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Streptococcus thermophilus as adjunct culture for a semi-hard cows' milk cheese

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Abstract — The influence of the addition of *Streptococcus thermophilus* as adjunct to the mesophilic starter on chemical, textural and sensory characteristics of a semi-hard cows' milk cheese was studied. Addition of the thermophilic adjunct increased α_{s1}-casein hydrolysis and the level of N soluble at pH 4.6 after 30 d of ripening, reduced the level of N soluble in TCA and did not affect the level of N soluble in PTA. Lower apparent elastic modulus, breaking force and hardness were recorded for cheeses with thermophilic adjunct, as a consequence of the lower dry matter and the slightly enhanced casein hydrolysis. Flavour quality, flavour intensity and cheese bitterness were not affected by *S. thermophilus* addition. In cheese made from milk with *Bacillus subtilis* neutral proteinase added to accelerate ripening and induce bitter flavour formation, the use of *S. thermophilus* as adjunct enhanced proteolysis and produced a considerably higher concentration of N soluble in PTA. Textural characteristics were not affected. Flavour quality was improved and cheese bitterness significantly reduced by the addition of the thermophilic adjunct. © Inra/Elsevier, Paris.

***S. thermophilus* / cheese / proteolysis / texture / bitterness**

Résumé — ***Streptococcus thermophilus* comme levain additionnel pour un fromage à pâte pressée fabriqué à partir de lait de vache.** L'influence de l'ajout de *Streptococcus thermophilus* comme complément à un levain mésophile sur les caractéristiques chimiques, texturales et sensorielles d'un fromage à pâte pressée fabriqué à partir de lait de vache a été étudiée. Le levain additionnel thermophile augmentait l'hydrolyse de la caséine α_{s1} et le niveau du N soluble à pH 4,6 après 30 jours d'affinage, diminuait le niveau du N soluble dans l'acide trichloroacétique et n'avait pas d'effet sur le niveau de N soluble dans l'acide phosphotungstique. Les fromages fabriqués avec le levain additionnel thermophile montraient des valeurs plus faibles du module d'élasticité, de la force au seuil de rupture et de la dureté comme conséquence du plus faible extrait sec et de l'hydrolyse de la caséine légèrement augmentée. La qualité et l'intensité de la saveur ainsi que le degré d'amertume n'étaient pas modifiés par l'addition de *S. thermophilus*. Dans des fromages fabriqués à partir du lait auquel on avait ajouté de la protéinase neutre de *Bacillus subtilis* pour accélérer l'affinage et induire l'apparition d'amertume, l'utilisation de *S. thermophilus* comme levain additionnel était capable d'augmenter la protéolyse et

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de produire une concentration de N soluble dans l'acide phosphotungstique notablement plus élevée. Le levain additionnel thermophile ne montrait pas d'influence sur la texture du fromage. La qualité de la saveur était améliorée et l'amertume du fromage considérablement réduite par le levain thermophile. Comme *S. thermophilus* ne réduisait pas le niveau des peptides hydrophobes, son action pourrait être due au masquage de la saveur amère des peptides hydrophobes par des composants azotés de faible poids moléculaire solubles dans l'acide phosphotungstique.

***S. thermophilus* / fromage / protéolyse / texture / amertume**

1. INTRODUCTION

Breakdown of caseins to peptides and amino acids during cheese making and ripening plays a major role in the development of cheese flavour. These changes are due to proteolytic and peptidolytic activities of starter lactic acid bacteria, rennet, plasmin and non-starter bacteria from adventitious contamination of cheese.

Addition of food grade proteinases to milk has shown to increase cheese flavour intensity. However, defects such as bitterness have been noted frequently. Bitterness has been attributed to the formation and accumulation of bitter peptides from the hydrophobic fragments of caseins. Peptidases from some strains of lactic acid bacteria can degrade bitter peptides [21, 25]. In bitter enzyme modified cheese slurries, Park et al. [16] demonstrated the hydrolysis of two hydrophobic peptides released from α_{s1}- and β-casein by crude enzyme extracts of *Lactobacillus casei* subsp. *casei* with active aminopeptidase and proline-specific peptidases.

Enhancement of peptidolysis by attenuated cultures of lactobacilli and decreased bitterness was observed in Gouda cheese by Bartels et al. [3, 4]. Synergistic effects of the addition of free Neutrase and heat-treated *L. helveticus* on the acceleration of Swedish cheese ripening was reported by Ardö and Petterson [1].

Non-starter lactic acid bacteria have been added as adjuncts to cheese milk. Increases in peptide degradation and enhancement of flavour intensity were observed by Broome et al. [6] and improved flavour quality by Trépanier et al. [22, 23] in Cheddar cheese made from milk with added *L. casei* subsp *casei*. Different mesophilic lactobacilli adjuncts increased the level of free amino acids and improved cheese quality [14]. According to Muir et al. [15] interactive effects between starter and lactobacilli adjuncts determined secondary aspects of sensory characteristics of Cheddar cheese. In cheese made from milk to which *Bacillus subtilis* neutral proteinase (BSNP) was added to induce flavour formation, the use of *L. plantarum* ESI 144 as adjunct starter gave rise to lower levels of hydrophobic peptides and lower bitterness scores [11].

