TeRiFiQ

Project no. 289397

Combining Technologies to achieve significant binary Reductions in Sodium, Fat and Sugar content in everyday foods whilst optimizing their nutritional Quality

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Deliverable D1.2
Report on influence of salt optimization on ripening fermentation in the European main cheeses

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Lead contractor/partner for this deliverable:
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Dissemination level	
PU Public (must be available on the website)	X
PP Restricted to other programme participants (including the Commission Services)	
RE Restricted to a group specified by the consortium (including the Commission Services)	
CO Confidential, only for members of the consortium (including the Commission Services)	

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1. Summary

1.1 Objectives of the deliverable

As described previously (Deliverable D1.1) we obtain a good reduction of salt level in four cheese types **(Brie, a soft model cheese; Trappist, a traditional semi-hard cheese; Raclette, a semi-hard cheese model and Bou d'Fâgnes, a Belgian smear-type cheese**). The lactic fermentation and composition of cheese were correctly managed as we showed in the deliverable D1.1.

This deliverable D1.2 has for objective to describe the influence of reduction of the salt content in experimental cheeses on three mechanisms implied in cheese maturation (*i.e.* ripening):

- proteolysis (production of large peptides (NS: water soluble nitrogen); small peptides (NPN: TCA soluble nitrogen and NPT: phosphotungstic acid soluble nitrogen)), proteolysis being an important factor of texture formation and of functional properties,

- lipolysis (production of caproic acids), lipolysis products being an important part of cheese aroma

- butyric fermentation (butyric acid), the source of the main defects in cheese (taste, aroma, aspect, etc.)

1.2 Main results obtained and next steps

The increase of proteolysis in low salt cheese is not important. In soft cheese we measure a worrying increase of lipolysis and in hard and soft cheese a possible increase of butyric acid: these two facts can lead to important taste and aroma defects in cheese. These two important risks must be taken into account when the decision of lowering salt content is taken by cheese makers.

The partner 1 will continue this work by studying in details (in laboratory and in hard cheese models) the influence of salt on lipolysis by propionibacteria and proteolysis.

The partners 2, 7 and 12 will study the influence of these modifications of ripening mechanisms on sensory properties of cheese (next deliverable D1.3, forecast in month 26.)

2. Introduction

2.1 Influence of salt on ripening processes

Cheeses are often salted by brining in salted water or/and by washing during the ripening time with a mix of water, salt and ripening flora. The reduction of salt level in cheese is generally obtained by the reduction of the brining time. The quantity S of salt intake by the cheese is in proportion with the concentration C of salt in brine, the ratio S/V between cheese surface and cheese volume, and the square root t1/2 of the brining time. That is why the reductions of salt level in cheese by –20% and –30% are obtained by reduction of the brining time by –36% and –50%.

The reduction of salt content in cheese is known for its influence on activity of water (a_w) which is one of the most important parameters of cheese maturation. Three main mechanisms of cheese ripening are influenced by the modification of a_w:

- The proteolysis of cheese proteins (caseins) to large and small peptides and aminoacids by milk proteases, starters and coagulating enzymes. The proteolysis is known for its influence on texture formation (especially the proteolysis of alpha s1 casein) and on cooking properties.

- The lipolysis of cheese fat by lipases (from starters and milk) is also influenced by a_w and contributes to the formation of aroma. Cheese lactates are transformed to CO_2 and free fatty acids (propionic and acetic acids for instance). But the butyric acid, formed by the lactates fermentation by *Clostridium tyrobutyricum* is the main defects in cheese. This fermentation is generally controlled by different actions among which the lowering of a_w by an optimum salt level.

2.2 Types of cheeses

We studied 4 types of cheese: a semi-hard cheese Trappist made in a semi-industrial company (Partner 12), a semi-hard cheese Raclette studied in a model-cheese level (Partner 2), a soft cheese with mould (*Pennicilium camemberti*) Brie studied in a model-cheese level (which was the reference model cheese for the Dream project; Partner 2) and a soft cheese ripened with yeasts and bacteria, washed during the ripening time Boû d'Fâgnes (Partner 7).

Note: In the case of Brie-cheese (cheese with moulds) the growth of *Pennicillium camemberti* is at the origin of lipolysis. This mould being unaffected by salt, there is no effect of salt on lipolysis in Brie-cheese (Kerjean, Richoux 2003).

