0.05±0.000 de	0.06±0.006 cde	0.08±0.004 bcd	0.07±0.005 cde	0.07±0.004
	90	0.06±0.002 cde	0.05±0.008 de	0.08±0.002 bcd	0.07±0.002 cde	0.07±0.003
	Mean	0.05±0.003	0.08±0.009	0.09±0.005	0.08±0.005	
Capric (C _{10:0})	1	0.16±0.003	0.21±0.003	0.22±0.010	0.23±0.017	0.21±0.009
	30	0.16±0.001	0.25±0.079	0.20±0.005	0.22±0.008	0.21±0.020
	60	0.18±0.002	0.17±0.002	0.22±0.010	0.23±0.024	0.20±0.010
	90	0.19±0.004	0.18±0.008	0.20±0.005	0.23±0.033	0.20±0.010
	Mean	0.17±0.005 b	0.20±0.020 ab	0.21±0.005 ab	0.23±0.010 a	
Lauric (C _{12:0})	1	0.37±0.010	0.48±0.003	0.51±0.021	0.52±0.018	0.47±0.019
	30	0.35±0.003	0.41±0.003	0.47±0.006	0.49±0.022	0.43±0.016
	60	0.38±0.008	0.33±0.077	0.49±0.023	0.53±0.062	0.43±0.032
	90	0.40±0.007	0.43±0.019	0.46±0.016	0.61±0.067	0.47±0.028
	Mean	0.38±0.006 b	0.41±0.023 b	0.48±0.010 a	0.54±0.024 a	
Myristic (C _{14:0})	1	1.81±0.060	2.22±0.010	2.46±0.048	2.50±0.007	2.25±0.084
	30	1.79±0.028	1.94±0.012	2.19±0.037	2.40±0.045	2.08±0.071
	60	1.90±0.048	2.05±0.048	2.24±0.104	2.57±0.292	2.19±0.101
	90	2.00±0.028	2.15±0.103	2.24±0.055	3.02±0.325	2.35±0.140
	Mean	1.88±0.030c	2.09±0.040bc	2.28±0.0420b	2.62±0.118a	
Palmitic (C _{16:0})	1	18.51±0.315 bcd	23.55±0.533 a	23.12±0.394 a	23.33±0.147 a	22.13±0.651
	30	16.36±0.275 cd	18.91±0.141 bc	21.19±0.457 ab	22.63±0.525 a	19.77±0.734
	60	15.35±0.767 d	23.10±1.478 a	22.86±0.938 a	21.02±0.580 a	20.58±1.034
	90	16.70±0.282 cd	21.26±0.852 ab	22.42±0.392 a	21.62±0.225 ab	20.50±0.708
	Mean	16.73±0.396	21.70±0.671	22.40±0.336	22.15±0.321	
Stearic (C _{18:0})	1	25.99±1.781 abc	28.75±0.531 ab	26.92±0.328 abc	26.29±0.598 abc	26.99±0.531
	30	20.21±0.209 e	22.80±0.644 cde	24.90±0.132 bcde	26.52±0.160 abc	23.61±0.729
	60	21.06±0.409 de	25.08±1.829 bcd	28.03±0.565 ab	26.66±1.382 abc	25.21±0.939
	90	20.92±0.156 de	28.62±0.750 ab	29.96±1.166 a	25.30±0.408 abcd	26.20±1.097
	Mean	22.04±0.798	26.31±0.883	27.46±0.622	26.19±0.371	
Oleic (C _{18:1})	1	49.41±2.275 bc	41.26±0.376 d	42.80±0.982 d	43.80±0.979 cd	44.32±1.091
	30	57.79±0.470 a	52.55±0.713 ab	46.32±0.264 bcd	43.11±0.318 cd	49.94±1.718
	60	57.25±0.224 a	46.67±2.028 bcd	42.79±1.363 d	45.05±1.715 cd	47.94±1.791
	90	56.51±0.441 a	44.02±1.937 cd	41.86±0.894 d	45.97±0.162 cd	47.09±1.761
	Mean	55.24±1.142	46.12±1.404	43.44±0.658	44.48±0.543	
Linoleic (C _{18:2})	1	3.59±0.214	2.70±0.368	3.22±0.223	2.68±0.288	3.05±0.166 ab
	30	3.17±0.139	2.36±0.139	3.80±0.588	3.77±0.399	3.27±0.236 a
	60	3.63±0.111	1.87±0.037	2.74±0.595	3.22±0.134	2.87±0.238 ab
	90	3.03±0.197	2.75±0.216	2.22±0.166	2.68±0.160	2.67±0.119 b
	Mean	3.35±0.108 a	2.42±0.143 b	2.99±0.258 ab	3.09±0.177 a	

