doi: 10.1111/j.1471-0307.2009.00546.x

ORIGINAL RESEARCH

A comparison of the physicochemical, microbiological and aromatic composition of Traditional and Industrial Leben in Tunisia

OLFA SAMET-BALI,[1] AMOR BELLILA,[2] MOHAMED-ALI AYADI,[1]* BRAHIM MARZOUK[2] and HAMMADI ATTIA[1]

[1]*Unité Analyses Alimentaires, Ecole Nationale d'Ingénieurs de Sfax, Route de Soukra 3038 Sfax, and* [2]*Unité des Plantes Aromatiques et Médicinales, INRST-BP 95, 2050 Hammam-Lif, Tunisia*

Traditional Tunisian Leben (TL) was produced according to the traditional method. Physicochemical and microbiological characteristics and major aromatic compounds were studied and compared to industrial Leben (IL) and experimental Leben (EL). The results show a decrease in lactose content and pH value and an increase in lactic acid during spontaneous fermentation. TL and EL were characterised by higher protein, lactose and ash contents but were less fatty and acidic than IL. Lactic acid bacteria (LAB) and yeasts present in TL were responsible for lactic acid fermentation and aroma development. The LAB and yeast counts in TL were higher than that in EL and IL. Dynamic headspace extraction procedure shows the existence of four major volatile compounds: acetaldehyde, ethanol, diacetyl and acetoin in TL, IL and EL. However, TL has the most important quantity of aroma.

Keywords Leben, Analytical data, Spontaneous fermentation, Physicochemical, Microbiological, Volatile compounds.

INTRODUCTION

The increasing interest in fermented dairy products has been due to the nutritional value and taste of these products. The food industry has introduced several types of new dairy products, but traditional fermented milks are still consumed, especially for their typical flavours and texture.

In Tunisia, traditional Leben (TL) is produced by spontaneous fermentation of milk with natural microflora. A weak protein gel develops due to a decrease in the pH of milk during the conversion of lactose to lactic acid by non-starter lactic acid bacteria (LAB). Microbiological studies have revealed that many species of mesophilic LAB, mostly of the genus *lactococcus* and *Leuconostoc*, and several yeasts species, especially species of the genera *Saccharomyces* and *Candida*, contribute to the spontaneous fermentation process (Tantaoui-Elaraki *et al.* 1983; Tantaoui-Elaraki and El Marrakchi 1987; Guizani *et al.* 2001; Benkerroum and Tamine 2004). Growth of LAB leads to production of many desirable aroma and flavour compounds in fermented milks (Steele and Unlu 1992). Among these compounds are nonvolatile acids, volatile acids, carbonyl compounds and a heterogeneous group of substances formed during degradation of proteins, fats and lactose (Tamine and Robinson 1990). Yeasts also synthesise volatile compounds that contribute to the flavour of traditional fermented milks (Tantaoui-Elaraki and El Marrakchi 1987).

Extraction of volatile compounds can be achieved using various techniques, such as distillation, solid phase micro-extraction and headspace techniques. Park (1993) reported that headspace analysis may include purging the sample and trapping volatile compounds into a porous polymer adsorbent prior to gas chromatographic (GC) separation. This analytical approach is known as dynamic headspace (DHS) sampling.

Although industrial processes are used to produce safe fermented milk and to provide the product with standard characteristics, consumers prefer TL due to its organoleptic quality (fresh and sour taste and characteristics aroma).

The objective of this study was the production of TL according to the traditional method and the determination of its physicochemical, microbiological and aroma characteristics (major volatile compounds) compared to IL and EL.

MATERIALS AND METHODS

Milk samples

Cows' milk (Holstein breed) was obtained from a private farm in the southern part of Tunisia. Samples of cows' milk were collected, kept refrigerated (4°C) and transported to our laboratory within 6 h. A sample was taken from 20 to 25 animals.

*Author for correspondence. E-mail: ayadimedali@yahoo.fr

SDT International Journal of Dairy Technology

Fermented milk preparation

TL production

Five litres of raw milk was left spontaneously at 25 ± 2°C for coagulation, requiring up to 18 h (Figure 1a). During the gelation step, the product is called rayeb. By churning for 40 min, the rayeb is separated into an aqueous fraction yielding Leben and a fatty fraction called raw butter. Churning takes place in a leather bag called a checoua. The checoua is manufactured from a goat in one piece; the openings of the leather are subsequently tied up with a string to avoid leakage when filled. The churning is achieved by hanging the checoua filled with rayeb to a wooden tripod or to a cottage roof and vigorously shaking it back and forth until the fat globules coalesce.

