components in cheese samples. The results for Manchego cheese obtained here can be considered as complementary to those obtained previously with SDE; the information obtained deals with highly volatile compounds, many of which are probably relevant for flavour. The main differences between the results found by the present method and by SDE can be explained in terms of properties such volatility and water solubility.

Acknowledgement

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Evaluation of commercial adjuncts for use in cheese ripening: 5. Effect of added freeze-shocked adjunct lactobacilli on proteolysis and sensory quality of reduced fat Cheddar cheese

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Reduced fat Cheddar cheese curds were made using *Lactococcus* starter and conventional milled curd cheesemaking procedure. After milling, the curds were inoculated with no (control) or one of 6 freeze-shocked adjunct cultures (~ 10^7 cfu/gram) of lactobacilli (4 strains of *Lb. helveticus* I, U, M and L or 2 strains of *Lb. casei* T and A). All adjunct-treated cheese had slightly higher levels of WSN than control. Adjunct treated cheeses reached higher levels of FAA than control with the progress of ripening. Greater increase of total FAA was observed in cheese inoculated with *Lb. helveticus* I followed by strain U and M. Organoleptic evaluation indicated that cheese inoculated with *Lb. helveticus* I rapidly developed the highest flavor score after 3 months of ripening compared to control cheese. Inoculation of *Lb. helveticus* strains in the cheese curds positively influenced flavor and texture attributes in reduced fat Cheddar by preventing bitterness and reducing firmness. Less influence on proteolysis and flavor intensity was observed in cheese inoculated with adjunct cultures with poor rate of autolysis and low peptidolytic activity such as *Lb. casei* T and A strains.

56 Cheese ripening (commercial adjuncts) **56 Käsereifung** (Mikrobenzusätze)

1. Introduction

Adjunct cultures of mesophilic lactobacilli are used to improve and accelerate flavor development during cheese ripening (1, 4, 8, 22). They may play an important role as a peptidolytic agent to prevent bitterness during Cheddar cheese ripening (5, 6, 8, 9).

Species studied as adjunct cultures for cheese include: *Lactobacillus plantarum*, *Lb. casei*, *Lb. helveticus*, *Lb. brevis* and to a lesser extent *Pedicoccus* or *Micrococcus* (14, 16, 18, 25). These organisms contribute to an increase in peptidase activity in cheese and enhance the production of free amino nitrogen during secondary and tertiary ripening (5, 18, 22). *Lactobacillus casei* and *Lb. helveticus* have been suggested as adjunct cultures with good potential, especially in reduced fat cheese (8, 17). However, positive results for flavor and texture improvement in cheese depends strongly on the strain used (11, 14, 28). The evidence that adjunct lactobacilli appear to have significant impact on Cheddar cheese maturation has encouraged culture research to select adjunct strains with appropriate proteolytic characteristics for cheese ripening (9,11, 17). Practically, bacterial strains to be used as adjunct cultures in reduced fat cheese should be carefully selected on the basis of their temperature sensitivity, autolytic properties and proteolytic/peptidolytic activities (9, 10, 23). In some reports specific catabolic activity of adjunct cultures may also be important (5 8, 16). In our previous studies (12, 23) several strains of laboratory and commercially available cultures of lactobacilli varied considerably in their enzymatic activities and their resistance to autolytic process either in buffer system or model cheese slurry system. Cheese slurries made with adjunct cultures selected for their high enzymatic potentials and high autolysis rate exhibited the highest levels of intracellular enzyme release and flavor development in cheese slurries. Other results from our laboratory (13, 24) also indicated that freeze shocked lactobacilli showed the highest rate of cell autolysis and enzyme release in model cheese slurry system compared to untreated cells or other physical treatments.

In this study we compare the effectiveness of selected strains of freeze shocked adjunct cultures of lactobacilli on the proteolysis and sensory attributes during ripening of reduced fat Cheddar cheese.

2. Materials and methods

2.1 Culture strains and freeze shocking treatment

Six commercial and laboratory culture strains of adjunct lactobacilli (4 strains of *Lb. helveticus*: I, U, M, L and 2 strains *Lb. casei*: T and A) were selected according to their enzymatic activities and autolytic properties as previously described (23). Cultures were cultivated in MRS broth at 37 °C and cells were harvested by centrifugation at 1500 g for 30 min at 4 °C. The cell pellet was washed twice with 0.01 M phosphate buffer (pH 7.0) and resuspended in the same buffer to obtain viable cell concentrations of approximately 10^9 cfu/ml. The cell suspension was frozen at –20 °C for 24 h and thawed in a water bath at 40 °C prior to use.

