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Original article

Impact of the proteolysis due to lactobacilli on the stretchability of Swiss-type cheese

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Abstract – Proteolysis is known to be a key factor for cheese stretchability. However, proteolysis in Swiss cheese is rather limited because of the inactivation of rennet during cooking. To determine the contribution of the main agents of Swiss cheese proteolysis, experimental cheeses were manufactured with various *Lactobacillus* cultures or with various amounts of plasmin added. Marked differences in stretchability were observed among the *Lactobacillus* cultures. *Lactobacillus helveticus* strains yielded higher stretchability than *Lactobacillus delbrueckii* subsp. *lactis* strains and than mixed cultures of both species. Plasmin addition improved the stretchability at the early stages of ripening (cold room ripening) only. Strong relationships were observed between the proportion of hydrophobic peptides in the fraction of pH 4.6-soluble nitrogen and cheese stretchability. These results show that *Lactobacillus* culture is a key factor for Swiss cheese stretchability and suggest the involvement of hydrophobic soluble peptides in Swiss cheese stretchability.

stretchability / Swiss-type cheese / proteolysis / *Lactobacillus helveticus* / *Lactobacillus delbrueckii* subsp. *lactis*

摘要 – 蛋白水解对瑞士干酪拉伸性的影响。蛋白质水解是干酪拉伸性能的一个关键参数。然而在瑞士干酪中由于凝乳酶失活使得蛋白质的水解程度较低。为了测定瑞士干酪中引起蛋白质水解的主要原因, 采用不同的乳杆菌发酵剂或者加入不同量的血纤维蛋白溶酶制成干酪样品。用不同乳杆菌发酵剂制成的干酪之间, 干酪的拉伸性能具有显著性的差异。使用 *Lactobacillus helveticus* 发酵剂的干酪拉伸性高于单独使用 *Lactobacillus delbrueckii* subsp. *lactis* 菌株和上述两株混合菌株的干酪拉伸性。血纤维蛋白溶酶的加入只能是在成熟初期 (低温成熟) 改进了干酪的拉伸性。pH 4.6 可溶性氮中疏水性肽的比例与干酪拉伸性能具有非常强的相关性。研究结果证明, 乳杆菌发酵剂是影响瑞士干酪拉伸性能的关键因素, 可溶性氮中疏水性肽含量也影响瑞士干酪的拉伸性。

拉伸性 / 瑞士干酪 / 蛋白质水解 / *Lactobacillus helveticus* / *Lactobacillus delbrueckii* subsp. *lactis*

Résumé – Impact de la protéolyse des lactobacilles thermophiles sur les propriétés filantes de l'emmental. La protéolyse des fromages joue un rôle déterminant dans leur aptitude à filer à chaud. Dans les fromages à pâte pressée cuite, la protéolyse est cependant limitée en raison de l'inactivation de l'enzyme coagulante pendant l'étape de cuisson. Pour déterminer la contribution des deux principaux agents protéolytiques de l'emmental, des fabrications expérimentales ont été réalisées en mettant en œuvre différentes cultures de lactobacilles thermophiles et différents niveaux d'addition de plasmine dans le lait. De fortes disparités de propriétés filantes ont été observées selon la culture de lactobacille utilisée. Les souches de *Lactobacillus helveticus* ont conduit à de fortes propriétés

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filantes comparativement aux souches de *Lactobacillus delbrueckiii* subsp. *lactis* ou aux cultures mixtes associant des souches de ces deux espèces. L'addition de plasmine n'a augmenté les propriétés filantes des fromages qu'aux premiers stades de l'affinage (cave froide). Pour ces deux types d'expérimentations, de fortes relations entre la longueur des fils et la proportion de peptides hydrophobes dans l'azote soluble ont pu être établies. Ces résultats montrent que le choix de la culture de lactobacille thermophile est un facteur clef des propriétés filantes de l'emmental. Ils suggèrent également la participation de peptides hydrophobes solubles dans le mécanisme de formation des fils d'emmental fondu.

