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RESEARCH ARTICLE

A study on chemical and microbiological properties of Kashar cheese produced without using starter culture

Abdullah Badem*, Gürkan Uçar

Department of Food Hygiene and Technology, Veterinary Faculty,
Selcuk University, 42075, Konya, Tukey
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*abdullah_badem@yahoo.com

Starter kültür kullanılmadan üretilen kaşar peynirlerinin olgunlaşma süresince kimyasal ve mikrobiyolojik özelliklerindeki değişim üzerine bir araştırma

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Öz

Amaç: Starter kültür kullanılmadan üretilen kaşar peynirlerin olgunlaşma süresince kimyasal ve mikrobiyolojik özelliklerinde meydana gelen değişimin araştırılması amaçlanmıştır.

Gereç ve Yöntem: Peynir üretimi için kullanılan çiğ inek sütü yağ oranı %3.0'e ayarlandı, 37°C'ye ısıtılıp enzim ilave edildi. Pıhtı işleme, baskı ve fermantasyon işlemi aşamasından sonra parçalama işlemi yapılarak %0.1 eritme tuzları ve %1.5 kaya tuzu ilave edildi. 65°C'de 5 dakika eritme işlemi yapılarak peynir üretildi. Peynir vakumla paketlendi, 8°C'de 90 gün süreyle olgunlaştırıldı. Peynir örneklerinde kurumadde gravimetrik yöntemle, yağ Gerber Metodu ile tuz Mohr yöntemi ile ve titrasyon asitliği % laktik asit olarak belirlendi. pH değerleri Nell marka pH metre ile ölçüldü. Bütün numuneler TAMB, LAB, koagulaz (+) *Staphylococcus aureus,* Koliform bakteri, Maya–Küf yönünden incelendi. Tüm örnekler istatistiksel olarak değerlendirildi.

Bulgular: Tüm peynirler 8°C'de 90 gün süreyle olgunlaştırıldı ve olgunlaştırmanın 1., 30., 60. ve 90. günlerinde peynirlerin kimyasal ve mikrobiyolojik analizleri yapıldı. Peynirlerin ortalama pH değerleri 5.62-5.76, asitlik %0.55-0.83 L.a., kurumadde %54.93-58.04, tuz %1.22-1.37, kurumaddede tuz %2,21-2,45, yağ %23,67-25,33 ve kurumaddede yağ %41.20-45.46 arasında tespit edildi. Mikrobiyolojik sayımlarda elde edilen veriler ise TAMB 5.17-5.75 log kob/g, LAB 5.49-6.98 log kob/g, Maya-Küf sayısı 3.66-4.88 log kob/g şeklinde bulundu. Araştırmada *Staphylococcus aureus* ve Koliform grubu bakteriye rastlanılmadı. Sonuç olarak üretilen peynirlerin titrasyon asitliğinde önemli farklılık bulundu (P<0.05). Fakat kurumadde, yağ, tuz ve mikrobiyolojik sonuçlar üzerinde olgunlaşma süresince istatistiki olarak fark tespit edilmedi.

Öneri: Kaşar peyniri üretiminde starter kültür kullanılmadan üretim yapılabileceği ve olgunlaşma sağlanabileceği ifade edilebilir.

Anahtar kelimeler: Kaşar peyniri, starter olmayan laktik asit bakterileri, kimyasal, mikrobiyolojik.

Abstract

Aim: It is aimed to research the change in chemical and microbiological properties of Kashar cheese produced without starter culture during ripening period.

Materials and Methods: Fat rate of the raw cow milk was set as 3.0% and was heated to 37°C and enzyme was added. After coagulum processing, press and fermantation stages, 0.1% of emulsifying salts and 1.5% of salt was added. Cheese was produced through a five-minute melting process at 65°C. Cheese was packaged by vacuuming and was ripened at 8°C for 90 days. In samples, dry matter was conducted with gravimetric, fat with Gerber, salt with Mohr and titration acidity with % lactic acidity. pH levels were tested by Nell pH meter. Samples are examined and evaluated statistically from the point of the TAMB, LAB, coagulase (+) *S. aureus,* coliform bacteria and mould and yeast.

