Lait (1999) 79, 3-22

Review

Advances in the biochemistry and microbiology of Swiss-type cheeses

Rémy Grappin[a]*, Éric Beuvier[a], Yvette Bouton[a,b], Sylvie Pochet[a]

[a] Station de recherches en technologie et analyses laitières, Inra, BP 89, 39801 Poligny cedex, France
[b] Comité interprofessionnel du comté, avenue de la Résistance, BP 26, 39801 Poligny cedex, France

Abstract — Microbial and biochemical characterization of the Swiss-type cheeses Emmental, Comté and Beaufort, and factors influencing the ripening and quality of cheeses were assessed. During the ripening of Comté, the numbers of thermophilic lactic acid bacteria decreased continuously, with simultaneous growth of non-starter bacteria. Facultatively heterofermentative lactobacilli (FHL) and propionic acid bacteria (PAB) increased from 10^3–10^4 to 10^8 cfu·g^{-1}, rapidly reaching a plateau at the end of the pre-ripening period in the case of FHL, and after the warm room in the case of PAB. Enterococci remained at levels of 10^3–10^4 cfu·g^{-1} throughout ripening. *Lactobacillus helveticus*, which originated only from the starter, died off rapidly during ripening. *L. delbrueckii* ssp. dominated the milk and cheese. Several genotypes of *L. delbrueckii* ssp. *lactis* were identified. Among FHL, *L. paracasei* ssp. *paracasei* dominated; *L. plantarum* and *L. rhamnosus* were also isolated. *Streptococcus thermophilus* was present in all milk samples and remained in the cheese for 1 month. Genotypic diversity was higher for starter and non-starter lactobacilli than for *S. thermophilus*. In model mini-Comté, 68 % of PAB were identified as *Propionibacterium freudenreichii*. Variability in the evolution of different species and subspecies of PAB and strong interactions between LAB and growth of PAB were observed during ripening. Overall casein hydrolysis of mature cheeses and particularly the level of small peptides and free amino acids (FAA) increased in the following order: Beaufort > Comté > Emmental. At the end of ripening, Emmental had the highest level of native caseins and the lowest level of γ-CN. RP-HPLC mapping of the water soluble fraction showed significant differences between the three cheese varieties. Pasteurization (Pa) or microfiltration (MF) of milk reduced the number of non-starter microorganisms in the initial stages of ripening but similar levels of FHL and PAB to those in the raw (Ra) milk cheeses were reached at the end of cheese maturation. Pa increased the activity of plasmin (PLM) with concomitant hydrolysis of β-CN. Ra cheeses had significantly higher levels of FAA and a more intense and diversified aroma than Pa or MF cheeses. Cooking the curd/whey mixture enhanced the activation of plasminogen into PLM. Increasing the cooking temperature decreased chymosin activity and increased both the concentration and activity of PLM in cheese. These findings explain the higher level of β-CN breakdown in Comté and Beaufort com-

---

Oral communication at the 2nd Symposium on Propionibacteria, Cork, Ireland, June 25–27, 1998.
* Correspondence and reprints. grappin@poligny.inra.fr

pared with Emmental. α_{s1}-CN degradation was also higher in the ripening conditions of Emmental. © Inra/Elsevier, Paris.

Swiss-type cheese / microorganism / proteolysis / ripening

Résumé — Progrès dans la biochimie et la microbiologie des fromages de type suisse. La caractérisation microbiologique et biochimique des fromages de type suisse, et l'étude de facteurs influençant l'affinage et la qualité des fromages ont été principalement basées sur des résultats expérimentaux obtenus sur l'emmental, le comté et le beaufort. Au cours de l'affinage du comté, le nombre de bactéries lactiques thermophiles a diminué continuellement et celui des bactéries non levains a augmenté. Les lactobacilles hétérofermentaires facultatifs (FHL) et les bactéries propioniques (PAB) sont passés de 10^3–10^4 à 10^8 ufc·g^{-1}, pour atteindre rapidement un plateau à la fin de la période de pré-affinage pour les FHL, et plus tard en cave chaude pour les PAB. Les entérocoques sont restés à 10^3–10^4 tout au long de l'affinage. Pendant l'affinage, *Lactobacillus helveticus* provenant uniquement du levain a rapidement disparu. *L. delbrueckii* ssp. étaient majoritaires dans le lait et le fromage. Plusieurs génotypes ont été identifiés pour *L. delbrueckii* ssp. *lactis*. Parmi les FHL, *L. paracasei* ssp. *paracasei* étaient majoritaires, avec trois biotypes identifiés par RAPD ; *L. plantarum* et *L. rhamnosus* ont été également isolés tout au long de l'affinage. *Streptococcus thermophilus* était présent dans tous les échantillons de lait et a été trouvé dans le fromage jusqu'à un mois. La diversité génétique était plus grande pour les lactobacilles que pour *S. thermophilus*. Dans des mini-fromages modèles de comté, 68 % des souches de PAB appartenaient à *Propionibacterium freudenreichii*. Les différentes espèces et sous-espèces de PAB ont évolué différemment pendant l'affinage, avec d'importantes interactions entre LAB et le développement des PAB, que ce soit au niveau des espèces ou des souches. L'hydrolyse globale des caséines en fin d'affinage, et plus particulièrement le nombre de petits peptides et d'acides aminés libres (FAA), a augmenté dans l'ordre suivant : emmental < comté < beaufort. En fin d'affinage, l'emmental avait le niveau de caséines natives et de α_{s1}-I-CN le plus élevé et celui de γ-CN le plus faible. La cartographie RP-HPLC de la fraction peptidique soluble dans l'eau a montré des différences importantes entre les trois variétés de fromages. La pasteurisation (Pa) ou la microfiltration (MF) du lait a réduit le nombre de microorganismes non-levains au stade initial de l'affinage, mais des niveaux en fin d'affinage identiques à ceux des fromages au lait cru (Ra) ont été observés pour FHL et PAB. La pasteurisation a augmenté l'activité de la plasmine (PLM) et induit une hydrolyse concomitante de β-CN. Les fromages Ra contenaient une importante quantité de FAA, avec des arômes plus intenses et plus diversifiés que les fromages Pa ou MF. Le chauffage du mélange caillé/lactosérum a entraîné l'activation du plasminogène en PLM. En augmentant la température de chauffage, l'activité de la chymosine diminue et la concentration et l'activité de la PLM augmentent. Ces résultats expliquent le niveau plus important de dégradation de β-CN dans le comté et le beaufort que dans l'emmental. Les conditions d'affinage utilisées pour l'emmental augmentent la dégradation de α_{s1}-CN. © Inra/Elsevier, Paris.

fromage de type suisse / microorganisme / protéolyse / affinage

1. INTRODUCTION

Swiss-type cheeses are classified as 'cheeses with eyes' [37, 47]. Reinbold [43] included 25 different types of cheeses with naturally occurring eyes but, traditionally, only the Emmental wheel and the rindless block were considered as Swiss cheese.

In their reviews on Swiss-type cheeses, Mocquot [39] and Steffen et al. [47] limited Swiss-type cheeses to Emmental, Gruyère and Appenzeller, mentioning Swiss Sbrinz and French Beaufort as similar types. Emmental has a Codex standard (Standard A-6 of Codex Alimentarius), and Comté and Beaufort have an 'appellation d'origine

protégée' (AOP). In both cases, the format, composition, sensory characteristics of the cheeses and manufacturing conditions are prescribed.

Swiss-type cheeses are usually considered to be made with thermophilic starters (*Streptococcus thermophilus*, *Lactobacillus helveticus* and *L. delbrueckii*), the curd/whey mixture is always cooked to between 50 and 56 °C, and the ripening time varies from 2 to 18 months. Cheeses usually are round or block shaped (30–80 kg), with a minimum of 60 % dry matter. Often, eyes are present due to the fermentation of lactate to propionate, acetate and CO_2. The texture is slightly elastic, and the flavour is mild, slightly sweet, with intensity increasing with age.

However, these characteristics are not typical of all Swiss-type cheeses. For instance, in Comté, propionic acid bacteria (PAB) are not intentionally added, Beaufort has no openness, and some of these characteristics are found in other cheese categories (e.g. propionic acid fermentation (PAF) in Leerdamer, which is made with mesophilic cultures, and high cooking temperature in Parmigiano-Reggiano, in which no PAF occurs).

This article will review recent published and unpublished results on the microbiology and biochemistry of Swiss-type cheeses, with special reference to studies carried out in our laboratory on Emmental, Comté and Beaufort.

2. CHARACTERIZATION OF SWISS-TYPE CHEESES

2.1. Microbiology of Comté cheese

Two aspects will be developed: the evolution of the different microorganisms during ripening, and the characterization of lactic acid bacteria (LAB) and PAB. Studies were carried out on traditional Comté wheels or on model mini-Comté cheeses of 1 kg, using biochemical tests and molecular typing methods such as Randomly Amplified Polymorphic DNA (RAPD) and Pulsed Field Gel Electrophoresis (PFGE).

2.1.1. Evolution of microflora during ripening

Figure 1 shows the average results for the major groups of microorganisms in 20 Comté cheeses made in five cheese plants (Bouton et al., unpublished). The same patterns were obtained in cheese from each plant, but large variations (up to 2 log cycles) occurred between plants (*figure 1*). The decrease of thermophilic LAB, particularly the streptococci, during ripening, as well as the growth of facultatively heterofermentative lactobacilli (FHL) during the early ripening period at 14 °C and of PAB in the warm room (17–18 °C), were also observed for model mini-Comté cheese [4, 15]. Enterococci did not grow but remained at low levels throughout ripening.

