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Original article

Monitoring proteolysis and cheese juice composition during ripening of Cheddar cheese made from microfiltered milk

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Summary — Cheddar cheeses were made from three types of cheese milks: HTST pasteurized milk (Past), thermized milk (TH) and MF milk, a mixture of skimmilk treated according to the Bactocatch® process and pasteurized cream. Bacterial flora development of MF and TH cheeses during ripening was similar with a high growth of species belonging to the *Lb casei* group and an increase of Lactococci enumeration. On the contrary, Lc counts in Past cheeses decreased by 3 log. As expected, the mesophilic spore count was much lower in MF cheeses than in the others. Proteolysis of the three categories of cheese was followed by analysis of peptidic fragments contained in cheese water extracts and in internal cheese juice obtained by pressing and by electrophoregrams of non-degraded caseins. TCA 12% soluble nitrogen contents were very similar for all cheeses except for a significant difference, at 6 months of ripening, between Past and MF cheeses. This difference was not found in the data obtained from cheese juices. On the other hand, comparison of cheese water extracts and juices showed that juices had always higher nitrogen content than extracts. β-Casein proteolysis was significantly more pronounced in MF cheeses than in the two other cheese types, maybe, because the plasmin-plasminogene system is activated by filtration treatment. All cheeses were graded in class I but slight better organoleptic qualities were found for MF cheeses (typical Cheddar cheese flavor).

Cheddar cheese / pasteurized milks / thermization / microfiltration / proteolysis / cheese juice / NSLAB / sensorial analysis

Résumé — Suivi de la protéolyse et de la composition du jus de fromage au cours de l'affinage de fromage cheddar, obtenu à partir de lait microfiltré. Des fabrications de fromages cheddar ont été réalisées à partir de trois types de laits de fabrication : lait pasteurisé HTST (LP), lait

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thermisé (LT) et mélanges de lait écrémé microfiltré selon le procédé Bactocatch® et de crème pasteurisée (MF). L'évolution de la flore bactérienne des fromages MF et LT était similaire, avec un fort développement des espèces appartenant au groupe *Lb casei* et une croissance de la population dénombrée en Lc (au contraire des fromages LP, où cette dernière flore décroissait de près de 3 log). Comme attendu, la teneur en spores mésophiles des fromages MF était très inférieure à celle des autres fromages. La protéolyse des trois catégories de fromages a été suivie à la fois par analyse des fragments peptidiques contenus dans l'extrait aqueux et dans le jus interne obtenu par pressage et par le suivi électrophorétique des caséines non dégradées. Les teneurs en fractions azotées solubles dans le TCA 12% étaient très similaires, avec toutefois une différence significative, à 6 mois d'affinage, entre fromages LP et fromages MF. Cette différence n'était pas retrouvée lors de l'analyse des composants azotés des jus. Sur un autre plan, la comparaison des extraits aqueux et des jus montrait que ces derniers contenaient systématiquement plus de matériel azoté que les extraits aqueux. La protéolyse de la caséine β était significativement plus élevée dans les fromages MF que dans les deux autres catégories de fromage, peut-être en raison d'une activation accrue du système plasmine–plasminogène résultant de l'opération de microfiltration. Tous les fromages étaient classés en catégorie I mais de légères différences de qualités organoleptiques en faveur des fromages MF étaient trouvées (flaveur considérée comme typique du fromage cheddar).

cheddar / laits pasteurisés / thermisation / microfiltration / protéolyse / jus de fromage / NSLAB / analyse sensorielle

INTRODUCTION

Pasteurization of milk (72°C, 16 s) prior to Cheddar cheese making influences both the extent and characteristics of proteolysis during cheese ripening (Lau et al, 1991). The pasteurization causes slight denaturation of whey proteins and may inactivate some native milk proteases and non-starter bacteria that play a role in proteolysis. The flavor intensity and character may be different in Cheddar cheese made from pasteurized and raw or thermized milks. The indigenous microflora of milk markedly affects the quality of Cheddar cheese made from raw milk. The NSLAB (non-starter lactic acid bacteria), especially lactobacilli dominate the flora of cheese throughout much of the ripening period (Mc Sweeney et al, 1993). In the USA and Canada, most aged Cheddar cheese is made of milk that is not fully pasteurized.

Microfiltration is a membrane separation technique which is very efficient for removing microorganisms from milk. Milk is first separated into cream and skimmilk. Milk proteins and smaller molecules of skimmilk pass through the microfilter whereas microorganisms, fat globules and somatic cells are retained by the microfilter. A 99.9% reduction in raw milk bacterial load is possible by microfiltration. The average decimal reduction of bacteria equals 2.6 and is independent of the initial level of the bacterial population (Trouvé et al, 1991). Microfiltration was used to assess the significance of the NSLAB in cheese ripening without concomitant heat-induced changes (Mc Sweeney et al, 1993). These authors found that differences observed between the raw and pasteurized milk cheese were related to the elimination of the NSLAB rather than heat-induced changes in indigenous enzymes. Differences in proteolysis observed were due to peptidases of the non-starter lactobacilli.

Bouton and Grappin (1995) also used microfiltered milk to study the influence of the indigenous microflora on the physicochemical and sensory characteristics of Comté cheese. The study showed that cheeses made from raw milk gave a better

organoleptic quality than cheeses made from microfiltered milk because the indigenous microflora influenced the flavor formation. Demarigny et al (1996) studied the evolution of the main microflora during ripening of Swiss-type cheeses. The propionibacteria and the facultatively heterofermentative lactobacilli, which were in very low numbers before ripening, increased rapidly to reach their final level after 6 weeks. Indigenous microflora enumerated in microfiltered milk cheeses evolved identically, except that they started at a lower level.

The aim of this study was to make Cheddar cheese from microfiltered (MF) milk which should be of higher or equivalent sensory quality than thermized and pasteurized milk cheeses. The ripening characteristics of Cheddar cheese made from MF, thermized and pasteurized milk were compared by following protein breakdown during aging. Characterization of cheese juice obtained by using a pressing unit was also performed to follow the ripening of Cheddar cheeses.

MATERIALS AND METHODS

Milk preparation

Raw milk was separated into three volumes. One volume was skimmed and microfiltered using an Alfa-Laval MFS-7 cross-flow filtration unit (Alfa-Laval, Aarhus, Denmark) with ceramic membranes (Membralox membrane, Société des Céramiques Techniques, Tarbes, France) (pore size of membrane, 1.4 μm; membrane area, 1.4 m^2; flux, 678 L h^{-1} m^{-1}; concentration factor, 20 (36 L h^{-1} m^{-2})). Raw milk was also thermized (63 °C, 16 s) or pasteurized (72 °C, 16 s).

Cheese manufacture

Three 250-L cheese vats were used to manufacture the Cheddar cheese. Cheeses were made from microfiltered (MF), thermized and pasteurized milks with three vats and these treatments were rotated for each replication to reduce experimental error. Pasteurized cream (72 °C, 16 s) was added to microfiltered (MF) milk to standardize the fat composition (3.6%). Calcium chloride solution (Marshall Products, Madison, WI, USA) was added at a rate of 0.2 g/kg of milk (except for MF milk) and the milk was heated to 30 °C. Starter culture was previously prepared as follows: frozen concentrated culture H102 (Christian Hansen, Madison, WI, USA) was added at a rate of 1 g/kg milk and incubated for 14–16 h at 20 °C until the titratable acidity reached 0.8–0.85% lactic acid. After incubation, starter culture was added at a rate of 15 g/kg of milk. The ripening period was 60 min. Single strength calf rennet extract (Rhône-Poulenc, Marshall Products) was added at a rate of 0.2 g/kg of milk. When the coagulum set to the desired consistency (approximately 25 min after rennet addition, pH 6.3) the curd was cut with vertical and horizontal wire knives. Curd was allowed to heat at 38 °C for 30 min. Whey was drained and curd was matted together. Matted curds were cut into 25 to 40 cm slabs and turned every 20 min, maintaining a curd temperature of 32 °C. After 1 h the slabs were stacked two high. After the whey reached a pH of 5.4, the curd was milled and salted at a rate of 2.6%. Curds were placed in 12-kg rectangular loops and pressed overnight. Cheese blocks were placed into vacuum-shrink bags, vacuum sealed by a Multivac (Koch, Kansas City, MO, USA) and ripened at 7 °C for 6 months. Fifteen Cheddar cheeses were manufactured (5 MF milk cheeses, 5 thermized milk cheeses and 5 pasteurized milk cheeses).

