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Original article

Contribution of propionic acid bacteria to lipolysis of Emmental cheese

Jean-François CHAMBA*, Éric PERREARD

Institut Technique Français des Fromages, 419 route des champs laitiers, BP 30
74801 La Roche-sur-Foron Cedex, France

Abstract — Compared to many cheeses, lipolysis of Emmental is generally low. It concerns about 1–2% of cheese fat and the amount of free fatty acids (FFAs) varies from 2 to 7 g·kg^{-1} of ripened cheese. In this work, we studied the extent of lipolysis due to propionic acid bacteria (PAB). The release of FFAs during the ripening of Emmental cheeses made from microfiltered milk, with or without propionic acid bacteria (PAB) was followed. The usual cooking step in the cheese process (up to 50 °C and 30 min) inactivated the lipoprotein lipase (EC 3.1.1.34) and the main factor in the lipolysis of Emmental cheese was shown to be the lipolytic activity of PAB. This activity was strain-dependent and not related to the production of propionic acid in cheese. The most abundant FFAs were palmitic acid, oleic acid, myristic acid and stearic acid as in milk fat and it was concluded that lipolytic activity of PAB is non-specific. Lastly, an interaction still not elucidated was evidenced between certain PAB strains and thermophilic lactobacilli strains regarding lipolysis extent in Emmental cheese.

propionic acid bacteria / lipolysis / free fatty acid / Emmental cheese

Résumé — **Contribution des bactéries propioniques à la lipolyse de l'emmental**. La lipolyse est comme la protéolyse et la glycolyse un phénomène majeur de l'affinage des fromages. Comparativement à de nombreux autres fromages, la lipolyse de l'emmental est généralement limitée. Elle porte sur environ 1 à 2% de la matière grasse du fromage et la teneur en acides gras libres (AGL) varie de 2 à 7 g·kg^{-1} dans le fromage affiné. Dans plusieurs expérimentations en fromagerie pilote, nous avons étudié l'intensité de la lipolyse générée par les bactéries propioniques. La libération des AGL au cours de l'affinage d'emmentals fabriqués à partir de lait microfiltré ensemencé ou non en bactéries propioniques, démontre que la cuisson habituelle du caillé en cuve (au moins 50 °C pendant 30 min) inactive la lipoprotéine lipase (EC 3.1.1.34) et que l'activité lipolytique des bactéries propioniques est majoritairement responsable de la lipolyse de l'emmental. Cette activité est souche dépendante et n'est pas corrélée avec l'intensité de la fermentation propionique. Des lactobacilles thermophiles homofermentaires interagissent avec les bactéries propioniques sur la lipolyse de l'emmental, par un

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* Correspondence and reprints
Tel.: (33) 4 50 03 33 03; fax: (33) 4 50 25 82 26; e-mail: itff.jfchamba@wanadoo.fr

mécanisme non élucidé. Les acides palmitique, oléique, myristique et stéarique sont les AGL les plus abondants au même titre que dans le lait. L'activité lipolytique des bactéries propioniques ne semble donc pas spécifique.

bactérie propionique / lipolyse / acide gras libre / emmental

1. INTRODUCTION

Lipolysis is an important phenomenon in cheese ripening as are proteolysis and glycolysis [17]. In Emmental cheese, the amount of free fatty acids (FFAs) varies from 2 to 7 $g \cdot kg^{-1}$. This lipolysis is low compared to many cheeses but FFAs are aromatic compounds by themselves and by their end products: alcohol, methyl-ketone, esters, and lactones [5, 8, 27, 28, 36, 40, 42, 43]. In this way, the lipolysis is generally recognised as necessary to produce the typical flavour of Emmental cheese [2, 29, 41, 44]. Nevertheless, higher FFAs content gives flavour defects [10, 34].