filant / emmental / protéolyse / *Lactobacillus helveticus* / *Lactobacillus delbrueckii* subsp. *lactis*

1. INTRODUCTION

A growing part of the cheeses produced worldwide is consumed in hot dishes [14] such as pasta, pizza, burgers, gratin, fondue, etc. Thus, the functional properties of melted cheese become a major criterion of cheese quality. Stretchability, which refers to the ability of melted cheese to form strings, is one of the main functional properties. Depending on the cheese variety and its utilisation, stretchability can be considered as a quality (Mozzarella and other *pasta-filata* cheeses, Swiss cheese) or a defect (Raclette cheese) [7, 8]. The control of the factors involved in this property is of practical relevance.

The stretchability of cheese depends on its gross composition, pH, mineralisation and proteolysis. Homogenisation or strong heat treatment of the cheesemilk as well as the incorporation of denatured whey proteins have also been shown to alter stretchability [8, 15]. It has been well established that cheese stretchability is modified during ripening [8]. Generally, the stretchability reaches a maximum after a few weeks and then decreases. These changes have been mainly related to proteolysis, and particularly to the action of residual rennet.

In hard cooked cheese such as Swiss cheese, the coagulating enzyme is inactivated during the cooking step (> 50 ˚C) and proteolysis is mainly due to plasmin and starter activities [5]. Thermophilic lactobacilli (*Lactobacillus helveticus* or *Lactobacillus delbrueckiii* subsp. *lactis* or both) are the most proteolytic starters used in Swiss cheese technology.

In Low Moisture Part Skim (LMPS) Mozzarella, the rod:coccus ratio and the use of specific strains of these species have been shown to influence proteolysis and stretchability [11, 16, 17, 23]. Hong et al. [11] found strong differences in stretchability when comparing three commercial strains of *L. helveticus* or *L. delbrueckii* subsp. *bulgaricus* with marked differences in proteinase and aminopeptidase activities. In this study, a strong proteolytic-low peptidasic *L. delbrueckii* subsp. *bulgaricus* strain gave the strongest stretchability. Oberg et al. [16] showed that proteinase-deficient strains of *L. delbrueckii* subsp. *bulgaricus* yielded to a weaker "stretchability" (helical viscosity) than proteinase-positive strains. Further, Oomen et al. [17] suggested that the specificity of cell-wall proteinase could be involved in the stretchability potential of *L. delbrueckii* subsp. *bulgaricus.* These authors found a "qualitative" effect of the strains on the number and the width of the strings, rather than their length.

The aim of this study was to investigate the influence of cultures of thermophilic lactobacilli and plasmin addition on the stretchability of Swiss-type cheese and to identify nitrogen fractions related to stretchability. In this way, this study aimed to contribute to the understanding of the mechanisms of stretchability.

Table I. Overview of lactobacilli cultures used for the production of the experimental Swiss-type cheeses.

Culture	Species	Commercial name	Supplier	Form
H1		LH77	Standa[1]	FD-SVI[6]
H2	*L. helveticus*	ISLC 5	ISLC[2]	FC[7]
H3		CNRZ 32	INRA[3]	FC[7]
L1		ITG LL14	ITFF[4]	FC[7]
L2	*L. delbrueckii* subsp. *lactis*	ITG LL45	ITFF[4]	FC[7]
L3		LL51	Standa[1]	FD-SVI[6]
M1	*L. helveticus* +	LH1-LL51	Standa[1]	FD-SVI[6]
M2	*L. delbrueckii* subsp. *lactis*	LH56-LL57	Standa[1]	FD-SVI[6]
M3		LH 100	Danisco[5]	FD-DVI[8]

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[2] Istituto Sperimentale Lattiero Caseario, Lodi, Italy.
[3] Institut National de la Recherche Agronomique, Jouy-en-Josas, France.
[4] Institut Technique Français des Fromages, Bourg-en-Bresse, France.
[5] Danisco, Dangé-Saint-Romain, France.
[6] Freeze-dried culture for Semidirect Vat Inoculation.
[7] Frozen culture.
[8] Freeze-dried for Direct Vat Inoculation.