Streptococcus thermophilus is generally used in combination with other thermophilic lactic acid bacteria in dairy products manufactured at high scald or incubation temperatures. However, commercial blends of *S. thermophilus* and *Lactococcus lactis* have been employed in Cheddar cheese manufacture, with technological and commercial advantages such as reduction in cell biomass, bacteriophage durability and cheese flavour profiles [19]. *S. thermophilus* possesses a number of peptidases with different specificities, sharing numerous activities with *L. lactis* [18].

The objective of the present work was to evaluate the effect of a commercial *S. thermophilus* culture added as adjunct to the mesophilic starter on the chemical, rheological and sensory characteristics of a semi-hard cows' milk cheese. Its influence on the levels of hydrophobic peptides in the water soluble fraction of cheeses enriched in bitter peptides by addition of BSNP was also investigated.

2. MATERIALS AND METHODS

2.1. Cheese manufacture

Cheeses were elaborated in two experiments on different days. In each experiment, pasteurized (15 s at 75 °C) cows' milk was divided into four 60 L vats. Milk at 30 °C was inoculated (1%) with a coagulated culture in milk of the mesophilic starter CH-N01 (Chr. Hansen-Lacta S.L., Madrid, Spain), composed of *L. lactis* subsp *cremoris*, *L. lactis* subsp *lactis*, *L. lactis* subsp *lactis* var diacetilactis and *Leuconostoc mesenteroides* subsp *cremoris* strains, grown for 18 h at 25 °C. In addition, vats 2 and 4 were inoculated (1%) with a coagulated culture in milk of the thermophilic starter TA 558 (Texel, Rhône-Poulenc, Madrid, Spain), composed of *S. thermophilus* strains grown for 8 h at 37 °C. Thirty minutes after starter and thermophilic adjunct inoculation, Neutrase L (Novo España S.A., Madrid, Spain) was added to vats 3 and 4 at a final concentration of 0.01 Anson units (AU)/L and rennet (8 mL Maxiren 1:15 000 strength vol/vol; Gist Brocades, Delft, The Netherlands) was added to each vat of milk. Coagulation took place in 40 min. Curds were cut into 6- to 8-mm cubes and scalded at 37 °C for 15 min. In each of the two experiments, four cheeses per vat were obtained, which were pressed for 4 h at 30 °C followed by 14 h at 20 °C, salted at 12 °C for 24 h in 15% NaCl brine and ripened at 12 °C for 60 d.

2.2. Chemical analysis

Cheese pH (in duplicate) and dry matter (in triplicate) were determined as previously described [17]. Homogenates were prepared from representative 20 g cheese samples, and amounts of N soluble at pH 4.6, in 12% TCA and in 1% PTA were determined in duplicate as previously described [10]. Soluble N fractions were expressed as percentages of total N. Residual casein fractions were determined in duplicate by PAGE and expressed as the percentage of the total amount of the respective casein fraction initially present in milk [17]. Water soluble fractions of cheese were prepared according to Aston and Creamer [2]. Hydrophobic and hydrophilic peptides in the water soluble fraction were determined in duplicate by reverse-phase HPLC as described by Lau et al. [13], with a Beckman System Gold chromatograph (Beckman Instruments España S.A., Madrid, Spain), equipped with a programmable solvent module 126, a diode array detector module 168 and an autosampler 502. Detection was at wavelengths of 214 and 280 nm. Results were expressed as units of chromatogram area per milligram of cheese dry matter [17].

2.3. Textural measurements

Apparent elastic modulus (calculated from the slope of the initial part of compression curves and expressed in Newtons per square millimeter), force at breaking point (breaking force expressed in Newtons) and hardness (work done on the cheese after 75% compression, expressed in Joules), were measured from compression curves obtained using an Instron Compression Tester 4301 (Instron, High Wycombe, Buckinghamshire, England) as described by Fernandez del Pozo et al. [9].

2.4. Sensory evaluation

Flavour quality, flavour intensity and cheese bitterness were evaluated by 13 trained panellists after 30 and 60 d of ripening on a 10-point scale as previously described [9].

2.5. Statistical treatment of results

Factorial analyses of variance were performed on data obtained from cheeses without BSNP, with addition of *S. thermophilus*, age of cheese and experiment (day of manufacture) as main effects, by using program BMDP8V (Department of Biomathematics, UCLA, Los Angeles, CA, USA). Means of cheeses with and without thermophilic culture were compared using the least significant difference test at $P = 0.05$ [20]. A similar statistical treatment was performed on data from cheeses with BSNP.

3. RESULTS

Age of cheese had a highly significant effect on all characteristics investigated, with the only exceptions of rheology in cheeses made with BSNP and bitterness in cheeses made with or without BSNP.

3.1. Cheese pH and dry matter

In cheeses made without BSNP, pH (*table I*) was significantly ($P < 0.001$) higher throughout the ripening period if the thermophilic adjunct was added to milk. The addition of BSNP did not affect cheese pH. In cheeses made with BSNP, higher pH values were also detected in presence of the thermophilic adjunct.

In cheeses without BSNP, dry matter values (*table I*) during ripening were significantly ($P < 0.001$) lower in cheese made with the thermophilic culture. The addition of BSNP increased cheese dry matter. In cheeses made with BSNP, dry matter values were significantly ($P < 0.001$) higher throughout the ripening period if the thermophilic adjunct was added to milk.

