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Original article

The effect of reduced salt content on the function of liposome-encapsulated Neutrase and heat-treated lactobacilli in rindless low-fat cheese*

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Summary — Low fat, rindless Dutch-type cheese (20% fat/dry matter) made from cheese milk with added heat-treated lactobacilli and/or Neutrase encapsulated in DRV liposomes was brined for 5 and 16 h, which resulted in a low-salt cheese and a normal-salt cheese. Compared to the cheese with the normal-salt content, low-salt cheese obtained at maturity a significantly lower percentage of dry matter; a higher pH; higher levels of pH 4.6 soluble nitrogen, acetoin, pyro-glutamic acid, pyruvic acid and uric acid; lower levels of acetone, ethanol and lactic acid; a less firm texture and a more bitter flavour. The effect of the addition of heat-treated lactobacilli to the cheese seemed to some extent to be salt-dependent. The bitter flavour was significantly more reduced in the low-salt cheese than in the normal-salt cheese with added heat-treated lactobacilli after 8 weeks of ripening. The amount of succinic acid was decreased in all cheeses with added heat-treated lactobacilli independent of salt content. The activity of Neutrase encapsulated in DRV liposomes was less influenced by the salt level of the cheese. The low-salt cheese with added liposome-encapsulated Neutrase was more bitter than the normal-salt cheese with the same addition.

salt content / low fat cheese / liposome / heat-treated lactobacilli

Résumé — Effet de la réduction de la teneur en sel sur l'activité de la Neutrase encapsulée dans des liposomes et de lactobacilles traités thermiquement dans du fromage sans croûte allégé en matière grasse. Un fromage de type Gouda, sans croûte, allégé en matière grasse (20 % de matière grasse/matière sèche) fabriqué à partir de lait, auquel avaient été ajoutés des lactobacilles traités thermiquement et/ou de la Neutrase encapsulée dans des liposomes, a été saumuré pendant 5 et 16 h pour obtenir un fromage à teneur réduite en sel et un fromage à teneur normale en sel. Comparé au fromage à teneur normale en sel, le fromage à teneur réduite en sel avait à maturité un pourcen-

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tage significativement plus faible de matière sèche, un pH plus élevé, des niveaux plus élevés d'azote soluble à pH 4,6, d'acétoine, d'acides pyro-glutamique, pyruvique et urique, des niveaux plus faibles d'acétone, d'éthanol et d'acide lactique, une texture moins ferme et une flaveur plus amère. L'effet de l'addition de l'actobacilles traités thermiquement au fromage semblait dans une certaine mesure être dépendant du sel. L'ajout de lactobacilles traités thermiquement réduisait la flaveur amère de manière significativement plus importante dans le fromage à teneur réduite que dans le fromage à teneur normale en sel, après 8 semaines d'affinage. La quantité d'acide succinique était réduite dans tous les fromages additionnés de lactobacilles traités thermiquement, quelle que soit la teneur en sel. L'activité de la Neutrase encapsulée dans des liposomes était moins influencée par la teneur en sel des fromages. Pour ces fromages avec Neutrase, l'amertume était plus élevée dans le fromage à teneur réduite en sel que dans celui à teneur normale en sel.

teneur en sel / fromage allégé en matière grasse / liposome / lactobacille traité thermiquement

INTRODUCTION

Low-fat Gouda-type cheese has been made with added heat-treated lactobacilli and liposome-encapsulated Neutrase in pilot plant cheesemaking (Skeie et al, 1995). It was shown that addition of Neutrase encapsulated in dehydrated rehydrated vesicles (DRV) to the cheese increased proteolysis from the first day after cheesemaking. The cheese with added heat-treated lactobacilli developed increased amounts of amino acid N and also increased amounts of acetaldehyde. The addition of heat-treated lactobacilli to low-fat Gouda-type cheese with rind contributed to a firmer but less cohesive cheese texture.

A too firm and too cohesive texture may be one of the factors influencing consumers to perceive low-fat cheese as an inferior product. A weak, pasty texture has been observed in cheese with low-salt concentration. The reduced firmness of low-salt cheese is believed to be attained by an increase in the proteolytic cleavage of the structure element, α_{S1} casein. Reducing the salt content of the low-fat cheese might therefore, result in a less firm structure. However, the reduced salt content may increase the bitter flavour of the cheese (Guinee and Fox, 1993).