Compositional analysis

The cheeses were analyzed at six different periods: after pressing (0 day) and 1, 2, 4, 12 and 24 weeks. A cross-sectional slice of cheese was removed from the middle of each block of cheese, cut into small blocks, placed into bag, vacuum sealed and stored at –45 °C until analysis, except for samples used for microbiological analysis. The total protein content of the cheeses was determined by the macro-Kjeldahl method (AOAC, 1990). Percentage of fat was determined by the Mojonnier method (APHA, 1985). Total solids and moisture were determined by drying the cheese samples (2 g) to a constant weight at 100 °C for 5 h. Other samples were heated for 2 days at 550 °C in a muffle furnace to determine the ash content (AOAC, 1990). Minerals were determined with an inductively coupled

plasma spectrophotometer (model 3510 ICP Spectrophotometer, Applied Research Laboratories, Sunland, CA, USA) according to the method described by St-Gelais et al (1992). Salt and chloride were measured by colorimetric titration (Chloride Analyzer 926, Corning, Medfield, MA, USA). pH of the cheeses was measured using a cheese electrode. Titratable acidity of the cheeses was determined by adding 10 g of cheese to 105 mL of distilled water, homogenized with a Stomacher (Seward Lab, London, UK). Twenty-five mL of the homogenate were titrated with 0.11 N NaOH using a pHmeter equipped with a titrator (Radiometer, Copenhagen, Denmark). Titratable acidity was expressed as percentage lactic acid content of cheese by weight. Sugars and organic acids in Cheddar cheeses were measured by HPLC (Biorad Laboratories, Richmond, CA, USA) using an Ion-300 column (Mandel Scientific Co, Rockwood, ON, Canada) with 0.02 N H_2SO_4 as the mobile phase at a flow rate of 0.4 mL /min using a refractive index detector and a UV detector (210 nm).

Microbiological analysis

Total bacteria, coliforms and non-starter lactic acid bacteria (NSLAB) counts in MF, thermized and pasteurized milks were determined in duplicate on the appropriate media. During ripening, counts of total flora, mesophilic spores, starters and NSLAB were determined in duplicate after the cheeses were removed from the press and during storage at 7 °C. At each time, 11 g of cheese were cut aseptically and added to 99 mL of peptone (0.1%) and saline (0.9%) water. The diluted sample was homogenized using a Stomacher for 2 min. Viable counts were determined as follows: total plate counts were determined in pour plates of Plate Count Agar (PCA, Difco Laboratories, Detroit, MI, USA) incubated at 30 °C for 36 h; coliforms: Violet Red Bile Agar (VRBA, Difco) aerobic incubation at 37 °C for 24 h; mesophilic spores: 11 g of cheese were added to 99 mL peptone-saline water, heated at 80 °C for 15 min, plate count agar supplemented with 0.1% starch (Difco), aerobic incubation at 30 °C for 48 h; starters (lactococci, medium M14, Difco), anaerobic incubation at 30 °C for 5 days; NSLAB: LBS, (BBL), anaerobic incubation at 30 °C for 5 days.

After a ripening period of 1, 3 and 6 months and for each cheese of six trials, twenty colonies were taken at random from the LBS agar plates containing around 100 colonies. Pure colonies were obtained by streaking on MRS (Difco) solidified with 1.5% agar, incubated at 30 °C for 48 h and the pure strains were then transferred into MRS broth. The characterization of NSLAB was performed by the means of the following tests: colony morphology, absence of catalase, fermentation of 29 different sugars (Roy et al, 1994). The results of 29 carbohydrate and catalase tests were treated as follows: a positive result was scored 1 and a negative result 0. The data were analyzed using the simple matching coefficient (Ssm) of Sokal and Michener (1958). Strains were grouped at 85% similarity level by unweighted pair-group average linkage analysis. All computations were performed on a Digital Equipment Corporation VAX computer using the IMSL package (IMSL Math/Library - IMSL Inc, Texas, USA, 1982, vol 2, chap III). The average probability of error, P, was calculated from 62 duplicate cultures of lactobacilli using the formula:

$$P = \frac{\left(1 - 1\sqrt{\left(1 - 4s^2\right)}\right)}{2}$$

of Sneath and Johnson (1972) where $s^2 = 0.020$ is the pooled variance for all tests and hence $P = 2.1\%$.

Extraction of juice from cheese

Cheese juice was extracted according to the procedure of Salvat-Brunaud et al (1995). Grated cheese (1800 g) was mixed with 3600 g sand (150–250 µm, Prolabo, France). The mixture was placed into a perforated stainless steel mould (Perfora type 5, APV Baker, Evreux, France) which was lined with disposable cheese cloths (Smith and Nephew Extruded Films Ltd, Hull, UK). The mould filled with the cheese-sand mixture was placed on the press plate (Mecalef Industrie, Rennes, France) and pressed using a hydraulic press (Transhydro, Morlaix, France) at room temperature. Pressure was increased gradually from 5 to 42 bars over 3 h (Salvat-Brunaud et al, 1995) and the expressed liquid was collected in a graduated cylinder. The cylinder containing the pressed liquid was cooled and held at 4 °C for 2 h. The layer of solidified fat was punctured to release the subnatant juice. The juice was filtered through Whatman No 541 filter. The cheese juice was stored at –20 °C until analysis.

Monitoring of proteolysis in cheese and juice

The water soluble nitrogen (WSN) of the cheese throughout ripening was determined according to the method of Kuchroo and Fox (1982) as follows: 100 mL distilled water were added to 50 g grated cheese. The mixture was homogenized in the blender (Warning Commercial Blender) for 4 min. After this, the extracts were held at 40 °C for 1 h and then centrifuged at 10 000 *g* for 30 min (4 °C). Trichloroacetic acid (TCA)-soluble N was prepared by mixing a volume of WSN (10 mL) with an equal volume of 24% TCA (Kuchroo and Fox, 1982). The mixture was left to stand for 15 min, followed by filtration throughout Whatman No 1 paper. The N content of the extracts was determined by the Kjeldahl method; WSN and TCAN were expressed as percentage of total N. Total free amino acids were determined by a modified Cd-ninhidrin reactive (CdNR) method (Folkerstma and Fox, 1992). A sample (20–100 µL) (depending on the concentration of amino acids expected) of WSN was diluted to 1 mL with distilled water. To this mixture, 2 mL of Cd-ninhydrin reagent (Cd-ninhydrin reagent: 0.8 g ninhydrin were dissolved in a mixture of 80 mL ethanol and 10 mL acetic acid, followed by the addition of 1 g $CdCl_2$ dissolved in 1 mL of distilled water) was added. The mixture was heated at 84 °C for 5 min, cooled and the absorbance at 507 nm determined. Total free amino acid content in the cheese juice was analyzed after hydrolysis with HCl (5 N) at 110 °C for 24 h and free amino acids were determined on the TCA filtrate by using a Pharmacia LKB-Alpha Plus Serial 2 (Pharmacia Biotech, Saint-Quentin-Yvelines, France).

Proteolysis was also monitored with SDS-PAGE using a 'Phastsystem' (Pharmacia) according to the method of Dalgleish and Banks (1991). Water-insoluble extract fraction (WISN) of Cheddar cheese was extracted as described by Kuchroo and Fox (1982) and freeze-dried. Freeze-dried samples were added to 10 mL of electrophoresis buffer solution (10 mM Tris-HCl, 1% SDS, 0.02% bromophenol blue tracking dye, pH 8.0). The mixture was homogenized with a Turbo Turrax (Type T25-S1, IKA-Labortechnik, Germany). The homogenate sample (0.1 mL) was added to 0.9 mL of electrophoresis buffer solution containing 0.8% (w/v) dithiothreitol (DTT), capped and placed in boiling water bath for 5 min. Boiling samples were cooled and 1 µL was loaded on a 20% homogeneous polyacrylamide gel and the electrophoresis was run according to the manufacturer's instructions (Pharmacia Biotech Inc, Baie d'Urfé, Québec, Canada). The bands on the gels were visualized by staining with silver stain. Gels were then scanned with a Image-Master Desk Top scanner (Pharmacia Biotech Inc) and analyzed using Image Master Software (1D/2D) (Pharmacia Biotech Inc). Protein peaks were identified and casein groups and breakdown products were calculated as percentages of total stained fractions (Basch et al, 1989). Three protein fractions were quantified. Fraction 1 consisted of α_{s1}-α_{s2}- and β-caseins; fraction 2 was the intermediate proteolytic breakdown product between β-casein and para-κ-casein; and fraction 3 contained proteolytic breakdown products with lower molecular mass than para-κ-casein (Brandsma et al, 1994).

Reverse-phase HPLC analysis

The chromatographic analysis of the WSN and cheese juice was performed according to Kaiser et al (1992): an HPLC system (Beckman Instruments Inc, San Ramon, CA, USA) consisting of a controller, two solvent pumps, a 50-µL sample loop, a variable wavelength UV monitor, and a Gold system software was used. Samples of WSN and cheese juice were analyzed using an ODS Hypersyl analytical column, 5 µm, 250 × 4 mm (Hewlett-Packard, Mississauga, ON, Canada). The column was equilibrated at 60 °C with elution solvent A (0.1% trifluoroacetic acid (TFA)). The samples (50 µL) previously filtered through a 0.45 µm sample filter (Millipore Canada Ltd, Nepean, ON, Canada) were injected and eluted with a linear solvent gradient from 0 to 80% elution solvent B (0.096% TFA in 80:20 (v/v) acetonitrile: water) in 60 min. The samples of each cheese at 1, 3, 6, and 9 months were analyzed by reverse-phase HPLC with detection at 214 nm.