3.2. Casein hydrolysis

The addition of *S. thermophilus* as adjunct significantly ($P < 0.001$) enhanced α_{s1}-casein degradation (*table II*) in cheeses without BSNP, whereas β-casein degradation was not affected. Addition of BSNP to milk increased α_{s1}- and β-casein degradation. In BSNP cheeses, α_{s1}-casein ($P < 0.05$) and β-casein ($P < 0.001$) were hydrolysed to a higher extent in presence of the thermophilic adjunct, with lower levels of the resi-

Table I. Values of pH[a] and dry matter[b] in cheeses manufactured with thermophilic adjunct (TA) and *Bacillus subtilis* neutral proteinase (BSNP).

Tableau I. Valeurs du pH[a] et de l'extrait sec[b] des fromages fabriqués avec et sans addition des streptocoques thermophiles (TA) comme levain additionnel et de la protéinase neutre de *Bacillus subtilis* (BSNP).

		0 AU BSNP/L		0.01 AU BSNP/L	
	Days	0 % TA	1 % TA	0 % TA	1 % TA
pH	1	4.97 ± 0.05	5.04 ± 0.07[c]	4.92 ± 0.01	5.04 ± 0.09[c]
	15	5.02 ± 0.09	5.08 ± 0.08[c]	4.95 ± 0.06	5.08 ± 0.06[c]
	30	4.98 ± 0.07	5.06 ± 0.02[c]	4.91 ± 0.01	5.11 ± 0.08[c]
	45	5.05 ± 0.04	5.10 ± 0.03[c]	5.01 ± 0.01	5.05 ± 0.05[c]
	60	5.00 ± 0.08	5.11 ± 0.02[c]	4.99 ± 0.09	5.08 ± 0.02[c]
Dry matter (%)	1	51.28 ± 1.30	50.59 ± 1.76	50.35 ± 1.17	52.15 ± 1.41[c]
	15	55.42 ± 1.40	52.39 ± 1.88[c]	53.89 ± 1.65	56.25 ± 0.25[c]
	30	57.22 ± 1.22	54.41 ± 1.98[c]	58.81 ± 0.82	59.81 ± 1.44
	45	59.19 ± 2.02	56.31 ± 1.72[c]	61.81 ± 0.65	63.00 ± 2.77[c]
	60	61.35 ± 2.10	58.33 ± 2.33[c]	65.82 ± 0.98	65.56 ± 1.28

[a] Mean ± SD of duplicate determinations in two cheesemaking experiments. [b] Mean ± SD of triplicate determinations in two cheesemaking experiments. [c] Significantly ($P < 0.05$) different from the respective value in cheese made without thermophilic adjunct.

[a] Moyenne ± écart type d'une double détermination lors de deux essais de fabrication de fromage. [b] Moyenne ± écart type d'une triple détermination lors de deux essais de fabrication de fromage. [c] Valeur significativement différente ($P < 0,05$) de celle du fromage fabriqué sans streptocoques thermophiles.

Table II. Residual α_{s1}- and β-casein[a] in cheeses manufactured with thermophilic adjunct (TA) and *Bacillus subtilis* neutral proteinase (BSNP).

Tableau II. Caséines α_{s1} et β résiduelles[a] dans des fromages fabriqués avec et sans addition des streptocoques thermophiles (TA) comme levain additionnel et de la protéinase neutre de *Bacillus subtilis* (BSNP).

		0 AU BSNP/L		0.01 AU BSNP/L	
	Days	0 % TA	1 % TA	0 % TA	1 % TA
α_{s1} -casein	1	67.5 ± 5.1	56.7 ± 6.4[b]	41.8 ± 7.5	55.0 ± 7.4[b]
	15	41.4 ± 5.2	43.1 ± 4.4	35.0 ± 5.9	32.7 ± 1.1[b]
	30	30.7 ± 5.1	30.7 ± 4.6	26.2 ± 8.5	21.3 ± 1.3[b]
	45	25.8 ± 6.3	18.7 ± 3.1[b]	19.8 ± 7.3	14.1 ± 1.2[b]
	60	24.2 ± 6.1	15.4 ± 0.5[b]	14.7 ± 5.9	10.0 ± 1.2[b]
β-casein	1	58.9 ± 7.4	65.7 ± 0.2	29.9 ± 9.5	42.1 ± 11.1[b]
	15	56.9 ± 5.2	62.7 ± 1.4	28.7 ± 7.6	18.7 ± 2.3[b]
	30	53.5 ± 5.8	59.0 ± 3.6	27.4 ± 8.2	18.1 ± 3.2[b]
	45	46.6 ± 8.3	41.0 ± 7.4	21.5 ± 5.8	8.4 ± 0.2[b]
	60	43.8 ± 7.4	39.3 ± 5.2	19.3 ± 6.1	6.8 ± 1.6[b]

[a] Mean ± SD of duplicate determinations in two cheesemaking experiments, expressed as percentage of α_{s1}- or β-casein contents of milk. [b] Significantly ($P < 0.05$) different from the respective value in cheese made without thermophilic adjunct.