Health authorities have recommended reduction of both fat and sodium intake (Norwegian Ministry of Social and Health Affairs, 1993). A low-fat, low-sodium cheese of acceptable quality is therefore of a certain nutritional interest.

The aim of the present experiment was to obtain a low-fat Dutch-type cheese with a less firm texture and a more pronounced flavour without bitterness, by reducing the salt content of the cheese and improving the ripening by the addition of liposome-encapsulated Neutrase and heat-treated lactobacilli.

MATERIALS AND METHODS

Experimental design and statistical analysis

Low-fat rindless Dutch-type cheese (20% fat/dry matter (F/DM), 10 kg cheeses), were made in a randomised experimental block design. Four cheese vats, three experimental and one control vat, were made in four replicate blocks. The four cheese vats were given the following sample codes according to their additions: Control (N0L0); N0L1, heat-treated lactobacilli (0.6 g/L cheesemilk); N1L0, liposome-encapsulated Neutrase (0.0168 Anson units (Anson, 1938) (Au)/kg cheese); N1L1, both heat-treated lactobacilli and liposome-encapsulated Neutrase (same levels as N0L1 and N1L0). Cheeses from each vat were divided in two batches and brined for either 5 or 16 h.

Analysis of variance (Snedecor and Cochran, 1989) was performed on the SAS/Stat (proc GLM) package (SAS Institute Inc, Cary, NC 27513, USA). The statistical model was constructed by the replicate block, the liposome-encapsulated Neutrase, the heat-treated lactobacilli and the brining-time with interaction between each of the three last factors and with the cheese vat nested under replicate block, liposome-encapsulated Neutrase and heat-treated lactobacilli. The statistical model was tested for cheese ripened for 8 and 16 weeks.

Chemicals

All chemicals used were of analytical grade. Unless other suppliers are denoted in the text they were obtained from Merck (Darmstadt, Germany).

Enzymes

Neutrase 0.5 L (Novo-Nordisk AS, Bagsvaerd, Denmark) a *Bacillus subtilis* neutral protease (EC 3.4.24.28) was encapsulated in dehydration-rehydration vesicles (DRV) as described by Skeie et al (1995). According to the amount of cheesemilk used, on average 1290 mL multilamellar vesicles (MLV) were required for each experimental block. Homogenisation of the MLVs was done at a microfluidizer pressure of 90 MPa instead of 50 MPa in order to increase the encapsulation efficiency.

Spray-dried heat-treated lactobacilli cells (Medipharm, Kågeröd Sweden) made from *Lactobacillus helveticus* CNRZ 303 (Inra, Jouy-en-Josas, France) were added to cheese milk to give about 2 10^{10} cells/L (Ardö and Petterson, 1988; Ardö et al, 1989; Skeie et al, 1995).

Cheese production

Rindless Dutch-type cheese (20% F/DM) was made from 2150 L milk in each vat according to a procedure described by Strand and Hulbækdal (1993). The following modifications were made: the milk was inoculated with a 20 mL/L mixed strain mesophilic starter culture type 'Visby 505/M1', (Laboratorium Visby, Tønder, Denmark) and ripened for 45 min at 30 °C. Liposome-encapsulated Neutrase and heat-treated lactobacilli were added 5 min before rennet addition. The renneting time was approximately 30 min. The stirring-time before whey drainage was 35 min. The cheese was pressed under whey at 2 MPa for 5 min and at 3.3 MPa for 15 min. Mould pressing of the 10 kg cheese loaves was done at 24 MPa for 5 min and 39 MPa for 15 min. Immediately after pressing the cheeses were divided in two groups and salted in brine (22%, 12 °C and pH 5.3) for either 5 or 16 h. The cheeses were wrapped in plastic film, and ripened at 9 °C (79% relative humidity (RH)) for 10 days, at 14 °C (70% RH) for 21 days, and at 4 °C (90% RH) for the remaining ripening period.