The influence of ripening temperature (10, 14, 18, 22 °C) and % salt-in-moisture (S/M) (0.2, 1.5, 2.1, 3.1 %) on the growth and activity of *P. freudenreichii* ssp. *shermanii* P93 in relation to the growth of starter and contaminant microorganisms was studied in 1-kg cheeses obtained from a single Comté-type loaf made from pasteurized milk [13]. All the cheeses had a pre-ripening period of 4 weeks at 10 °C. The S/M had a limited influence on the growth rate of PAB during ripening, particularly at S/M levels < 2.1 %. At 3.1 % S/M, the growth rate declined significantly. Increasing the ripening temperature, particularly above 14 °C, enhanced the growth rate of PAB. It appeared that the S/M determined the initial level of PAB, and the differences in numbers of PAB remained more or less the same throughout ripening, irrespective of the temperature of ripening.

Increasing the S/M level from 1.0 to 3.2 % in mini-Emmental cheeses delayed the propionic acid fermentation, particularly

the production of gas [44]. The influence of salt concentration on 13 strains of *P. freudenreichii* was strain dependent. Propionic acid fermentation was also strongly influenced by temperature and a_w: lactate consumption increased up to 18 °C and decreased with salt level with concomitant changes in acetate and propionate concentration [13]. Concerning the growth of other groups of microorganisms at the end of the pre-ripening period [13], the level of enterococci and *Micrococcaceae* correlated positively with the S/M, whereas FHL and thermophilic streptococci correlated negatively and thermophilic lactobacilli were not influenced by the S/M.

2.1.2. Characterization of thermophilic and mesophilic LAB in Comté

From one Comté cheese, made with a mixture of 2 strains of *S. thermophilus* and 3 strains of *L. helveticus*, 70 strains were isolated from MRS and LM17 plates [6] (*table I*). All strains isolated at the beginning of ripening from LM17 were *S. thermophilus* and/or enterococci. In the later stages, *S. thermophilus* was almost absent, whereas enterococci and pediococci dominated (*table I*). Discrimination between *S. thermophilus*, *Enterococcus* and *Pediococcus* was based on microscopic examination, hydrolysis of arginine, growth at 10

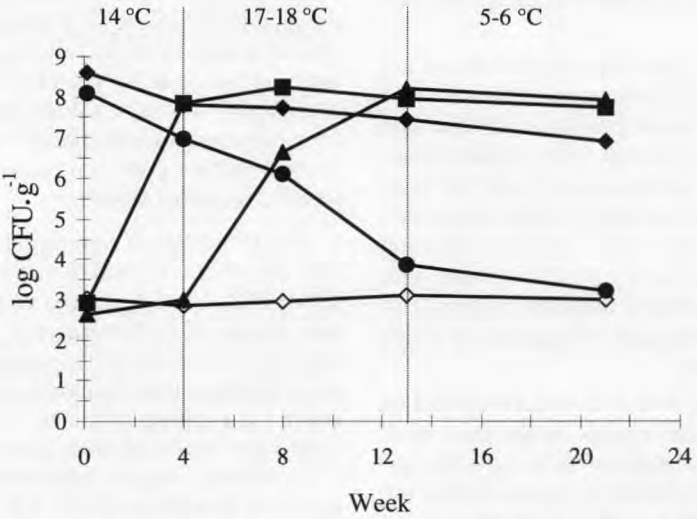

Figure 1. Evolution of the major groups of microorganisms in Comté cheeses during ripening (Bouton et al., unpublished). Mean values of 20 cheeses (curves). The minimum and maximum standard-deviations over the 5 time points are shown in parenthesis.
—■— (0.18–0.60) facultatively heterofermentative lactobacilli [Isolini D. et al., 33]
—▲— (0.32–0.94) propionibacteria [Drinan F.D. and Cogan T.M., 16]
—◆— (0.36–0.55) thermophilic lactobacilli [De Man J.C. et al., 12]
—●— (0.49–0.95) thermophilic streptococci [Therzaghi B.E. and Sandine W.E., 49]
—◇— (0.74–0.91) enterococci [Isenberg H.D. et al., 32].

Figure 1. Évolution des principaux groupes de microorganismes du comté pendant l'affinage (Bouton et al., non publié). Valeurs moyennes de 20 fromages (courbes) et écarts type minimum et maximum (entre parenthèses).
—■— (0,18–0,60) lactobacilles hétérofermentaires facultatifs [Isolini D. et al., 33]
—▲— (0,32–0,94) bactéries propioniques [Drinan F.D., Cogan T.M., 16]
—◆— (0,36–0,55) lactobacilles thermophiles [De Man J.C. et al., 12]
—●— (0,49–0,95) streptocoques thermophiles [Therzaghi B.E., Sandine W.E., 49]
—◇— (0,74–0,91) entérocoques [Isenberg H.D. et al., 32].

Table I. Identification of presumptive thermophilic lactobacilli and streptococci strains ($n = 70$) in one Comté throughout ripening [6].

Tableau I. Identification des souches ($n = 70$) de lactobacilles et streptocoques thermophiles dans un comté au cours de l'affinage [6].

	Time of ripening			
	1 d	1 mo	3 mo	5 mo
	Strains isolated from MRS agar incubated anaerobically at 42 °C			
Organism	12	13	9	5
L. helveticus	0	0	0	0
L. delbrueckii ssp. *lactis*	12	11	4	4
L. fermentum	0	1	0	0
L. paracasei ssp. *paracasei*	0	1	1	0
L. rhamnosus	0	0	1	1
Pediococci	0	0	3	0
	Strains isolated from LM17 agar incubated aerobically at 42 °C			
Organism	12	6	7	6
S. thermophilus	10	0	1	0
Enterococci	2	0	2	3
Pediococci	0	6	4	3

and 45 °C and in 6.5 % NaCl, hydrolysis of aesculin, production of lactic acid enantiomers, and carbohydrate fermentation patterns. Of 11 strains of *S. thermophilus* characterized by PFGE, 4 had similar patterns to the 2 strains used as starter. The other strains originated from the environment, and very likely from the milk.

L. helveticus was not found at day one, but wild strains of *L. delbrueckii* ssp. *lactis* dominated throughout ripening. After 1 month of ripening, the population changed to one dominated by FHL (*L. paracasei* ssp. *paracasei* and *L. rhamnosus*) and obligately heterofermentative lactobacilli (*L. fermentum*).

In 4 model mini-Comté cheeses, *L. plantarum* and *L. rhamnosus* were isolated during ripening [15], but the most dominant species was *L. paracasei* ssp. *paracasei,* with three different clusters obtained by RAPD analysis and Fourier transform infrared spectroscopy (Beuvier and Lefier, unpublished). *L. brevis,* an obligately heterofermentative lactobacilli, was found at all stages of ripening.

In another series of experiments, isolates were obtained from milks, starters and Comté cheeses at different stages of ripening (*table II*). The 4 cheeses were produced in 2 local cheese plants, either with a mixture of 2 strains of *S. thermophilus*, 1 strain of *L. helveticus* and 1 strain of *L. delbrueckii* ssp. *lactis*, or with local wild starters obtained after incubation of whey at 42 °C. The genetic diversity of *S. thermophilus* and lactobacilli was studied by PCR and RAPD fingerprinting (Bouton et al., unpublished).

S. thermophilus was present in all milk samples and in cheese at 1 d and 1 month, but was nearly absent in the later stages of ripening. Similar RAPD pattern (> 60 %

Table II. Identification of *Lactobacillus* sp. strains isolated from milk, thermophilic wild starter, and Comté cheeses collected in two cheese plants. Lactobacilli were isolated on MRS agar incubated anaerobically at 42 °C.

Tableau II. Identification de souches de *Lactobacillus* sp. provenant de laits, de levains thermophiles sauvages et de fromages de comté collectés dans deux fromageries. Les lactobacilles ont été isolés sur milieu MRS en anaérobie incubés à 42 °C.

Group	Species	Milk	Cheese ($n = 4$)				'Wild' starter
		($n = 5$)	1 d	1 mo	3 mo	5 mo	($n = 2$)
Obligately homofermentative	*L. helveticus*		5	–	–	–	10
	L. delbrueckii ssp	19	19	22	11	12	
	L. acidophilus	3	–	–	–	–	–
	n.i						1
Facultatively heterofermentative	*L. paracasei*	–	–	–	5		
	L. rhamnosus	1	–	–	6	9	
	L. plantarum	2	–	–	–	–	
Obligately heterofermentative	*L. fermentum*	4	–	–	–	–	

n.i = non identified / non identifié.

similarities) were found for all strains of *S. thermophilus*, and the group could be divided into 7 clusters with a coefficient of similarity over 80 %.

Lactobacilli isolated from MRS plates incubated at 42 °C, showed considerable diversity. *L. helveticus* was absent in milk and in cheese after 1 d (*table II*). *L. fermentum*, which was isolated from the milk, was not found in the cheese, whereas *L. delbrueckii* was the dominant species throughout ripening. *L. paracasei* and *L. rhamnosus* were found at later ripening stages (*table II*). Concerning the genetic diversity of the lactobacilli isolated from cheese, several groups, with a coefficient of similarity of 45 %, were identified. Homofermentative lactobacilli were divided into 7 clusters, 5 of *L. delbrueckii* ssp. and 2 of *L. helveticus*. Several strains exhibiting a unique RAPD pattern were also detected.