Results: Cheeses were ripened at 8°C for 90 days and analyses were exercised on ripening period. According to results, pH value was found between 5.62-5.76, acidity 0.55-0.83L.a., dry matter 54.93-58.04%, salt 1.22-1.37%, salt in dry matter 2.21-2.45%, fat 23.67-25.33% and fat in dry matter 41.20-45.46%. Mean values of the enumeration of TAMB were determined between 5.17-5.75 log cfu/g, LAB 5.49-6.98 log cfu/g, mould-yeast 3.66-4.88 log cfu/g. *S. aureus* and Coliform group microorganisms were also analyzed, but weren't detected. In this study samples were assessed and a difference was determined on titratable acidity (P<0.05), but no differences were determined on the others.

Conclusion: It may be concluded that Kashar cheese can be produced without using starter culture and can be ripened.

Keywords: Kashar cheese, non-starter lactic acid bacteria, chemical, microbial.

Introduction

It is estimated that there are more than 4000 types of cheese all over the world, some of which are produced commercially in large amount and some are produced and marketed locally. In Turkey, there are nearly fifty types of cheese and with local production this number increase to a hundred. Kashar cheese is the second most produced cheese type after the white pickled cheese in Turkey (Üçüncü 2005). Kashar cheese is a solid product obtained from cow, sheep, goat or water buffalo milk or a mixture of them after they are pasteurized by a suitable technique and enriched by some additives if necessary and it has a distinctive flavor, taste, smell and colour and can be consumed after or before ripening process (Anonymous 2006). Besides, it is described as ripen (old) Kashar cheese and unripe (fresh) Kashar cheese. It is also divided into three types as whole-fat cheese, fat cheese, low-fat cheese according to its fat rate.

Proteolysis, the most complicated process of three main biochemical reactions occurring during the ripening of cheese, is probably the most significant for the flavor and texture development. During ripening, proteinases and peptidases which have a role in the proteolysis come from six main sources. These are coagulators, original enzymes of milk, starter lactic acid bacteria (LAB), non-starter lactic acid bacteria (NSLAB), secondary starters and proteinases and peptidases added into the milk or the curd in order to accelerate ripening (Fox 1989).

Microorganisms related to the ripening of cheese can be divided into two as starter LAB and secondary microflora. Secondary microflora can be divided mainly as non-starter *Lactobacilli, Pediococcus, Enterococcus* and *Leuconostoc,* propionic acid bacteria, moulds and yeasts and bacteria (Beresford and Williams 2004, Fox and McSweeney 2004).

Non-starter lactic acid bacteria include non-starter *Lactobacilli, Leuconostoc, Pedicocci* and *Enterococci* species. Non-starter *Lactobacilli* are the microorganisms which form much of the cheese population during the ripening time. NSLAB grow at a temperature of 2-53°C and at optimum 5.5-6.2 pH level and the acid tolerance of NSLAB is very high (Beresford et al 2001).

When starter LAB is not used, NSLAB population found in the milk demonstrates an increase. Studies conducted to research the role of NSLAB on Cheddar cheese have indicated that an increase in typical flavor growth and improvement and also flavor defects result directly from NSLAB (Lee et al. 1990a, b, Wilkinson et al. 1994, Fox et al 1998, Beresford et al 2001, Banks and Williams 2004).

NSLAB amount was 101 cfu/g at the beginning, but it reached to 107-108 cfu/g at the end of third month of the ripening period in Cheddar cheese. NSLAB in Cheddar cheese consisted of mesophilic homo and heterofermentative Lactobacillus spices and in addition to this, it was stated that *Pediococcus* and *Leuconostoc* species had been found in it (Peterson and Marshall 1990). Decreasing the ripening temperature of Cheddar cheese has a significant effect on NSLAB growth (Martley and Crow 1993, Shakeel-Ur-Rehmann et al 2000). When the ripening temperature was decreased to 1°C from 8°C, the amount of non-starter lactobacillus population also decreased 2 log.

NSLAB need an energy source to grow in cheese. Lactose amount in Cheddar cheese is limited, so it was detected that NSLAB consumed all of the lactose in the curd in 8-20 days. Although lactose was finished at the further stages of ripening period, the amount of NSLAB still decreased, which has revealed that lactose was not the only energy source. Other energy sources used by NSLAB were bacterial metabolites such as fat acids, amino acids and peptides appearing during the cheese ripening (Martley and Crow 1993, Waldron 1997, Fox et al 1998). In aseptic environment, flavor development of Cheddar cheese occurred without NSLAB. However, it was observed that the cheese lacked ripen flavor development and an undesirable flavor profile occurred (Crow et al 2001).