Analyses

During cheesemaking, pH was measured in cheesemilk, at cutting, at the end of stirring, after pressing and in 1-day-old cheese (cheese brined for 16 h). The number of clostridia in cheese milk, the number of coliforms in cheese milk, whey and 1-day-old cheese was observed. Salt concentration was analysed after 8 weeks of ripening. After 8 and 16 weeks of ripening dry matter, pH, pH 4.6 soluble nitrogen (SN 4.6), amino acid N (formol titration), volatile flavour compounds, total bacterial count and number of lactobacilli were analysed as described earlier (Skeie et al, 1995). Organic acids were analysed by high performance liquid chromatography (HPLC) after a modified method of Marsili et al (1981). To cheese (5.0 g) was added water (5 mL), 1 mol/L H_2SO_4 (0.7 mL) and acetonitrile (20 mL). The mixture was shaken for 30 min and centrifuged at 7000 *g* for 5 min. The clear supernatant was filtered through a 0.45 μm Millex filter (Millipore, Bedford, USA) and 25 μL was injected into a Perkin Elmer series 4 HPLC (Perkin Elmer, Norwalk, USA) equipped with an Aminex HPX 87 H strong anion-exchange column (Biorad, Richmond, USA) and a PE LC-95 UV/VIS detector (Perkin Elmer, Norwalk, USA). The mobile phase was 30 mmol/L H_2SO_4. The column was run at 40 °C and 0.5 mL/min. The separated organic acids were measured at 210 nm and the area was integrated using a Maxima 820 data-chromatography-station (Waters, Ventura, USA).

Sensory quality grading of texture, internal appearance, flavour and general impression of the cheese was done by a panel of five experienced cheese graders after 8 and 16 weeks of ripening. A hedonic scale from 1 to 5 was used, with 1 as very low quality and 5 as very good quality. Sensory profiling was done as described earlier (Skeie et al, 1995) after 8 and 16 weeks of cheese ripening. The profiling attributes were: firmness, elasticity, doughtiness, dryness, total odour, sharp odour, total flavour, bitterness, off-flavour and the number and size of eyes.

RESULTS

Encapsulation efficiency

The encapsulation efficiency of Neutrase in liposomes was 30% for three of the replicate blocks, which was in accordance with the results reported by Kirby et al (1987). The fourth replicate block had a lower encapsulation efficiency of 24%.

Influence of the brining level

The time in brine significantly influenced the ripening characteristics and the sensory quality of the cheese (table I).

As expected, the salt content was significantly lower in cheese brined for 5 h than in cheese brined for 16 h as measured after 8 weeks of ripening, and the cheese from the two brining levels will further be referred to as low-salt cheese and normal-salt cheese, respectively. The low-salt cheese obtained a significantly lower level of dry matter, acetone and ethanol and a significantly higher pH than the normal-salt cheese, as determined both after 8 and 16 weeks of ripening. The difference in pH between the two salt-levels was however reduced, while the difference in the level of acetone increased from 8 to 16 weeks of ripening. After 8 weeks of ripening the levels of acetoin and pyro-glutamic acid were significantly higher in the low-salt cheese than that of the normal-salt cheese, while the level of lactic acid was significantly lower. After 16 weeks of ripening the low-salt cheese obtained a level of SN 4.6, pyruvic acid and uric acid which were significantly higher than that of the normal-salt cheese.

No significant difference was found in bacterial count at any stage of ripening tested.

The low-salt cheese obtained a significantly better texture quality grading than the normal-salt cheese after 8 weeks of ripening (table I), while no significant difference was found on these parameters between the two salt levels after 16 weeks of ripening. The internal appearance of the low-salt cheese was however graded significantly lower than that of the normal-salt cheese after 16 weeks of ripening. The lower score of the low-salt cheese was mainly caused by too many and small eyes by the cheese graders.

From the sensory profiling the texture of the low-salt cheese was graded significantly less firm, less dry and more doughy than the normal-salt cheese, both after 8 and 16 weeks of ripening. However, after 16 weeks of ripening, the low-salt cheese was also significantly more bitter, and obtained a significantly lower total flavour score than the normal-salt cheese.

Influence of the heat-treated lactobacilli

During cheesemaking, the pH of cheese with added heat-treated lactobacilli was significantly lower than the pH of the control cheese; 0.02 pH units lower ($P < 0.05$) at the time of cutting, 0.06 pH units lower ($P < 0.05$) after pressing and 0.03 pH units lower ($P < 0.01$) 1 day after cheesemaking.

As shown in table II, the addition of heat-treated lactobacilli to the cheese was associated with a significantly increased level of amino acid N in percentage of total N after 8 and 16 weeks of ripening and a significantly higher pH and dry matter content after 16 weeks of ripening than the cheese without such addition. Cheese added heat-

Table I. Influence of brining time on chemical composition and sensory attributes during ripening of low-fat Dutch-type cheese[a].