Three aromatic amino acids, namely, tyrosine (6.24 min), phenylalanine (9.57 min), and tryptophan (13.96 min), were also run individually on the HPLC to locate their retention time in the elution pattern. Changes in the relative proportion of hydrophilic and hydrophobic peptides in the cheese juice samples of Cheddar cheeses throughout ripening were measured according to Lau et al (1991). After each run, the total integration area of peptides and aromatic acids detected at 214 nm were determined. The UV absorption peaks observed for the HPLC runs

were arbitrarily divided into two groups (hydrophilic and hydrophobic). The first group (hydrophilic) of peptides consisted of the peaks with retention times from 6.2 min to 13.90 min. The second group of peptides (hydrophobic) consisted of the peaks from 13.90 min to 45 min.

Separation of α_{s1}-α_{s2}-, β- and para-κ-caseins was achieved by HPLC according to Jaubert and Martin (1992). Separations were carried out on a 15-cm Vydac C4 column (Vydac, Hesperia, CA, USA). Solvents used were: solvent A, 0.1% (v/v) trifluoroacetic acid in water; and solvent B, 0.096% (v/v) trifluoroacetic acid in 80% (v/v) acetonitrile. Elution was achieved using a linear gradient from 37 to 57% solvent B for 38 min at a flow rate of 1 mL /min, and the absorbance at 214 nm.

Before analysis, freeze-dried samples of WISN (0.1 g) were added to 4 mL of buffer solution (100 mM Tris-HCl, 8 M, 1.3% trisodium citrate, pH 7.0). The mixture was reduced with 10 mM-DTT, homogenized with a Turbo Turrax (IKA-Labortechnik, Germany) and maintained for 1 h at 37 °C. Before injection, the homogenate sample was diluted 10 × with solvent A. The amount of sample injected was 50 µL.

For all cheese samples, para-κ-casein which is not broken down during cheese ripening, was selected as an internal standard (Lau et al, 1991). The areas for α_{s1}-α_{s2}-β- and para-κ-caseins were determined for each sample. Data from HPLC analysis are presented as the ratio for α_{s1}-casein and β-casein with respect to area of para-κ-casein at each age of ripening. The change of these ratios throughout ripening with respect to the original ratio was expressed as percentage of ratio at 0 day. As intact α_{s1}- and β-caseins break down with increasing cheese age, their ratio to para-κ-casein will decrease. Thus, the change in ratio expressed as a percentage of the original ratio at day 0 will reflect the percentage of degradation of the original amount of α_{s1}- and β-caseins present at day 0 (Lau et al, 1991).

Sensorial evaluation

After 6 months of ripening, each cheese (coded) was presented to a panel of five expert graders of Agriculture and Agri-Food Canada. The cheeses were scored according to the Agriculture and Agri-Food Canada procedure. The cheeses were graded on the basis of flavor and overall quality (taste, texture, color) with maximum scores of 41 and 93, respectively.

Statistical analysis

Five replicates were conducted and data were analyzed using the general linear model procedure from SAS (1990).

RESULTS AND DISCUSSION

Cheese composition

The composition of the cheeses is summarized in table I. No significant difference was found between cheese made from microfiltered milk and those made from thermized and pasteurized milk, except for fat composition. The calculated parameters: salt in moisture (S/M), fat in dry matter (FDM) and moisture-in-non-fat solids (MNFS), are within values typical of Cheddar cheese although FDM values of microfiltered (MF) milk cheese were higher (53.0 g/100 g) than those of thermized and pasteurized milk cheeses. Low values of moisture, MNFS and S/M were within values associated to those of high quality Cheddar cheese (Barlow et al, 1989; Kelly et al, 1996).

Microbiological analysis

Table II shows total bacteria, coliform and non-starter lactic acid bacteria (NSLAB) counts for the cheese milks. Total bacteria and coliform counts of MF and pasteurized milks were similar whereas thermized milk contained higher populations of total bacteria, coliforms and NSLAB. After microfiltration, Demarigny et al (1996) noted that total count in milk did not exceed 2×10^3 cfu/mL. Mc Sweeney et al (1993) indicated that microfiltration reduced the total bacterial count by more than 99% and MF cheese milk had a lower count than pasteurized milk in agreement with our results. It must be remembered that MF cheese milk was fat standardized by addition of cream submitted to a classical pasteurization. Heat

Table I. Mean values of compositional analysis of Cheddar cheeses made from filtered (MF), thermized (TH) and pasteurized (Past) milk.

Valeurs moyennes de l'analyse de composition des fromages cheddar réalisés à partir de laits microfiltré (MF), thermisé (TH) et pasteurisé (Past).

	Value for cheese[1]					
	MF		*TH*		*Past*	
Fat (%)	35.6	1.1[2]	34.2	1.0[2]	34.0	1.4[2]
Moisture (%)	32.8	0.7	33.5	1.0	33.7	0.3
Salt (%)	1.54	0.19	1.59	0.20	1.61	0.08
Protein (%)	26.4	0.7	26.9	0.8	28.1	1.7
pH	5.34	0.05	5.37	0.07	5.33	0.06
Ash (%)	3.22	0.15	3.27	0.07	3.24	0.17
Calcium (%)	0.65	0.03	0.67	0.03	0.65	0.06
Phosphorus (%)	0.45	0.03	0.47	0.02	0.46	0.04
Salt in moisture (%)	4.7		4.7		4.8	
Fat in dry matter (%)	53.0		51.4		51.3	
Moisture in non-fat solids (%)	50.9		50.9		51.1	
Lactose (%)	0.38	0.17	0.28	0.06	0.38	0.16
Galactose (%)	0.02	0.01	0.01	0.01	0.01	0.01
Citric acid (%)	0.13	0.02	0.13	0.01	0.14	0.03
Lactic acid (%)	1.08	0.17	1.09	0.09	1.08	0.14

[1] Mean of five replicates. [2] Standard deviation.

[1] *Chaque valeur est la moyenne de cinq répétitions.* [2] *Écart type.*

treatment of cream could be increased if lower counts were necessary.

Changes in the numbers of total bacteria, mesophilic spores, lactococci and NSLAB in the cheeses during ripening are shown in figure 1. The total bacteria, lactococci and NSLAB counts in pasteurized milk cheese were lower than those in MF and thermized milk cheese. Total bacteria and lactococci counts (fig 1A, C) decreased during ripening of pasteurized milk cheese whereas these counts increased in the other cheeses due to the higher number of NSLAB (fig 1D). Indeed, NSLAB could also grow on the M17 medium used to count lactococci resulting in overestimations (Mc Sweeney et al, 1993). Mesophilic spores in MF milk cheese were lower than those in the other cheeses (fig 1B). Microfiltration of raw milk was effective to control the presence of mesophilic spores in Cheddar cheese.

Table II. Bacterial populations (cfu/mL) in microfiltered (MF), thermized and pasteurized cheese milk.

Populations bactériennes (ufc/mL) dans les laits de fromagerie microfiltré (MF), thermisé et pasteurisé.

Milk	*Total bacteria*[1]	*Coliform*	*NSLAB*
MF	371	< 1	4
Thermized	1 205	8	23
Pasteurized	383	1	< 1

[1] Mean of five replicates.

[1] *Chaque valeur est la moyenne de cinq répétitions.*

1051 isolates selected from the LBS agar plates used to count the NSLAB population after 1, 3 and 6 months of ripening (including 16 reference strains) were identified by using phenotypic tests. Their fermentative patterns were analyzed by numerical ana-

lysis to discriminate the secondary flora which resulted in the determination of 13 clusters (results not shown). The use of phenotypic tests should be interpreted in terms of tendencies (Demarigny et al, 1996). The isolates were thus identified according to large groups and specific criteria obtained by numerical analysis instead of only using characteristics described by Dellaglio et al (1994) and Kandler and Weiss (1986). More than 78% of lactobacilli were identified as belonging to *Lactobacillus casei* group due to the presence of *Lb casei*, *Lb rhamnosus*, *Lb paracasei* reference strains in the same cluster than isolates. These results are in agreement with Litopoulou-Tzanetaki et al (1989). The majority of the isolates found in American Cheddar cheese were lactobacilli (*casei* group). They identified two subspecies which differed by their ability to metabolize rhamnose and a third species which could not metabolize lactose.