[a] Moyenne ± écart type d'une double détermination lors de deux essais de fabrication de fromage, exprimés en pourcentage de caséines α_{s1} ou β dans le lait. [b] Valeur significativement différente ($P < 0,05$) de celle du fromage fabriqué sans streptocoques thermophiles.

dual fractions throughout the ripening period (*table II*).

3.3. Formation of soluble N

In cheeses made without BSNP, the formation of pH 4.6 soluble N (*table III*) was reduced ($P < 0.05$) in presence of the thermophilic adjunct up to 30 d of ripening, whereas values of this fraction were significantly higher ($P < 0.05$) in 45 d and 60 d cheeses. These results are in agreement with levels of residual α_{s1}-casein, that were significantly lower in cheese with thermophilic adjunct after 45 d of ripening. The TCA soluble N (*table III*) concentration was significantly ($P < 0.001$) lower in cheese with *S. thermophilus* as adjunct throughout ripening. No significant differences were detected in PTA soluble N (*table III*) between cheeses with or without adjunct.

The addition of BSNP increased the levels of the three soluble nitrogen fractions studied (*table III*). In cheeses with BSNP and *S. thermophilus*, pH 4.6 soluble N ($P < 0.001$), TCA soluble N ($P < 0.01$) and PTA soluble N ($P < 0.001$) reached values significantly higher than in cheeses without *S. thermophilus*.

3.4. Hydrophobic and hydrophilic peptides

In cheeses made without BSNP, the amount of hydrophobic and hydrophilic peptides determined at 214 nm and 280 nm (*tables IV* and *V*) was lower ($P < 0.001$) when thermophilic adjunct was added. Addi-

Table III. Soluble N fractions[a] in cheeses manufactured with thermophilic adjunct (TA) and *Bacillus subtilis* neutral proteinase (BSNP).

Tableau III. Fractions d'azote soluble[a] dans des fromages fabriqués avec et sans addition des streptocoques thermophiles (TA) comme levain additionnel et de la protéinase neutre de *Bacillus subtilis* (BSNP).

		0 AU BSNP/L		0.01 AU BSNP/L	
N soluble	Days	0 % TA	1 % TA	0 % TA	1 % TA
At pH 4.6	1	12.89 ± 1.44	11.87 ± 0.73[b]	16.15 ± 0.19	21.72 ± 0.45[b]
	15	14.58 ± 0.91	12.43 ± 0.67[b]	20.83 ± 1.34	22.52 ± 1.69[b]
	30	15.96 ± 0.86	14.37 ± 1.28[b]	23.84 ± 1.92	23.30 ± 0.95[b]
	45	19.76 ± 2.21	21.84 ± 0.48[b]	24.43 ± 1.56	24.67 ± 2.18
	60	21.31 ± 1.05	22.88 ± 0.47[b]	25.05 ± 0.11	27.58 ± 0.61[b]
In TCA	1	4.71 ± 0.49	5.21 ± 0.85[b]	6.61 ± 1.03	5.58 ± 1.18[b]
	15	7.30 ± 0.76	6.14 ± 0.26[b]	9.02 ± 0.33	10.72 ± 0.50[b]
	30	8.08 ± 1.04	7.11 ± 0.35[b]	10.33 ± 0.87	11.46 ± 0.10[b]
	45	10.83 ± 1.10	10.02 ± 0.52[b]	12.96 ± 0.55	12.43 ± 0.64[b]
	60	11.92 ± 0.43	10.95 ± 1.36[b]	13.58 ± 0.14	13.04 ± 0.64[b]
In PTA	1	2.21 ± 1.03	2.19 ± 1.00	2.51 ± 1.13	1.57 ± 0.70[b]
	15	3.05 ± 0.70	2.41 ± 0.54	3.49 ± 0.78	4.18 ± 0.95[b]
	30	3.18 ± 0.70	2.92 ± 0.65	4.53 ± 1.05	5.90 ± 1.34[b]
	45	5.48 ± 1.22	4.85 ± 1.12	5.46 ± 1.22	8.98 ± 2.03[b]
	60	5.77 ± 1.30	6.72 ± 1.50	6.58 ± 1.48	10.64 ± 2.40[b]

[a] Mean ± SD of duplicate determinations in two cheesemaking experiments, expressed as percentage of total N. [b] Significantly ($P < 0.05$) different from the respective value in cheese made without thermophilic adjunct.

[a] Moyenne ± écart type d'une double détermination lors de deux essais de fabrication de fromage, exprimés en pourcentage du total N. [b] Valeur significativement différente ($P < 0,05$) de celle du fromage fabriqué sans streptocoques thermophiles.

tion of BSNP to milk increased the level of hydrophobic and hydrophilic peptides at 214 nm and 280 nm. In cheeses made with BSNP, significantly ($P < 0.001$) higher levels of hydrophobic and hydrophilic peptides were detected at both wavelengths if the thermophilic adjunct was added to milk (*tables IV* and *V*).

3.5. Textural characteristics

The addition of thermophilic adjunct in cheeses without BSNP led to a decrease of the three rheological parameters studied (*table VI*). Apparent elastic modulus, breaking force and hardness were lower ($P < 0.001$) in cheeses with *S. thermophilus* as adjunct at 30 and 60 d of ripening. The addition of BSNP also produced softening in cheese texture. However, in cheeses with BSNP the addition of *S. thermophilus* did not affect rheological characteristics (*table VI*).