Influence du temps de saumurage sur la composition chimique et les caractéristiques sensorielles de fromage allégé en matière grasse de type Gouda au cours de l'affinage[a].

Age (weeks)	*8*		*16*	
Brining time, h[b]	*5*	*16*	*5*	*16*
Dry matter%	47.5**	48.3	47.2***	48.5
Salt%	0.6***	0.9		
pH	5.75*	5.65	5.81***	5.78
SN 4.6,% of total N	22.24	21.63	25.9*	24.09
Volatile compounds, integrated area (headspace GC analysis)				
Acetoin	4918*	3990	4743	3934
Acetone	8932**	9805	8550**	10125
Ethanol	86940**	118607	83807**	115133
Organic acids, ppm (HPLC analysis)				
Pyro-glutamic acid	184.7**	158.6	258.1	159.2
Lactic acid	14558**	16046	15020	13358
Pyruvic acid	162.6	156.4	93.4**	72
Uric acid	38	38.8	30.5**	23.2
Sensory quality grading (scale 1 to 5)				
Texture	3.6*	3.2	3.6	3.5
Internal appearance	3.1	3.4	2.9**	3.2
Sensory profiling (scale 1 to 7)				
Firm	3.9***	4.8	3.6***	4.4
Dry	2.4**	2.9	1.6*	1.9
Doughy	1.6***	1.2	1.6**	1.2
Bitter	1.5	1.3	2.4*	2.1
Total flavour	3.8	3.9	3.8*	4.1

[a] Values are mean of all cheeses with the same brining level and age ($n = 16$). [b] Significant differences influenced by the brining time are shown within each age group in the column representing 5 h of brining: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

[a] Les valeurs sont la moyenne de tous les fromages ayant les mêmes temps de saumurage et âge (n = 16). [b] Les différences significatives influencées par le temps de saumurage sont indiquées pour chaque groupe d'âge dans la colonne représentant un saumurage de 5 heures : * p < 0,05; ** p < 0,01; *** p < 0,001.

treated lactobacilli obtained significantly higher levels (table III) of acetaldehyde after 8 and 16 weeks of ripening, however, the level of acetaldehyde was higher in the low salt cheese than in the normal salted cheese.

The heat-treated lactobacilli had an significant influence on the amount of several organic acids in the cheese, as shown in table III. After 8 weeks of ripening, cheese with added heat-treated lactobacilli had a significantly higher level of acetic acid and formic acid compared to cheese without this addition. The level of pyro-glutamic acid was significantly higher in cheese with added heat-treated lactobacilli both after 8 and 16 weeks of ripening. The amount of succinic acid was reduced in the cheese from

Table II. Influences on dry matter, salt, pH and proteolysis by the addition of heat-treated lactobacilli and liposome-encapsulated Neutrase[a] during ripening of low-fat rindless Dutch-type cheese brined for 5 and 16 h[b].

Influence de l'ajout de lactobacilles traités thermiquement et de Neutrase encapsulée dans des liposomes[a] sur la teneur en matière sèche, le pH et la protéolyse de fromage allégé en matière grasse de type Gouda, sans croûte, saumuré pendant 5 et 16 heures[b], au cours de l'affinage.

	Age (weeks)	*Brining time*	*Control*	*N0L1*	*N1L0*	*N1L1*
Dry matter	16	5^{***}	46.86 (0.9)	47.7^{**}(0.8)	46.8 (0.9)	47.42 (0.5)
	16	16	48.2 (0.4)	48.6^{**}(0.3)	48.4 (0.9)	49.0 (0.6)
pH	16	5^{***}	5.79 (0.12)	5.82^{***}(0.12)	5.81 (0.12)	5.83 (0.13)
	16	16	5.76 (0.11)	5.80^{***}(0.11)	5.77 (0.1)	5.81 (0.14)
SN 4.6						
% of total N	8	5	20.16 (1.02)	20.12 (1.09)	25.56^{***}(4.47)	23.11 (1.27)
	8	16	19.19 (1.77)	21.23 (3.79)	21.55^{***}(1.58)	24.58 (1.7)
	16	5^{***}	24.62 (1.94)	23.50 (1.42)	28.48^{***} (0.7)	26.84 (2.73)
	16	16	20.70 (1.61)	22.09 (0.93)	26.7^{***} (0.89)	26.66 (0.51)
Amino acid N						
% of total N	8	5	4.94 (0.45)	7.24^{***}(0.66)	6.8 (1.84)	6.93 (0.88)
	8	16	4.66 (0.64)	7.34^{***}(1.11)	5.06 (0.53)	8.09 (1.39)
	16	5	6.07 (0.39)	9.30^{***} (0.5)	7.52 (2.27)	10.15 (1.02)
	16	16	5.93 (0.82)	7.98^{***}(1.73)	6.27 (0.34)	9.98 (0.99)