Two sources of lipolytic activity can occur in Emmental cheese. The first one is lipoprotein lipase (EC 3.1.1.1.34), about 1–2 $mg \cdot L^{-1}$ of milk, essentially linked to casein [10]. This enzyme is thermolabile and according to Driessen [12] the cooking of the curd used in Emmental cheesemaking leads to a strong inactivation of lipoprotein lipase, by 3 $\log_{10}$ under minimal cooking conditions such as 50 °C for 20 min, and by 7 $\log_{10}$ under traditional cooking conditions at 53 °C for 45 min. Consequently, this lipolytic enzyme should not be "active" in Emmental cheese. The second source is bacterial lipases [8, 17, 32, 39]. The microbial ecosystem of Emmental cheese contains several microorganisms: mesophilic and thermophilic lactic acid bacteria, homofermentative or heterofermentative bacteria and propionic acid bacteria. In Emmental cheese lactococci are normally low, about 10^3 $cfu \cdot g^{-1}$ [6]. Among thermophilic lactic acid bacteria *S. thermophilus* exhibits in vitro noticeable intracellular lipolytic activity, whereas lactobacilli have a weak lipolytic activity [19, 38]. Lipolytic activities of non-starter lactic acid bacteria (NSLAB), such as heterofermentative lactobacilli and pediococci, are variable [20–22]. Propionic acid bacteria (PAB) are well known for their high lipolytic activity, 10 to 100 times more than lactic acid bacteria [15, 26]. In vitro studies as well as experimental cheeses have shown the release of FFAs by PAB [14, 24, 31, 37]. The location of lipase in PAB remains controversial: intracellular, parietal and extracellular, or extracellular [13, 30].

This study delineates the contribution of PAB to lipolysis of Emmental cheese. In order to achieve this we have performed several experiments in the ITFF cheese pilot plant. Firstly, as a reference, we followed the lipolysis during the ripening of Emmental cheeses made from raw milk without the addition of PAB. Then, we used microfiltration to remove natural flora of milk, especially PAB [35]. Cheeses were made without PAB or with three different PAB strains selected from previous results obtained in minifabrication and in cheese pilot plant [35]. Finally, we compared several associations between PAB strains and thermophilic lactobacilli strains (LAB) used in cheeses made from thermised milk [25]. These last experiments give results that are closest to an industrial scale.

2. MATERIELS AND METHODS

2.1. Strains

Five strains of *Propionibacterium freudenreichii* were used: the ITGP1, ITGP9, ITGP18 and ITGP23 strains come from our collection, and the VP131 strain comes from VALIO AS Helsinki, Finland

kindly provided by T. Suomalainen. Characteristics of PAB-ITG strains have been described previously [18, 25, 35]. *Lactobacillus helveticus* ITGLH56 and ITGLH192, *Lactobacillus delbrueckii* subsp. *lactis* ITGLL57 and ITGLL229 also come from our collection (ITFF, La Roche-sur-Foron, France). For all experiments, we have used the same *Streptococcus thermophilus* starter PAL 82-87 (Standa Industrie, Caen, France). All strains were conserved under gaseous N_2 (≈ -160 °C) in skim milk containing 10% glycerol. Starter of *S. thermophilus* was obtained by sub-cultivation in Marstar 412A medium (Rodhia Food, Dangé Saint Romain, France) and starter of lactobacilli strains was obtained by sub-cultivation in Phagex LBH medium (Standa Industries, Caen, France); PAB strains were sub-cultured according to Richoux and Kerjean [35].

2.2. Experimental design

A single comparison between PAB strains and control without PAB was performed three times for the two experiments from microfiltered milk. For the interaction experiment we used thermised milk, and a full balanced and randomised plan with three repetitions for each association between PAB and LAB.

Data on six cheeses made from raw milk come from the lipolysis project performed in our cheese pilot plant during a grazing season (spring) within the same period of PAB experiments. In these cheeses, we have used the same starters of lactic acid bacteria which were used in experiments from microfiltered milk.

2.3. Cheese making

From the same milk, two or three vats of Emmental cheese were made daily. According to experiments, the milk was used raw or thermised (63 °C, 30 s) or microfiltered and it was always standardised (fat $\approx$ 2.8%). Before microfiltration, raw milk was entirely skimmed, the cream was pasteurised (78 °C, 30 s) and then a part was added to skim milk to standardise its fat content. The microfilter device used was MFS7 (Tetra Pak, Aarhus, Denmark) with ceramic membrane 1,4 µ Membralox (Société des Céramiques, Tarbes, France). The same quantity of starters and rennet was added to each vat ($\approx$ 900 kg of milk). Before renneting, the milk was ripened in the vat for 1 h at 32 °C with 0.2% of *Streptococcus thermophilus* starters. After the addition of 0.05% lactobacilli starter, PAB and rennet (25 mL·100 L^{-1}), the coagulation time was about 25 min. The curd was cut into grains (diameter: 2.5 to 3 mm) and stirred for 10 min before cooking to 53 °C in 30 min. After cooking, the stirring continued for approximately 30 min, followed by cooling the curd to 50 °C for moulding.