Significant differences in the genetic diversity of lactobacilli were found between plants, with particular diversity in one plant. On the other hand, RAPD patterns of *S. thermophilus* were much more homogenous within and between plants.

From these studies, it is concluded that *L. helveticus*, which originated from the starter, died off rapidly or at least was unable to grow significantly, whereas *L. delbrueckii*, which was found in milk and was added as starter, remained in the cheese throughout ripening. Besides competition between species, sensitivity to pH and differences in nutrient requirements, autolytic properties and heat sensitivity may partly explain the differences of behaviour between the two species. For instance, we found a 10–40 % variability in the autolytic properties of 15 strains of *L. helveticus*. It is therefore possible that the *L. helveticus* strain used in our studies, which was resistant to 9 phages, was highly autolytic. Concerning heat sensitivity, Giraffa et al. [27] found that the temperature gradient across Grana Padano cheese, which is cooked at 53 °C for about 140 min before moulding, affects the distribution of the species of lactobacilli within the cheese. Forty-eight hours after

Lait (1999) 79

Foreword

Swiss-type cheeses, i.e. cheeses with large eyes, are an important part of cheese production in the European Union: France produces 275 000 t of this type of cheese annually; the Netherlands 89 400 t, Germany 88 300 t, Sweden 28 400 t, Finland 26 400 t, Austria 12 800 t, Denmark 6 600 t and Ireland 5 000 t. The bacteria responsible for eye formation in these cheeses are members of the genus, *Propionibacterium*. They have no function in the manufacture of cheese but grow during ripening, fermenting lactate to propionate, acetate and CO_2, which is responsible for the formation of the eyes.

Compared with the lactic acid bacteria used as starter cultures in cheesemaking, relatively little is known about propionibacteria. The current status of research on these bacteria was recently examined at the 2nd Symposium on *Propionibacteria*, held at University College, Cork, Ireland from June 25th to June 27th 1998. The Symposium was attended by 105 delegates from 16 countries. This volume of Le Lait contains the plenary papers presented. These reports provide up-to-date, authoritative reviews of the current state of knowledge on important aspects of the use of these bacteria in cheesemaking.

Several new uses of propionibacteria were suggested, e.g. improving the keeping and nutritional quality of fermented vegetable and the keeping quality of bread, protecting bacteria and yeast from various stresses and their potential as probiotics. Further research is required before these bacteria could be used commercially in these applications but the results to-date are quite promising.

The organisers of the Symposium are particularly grateful for the financial support of several sponsors including the Society of General Microbiology, Reading, United Kingdom, the Federation of European Microbiological Societies, Utrecht, the Netherlands, Tipperary Co-Operative, Tipperary, Ireland and the European Commission, Brussels, Belgium.

The 3rd Symposium on Propionibacteria will be held in Zurich, Switzerland in 2001 and is being organised by Dr Leo Meile, Laboratory of Food Microbiology, Eidgenössische Technische Hochschule, Zurich, Switzerland.

T. M. Cogan, S. Condon

Fax: +41 1 632 1266
E-mail: meile@ilw.agrl.ethz.ch

moulding, *L. helveticus* dominates the outer zone, which cools off rapidly, while *L. delbrueckii* dominates in the core of the cheese reflecting the higher heat sensitivity of *L. helveticus* compared with *L. delbrueckii*.

2.1.3. Characterization of propionibacteria

In model mini-Comté, 68 % of the 81 strains isolated belonged to *P. freudenreichii* sp., 16 % to *P. thoenii* and 16 % to *P. jensenii; P. freudenreichii* subsp. *freudenreichii* dominated over *P. freudenreichii* subsp. *shermanii* [13]. Different growth rates were observed during ripening for the various species and subspecies of PAB: the number of *P. freudenreichii* ssp. *shermanii* decreased in the warm room and remained stable. *P. thoenii* increased in the warm room then decreased during storage at 6–7 °C while *P. jensenii* increased slightly throughout ripening.

Other studies have shown an extraordinary large diversity of species and strains among 474 strains of PAB isolated from Swiss lowland and alpine milk, with significant differences between regions and between milks [20]. In both types of milk, *P. freudenreichii* predominated. No *P. acidipropionici* and a higher proportion of *P. thoenii* were found in alpine milk. Among *P. freudenreichii* sp., 79 different strains were identified. Similar diversity in PFGE patterns of *P. freudenreichii* (40 different patterns among 48 strains) was also observed in PAB from international and private collections [24].

2.1.4. Interaction between PAB and LAB

Interactions between 6 strains of thermophilic LAB (*L. helveticus, L. delbrueckii* ssp. *lactis* and *S. thermophilus*) and 3 strains of PAB isolated from Comté (*P. freudenreichii* ssp. *freudenreichii, P. jensenii, P. thoenii*) with one reference strain, was assessed by measuring the growth of PAB on filter-sterilized whey obtained after growth of LAB in milk (Marmot and Bouton, unpublished). Results (*figure 2*) showed a strong interaction between LAB and PAB; *L. helveticus* showed the greatest stimulation. Growth of *P. jensenii* was only slightly influenced or not affected by the LAB. These results are in agreement with earlier studies [42] which showed that only *L. helveticus* strains had a positive interaction on the growth of the 4 PAB strains of *P. freudenreichii* and *P. acidipropionici* tested. For the other strains of LAB (*L. acidophilus, L. delbrueckii* ssp. *lactis* and *S. thermophilus*), no inhibition of PAB was found but there was always at least one combination for which there was no stimulation. The stimulation of PAB by thermophilic lactobacilli has a direct influence on the propionic acid fermentation in Emmental [9].

Analysis of the medium used for the growth of PAB (N fractions, amino acid composition, lactate isomers, NaCl), showed no direct relationship between the composition of the medium and the interaction between LAB and PAB [46].

FHL (*L. rhamnosus* and *L. casei)* had an inhibitory effect on the growth of *P. freudenreichii* in Emmental cheese, probably due to some metabolites (e.g. diacetyl, acetate and formate) produced by the activity of FHL [36].

2.2. Proteolysis of Emmental, Comté and Beaufort

2.2.1. Proteolytic profiles

Four Emmental, Comté and Beaufort cheeses made from raw milk were collected in winter and summer in two consecutive years and were analysed using the fractionation scheme shown in *figure 3* [31].

The average proportion of the different nitrogen fractions for each type of cheese showed that Emmental had the highest proportion (33.0 %) of native caseins, followed

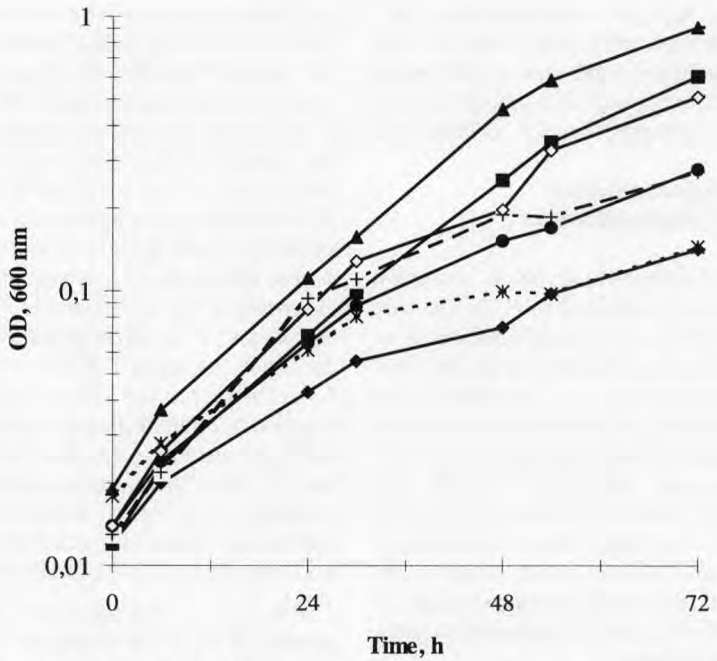

Figure 2. Growth of *P. freudenreichii* ssp. *freudenreichii* F 71433 in wheys produced from growth of different strains of LAB (Marmot and Bouton, unpublished).
—▲— *L. helveticus* CNRZ 32, —◇— *L. helveticus* L 116, —■— *L. helveticus* L 80, —+— *L. delbrueckii* ssp. *lactis* L 85, —✳— *S. thermophilus* S 18, —●— *S. thermophilus* S 79, —◆— Control whey.

Figure 2. Courbe de croissance de *P. freudenreichii* ssp. *freudenreichii* F 71433 dans des lactosérums fermentés par différentes souches de bactéries lactiques (Marmot et Bouton, non publié).
—▲— *L. helveticus* CNRZ 32, —◇— *L. helveticus* L 116, —■— *L. helveticus* L 80, —+— *L. delbrueckii* ssp. *lactis* L 85, —✳— *S. thermophilus* S 18, —●— *S. thermophilus* S 79, —◆— lactosérum témoin.

by Comté (21.6 %) and Beaufort (19.0 %) (*table III*). The low level of γ-CN [β-CN f 29–209 (γ_1-CN) + β-CN f 106–209 (γ_2-CN) + β-CN f 108–209 (γ_3-CN)] and high level of α_{s1}-I-CN (α_{s1}-CN f 24–199) in Emmental, indicate lower plasmin (PLM) and higher chymosin (CHY) activity in Emmental compared to Comté and Beaufort. Similar results were also found in Emmental by Fox and McSweeney [21] who compared the water insoluble peptides of a large variety of hard and semi-hard cheeses. Because of the denaturation of CHY during cooking, the presence of α_s-I-CN in Emmental was attributed to cathepsin D activity which has similar proteolytic activities to CHY. Comparison of the isoelectric focussing electrophoregram patterns of a Swiss cheese at 3 and 9 months on one hand, and of milks incubated with combinations of various proteases (rennet, PLM and proteases from *L. helveticus*) on the other hand, showed that the presence and activity of PLM was necessary to obtain identical electrophoregram patterns between cheese and milks with added enzymes [41].