[a] For explanation of sample codes, see text. [b] Values are mean (SD) for four replicate blocks). A significant influence of the brining time, is marked in the column showing the brining time. A significant influence by the addition of heat-treated lactobacilli or liposome-encapsulated Neutrase is marked in the column where this factor alone appears: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

[a] *Voir texte pour l'explication des codes des échantillons.* [b] *Les valeurs sont la moyenne (écart type) de quatre répétitions. Une influence significative du temps de saumurage est indiquée dans la colonne montrant le temps de saumurage. Une influence significative de l'ajout de lactobacilles traités thermiquement ou de Neutrase encapsulée dans des liposomes est indiquée dans la colonne où ce facteur seul apparaît :* * p < *0,05 ;* ** p < *0,01 ;* *** p < *0,001.*

8 to 16 weeks of ripening. However, in the low-salt cheese with only added heat-treated lactobacilli, the level of succinic acid increased from 8 to 16 weeks of ripening. Cheese with the addition of heat-treated lactobacilli also obtained a significantly lower level of succinic acid at both stages of ripening evaluated. The reduction of pyruvic acid (150–170 ppm in all cheeses after 8 weeks of ripening) were less expressed in the low-salt cheeses without addition of heat treated lactobacilli.

As shown by the sensory quality grading (table IV) the internal appearance score of the cheese after 8 weeks of ripening was significantly lower in cheese with added heat-treated lactobacilli. The lower internal appearance grading was mainly explained by many and small eyes, in accordance with earlier findings (Ardö and Lindmark Månsson, 1990). However, the cheese with added heat-treated lactobacilli had a higher flavour score than the cheese without such addition after both 8 and 16 weeks of ripening. After 16 weeks of ripening the cheese with added heat-treated lactobacilli had a significantly better general impression grading than the cheese without such addition.

As assessed by profiling (table V) the cheese with added heat-treated lactobacilli obtained a significantly firmer texture and significantly more eyes than cheese without this addition after 16 weeks of ripening. At this stage of ripening the assessors in the profiling panel were asked to rate their general impression of each cheese on a scale from 1 to 5, with 5 as the best. In accordance with the cheese graders, the profiling panel graded the cheese with added heat-treated lactobacilli to be significantly better than cheese without such addition.

The heat-treated lactobacilli had a significant effect in reducing the bitter flavour which was significantly more reduced in the low-salt cheese than in the normal-salt cheese after 8 weeks of ripening.

Table III. Acetaldehyde (integrated area, HSGC analysis) and organic acids (ppm, HPLC analysis) influenced by the addition of heat-treated lactobacilli and liposome-encapsulated Neutrase[a] during ripening of low-fat rindless Dutch-type cheese brined for 5 and 16 h[b].

Influence de l'ajout de lactobacilles traités thermiquement et de Neutrase encapsulée dans des liposomes[a] sur l'acétaldéhyde (aire intégrée, analyse HSGC) et les acides organiques (ppm, analyse HPLC) de fromage allégé en matière grasse de type Gouda, sans croûte, saumuré pendant 5 et 16 heures[b], au cours de l'affinage.