EL production

The stages of the manufacture of experimental Leben were identical to the manufacture of TL. The only difference lies in the churning of the fermented milk which was carried out in a flask.

IL production

Industrial Leben samples were produced by adding mesophilic starter cultures (industrial starter cultures) of *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *diacetylactis* and *Lactococcus lactis* subsp. *cremoris* (Rhodia, France) to pasteurised (82°C/3 min) and standardised milk (15 g/L of fat) at 27°C during 12 h of fermentation (Figure 1b).

After production, samples were stored at 4°C and packed in glass. The fermentation process was triplicated for each sample.

Physicochemical analysis

Total nitrogen (TN) and noncasein nitrogen (NCN) contents of the Leben were determined using the Kjeldahl method (AFNOR 1993) using a Büchi 325 apparatus (Büchi, Flawil, Switzerland). The total casein content was calculated by difference between TN and NCN after separation according to Rowland (1938). Dry matter, ash, lactose and fat contents were determined according to standard methods (AFNOR 1993). Titratable acidity, expressed in Dornic degrees, was determined by titration of the sample with N/10 sodium hydroxide to the pink endpoint using phenolphthalein as indicator (AFNOR 1993). The pH was determined using a pH meter (METTLER TOLEDO MP 220) calibrated with standard buffer solutions at pH 4.0 and 7.0.

Enumeration of micro-organisms

The number of viable mesophilic LAB and yeasts expressed as colony-forming units per millilitre (cfu/mL) was estimated. Sample preparation and decimal dilutions were made according to the International Dairy Federation (IDF) Standard method (IDF 1992). MRS medium (Difco, Detroit, MI, USA) was used for counting LAB of Leben: *Lactococcus lactis* and *Leuconostoc*. Plates were incubated at 30°C for 48 h (Garrote *et al.* 2001). Yeasts were enumerated in Sabouraud dextrose agar after incubation at 30°C for 3 days (Tantaoui-Elaraki *et al.* 1983).

Extraction and optimisation of volatile compounds

Volatile compound extraction was performed by the DHS procedure (Dhifi *et al.* 2005), whose operating conditions needed to be optimized. IL was used to perform this optimisation due to its standard characteristics as an industrial product. Different quantities (35, 40 and 50 mL) of the sample were put into a 120 mL Drechel gas washing bottle with a porous distributor. Volatiles were stripped with nitrogen (0.2 bar, 36°C) for 30, 60, 90 and 120 min, trapped on 40, 50 and 60 mg of activated charcoal (0.5–0.85 mm, 20–35 mesh

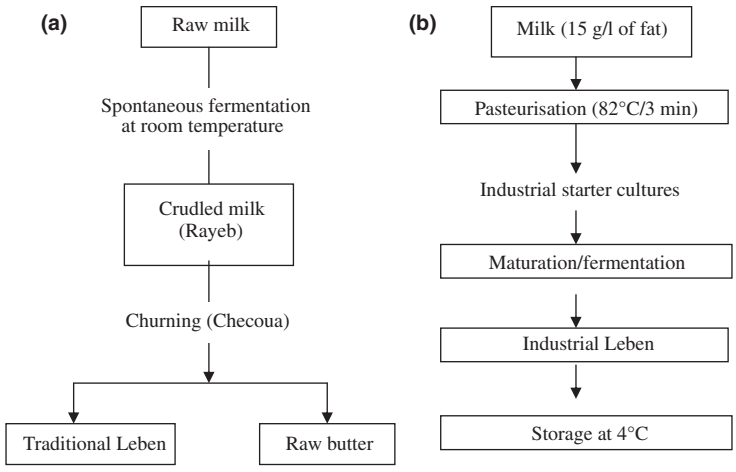

Figure 1 Schematic preparation of fermented milk in Tunisia; (a) traditional process, (b) industrial process.

ASTM) from E. Merck (Schuchardt, Germany), at 34, 36, 38 and 40°C and eluted with 1 mL of diethyl ether. Ten microlitre of hexanol was used as internal standard and added for the quantification of volatile compounds. After the optimisation of the DHS extraction process with IL sample, this procedure was used to detect major volatile compounds present in TL and EL.