All the fractions representing secondary proteolysis (fractions II, III, IV and V, *table III*) were lower in Emmental than in

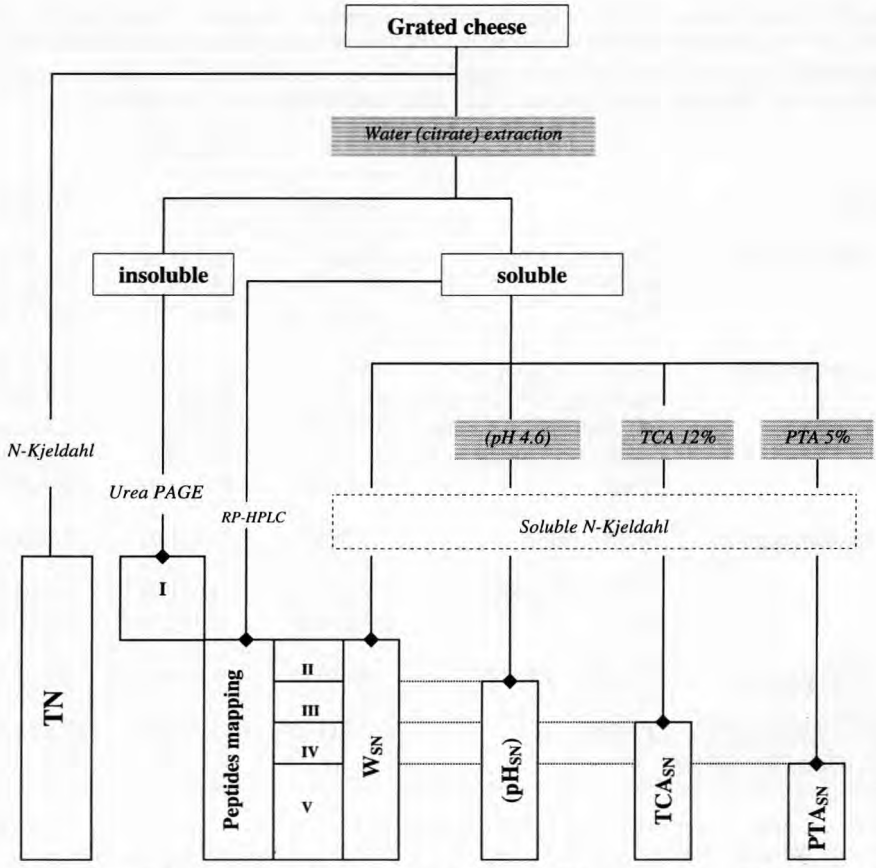

Figure 3. Scheme for the fractionation of nitrogen in cheese. TN: total nitrogen; WSN: water soluble nitrogen; pHSN: pH 4.6 soluble nitrogen; TCASN: 12 % TCA soluble nitrogen; PTASN: 5 % phosphotungstic acid soluble nitrogen.

Figure 3. Schéma de fractionnement de l'azote dans les fromages. TN : azote total ; WSN : azote soluble dans l'eau ; pHSN : azote soluble à pH 4,6 ; TCASN : azote soluble dans l'acide trichloroacétique à 12 % ; PTASN : azote soluble dans l'acide phosphotungstique à 5 %.

Comté and Beaufort. Except for fraction III which was nearly absent in Beaufort, probably because of a high aminopeptidase activity, the concentration of the different fractions increased in the following order: Beaufort > Comté > Emmental. Comté had the highest amount of medium size peptides (fractions II and III).

RP–HPLC profiles of the WSN fraction showed large differences between the 3 varieties (*figure 4*). Comté had the largest number of peaks in the hydrophobic-hydrophilic zone (> 35 min elution). In Beaufort, there was a low concentration of fraction III and high level of PTASN (*table III*), while the last fraction (> 70 min elution) was nearly absent. On the other hand, this fraction was quantitatively important in Emmental and Comté, but no clearly identified peak was present in Emmental.

The concentration of free amino acids (FAA) in the WSN was 18.2 for Emmen-

Table III. Quantification of the various N fractions in mature Emmental, Comté and Beaufort cheeses ($n = 4$). Average (standard deviation) in % of total nitrogen (Pochet et al., unpublished).

Tableau III. Approche quantitative de la dégradation des caséines dans l'emmental, le comté et le beaufort ($n = 4$). Moyenne (écart type) en % de l'azote total (Pochet et al., non publié).

Fraction			% Total N in:		
			Emmental	Comté	Beaufort
I	Native caseins	$(\alpha_{S1} + \alpha_{S2})$-CN	14.9 (1.7)	11.1 (1.5)	10.2 (0.9)
		β-CN	18.1 (0.9)	10.5 (0.7)	8.8 (1.3)
		Total	33.00 (2.3)	21.6 (2.1)	19.0 (2.0)
	Large peptides	γ-CN ($\gamma_1 + \gamma_2 + \gamma_3$)	15.3 (0.4)	18.5 (0.3)	18.4 (0.9)
		α_{S1}-I (α_{S1}-CN f 24–199)	15.3 (1.0)	12.2 (0.7)	11.4 (0.9)
		α_{S1} degradation products	10.9 (0.4)	12.3 (0.3)	11.7 (1.0)
		Unidentified products	8.1 (0.4)	8.2 (0.4)	7.1 (0.5)
		Total	49.6 (0.4)	51.1 (1.3)	48.7 (2.5)
II	Medium peptides	WSN – pHSN	1.7 (0.8)	3.4 (1.2)	5.0 (3.4)
III		PHSN – TCASN	2.5 (0.9)	4.0 (1.2)	0.2 (0.2)
		Total	4.3 (0.6)	7.4 (1.7)	5.2 (2.9)
IV	Small peptides	TCASN – PTASN	6.0 (0.7)	8.5 (0.4)	9.2 (0.3)
V	Free amino acids + very small peptides	PTASN	7.2 (1.6)	11.4 (1.3)	17.9 (1.2)

tal, 23.5 for Comté and 34.9 $mg \cdot g^{-1}$ for Beaufort [31]. Significant differences were observed in the amount of the different FAA in the cheeses, irrespective of the ripening conditions; season had only a limited influence. Asp, Thr, Ser, Glu, Pro, Ala, Val, Met, His and Lys followed a similar order in each cheese variety.

Using linear regression analysis, the rate of formation of 22 FAA during the first 150 d of ripening of Emmental ($n = 58$), Gruyère ($n = 184$) and Appenzeller ($n = 85$) was in decreasing order: Emmental < Appenzeller < Gruyère (173, 245, 293 $mg \cdot kg^{-1} \cdot d^{-1}$). The concentration of FAA decreased after 200 d of ripening because of decarboxylation [7].

Even though thermophilic lactobacilli in Emmental possess significant peptidase activities [22], the low concentration of FAA which was observed in Emmental, but also the differences in native caseins and proportions of peptides between the three cheese varieties (*table III*), can be explained by differences in the peptidases and cell-wall proteinase activities of the mesophilic and thermophilic lactobacilli populations of the cheeses [40, 51]. However, the extent of primary proteolysis by CHY and PLM should not be overlooked. For instance, hydrolysis of α_{s1}-CN by CHY, which occurred before heating, seems necessary for further degradation into FAA by LAB peptidases. These peptides enhance significantly the growth of *L. delbrueckii* ssp. *lactis*, even though this species preferentially utilizes β-CN [2]. Hydrolysis of β-CN by PLM during milk storage (3 d at four-fold the normal concentration of PLM) also stim-

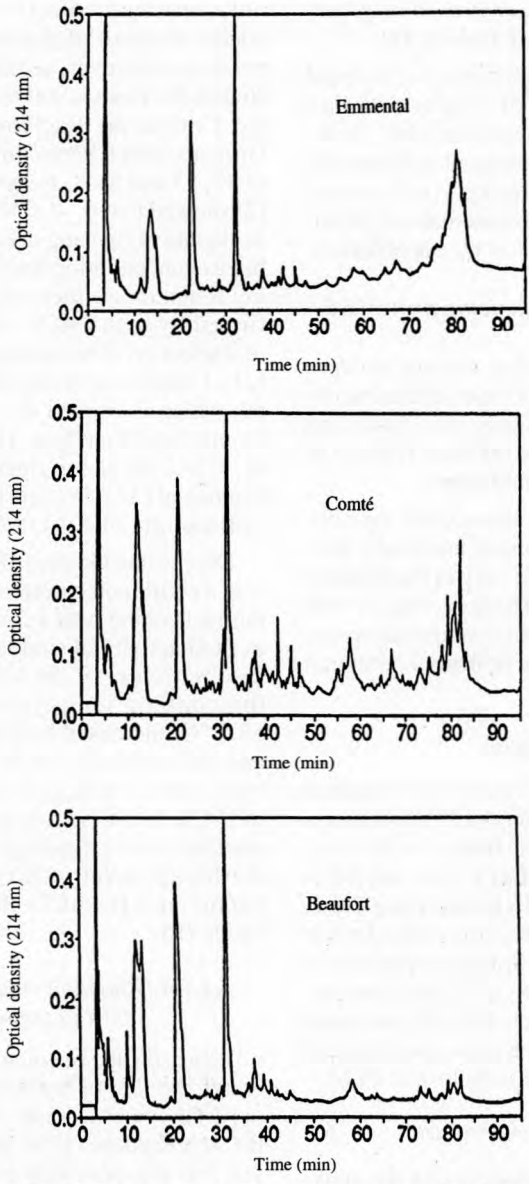

Figure 4. Examples of RP–HPLC peptide profiles of the water soluble fraction of Emmental, Comté and Beaufort cheeses (Nucleosil C18, 5 µm, 30 nm, 250 × 4.6 mm; Solvent A = TFA 0.115 % (v/v), solvent B = acetonitrile (60)/water (40) / TFA (0.1) (v/v/v); gradient: 0 % B for 10 min, then 0 to 80 % B in 80 min, then 100 % B for 10 min, then back to initial conditions for 15 min; flow rate: 1 mL·min^{-1}; detection: 214 nm) [31].