Figure 2 shows that a difference was found between the species found in MF and

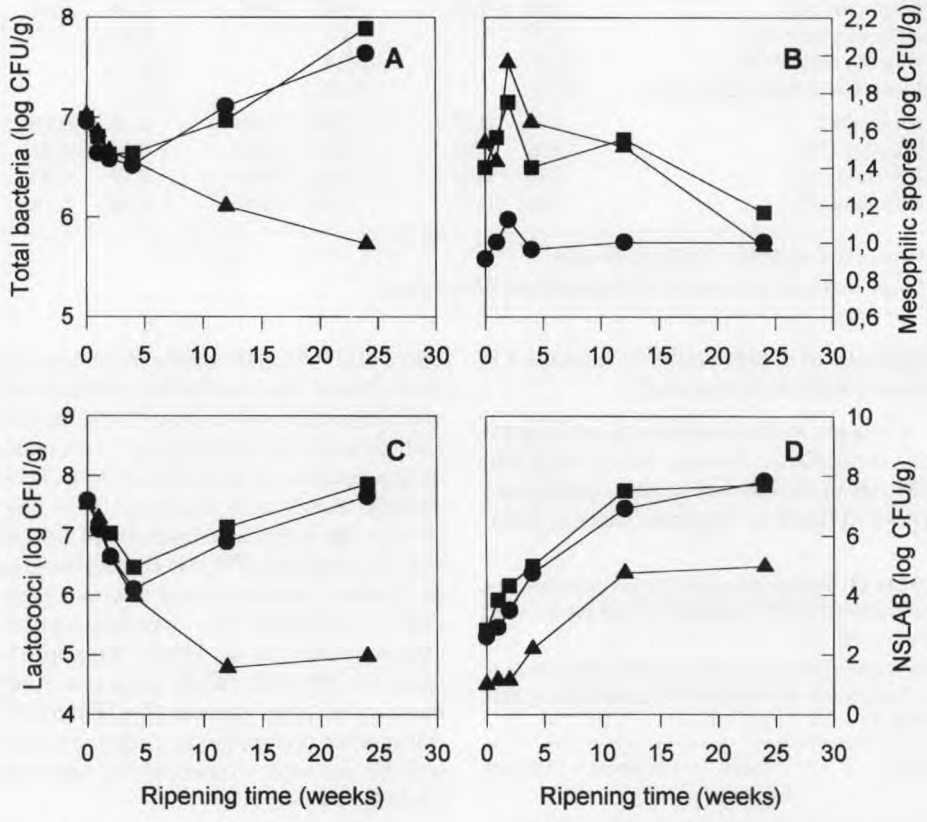

Fig 1. Counts of total bacteria (**A**), mesophilic spores (**B**), lactococci (**C**) and NSLAB (**D**) during ripening of Cheddar cheeses made from microfiltered, thermized and pasteurized milk. ●, microfiltered milk; ■, thermized milk; and ▲, pasteurized milk. Means of five replicates.

*Dénombrement de la flore totale (**A**), des bactéries sporulées mésophiles (**B**), des lactocoques (**C**) et des bactéries lactiques non levain (**D**) au cours de l'affinage des fromages cheddar. ● Lait microfiltré, ■ lait thermisé et ▲ lait pasteurisé. Moyennes de cinq répétitions.*

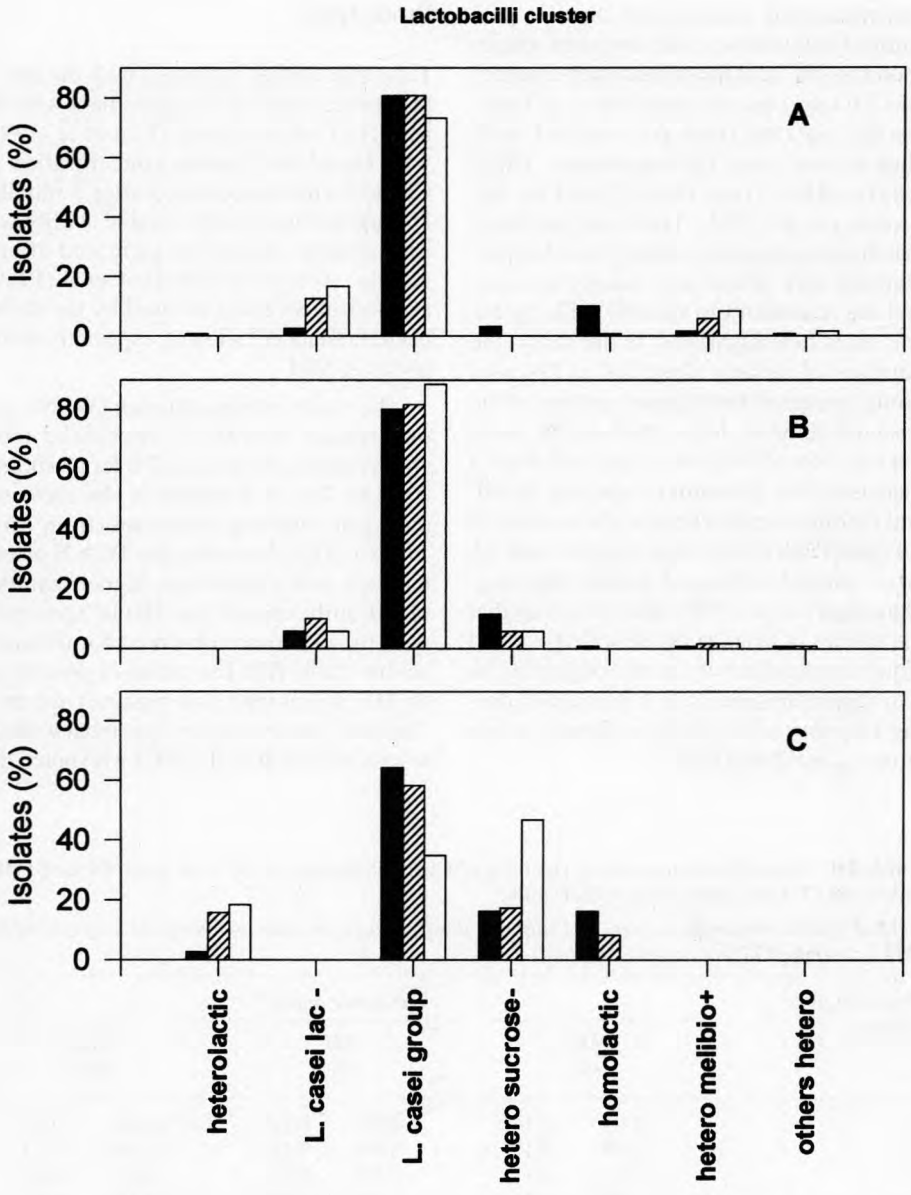

Fig 2. Classification of lactobacilli isolated during ripening of Cheddar cheeses made from microfiltered (**A**), thermized (**B**) and pasteurized (**C**) milk. Column identification within each lactobacilli cluster, from left to right: percentage of isolates selected at random at 1, 3 and 6 months of ripening.

*Classification des lactobacilles isolés au cours de l'affinage de fromages cheddar faits de lait microfiltré (**A**), thermisé (**B**) et pasteurisé (**C**). Identification des colonnes à l'intérieur de chaque groupement de lactobacilles, de gauche à droite: pourcentage des isolats sélectionnés au hasard à 1, 3 et 6 mois d'affinage.*

thermized milk cheeses and those in pasteurized milk cheeses. The dominant species found in MF and thermized milk cheeses was *Lb casei* but the population of lactobacilli isolated from pasteurized milk cheeses was more heterogeneous. These results differ from those found by Mc Sweeney et al (1993). These authors noted that the predominantly species found in pasteurized milk cheese was mainly *Lb casei* and the microflora in the raw milk cheese was more heterogeneous. In our study, the numbers of isolates identified as *Lb casei* group decreased throughout ripening of the pasteurized milk cheese whereas the numbers of heterolactic bacteria (sucrose-) increased after 6 months of ripening. In MF and thermized milk cheeses, the number of *Lb casei* (*Lb casei* lactose negative and *Lb casei* group) increased during ripening. Demarigny et al (1996) also observed that the species of lactobacilli identified evolved from a great diversity in the beginning to two major subspecies of *L paracasei* during ripening of Swiss-type cheeses made from microfiltered milk.

Proteolysis

Titratable acidity increased with the age of the cheese (table III) in agreement with the results of other authors (Lau et al, 1991) who found that lactate concentration in Cheddar cheese increased after 3 months. During the first month, titrable acidity was independent of age but increased after 3 months. According to Barlow et al (1989), all the lactose could be used by the starter bacteria (data not shown), especially at low levels of S/M.

The water soluble nitrogen (WSN), as a percentage of total N, increased from approximately 3.1% to 20.2% for all cheeses (fig 3A). The TCA soluble N also increased during the ripening of cheeses (from 1.4 to 11.2%). After 3 months, the TCA N of pasteurized milk cheese was higher than that of MF milk cheese (fig 3B) in agreement with the difference observed for titratable acidity (table III). The extent of proteolysis of MF, thermized and pasteurized milk Cheddar cheeses was in agreement with the results of Fritsch et al (1992) who noted that

Table III. Titratable acidity during ripening of Cheddar cheeses made from microfiltered (MF), thermized (TH) and pasteurized (Past) milk.