After 6 h under press, the cheese was stored in an acidifying room for 18 h at 20 °C. Then, the cheese was placed in a cold room for 12 h at 12 °C before salting in saturated brine (48 h at 12 °C).

The ripening of Emmental cheese begins in a cold room for 15 d at 12 °C and 80% relative humidity, followed by 15 d at 15 °C and then, by setting in a warm room (23 °C, 80% relative humidity) for 4 to 6 weeks according to the eye development. The cheeses were then cooled to 8 °C and stored until they were 90 d old, in the traditional way, without plastic film.

2.4. Analysis of cheeses

Acidification, moisture, propionic acid fermentation and FFAs content were analysed. A penetration pH electrode was introduced into grated cheese at 20 °C. After 30 s, stability was reached and the pH was measured The dry matter of cheese was determined at 103 °C ± 2 °C for 15 h on 5 g of grated cheese. Fat was measured on grated cheese according to the Heiss butyrometric method [23].

Acetic acid and propionic acid were measured by gas chromatography according to Berdagué [4]. The FFAs content of cheese was determined by the ITERG - Institut des Corps Gras, Pessac, France, according to De Jong and Badings [11], PAB was enumerated after 7 d on PAL Propiobac Agar (Standa Industrie, Caen, France) incubated at 30 °C under anaerobic growth conditions.

2.5. Statistical analysis

This was done with the STAT-ITCF software (1991) for variance comparison, regression study an ANOVA. These tests enabled us to quantify the influence of the various PAB and LAB strains on the characteristics of the experimental cheeses.

3. RESULTS

3.1. Evolution of lipolysis during the ripening of Emmental cheese made from raw milk

At the end of the ripening (90 d old) average values were 63.5% (± 0.4) for dry matter (DM), 46.8% (± 1.3) for fat in DM, 5.72 (± 0.05) for cheese pH, 2.33 (± 0.4) g·kg^{-1} for acetic acid and 4.3 (± 0.5) g·kg^{-1} for propionic acid (Tab. I).

Table I. Evolution of FFAs during ripening of Emmental cheeses made from raw milk, average values and standard deviation in parentheses.

FFAs mg·kg^{-1}	Ripening time		
	7 d	50 d[(1)]	90 d
Butyric acid (C4)	8 (1)	40 (10)	84 (8)
Caproïc acid (C6)	6 (0.1)	14 (2)	32 (5)
Caprylic acid (C8)	9 (0.5)	12 (0.5)	22 (3)
Capric acid (C10)	48 (11)	46 (2)	62 (11)
Lauric acid (C12)	52 (5.5)	62 (2.5)	86 (20)
Myristic acid (C14)	118 (8.5)	149 (7)	205 (50.5)
Myristoleïc acid (C14:1)	14 (1)	16 (0.5)	28 (13)
Pentadecanoïc acid (C15)	[(2)]	[(2)]	29 (6)
Palmitic (C16)	355 (19.5)	453 (20)	610 (150)
Palmitoleic acid (C16:1)	30 (2)	32 (4)	51 (8)
Heptadecanoïc acid (C17)	24 (2.5)	20 (4)	39 (13)
Steareic acid (C18)	106 (5)	153 (14)	153 (46)
Oleïc acid (C18:1)	315 (16)	360 (31)	505 (104)
Linoleïc acid (18:2)	37 (7)	43 (6)	55 (13.5)
Conjugated linoleïc acid (18:2c)	7 (0.5)	9 (0.5)	16 (4.5)
Linolenic acid (C18:3)	12 (4)	16 (1)	12 (2.5)
Arachidic acid (C20)	2 (0.5)	2 (2)	2 (0.7)
Gadoleïc acid (C20:1)	3 (0.5)	4 (1.5)	5 (2.5)
Total	**1 146 (26.5)**	**1 431 (215)**	**1 996 (419)**
Short chain FFAs, %	6.2	7.8	10
Medium chain FFAs, %	16	15.9	17.4
Long chain FFAs, %	77.8	76.3	72.6

(1) After 20 d in warm room.
(2) Undetectable.