3.6. Sensory characteristics

Flavour quality, flavour intensity and bitterness in cheeses without BSNP were not affected by thermophilic adjunct (*table VII*). The addition of BSNP reduced flavour qua-

Table IV. Hydrophobic and hydrophilic peptides[a] in the water soluble fraction of cheeses manufactured with thermophilic adjunct (TA) and *Bacillus subtilis* neutral proteinase (BSNP) determined by reverse-phase HPLC at a wavelength of 214 nm.

Tableau IV. Peptides[a] hydrophobes et hydrophiles dans la fraction hydrosoluble des fromages fabriqués avec et sans addition des streptocoques thermophiles (TA) comme levain additionnel et de la protéinase neutre de *Bacillus subtilis* (BSNP) déterminés par CLHP en phase inverse à une longeur d'onde de 214 nm.

		0 AU BSNP/L		0.01 AU BSNP/L	
Peptides	Days	0 % TA	1 % TA	0 % TA	1 % TA
Hydrophobic	1	39.39 ± 13.91	30.03 ± 9.84[b]	67.80 ± 1.88	70.98 ± 7.37
	15	72.29 ± 7.37	63.77 ± 5.81[b]	106.78 ± 15.62	136.55 ± 4.48[b]
	30	89.87 ± 1.17	96.67 ± 18.94[b]	138.77 ± 16.34	180.38 ± 11.89[b]
	45	122.16 ± 17.30	112.44 ± 6.89[b]	155.47 ± 19.68	176.00 ± 14.49[b]
	60	76.29 ± 12.13	69.27 ± 10.89[b]	113.55 ± 19.21	148.12 ± 22.79[b]
Hydrophilic	1	38.65 ± 7.42	34.01 ± 9.02[b]	56.25 ± 0.56	62.76 ± 10.05
	15	112.92 ± 14.89	93.78 ± 2.86[b]	131.69 ± 13.88	175.19 ± 15.79[b]
	30	140.85 ± 18.82	159.82 ± 7.17[b]	205.74 ± 14.46	277.73 ± 6.84[b]
	45	202.76 ± 22.06	193.53 ± 4.27[b]	273.31 ± 15.80	311.37 ± 10.04[b]
	60	154.25 ± 10.93	146.88 ± 15.03[b]	275.35 ± 54.10	269.28 ± 33.27

[a] Mean ± SD of duplicate determinations in two cheesemaking experiments, expressed as chromatogram area units per milligram of dry cheese. [b] Significantly ($P < 0.05$) different from the respective value in cheese made without thermophilic adjunct.

[a] Moyenne ± écart type d'une double détermination lors de deux essais de fabrication de fromage, exprimés en unités chromatographiques/mg de fromage sec. [b] Valeur significativement différente ($P < 0,05$) de celle du fromage fabriqué sans streptocoques thermophiles.

lity, whereas flavour intensity and bitterness were enhanced. In BSNP cheeses, inoculation with *S. thermophilus* as adjunct resulted in the improvement of flavour quality ($P < 0.01$) whereas flavour intensity was not affected. Cheese bitterness was significantly ($P < 0.01$) reduced by the addition of the adjunct culture (*table VII*).

4. DISCUSSION

In Cheddar cheesemaking with blends of mesophilic and *S. thermophilus* cultures, mesophilic cultures begin the acidification process, whereas *S. thermophilus* participate at the late stages of acid production. At mesophilic cheesemaking temperatures (32–33 °C) *S. thermophilus* does not offer any increase in acid producing activity over the mesophilic component. Initial acidities are low with reduced whey expulsion, giving rise to cheeses with high moisture [19]. In the present work, milk inoculation with the thermophilic adjunct was responsible for higher pH values and lower dry matter level, in cheeses made without BSNP. *S. thermophilus* may compete with the mesophilic starter for essential nutrients present in milk, retarding the acidification of the curd, as already shown by Stanley [19]. In cheeses made with BSNP, acidification was also slower when the thermophilic adjunct was added. The higher pH would enhance the activity of BSNP, thus favouring whey expulsion and increasing cheese dry matter. A significantly higher dry matter was recorded for Manchego cheese made from

Table V. Hydrophobic and hydrophilic peptides[a] in the water soluble fraction of cheeses manufactured with thermophilic adjunct (TA) and *Bacillus subtilis* neutral proteinase (BSNP) determined by reverse-phase HPLC at a wavelength of 280 nm.

Tableau V. Peptides[a] hydrophobes et hydrophiles dans la fraction hydrosoluble des fromages fabriqués avec et sans addition des streptocoques thermophiles (TA) comme levain additionnel et de la protéinase neutre de *Bacillus subtilis* (BSNP) déterminés par CLHP en phase inverse à une longeur d'onde de 280 nm.