Figure 4. Exemples de profils peptidiques par CLHP–PI de la fraction soluble dans l'eau de fromages emmental, comté et beaufort (nucleosil C18, 5 µm, 30 nm, 250 × 4,6 mm ; solvant A = TFA 0,115 % (v/v), solvant B = acétonitrile (60)/eau (40) / TFA (0,1) (v/v/v) ; gradient : 0 % B pendant 10 min, puis de 0 à 80 % de B en 80 min, puis 100 % de B pendant 10 min, puis retour aux conditions initiales pendant 15 min ; débit : 1 mL·min^{-1} ; détection : 214 nm) [31].

ulates the growth of *L. delbrueckii* ssp. *lactis* by simulating cheese making [2].

Highly significant differences were found in the concentration of biogenic amines within and between cheese varieties. Comté had the lowest level compared to Emmental and Beaufort, i.e. 125 mg·kg^{-1} of dry matter vs. 873 and 1240 for Emmental and Beaufort respectively (Pochet et al., unpublished).

2.2.2. Factors influencing proteolysis

Cooking the curd/whey mixture between 50 and 55 °C for 20–30 min, limited acidification at draining (usually pH > 6.40), and long ripening times are the main features of Swiss-type cheese manufacture.

The pH at draining shows little variability, but cooking temperature and time × temperature ripening cycles vary in the different Swiss-type cheeses. These factors as well as the variability in gross composition may influence the activity of proteolytic enzymes during ripening.

2.2.2.1. pH at draining

Inconsistant results have been obtained concerning the retention of CHY by caseins at different pH values. However, it seems that the adsorption of CHY to α_s and β-CN increased as the pH decreased from 7.2 to 5.2 [38]. In addition, because of the lack of acidification, only a small proportion of CHY is retained in the curd [21]. Concerning PLM, several authors [19, 30] concluded that at the usual pH of Swiss-type cheese at draining (> 6.4), there is no loss of PLM.

2.2.2.2. Cooking temperature

It has been acknowledged that the activity of CHY, assessed either by the floculation time of the supernatant after centrifugation [23] or by the presence of α_{s1}-I-CN [10], was extensively diminished with increasing cooking temperature. Heat inactivation of CHY is enhanced at higher pH values, but both protein and Ca^{2+} have a protective effect [23]. It has been claimed for a long time that CHY had either no or only very limited activity in Swiss-type cheese because of the high cooking temperature; however, recent studies demonstrated that there is still residual CHY activity. Comparing Raclette, Emmental and Gruyère, which have cooking temperatures of 42, 53 and 56 °C respectively, Baer et al. [2] showed that α_{s1}-I-CN was present at the beginning of ripening in each type of cheese, but its concentration decreased as the cooking temperature increased. This was confirmed by urea-PAGE electrophoregrams of a selection of hard and semi-hard cheeses [21]. Comparing the proteolysis of Emmental, which is cooked at 2–3 °C lower than Comté and Beaufort, the breakdown of α_{s1}-CN at the end of ripening was higher in Emmental (37.2 %) than in Comté (34.4 %) or Beaufort (34.1 %) (*table IV*).

Dupont and Grappin [17] found that there was a continuous increase in PLM activity during cooking with a concomitant decrease in PLG activity in simulated Comté manufacture (*figure 5*). In addition, increasing the cooking temperature from 49.5 to 55.5 °C, increased both the concentration and the activity of PLM in mature Swiss-type cheeses with concomitant hydrolysis of β-CN to γ-CN (*figure 6*). These results confirm earlier findings [10] and explain the higher level of β-CN breakdown in Comté and Beaufort than in Emmental (*table IV*).

2.2.2.3. Ripening conditions and salt content

Increasing the ripening temperature accelerates the rate of hydrolysis of α_{s1}-CN to α_{s1}-I-CN, but has only a limited effect on β-CN breakdown [29]. Secondary proteolysis was also positively correlated with temperature. Emmental, Comté and Beaufort curds which were ripened according to the time × temperature ripening cycles of these three types of cheeses confirmed these earlier findings (*table IV*). Irrespective of the type of cheese, ripening conditions significantly influenced the degradation of α_{s1}-CN to α_{s1}-I-CN in the following order Beaufort = Comté < Emmental but had little

Table IV. Influence of cheese making and ripening conditions on the hydrolysis of α_{s1} and β caseins of Emmental, Comté and Beaufort at the end of ripening (Pochet et al., unpublished). Three unripened cheeses from the same vat were collected in Emmental, Comté and Beaufort cheese plants (curd origin) and ripened according to the following conditions:
Emmental (E): 7 °C / 1 wk; 11 °C / 3 wk; 23 °C / 5 wk; 5 °C / 5 wk
Comté (C): 13 °C / 1 wk; 18 °C / 12 wk; 6 °C / 8 wk
Beaufort (B): 10–11 °C / 31 wk
– (α_{s1}-CN proteolytic index = (α_{s1}-I-casein) 100 / (α_{s1} + α_{s1}-I + α_{s1} unidentified peptides)
– (β-CN proteolytic index = (casein·100 / (γ + β)
– Figures in bold correspond to genuine Emmental, Comté and Beaufort cheeses.
– Letters represent values that are statistically different at the 5 % level; superscripts and subscripts correspond to different comparisons of means.

Tableau IV. Influence des conditions de fabrication et d'affinage du fromage sur l'hydrolyse des caséines α_{s1} et β de l'emmental, du comté et du beaufort en fin d'affinage (Pochet et al., non publié). Trois fromages non affinés provenant de la même cuve ont été collectés dans des fromageries fabriquant de l'emmental, du comté et du beaufort (origine du caillé) et ont été affinés dans les conditions suivantes :
Emmental (E) : 7 °C / 1 semaine ; 11 °C / 3 semaines ; 23 °C / 5 semaines ; 5 °C / 5 semaines.
Comté (C) : 13 °C / 1 semaine ; 18 °C / 12 semaines ; 6 °C / 8 semaines.
Beaufort (B) : 10–11 °C / 31 semaines.
– α_{s1}–CN proteolytic index = indice de protéolyse de la caséine α_{s1} = (caséine α_{s1}–I) 100 / (α_{s1} + α_{s1}–I + peptides α_{s1} non identifiés.
– β–CN proteolytic index = indice de protéolyse de la caséine = (caséine g·100 / (γ + β)).
– Les chiffres en caractères gras correspondent à de vrais fromages d'emmental, comté et beaufort.
– Les valeurs affectées de lettres différentes sont statistiquement différentes au seuil de 5 % ; les lettres utilisées en exposant ou en indice correspondent à des comparaisons distinctes de moyennes.

Casein hydrolysis index	Ripening conditions (n = 4)	Curd origin (n = 4)			
		E	C	B	Total (n = 12)
α_{s1}–CN	E	**37.2**^{a}	37.6^{a}	36.9^{a}	*37.3*$_{a}$
proteolytic index	**C**	35.4^{b}	**34.4**$^{b, c}$	32.9^{d}	*34.3*$_{b}$
	B	32.8^{d}	33.8$^{c, d}$	**34.1**$^{c, d}$	*33.6*$_{b}$
	Total (n = 12)	*35.3*	*35.2*	*34.7*	
β–CN	E	**45.8**^{a}	60.3^{c}	64.0^{d}	*56.7*
proteolytic index	C	47.6$^{a, b}$	**64.0**^{d}	67.5	*59.7*
	B	50.5^{b}	63.6$^{c, d}$	**67.7**	*60.6*
	Total (n = 12)	*47.9*$_{a}$	*62.6*$_{b}$	*66.4*$_{c}$	

influence on the degradation of β-CN into γ-CN, except in Emmental in which lowered β-CN breakdown was found.

In addition to a lower cooking temperature, the low S/M content of Emmental (0.9 % vs 2.8 and 2.3 % for Comté and Beaufort, respectively) partly explains the greater breakdown of α_{s1}-CN by chymosin. Increasing the S/M level in model minicomté resulted in a linear decrease in the breakdown of α_{s1}-CN to α_{s1}-I-CN. The influence of salt on β-CN hydrolysis is more complex with a maximum activity at 2.3 % S/M and minima at 0 and 4.0 % [11].