These characteristics are usual for French Emmental cheese made from raw milk [3].

At the start of the ripening, cheeses already contained 1.15 g·kg^{-1} of FFAs: the release of FFAs started in the warm room (Tab. I) and they increased by 1.25 times between day one and day 50 (20 d in the warm room) and by 1.74 times at the end of the ripening (90 d old). Nevertheless, the level of FFAs, 2 g·kg^{-1}, indicates a low lipolysis in these cheeses. The most abundant FFAs were palmitic acid (C16), oleic acid (C18: 1), myristic acid (C14) and stearic acid (C17) as in milk [9]. The pattern of FFAs was modified noticeably during the ripening. The ratio of long chain FFAs (C16 to C20) decreased from 77.8% to 72.6%, whereas the ratio of short chain FFAs increased from 6.2% to 10%. The amount of butyric acid was 10 times higher but the low level, 84 mg·kg^{-1}, and the low ratio between C4 and C6, 2.6, showed that it came only from lipolysis and was not produced by butyric fermentation.

3.2. Effect of PAB on lipolysis of Emmental cheeses made from microfiltered milk

In the first experiment (Tab. II, Fig. 1), the milk used after microfiltration contained less than 10 cfu·mL^{-1} of PAB; the

Table II. Composition of ripened Emmental cheeses (90 d old) made from microfiltered milk without or with PAB strain ITGP9.

Characteristics	Without PAB	With PAB
Dry matter, %	63.5	63.1 ^{NS}
Fat in dry matter, %	46.3	45.8 ^{NS}
pH	5.58	5.64 ^{NS}
Acetic acid, g·kg^{-1}	0.94	2.30 **
Propionic acid, g·kg^{-1}	1.10	7.20 **
Free fatty acids (mg·kg^{-1})		
Butyric acid	58	99 *
Caproïc acid	23	61 *
Caprylic acid	17	33 *
Capric acid	59	111 *
Lauric acid	16	171 *
Myristic acid	227	454 *
Myristoleïc acid	25	43 ^{NS}
Pentadecanoïc acid	26	56 *
Palmitic acid	684	1 446 **
Palmitoleïc acid	54	91 **
Heptadecanoïc acid	28	76 **
Stearic acid	190	337 **
Oleïc acid	500	920 *
Linoleïc acid	52	92 **
Conjucated linoleïc acid	17	21 ^{NS}
Linolenic acid	19	31 *
Arachidic acid	1	3 ^{NS}
Gadoleïc acid	1	9 *
Total FFAs (mg·kg^{-1})	**2 080**	**4 062 ** **

^{NS} : Non significant difference ($P > 0.05$).
*, ** : Significant difference (* $P < 0.05$, ** $P < 0.01$).

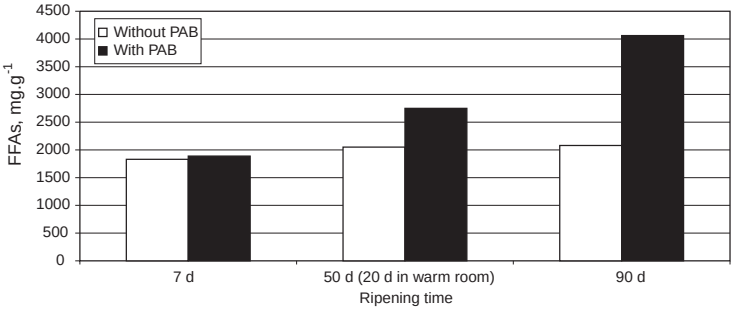

Figure 1. Evolution of FFAs content during ripening of Emmental cheeses, made from microfiltered milk without or with PAB strain ITGP9.

PAB strain ITGP9 was added before renneting to 7.7×10^4 cfu·mL^{-1}. This led to very different propionic fermentation in Emmental cheeses. With the addition of PAB the content of propionic acid was about seven times more than in cheeses made without PAB. Without PAB the content in FFAs increased slightly during ripening (+13.7%) whereas with PAB it was about twice as much. In these cheeses, the release of FFAs is noticeable (+ 45.6%) already after 20 d in a warm room. These results demonstrated the strong contribution of PAB to lipolysis of Emmental cheese. Moreover, these results pointed out that other lipolytic enzymes such as lipoprotein lipase or lipases of lactic acid bacteria seemed ineffective during the ripening of Emmental cheese.