		0 AU BSNP/L		0.01 AU BSNP/L	
Peptides	Days	0 % TA	1 % TA	0 % TA	1 % TA
Hydrophobic	1	0.76 ± 0.30	0.41 ± 0.07[b]	1.85 ± 0.01	2.01 ± 0.19[b]
	15	1.22 ± 0.35	1.03 ± 0.28[b]	1.81 ± 0.17	2.68 ± 0.45[b]
	30	1.17 ± 0.34	1.40 ± 0.25[b]	2.37 ± 0.16	2.91 ± 0.44[b]
	45	2.23 ± 0.53	1.70 ± 0.07[b]	3.04 ± 0.54	3.05 ± 0.42
	60	0.78 ± 0.18	1.00 ± 0.26[b]	1.80 ± 0.24	2.73 ± 0.25[b]
Hydrophilic	1	2.80 ± 0.21	2.36 ± 0.35[b]	3.19 ± 0.28	3.35 ± 0.41
	15	4.98 ± 0.55	2.89 ± 0.21[b]	6.05 ± 0.61	7.39 ± 0.49[b]
	30	6.48 ± 0.28	4.52 ± 0.15[b]	9.41 ± 0.55	10.63 ± 0.62[b]
	45	9.66 ± 1.27	7.32 ± 0.40[b]	14.16 ± 0.94	15.78 ± 1.67[b]
	60	6.48 ± 0.67	4.68 ± 0.41[b]	10.67 ± 1.20	12.19 ± 1.40[b]

[a] Mean ± SD of duplicate determinations in two cheesemaking experiments, expressed as chromatogram area units per milligram of dry cheese. [b] Significantly ($P < 0.05$) different from the respective value in cheese made without thermophilic adjunct.

[a] Moyenne ± écart type d'une double détermination lors de deux essais de fabrication de fromage, exprimés en unités chromatographiques/mg de fromage sec.[b] Valeur significativement différente ($P < 0,05$) de celle du fromage fabriqué sans streptocoques thermophiles.

milk to which encapsulated BSNP had been added [17].

Breakdown of caseins in Feta cheese elaborated with *S. thermophilus* as adjunct starter was more extensive than in control cheese although the proteolytic pattern was similar to the control [24]. Similarly, when the thermophilic adjunct was added to our BSNP cheeses the hydrolysis of α_{s1}- and β-casein was significantly increased, probably due to an additive effect. The higher pH of cheese made with *S. thermophilus* would also favour the activity of most of the proteolytic enzymes, especially of BSNP, present in cheese throughout ripening.

In Feta cheese manufactured with heat-shocked *S. thermophilus,* TCA soluble N and PTA soluble N values were significantly increased. However, Bartels et al. [3] did not detect enhancement of TCA soluble N or PTA soluble N formation in Gouda cheese elaborated with a heat-shocked culture of *S. thermophilus* as adjunct to the lactic starter. Our results show a different pattern for N soluble at pH 4.6 and in PTA, with higher levels at the end of ripening in cheeses made with the thermophilic adjunct, from that of N soluble in TCA, with slightly lower levels at 60 d in the presence of the thermophilic adjunct. The N soluble in TCA served as substrate for *S. thermophilus* proteolytic system, which action resulted in high levels of N soluble in PTA.

In cheeses without BSNP, the lower levels of dry matter and residual α_{s1}-casein

Table VI. Textural characteristics[a] of cheeses manufactured with thermophilic adjunct (TA) and *Bacillus subtilis* neutral proteinase (BSNP).

Tableau VI. Caractéristiques texturales[a] des fromages fabriqués avec et sans addition des streptocoques thermophiles (TA) comme levain additionnel et de la protéinase neutre de *Bacillus subtilis* (BSNP).

		0 AU BSNP/L		0.01 AU BSNP/L	
	Days	0 % TA	1 % TA	0 % TA	1 % TA
Apparent elastic modulus (Newtons per mm^2)	30	0.503 ± 0.224	0.296 ± 0.164[b]	0.525 ± 0.224	0.329 ± 0.128
	60	0.841 ± 0.201	0.319 ± 0.080[b]	0.439 ± 0.165	0.549 ± 0.231
Breaking force (Newtons)	30	20.60 ± 5.95	10.85 ± 1.69[b]	18.74 ± 6.90	12.67 ± 4.19
	60	28.53 ± 4.43	14.83 ± 4.68[b]	15.85 ± 4.62	17.39 ± 5.84
Hardness (Joules)	30	0.223 ± 0.045	0.127 ± 0.014[b]	0.204 ± 0.073	0.150 ± 0.048
	60	0.326 ± 0.051	0.146 ± 0.043[b]	0.185 ± 0.047	0.196 ± 0.061

[a] Mean ± SD of six determinations per cheese in two cheesemaking experiments. [b] Significantly ($P < 0.05$) different from the respective value in cheese made without thermophilic adjunct.

[a] Moyenne ± écart type de six déterminations par fromage lors de deux essais de fabrication de fromage. [b] Valeur significativement différente ($P < 0,05$) de celle du fromage fabriqué sans streptocoques thermophiles.

Table VII. Sensory characteristics[a] of cheeses manufactured with thermophilic adjunct (TA) and *Bacillus subtilis* neutral proteinase (BSNP).

Tableau VII. Caractéristiques sensorielles[a] des fromages fabriqués avec et sans addition des streptocoques thermophiles (TA) comme levain additionnel et de la protéinase neutre de *Bacillus subtilis* (BSNP).