	Age (weeks)	*Brining time*	*Control*	*N0L1*	*N1L0*	*N1L1*
Acetaldehyde	8	5	2377 (562)	3524*** (680)	2396*** (458)	4271 (655)
	8	16	2016 (505)	2925* (235)	2804* (404)	3968 (676)
	16	5	2316 (319)	3700** (576)	2773* (476)	4010 (1144)
	16	16	2643 (282)	3467 (1001)	3819 (1414)	4018 (1833)
Acetic acid	8	5	1809.2 (401)	1903.3*** (299)	1903.9* (421)	1989.0 (496)
	8	16	1870.9 (360)	2016.9*** (353)	1874.2* (420)	2043.8 (381)
Formic acid	8	5	19.6 (29.7)	55.2*** (40.9)	9.6** (14.6)	32.5 (7.9)
	8	16	5.2 (10.4)	58.6*** (75.6)	3.3** (6.6)	27.2 (6.5)
Pyro-glutamic acid	8	5**	93.4 (29.2)	239.5*** (61.7)	112.6* (39.2)	239.2 (114.2)
	8	16	73.0 (19.9)	231.5*** (58.7)	80.5* (18.1)	249.2 (61.7)
	16	5	104.9 (12.5)	290.1** (132.7)	117.1 (19.3)	520.2 (376.4)
	16	16	60.7 (28.2)	309.3** (98)	99.6 (37.8)	170 (136.2)
Succinic acid	8	5	439.9 (133.6)	189.7*** (81.7)	465.9 (159.7)	230.9 (123.7)
	8	16	462.2 (171.7)	184.0*** (64.5)	453.6 (148.9)	195.6 (80.9)
	16	5	350.6 (104.5)	260.0*** (69.9)	313.1 (55.5)	200.8 (68.0)
	16	16	327.1 (87.8)	170.8*** (105.3)	307.3 (92.2)	177.0 (70.5)
Pyruvic acid	16	5**	117.4 (29.3)	71.5** (15.1)	107.1 (19.0)	76.9 (31.2)
	16	16	93.3 (36.2)	73.5** (13.8)	64.6 (20.8)	51.4 (37.3)

[a] For explanation of sample codes, see text. [b] Values are mean (SD) for four replicate blocks. A significant influence of the brining time, is marked in the column showing the brining time. A significant influence by the addition of heat-treated lactobacilli or liposome-encapsulated Neutrase is marked in the column where this factor alone appears: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

[a] Voir texte pour l'explication des codes des échantillons. [b] Les valeurs sont la moyenne (écart type) de quatre répétitions. Une influence significative du temps de saumurage est indiquée dans la colonne montrant le temps de saumurage. Une influence significative de l'ajout de lactobacilles traités thermiquement ou de Neutrase encapsulée dans des liposomes est indiquée dans la colonne où ce facteur seul apparaît : * p < 0,05 ; ** p < 0,01 ; *** p < 0,001.

Table IV. Sensory quality grading[a] of low-fat rindless Dutch-type cheese made from milk added liposome-encapsulated Neutrase and/or heat-treated lactobacilli[b] brined for 5 and 16 h[c].

Classement[a] selon la qualité sensorielle de fromage allégé en matière grasse de type Gouda, sans croûte, fabriqué à partir de lait additionné de Neutrase encapsulée dans des liposomes et/ou de lactobacilles traités thermiquement[b], saumuré pendant 5 et 16 heures[c].

	Age (weeks)	*Brining-time*	*Control*	*N0L1*	*N1L0*	*N1L1*
Internal appearance	8	5	3.1 (0.3)	3.0* (0.2)	3.5 (0.1)	3.0 (0.0)
	8	16	3.6 (0.5)	3.2* (0.4)	3.5 (0.5)	3.3 (0.5)
	16	5**	2.8 (0.4)	2.9 (0.6)	3.0* (0.3)	3.2 (0.4)
	16	16	3.1 (0.1)	3.2 (0.6)	3.2* (0.3)	3.3 (0.3)
Flavour	8	5	2.9 (0.1)	3.5* (0.2)	3.0 (0.4)	3.3 (0.1)
	8	16	3.4 (0.4)	3.5* (0.2)	3.4 (0.4)	3.6 (0.4)
	16	5	3.1 (0.1)	3.5*** (0.2)	3.0 (0.2)	3.5 (0.2)
	16	16	3.2 (0.3)	3.7*** (0.2)	3.2 (0.6)	3.6 (0.4)
General impression	16	5	2.9 (0.2)	3.1* (0.3)	3.0 (0.3)	3.2 (0.3)
	16	16	3.1 (0.1)	3.3* (0.1)	3.0 (0.3)	3.2 (0.3)

[a] Scale from 1–5, with 5 as the best. [b] For explanation of sample codes, see text. [c] Values are mean (SD) for four replicate blocks. A significant influence of the brining time is marked in the column showing the brining time. A significant influence by the addition of heat-treated lactobacilli or liposome-encapsulated Neutrase is marked in the column where this factor alone appears: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