Acidité titrable mesurée au cours de l'affinage des fromages cheddar composés de laits microfiltré (MF), thermisé (TH) et pasteurisé (Past).

Ripening time (weeks)	*Titratable acidity*[1]					
	MF (%)		*TH (%)*		*Past (%)*	
0	0.94	0.14[2]	1.05	0.10[2]	1.03	0.12[2]
1	0.95	0.17	1.04	0.15	1.10*	0.23
2	1.01	0.12	0.97	0.07	1.02	0.08
4	0.99	0.20	1.10	0.09	0.18*	0.17
12	1.38	0.15	1.47	0.13	1.60*	0.14
24	1.78	0.25	1.81	0.27	1.91	0.22

* Means of cheese made from thermized and pasteurized milk in column with similar superscripts differ from that made from microfiltered milk ($P < 0.05$). [1] Mean of five replicates. [2] Standard deviation.

**Les valeurs des moyennes pour les fromages composés de laits thermisé et pasteurisé, dans une même colonne, avec un tel indice, diffèrent de celle des fromages composés de lait microfiltré ($P < 0,05$). [1] Chaque valeur est la moyenne de cinq répétitions. [2] Écart type.*

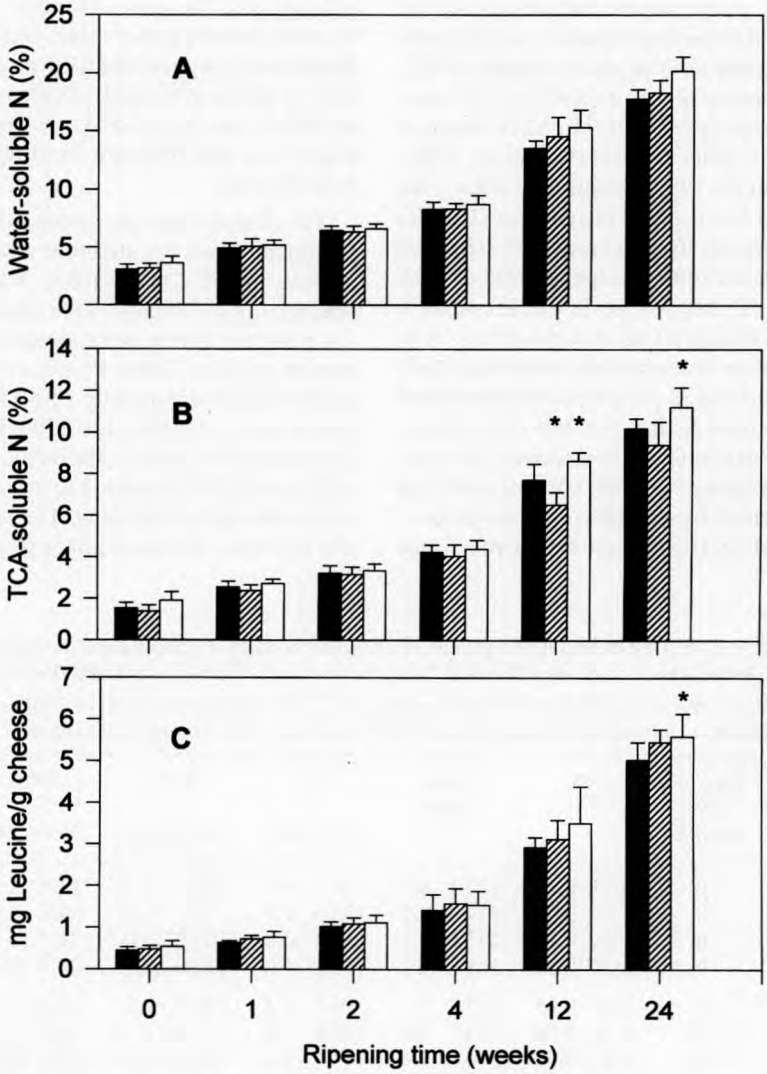

Fig 3. Development of water-soluble nitrogen (**A**), TCA-soluble nitrogen (**B**) and free leucine (**C**) during ripening of Cheddar cheeses made from microfiltered, thermized and pasteurized milk. Column identification within each ripening time, from left to right: microfiltered, thermized ans pasteurized milk cheeses, respectively. Bars = standard deviation. *Means of cheeses made from thermized and pasteurized milk with similar superscripts differ from that made from microfiltered milk ($P < 0.05$). Means of five replicates.

*Évolution de l'azote soluble dans l'eau (**A**), l'azote soluble dans TCA (**B**) et la leucine libre (**C**) au cours de l'affinage de fromages cheddar faits de lait microfiltré, thermisé et pasteurisé. L'identification des colonnes à l'intérieur de chaque temps d'affinage, de gauche à droite : fromages au lait microfiltré, thermisé et pasteurisé, respectivement. Barres : écart type. * La valeur des moyennes des fromages faits de lait thermisé et pasteurisé avec un tel indice diffèrent de ceux faits de lait microfiltré (p < 0,05). Moyenne de cinq répétitions.*

18–24% of the protein initially present in the cheeses was hydrolyzed to soluble peptides and amino acids after 6 months (4 °C). The amount of nitrogen soluble in TCA was approximately 50% of the WSN which is similar to other reports (Lau et al, 1991; Fritsch et al, 1992). Figure 3C shows the levels of Cd-ninhydrin reactive (CdNR) amino groups in the cheeses. Pasteurized milk cheese exhibited a higher level of TCA soluble N than MF milk cheese after 6 months of ripening. Mc Sweeney et al (1993) also observed that pasteurized milk cheese contained a higher concentration of TCA soluble N than the MF milk cheese throughout ripening. The difference between pasteurized and MF milk cheeses could not be explained by the difference in terms of S/M and MNFS which affect the water activity and thus bacteria and enzymes because their respective values were similar. These results suggest that the type of proteolysis in pasteurized milk cheeses might be different due to the NSLAB population which was also different from that of MF milk cheeses.

The cheeses were also analyzed by using a hydraulic press to extract the serum phase (cheese juice). Cheese juices throughout ripening, up to 9 months were characterized for nitrogen, amino acid composition and peptide profiles. Table IV shows that total solids which were initially 120–130 g/kg of cheese juice, reached 260–270 g/kg for the cheeses aged 9 months. The increase of total solids could be related to the proteolysis of casein throughout ripening. The total nitrogen fractions of cheese juices were mainly

Table IV. pH, total solids and development of nitrogen (in g/kg) of cheese juice during ripening of Cheddar cheeses made from microfiltered (MF), thermized (TH) and pasteurized (Past) milk.

pH, matière sèche et évolution de l'azote (en g/kg) du jus de fromage au cours de l'affinage de fromages cheddar composés de laits microfiltré (MF), thermisé (TH) et pasteurisé (Past).

Type of cheese	*Ripening time (months)*	*pH*		*Total solids*		*NT (N × 6.38)*		*NCN (N × 6.38)*		*NPN (N × 6.38)*		*NJ*[3] *(%)*
MF	0	5.30	0.11[2]	127.1	4.9[2]	31.3	4.0[2]	25.8	3.0[2]	13.0	1.1[2]	4.4
	3	5.24	0.08	219.3	8.1	123.6	1.5	116.1	2.6	59.2	1.8	19.5
	6	5.26	0.10	241.6	11.2	151.8	0.8	146.2	0.0	74.7	3.2	24.6
	9	5.24	0.14	265.8	9.3	172.2	4.0	170.0	0.2	105.4	0.2	28.8
TH	0	5.26	0.05	125.7	4.3	29.2	2.4	26.4	0.1	12.2	1.1	4.3
	3	5.18	0.06	217.7	1.0	119.8	1.2	114.4	1.1	59.2	1.6	19.7
	6	5.20	0.08	248.7	2.4	144.6	2.7	141.7	5.4	77.9	0.6	24.8
	9	5.24	0.16	267.9	3.5	171.3	0.1	165.6	4.7	106.7	0.1	30.2
Past	0	5.24	0.01	126.1	6.4	30.4	0.9	27.3	1.7	13.6	2.5	4.0
	3	5.16	0.03	231.3	0.6	130.4	0.5	126.3	0.2	63.0	6.2	19.6
	6	5.17	0.04	255.9	7.4	152.2	0.4	151.4	0.5	86.6	0.6	23.6
	9	5.14	0.03	271.0	0.6	168.5	0.1	168.6	1.6	112.7	0.8	26.6

[1] Mean of two replicates (trials 4 and 5). [2] Standard deviation.[3] NJ = Percentage of nitrogen in cheese juice/total nitrogen in cheese calculated as follows: $100 \times n \times V / N \times (100 - t)$ where n, nitrogen in cheese juice; V, percentage of water in cheese; N, total nitrogen in cheese; and t, total solids in cheese juice.