2. MATERIAL AND METHODS

2.1. Bacterial strains and culture conditions

A total of 9 commercial cultures including pure and mixed strains of *L. helveticus* and *L. delbrueckii* subsp. *lactis* were tested in experimental cheesemaking. The bacterial composition, commercial name, supplier and application form of the tested culture are listed in Table I.

Frozen cultures were thawed overnight at 4 ˚C and grown at 44 ˚C until 0.65% acidity in ionised skim milk (LaitG, Laboratoires Standa, Caen, France) reconstituted (10% w/w) in autoclaved water with 0.5% yeast extract. A subculture was made at 44 ˚C until 1.1% acidity in a specific medium (Phagex LB, Laboratoires Standa) reconstituted at 13.5% and heat-treated at 85 ˚C for 30 min. Freeze-dried cultures for semidirect vat inoculation (FD-SVI) were directly grown in Phagex medium as described above. For both types of cultures, 3 mL of Phagex culture were added to cheesemilk (about 10^5 cfu·mL^{-1}). Freeze-dried cultures for direct vat inoculation (FD-DVS) were directly added to the vat.

A FD-SVI culture of *Streptococcus thermophilus* (PAL-ITG ST20-87, Laboratoires Standa) was grown in 13.5% reconstituted Marstar 412A (Danisco, Dangé-Saint-Romain, France) until 0.95% acidity. Then 12 mL of this culture (about 10^6 cfu·mL^{-1}) were added per litre of cheesemilk.

2.2. Cheesemaking

Small-scale Swiss-type cheeses (800 g) were manufactured from microfiltered skim milk standardised with heat-treated cream (115 ˚C for 20 s) using a standardised process as described by Richoux and

Kerjean [19]. Briefly, the milk was ripened with starters at 32 °C for 60 min and coagulated by the addition of calf rennet. The gel was cut into curd grains of 4 mm mean diameter. After 15 min of foreworking, the whey-curd mixture was cooked at 53.5 °C for 33 min. The duration of stir-out was 45 min. The curd was then moulded into forms with a diameter of 12 cm.

The pressing step (6 h) and the acidification step (14 h) were conducted in thermostated ovens at 48 °C and 36 °C, respectively, in order to mimic the cooling rate of the centre of an 80-kg full-size Emmental wheel. The cheeses were then cooled and salted for 5 h in a NaCl-saturated brine at 7 °C. The cheeses were wrapped under vacuum in BK1L-ripening bags (Cryovac, Epernon, France) and ripened at 12 °C for 21 days, at 24 °C for 21 days and then at 4 °C for 5 days.

Porcine plasmin (EC3.4.21.7, Sigma-Aldrich, Saint Quentin Fallavier, France) was added to the cheesemilk (1.1 or 2.2 $U{\cdot}L^{-1}$) in some experiments made with M1 culture to test its effect on cheese stretchability.

2.3. Cheese analysis

The cheeses were analysed for moisture [13], fat [10], NaCl (potentiometric method) and calcium [18]. Total nitrogen (TN), pH 4.6-soluble nitrogen (SN) and 12% TCA-soluble nitrogen (NPN) were determined according to Gripon et al. [6] using the Kjeldahl method [12]. Casein breakdown was evaluated by urea-PAGE of the pH 4.6-insoluble nitrogen according to Collin et al. [3].

Cheese stretchability (3 replicates per sample) was assessed by a method involving vertical traction of melted cheese (82 °C) according to Richoux et al. [20]. The length (mm) of the cheese strands was measured. The coefficient of variation of repeatability was about 15%.