The influence of ripening temperature and salt concentration on FAA production was studied by Demarigny [13]. The total amount of FAA, at the end of ripening, decreased slightly with the S/M level, with significant differences observed only

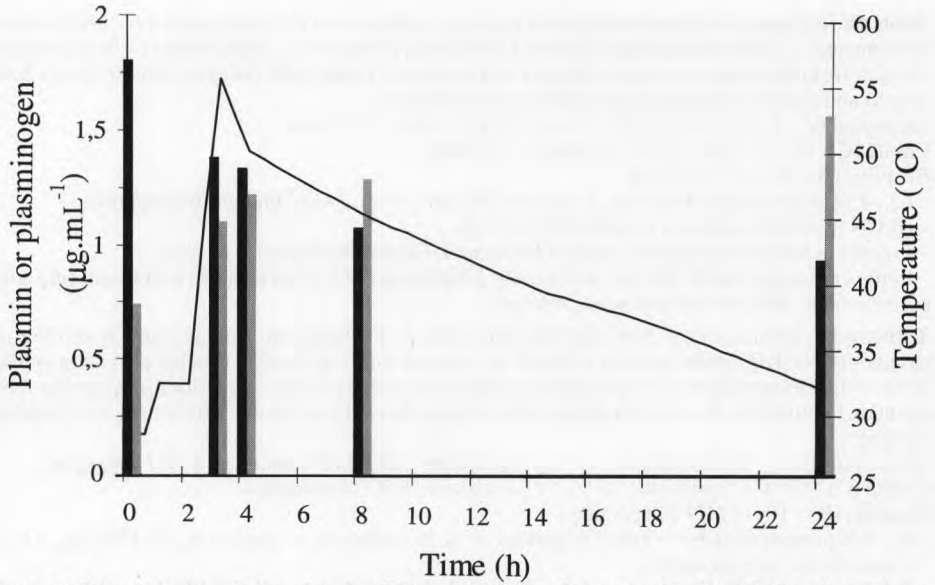

Figure 5. Plasmin (■) and plasminogen (■) activities in milk, determined by ELISA, during heat-treatment used for Swiss-type cheese manufacture. Temperature is represented by the continuous line [17].

Figure 5. Évolution de la concentration en plasmine (■) et en plasminogène (■) du lait, déterminée par ELISA, pendant le cycle de chauffage de la fabrication de fromage de type suisse. La température est représentée par une ligne continue [17].

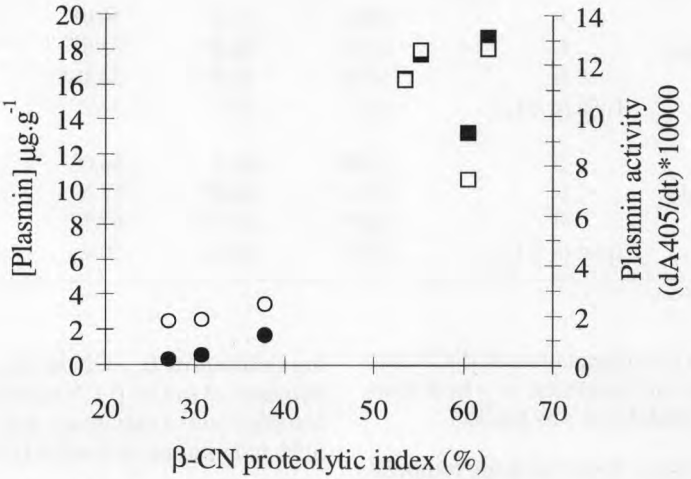

Figure 6. Influence of cooking temperature of the curd/whey mixture on the concentration and activity of plasmin of mature Swiss-type cheeses ($n = 7$). open symbol: plasmin activity [45], full symbol: plasmin concentration.

Figure 6. Influence de la température de cuisson du mélange caillé / lactosérum sur la concentration et l'activité de la plasmine dans des fromages affinés de type suisse. Symbole vide : activité de la plasmine [45]. Symbole plein : concentration en plasmine.

between 0.2 % and higher salt levels (1.5, 2.1 and 3.1 %). Ripening at temperatures > 14 °C had a more marked effect on the rate of FAA production with some differences in individual amino acids. The concentrations of total FAA increased from 781 mg·100 g^{-1} dry matter and 882 at 10 and 14 °C respectively to 1 407 at 18 °C and 1 992 at 22 °C [13].

2.3. Pasteurization and microfiltration of milk

During the last five years, several studies have been carried out on the influence of raw milk microflora on the ripening and quality of cheeses, comparing cheese made from raw milk (Ra) to that made with pasteurized (Pa) or microfiltered (MF) milk [28]. Studies on Swiss-type cheeses were carried out mainly on model mini-Comté [4, 5, 14, 15], and on Bergkäse [18, 25, 26], a traditional raw milk Swiss-type cheese, made in the alpine regions of Austria, with thermophilic starters. Bergkäse is cooked at 52 °C and ripened for 6 months at 12 °C and has a surface smear.

2.3.1. Microbiology

Pa or MF only delayed the growth of non-starter microorganisms (FHL, PAB, enterococci and *Micrococcaceae*) during the initial ripening stages of mini-Comté cheese. Except enterococci, which remained at a lower level than in Ra cheeses, the other microbial groups usually reached the same levels at the end of ripening in MF cheeses and slightly lower levels in Pa cheeses [4, 15]. On the other hand, the numbers of thermophilic lactobacilli and streptococci which were usually at levels of 10^8–10^9 cfu·g^{-1} at the beginning of ripening, decreased during ripening, but more rapidly in Pa and MF cheeses than in Ra cheeses. The decline of thermophilic lactobacilli in MF and Pa cheeses can be explained by the fact that *L. helveticus* from the starter dominated Pa and MF cheeses and disappeared rapidly, whereas *L. delbrueckii* ssp., which is commonly found in milk, and is more heat-resistant than *L. helveticus*, could grow to higher levels in Ra cheeses during ripening. Besides modifications in the level and growth rate of microorganisms, Pa or MF led to a lower diversity of species and strains [13]. In Bergkäse, FHL rapidly reached levels of 10^7–10^8 cfu·g^{-1} in Ra cheeses, and were not detected or remained at low levels in Pa cheeses. FHL growth was closely related to citrate metabolism which decreased from ~10 mmol·kg^{-1} to between 0.20 and 2.00 mmol·kg^{-1} during maturation, with concomitant increases in acetic and formic acids [18].

2.3.2. Proteolysis

Because pasteurization partially inactivates inhibitors of plasminogen (PLG) activators and PLM inhibitors, an increase of the hydrolysis of β-CN into γ-CN by PLM is found in Pa cheeses [4, 26]. The lower level of β-CN in MF cheeses [4, 5] was certainly the consequence of the retention of a small amount of β-CN by the MF membrane [50]. Even if the amount of α_{s1}-I-CN was not statistically different between the cheeses, the lower hydrolysis of α_{s1}-CN in Pa cheeses might be the result of heat denaturation of cathepsin D.

Secondary proteolysis, particularly the amount of FAA and very small peptides, measured in the PTASN fraction, was strongly influenced by raw milk microflora. Except in Bergkäse [26], all studies [4, 5, 14], showed significant higher levels of PTASN/TN in Ra cheeses compared to the Pa or MF cheeses. Comparison of cheeses made from MF milk, with or without addition of different retentates, showed lower levels of all the major FAA in MF cheeses at the end of ripening [13]. RP-HPLC profiles of the WSN fraction and particularly the hydrophobic peptides were also influenced by the presence and the origin of milk microflora [4, 5, 14], with significantly higher concentrations of hydrophobic pep-

tides [26]. Tyramine was found in mature Ra Bergkäse cheeses indicating FAA decarboxylation of tyrosine by the raw milk microflora [26].

2.3.3. Sensory properties

The lower level of FAA and the major reduction in propionic acid fermentation due to Pa or MF of milk, will induce significant changes in the sensory properties of the cheese. In model mini-Comté cheeses [4], made following standardized procedures from Ra, MF, Pa and Pa milk with added microfiltration retentates, clear discrimination between cheeses made with or without Ra milk flora, was obtained on the Analysis of Principal Component first axis of microbiological and biochemical data (*figure 7*). The second axis, which is correlated with α_{s1} and β-CN hydrolysis, discriminates cheeses according to whether the milk was pasteurized. The sensory properties used as additional variables showed that Ra cheeses had a more intense overall aroma, were more pungent, firmer and more granulous than MF or Pa cheeses both of which were bitter. Significant lower smell and aroma intensities with important bitterness were also observed in Bergkäse made from Pa milk [25]. These characteristics, together with a more intense buttery or lactic aroma in the Pa cheeses have usually been observed in all studies on Swiss-type cheeses. Part of the differences between Ra and Pa cheeses could be the low level and activity of PAB in MF and Pa cheeses. Emmental MF cheese was firmer, more granulous, with a lower nutty flavour than Ra cheeses, even on addition of PAB to the MF milk; greater openness was also a problem [8]. In addition, the higher casein breakdown arising from more intense PLM activity in Pa cheeses, with concomitant lower levels and activities of FHL explains the bitterness of Pa cheeses [4, 25].

It is also noteworthy that physico-chemical, microbial and sensory characteristics of cheese made with Ra were less uniform than Pa or MF cheeses (*figure 7*). When different microfiltration retentates, corresponding to different microfloras, were added to a single MF milk, the physicochemical characteristics and the sensory properties of model mini-Comté cheeses varied according to the origin of the milk microflora [14]. It is therefore possible that the diversity of aroma observed in Comté cheese [48], which is produced in about 200 small units, could partially originate from the variability of the indigenous milk microflora.