[1] Chaque valeur est la moyenne de deux répétitions (essais 4 et 5). [2] Écart type. [3] NJ = Pourcentage d'azote dans le jus de fromage/azote total dans le fromage calculé comme suit : $100 \times n \times V / N \times (100 - t)$ avec n, azote dans le jus de fromage; V, pourcentage d'eau dans le fromage; N, azote total dans le fromage et t, matière sèche dans le jus de fromage.

constituted of water soluble nitrogen. More than 50% of soluble nitrogen fraction was also soluble in TCA 12% (table IV). These results are in agreement with Salvat-Brunaud et al (1995) although obtained on a very different cheese variety. These authors observed that the nitrogen content of cheese juice of Emmental cheese was almost constituted of soluble nitrogen in which 50% was soluble in TCA. The calculated values of nitrogen in cheese juice in percentage of total nitrogen in cheese (NJ) increased from 4.0 to 30.2%. These NJ values (table IV) were higher throughout ripening than the WSN values (fig 3A) obtained by using the extraction with water which could be explained by the presence of components not dissolved during the extraction with

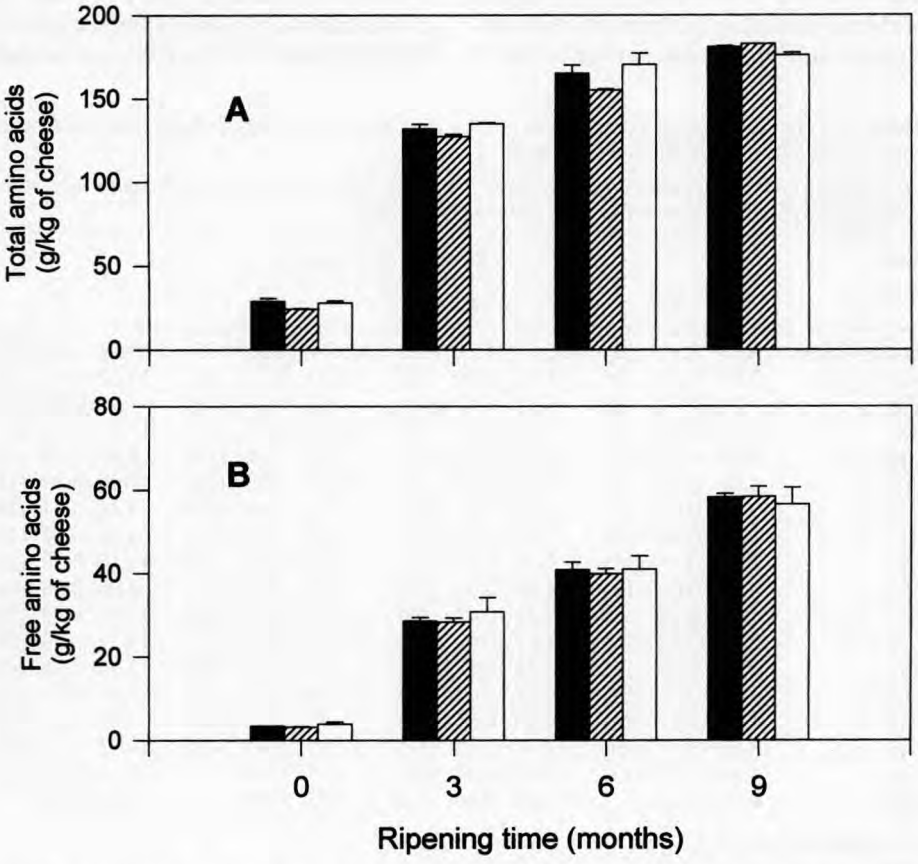

Fig 4. Total **(A)** and free amino acid **(B)** concentrations of cheese juice during ripening of Cheddar cheeses made from microfiltered, thermized and pasteurized milk. Column identification within each ripening time, from left to right: Cheddar cheeses made from microfiltered, thermized and pasteurized milks. Bars = standard deviation.

*Concentrations en acides aminés totaux (**A**) et sous la forme libre (**B**) au cours de l'affinage de fromages cheddar faits de lait microfiltré, thermisé et pasteurisé. Identification des colonnes à l'intérieur de chaque temps d'affinage, de gauche à droite : fromages cheddar faits de lait microfiltré, thermisé et pasteurisé. Barres : écart type.*

water (Sandberg et al, 1930). Such result must likely be the balance of two mechanisms: water extraction increases solubilization of hydrophobic peptides because it decreases hydrophobic interactions but on the other hand, numerous peptides and amino acids have a low water solubility.

Figure 4 shows that total and free amino acid concentrations in cheese juice increased throughout ripening. No apparent difference was observed between the different types of cheese, indicating that proteolysis of pasteurized milk cheese was similar to that of the other cheeses. Free amino acids/total amino acids ratio increased from an average value of 13% at day 0 to 33% after 9 months, suggesting that individual amino acid were released at the same rate throughout ripening. Moreover, table V indicated that amino acid composition of cheese juice of MF milk cheese was similar between the two trials, except for ornithine which was present in trial 4.

Table VI indicates that P, Ca and Mg concentrations of juice expressed from all Cheddar cheeses increased throughout ripen-

Table V. Total amino acids composition in g/kg (free amino acid %) of cheese juice of Cheddar cheeses made from microfiltered (MF) milk.

Composition en acides aminés totaux en g/kg (% sous la forme libre) du jus de fromage au cours de l'affinage de fromages cheddar faits de lait microfiltré (MF).

Amino acids	*Ripening time (months)*[1]											
	0			*3*			*6*			*9*		
Total (% free)	29.05	2.33[2]	(12)	132.07	4.21[2]	(22)	165.44	6.95[2]	(25)	180.98	1.04[2]	(32)
Asp	2.60	0.23	(4)	11.76	0.30	(3)	14.70	0.62	(4)	15.57	0.94	(6)
Thr	1.20	0.02	(2)	3.27	0.17	(13)	4.03	0.28	(14)	4.32	0.09	(28)
Ser	1.47	0.06	(3)	7.59	0.19	(7)	10.38	0.77	(7)	11.73	0.42	(11)
Glu	6.39	0.78	(12)	34.56	1.08	(16)	43.67	1.80	(18)	48.93	0.15	(23)
Pro	2.12	0.15	(9)	8.60	0.45	(9)	10.35	0.92	(10)	10.96	0.39	(15)
Gly	0.68	0.07	(2)	2.54	0.17	(17)	3.21	0.18	(18)	3.48	0.04	(28)
Ala	1.01	0.06	(10)	2.96	0.16	(18)	3.90	0.08	(40)	4.30	0.02	(54)
Val	1.49	0.13	(12)	7.30	0.15	(23)	9.34	0.21	(19)	10.16	0.30	(27)
Met	0.26	0.06	(19)	1.79	0.04	(24)	1.87	0.47	(37)	2.00	0.28	(64)
Ile	1.48	0.16	(3)	7.25	0.02	(5)	9.38	0.05	(40)	10.20	0.27	(52)
Leu	2.49	0.12	(16)	11.12	0.67	(43)	12.99	0.76	(30)	14.25	0.30	(36)
Tyr	0.68	0.05	(16)	2.75	0.18	(27)	3.47	0.32	(46)	3.74	0.16	(49)
Phe	1.04	0.06	(13)	4.51	0.03	(27)	6.08	0.24	(5)	6.18	0.33	(20)
His	1.03	0.11	(4)	4.70	0.13	(3)	5.51	0.16	(45)	5.83	0.23	(39)
Lys	3.06	0.19	(10)	13.38	1.22	(11)	17.25	1.97	(8)	19.55	1.74	(15)
Arg	1.30	0.17	(13)	4.97	0.47	(16)	5.59	0.84		5.62	1.64	
Cys	0.20	0.08		0.62	0.06		0.75	0.08		0.84	0.15	
Free amino acids (g/kg)												
Trial	4	5		4	5		4	5		4	5	
Pser	0.1	0.2		1.0	1.5		1.8	2.2		2.8	3.6	
Asn	0.2	0.2		3.4	3.7		4.3	4.1		5.6	5.8	
Gln	0.1	0.1		0.7	0.9		1.7	1.1		2.4	1.8	
Orn	0.01	nd		0.5	nd		1.0	nd		2.0	nd	
Cit	0.2	0.1		1.7	1.0		2.7	1.3		2.4	2.2	

[1] Mean of two replicates (trials 4 and 5). [2] Standard deviation.

[1] Chaque valeur est la moyenne de deux répétitions (essais 4 et 5). [2] Écart type.

Table VI. Ash and minerals in g/kg of cheese juice during ripening of Cheddar cheeses made from microfiltered (MF), thermized (TH) and pasteurized (Past) milk.

Cendre et minéraux en g/kg du jus de fromage au cours de l'affinage de fromages cheddar faits de lait microfiltré (MF), thermisé (TH) et pasteurisé (Past).