2.4. Experimental design

The results presented in this study were obtained from three independent experiments conducted within a short period (2 weeks) and with the same batch of microfiltered milk. In the first experiment, four *Lactobacillus* cultures (H1, L1, L2 and M1) were tested (three replicates per strain). In the second experiment, cheeses were made with the M1 culture with or without plasmin addition (3 replicates). In the third experiment, various lactobacillus cultures were tested : H1 ($n = 8$), H2 ($n = 2$), H3 ($n = 2$), L3 ($n = 3$), M2 ($n = 8$) and M3 ($n = 9$). Cheeses without lactobacilli addition were also made during the third experiment ($n = 2$).

Cheese proteolysis and stretchability were measured at day 7, day 21 (end of cold room ripening) and day 49 (ripened cheeses), except in the third experiment, in which cheeses were analysed at day 49 only.

2.5. Statistical analysis

Analyses of variance (ANOVA) were performed using the General Linear Model procedure of Statbox V6.3 (GrimmerSoft, Paris, France). Differences between the treatment means were compared at the 5% level of significance using Fisher's least significance difference (LSD) test.

3. RESULTS

3.1. Cheesemaking and composition of cheeses

Lactobacillus culture or plasmin addition had little impact on the cheesemaking parameters (data not shown). The cheeses manufactured with *L. helveticus* strains alone (H cheeses) showed a significantly ($P < 0.05$) slower acidification at

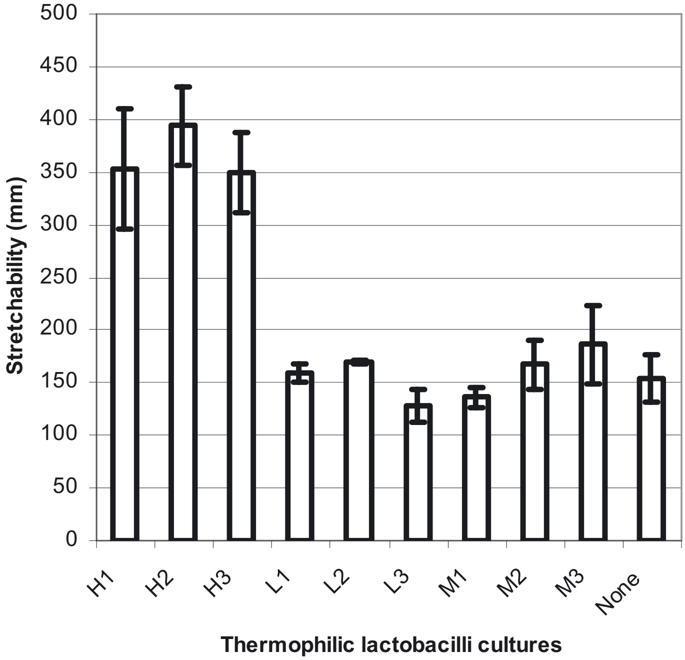

Figure 1. Influence of the *Lactobacillus* culture on the stretchability (mean ± SD) of ripened Swiss-type cheeses (d49). H1, H2, H3: *L. helveticus* cultures; L1, L2, L3: *L. delbrueckii* subsp *lactis* cultures; M1, M2, M3: *L. helveticus* + *L. delbrueckii* subsp. *lactis* cultures; None: no lactobacilli added.

the latest stages of cheesemaking (ΔpH = 0.08 after 6 h and 0.04 after 24 h, respectively). As expected, the "L(+):D(–)-lactate ratio" of the green cheeses differed depending on the *Lactobacillus* cultures: 1.0 (± 0.17) for *L. delbrueckiii* subsp. *lactis* (L-cheeses), 4.1 (± 0.3) for *L. helveticus* strains (H-cheeses) and 1.8 (± 0.22) for mixed cultures (M-cheeses). For the mixed cultures, it can be estimated that the "*L delbrueckii*:*L. helveticus* population ratio" in green cheeses was around 2:1 since *L. helveticus* strains produce about 70% L(+)-lactate under Swiss cheese conditions [1].