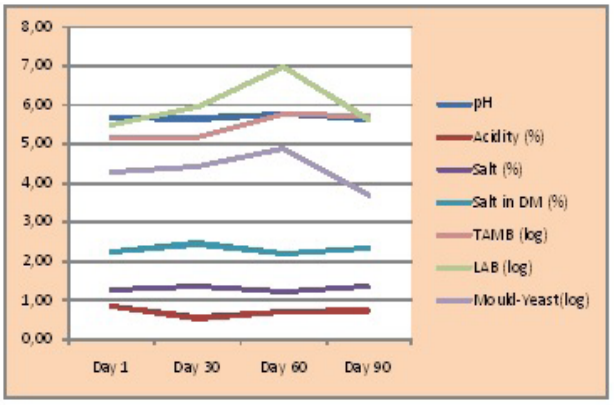

Figure 1. Changes in chemical and microbiological parameters in storage period of Kashar cheese produced without using starter culture.

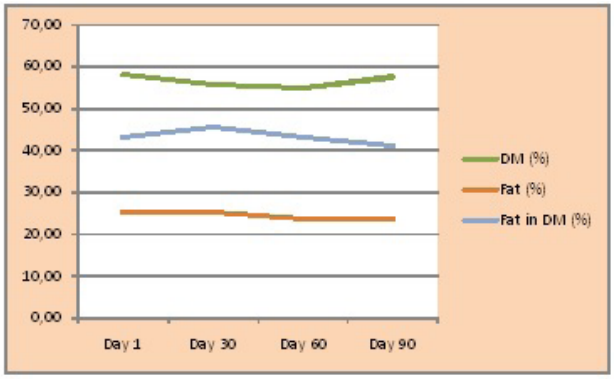

Figure 2. Changes in chemical and microbiological parameters in storage period of Kashar cheese produced without using starter culture.

Table 1. Chemical and microbiological parameters in storage period of Kashar cheese produced without using starter culture (n=3).

Parameters	Day 1	Day 30	Day 60	Day 90	F
pH	5.66±0.12	5.65±0.69	5.76±0.04	5.62±0.05	0.59
Acidity (%)	$0.83±0.11^{X}$	$0.55±0.06^{Y}$	$0.72±0.03^{XY}$	$0.73±0.07^{XY}$	2.57*
DM (%)	58.04±1.15	55.72±1.37	54.93±1.10	57.53±1.39	1.36
Salt (%)	1.28±0.13	1.37±0.09	1.22±0.09b	1.34±0.01	0.53
Salt in DM (%)	2.22±0.27	2.45±0.11	2.21±0.12c	2.32±0.07	0.50
Fat (%)	25.33±1.67	25.33±0.88	23.67±0.88	23.67±0.88	0.73
Fat in DM (%)	43.10±3.13	45.46±0.90	43.06±0.78	41.20±2.04	0.79
TAMB (log)	5.18±0.59	5.17±0.24	5.75±0.65	5.71±0.29	0.47
LAB (log)	5.49±0.48	5.95±1.05	6.98±0.31	5.64±0.16	1.23
Mould-Yeast (log)	4.26±0.01	4.43±0.20	4.88±0.45	3.66±0.83	1.10

*X,Y,Z: Different letters in the same line refers significant differences between the averages (P<0.05).

Studies on Cheddar cheese have revealed that NSLAB amount in cheese was highly affected in cheese with a cold curd and in ripen cheese. With the cooling of the cheese to 4°C after its pressing and being shaped, the number of NSLAB reached to 10^7 cfu/g in the ripening period. When NSLAB development is not desired, ripening temperature can be increased to 1°C and thus the desired result can be observed. It has been stated that the extent of the relationship between NSLAB development and cheese composition (moisture, salt, pH etc.) hasn't been determined yet (Fox and Cogan 2004).

In this study, changes occurring in the chemical and microbiological properties of cheese produced without starter culture were searched.