An aroma extraction kinetic curve shown in Figure 2 was obtained using different extraction times. Increasing volatile compound extraction during the first 90 min was observed. Based on this optimisation process, the optimal conditions of volatile compound extraction were fixed as follows: 50 mL of Leben samples, headspace extraction time: 90 min, extraction temperature: 36°C and activated charcoal amount: 50 mg.

Gas chromatography analysis

Volatile compounds were analysed by GC, using a Hewlett-Packard 6890 gas chromatograph (Palo Alto, CA, USA) equipped with a flame-ionisation detector and an electronic pressure control injector. A polyethylene glycol fused silica capillary column (HP-Innowax: 30 m × 0.25 mm ID, 0.25 μm film thickness) purchased from Agilent (Wilmington, DE, USA) was used. The carrier gas flow (N_2) was 1.6 mL/min. The split ratio in the injector was 60:1. The detector and injector temperatures were held at 275 and 250°C respectively. GC oven temperature was kept at 35°C for 10 min. Aroma compounds were identified by comparing their retention time with those of authentic standards analysed under the same analytical conditions.

Gas chromatography-mass spectrometry (GC-MS) analysis

The volatiles were analysed by GC-MS (HP 5890 (II) gas chromatograph). The compounds were separated by HP-5MS 5% phenyl methyl silicone and 95% dimethyl polysiloxane capillary column (30 m × 0.25 mm, 0.25 μm). The oven temperature was programmed to rise from holding times at 50°C and 240°C at a rate of 5°C/min. The injection port was heated at 250°C. The carrier gas was He with a flow ratio of 1.2 mL/min; the split ratio was 60:1. Detection was performed with the mass spectrometer detector (HP 5972, mass spectrometer) operating at a scan rate of 3.81 scans/s and the ionisation energy set at 70 eV. The temperatures of the ion source and the quadrupole mass analyser were held at 230°C and 150°C respectively. The identification of volatile compounds was made by their retention times and by comparison of their mass spectra with those in the Wiley Mass Spectral database (Wiley & Sons Inc., New York, NY, USA).

Statistical analysis

The fermentation process was triplicated and duplicate analyses were performed on each replicate. Values of different tests were expressed as the mean ± standard deviation (x ± SD). The SPSS packet program for Windows (SPSS, version 11, Chicago, IL, USA) was used for the statistical analysis. Significant differences between mean ($P < 0.05$) were determined by using a one-way ANOVA (Duncan's test).

RESULTS AND DISCUSSION

Physicochemical and microbiological analysis

Table 1 presents the physicochemical analysis of milks TL, IL and EL. The chemical characteristics of the milks show suitable technological properties. Table 1 shows that for chemical composition, significant ($P < 0.05$) differences were observed between TL, IL and EL. Indeed, TL and EL were characterised by higher protein, lactose and ash contents but were less fatty and acidic than IL. This distinction could be explained by the difference in the method employed for the production of TL, IL and EL. The results show also that lactose content and pH value decrease significantly ($P < 0.05$), whereas lactic acid value increases during spontaneous fermentation of milk. These results were similar to those reported by Tantaoui-Elaraki *et al.* (1983) in the traditional Marroccan Leben and Abd-El-Malek (1978) in the traditional Egyptian one. The decrease in fat content in TL and EL

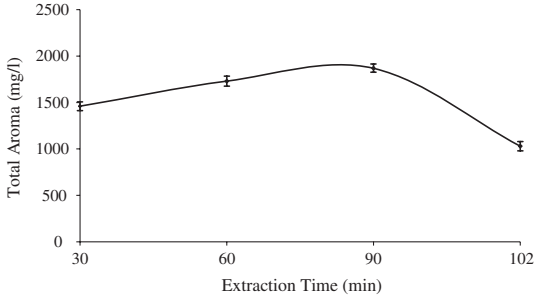

Figure 2 Aroma extraction kinetic of Industrial Leben (IL) Sample volume: 50 mL, Headspace extraction time: 30, 60, 90 and 120 min, extraction temperature: 36°C, activated charcoal amount: 50 mg.

samples is due to the churning process of the fermented milks. The pH was about 4–4.5, within the range considered optimal for aroma development in fermented milks as reported by Murti *et al.* (1992).

