oscillatory shear. The optimisation of the method showed a linear visco-elastic area up to a deformation of 1%. Continuous oscillation tests were performed on samples taken at different positions in cheese of different age. The elastic, viscous, and complex modulus of old Gouda cheese were significantly higher than those of young and medium Gouda cheese, indicating that old Gouda cheese is more consistent. The phase angle was not influenced by ageing: the simultaneous changes of both water content and degree of proteolysis caused δ to be constant during ripening. A positive gradient in elasticity related to position within the cheese was noticed for all Gouda cheeses. In this case, the decreasing water content towards the crust had a more pronounced effect than any proteolytic differences.

DEWETTINCK, K., DEROO, L., MESSENS, W., HUYGHEBAERT, A.: **Einfluß von Alter und Position auf die dynamischen rheologischen Eigenschaften von Goudakäse**. Milchwissenschaft **54** (5) 258–262 (1999).

55 Goudakäse (dynamische rheologische Eigenschaften)

Es wurde das dynamische rheologische Verhalten von jungem, mittelaltem und altem Goudakäse mithilfe von „small amplitude oscillatory shear" untersucht. Die Optimierung des Verfahrens zeigte einen linearen viskoelastischen Bereich bis zu einer Verformung von 1%. Kontinuierliche Oszillationstests wurden an Proben durchgeführt, die an unterschiedlichen Stellen des unterschiedlich alten Käses genommen worden waren. Der elastische, viskose und komplexe Modulus von altem Goudakäse lag signifikant höher als bei jungem und mittelaltem Gouda und zeigte, daß alter Gouda konsistenter ist. Der Phasenwinkel wurde nicht durch das Altern beeinflußt: die gleichzeitigen Veränderungen von Wassergehalt und Proteolysegrad hielten δ während der Reifung konstant. Bei allen Goudakäsen wurde ein positiver Elastizitätsgradient im Verhältnis zur Position innerhalb des Käses festgestellt. Der sinkende Wassergehalt in Richtung Rinde hatte in diesem Fall einen deutlicheren Einfluß als alle Proteolyseunterschiede.

Evaluation of commercial adjuncts for use in cheese ripening: 3. Properties of heat-shocked adjuncts in buffer and cheese slurry systems

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1. Introduction

Different methods described in the literature to modify selected cultures of lactic acid bacteria or other cheese related microorganisms in such a way that they would not produce lactic acid, would not compete with normal starter growth and still deliver active intracellular peptidases and esterases in the cheese matrix. These methods include heat shocking, freeze shocking, and spray drying of the bacterial cells, lysozyme treatment and solvent treatment (8, 11, 17). Lysozyme treatment is considered to be too expensive for industrial applications (12) while solvent treatment of cells may present concerns about the toxicity of the residual solvents. For heat shocking, temperatures ranging from 56–75 °C for 15–22 s were used. These treatments led to drastic reduction in acid production ranging from 89–97% with a limited decrease (10–37%) in proteinase and peptidase activity (1, 2, 9, 15, 16). However, none of the studies characterize the influence of heat shocking on the autolytic properties of the cells despite the importance of autolysis as a prerequisite event to enzyme release during cheese ripening.

The aim of the present work was to study the impact of the heat shocking treatment of commercially available adjunct cultures on their autolytic properties and intracellular enzyme activities. In addition Cheddar cheese slurries with added heat shocked and freeze shocked cells were made to evaluate and compare the impact of these two methods on the levels of intracellular peptidase and esterase release as well as the rate of protein and fat degradation in the slurries.

2. Materials and methods

2.1 Culture cultivation and preparation of cell free extract (CFE)

Commercial adjunct cultures used in our previous studies (7, 14) were divided into 3 groups according to their peptidase and esterase activities and autolytic properties. *Lactobacillus helveticus* I and M were high in both enzymatic activities and autolysis while *Lb. casei* A and *Lb. helveticus* L were high in enzymatic activities and low in autolysis. *Lb. helveticus* U and *Lb. casei* T showed relatively high autolysis and low peptidase activity. Cultivation of microorganisms, preparation of culture suspension (~10^7 CFU/ml) and the CFE were previously described (7).