2.2 Cheese manufacturing

Reduced fat Cheddar cheeses were made from standardized (protein/fat ratio: ~1.60) pasteurized (77.3 °C for 16 s) milk in 2000 liter vat according to a conventional protocol for milled curd Cheddar cheese-making (19). A commercial "direct to vat set" mesophilic *lactococcus* starter culture (type DVS 850, Chr. Hansen Lab, Milwaukee, WI) was added to milk (0.015% w/w) and ripened for 45 min at 30 °C. After renneting (Chymax, Chr. Hansen Lab, Milwaukee, WI, 0.09% v/w), the coagulum was cut and cooked to 39 °C over 30 min and held at this temperature for an additional 30 min stir out. After whey drainage, the curds were cheddared until the pH reached 5.4 and milled. NaCl (1.7% w/w) was added to the curd in 3 equal applications over 15 min. Portions of the cheese curd (5 kg) were individually inoculated with no adjunct (control cheese) or with one of 6 freeze shocked culture suspensions of adjunct lactobacilli (~10^7 cfu/g cheese). While cultures are normally added to the cheese milk, adjunct cultures were added directly to cheese curd in this study to insure equal numbers of adjunct culture per gram of cheese curd. The cheese curd were mixed thoroughly to ensure even distribution of culture cells, hooped in rectangular blocks, pressed at 40 psi for 3 h, vacuum packaged and ripened at 10 °C for 6 months.

2.3 Chemical and microbiological analysis of cheese

Duplicate samples of cheese were analyzed for moisture, fat, salt, total protein and pH (3). Proteolysis was assessed by measuring water-soluble nitrogen (20), total free amino acids using the cd-ninhydrin method (15) and urea-PAGE (2). The counts of viable cells of lactobacilli in cheese were determined by enumeration on MRS plate agar and incubation at 32 °C for 72 h.

2.4 Sensory evaluation

Cheese samples were graded for flavor/aroma and body/texture at 3 and 6 month by 5 experienced Cheddar cheese taste panelists on a 1–10-point scale with more than 7.0 indicating high quality Cheddar cheese flavor or texture and less than 4 indicating poor quality Cheddar cheese flavor or texture (24). Panelists commented on Cheddar flavor intensity, bitterness, off-flavor and firmness.

3. Results and discussion

3.1 Compositional and microbiological analysis

The composition of the 2 trials were found to be within an average for moisture, fat, protein and salt of 43.65±1.15%, 17.6±0.30%, 29.85±0.82 and 1.55±0.08, respectively. One-day old cheese displayed an average pH of 5.18±0.06. In fact, the experimental cheese pH's were not affected by inoculation of adjunct lactobacilli which may due to the low residual lactose in curd or the low ability of the added freeze shocked adjunct lactobacilli to maintain activity to ferment the residual lactose and produce acid (14, 21).

In both trials, the initial counts of non-starter lactobacilli (Fig. 1) in the experimental cheese after one day of ripening were between 10^4–10^6 cfu/g cheese. The number is lower than the number added to cheese curd possibly due to the loss of some adjunct cells when mixed with cheese curd, losses due to pressing and

loss of viability. Very low numbers of non-starter lactobacilli were detected on MRS media of control cheese ($<10^2$ cfu/g). This number of advantageous non-starter lactobacilli in control cheese reached a level of ~10^4 after 3 months of ripening and remained constant up to the end of ripening. However, the non-starter lactobacilli counts in the experimental cheese were most likely those recovered from inoculation of adjunct cultures in cheese curd. Cheese inoculated with *Lb. casei* strain showed dramatic increases in the number of lactobacilli, reaching 10^9 cfu/g cheese after about 3 month of ripening and then slightly decreased at the end of ripening. The corresponding number of viable cells retained in cheese with *Lb. helveticus* strains was between 10^6–10^8 after 3 months of ripening and decreased with the progress of ripening. A decrease in cell counts of adjunct lactobacilli after 2–6 month of ripening was also noted in several studies (17, 21, 22) which may have been due to autolysis of the adjunct cells (14). The decreasing rate of cell viability was more pronounced in some cheese. In particular, those inoculated with *Lb. helveticus* I had a high extent of cell lysis (14, 23). Reported data in our proceeding work (13, 26) demonstrated that cell lysis is strain specific and markedly enhanced by freeze shocking treatment.