[a] Échelle de 1 à 5, 5 étant le meilleur. [b] Voir texte pour l'explication des codes des échantillons. [c] Les valeurs sont la moyenne (écart type) de quatre répétitions. Une influence significative du temps de saumurage est indiquée dans la colonne montrant le temps de saumurage. Une influence significative de l'addition de lactobacilles traités thermiquement ou de Neutrase encapsulée dans des liposomes est indiquée dans la colonne où ce facteur seul apparaît : * p < *0,05 ;* ** p < *0,01 ;* *** p < *0,001.*

Influence of liposome-encapsulated Neutrase

In accordance with earlier findings (Skeie et al, 1995) liposome encapsulated Neutrase significantly increased the amount of SN 4.6 in the cheese (table II).

Cheese with added liposome-encapsulated Neutrase obtained significantly higher levels of acetaldehyde than the control cheese measured both after 8 and 16 weeks of ripening (table III), however the level of acetaldehyde was higher in the normal salted cheese than in the low salt cheese. The cheese with added liposome-encapsulated Neutrase obtained, after 8 weeks of ripening, a higher level of acetic acid and pyro-glutamic acid, and a lower level of formic acid than the control cheese (table II).

The quality grading panel graded internal appearance of the cheese with added liposome-encapsulated Neutrase significantly better than the cheese without such addition after 16 weeks of ripening (table IV). At this stage of ripening, the cheese with added liposome-encapsulated Neutrase also had a significantly more bitter taste than the cheese without this addition (table V). However, although all cheese with added liposome-encapsulated Neutrase was significantly more bitter, the low-salt cheese obtained a much more bitter taste than the normal-salt cheese.

Table V. Sensory profiles[a] of low-fat rindless Dutch-type cheese made from milk added liposome-encapsulated Neutrase and/or heat-treated lactobacilli[b] brined for 5 and 16 h[c].

Profils sensoriels[a] de fromage allégé en matière grasse de type Gouda, sans croûte, fabriqué à partir de lait additionné de Neutrase encapsulée dans des liposomes et/ou de lactobacilles traités thermiquement[b], saumuré pendant 5 et 16 heures[c].

	Age (weeks)	*Brining time*	*Control*	*N0L1*	*N1L0*	*N1L1*
Firmness	16	5^{***}	3.5 (0.5)	3.7^{*} (0.3)	3.4 (0.5)	3.9 (0.3)
	16	16	4.0 (0.3)	4.7^{*} (0.4)	4.3 (0.3)	4.6 (0.1)
Amount of eyes	16	5	2.6 (0.4)	4.0^{***} (0.4)	2.7 (0.4)	3.8 (0.3)
	16	16	3.0 (0.8)	4.1^{***} (1.0)	2.9 (0.6)	3.3 (0.2)
Bitterness	8	5	1.5 (0.3)	1.1^{**} (0.1)	2.0^{*} (0.4)	1.3 (0.2)
	8	16	1.2 (0.2)	1.2^{**} (0.1)	1.4^{*} (0.3)	1.2 (0.1)
	16	5^{*}	2.5 (0.5)	1.7^{***} (0.3)	3.6^{**} (0.4)	1.9 (0.3)
	16	16	2.3 (0.4)	1.6^{***} (0.2)	2.6^{**} (0.6)	1.6 (0.7)
General impression[d]	16	5	2.9 (0.2)	3.4^{**} (0.3)	2.6 (0.3)	3.4 (0.3)
	16	16	3.1 (0.2)	3.4^{**} (0.2)	3.2 (0.2)	3.2 (0.3)

[a] Scale: 1 to 7. [b] For explanation of sample codes, see text. [c] Values are mean (SD) for four replicate blocks. [d] Scale: 1 to 5, with 5 as the best. A significant influence of the brining time, is marked in the column showing the brining time. A significant influence by the addition of heat-treated lactobacilli or liposome-encapsulated Neutrase is marked in the column where this factor alone appears: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

[a] Échelle de 1 à 7. [b] Voir texte pour l'explication des codes des échantillons. [c] Les valeurs sont la moyenne (écart type) de quatre répétitions. [d] Échelle de 1 à 5, 5 étant le meilleur. Une influence significative du temps de saumurage est indiquée dans la colonne montrant le temps de saumurage. Une influence significative de l'ajout de lactobacilles traités thermiquement ou de Neutrase encapsulée dans des liposomes est indiquée dans la colonne où ce facteur seul apparaît : * p < *0,05* ; ** p < *0,01* ; *** p < *0,001.*