2.3 Heat shocking and freeze shocking

The method described by THIBOUTOT *et al.* (16) was used to accomplish the heat shocking treatment. Freeze shocking was carried out by freezing the cultures at –20 °C for 24 h (7).

2.4 Intracellular enzyme assays

Aminopeptidase and dipeptidylaminopeptidase activities were measured by the method of El ABBOUDI *et al.* (4). The esterolytic activity was measured as described by BRANDL and ZIZER (3). One unit of enzymatic activity was defined as the increases in 0.01 absorbance at 410 nm/min; the specific activity was defined as the number of activity units per mg of protein of the CFE.

The protein content of the CFE was determined by the method of LOWRY *et. al.* (13).

2.5 Measurement of the rate of autolysis

A portion of cell suspension (~10%, v/v) was added to 0.01 M phosphate buffer pH 5.2 containing 0.5 M sodium chloride to obtain an optical density of 0.8 to 1.0 at 650 nm and incubated at 30 °C. After set time intervals the percentage decrease in optical density was measured and expressed as % autolysis (16).

2.6 Preparation and analysis of cheese slurries

Cheese slurries were prepared under controlled microbiological conditions following the procedure described by FARKYE *et al.* (10). The methods used to determine compositions, chemical ripening indices and enzymatic activities in cheese slurry were those described in our previous paper (14).

3. Results and discussion

3.1 Effect of heat shocking on autolytic properties and intracellular enzyme activity

Fig. 1 shows the effect of the heat shocking treatment on the rate of autolysis of the different cultures. The autolysis rate of all the cultures is affected at different levels by the heat shocking treatment when compared to the untreated cells. In the case of strain I, the rate of autolysis after 48 h was 80% for the untreated cells while it was 60, 50 and 30% after the heat shocking treatment at 60, 65 and 70 °C, respectively. Similar trends were also observed for the other strains. These results indicate that heat shocking has a negative effect on the rate of autolysis when compared to freeze shocking (6, 8). Our results are comparable to the findings of EL-KHOLY *et al.* (5) for *Lb. helveticus* and *Lb. casei* strains. The decrease in activity at higher heat shocking treatments may be attributed to a partial denaturation of the autolytic system of the cultures.

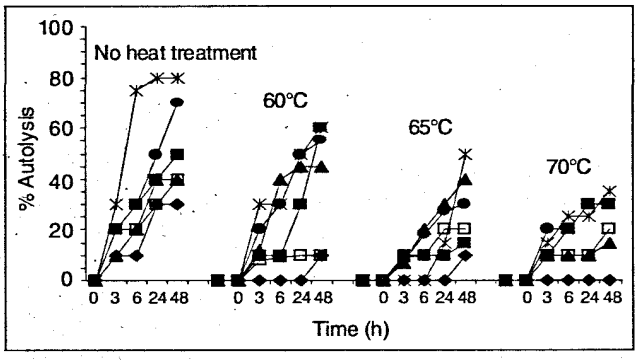

Fig. 1: Effect of heat shocking temperature (60, 65 or 70°C/15 s) on the rate of autolysis of adjunct cultures of *Lb. helveticus* strains I, M, U and L and *Lb. casei* strains A and T after incubation for 48 h at 30°C.
■ U; ● T; ▲ A; ◆ L; □ M; ✳ I

The results in Fig. 2 illustrate the influence of heat shocking temperature on the % decrease in aminopeptidase, dipeptidyl-aminopeptidase and esterase activities of the different commercial adjuncts. As expected, the % decreases in specific activity increased with the increase in the heat shocking temperature. The values obtained for % decrease in aminopeptidase activity for *Lb. helveticus* I were 93, 62 and 30% after a heat shocking treatment of 70, 65 and 60 °C, respectively. The corresponding values for the dipeptidyl-aminopeptidase activity of this culture were 64, 40 and 0%, while they were 85, 77 and 24% for the esterolytic activity. Considerable variations in the resistance of the different enzymes from the different microorganisms could be noticed. Although the % decrease in aminopeptidase activity for strain U was 95%, it was 64 and 40% for *Lb. helveticus* I and *Lb. helveticus* L. The *p*-nitrophenyl butyrate hydrolyzing activity of *Lb. casei* T was not affected by the heat treatment at 60 °C while 17% of its aminopeptidase activity was inactivated at this temperature. Variations among strains in their resistance to heat shocking treatment were reported by other authors (15, 16).