At the end of the ripening (49 d) the experimental cheese had on average the following composition: Total solids 62.7 (± 0.5%), Fat-in-Dry-Matter 47.6 (± 0.3)%, Moisture in Non-Fat Substance 53.6 (± 0.5)%, Calcium/Non-Fat Solid 3.15 (± 0.1)% and Salt-in-Moisture 1.1 (± 0.15)%. These values are in agreement with the data of Richoux et al. [20] for French Emmental cheese. There were no statistical differences between treatments.

3.2. Influence of thermophilic lactobacilli cultures on the stretchability of the ripened cheeses

The stretchability of the ripened cheeses ranged from 120 to 400 mm, depending on the thermophilic strains and species (Fig. 1). Cheeses made with *L. helveticus* had a higher stretchability ($P < 0.001$) than *L. delbrueckii* subsp. *lactis* or mixed

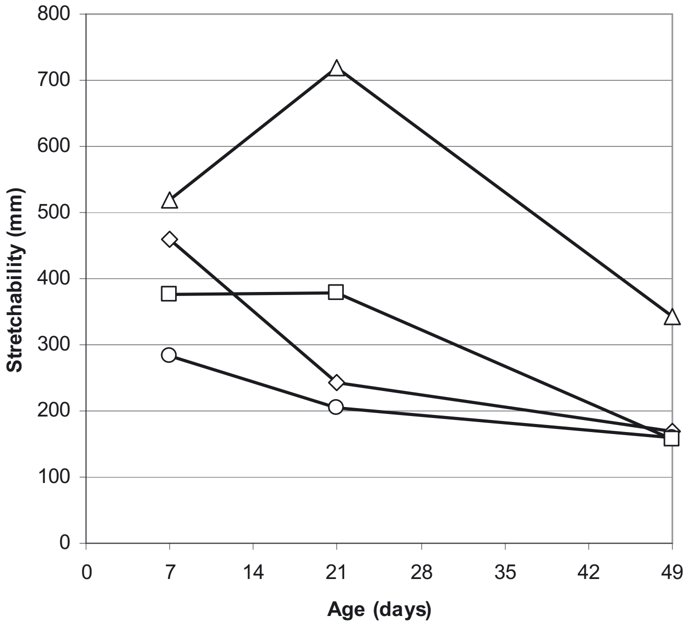

Figure 2. Changes in the stretchability of Swiss-type cheeses made with various lactobacillus cultures during ripening (mean values, n = 3); △ H1, ◊ L1, ○ L2, □ M1.

cultures: 366 (± 25) mm vs. 152 (± 21) mm and 163 (± 25) mm, respectively. The stretchability of Swiss-type cheeses manufactured without lactobacilli addition was similar to that of cheese containing *L. delbrueckii* subp. *lactis*. However, as expected, pH and casein breakdown were very different in these cheeses (data not shown).

3.3. Evolution of the stretchability during ripening

A kinetic study was performed with four cultures of thermophilic lactobacilli: H1, L1, L2 and M1. The changes in stretchability during ripening depended on the thermophilic lactobacilli used (Fig. 2). Both *L. delbrueckii* subsp. *lactis* strains yielded a progressive decrease in stretchability, the rate of decrease being more pronounced during cold room ripening. On the contrary, the stretchability of the H1-cheeses increased markedly during cold room ripening and decreased at a high rate thereafter.

3.4. Relationships between stretchability and proteolysis

The results of proteolysis of the cheeses from the kinetic study are summarised in Table II. The variations between replicate trials were rather limited since standard deviations were below 0.3 g·100 g^{-1} for SN/TN and NPN/TN and below 0.9 g·100 g^{-1} for casein fractions. As expected, the age of cheeses was the main factor affecting the level of proteolysis. *Lactobacillus* strains had no measurable effect on the casein breakdown. Similar concentrations (calculated as % of TN or % of pH 4.6-insoluble nitrogen) of non-degraded caseins, main macropeptides

Table II. Proteolysis in Swiss-type cheese manufactured with various lactobacilli cultures (mean values, $n = 3$).

