TeRiFiQ

Project no. 289397

Combining **Te**chnologies to achieve significant binary **R**eductions **i**n Sodium, **F**at and Sugar content **i**n everyday foods whilst optimizing their nutritional **Q**uality

Start date of project: 1 January 2012

Duration of project: 4 years

Call: FP7-KBBE-2011-5

Theme: KBBE.2011.2.3-05 [Processed foods with a lower salt, fat and sugar content]

Funding Scheme: Collaborative Project (small or medium-scale focussed research project targeted to SMEs)

Deliverable D1.4: Report on corrective means which can be used by cheese makers for cheese with low salt

Due date of deliverable: M32
Actual submission date: M33
Lead contractors/partners for this deliverable: ACTIA (ACTALIA)
WP1 Leader: ACTIA (ACTALIA)
Contributors: ACTIA (ACTALIA), HERVE, ORVAL

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)	[ ]

Table of Contents

1. Summary

1.1. Objectives of the deliverable

As described previously (Deliverable D1.3), a good quality was obtained by ACTIA (ACTALIA) after reduction (- 20% and - 30%) of salt level in two cheese types (Brie, a soft model cheese; Raclette, a semi-hard model cheese).

The salt-level reduction did not modify the smell and aromatic richness of Bou d'Fagne (a soft cheese with smear made by the industrial partner HERVE) and of Trappist semi-hard cheese (a traditional semi-hard cheese made by the partner ORVAL).

But it is known that the NaCl content reduction in cheese, which causes a proportional increase of activity of water aw, leads to lower the protection against some microbiological defects. This is the case in two cheese-types which were studied in the WP1: semi-hard cheeses and soft cheese with smear.

For example, the low salt semi-hard cheeses are less protected against the butyric fermentation by the *Clostridium tyrobutyricum* present in milk, especially in winter milks when cow are fed with maize or grass silage. This important defect related to butyric acid fermentation appears in some low-salt Trappiste cheese made by ORVAL, giving a unacceptable appearance (presence of holes, bad butyric taste with more than 600 ppm of butyric acid in cheese). The first objective of the deliverable 1.4 was to limit this butyric acid fermentation.

Another classical example is the biological repression by salt of the moulds (i.e. *Penicillium camemberti*) which threshold activity of water (a_wt) is 0.86 against useful yeasts like *Geotricum candidum* that are the normal ripening microorganisms for washed soft cheeses with smear (yeasts a_wt being higher: 0.90 – 0.95) or useful ripening bacteria like *Brevibacterium linens* (a_wt = 0.88).
This important defect of the presence of white moulds (*Penicillium camemberti*) appeared in a notable proportion of low-salt Bout d'Fagne cheese. This is a severe defect of presentation for the company Herve. The second objective for the deliverable 1.4 was to reduce this "Penicillium" defect.

1.2. Main results obtained and next steps

The butyric acid fermentation in Trappist cheese was correctly controlled and limited by the addition of lysozyme in milk.

The modification of the ecosystem by lactic acid bacteria is not efficient against the defect "*Penicilium camemberti*" in Bou d'Fagne. It is likely that the development of a low salt soft cheese like Bou d'Fagne is difficult and needs other technological improvements.
The way chosen to reduce salt level in Bou d'Fagne in the future by Herve is to consider in parallel the reduction of cheese contaminants by working on water and milk microbial quality and biofilm on equipments.

2. Introduction

2.1. State of knowledge

Salt is well known to regulate water activity (aw) in cheese. This aw is the main tool for controlling desirable (lactic, ripening starters) and undesirable fermentations. Two examples are well known (Guinee and *al* 2007):

1. the control of the butyric acid fermentation in hard cheeses, due to the milk spoilage by *Clostridium tyrobutyricum* from silage (JF Chamba, JR Kerjean, Butyric acid bacteria a review. Edition ITG-ACTALIA 82/B00, 210 pages)

2. the repression of mould growth in soft smear cheeses.

Different methods were studied to control and limit the butyric acid fermentation :

1. to replace maize and grass silage by other forages,

2. to reduce the milk contamination at milking,

3. to remove the *C.tyrobutyricum* spores from milk by centrifugation (bactofugation) (JF Chamba, The bactofugation, studies at different temperature. Edition ITG-Actalia 82-94/01B 54p) or microfiltration (JR Kerjean, JP Quiblier 1991, Du lait microfiltré pour l'emmental. Parlons technologie V, 6, Edition ITG-ACTALIA),

4. to inhibit the germination of *C.tyrobutyricum* spores by inhibitory starters (JR Kerjean 1988, Inhibition of butyric fermentation by lactobacilli. Parlons Technologie III, Edition ITG-ACTALIA ; JR Kerjean 1995 Natural Antimicrobial systems. Lactic acid bacteria against butyric acid fermentation. Final report on the R&D project FLAIR/2/AGRF0048) or by lysozyme (JF Chamba, JR Kerjean 1984, Use of lyzosyme to limit the butyric acid fermentation, Edition ITG-ACTALIA 84/B 35 p).