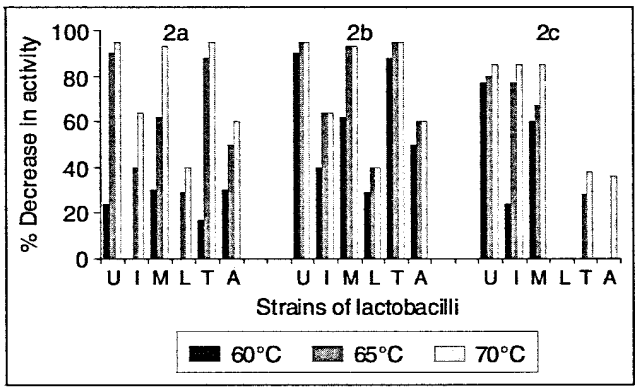

Fig. 2: Effect of heat shocking temperature on % decrease of the levels of aminopeptidase (2a), dipeptidylpeptidase (2b) and esterase (2c) activity of adjunct cultures of *Lb. helveticus* strains I, M, U and L and *Lb. casei* strains A and T

3.2 Effect of added heat shocked and freeze shocked cultures to cheddar cheese slurries on the levels of enzymes and on proteolysis

The results illustrated in Table 1 clearly indicate that freeze shocking of the commercial adjuncts lead to more enzyme release in the slurries when compared to heat shocking. In fact the number of enzyme units /g of cheese in the case of *Lb helveticus* I were 28 for the freeze shocked cells while it was 4-fold lower in the case of the heat shocked cells. Similar observations can also be noticed in the case of the dipeptidyl-aminopeptidase and esterase activity. The same trend in the results is observed for *Lb. casei* T.

The results of the ripening indices of the cheese slurry illustrated in Table 2 confirm the foregoing findings described in Table 1. As a matter of fact, higher levels of protein breakdown could be measured after 5 d of ripening in the slurries made with the freeze shocked *Lb. helveticus* I as compared to the heat shocked cells or the control. The concentration of cd-ninhydrin reactive amino groups in the slurries made with the freeze shocked *Lb helveticus* I was 4.9. The value obtained for the control was 0.9 while it was 3.1 for the slurries made with the heat shocked cells. As far as fat degradation is concerned the results in Table 2 reveal higher rates of lipolysis in the attenuated cells treated slurries as compared to the control, The increase in FFA was almost two-fold higher in the case of slurries with added freeze shocked *Lb. helveticus* I.

Slurries made with heat shocked cells showed higher amounts of free fatty acids when compared to those made with heat shocked cells. In fact the free fatty acids levels were 60 and 12% higher in the slurries made with freeze shocked *Lb. helveticus* I and *Lb. casei* T, respectively. These results confirm our findings related to the levels of esterase in the cheese slurries (Table 1) where about 50% more esterase activity could be measured in slurries made with freeze shocked cell when compared to those made with heat shocked cells. It is expected that cheese made with freeze shocked cells will develop stronger flavor. The higher level of protein and fat breakdown in the slurries made with freeze-shocked cells can be attributed to ruptures in the envelopes of the adjunct cells, which make them more susceptible to the autolytic process. On the other hand, if the autolysins of the cells are inactivated by the heat shocking treatment they will be more resistant to autolysis and, therefore, will need a longer time to release their enzymes in the cheese matrix.