		Nitrogen fractions (% of total nitrogen)					
	Culture	SN/TN	NPN/TN	γ-CN	β-CN	αs_1-CN	αs_1-I-CN
Day 7	H1	7.7^{a}	2.7^{a}	8.3^{a}	22.0^{c}	36.7^{c}	2.0^{a}
	L1	7.7^{a}	4.3^{c}	7.9^{a}	21.9^{c}	36.9^{c}	2.5^{ab}
	L2	7.6^{a}	3.9^{b}	8.1^{a}	22.3^{c}	36.7^{c}	2.5^{ab}
	M1	7.4^{a}	3.9^{b}	8.1^{a}	22.7^{c}	36.1^{c}	3.3^{b}
Day 21	H1	10.3^{b}	3.3^{ab}	10.0^{b}	18.9^{b}	28.1^{b}	3.4^{b}
	L1	10.6^{b}	6.5^{e}	9.9^{b}	18.9^{b}	27.3^{b}	3.9^{bc}
	L2	10.3^{b}	5.3^{d}	9.8^{b}	19.0^{b}	28.4^{b}	3.7^{bc}
	M1	9.6^{b}	5.0^{d}	9.8^{b}	18.9^{b}	27.9^{b}	4.2^{c}
Day 49	H1	21.9^{c}	11.0^{f}	13.8^{c}	12.3^{a}	15.0^{a}	12.1^{d}
	L1	23.1^{d}	19.4^{h}	13.8^{c}	12.4^{a}	15.5^{a}	13.0^{d}
	L2	23.1^{d}	16.6^{g}	14.4^{c}	11.2^{a}	16.4^{a}	12.5^{d}
	M1	21.2^{d}	15.2^{g}	13.9^{c}	12.4^{a}	15.2^{a}	12.2^{d}

$^{a-h}$: Means in a column with common superscripts do not differ ($P > 0.05$).

(γ-casein and αs_1-I-casein) and secondary non-identified peptides (not shown) were obtained for all four cultures. However, small differences in secondary proteolysis (SN/TN, NPN/TN) occurred. Neither mean value per strain nor individual results of proteolysis were linked to the stretchability (data not shown). However, the stretchability was exponentially linked to the (SN–NPN)/SN ratio (or its better-known complement NPN/SN), by the following equation (Fig. 3):

$$\text{Stretchability (mm)} = 80.6\ e^{-3.0\frac{(SN-NPN)}{SN}} \quad (1)$$

$$r^2 = 0.78,\ n = 36,\ P < 0.001.$$

3.5. Influence of the increase in primary proteolysis on the stretchability

As expected [5], the hydrolysis of β-casein and the production of pH 4.6-SN were increased by porcine plasmin addition to cheesemilk (Tab. III), while αs_1-casein breakdown and NPN concentration remained unaffected. Stretchability of the cheeses with added plasmin was enhanced at d7 and d21. However, at the end of ripening (d49) a similar stretchability was obtained as in the control cheeses.

In this set of data, stretchability was also exponentially linked to the (SN–NPN)/SN ratio:

$$\text{Stretchability (mm)} = 53.5\ e^{-4.0\frac{(SN-NPN)}{SN}} \quad (2)$$

$$r^2 = 0.70,\ n = 27,\ P < 0.001.$$

4. DISCUSSION

Stretchability is a major functional property of melted cheese. Proteolysis, and especially the proteolysis due to rennet activity, has been identified as the main factor which affects the evolution of the stretchability of Cheddar or Mozzarella cheeses during ripening. In Swiss cheese, however, the coagulant is denatured by cooking and the proteolysis is mainly due to plasmin and starter proteolytic activities. The aim of our study was to investigate the contribution of these factors to the stretchability of Swiss cheese.