Different modifications in technology can be used to control the defect “presence of moulds” in the soft cheese with smear Bou d'Fagne:

1. modification of the ecosystem by the use of specific lactic acid bacteria in a cross-functional solution allowing sodium reduction in cheese;

2. use of very active *Geotrichum candidum* strains + yeasts cocktail that can consumes the lactates in cheese.

2.2. Solutions chosen for controlling cheese defects

-As bactofugation and microfiltration are not adapted to small cheese plants, the use of lysozyme or inhibitory starters was chosen by ORVAL to combat the butyric acid fermentation.

Lysozyme was added to milk before cheese making at the amount of 100 ppm.

A *Lactobacillus rhamnosus* strain known for inhibitory properties was used at 60 units by ml.

-The chosen corrective mean for low salt Bou d'Fagne is the modification of ecosystem by use of specific lactic acid bacteria.

The use of *Geotrichum candium* strain was not chosen because it changed too much the product.

3. Results and Discussion

3.1. Control of the butyric acid fermentation in low-salt Trappist cheese

Nineteen cheese vats (N°1 to 19) were studied by ORVAL to understand and control the butyric acid fermentation between August 2012 and June 2014.

The salt level was reduced to two levels : reduction by 10 to 14% (Reduction 1 R1) and reduction by 18 to 27% (Reduction 2 R2).

100 ppm of lysozyme was added to milk in fifteen vats.

L.rhamnosus was present in all vats but the level was doubled from 30 units to 60 units in the vat 12.

No butyric acid fermentation was noticed on vats 4, 5, 8, 10, 18, 19 without salt reduction.

The butyric acid fermentation leading to cheese blowing and bad taste was present in four vats 1, 3 11 and 12 which was made with salt reduction and no lysozyme addition.

The vat with high level of inhibitory Lactbacillus rhamnosus was characterised by a butyric fermentation.

There was no butyric acid fermentation in all the 15 vats with lysozyme addition.

These results clearly show that lysozyme is efficient – as we know in emmental cheese – on the butyric acid fermentation in low salt Trappist cheese.

L Rhamnosus does not show here any efficiency against butyric acid fermentation.

Table 1 Observation of the butyric defect ("butyric swelling"= blowing + butyric odour) on Trappist cheese with different salt reduction (R1: 10-14%, R2: 18-27%, T: control no reduction) with use of inhibitory *L.Rhamnosus* and/or 100ppm of lysozyme or not.

Date	Batch n°	Salt reduc %	butyric swelling	lysozyme ppm	L. Rhamnosus	NUMERO
19/08/2012	**2012/233**	**R2**	**yes**	**0**	**T**	1
14/11/2012	2012/332	R1	no	0	T	2
23/01/2013	**2013/028**	**R1**	**yes**	**0**	**T**	3
24/02/2013	2013/052	T	no	100	T	4
28/03/2013	2013/088	T	no	100	T	5
17/04/2013	2013/114	R1	no	100	T	6
16/05/2013	2013/144	R2	no	100	T	7
30/05/2013	2013/160	T	no	100	T	8
19/06/2013	2013/182	R2	no	100	T	9
25/07/2013	2013/226	T	no	100	T	10
17/10/2013	**2013/336**	**R1**	**yes**	**0**	**T**	11
04/12/2013	**2013/394**	**R1**	**yes**	**0**	**E**	12
09/01/2014	2014/010	R2	no	100	T	13
20/02/2014	2014/058	R1	no	100	T	14
13/03/2014	2014/088	R1	no	100	T	15
01/04/2014	2014/110	R2	no	100	T	16
08/05/2014	2014/150	R2	no	100	T	17
22/05/2014	2014/170	T	no	100	T	18
12/06/2014	2015/189	T	no	100	T	19

3.2. Improving the quality of low-salt soft cheese with smear

3.2.1- Lactic acid bacteria modification

The use of two types (Lactics 1 and Lactics 2) of special strains of lactic acid bacteria did not show efficient result. On the table 2, it can be seen that the control (Temoin) with no salty reduction is correct (No PC=no Penicilium camemebrti). But on the trials with lactic acid bacteria a salt reduction, defaults are still very important: the pigmentation is still low, the colour of the rind is unsatisfactory, contaminations and proteolysis are noticed on the rind.