Table 1: Peptidase and esterase activity in cheese slurries with added heat shocked (65°C/15 s) or freeze shocked adjunct culture of lactobacilli (unit/g cheese)

Treatment	Amino-peptidase	Diamino-peptidase	Esterase
Heat shocking			
Lb. helveticus I	6.00 ± 0.42	5.00 ± 0.13	0.10 ± 0.004
Lb. casei T	0.01 ± 0.003	0.27 ± 0.014	0.10 ± 0.008
Freezeshocking			
Lb. helveticus I	28.0 ± 1.46	7.00 ± 0.36	0.20 ± 0.013
Lb. casei T	0.25 ± 0.025	0.40 ± 0.18	0.20 ± 0.016
Control	0.01 ± 0.006	0.32 ± 0.016	0.04 ± 0.003

Results are means of duplicate determination on 2 replicates, n = 4

Table 2: Protein and fat hydrolysis in cheese slurries inoculated with heat shocked (65°C/15 s) or freeze shocked adjunct culture of lactobacilli

Treatment	Free amino acids (mM leucine equiv	Water-soluble N (as % of total N)	Free fatty acids (mol equiv/g)
Heat shocking			
Lb. helveticus I	3.1 ± 0.16	17.6 ± 0.84	13.4 ± 0.93
Lb. casei T	1.2 ± 0.08	17.2 ± 1.05	16.5 ± 1.07
Freezeshocking			
Lb. helveticus I	4.9 ± 0.26	21.2 ± 1.32	21.5 ± 1.22
Lb. casei T	1.6 ± 0.10	15.5 ± 1.10	18.2 ± 0.96
Control	0.1 ± 0.024	3.3 ± 0.20	8.8 ± 0.66
Control 5d	0.9 ± 0.057	11.3 ± 0.26	11.6 ± 1.14

Results are means of duplicate determination on 2 replicates, n = 4

This study reveals that attenuated selected commercial adjuncts cells lead to enhanced proteolysis and lipolysis in cheese slurries. However, the extent of proteolysis depends on the methods used to attenuate the cells. Freeze shocking seems to be more effective in that respect. Cheesemaking trials are in progress to confirm these results.

Acknowledgements

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5. Summary

EL-SODA, M. MADKOR, S.A. TONG, P.S. **Evaluation of commercial adjuncts for use in cheese ripening: 3. Properties of heat-shocked adjuncts in buffer and cheese slurry systems.** Milchwissenschaft **54** (5) 262–265 (1999).

56 Cheese ripening (heat-shocked adjuncts)

Heat shocking of selected commercial adjunct cultures led to a decrease in the rate of cell autolysis and enzymatic activities. Changes of both parameters were related to the temperature used in heat-shocking process. The higher the temperature used the greater the decrease in autolysis and activity. Cheese slurries made with freeze shocked cells revealed higher enzyme activity as well as more protein and fat breakdown when compared to cheese slurries inoculated with heat shocked adjuncts. This was attributed to the higher release of enzyme activity in case of freeze shocked adjunct cultures.

EL-SODA, M. MADKOR, S.A. TONG, P.S. **Bewertung handelsüblicher Zusätze für die Käsereifung. 3. Eigenschaften von hitzeschockbehandelten Zusätzen in**

Puffer- und Käseaufschwemmungssystemen. Milchwissenschaft **54** (4) 262–265 (1999).

56 Käsereifung (hitzeschockbehandelte Zusätze)

Hitzeschockbehandlung von ausgewählten handelsüblichen Zusatzkulturen führte zu einer Abnahme des Zellautolysegrades und der enzymatischen Aktivitäten Die Veränderungen beider Parameter waren abhängig von beim Hitzeschockverfahren verwendeten Temperatur. Je höher die Temperatur, desto mehr sanken Autolyse und Aktivität. Käseaufschwemmungen aus gefriergeschockten Zellen zeigten eine höhere Enzymaktivität und einen stärkeren Protein- und Fettabbau verglichen mit solchen, die mit hitzeschockbehandelten Zusätzen inokuliert worden waren. Dies wird der höheren Freisetzung von Enzymaktivität bei gefriergeschockten Zusatzkulturen zugeschrieben.

Production of ice cream containing probiotic bacteria

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1. Introduction

Frozen yoghurt has become popular and is continuously reaching new markets. Its popularity is associated with the healthy image of yoghurt, delicate taste and its low fat content. Using the same technology it is possible to produce a probiotic ice cream. This ice cream contains cultures selected for dietary benefits. A probiotic can be defined as "a mono- or mixed culture of live microorganisms which, applied to man or animal, beneficially affects the host by improving the properties of the indigenous microflora" (4). HUIS IN'T VELD and HAVENAAR (4) also describe the possible dietary benefits of probiotic bacteria.