Materials and Methods

Fat rate of the raw cow milk used for cheese production was set as 3.0% and it was heated to 37˚C and enzyme was added. After coagulum processing, press and fermentation stages, 0.1% emulsifying salts, 1.5% salt was added. Kashar cheese was produced through a five-minute melting process at 65˚C in the process tank. After it was waited at room temperature for one day, Kashar cheese was packaged by vacuuming. The cheese which was produced monotype was ripened at 8˚C for 90 days.

In cheese samples, dry matter analysis was conducted with gravimetric method, fat analysis with Gerber method butyrometer (Van Gulik) and salt analysis with Mohr titration. Titration acidity was determined as % lactic acidity (Anonymous 2000). pH levels were tested by Nell pH meter. For TAMB count, PCA (Oxoid); for lactic acid bacteria count, MRS (LabM, Lab93); for Staphylococcus aureus, BPA (bioMerieux); for coliform bacteria group, VRBA (LabM) and for enumeration of yeast and mould, DRBC (Merck) was employed. Coagulase test (staphylase test; bioMerieux) was applied to typical colonies test for *Staphylococcus aureus,* Coagulase-positive samples were confirmed by the identification system device, VITEK II (bioMerieux, France) and counting was performed (Anonymous 2001).

For statistical analysis, SPSS 21.0 program was used. Chemical and microbiological analysis was made with Duncan test and sensory analysis was performed with Kruskal-Wallis H test. Study was conducted in three replications. P<0.05 level was accepted statistically significance.

Results

Results of chemical and microbiological analysis are demonstrated in Table 1 and the changes in the data are represented with graphics in Figure 1 and Figure 2.

Cheese samples were ripened at 8˚C for 90 days and analysis was exercised on ripening period. According to results, pH values were found between 5.62-5.76, acidity 0.55-0.83L.a., dry matter 54.93-58.04%, salt 1.22-1.37%, salt in dry matter 2.21-2.45%, fat 23.67-25.33% and fat in dry matter 41.20-45.46%. Mean values of the enumeration of TAMB were determined between 5.17-5.75 log cfu/g, LAB 5.49-6.98 log cfu/g, mould-yeast 3.66-4.88 log cfu/g. *Staphylococcus aureus* and coliform group microorganisms were also analyzed, but were not detected. In this study, samples were assessed and a significance difference was determined on titratable acidity (P<0.05), but no significance differences were determined on the others.

Discussions

Acidity development in cheese commences with coagulation and filtration and goes on during the ripening period. Acidity in cheese take shapes mostly from nitrogenous substances such as casein and paracasein, and the rest of the acidity

from the acids (lactic acid, formic acid and acetic acid) which originate from lactose and nitrogenous substances fragmented by the microbial activity of LAB and proteolytic bacteria (Şimşek 1995, Akın and Şahan 1998). NSLAB in cheese may become dominant and its amount can reach to 10^7-10^8cfu/g (Fox ve Cogan 2004). In the research, pH levels in Kashar cheese was measured as between 5.62-5.76 and acidity levels between 0.55-0.83% L.a. It has been stated that this fluctuation at pH levels was caused by acidity and proteolysis occurring after the microbial activity and the formation of buffering molecules originating from the fragmentation of macromolecule proteins (Guinee and Fox 1993).

According to the results TAMB number was between 4.67-6.25 log cfu/g and the TAMB number in the first day was observed lower than the TAMB number determined by Nizamlıoğlu et al (1996), Atasever et al (2003), Çetinkaya and Soyutemiz (2006), Sert et al. (2007), Temiz (2010) and Tunçtürk et al. (2010). Later, the number of TAMB was reached determined by the researchers during the ripening period. It was determined that pH levels measured in the research was related to not using starter culture in the process and the number of microorganism of the raw milk used and these results were also confirmed by Üçüncü (2005).

NSLAB results potentially from air-born transmission after pasteurization, NSLAB which do not inhibit after heating operations (Jordan and Cogan 1999) or NSLAB resistant to cleaning and disinfection on production equipment (Somers et al 2001). It is stated that NSLAB number which infects the curd produced by using the milk pasteurized appropriately through modern production technique is $<10^3$ cfu/g on average in 1 day, but after a 3-month ripening period, NSLAB becomes dominant and reaches to the values of 10^7-10^8 cfu/g (Fox and Cogan 2004).