3. CONCLUSION

With continuous improvement in the microbiological quality of raw milk, there is a need for a better identification and characterization of microorganisms which participate in the ripening of cheese. For instance, the evolution of the major groups of starter and non-starter microorganisms in Comté during ripening, as well as the identification and biodiversity of lactobacilli is now clearer. Even though it is well established that non-starter microorganisms, especially FHL, usually induce higher proteolysis, the role of different groups of microorganisms on the quality of the final product, in particular in the case of Ra cheeses, is still in its infancy.

Besides bacterial proteinases, PLM and CHY, and probably cathepsin D, are the major proteases which contribute to casein breakdown during ripening. There is either a contradictory trend between the two enzymes (i.e. negative for CHY and positive for PLM for cooking temperature and S/M for Comté and Emmental), or no influence for one of the enzymes (i.e. milk pasteurization, addition of water [34], ripening temperature, ripening conditions for Comté and Emmental, and cheese variety for Beaufort and Comté). Little information is available on the specific consequence of the hydrolysis of α_{s1} and β-CN on the quality of cheese. However, it has been recognized

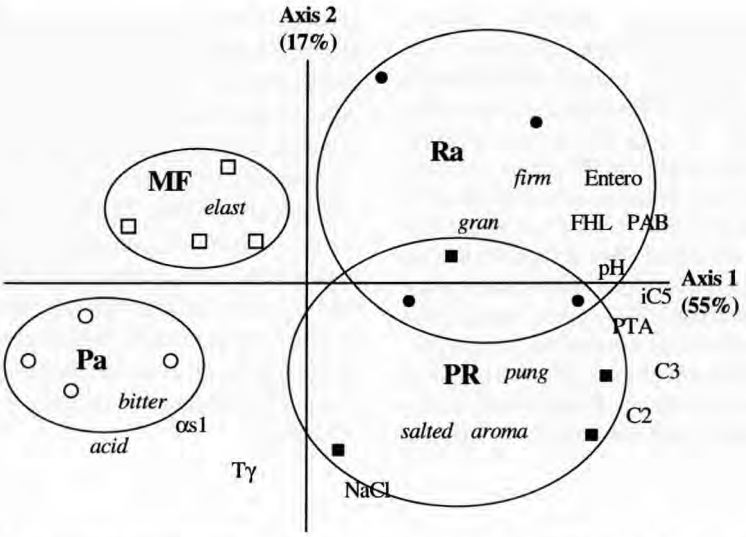

Figure 7. Principal components analysis of physico-chemical, microbiological and sensory measurements of model mini-Comté cheeses made from raw, Ra (●), microfiltered, MF (□), pasteurized, Pa (○), or pasteurized + MF retentate (■) milk.
Active variables: C2: acetic acid; C3: propionic acid; iC5: isovaleric acid; PTA: phosphotungstic acid-soluble nitrogen; PAB: propionibacteria; α_{s1}: α_{s1}-casein; Tγ: γ-caseins; FHL: facultatively heterofermentative lactobacilli; Entero: enterococci.
Additional variables: *aroma*: overall aroma intensity; *pung*: pungent; *gran*: granular; *elas*: elastic; *salted*; *bitter*; *acid*; *firm*.

Figure 7. Analyse en composantes principales des mesures physico-chimiques, microbiologiques et sensorielles effectuées sur des mini-fromages de Comté fabriqués à partir de lait cru, Ra (●), microfiltré, MF (□), pasteurisé, Pa (○) et pasteurisé + rétentat de microfiltration, PR (■).
Variables actives : C2 : acide acétique ; C3 : acide propionique ; iC5 : acide isovalérique ; PTA : azote soluble dans l'acide phosphotungstique ; PAB : bactéries propioniques ; α_{s1} : caséine α_{s1} ; Tγ : caséines γ ; FHL : lactobacilles hétérofermentaires facultatifs ; Entero : entérocoques.
Variables supplémentaires : *aroma* : intensité de l'arôme ; *pung* : piquant ; *gran* : granuleux ; *elas* : élastique ; *salted* : salé ; *bitter* : amer ; *acid* : acide ; *firm* : ferme.

that low α_{s1}-CN breakdown induces higher mechanical resistance of cheese to fracture (higher fracture stress) with higher deformability before fracture (higher fracture strain). Primary proteolysis may also influence the growth and activity of microorganisms. Sensory evaluation of Swiss cheese made with urokinase which increases PLM activity, produced cheese with a more intense propionic acid flavour [3]. This is likely to be due to stimulation of the growth of PAB following plasmin-induced hydrolysis of casein [1].

In the introduction, it was pointed out that it was almost impossible to use compositional and technological parameters to define Swiss-type cheese varieties. However, several attempts have been made to classify them either according to their variety or their origin, on the basis of their analytical characteristics. For instance, large numbers of Emmental (n = 116) and Gruyère (n = 320) were correctly classified, 97 and 93 % respectively, on the basis of their FAA composition and multivariate analysis [7]. Similarly, on the basis of sensory evalua-

tion (smell, elasticity, friability, flavour, saltiness and age-related pungency) of Bergkäse cheeses, a correct classification by cluster analysis was obtained according to the origin (cheese plant, and whether cheeses were made on the plains or in the mountains), and to the season of production of the cheeses [35]. In an experiment carried out on Comté cheeses made in five cheese plants, with either wild starters made locally or with the same starter, and cheeses ripened in the same conditions, it was possible to discriminate correctly the origin of 20 cheeses according to their physico-chemical variables, microbiological counts and sensory characteristics (*figure 8*). These results show the primary importance of milk characteristics and cheese making conditions on the characteristics of cheese. It also illustrates that the starter had almost no influence on the classification of cheeses. These examples show that, instead of relying on the gross composition and technological parameters of the cheese, it would be more useful to use the final characteristics of the cheese to define and classify cheese varieties. However, none of the models used have been validated on unknown samples of cheese.

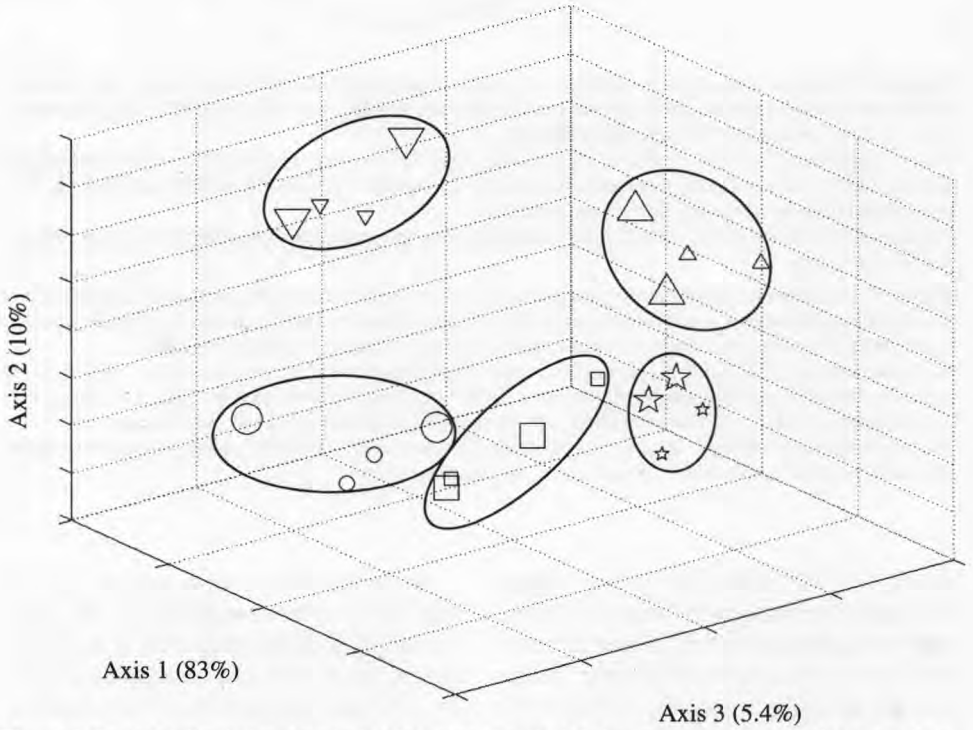

Figure 8. Factorial discriminant analysis of physico-chemical (L-lactate, propionic acid, fat, proteolytic index), microbial (enterococci) and sensory (acid, rancid, pungent, flavour intensity, sweet) characteristics of 20 Comté cheeses made in five cheese plants (▽, △, ○, □, ☆) made with local thermophilic starters (large symbols) or common thermophilic starter (small symbols) (Bouton, unpublished).

Figure 8. Analyse factorielle discriminante des caractéristiques physico-chimiques (Lactate L, acide propionique, matière grasse, indice de protéolyse), microbiologiques (entérocoques) et sensorielles (acide, rance, piquant, intensité de l'arôme, sucré) de 20 fromages de Comté fabriqués dans cinq fromageries (▽, △, ○, □, ☆) avec des levains thermophiles sauvages (grands symboles) ou des levains thermophiles identiques (petits symboles) (Bouton, non publié).

ACKNOWLEDGEMENTS

The excellent assistance of S. Fleuriot in preparing the manuscript was greatly appreciated.

REFERENCES

[1] Baer A., Influence of casein proteolysis by starter bacteria, rennet and plasmin on the growth of propionibacteria in Swiss-type cheese, Lait 75 (1995) 391–400.

[2] Baer A., Ryba I., Casey M., Influence of coagulating enzymes and plasmin on the growth of starter bacteria at the start of Emmental cheese manufacture. Preliminary report, Lait 77 (1997) 189–199.