The aim of this study was to produce a probiotic ice cream containing high levels of probiotic bacteria ($>10^6$ cfu/g, which is the recommended minimum daily intake (5)), and to determine their survival immediately after freezing and during frozen storage. In an attempt to improve the survival of the cells during freezing, one of the batches was added 2% glycerol.

2. Materials and methods

2.1 Probiotic strains

Four different cultures were used. *Lactobacillus reuteri* (LR) obtained from BioGaia Biologics AB, Stockholm, Sweden. *Lactobacillus acidophilus* (LA-5) and *Bifidobacterium bifidum* (BB-12) obtained from Christian Hansen AS, Norway, Oslo, and *Lactobacillus rhamnosus "GG"* (ATCC 53103) obtained from Valio Ltd., Helsinki, Finland.

2.1.1 Storage of strains

Concentrated freeze-dried cultures were prepared in the following way. The cells were routinely propagated on 3 successive days, at the rate of 1% in MRS-broth (Oxoid) and incubated at 37°C for 24 h. For the concentrate preparation, the cultures were grown in 300 ml MRS and the cell mass was harvested by centrifugation at ca 1400xg for 10 min (Sorvall RC-5B refrigerated Superspeed centrifuge, Du Pont Instrument). After centrifugation the cell pellets were resuspended in 30 ml UHT (Ultra High Treatment) semi skimmed milk, distributed into 10 tubes, and stored at –80°C until required.

2.2 Procedure for ice cream manufacturing

Batches of ice cream mix based on unsalted sweet cream butter and skimmed milk powder (Norwegian Dairies, Oslo) were prepared with 10% fat, 12% milk solids not fat, 12.5% sucrose, 0.8% stabilizer/emulgator (Cremodan SE 38 veg. Danisco Ingredients, Denmark), 0.3% vanilla and 2% glycerol (added to one of the batches). The flow chart is shown in Fig. 1. Water and unsalted sweet cream butter were heated to 75°C. During this heating, skim milk powder and sucrose were added at 40–50°C and the combined stabilizer/emulgator was added at 70°C. The mix was pasteurized at 75°C for 2 min, homogenized at 175 kp/cm^2 and then aged overnight at 4°C.

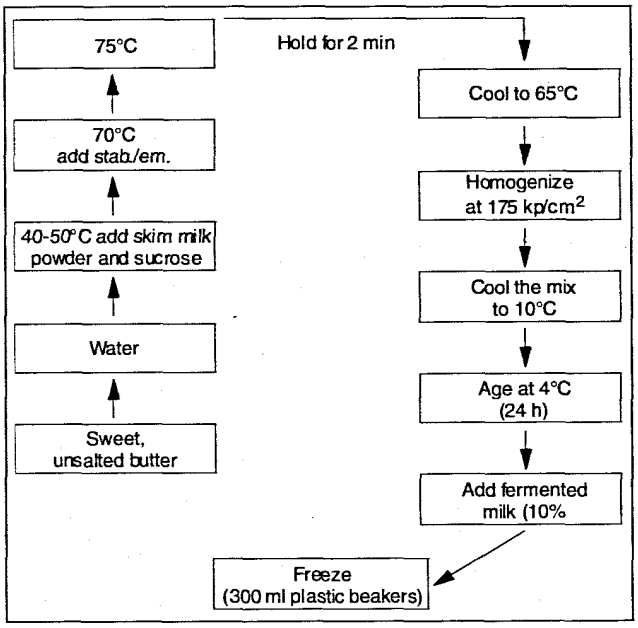

Fig. 1: Production of ice cream

Each strain was cultured for 12 h at 37°C in UHT semi-skimmed milk, fortified by the addition of 1% D-glucose (puriss, Kebo lab AB, 50% w/v filter sterilised solution) and 1% tryptone (Oxoid, 25% w/v filter sterilised solution). This fermented milk was then added