Type of cheese	*Ripening time (months)*	*Ash*		*P*	*Ca*	*Mg*	*K*	*Na*	*Cl*
MF	0	61.1	1.5[2]	6.32	4.56	0.39	2.04	10.68	27.3
	3	62.7	2.6	7.15	7.33	0.53	1.88	9.63	24.9
	6	62.2	3.7	8.85	7.57	0.54	1.80	9.20	23.6
	9	62.6	3.0	10.54	7.83	0.56	1.76	9.16	24.9
TH	0	60.6	4.4	6.30	4.73	0.40	2.11	10.58	26.1
	3	60.7	6.5	7.53	7.35	0.52	1.91	8.82	22.4
	6	58.5	2.9	9.11	7.00	0.54	1.87	8.68	25.3
	9	59.0	7.1	10.73	6.92	0.57	1.83	8.60	25.6
Past	0	60.7	0.9	7.26	4.98	0.38	2.15	10.86	26.8
	3	62.6	0.3	8.47	7.52	0.52	1.88	8.99	23.6
	6	62.2	1.3	10.00	7.83	0.54	1.77	8.73	24.7
	9	61.4	0.3	10.95	8.03	0.57	1.74	8.47	23.1

[1] Mean of two replicates (trials 4 and 5). [2] Standard deviation.

[1] *Chaque valeur est la moyenne de deux répétitions (essais 4 et 5).* [2] *Écart type.*

ing. Such increases might likely be due to a constant release of caseinphosphopeptides binding Ca and Mg as it can be hypothesized from the P Ser data of table V. The decrease of Na and K levels in juices during ripening is astonishing and more studies are requested for understanding why the paracasein network is binding increasing amounts of monovalent ions during ripening.

Finally, the total water-soluble peptides in the WSN and cheese juice samples were estimated by using reverse-phase HPLC. At 214 nm, the total area under the peaks on the HPLC chart represents the light absorbed by aromatic amino acids and peptide bonds present in the WSN and cheese juice samples of cheese (Lau et al, 1991). Throughout ripening, the total water-soluble peptide content of the cheeses increased (fig 5), especially between 0 and 3 months. After this stage of ripening, only a slight increase was observed. These results are in agreement with those of Lau et al (1991) and Bican and Spahni (1993). Lau et al (1991) indicated that the formation of decomposition products from caseins and high molecular mass peptides which are broken down into smaller peptides, could be the origin of the peptide water soluble material seen throughout ripening. The total water-soluble peptide content of cheeses made from MF, thermized and pasteurized milk were similar throughout ripening except that the peptide contents estimated in the WSN samples were higher than in the cheese juice samples.

Figure 6 shows that hydrophilic and hydrophobic peptides in the cheese juice samples increased during aging. During ripening, the production of hydrophobic peptides in the cheese was faster than hydrophilic peptides. Lau et al (1991) gave two possible explanations for the higher level of hydrophobic peptides than

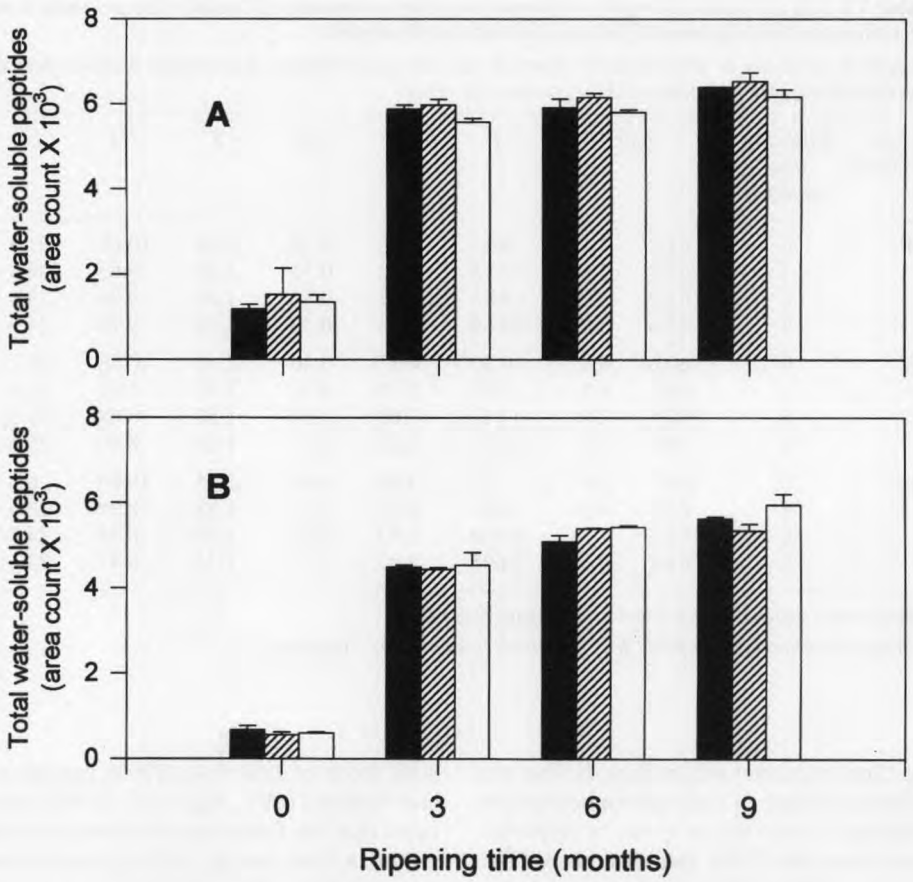

Fig 5. Area count of total water-soluble peptides of water-soluble fraction (**A**) and cheese juice (**B**) during ripening of Cheddar cheeses made from microfiltered, thermized and pasteurized milk; detection at 214 nm. Column identification within each ripening time, from left to right: Cheddar cheeses made from microfiltered, thermized and pasteurized milks. Bars = standard deviation.

*Aire totale des peptides totaux solubles dans l'eau de la fraction soluble dans l'eau (**A**) et dans le jus de fromage (**B**) au cours de l'affinage de fromages cheddar faits de lait microfiltré, thermisé et pasteurisé; détection à 214 nm. Identification des colonnes à l'intérieur de chaque temps d'affinage, de gauche à droite : fromages cheddar faits de lait microfiltré, thermisé et pasteurisé. Barres : écart type.*

hydrophilic peptides in Cheddar cheeses made from raw and pasteurized milk. It is likely that some water soluble peptides were broken during aging to yield smaller peptides which were hydrophobic. In addition, no apparent difference was observed between MF milk cheeses and the other cheeses (fig 6). These results are in agreement with the previous results obtained by using other methods.

Typical electrophoretograms (not shown) of all cheeses revealed that the concentration

of the caseins decreased throughout ripening (table VII). Casein identified through densitometry of the SDS-PAGE gels decreased from approximately 49% of the total protein to 30% after 6 months. Caseolysis was essentially the same in all cheeses (table VII). The protein breakdown fraction 1 which consists of casein breakdown (protein peaks from β-casein up to para-κ-casein) increased from approximately 13% to 24% as cheese aged. Table VII also shows that the third protein group (protein breakdown fraction 2) with a lower molecular mass than para-κ-casein increased throughout ripening. Basch et al (1989) indicated that when Cheddar cheese is young, the per-

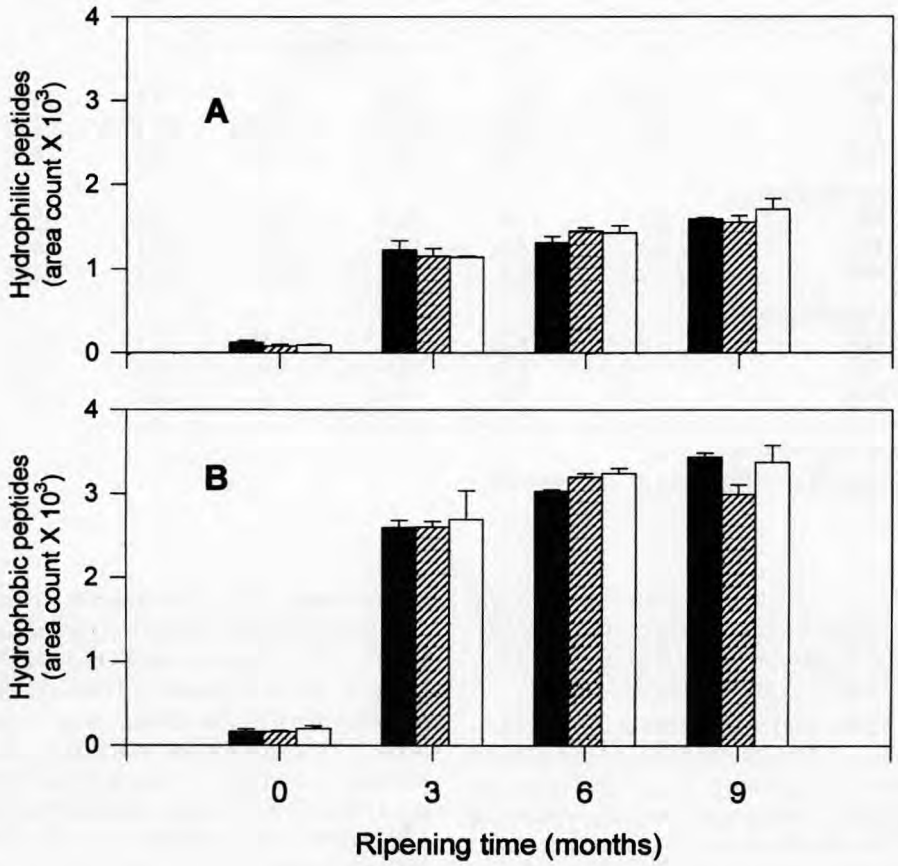

Fig 6. Area count of hydrophilic peptides (**A**) and hydrophobic peptides (**B**) of cheese juice during ripening of Cheddar cheeses made from microfiltered, thermized and pasteurized milk; detection at 214 nm. Column identification within each ripening time, from left to right: Cheddar cheeses made from microfiltered, thermized and pasteurized milk. Bars = standard deviation.