DISCUSSION

By the reduction of the salt content, a less firm and a less dry but slightly more doughy texture of the cheese was achieved. The texture changes of the low-salt cheese could partly be explained by an altered chemical composition compared to the cheese with the normal-salt content. As salt penetrates into the cheese during brining, water migrates out (Geurts et al, 1974, 1980), which contributes to a higher amount of dry matter as found in the normal-salt cheese compared to the low-salt cheese. The higher pH and the higher SN 4.6 level indicate a more active proteolytic activity in the low-salt cheese than in the normal-salt cheese. Exterkate et al (1995) reported increased production of amino N in unsalted cheese compared to salted cheese. Our results showed, however, no differences in amino N dependent on the salt-level of the cheese. The level of pyro-glutamic acid was, however, higher in the low salt cheese. According to Tschager and Jager (1988), pyro-glutamic acid is a product of peptidolysis, and cyclization of glutamic acid. The higher level of pyro-glutamic acid in cheese with added heat-treated lactobacilli is therefore also in accordance with the higher level of amino acid N observed. Many of the amino acids found in cheese have been reported to contribute to the cheese flavour by themselves or as precursors for other flavour compounds (Dumont and Adda, 1979).

The differences found in metabolic products (volatile compounds and organic acids) may be connected with the difference in proteolytic products but may as well indicate differences in microbiological activity in low-salt and normal-salted cheese during ripening, although no significant difference in bacterial count was found.

The effects on the amount of proteolytic/peptidolytic products and volatile flavour compounds by the addition of heat-treated lactobacilli and liposome-encapsulated Neutrase on a low-fat cheese were in accordance with earlier results (Skeie et al, 1995). The significant slightly lower pH during cheesemaking caused by the addition of heat-treated lactobacilli was in accordance with earlier results, however the significant effect of the addition of heat-treated lactobacilli after 1 day did not occur in the earlier experiments (Ardö et al, 1989; Skeie et al, 1995). Contrary to earlier results (Skeie et al, 1995) the addition of liposome-encapsulated Neutrase influenced the production of acetaldehyde in low-fat Dutch-type cheese.

In accordance with earlier results and conclusions (Skeie et al, 1995) few interaction effects were found between the liposome-encapsulated Neutrase and heat-treated lactobacilli. Those found had very small effects compared to the main effects of the treatment factors and are therefore not taken into further consideration.

The ability of the added heat-treated lactobacilli to reduce the intensity of the bitter flavour suggests its suitability as a debittering agent, which is in accordance with earlier findings (Bartels et al, 1987; Ardö and Pettersson, 1988; Ardö et al, 1989). This was particularly obvious in the low-salt cheese with added heat-treated lactobacilli, of which the bitter score was lower than that of the control cheese with the normal salt content. A decrease in bitterness associated with increased salt levels in Gouda cheese has also been shown by others (Stadhouders and Hup, 1975; Stadhouders et al, 1983; Visser et al, 1983). According to Stadhouders et al (1983), salt inhibits the bitter flavour enhancing enzymes and represses the formation of bitter peptides. The debittering effect of the heat-treated lactobacilli has been shown to be connected to increased degradation of bitter peptides. However, according to Whiting (1975) succinic acid has a strong salty bitter flavour. The cheese with added heat-treated lactobacilli had a lower level of succinic acid which might also be of importance for the reduced bitter flavour. The influence of succinic acid on cheese flavour should be of interest for further studies.

McGregor and White (1990) found that addition of proteolytic and lipolytic enzymes influenced the amount of several organic acids in low-fat Cheddar cheese after 4 months of ripening. The relations between amounts of proteolytic and peptidolytic enzymes and the levels of organic acids in cheese have been shown in this experiment. The way these organic acids and volatile compounds are produced or reduced during ripening of cheese should receive increased interest since their development may predict the cheese quality during ripening.

CONCLUSIONS

The acceptability of low fat cheese by the consumers depends on the sensory properties of the cheese. The effect of reducing the brining level and thereby the salt content of the cheese to achieve a less firm and more acceptable texture in low fat Dutch-type cheese has been demonstrated. However, the reduced salt level increased the level of bitter flavour in the cheese. This problem could, however, be reduced by the addition of heat-treated lactobacilli, which reduced bitterness and increased the flavour of the cheese.

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