Table 2 : Observation of the effect of Lactic acid bacteria modification (Lactic 1 and 2 against control) on Bou d'Fagne low salt cheese (Temoin=Control with no salt reduction)

	Observations in ripening		
	TEMOIN	**LACTICS 1**	**LACTICS 2**
J+7	PC **(-)**	PC **(+)**	PC **(+/-)**
J+12	NO PC	Pc **(-)**	PC **(+++)**
J+14	ORANGE RIND (B. *linens*) NO PC	LIGHT YELLOW RIND PC **(-)**	LIGHT YELLOW RIND Pc **(+)**
J+21)	ORANGE RIND (B. *linens*) NO PC	LOW PIGMENTATION PROTEOLYSIS ON THE RIND **(+++)** BUT NOT IN THE BODY	LOW PIGMENTATION CONTAMINATIONS ++

4. Conclusion

In low salt Raclette and Brie type cheese, we did not notice very important defects linked to NaCl reduction.

In semi-hard cheese like Trappist cheese, the salt reduction can increase the hazard of butyric acid fermentation and cheese blowing. In small factories, addition of 100 ppm of lysozyme could be a good solution to prevent this risk. In other cases (industry) bactofugation or microfiltration could be used. In all cases, the reduction of Clostridium tyrobutyricum spoilage of milk is necessary to avoid the butyric defect.

The main fact concerning low-salt Bou d'Fagne - a soft cheese-type with smear - was the presence of cheese pieces with the white mould *P. camemberti*, which is considered by professionals and by consumers as an important defect. In the task 1.4 we did not find a good method to limit this defect. The conclusion is that the salt reduction is not easy to carry out in this type of cheese. The way chosen to reduce salt level is to consider in parallel the reduction of cheese contaminants by working on water and milk microbial quality and biofilm on equipment.

The overall conclusion of this task is that the salt reduction leads to a modification of activity of water which, depending on the cheese-type, can bring modifications of fermentation and emergences of defects linked to this fermentation. Some of these defects, like butyric acid fermentation in semi-hard cheese, can be limited. Other defects, like mould defect in soft cheese with moulds, are impossible - or at least very difficult - to restrict.

In a general point of view, the Actalia experts' main pieces of advice on this defects are:

-1) to check that the air movements do not bring on the cheese before ripening and in the ripening room a contamination of *Penicillium* from other rooms or other cheeses, but this is not always easy to obtain in plants which make different types of soft cheese types ;

-2) to use quick strains of *Geothricum candidum* which can be able to inhibit the growth of *Penicillium camemberti,* but these strains are not always well adapted to the quality objectives,

-3) to add solution of KCl for cheese washing during ripening, but this is not authorized for different cheese types, and for example are forbidden for some labels (ex: foods from organic milk) or protected designation of origin or are not possible for some traditional cheeses. This solution is often considered also as too expensive for small companies.

5. References

TP Guinee & BT O'Kennedy (2007) Reducing Salt in Cheese and Dairy Spreads in Reducing Salt in Foods: Practical Strategies, Woodhead PTD edEd., London

ITG-ACTALIA (1980-2000) Studies on Emmental technology, internal reports available in ACTALIA (actalia74@actalia.eu) :

- JR Kerjean & R Richoux 2003 Manuel du Salage en Fromagerie, Editions Arilait, Paris, 69 pages
- JF Chamba & JR Kerjean 1982 Butyric acid bacteria a review. Edition ITG-ACTALIA 82/B00, 210 pp
- JF Chamba 1984 The bactofugation, studies at different temperature. Edition ITG-Actalia 82-84/01B 54p
- JR Kerjean & JP Quiblier 1991, Du lait microfiltré pour l'emmental. Parlons technologie V, 6, Edition ITG-ACTALIA
- JR Kerjean 1988 Inhibition of butyric fermentation by lactobacilli. Parlons Technologie III, Edition ITG-ACTALIA 3p
- JR Kerjean 1995 Natural Antimicrobial systems. Lactic acid bacteria against butyric acid fermentation. Final report on the R&D project FLAIR/2/AGRF0048 22 pp
- JF Chamba & JR Kerjean 1984, Use of lyzosyme to limit the butyric acid fermentation, Edition ITG-ACTALIA 84/B 35 pp