In the second experiment (Tab. III), the milk after microfiltration contained 20 cfu·mL^{-1} of PAB. PAB strains ITGP14 and ITGP18 were added before renneting to 1×10^5 cfu·mL^{-1}. This higher number of PAB in the milk explains the higher propionic fermentation in control cheese compared to the first experiment. Otherwise, the PAB strains ITGP9, P14 and P18 produced generally in experimental cheeses a similar amount of propionic acid (data not shown). Moreover, the pattern of FFAs differed between experiments because cheeses were made during two different seasons (March and June). Nevertheless, the results were similar, at least for the comparison between control and cheeses made with PAB strain ITGP14. The FFAs content of cheese made with this strain is more than twice the amount in control cheese made without PAB. In contrast, the strain ITGP18 shows a very weak lipolytic activity.

Lipolytic activity of PAB releasing all fatty acids appeared unspecific (Tabs. II and III). However, the evolution of the FFAs pattern (Fig. 2) in these experiments from microfiltered milk show some modifications during ripening. At the start of the ripening, this pattern was similar to the milk whereas in the warm room the ratio of long chain FFAs increased and medium chain FFAs decreased.

This second experiment confirmed the first one and pointed out that the lipolytic activity of PAB was strain-dependent.

3.3. Effect of PAB and LAB associations on lipolysis of Emmental cheese made from thermised milk

Figure 3 shows the effect of PAB strains on FFAs and propionic acid of ripened cheeses; no correlation between propionic

Table III. Composition of ripened Emmental cheeses (90 d old) made from microfiltered milk without or with PAB strains ITGP14 and ITGP18.

Characteristics	Without PAB	With PAB strains	
		P18	P14
Dry matter, %	63.4	63.2	62.9
Fat in dry matter, %	45.7	45.9	46.1
pH	5.53	5.59	5.54
Acetic acid, g·kg^{-1}	1.24 ^{b}	2.32 ^{ab}	2.88 ^{a}
Propionic acid, g·kg^{-1}	2.80 ^{b}	7.07 ^{a}	8.18 ^{a}
Free fatty acids (mg·kg^{-1})			
Butyric acid	81 ^{b}	83 ^{b}	149 ^{a}
Caproïc acid	52 ^{b}	56 ^{b}	132 ^{a}
Caprylic acid	27	56	73
Capric acid	77 ^{b}	112 ^{ab}	189 ^{a}
Lauric acid	98 ^{b}	143 ^{ab}	268 ^{a}
Myristic acid	197 ^{b}	202 ^{b}	707 ^{a}
Myristoleïc acid	ND	ND	ND
Pentadecanoïc acid	52 ^{b}	64 ^{b}	195 ^{a}
Palmitic acid	848 ^{b}	800 ^{b}	1 932 ^{a}
Palmitoleïc acid	36 ^{b}	40 ^{b}	102 ^{a}
Heptadecanoïc acid	26 ^{b}	19 ^{b}	58 ^{a}
Stearic acid	396 ^{b}	369 ^{b}	728 ^{a}
Oleïc acid	707 ^{b}	889 ^{b}	1 802 ^{a}
Linoleïc acid	154 ^{b}	106 ^{b}	255 ^{a}
Conjucated linoleïc acid	ND	ND	ND
Linolenic acid	33 ^{b}	28 ^{b}	53 ^{a}
Total FFAs, (mg·kg^{-1})	**2 784** ^{b}	**2 967** ^{b}	**6 643** ^{a}

$^{a, b}$ Means followed by different superscripts on the same row differ ($P < 0.05$).
ND Non-detected.