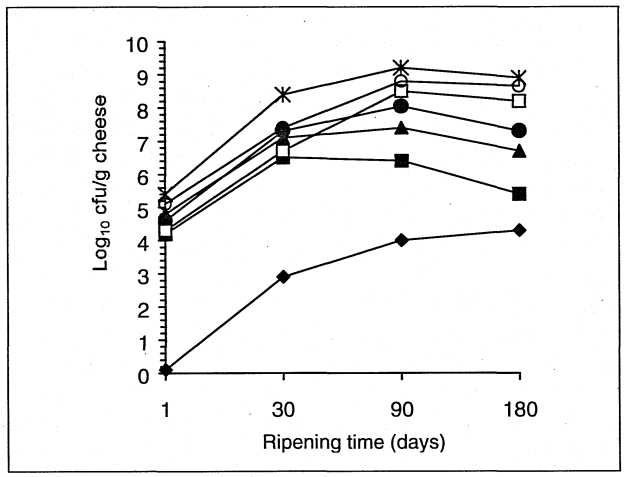

Fig. 1: Counts of viable lactobacilli during ripening of reduced-fat Cheddar cheese made without adjunct (control cheese: ◆) or with added freeze-shocked adjunct cultures of *Lb. helveticus* I (■), U (▲), M (●) and L (□) or *Lb. casei* T (∗) and A (○)

3.2 Proteolysis

Proteoysis as assessed by the amount of WSN (Fig. 2) increased linearly with time for all cheese up to the end of ripening period. All adjunct treated cheeses showed somewhat slightly higher levels of WSN than control. This trend was manifested more in *Lb. helveticus* strains I when compared to *Lb. casei*. Also, the proteolytic activity of *Lb. helveticus* I may have also been responsible for some qualitative differences in peptide profiles which were detected in gel electrophoretograms of 3 and 6 month old cheese (Fig. 3).

As in our preceding report (24) the levels of total free amino acids (FAA) was significantly affected by adjunct cultures. The corresponding levels of total FAA as measured by the cd-ninhydrin method (Fig. 4) reached considerably higher levels of FAA than control. There were only slight differences in the levels of FAA between all cheeses up through 2 month of ripening, however, higher concentration of total FAA developed thereafter in adjunct treated cheeses than control cheese through the end of ripening. A greater increase in the level of total FAA was observed in cheese inoculated with *Lb. helveticus* I. Notably, in cheese inoculated with *Lb. helveticus* I the total free amino acids increased considerably with time and was higher than for all other strains at 6 month of ripening. It is interesting to notice that the level of FAA in 3-month old cheese inoculated with *Lb. helveticus* I reached a value similar to that in 6-month old cheese without adjunct.

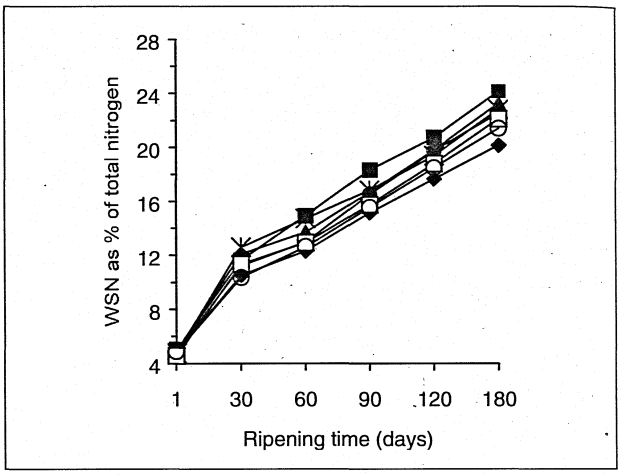

Fig. 2: Formation of water-soluble nitrogen (WSN) during ripening of reduced-fat Cheddar cheese made without adjunct (control cheese: ◆) or with added freeze-shocked adjunct cultures of *Lb. helveticus* I (■), U (▲), M (●) and L (□) or *Lb. casei* T (∗) and A (○)

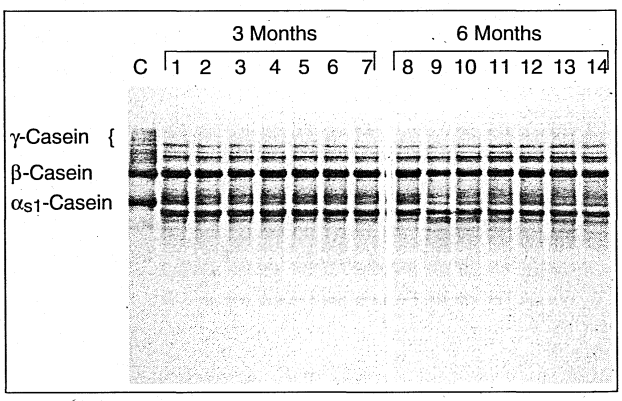

Fig. 3: Gel electrophoretograms of Cheddar cheese at 3 (lane 1–7) and 6 (lane 8–14) month of ripening inoculated with freeze shocked adjunct strains of *Lb. helveticus* I (lane 2, 9); M (lane 3, 10); U (lane 4, 11); L (lane 6, 13) or *Lb. casei* T (lane 5, 12) and A (7, 14). Control (no adjunct, lane: 1, 8). Na-caseinate (lane C)