Fırat (2006) examined some properties of fresh and old Kashar cheese during ripening period and determined that the number of LAB of cheese produced without starter culture is 6.90 log cfu/g. Keçeli et al (2006) have stated that LAB amount in the two types of Kashar cheese produced from raw milk by traditional techniques are 7.08-7.40 log cfu/g and 5.25-5.76 log cfu/g. Sert et al (2007) produced Kashar cheese from raw milk without using starter culture, in this cheese mesophilic bacteria number was measured as between 3.58-4.57 log cfu/g for 1st day and between 3.58-4.64 log cfu/g for 90th day. According to Poullet et al (1991) and Tornadijo et al (1995), LAB activity was higher at the beginning of ripening because of the abundance of food, high water content and low acid concentration. Water amount decreases as a result of microbial activity, salt and acidity concentration increases and also LAB number decreases towards the end of the ripening period. In this present research mentioned situation occurred during the ripening period and LAB number decreased in 90th day.

In this research, a significant difference found in terms of titration acidity ($P<0.05$). As starter culture wasn't used in Kashar cheese production, it was observed that microbiological values were lower when compared to the values found in other studies.

Conclusion

It can be stated that Kashar cheese can be produced without using starter culture. Besides, non-starter lactic acid bacteria transmitted in the obtaining process of milk and from the atmosphere of production place have a positive effect on the ripening period.

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References

Akın SM, Şahan N. Şanlıurfa'da üretilen taze urfa peynirlerinin kimyasal ve duyusal özelliklerinin belirlenmesi üzerine bir araştırma. V. Süt ve Süt Ürünleri Sempozyumu, 21-22 Mayıs 1998, Ankara,Türkiye, pp:315.

Anonymous 2000. Official Methods of Analysis of the Association of Official Chemists. Acidity of Milk AOAC Official Method 947.05, Chapter 33, 17th edition, USA.

Anonymous 2001. Food and Drug Administration, Bacteriological Analytical Manual, New Hampshire, USA, pp:222-228.

Anonymous 2006. The Turkish Standards Institute. TS-3272:2006, Kashar cheese standard, Ankara, Türkiye, pp:2.

Atasever M, Uçar G, Keleş A, Köse Z, 2003. The effects of natural smoke and liquid smoke applications on the quality factors of Kashar cheese. Turk J Vet Anim Sci, 27, 781-787.

Banks JM, Williams AG, 2004. The role of the nonstarter lactic acid bacteria in Cheddar cheese ripening. Int J Dairy Technol, 57, 145-152.

Beresford T, Williams A, 2004. The microbiology of cheese ripening, In: Cheese: Chemistry, Physics and Microbiology, Volume 1: General Aspects, 3th edition, Elsevier Ltd, USA, pp: 287-317.

Beresford TP, Fitzsimons NA, Brennan, NL, Cogan TM, 2001. Recent advances in cheese microbiology. Int Dairy J, 11, 259-274.

Çetinkaya F, Soyutemiz GE, 2006. Microbiological and chemical changes throughout the manufacture and ripening of Kashar: A traditional Turkish cheese. Turk J Vet Anim Sci, 30, 397-404.

Crow V, Curry B, Hayes M, 2001. The ecology of non-starter lactic acid bacteria and their use as adjuncts in New Zea-

land Cheddar. Int Dairy J, 11, 275-283.

Fırat N, 2006. Çiğ ve pastörize sütten üretilen kaşar peynirlerinin olgunlaşma süresince bazı mikrobiyolojik, fiziksel ve kimyasal özelliklerinin belirlenmesi. Yüksek Lisans Tezi, Atatürk Üniversitesi Fen Bilimleri Enstitüsü, Erzurum, Türkiye.

Fox PF, 1989. Proeolysis during cheese manufacture and ripening. J Dairy Sci, 72, 1379-1400.

Fox PF, Cogan TM, 2004. Factors that affect the quality of cheese, In: Cheese: Chemistry, Physics and Microbiology, Volume 1: General aspects, 3th edition, Elsevier Ltd, USA, pp: 583-608.

Fox PF, McSweeney PLH, 2004. Cheese: An overview, In: Cheese: Chemistry, Physics and Microbiology, Volume 1: General aspects, 3th ed., Elsevier Ltd, USA, pp: 1-18.