*Aire totale des peptides hydrophiles (**A**) et des peptides hydrophobes (**B**) du jus de fromage au cours de l'affinage de fromages cheddar faits de lait microfiltré, thermisé et pasteurisé; détection à 214 nm. Identification des colonnes à l'intérieur de chaque temps d'affinage, de gauche à droite : fromages cheddar faits de lait microfiltré, thermisé et pasteurisé. Barres : écart type.*

Table VII. Specific casein fraction (α_{s1}-α_{s2}- and β-caseins), protein breakdown fraction 1 (component between β-casein and para-κ-casein) and protein breakdown fraction 2 (component of lower molecular mass than para-κ-casein) percentages during ripening of Cheddar cheeses made from microfiltered (MF), thermized (TH) and pasteurized (Past) milk.

Pourcentage des fractions caséines (α_{s1}-α_{s2}- et β-caséines), des peptides de haut poids moléculaire (compris entre la caséine β et la para-κ-caséine) et des peptides de bas poids moléculaire (composés de poids moléculaire plus petits que la para-κ-caséine) au cours de l'affinage de fromages cheddar faits de lait microfiltré (MF), thermisé (TH) et pasteurisé (Past).

Fractions[1]	*Ripening time (weeks)*					
	0	1	2	4	12	24
	Percentages					
Caseins						
MF	50.0	48.5	45.6	42.7	35.9	31.0
TH	48.4	46.6	46.8	41.8	35.1	31.0
Past	49.4	47.8	45.8	41.2	34.4	28.9
Protein breakdown 1						
MF	12.5	12.6	14.3	16.9	20.8	22.7
TH	13.2	14.3	13.5	17.8	21.1	24.5
Past	12.7	14.9	14.8	19.2	22.4	24.7
Protein breakdown 2						
MF	8.4	9.0	11.0	11.8	15.7	19.0
TH	6.9	8.3	8.9	9.9	12.4	16.0
Past	7.7	8.2	9.0	11.4	14.7	18.1

[1] Mean of five replicates.

[1] *Chaque valeur est la moyenne de cinq répétitions.*

cent total casein is high and other protein groups are low. As the cheese became older, the percent total casein decreases, whereas the other two groups increase.

Table VIII shows that α_{s1}-casein was almost completely hydrolyzed after 6 months of ripening. The degradation pattern of α_{s1}-casein was essentially identical in all cheese whereas the β-casein pattern was different. Although β-casein underwent slight proteolysis throughout ripening, as determined by HPLC analysis, MF milk cheese exhibited higher percentage of degradation than pasteurized milk cheese after 4 and 12 weeks, respectively. The high percentage of degradation of α_{s1}-casein was within the range expected for Cheddar cheeses with a lower level of S/M since approximately 85% of the intact α_{s1}-casein was degraded within 6 months (Thomas and Pearce, 1981; Creamer and Olson, 1982; Lau et al, 1991). Creamer and Olson (1982) observed that Cheddar cheese samples aged between 10 and 44 weeks were quite similar with most of the α_{s1}-casein degraded to α_{s1}-I but with the β-casein essentially intact. The higher level of cleavage of β-casein in MF milk cheese as compared to pasteurized milk cheese suggested that indigenous enzyme activity was higher than in the ones made from pasteurized milk. Indigenous enzymes such as plasmin could be responsible for the difference observed in our study in MF milk cheeses as compared with the other Cheddar cheeses. Such a result can be compared with that of Bouton and Grappin

Table VIII. Percentage of initial level of α_{s1}- and β-caseins that was degraded during ripening of Cheddar cheeses made from microfiltered (MF), thermized (TH) and pasteurized (Past) milk as determined by HPLC analysis.

Pourcentage du niveau initial de caséines α_{s1} et β qui a été dégradé au cours de l'affinage des fromages cheddar faits de lait microfiltré (MF), thermisé (TH) et pasteurisé (Past).

Casein	*Treatment*	*Ripening time (weeks)*										
		0	*1*		*2*		*4*		*12*		*24*	
		(Percentage degraded)										
α_{s1}-	MF	0	21.6	6.0[2]	32.7	18.0[2]	55.9	6.0[2]	79.7	2.4[2]	85.6	1.3[2]
	TH	0	18.7	8.6	37.4	7.0	51.9	12.7	81.6	2.1	87.2	1.4
	Past	0	26.7	5.8	44.3	7.6	63.8	5.7	81.1	3.6	87.9	1.2
Beta-	MF	0	4.3	2.7	8.6	8.9	8.5	7.0	17.2	6.4	15.1	3.5
	TH	0	0.2	7.1	–0.3*	6.3	5.5	6.7	11.7	3.8	7.8*	6.0
	Past	0	–2.3	5.1	0.1	4.5	4.0*	5.8	8.7*	4.7	12.5	3.9

* Means of cheese made from thermized and pasteurized milk in column with similar superscripts differ from that made from microfiltered milk ($P < 0.05$). [1] Mean of five replicates. [2] Standard deviation.

* *Les valeurs des moyennes pour les fromages faits de lait thermisé et pasteurisé dans une même colonne avec un tel indice diffèrent de celle pour les fromages faits de lait microfiltré* ($p < 0,05$). [1] *Chaque valeur est la moyenne de cinq répétitions.* [2] *Écart type.*

(1995) who observed a lower proportion of β-casein in MF milk Comté cheese than in raw milk cheese. Amongst their different hypotheses to explain this result, the more convincing could be an activation of plasmin-plasminogen complex by inactivation of the inhibitor of the plasminogen activator related to the heating of cream (85 °C, 30 s) and skimmilk during MF (50 °C, 20 min).

The scores for flavor and overall quality (flavor quality and texture) for the cheeses are summarized in table IX. After 24 weeks of ripening, all cheeses were graded as class I cheeses (scores of 39 and 92 and more for flavor and overall quality, respectively). The MF milk cheeses were considered to be of high quality, especially for trials 4 and 5 (typical flavor of Cheddar cheese). The pasteurized milk cheeses were considered by graders as fair, mainly on the basis of flavor. Difference between MF milk cheeses and pasteurized milk cheeses might be partly due to the percentage of milk fat although only small differences were observed, especially for trial 5 (table IX).

CONCLUSION

Cheddar cheeses made from microfiltered milk exhibited the same pattern throughout ripening than thermized and pasteurized milk cheeses. This is in agreement with previous reports that indicated that the coagulant is one of the principal proteolytic agents responsible for the proteolysis detectable by WSN and PAGE (Lane and Fox, 1996). Difference in sensorial evaluations between MF and pasteurized milk cheeses might be due to the composition in NSLAB. The presence of a more homogeneous population of lactobacilli, particularly *Lb casei*, in MF and thermized milk cheeses, than in pasteurized milk cheese might be responsible for the typical Cheddar cheese flavor. Strains of species of lactobacilli isolated from pas-

Table IX. Grades for quality of Cheddar cheeses made from microfiltered (MF), thermized (TH) and pasteurized (Past) milk after 6 months of ripening.

Classement pour la qualité des fromages cheddar faits de lait microfiltré (MF), thermisé (TH) et pasteurisé (Past) après 6 mois de maturation.

Attribute	*Cheese replicate*														
	MF					*TH*					*Past*				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Fat (%)	36.3	35.8	33.7	36.5	35.7	34.7	34.4	32.4	35.0	34.6	34.4	34.0	32.0	33.8	35.9
Flavor	39	39	39	39	40	39	39	39	39	39	39	39	39	39	39
Overall quality	92	92	92	92	93	92	92	92	92	92	92	92	92	92	92

teurized milk cheeses due to post-pasteurization contamination might be responsible for split defects (Khalid and Marth, 1990). In conclusion, the MF milk Cheddar cheese was similar in all respects to thermized milk cheese and microfiltration could be used to produce high quality cheese with typical Cheddar cheese flavor.

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