		0 AU BSNP/L		0.01 AU BSNP/L	
	Days	0 % TA	1 % TA	0 % TA	1 % TA
Flavour quality	30	5.88 ± 1.60	6.25 ± 1.16	5.22 ± 1.70	5.87 ± 1.56[b]
	60	6.63 ± 1.61	6.78 ± 1.26	5.99 ± 1.48	6.54 ± 0.90
Flavour intensity	30	5.40 ± 1.59	5.57 ± 1.23	5.64 ± 1.88	6.03 ± 1.27
	60	6.69 ± 0.94	6.57 ± 0.85	7.21 ± 1.14	7.03 ± 1.07
Bitterness	30	0.10 ± 0.21	0.26 ± 0.85	0.76 ± 0.85	0.43 ± 0.55[b]
	60	0.01 ± 0.04	0.02 ± 0.07	0.55 ± 0.81	0.21 ± 0.38[b]

[a] Mean ± SD of scores from 13 trained panellists in two cheesemaking experiments using a 10-point scale. [b] Significantly ($P < 0.05$) different from the respective value in cheese made without thermophilic adjunct.

[a] Moyenne ± écart type des résultats de 13 examinateurs, exprimés sur une échelle de 10 points, lors de deux essais de fabrication de fromage. [b] Valeur significativement différente ($P < 0,05$) de celle du fromage fabriqué sans streptocoques thermophiles.

observed at 45 and 60 d in cheeses elaborated with the thermophilic adjunct could contribute to their softening, as hydrolysis of α_{s1}-casein loosens the protein net in the cheese [8]. In cheeses with BSNP, the use of *S. thermophilus* as adjunct did not affect the rheological characteristics, probably because the higher level of dry matter in these cheeses counteracted the enhanced proteolysis.

No significant differences were detected in sensory characteristics of cheeses made without BSNP between cheeses elaborated with and without *S. thermophilus*. However, addition of the thermophilic adjunct in cheeses made with BSNP significantly improved flavour quality. Heat-shocked cultures of *S. thermophilus* and *L. bulgaricus* added as adjuncts gave rise to Feta cheeses with higher flavour, body and texture scores, cultures contributing to flavour more than neutral or acid proteinases [24]. On the contrary, *S. thermophilus* as adjunct had a negative effect on flavour quality of Gouda cheese, with higher bitterness scores in experimental cheese than in control cheese [3].

Cheese slurries treated with BSNP showed an increase in the number and height of HPLC peaks which was related to the appearance of bitterness [7]. In the present work, levels of hydrophobic peptides and bitterness scores in cheeses with BSNP were lower than those previously observed in semi-hard cows' milk cheese made with lactobacilli adjunct [11]. As previously reported [12], the level of hydrophobic peptides in the water soluble fraction determined by reverse-phase HPLC was a reliable indicator of bitter defect in semi-hard cheese made from pasteurized milk. Differences in technology employed in this work, with a higher pressing temperature to favour the growth of the thermophilic culture during the first hours, could result in a less intense development of bitterness or even in an increase of the threshold level required by the tasting panel to detect this defect in cheese. Another fact that might influence bitterness detection is the high level of PTA soluble N (small peptides and amino acids) found in cheese with BSNP and *S. thermophilus* (*table III*) that could mask the presence of undesirable bitter compounds. *S. thermophilus* lysis throughout ripening in cheeses with BSNP could lead to a higher peptidasic activity producing a more intense degradation of peptides. Differences in PTA soluble N between BSNP cheeses with or without thermophilic adjunct tended to increase as ripening proceeded, with higher differences in this nitrogen fraction after 60 d of ripening.

Small peptides and amino acids in the water soluble fraction of Cheddar cheese are the major contributors to cheese flavour [2]. Bouton et al. [5] showed the negative correlation found in Comté cheese between bitterness and the ratio PTA soluble N/water soluble nitrogen. Ardö and Petterson [1] reported increased hydrolysis of casein and enhanced bitterness in cheese made with BSNP. Bitter taste was eliminated by the simultaneous addition of a heat-treated culture of *L. helveticus*.

According to Rul and Monnet [18], the peptidase equipment of *S. thermophilus* would be implied in the development of cheese flavour. Thirteen different peptidases were found in a strain of *S. thermophilus* with biochemical homologies with *L. lactis* peptidases.

The use of *S. thermophilus* in the present work as adjunct culture for the manufacture of a semi-hard cheese resulted in enhanced α_{s1}-casein degradation and a softer texture, which may be desirable in some varieties. Appearance of bitter flavour because of abnormal cheese proteolysis may be prevented by the use of *S. thermophilus* as adjunct to the mesophilic starter. The enhanced formation of low molecular weight nitrogen compounds by *S. thermophilus* would mask the presence of bitter hydrophobic peptides by rising their detection threshold.

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REFERENCES

[1] Ardö Y., Pettersson H-E., Accelerated cheese ripening with heat treated cells of *Lactobacillus helveticus* and a commercial proteolytic enzyme, J. Dairy Res. 55 (1988) 239–245.

[2] Aston J.W., Creamer L.K., Contribution of the components of the water-soluble fraction to the flavour of Cheddar cheese, N. Z. J. Dairy Sci. Technol. 21 (1986) 229–248.