Table 2 summarises the microbial counts obtained from TL, EL and IL. The LAB and yeast counts in TL were higher ($P < 0.05$) than those in EL and IL. Mesophilic LAB are the main microflora responsible for lactic acid fermentation and aroma development in fermented milks (Stien *et al.* 1999). Menéndez *et al.* (2000) reported that mesophilic *lactococci* have been used to improve the acidification and organoleptic characteristics of fermented milk. Benkerroum and Tamine (2004) reported that mesophilic LAB: *Lactococcus* and *Leuconostoc* species, predominant in traditional Moroccan Leben, contribute the strong buttermilk flavour that characterises the product. The predominance of mesophilic LAB (lactococci) was also reported in Omani traditional Leben (Guizani *et al.* 2001). The yeast counts were lower than the counts for LAB bacteria. Jacobsen and Narvhus (1996) reported that yeasts produce valuable nutrients (vitamins, essential amino-acids) and various aroma compounds such as diacetyl, acetaldehyde, methyl ketone, and ethanol. Benkerroum and Tamine (2004) reported that in traditional Moroccan Leben, yeasts were recovered towards the end of the fermentation stage, and suggest that they play a role in the aroma development in the product.

Physicochemical and microbiological properties of TL revealed that mesophilic LAB and different yeast strains present in Leben were the main agent of milk acidification. In this fermentation process, lactic acid was the major compound found, and a variety of aroma compounds could be produced**.**

Aroma compounds

Four major volatile compounds were found: ethanol, acetaldehyde, acetoin and diacetyl in the three fermented milks, but at different levels (Table 3). TL has the most important quantity of aroma (1684.74 mg/L) compared to the other samples: 1246.06 mg/L in EL and 696.51 mg/L in IL. This difference could be explained by the role of the checoua in the liberation of Leben volatile compounds. Ethanol was the first principal component present in all Leben types. The mean concentration of acetaldehyde was higher in TL (45.64 mg/L) and IL (38.62 mg/L) than in EL (26.98 mg/L). On the other hand, the level of diacetyl was higher ($P < 0.05$) in EL (86.26 mg/L) than in TL (10.92 mg/mL) and IL (13.6 mg/L). All Leben samples showed a significant acetoin content. In

Table 1 Physicochemical composition (g/kg) of milk, industrial Leben, traditional Leben and experimental Leben (mean[a] ± SD)

		Leben		
Parameters	*Milk*	*Industrial*	*Traditional*	*Experimental*
Dry matter	117.13 ± 0.28[a]	73.97 ± 0.27[b]	70.54 ± 1.46[b]	72.90 ± 0.6[b]
Total nitrogen	33.41 ± 0.65[a]	22.36 ± 0.40[b]	32.10 ± 0.40[c]	31.30 ± 0.28[c]
Caseins	26.71 ± 0.53[a]	18.65 ± 0.13[b]	25.62 ± 1.38[a]	22.84 ± 0.47[a]
Fat	34.50 ± 0.54[a]	14.83 ± 0.98[b]	3.50 ± 0.31[c]	7.20 ± 0.80[c]
Lactose	41.37 ± 0.48[a]	19.01 ± 0.15[b]	25.90 ± 0.41[c]	24.84 ± 0.86[c]
Ash	8.25 ± 0.08[a]	6.77 ± 0.01[b]	7.28 ± 0.14[c]	7.54 ± 0.37[c]
pH	6.70 ± 0.03[a]	4.29 ± 0.06[b]	4.45 ± 0.04[b]	4.40 ± 0.60[b]
Lactic acid	1.66 ± 0.03[a]	7.05 ± 0.36[b]	6.55 ± 0.10[c]	6.94 ± 0.47[c]

[a]Mean are average from two independent trials.

Different superscript alphabets indicate significant differences ($P < 0.05$) between samples.

Table 2 Counts of the different microbial groups (cfu/mL) in industrial Leben, traditional Leben and experimental Leben (Mean[a] ± SD)

	Leben		
Parameters	*Industrial*	*Traditional*	*Experimental*
Lactic acid bacteria (10^6)	1.58 ± 0.12[a]	117.48 ± 0.33[b]	12.02 ± 0.02[c]
Yeasts (10^4)	2.18 ± 0.32[a]	1995.26 ± 0.21[b]	478.63 ± 0.40[c]

[a]Mean are average from two independent trials.