2.3 Targets of the experimental work

a. To measure the influence of salt level reduction on proteolysis;

b. To evaluate the influence of salt level reduction on lipolysis;

c. To check the influence of salt level reduction on butyric acid fermentation;

in order to understand the sensory characteristics modifications which will be evaluated in the next deliverable.

2.4 Analytical methods

Main analytical methods for cheese are from R Grappin and JL Berdague 1987 *Le Lait-Dairy Sci Technol* 67, 219.

The index NS/NT (non casein nitrogen/total nitrogen %) expresses the percentage of large peptides from casein proteolysis.

The index NPN/NT (non protein nitrogen/total nitrogen %) corresponds to the percentage of small peptides from casein and large peptide proteolysis.

Analysis	Method
Total Nitrogen NT	Kjeldahl (FIL 20B)
Non Casein Nitrogen NS	Kjeldahl on pH 4.6 filtrate (FIL 29)
Non Protein Nitrogen NPN	Kjeldahl on TCA12% filtrate (FIL 20B)
Total Calcium	Complexometry (Pearce, 1977)
Soluble Calcium	Complexometry (Pearce, 1977)
Lactose	Enzymatic

Curd and cheese

Total solids	Oven drying
Fat	Acido-Butyrometric
Total Nitrogen NT	Kjeldahl
pH 4.6 Soluble Nitrogen NS	Kjeldahl (adapted from FIL 20B)
12%TCA Soluble Nitrogen NPN	Kjeldahl (adapted from FIL 20B)
Casein hydrolysis (alpha, beta, gamma caseins)	Urea PAGE of pH 4.6 insoluble N
Galactose	Enzymatic
L(+) and D(-) Lactate	Enzymatic
NaCl (*)	Chloride meter
Total Calcium	Complexometry
Acetic C2, propionic C3, butyric C4, isovaleric C5, and caproic C6 volatile acids	Gas chomatrophy according to JL Berdague 1986 *Le Lait-Dairy Science Technol* 66 243

(*) NaCl is generally given by the index S/M% (NaCl/cheese moisture)

3. Results and discussion in terms of proteolysis, lipolysis and butyric acid fermentation

3.1 Trappist cheese

In Trappist cheese, the NaCl levels in ripened cheeses were 2% (g NaCl / 100 g cheese), 1.48% and 1.29% corresponding to the reductions 0%, 26.8% and 36.8% reaching the objective (N=10). Proteolysis is increased by salt reduction: this increase is low (0.7%) but steady and consistent. Lipolysis (C6) is also put up to +50% (index). The butyric acid fermentation (C4) is clearly made worse by salt reduction, which constitutes a real danger for this type of cheese.

Trappist cheese		Control	–20%	–30%
Salt %	g/100g	2,02	1,48	1,29
Salt Reduction	%	0,00	26,8	36,4
NS/NT	%	12,7%	13,4%	13,6%
NPN/NT	%	6,4%	6,7%	6,9%
NPT/NT	%	1,3%	1,4%	1,3%
C2	mg/100g	80,16	75,46	79,01
C3	mg/100g	1,38	0,88	1,31
C4	mg/100g	3,29	5,51	4,51
iC5	mg/100g	3,65	3,52	3,38
C6	mg/100g	1,47	2,38	2,24
Lactates L	mg/100g	835,20	978,67	988,80
Sodium	mg/100g	630,10	493,89	410,20

3.2 Raclette cheese model

In Raclette cheese model cheeses, the NaCl contents were (mean, standard-deviation, N=3) 2.32 % (0.06%), 2.06% (0.19%), 1.86% (0.05%) corresponding to the reductions –14% and –29% not far from the objective. The global proteolysis (NS/NT; NPN/NT) is not significantly modified, but we observe the increase of one specific peptide (alpha S1-I) from 13 to 18%. As this peptide, produced by the proteolysis of alpha s1 casein by coagulating enzyme and/or starter protease(s), is important for texture and cooking properties, theses characteristics will be observed in details further.