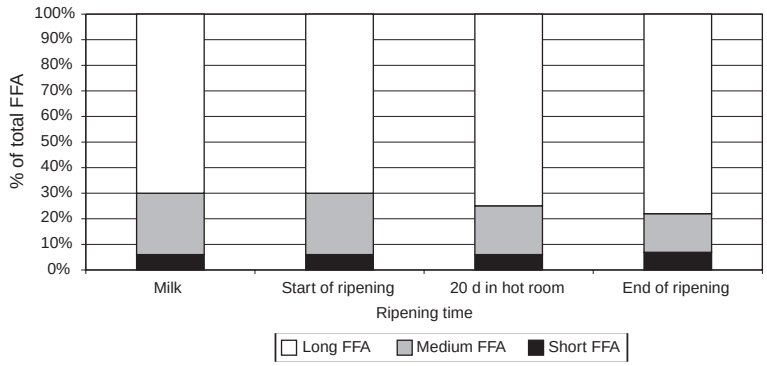

Figure 2. Evolution of FFAs ratio in Emmental cheese made from microfiltered milk with PAB strains ITGP9 and ITGP14.

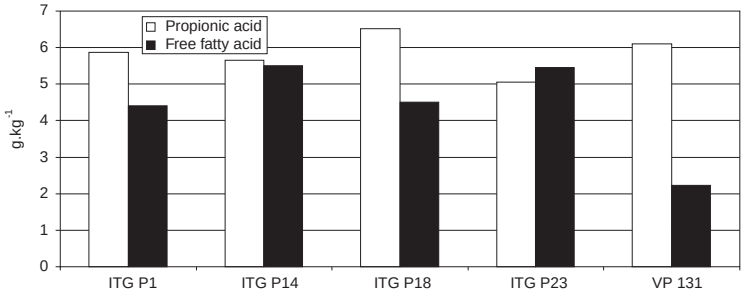

Figure 3. Effect of PAB strains on propionic acid and free fatty acids content in ripened Emmental cheese.

acid and FFAs was found (r = – 0.11). This absence of relation is also the case for the number of PAB. For example, the PAB in cheese made with strain LH56 after 20 d in the warm room was 7.85 ufc·g^{-1} for PAB strain ITGP1, 6.85 ufc·g^{-1} for ITGP23 and 6.91 ufc·g^{-1} for VP131. Only the FFAs content of Emmental cheeses made with PAB strain VP131 was significantly lower ($P < 0.05$). This strain has no, or a weak, lipolytic activity. Surprisingly PAB strain ITGP18 produced a slightly lower lipolysis than PAB strain ITGP14. These results from thermised milk are in conflict with those obtained previously from microfiltered milk.

Only cheeses made with PAB-LAB pairs ITGP23-LH56, VP131-LL57, VP131-LH56 and VP131-LH192 differed significantly in their FFAs contents (Tab. IV). Otherwise, the pair ITGP18-LL229 gave a high amount of FFAs close to pair ITGP14-LL229 whereas the other PAB-LAB pairs with ITGP18 led to lower values than corresponding ITGP14 pairs. Moreover, the LAB strain LH56 gave the highest amount of FFAs in cheeses made with ITGP23 and reduced comparatively lipolysis in cheeses made with PAB strain ITGP1. These results showed an interaction between PAB and LAB on lipolysis of Emmental cheese.

Table IV. FFAs content (g·kg^{-1}) of ripened Emmental cheeses (90 d old) made from thermized milk with PAB and LAB associations.

PAB strains	Thermophilic lactobacilli strain (LAB)			
	LL57	**LL229**	**LH56**	**LH192**
ITGP1	5.20 ^{abc}	5.21 ^{abc}	3.14 ^{bc}	4.09 ^{abc}
ITGP14	4.85 ^{abc}	6.03 ^{ab}	5.97 ^{ab}	5.23 ^{abc}
ITGP18	3.80 ^{abc}	5.97 ^{ab}	3.91 ^{abc}	4.33 ^{abc}
ITGP23	4.90 ^{abc}	5.20 ^{abc}	6.93 ^{a}	4.74 ^{abc}
VP131	1.81 ^{c}	2.89 ^{bc}	2.01 ^{c}	2.19 ^{c}

$^{a, b, c}$ Means followed by different superscripts differ ($P < 0.05$).

4. DISCUSSION

The level of lipolysis in Emmental cheeses just after cheesemaking and before ripening is variable. It depends mainly on the milk used. The stage of lactation of the herds, milking collection, and the cooling and agitation of milk in farm bulk tanks act on the activity of lipoprotein lipase on milk fat [7]. Then, the thermisation (63 °C , 30 s) of milk reduces by a factor of two the activity of lipoprotein lipase [12]. These facts explain the variation noticed in levels of lipolysis in young cheeses during our study.