Different superscript alphabets indicate significant differences ($P < 0.05$) between samples.

fermented dairy products, the variation in levels of flavour compounds has been attributed to the use of different strains of LAB, as reported by Chammas *et al.* (2006). Indeed, IL was prepared with selected starters such as *Lactococcus lactis lactis*, *Lactococcus lactis diacetylactis* and *Lactococcus cremoris*; whereas TL and EL were obtained after spontaneous fermentation with natural microflora such as *Lactococcus lactis*, *Lactococcus cremoris* and *Leuconostoc* strains (results submitted for publication). Benkerroum and Tamine (2004) reported that ethanol, acetaldehyde, acetoin and diacetyl were also the main aroma compounds in Moroccan Leben.

Bottazi and Dellaglio (1967) reported that diacetyl, lactic acid and acetaldehyde contribute most to the final flavour. Their concentration and relative levels determine the quality of the product and its acceptance by consumers. This could explain the preference of Tunisian consumers for the traditional product (TL).

Evolution of volatile compounds during the manufacture of TL

Table 4 shows the evolution of volatile compounds during the ripening periods of TL. Several quantitative changes were observed. Acetaldehyde began to appear after 15 h of fermentation time and increased slightly. This variation was probably attributable to the fact that the acetaldehyde originated from different reactions. This result confirmed that reported by Valero *et al.* (2001). Ethanol increased considerably during the manufacture of TL. This could be formed not only by reduction from the corresponding aldehydes (Nursten 1997) but also by lactose fermentation with LAB and yeasts. The amounts of diacetyl and acetoin were important at the beginning (20.86 and 20.35 mg/L respectively), increased (43.62 and 762.40 mg/L respectively) during fermentation then decreased (10.92 mg/L for diacetyl and 290.97 mg/L for acetoin) in the final product. This result could be explained by the passage of diacetyl and acetoin, characterised by buttery notes, during the churning by the checoua in the fatty fraction (raw butter).

Effects of LAB and yeasts on the production of volatile compounds

Acetaldehyde (ethanal) was likely to have an important impact on the aroma of investigated Leben due to its low threshold values (Nogueira *et al.*

Table 3 Major volatile compounds of industrial Leben, traditional Leben and experimental Leben (Mean[a] ± SD)

	Leben		
Compound (mg/L)	*Industrial*	*Traditional*	*Experimental*
Ethanol	343.23 ± 12.6[a]	1337.21 ± 201.81[b]	781.06 ± 132.5[c]
Acetaldehyde	38.62 ± 2.1[a]	45.64 ± 9.59[b]	26.98 ± 5.12[c]
Diacetyl	13.6 ± 1.2[a]	10.92 ± 1.31[b]	86.26 ± 5.45[c]
Acetoin	301.06 ± 57.20[a]	290.97 ± 85.25[b]	351.76 ± 7.25[c]
Total aroma	696.51[a]	1684.74[b]	1246.06[c]

[a]Mean are average from two independent trials.

Different superscript alphabets indicate significant differences ($P < 0.05$) between samples.

Table 4 Evolution of the major volatile aroma compounds during the manufacture of traditional Leben (Mean[a] ± SD)

Compound (mg/L)	*A*	*B*	*C*	*D*
Ethanol	+	50.76 ± 1.14[a]	866.87 ± 18.36[b]	1337.21 ± 201.81[c]
Acetaldehyde	−	+	38.05 ± 3.35[a]	45.64 ± 9.59[a]
Diacetyl	−	20.86 ± 4.96[a]	43.62 ± 5.73[b]	10.92 ± 1.31[c]
Acetoin	+	20.35 ± 6.46[a]	762.40 ± 33.24[b]	290.97 ± 85.25[c]

A: milk after 10 h fermentation time.

B: milk after 15 h fermentation time.

C: fermented milk just before churning (18 h fermentation time).

D: TTL (fermented milk after churning).

+: compound detected as traces.

−: compound was absent.

[a]Mean are average from two independent trials.

Different superscript alphabets indicate significant differences ($P < 0.05$) between samples.

2005). Acetaldehyde production, resulting from the activity of LAB, depends not only on total cell numbers, but rather on the capacity of cells to utilise certain precursors. Acetaldehyde is produced by LAB from lactose and threonine (Marshall and Tamine 1997), and threonine aldolase can transform threonine to acetaldehyde.