[3] Bastian E.D., Lo C.G., David K.M.M., Plasminogen activation in cheese milk: Influence on Swiss cheese ripening, J. Dairy Sci. 80 (1997) 245–251.

[4] Beuvier E., Berthaud K., Cegarra S., Dasen A., Pochet S., Buchin S., Duboz G., Ripening and quality of Swiss-type cheese made from raw, pasteurized or microfiltrated milk, Int. Dairy J. 7 (1997) 311–323.

[5] Bouton Y., Grappin R., Comparaison de la qualité des fromages à pâte pressée cuite fabriqués à partir du lait cru ou microfiltré, Lait 75 (1995) 31–44.

[6] Bouton Y., Guyot P., Grappin R., Preliminary characterization of microflora of Comté cheese, J. Appl. Microbiol. 85 (1998) 123–131.

[7] Bütikofer U., Fuchs D., Development of free amino acids in Appenzeller, Emmentaler, Gruyère, Raclette, Sbrinz and Tilsiter cheese, Lait 77 (1997) 91–100.

[8] Chamba J.F., Perréard E., Effect of microfiltration of milk on Emmental cheese characteristics, Bull. Int. Dairy Fed. 317 (1996) 64.

[9] Chamba J.F., Perréard E., Kerjean J.R., Interaction between thermophilic lactobacilli and propionic acid bacteria in Emmental cheese, Bull. Int. Dairy Fed. 317 (1996) 58.

[10] Delacroix-Buchet A., Fournier S., Protéolyse et texture des fromages à pâte cuite pressée. II. Influence de la chymosine et des conditions de fabrication, Lait 72 (1992) 53–72.

[11] Delacroix-Buchet A., Trossat P., Protéolyse et texture des fromages à pâte cuite pressée. I. Influence de l'activité de l'eau, Lait 71 (1991) 299–311.

[12] De Man J.C., Rogosa M., Sharpe M.E., A medium for the cultivation of lactobacilli, J. Appl. Bacteriol. 23 (1960) 130–135.

[13] Demarigny Y., Rôle de la flore naturelle du lait cru et des paramètres technologiques (teneur en sel et température d'affinage) sur l'évolution des caractéristiques biochimiques, microbiologiques et sensorielles des fromages à pâte pressée cuite, thèse université de Bourgogne, ENSBANA, Dijon, 1997.

[14] Demarigny Y., Beuvier E., Buchin S., Pochet S., Grappin R., Influence of raw milk microflora on the characteristics of Swiss-type cheeses. II. Biochemical and sensory characteristics, Lait 77 (1997) 151–167.

[15] Demarigny Y., Beuvier E., Dasen A., Duboz G., Influence of raw milk microflora on the characteristics of Swiss-type cheeses. I. Evolution of microflora during ripening and characterization of facultatively heterofermentative lactobacilli, Lait 76 (1996) 371–387.

[16] Drinan F.D., Cogan T.M., Detection of propionic acid bacteria in cheese, J. Dairy Res. 59 (1992) 65–69.

[17] Dupont D., Grappin R., ELISA for differential quantitation of plasmin and plasminogen in cheese, J. Dairy Res. 65 (1998) 643–651.

[18] Eliskases-Lechner F., Ginzinger W., Rohm H., Tschager E., Raw milk flora affects composition and quality of Bergkäse. 1. Microbiology and fermentation compounds, Lait (accepted for publication).

[19] Farkye N.Y., Fox P.F., Observations on plasmin activity in cheese, J. Dairy Res. 57 (1990) 413–418.

[20] Fessler D.S., Characterization of propionibacteria in Swiss raw milk by biochemical and molecular biological methods, thesis, Swiss Federal Institute of Technology, Zurich, 1997.

[21] Fox P.F., McSweeney P.L.H., Proteolysis in cheese during ripening, Food Rev. Int. 12 (1996) 457–509.

[22] Gagnaire V., Lortal S., Léonil J., Free active peptidases are detected in Emmental juice extracted before ripening in the warm room, J. Dairy Res. 65 (1998) 119–128.

[23] Garnot P., Mollé D., Heat-stability of milk-clotting enzymes in conditions encountered in Swiss cheese making, J. Food Sci. 52 (1987) 75–77, 87.

[24] Gautier M., de Carvalho A., Renault A., DNA fingerprinting of dairy propionibacteria strains by Pulsed-Field Gel Electrophoresis, Current Microbiol. 32 (1996) 17–24.

[25] Ginzinger W., Jaros D., Lavanchy P., Rohm H., Raw milk flora affects composition and quality of Bergkäse. 3. Physical and sensory properties, Lait (accepted for publication).

[26] Ginzinger W., Jaros D., Mayer H.K., Rohm H., Tschager E., Raw milk flora affects composition and quality of Bergkäse. 2. Chemical composition, Lait (accepted for publication).

[27] Giraffa G., Rossetti L., Muccetti G., Addeo F., Neviani E., Influence of the temperature gradient on the growth of thermophilic lactobacilli used as natural starters in Grana cheese, J. Dairy Sci. 81 (1998) 31–36.

[28] Grappin R., Beuvier E., Possible implications of milk pasteurization on the manufacture and sensory quality of ripened cheese: a review, Int. Dairy J. 7 (1997) 751–761.

[29] Grappin R., Lefier D., Dasen A., Pochet S., Characterizing the ripening of Gruyère de Comté: Influence of time × temperature and salting conditions on eye and slit formation, Int. Dairy J. 3 (1993) 313–328.

[30] Grufferty M.D., Fox P.F., Factors affecting the release of plasmin activity from casein micelles. N. Z. J. Dairy Technol. 23 (1988) 153–163.

[31] Guichard H., Contribution au développement de méthodes instrumentales pour l'étude des fractions volatiles et azotées solubles dans l'eau des fromages à pâte pressée cuite. Relation avec l'analyse sensorielle, thèse université de Bourgogne, ENSBANA, Dijon, 1995.

[32] Isenberg H.D., Goldberg D., Sampson J., Laboratory studies with a selective *Enterococcus* medium, Appl. Microbiol. 20 (1970) 443–446.

[33] Isolini D., Grand M., Glättli H., Selektivmedien zum Nachweis von obligat und fakultativ heterofermentativen Laktobazillen, Schweiz. Milchw. Forschung 19 (1990) 57–59.

[34] Jaros D., Ginzinger W., Tschager E., Mayer H.K., Rohm H., Effects of water addition on composition and fracture properties of Emmental cheese, Lait 77 (1997) 467–477.

[35] Jaros D., Jenny B., Rohm H., Characteristics and description of Voralberger Bergkäse. 3. Sensory properties, Milchwissenschaft 53 (1998) 143–147.

[36] Jimeno J., Lazaro M.J., Sollberger H., Antagonistic interactions between propionic acid bacteria and non-starter lactic acid bacteria, Lait 75 (1995) 401–413.

[37] Kosikowski F.V., Cheese with eyes, in: Cheese and Fermented Milk Foods, Edward Brothers Inc., Ann Arbor, Michigan, 1977, pp. 261–291.

[38] Larsson K.I., Andrén A., Affinity between chymosin and individual caseins at varying pH values, Int. Dairy J. 7 (1997) 615–618.

[39] Mocquot G., Reviews of the progress of Dairy Science: Swiss-type cheese, J. Dairy Res. 46 (1979) 133–160.

[40] Naes H., Nissen-Meyer J., Purification and N-terminal amino acid determination of the cell wall bound proteinase from *Lactobacillus paracasei* subsp. *paracasei*, J. Gen. Microbiol. 138 (1992) 313–318.

[41] Ollikainen P., Kivelä T., The importance of plasmin in Swiss-type cheese ripening, Milchwissenschaft 44 (1989) 204–206.

[42] Piveteau P.G., Condon S., Cogan T.M., Interactions between lactic and propionic acid bacteria, Lait 75 (1995) 331–343.

[43] Reinbold G.W., Swiss Cheese Varieties, Pfizer Inc., New York, 1972.

[44] Richoux R., Kerjean J.R., Study of relationships between salt level in Emmental cheeses and propionic acid fermentation: Test of 13 strains of propionibacteria, Bull. Int. Dairy Fed. 317 (1996) 61.

[45] Rollema H.S., Visser S., Poll J.K., Spectrophotometric assay of plasmin and plasminogen in bovine milk, Milchwissenschaft 38 (1983) 214–217.

[46] Salvat-Brunaud D., Thierry A., Les bactéries lactiques thermophiles influençant la fermentation propionique. Étude en milieu simulant la phase aqueuse de l'Emmental, 8e colloque club bactéries lactiques, 28–29 Dijon, 1997, Affiche T 1.

[47] Steffen C., Eberhard P., Bosset J.O., Rüegg M., Swiss-type varieties, in: Fox P.F. (Ed.), Cheese: Chemistry, Physics and Microbiology, vol 2 Major cheese groups, Chapman & Hall, London, 1993, pp. 83–110.

[48] Stévenot C., Bérodier F., Schlich P., Typologie aromatique des fromages de Comté, Sci. Aliments. 17 (1997) 547–553.

[49] Therzaghi B.E., Sandine W.E., Improved medium for lactic streptococci and their bacteriophages. Appl. Microbiol. 29 (1975) 807–813.

[50] Tziboula A., Steele W., West I., Muir D.D., Microfiltration of milk with ceramic membranes: Influence on casein composition and heat stability, Milchwissenschaft 53 (1998) 8–11.

[51] Zevaco C., Gripon J.-C., Properties and specificity of a cell-wall proteinase from *Lactobacillus helveticus*, Lait 68 (1988) 393–408.