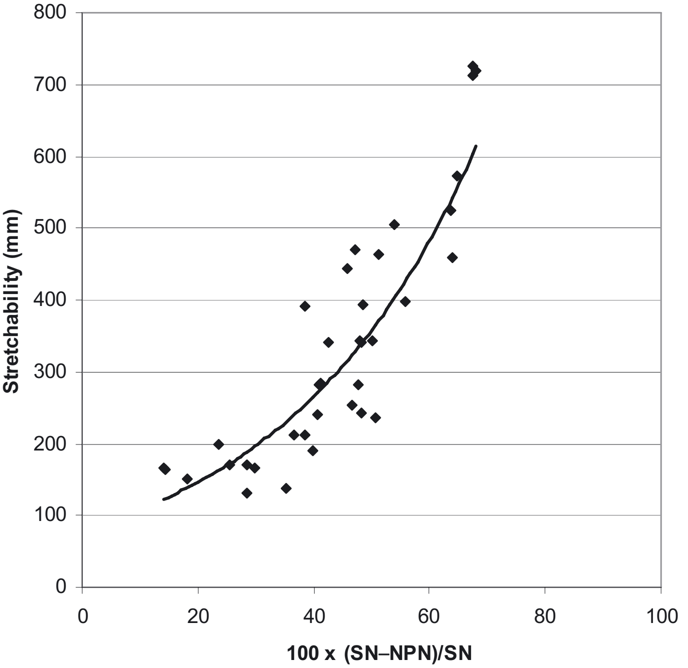

Figure 3. Relationships between stretchability and the proportion of hydrophobic peptides in pH 4.6-soluble nitrogen [(SN–NPN)/SN]. Each dot represents an individual value (n = 36; 4 strains, 3 ripening times, 3 replicates).

Table III. Proteolysis of Swiss-type cheeses made with or without added plasmin (mean values, n = 3).

		Nitrogen fractions (% of total nitrogen)					Stretchability (mm)
		SN	NPN	γ-CN	β-CN	αs$_1$-CN	
Day 7	Control	7.2^a	3.9^a	7.6^a	25.1^e	33.1^c	377^c
	1.1 U·L^{-1} plasmin	8.5^b	4.4^{ab}	9.7^b	23.1^d	32.7^c	434^d
	2.2 U·L^{-1} plasmin	9.4^c	4.5^{ab}	10.4^b	22.3^d	32.5^c	535^e
Day 21	Control	9.4^c	5.0^{bc}	10.0^b	23.5^d	27.3^b	297^b
	1.1 U·L^{-1} plasmin	10.7^{cd}	5.2^{bc}	10.4^b	21.1^c	27.1^b	333^{bc}
	2.2 U·L^{-1} plasmin	11.3^d	5.5^c	12.0^c	20.1^c	26.9^b	535^e
Day 49	Control	21.2^e	15.1^d	12.4^c	15.4^b	11.4^a	156^a
	1.1 U·L^{-1} plasmin	21.5^e	15.0^d	13.6^d	14.9^b	10.7^a	174^a
	2.2 U·L^{-1} plasmin	23.2^f	15.3^d	15.1^e	12.6^a	10.6^a	163^a

$^{a-f}$: Means in a column with common superscripts do not differ (P > 0.05).

The stretchability of our experimental cheeses was in the range of that of the commercial French Emmental cheeses analysed by Richoux et al. [20]. In this survey, the stretchability ranged from 80 to 950 mm and 90% of the values were lower than 400 mm. As described in other cheese varieties [8], the stretchability of our experimental cheeses globally decreased during ripening, showing eventually an optimum.

Our results show that thermophilic lactobacilli greatly influence the stretchability of Swiss cheese, since a three-fold variation can be seen among the strains. In agreement with the results of Wyder [22] in Raclette cheese, *L. helveticus* yielded higher stretchability than *L. delbrueckii* subsp. *lactis* and mixed culture of both species. The association of *L. delbrueckii* subsp. *lactis* with *L. helveticus* decreased the cheese stretchability (Fig. 1). Based on the "L(+):D(-)-lactate ratio" of the green cheeses, it can be estimated that the *L. helveticus* counts were three-fold lower in mixed cultures than in *L. helveticus* cheeses. Specific peptides involved in stretchability could have been produced in lower amounts due to a weaker *L. helveticus* population. More probably, these peptides could have been hydrolysed by aminopeptidases of *L. delbrueckii* subsp.. *lactis.*