Other strains of *Lb. helveticus* have been reported to contribute to the levels of FAA in reduced fat Cheddar cheese (8, 17). The data revealed that all adjunct cultures evaluated herein would influence development of FAA but to different degree. Consequently, substantial increase in free amino group content of cheese made with *Lb. helveticus* I strain may have been due to their high peptidolytic activity and increased cell lysis. In our

preceding studies (12, 13, 23), certain strains of lactobacilli such as *Lb. casei* T and A were expected to have less influence in promoting proteolysis due to their low rate of enzyme release and proteolytic activity. Thus, depending on strain type, the extent of enzyme release and the effectiveness of peptidase activities of selected lactobacilli added to cheese, the WSN peptides can be degraded extensively to elevated levels of FAA (5, 8, 22, 24). In fact, enhanced cell lysis and high aminopeptidase activities of selected lactobacilli can be a limiting factor in the rapid formation of flavor constituents during ripening of Cheddar cheese with reduced fat content. A strong correlation between the extent of aminopeptidolysis and cheese flavor intensity was revealed in several other reports (6, 8, 21, 25).

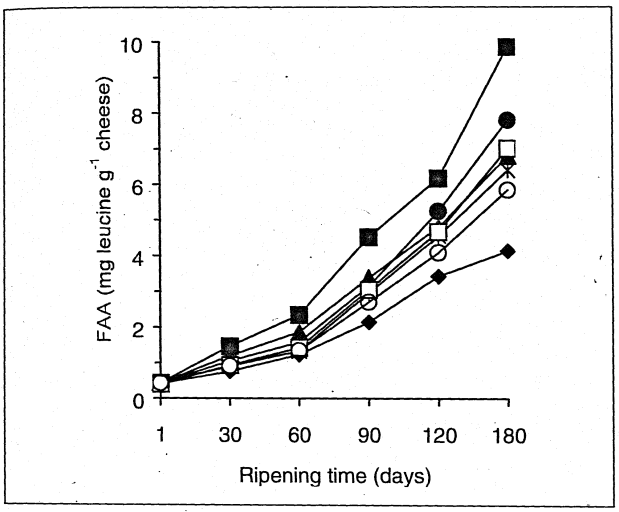

Fig. 4: Development of total free amino acids during ripening of reduced-fat Cheddar cheese made without adjunct (control cheese: ◆) or with added freeze-shocked adjunct cultures of *Lb. helveticus* I (■), U (▲), M (●) and L (□) or *Lb. casei* T (✳) and A (○)

3.3 Sensory evaluation

The sensory score of cheese (Fig. 5) indicates that highest scores for flavor and texture were received for cheese inoculated with *Lb. helveticus* I followed by *Lb. helveticus* M and U. Taste panelists noted that adjunct treated cheese, in particular cheese inoculated with *Lb. helveticus* I, rapidly developed the highest flavor score after 3 month of ripening compared to control cheese. Cheeses treated with some adjuncts such as *Lb. casei* T or A were downgraded because the panelist indicated that the cheese contained a sour-milky taste, which remained constant in these cheeses up to the end of ripening. The panelist comments also indicated that control cheese lacked flavor after 3 month of ripening and with extended ripening tended to have bitterness. Flavor scores in cheese made with *Lb. helveticus* I was highest at 3 and 6 month of ripening period. Other reported studies revealed that adjunct cultures of *Lb. helveticus* positively influenced flavor and prevented bitterness during ripening of reduced fat Cheddar cheese through their high amino peptidolytic activities (8, 17, 28).

Cheeses inoculated with *Lb. helveticus* I and M tended to have higher texture scores, relatively softer body and less firmness compared to control. The higher proteolysis rates of some adjunct treated cheese evaluated herein may be associated with the decrease in firmness particularly in low fat cheese (8). Enhanced breakdown of the casein matrix especially α-casein has been associated with improved texture and smooth body in reduced fat cheese (8, 7, 28). However, uncontrolled proteolysis can negatively impact cheese body and machinability.