Fox PF, McSweeney PLH, Lynch CM, 1998. Significance of non-starter lactic acid bacteria in Cheddar cheese. Aust J Dairy Technol, 53, 83-89.

Guinee TP, Fox PF, 1993. Cheese: Chemistry, Physics and Microbiology, General Aspects, Volume 1, Chapman and Hall, USA, pp: 257-302.

Jordan KN, Cogan TM, 1999. Heat resistance of Lactobacillus spp. isolated from Cheddar cheese. Lett Appl Microbiol, 29, 136-140.

Keçeli T, Şahan N, Yaşar K, 2006. The effect of pre-acidification wilt citric acid on reduced-fat kashar cheese. Aust J Dairy Technol, 61, 32-36.

Lee BH, Laleye LC, Simard RE, Holley RA, Emmons DB, Giroux RN, 1990a. Influence of homofermentative lactobacilli on physicochemical and sensory properties of Cheddar cheese. J Food Sci, 55, 386-390.

Lee BH, Laleye LC, Simard RE, Munsch MH, Holley RA, 1990b. Influence of homofermentative lactobacilli on the microflora and soluble nitrogen components in Cheddar cheese. J Food Sci, 55, 391-397.

Martley FG, Crow VL, 1993. Interactions between nonstarter microorganisms during cheese manufacture and ripening. Int Dairy J, 3, 461-483.

Nizamlıoğlu M, Gürbüz Ü, Doğruer Y, 1996. The effects of potassium sorbate on the chemical and microbiological quality in Kashar Cheese. Eurasian J Vet Sci, 12, 23-29.

Peterson SD, Marshall RT, 1990. Nonstarter lactobacilli in cheddar cheese: A review. J Dairy Sci, 73, 1395-1410.

Poullet B, Huertas M, Sa'nchez A, Ca'ceres P, Larriba G, 1991. Microbial study of Casar de a'ceres cheese throughout ripening. J Dairy Res, 58, 231-238.

Sert D, Ayar A, Akın N, 2007. The effects of starter culture on chemical composition, microbiological and sensory characteristics of Turkish Kashar cheese during ripening. Int J Dairy Technol, 60, 245-252.

Shakeel-Ur-Rehmann, Banks JM, McSweeney PLH, Fox PF, 2000. Effect of ripening temperature on the growth and significance of non-starter lactic acid bacteria in Cheddar cheese made from raw or pasteurised milk. Int Dairy J, 10, 45–53.

Şimşek B, 1995. Ankara piyasasında satılan beyaz peynirlerin proteoliz düzeyi üzerinde bir araştırma. Yüksek Lisans Tezi, Ankara Üniversitesi Fen Bilimleri Enstitüsü, Ankara, Türkiye.

Somers EB, Johnson ME, Wong ACL, 2001. Biofilm formation and contamination of cheese by nonstarter lactic acid bacteria in the dairy environment. J Dairy Sci, 84, 1926-1936.

Temiz H, 2010. Effect of modified atmosphere packaging on characteristics of sliced Kashar cheese. J Food Process Pres, 34, 926-943.

Tornadijo ME, Fresno JM, Bernardo A, Martin R, Carballo J, 1995. Microbiological changes throughout the manufacturing and ripening of Spanish goat's raw milk cheese (Armada variety). Lait, 75, 551-570.

Tunçtürk Y, Ocak E, Zorba Ö, 2010. The effect of different homogenization pressures on chemical, biochemical, microbiological, and sensorial properties of Kashar cheese. YYÜ J Agr Sci, 20, 88-99.

Üçüncü M, 2005. Süt ve Mamulleri Teknolojisi, Ege Üniversitesi Mühendislik Fakültesi Gıda Mühendisliği Bölümü Yayınları, İzmir, Türkiye, pp:185-191.

Waldron DS, 1997. Effect of lactose concentration on the quality of Cheddar cheese. MSc Thesis. National University of Ireland, University College Cork, Ireland.

Wilkinson MG, Guinee TP, O'Callaghan DM, Fox PF, 1994. Autolysis and proteolysis of different trains of starter bacteria during cheese ripening. J Dairy Res, 61, 249-262.