A few studies have described the effect of the thermophilic lactobacilli on cheese stretchability, mainly LMPS Mozzarella cheese. These works suggested that the "stretchability potential" of the strains depends on (i) their quantitative proteinasic activity, (ii) the specificity of their cell-wall proteinase, and (iii) their peptidasic activity [11, 16, 17]. This specificity was not studied in our experiments and its determination could be helpful to understand the mechanisms involved in stretchability. However, *L. helveticus* and *L. delbrueckii* subsp. *lactis* differ in both their proteinase and peptidase activities, as illustrated by the RP-HPLC pattern of peptides observed in Swiss cheese [2]. A complete characterisation of the proteolytic equipment and of the autolytic properties of the strains would probably be required to understand their potential to enhance stretchability.

Plasmin addition did not alter the stretchability of the ripened Swiss-type cheeses although it was affected at the early stages of ripening. Similarly, Somers et al. [21] found little effect of the stage of lactation on the stretchability of LMPS-Mozzarella. However, only M1 culture was tested in our study and interactions between plasmin activity and lactobacillus culture have to be studied.

In agreement with the findings of other authors [9], we did not establish direct relationships between stretchability and classical nitrogen fractions of cheeses. However, the stretchability of Swiss cheese was linked to the hydrophobic moiety of pH 4.6-soluble peptides since 12% TCA-fractionation excludes long hydrophobic peptides [24]. Similar relationships between the (SN–NPN)/SN ratio and stretchability were obtained when testing either four lactobacillus cultures with constant milk plasmin activity or three levels of plasmin activity with one lactobacillus culture. Similarly, the (12% TCA-SN)/(Water-SN) ratio has been correlated to strain at fracture during uniaxial compression of various Swiss-type varieties [4]. High compression rates at fracture (i.e. the empirical longness of cheese) was associated with weak (12% TCA-SN)/(Water-SN). More recently, Wyder [22] related differences in sensorial characteristics (viscosity, longness and firmness) of melted Raclette cheese made with various lactic starters to the content of (Water-SN)–(12% TCA-SN) of cheeses.

From these results, we suggest that, besides mineral bonds [8], hydrophobic bonds could be involved in the mechanism of stretchability. Considering their size and concentration in cheese, it seems very unlikely that such hydrophobic peptides could form the backbone of the strands of cheese. However, they could be involved in the formation of the fibres of protein by helping the establishment of hydrophobic bonds between intact caseins or macropeptides. Alternatively, they could be only an indicator of the presence of specific macropeptides that would form the strings, although urea-PAGE of pH 4.6-insoluble nitrogen did not reveal differences between strong and weak stretching cheeses (data not shown). The hydrophobic pH 4.6-soluble peptides could also reflect an optimal microstructure for strand formation.

However, the hydrophobic pH 4.6-soluble fraction was not correlated to stretchability when expressed as percent of total nitrogen. For example, some ripened cheeses with weak stretchability showed the same [(SN – NPN)/TN] ratio as some green cheeses with strong stretchability. This suggests that the peptide composition of this nitrogen fraction plays a crucial role in the stretchability. Alternatively, the balance between hydrophobic and hydrophilic peptides could be determinant.

The answer to these questions will probably help to explain the fundamental mechanisms of string formation in the future. Although our study does not permit this explanation, it gives, besides the well-known impact of cheese mineralisation [8], operating levers to modulate the stretchability of Swiss cheese.

5. CONCLUSIONS

These preliminary results show that the choice of thermophilic lactobacilli culture is a key factor for the stretchability of Swiss cheese, stronger stretchability being observed with *L. helveticus* cultures. Qualitative rather than quantitative aspects of proteolysis are determinant for the development of this functional property. In particular, hydrophobic soluble peptides or the balance between hydrophobic and hydrophilic peptides seem to be a critical point. Further studies are now required to understand the mechanisms involved in the formation of these peptides and their contribution to cheese stretchability.

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