¹ Values are means of 3 replicates ±SEM.

² Cheese: A= full-fat control; B= low-fat control; C= Low-fat cheese with freeze-shocked *L.casei* D= low-fat cheese with freeze-shocked *L. helveticus*

a-g; means in the same row or column with different letters for each fatty acid differs statistically significant (P<0.05).

Table 4. Effects of fat content and storage time on the appearance points¹ of Kaşar cheese samples²

Ripening (days)	Cheese ²			
	A	B	C	D
1	4.77±0.05 aA	4.61±0.06 aA	4.64±0.03 aA	4.30±0.16 aA
30	4.72±0.07 aA	3.88±0.34 bB	3.81±0.18 bB	3.17±0.17 cB
60	4.74±0.09 aA	4.20±0.19 bAB	4.02±0.15 bcB	3.53±0.34 cB
90	4.72±0.06 aA	3.30±0.15 bC	3.73±0.27 bB	3.42±0.30 bB

¹ Ranges from 0 to 5, 0: unfavourable 5: most favourable, and presented values are means of 3 replicates ±SEM.

² Cheese: A= full-fat control; B= low-fat control; C= Low-fat cheese with freeze-shocked *L.casei* D= low-fat cheese with freeze-shocked *L. helveticus*.

a-c; means in the same row with different letters were significantly affected by cheese type,

A-C; means in the same column with different capital letters were significantly affected by ripening period (P<0.05)

Table 5. Effects of fat content and storage time on sensory properties of cheese samples¹

Main factors	Sensory parameters		
	Body and texture points ³	Flavour points ³	Overall score points ⁴
Cheese ² (n=12)			
A	4.30±0.16 a	4.21±0.15 a	4.41±0.10 a
B	3.75±0.16 b	3.10±0.18 b	3.62±0.15 b
C	3.97±0.15 ab	3.17±0.21 b	3.73±0.14 b
D	3.61±0.16 c	2.67±0.15 c	3.34±0.12 c
Ripening time (n=12)			
1	4.44±0.10 a	3.98±0.13 a	4.33±0.09 a
30	3.88±0.16 b	3.39±0.24 b	3.72±0.18 b
60	3.84±0.09 b	3.04±0.20 c	3.67±0.12 b
90	3.61±0.18 b	2.75±0.22 c	3.38±0.15 c

¹ Values are means of 3 replicates ±SEM.

² Cheese: A= full-fat control; B= low-fat control; C= Low-fat cheese with freeze-shocked *L.casei* D= low-fat cheese with freeze-shocked *L. helveticus*.

a-c; means in the same column with different letters are statistically significant for each factor (P<0.05)

³Ranges from 0 to 5, 0: unfavourable 5: most favourable

⁴Means of appearance, body and texture and flavour points

Conclusions

This study has revealed that low-fat cheese can be manufactured using the freeze-shocked *L. helveticus* or *L. casei*. These attenuated cultures have not affected the general composition of the cheeses, but accelerated the ripening of cheese samples more distinct effect with *L. helveticus*. When sensory properties are considered, *L. casei* has provided more favourable results for the manufacture of low-fat Kaşar cheese.

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