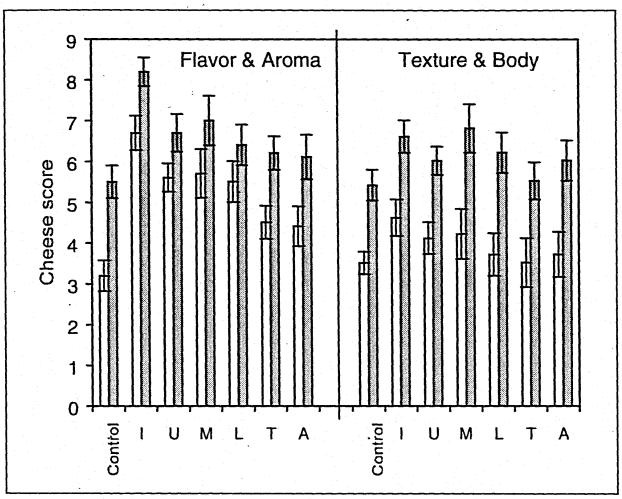

Fig. 5: Sensory scores of 3 (□) and 6 (■) month old reduced-fat Cheddar cheese made without adjunct (control cheese) or with added freeze-shocked adjunct cultures of *Lb. helveticus* I, U, M and L or *Lb. casei* T and A

4. Conclusion

The present study confirms and expands upon the findings of our previous studies (23, 24) that freeze shocked *Lb. helveticus* in particular strain I can be used successfully to enhance flavor development and improve quality of reduced fat Cheddar cheese. The addition of freeze shocked *Lb. helveticus* I has the important advantage of supplementing whole cell enzyme system which increases the proteolytic potential in cheese. Inoculation of *Lb. helveticus* with high autolytic properties and high peptidolytic activity led to substantial increase in tertiary proteolysis rate resulting in elevated levels of small peptides and free amino acids. Consequently, cheese maturity and quality were enhanced as determined by the high flavor score after 3 and 6 month of ripening. Also, *Lb. helveticus* I positively influenced flavor and texture attributes in reduced fat Cheddar by preventing bitterness and reducing firmness. Less influence on proteolysis and flavor intensity were observed when adjunct cultures with low rates of autolysis and low peptidolytic activity was used (*e.g. Lb. casei* T and A strains).

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Stabilisation of calcium phosphate using denatured whey proteins

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Denatured whey proteins (3% w/w) were shown to stabilise 30 mM calcium and phosphate. Subsequent stability to heat was dependent on the initial pH of the calcium phosphate/whey protein mixture. At 6.4 < pH < 6.7, stand-up gels were formed on heating to 90 °C for 30 min. At pH > 6.7 no gelation was evident. The degree of unfolding/aggregation of whey protein prior to addition of calcium and phosphate had a significant effect on the strength of the gel observed after subsequent heating. The duration of heating at 70 °C showed major effects on gel strength following calcium and phosphate addition, at pH 6.5, and subsequent re-heating. The strength of the gels following pre-heating at 80 °C was also affected by duration of heating but less so than at 70 °C. Pre-heating at 90 °C resulted in gels which decreased in strength with increased duration of heating. The strength of the gels formed from whey protein which had been pre-heated at 90 °C, especially for the longer times, were weaker than gels formed from the 80 °C pre-heat treatment. The secondary heating step was shown to induce a major release of protons due to secondary calcium phosphate formation, and the accompanying decrease in pH aided in gel formation.

66 Whey proteins (phosphate stabilisation) **66 Molkenproteine** (Phosphatstabilisierung)

1. Introduction

Whey proteins are globular proteins and are denatured by heat. They are widely used to enhance the nutritional value of formulated foods and are often cited as being highly functional, due to their ability to gel on heating and immobilise large quantities of water (1, 2, 3). Whey proteins can form either thermo-reversible or thermo-irreversible gels when heated above 65 to 70 °C, depending on the initial pH and composition of the protein solution (4). Thermal gelation of whey proteins is a two-stage process, involving an initial unfolding and a subsequent aggregation of the protein molecules (5). Any action that changes the native conformation of the protein, has, in principle, the potential to induce gelation. This may include heat treatment (6), addition of calcium, sodium or acid (7, 8, 9) or enzymatic treatment (10, 11). A major force which opposes protein aggregation is electrostatic repulsion between similarly charged protein molecules (12), therefore, protein aggregation is particularly sensitive to pH, the ionic species present and ionic strength. In this study, the possibility of introducing