Diacetyl (2, 3-butanedione) is considered as a main flavour compound of many fermented dairy products (El Attar *et al.* 2000). Most of the knowledge of the metabolic pathways involved in citrate metabolism in LAB has been derived from *Leuconostoc* species and *Lactococcus lactis* subsp. *diacetylactis* (Bekal *et al.* 1998). Bourel *et al.* (2001) reported that the most important function of *Leuconostoc* bacteria was their ability to produce CO_2 and flavour compounds though lactose hetero-fermentation and citrate utilisation. However, α-acetolactic acid oxidative decarboxylation is thought to be the dominant mechanism for the characteristic dairy flavour of diacetyl produced by LAB of the *Leuconostoc* and *Lactococcus* genus (Rondags *et al.* 1998).

Acetoin (Methyl ketones), characterised by buttery notes, was found in Leben as it has been reported by Boubekri *et al.* (1984). The acetoin, also referred to in the literature as 3-hydroxy-2-butanone or acetyl methyl carbinol, can be derived from diacetyl metabolism (Rehman *et al.* 2000) or from pyruvate metabolism during the conversion of lactose to lactic acid. Methyl ketones were recognised as key components in the flavour of different fermented dairy products (Nogueira *et al.* 2005).

Ethanol, which was found in relatively large quantities, has been described as a flavour component of Leben. Indeed, high levels of ethanol have been reported in Leben, and quantitatively it is an important volatile compound that might contribute a typical aroma and flavour to the product (Benkerroum and Tamine 2004). Ethanol can be produced through lactose fermentation by LAB (Fox *et al.* 1995) and by yeasts (Fernandez-Garcia 1996). O'Riordan and Delahunty (2003) reported that ethanol was produced also by enzymatic reduction of acetaldehyde by LAB.

CONCLUSIONS

Mesophilic LAB were the main microflora responsible for lactic acid fermentation and aroma development in TL. However, a symbiosis between yeast and LAB has been suggested for the production of four major aroma compound detected in Leben: acetaldehyde, diacetyl, acetoin and ethanol.

Traditional fermented milks are apparently considered to be more aromatic than similar industrial products. The difference could be attributed to the types of micro-organisms, which produce different flavour compounds.

REFERENCES

Abd-El-Malek Y (1978) Traditional Egyption Dairy Fermentations. In *Global Impact of Food Microbiology (GIAM) State of the Art: GIAM and its Relevance to Development in Developing Countries*, pp 198–208. Stantan W R and Da Silva E J, eds. Kuala Lumpur: University of Malaysia Press.

AFNOR (1993) Contrôle de la qualité des produits alimentaires. In *Laits et produits laitiers*, ed. Paris: Association Française de Normalisation.

Bekal S, Diviès C and Prévost H (1998) Citrate lyases of lactic acid bacteria. *Le Lait* **78** 3–10.

Benkerroum N and Tamine A Y (2004) Technology transfer of Maroccan traditional dairy products (Lben, jben and smen) to small industrial scale. *Food Microbiology* **21** 399–413.

Bottazi V and Dellaglio F (1967) Acetaldehyde and diacetyl production by *Streptococcus thremophilus* and other lactic streptococci. *Journal of Dairy Research* **34** 109–112.

Boubekri C, Tantaoui-Elaraki A, Berrada M and Ben Kerroum N (1984) Cractérisation physico-chimique du Lben marocain. *Le Lait* **64** 436–447.

Bourel G, Henini S, Krantar K, Oraby M, Diviès C and Garmyn D (2001) Métabolisme sucre-citrate chez *Leuconostoc mesenteroides*. *Le Lait* **81** 75–82.

Chammas G I, Saliba R, Corrieu G and Béal C (2006) Characterisation of lactic acid bacteria isolated from fermented milk « laban ». *International Journal of Food Microbiology* **110** 52–56.

Dhifi W, Angerosa F, Serraiocco A, Oumar I, Hamrouni I and Marzouk B (2005) Virgin olive oil aroma: characterization of some Tunisian cultivars. *Food Chemistry* **93** 697–701.

El Attar A, Monnet C and Corrieu G (2000) Metabolism of lactose and citrate by mutants of *Lactococcus lactis* producing excess carbon dioxide. *Journal of Dairy Research* **67** 571–583.