Raclette cheese		Control		Salt Reduced 1		Salt Reduced 2	
		Mean	SD	Mean	SD	Mean	SD
NaCl	g/100g	2,32	0,06	2,06	0,19	1,86	0,05
S/M	%	5,04	0,17	4,67	0,44	4,14	0,08
NS/NT	%	26,7	0,5	26,4	0,3	26,2	0,5
NPN/NT	%	15,4	0,5	15,0	0,8	16,3	1,5
gamma-cn	% of NT	16.4	1.0	14.7	0.7	14.8	1.0
beta-cn	% of NT	24.4	2.1	24.5	3.7	21.8	2.5
alpha s1-cn	% of NT	2.5	0.3	3.2	0.5	2.8	0.4
alpha s1-I	% of NT	13.0	2.4	15.1	3.9	18.3	0.4
C2	mg/100g	36,9	7,8	46,8	12,6	40,6	15,0
C3	mg/100g	0,3	0,3	0,3	0,2	0,0	0,0
C4	mg/100g	4,0	1,4	3,8	1,1	4,4	0,8
iC5	mg/100g	6,8	3,2	6,1	1,3	5,5	0,4
C6	mg/100g	1,2	0,5	0,8	0,6	1,2	0,3

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3.3 Brie cheese model

In Brie cheese model cheeses, the NaCl levels in different trials at the end of ripening were (mean, standard deviation, N=3): 1.71% (0.13%) ; 1.43 (0.04%) ; 1.31 (0.01%). This corresponds to the reductions: 0% (control), -16% and -24% not far from the target. Proteolysis increases slightly with salt levels reduction (by 1% in index) but this is not clearly technologically or statistically significant.

Brie cheese model		Control		Reduction 1		Reduction 2	
		Mean	SD	Mean	SD	Mean	SD
lactate	mg/100g	241	33	268	68	219	13
NaCl (viaCl)	g/100g	1,71	0,13	1,43	0,04	1,31	0,01
Na	mg/100g	605	54	523	6	486	4
Salt/Moisture	%	3,64	0,17	3,04	0,07	2,83	0,04
NS/NT	%	37,13	2,01	42,99	2,36	41,85	5,99
NPN/NT	%	16,19	1,05	18,87	0,78	17,52	2,72

3.4 Boû d'Fâgnes cheese

In Boû d'Fâgnes cheese (N=10) the salt reductions were obtained by brining times of 3h, 1h30 and 1h. In this cheese, the final NaCl levels in ripened cheeses were: 2.4%, 2.3%, 2.2%, leading to a salt reduction lower than the target.

Proteolysis increases slightly by about 1% or 2% in index but this increase is not statistically significant. The lipolysis (caproic acid C6 from 2.8 to 5) is increased by salt lowering and the other fermentations (C, iC5) are not modified except butyric acid fermentation. This point must be observed as the presence of butyric acid over 20 mg/100 g can cause aroma defects.

		TRIALS		TRIALS		CONTROL	
Brining time		1h00		1h30		3h00	
	Nacl %	2,2		2.3		2.4	
	NaCl/H_2O	4,62	0,2	4,79	0,3	5,07	0,4
NH3 (mg/100g)		0,1	0	0,1	0	0,1	0
NS/NT (%)		44,80%		44,60%		39,80%	
NPN/NT (%)		24,90%		27,60%		22,80%	
NPT/NT (%)		8,90%		10,30%		9,30%	
(mg/100g) C3		2,9	2,5	2,9	2,1	2,2	1,3
(mg/100g) C4		18,5	7,7	19,7	12	12,1	8,9
(mg/100g) iC5		43,8	12	56,1	7,9	49,4	12
(mg/100g) C6		5	2,4	4,7	3,1	2,8	1,8

4. Conclusion

The technological and interpretation of cheese making (deliverable D1.1) allowed us to compare the ripening processes (proteolysis and lipolysis).

We observed a very low increase of proteolysis by 30% salt reduction, with the exception of the alphas1-I peptide in Raclette cheese. Compared to literature reviewed by Kerjean and Richoux 2003, Guinee and O'Kennedy 2007, this exception and these increases are difficult to understand and must be linked to the action of proteinases and peptidases which can be reduced in lower extent than previously observed. The partner 1's specific work will focus on this specific issue concerning starter proteinases.

The partners noted an increase in lipolysis associated with salt reduction, for example in soft cheeses and in Trappist cheeses. We will try to compare this analytical fact to sensory analysis presented in the next deliverable D1.3.

In Bou d'Fâgne and in Trappist cheeses, a slight increase of butyric acid is measured. This is a slight but consistent and steady increase. The cheese makers always worry when this fact is observed. The butyric acid fermentation is the main cheese defects leading to bad tastes, bad aroma, blowing, defects in cheese aspects etc. Generally these defects are present when a level of butyric acid over 20 mg/100 g is measured. This is not the case here; in the next trials a special attention will be given to this important aspect.

5. References

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