[3] Bartels H.J., Johnson M.E., Olson N.F., Accelerated ripening of Gouda cheese. 1. Effect of heat-shocked thermophilic lactobacilli and streptococci on proteolysis and flavor development, Milchwissenschaft 42 (1987a) 83–88.

[4] Bartels H.J., Johnson M.E., Olson N.F., Accelerated ripening of Gouda cheese, 2. Effect of freeze-shocked *Lactobacillus helveticus* on proteolysis and flavor development, Milchwissenschaft 42 (1987b) 139–144.

[5] Bouton Y., Guyot P., Dasen A., Grappin R., Activité protéolytique de souches de lactobacilles thermophiles isolées de levains et de Comté, II. Applications en sites industriels, Lait 74 (1994) 33–46.

[6] Broome M.C., Krause D.A., Hickey M.W., The use of non-starter lactobacilli in Cheddar cheese manufacture, Aust. J. Dairy Technol. 45 (1990) 67–73.

[7] Cliffe A.J., Law B.A., Peptide composition of enzyme-treated Cheddar cheese slurries determined by reverse phase high performance liquid chromatography, Food Chem. 36 (1990) 73–80.

[8] Creamer L.K., Olson N.F., Rheological evaluation of maturing Cheddar cheese, J. Food Sci. 47 (1982) 631–636, 646.

[9] Fernandez del Pozo B., Gaya P., Medina M., Rodriguez-Marin M.A., Nuñez M., Changes on chemical and rheological characteristics of La Serena ewes' milk cheese during ripening, J. Dairy Res. 55 (1988) 457–464.

[10] Gaya P., Medina M., Rodriguez-Marin M.A., Nuñez M., Accelerated ripening of ewes' milk Manchego cheese: the effect of elevated ripening temperatures, J. Dairy Sci. 73 (1990) 26–32.

[11] Gomez M.J., Gaya P., Nuñez M., Medina M., Effect of *Lactobacillus plantarum* as adjunct starter on the flavour and texture of a semi-hard cheese made from pasteurised cows' milk, Lait 76 (1996) 461–472.

[12] Gomez M.J., Garde S., Gaya P., Nuñez M., Medina M., Relationship between level of hydrophobic peptides and bitterness in cheese made from pasteurized and raw milk, J. Dairy Res. 64 (1997) 289–297.

[13] Lau K.Y., Barbano D.M., Rasmussen R.R., Influence of pasteurization of milk on protein breakdown in Cheddar cheese during aging, J. Dairy Sci. 74 (1991) 727–740.

[14] Lynch C.M., McSweeney P.L.H., Fox P.F., Cogan T.M., Drinan F.D., Manufacture of Cheddar cheese with and without adjunct lactobacilli under controlled microbiological conditions, Int. Dairy J. 6 (1996) 851–867.

[15] Muir D.D., Banks J.M., Hunter E.A., Sensory properties of Cheddar cheese: effect of starter type and adjunct, Int. Dairy J. 6 (1996) 407–423.

[16] Park S.Y., Gibbs B.F., Lee B.H., Effects of crude enzyme of *Lactobacillus casei* LLG on water-soluble peptides of enzyme-modified cheese, Food Res. Int. 28 (1995) 43–49.

[17] Picon A., Gaya P., Medina M., Nuñez M., The effect of liposome-encapsulated *Bacillus subtilis* neutral proteinase on Manchego cheese ripening, J. Dairy Sci. 78 (1995) 1238–1247.

[18] Rul F., Monnet V., Presence of additional peptidases in *Streptococcus thermophilus* CNRZ 302 compared to *Lactococcus lactis*, J. Appl. Microbiol. 82 (1997) 695–704.

[19] Stanley G., Introduction of *Streptococcus thermophilus* in Cheddar cheese using freeze dried direct vat inoculation cultures, Actes du Colloque LACTIC 94, Les bactéries lactiques, Centre de Publications de l'Université de Caen, France, 1994, pp. 257–266.

[20] Steel R.G.D., Torrie J.H., Principles and Procedures of Statistics, a Biometrical Approach, 2nd Ed, in: Napier C., Maisel J.W., Eds., Multiple comparisons, McGraw-Hill International Book Co, N. Y., USA, 1980, pp. 172–194.

[21] Tan P.S.T., Poolman B., Konings W.N., Proteolytic enzymes of *Lactococcus lactis*, J. Dairy Res. 60 (1993) 269–286.

[22] Trépanier G., Simard R.E., Lee B.H., Effect of added lactobacilli on composition and texture of Cheddar cheese during accelerated maturation, J. Food Sci. 56 (1991a) 696–700.

[23] Trépanier G., Simard R.E., Lee B.H., Lactic acid bacteria relation to accelerated maturation of Cheddar cheese, J. Food Sci. 56 (1991b) 1238–1240, 1254.

[24] Vafopoulou A., Alichanidis E., Zerfiridis G., Accelerated ripening of Feta cheese with heat-shocked cultures or microbial proteinases, J. Dairy Res. 56 (1989) 285–296.

[25] Visser S., Hup G., Exterkate F.A., Stadhouders J., Bitter flavour in cheese. 2. Model studies on the formation and degradation of bitter peptides by proteolytic enzymes from calf rennet, starter cells and starter cell fractions, Neth. Milk Dairy J. 37 (1983) 169–180.