In our Emmental cheeses made from raw milk the lipolysis was low, about 2 $g \cdot kg^{-1}$ of FFAs or 0.7% of fat, which is closed to the value obtained by Berdagué et al. [3], Langsrud and Reinbold [29] and Woo et al. [42]. The release of FFAs started in the warm room simultaneously with the growth of PAB. In these cheeses, PAB is a natural flora which is very diversified as shown in Switzerland by Fessler et al. [16]. It could be that this natural flora contained strains with low lipolytic activity since the level of propionic fermentation was normal.

By using microfiltration of milk and pasteurisation of cream, we verified that the normal cooking in the Emmental cheeses process, up to 50 °C for 30 min, inactivates the lipoprotein lipase during ripening as established by Driessen [12]. By the same means, we showed that the lipolytic activity of PAB was the main factor in the lipolysis of Emmental cheese, in accordance with Paulsen et al. [32]. Thus, the very slight release of FFAs during ripening of cheeses made without PAB from microfiltered milk confirmed the weak contribution of *S. thermophilus* and thermophilic lactobacilli in the lipolysis of Emmental cheese. However, concerning *S. thermophilus* frequently exhibiting an intracellular lipolytic activity [15], it should be pointed out that only one starter was used during all the experiments. This starter could have weak lipolytic activity or weak autolytic capability limiting the release of intracellular lipase. The strong contribution of PAB in lipolysis of Emmental cheese was confirmed in cheeses made from thermised milk with the addition of the PAB starter. Compared to the beginning of the warm room ripening, the ratio of long chain FFAs was higher in ripened cheese. As results obtained in vitro did not show any specific activity of PAB regarding hydrolysis of long chain FFAs, it could be that short and medium chain FFAs were transformed quickly by β-oxydation and esterification [8]. In fact, many volatile compounds found in Emmental cheese by Bosset et al. [5] originate from the catabolism of FFAs.

For Bachman [2], the difference in lipolysis of Emmental cheese comes mainly from the level of PAB count and less from the lipolytic potential of PAB strains. Our results showed different amounts of FFAs released according to PAB strains with a similar count of PAB in cheeses. The FFAs content depended thus mainly on the PAB strain activity and varied from 1 to 3 in the Emmental cheeses made from thermised milk. These results were in accordance with many researchers which demonstrated in vitro the variability of lipolytic activities among PAB strains [13, 14, 24, 31]. The frequent interaction between PAB and LAB on propionic fermentation is now well known [25, 33]. In contrast, their interaction regarding lipolysis seems less strong but still exists. The mechanism could be the effect of the lipolytic or esterasic activity of LAB strains [19, 31]. Even weak, these activities could complete the lipolytic activity of PAB.

We noticed a lack of consistency between results obtained in cheeses made from thermised milk and from microfiltered milk with PAB strains ITGP14 and ITGP18. Using microfiltered milk, the lipolysis produced by ITGP18 was very low, whereas in cheese made from thermised milk it was higher but remained

lower than lipolysis produced by ITGP14. This inconsistency could be explained in two ways. Firstly, the cream used in microfiltered milk was pasteurised (78 ºC, 30 s). Consequently, a part of the alkaline phosphatase (EC3131) was inactivated and the capability of fat globules to absorb microorganisms was drastically reduced [1]. Thus, the accessibility of fat by PAB could be modified. Secondly, it is possible that the enzymatic equipment of PAB strain ITGP18 was defective in hydrolysing the membrane of the fat globule. This hydrolysis of phosphoglycerid should be reached by other microorganisms such as NSLAB which can survive thermisation [6], but they are removed from milk by microfiltration.

5. CONCLUSION

This study showed that propionic acid bacteria are the main agent in lipolysis of Emmental cheese. This lipolytic activity was shown to be strain-dependant and an interaction between propionic acid bacteria and thermophilic lactobacilli can occur, influencing lipolysis. The conditions of cheesemaking, especially the milk treatment, affected the expression of lipolysis by PAB. Consequently, results obtained in vitro or in experimental conditions should be adapted carefully for industrial purposes. Nevertheless, the lipolytic activity must be controlled for selection of propionic acid bacteria as cheese starter after a necessary improvement of laboratory screening methods.

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