Fernandez-Garcia E (1996) Use of headspace sampling in the quantitative analysis of artisanal cheese aroma. *Journal of Agricultural and Food Chemistry* **44** 1839–1883.

Fox P F, Singh T K and McSweeney P L H (1995) Biogenesis of flavour compounds in cheese. In *Chemistry of structure Function Relationships in Cheese*, pp 59–98. Malin E L and Tunick M H, eds. New York: Plenum Press.

Garrote L G, Abraham A G and De Antoni G L (2001) Chemical and microbiological characterisation of Kefir. *Journal of Dairy Research* **68** 639–652.

Guizani N, Kasapis S and Al-Ruzeiki M (2001) Microbiol, chemical and rheological properties of Laban (cultured milk). *International Journal of Food Science and Technology* **36** 199–205.

IDF (1992) Milk and milk products. In *Preparation of Samples and Dilutions for Microbiological Examination*, ed. Brussels: International Dairy Federation.

Jacobsen M and Narvhus J (1996) Yeasts and their possible role beneficial and negative effects on the quality of dairy products. *International Dairy Journal* **6** 755–768.

Marsall V M E and Tamine A Y (1997) Physiology and biochemistry of fermented milks. In *Microbiology and Biochemistry of Cheese and Fermented Milk*, pp 153–192. Law B A, ed. London: Blackie Acad. & Profess.

Menéndez S, Centeno J A, Godinez R and Rodriguez-Otero J L (2000) Effects of *Lactobacillus* strains on the ripening and organoleptic characteristics of Arzua-Ulloa cheese. *International Journal of Food Microbiology* **59** 37–46.

Murti T W, Bouillanne C, Landon M and Desmazeaud M J (1992) Bacterial growth and volatile compounds in yoghurt-type products from soymilk containing *Bifidobacterium* ssp. *Journal of Food Science* **58** 153–157.

Nogueira M C L, Lubachevsky G and Rankin S A (2005) A study of the volatile composition of Minas cheese. *LWT-Food Science and Technology* **38** 555–563.

Nursten H E (1997) The favour of milk and dairy product: I. Milk of different kinds, milk powder, butter and cream. *International Journal of Dairy Technology* **50** 48–56.

O'Riordan P J and Delahunty C M (2003) Characterisation of commercial Cheddar cheese flavour. 2: study of Cheddar cheese discrimination by composition, volatile compounds and descriptive flavour assessment. *International Dairy Journal* **13** 371–389.

Park P S W (1993) Los of volatile lipid oxidation products during thermal desorption in dynamic headspace-capillary gas chromatography. *Journal of Food Science* **58** 220–222.

Rehman S, McSweeney P L H, Banks J M, Brechany E Y, Muir D and Fox P F (2000) Ripening of Cheddar cheese made from blends of raw and pasteurized milk. *International Dairy Journal* **10** 33–44.

Rondags E, Germain P and Marc I (1998) Cinétiques de décarboxylation oxydative extra- et intracellulaire d' α-acétolactate par un lactococcus lactis ssp. var. diacetylactis. *Le Lait* **78** 135–143.

Rowland S J (1938) The determination of the nitrogen distribution of milk. *Journal of Dairy Research* **9** 42–46.

Steele J L and Unlu G (1992) Impact of lactic acid bacteria on cheese flavor development. *Food Technology* **46** 128–136.

Stien G, Blanchard F, Rondags E and Marc I (1999) Une méthode de dosage en ligne du diacétyle et de l'acétaldéhyde dans les yaourts, laits fermentés, beurres et margarines. *Le Lait* **79** 615–624.

Tamine A Y and Robinson R K (1990) *In Yogur: ciencia y Tecnologia. Acridia S. A.*, pp. 55–87. Spain: Zaragoza.

Tantaoui-Elaraki A and El Marrakchi A (1987) Study of the Maroccan dairy product: Lben and smen. *MIRCEN Journal* **3** 211–220.

Tantaoui-Elaraki A, Berrada M, El Marrakchi A and Berramou A (1983) Etude sur le Lben marocain. *Le Lait* **63** 230–245.

Valero E, Villamiel M, Miralles B, Sanz J and Martinez-Castro I (2001) Changes in flavour and volatile components during storage of whole and skimmed UHT milk. *Food Chemistry* **72** 51–58.