Evaluation of commercial adjuncts for use in cheese ripening: 4. Comparison between attenuated and not attenuated lactobacilli

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The influence of heat-shocking and freeze-shocking on cell viability, autolytic properties, aminopeptidase, esterase activities and acid production of Lb. helveticus and Lb. casei was studied in buffer and in a cheese slurry system. Heat-shocking had the most destructive effect on cell viability. The rate of acid production in milk was a function of the attenuation method. Among all strains of lactobacilli, Lb. helveticus I had the least acid production. Heat-shocking was the most effective treatment in reducing acid production. Two cycles of freeze thawing were the most effective treatment in promoting cell autolysis. Heat-shocking had the most detrimental effect on the % autolysis and on the levels of enzymatic activity. The not attenuated cells showed the highest levels of enzymatic activities followed by the freeze-shocked cells and the heat-shocked cells. Cheese slurry containing added freeze-shocked cells of Lb. helveticus I showed considerably higher levels of peptidase activity release and higher rate of proteolysis compared to the Lb. casei strains. The cheese slurry inoculated with freeze-shocked cells of Lb. helveticus I had the most Cheddar flavor.

1. Introduction

Adjunct cultures are important ingredients in cheese-making due to their contribution to texture and flavor development during the ripening process. The type of adjunct cultures and their role during ripening has been described in several review papers (5, 8, 9, 10). Both, attenuated and not attenuated adjunct cultures have been utilized successfully in cheese manufacture (8). Despite the information available in the literature, it is unclear what the advantages and disadvantages are in using attenuated over not attenuated adjunct cultures. In previous work, (6, 7) we studied the influence of freeze-shocking and heat-shocking cell attenuation treatments on peptidase and esterase activities and on the autolytic properties of adjunct lactobacilli. Selected cultures were then evaluated in a slurry system (7, 14) as well as in Cheddar cheese (15). In this communication we compared the enzymatic activities, autolysis and the ability of the cells to produce acid of not attenuated, freeze-shocked and heat-shocked attenuated adjunct cultures of lactobacilli. Evaluation of the different adjuncts in a slurry system was also conducted.

2. Materials and methods

2.1 Cultures, cell cultivation and cell counts

Three Lactobacillus cultures were used for this study: Lb. helveticus I, Lb. casei A and Lb. casei T. Criteria for selection of these cultures, cultivation of the microorganisms and preparation of the crude cell free extracts were as described previously (6). The viable cell counts of lactobacilli were determined by enumeration on MRS plate agar and incubation at 35 °C for 48 h.

2.2 Heat-shocking and freeze-shocking

Heat-shocking the cells at 65 °C/15 s was carried out as described by THIBOUTOT et al. (17). The heat-shocked cells were then frozen at -20 °C for 24 h before use. Freeze-shocked cells were produced by freezing the cell suspension at -20 °C for 24 h. Thawing of each culture was at 40 °C in a water bath. The cells were subjected to 1 or 2 freeze/thaw cycles.

2.3 Intracellular enzyme assays and measurement of the % of autolysis

Aminopeptidase, dipetidylaminopeptidase and esterase activities as well as the % of autolysis were measured as described previously (6). The protein content of the crude cell free extract was determined using the method of LOWRY et al. (13).

2.4 Determination of acid production

The ability of attenuated heat-shocked and freeze-shocked cells to produce acid in milk was determined by inoculating sterilized skim milk with 2% of the cell suspension of the adjunct culture. Acid development was followed by measuring the decrease in the pH of the milk after incubation at 35 °C for 3, 6, 9, 12 and 24 h.

2.5 Preparation and analysis of the cheese slurries

Methods for cheese slurry preparation, determination of cheese composition, enzyme release from the cells, ripening indices and sensory evaluation of the cheese slurries were described in our previous publication (14).

3. Results and discussion

3.1 Effect of attenuation method on cell viability

Fig. 1 reveals that heat-shocking resulted in a marked reduction in Lactobacillus numbers. A decrease of approximately 3 log cycles could be measured after the heat-shocking treatment. On the other hand 1 to 2 log cycle reduction was noticed after freeze-shocking. These results are comparable to previous work on the effect of attenuation method on cell viability (1, 2, 11, 17).
3.2 Effect of attenuation method on the rate of cell autolysis

Fig. 2 illustrates the % autolysis of not attenuated cells of Lb. helveticus, Lb. casei A and T. It also indicates the influence of attenuation methods on cell autolysis. Heat-shocking had the most detrimental effect on autolysis. The % autolysis did not exceed 40% after 48 h of incubation. Freeze-shocking on the other hand led to enhancement of cell autolysis. The enhancement was greater when cells were subjected to 2 cycles of freezing and thawing compared to not attenuated cells. For example, the % autolysis was 65 and 70% for Lb. helveticus I after 1 and 2 freeze/thaw cycles, respectively. The % autolysis of not attenuated cells was slightly lower than the freeze-shocked cells. Similar observations were reported by EL KHOLI et al (4) for several Lactobacillus species including Lb. helveticus, Lb. delbruckii subsp. bulgaricus, Lb. delbruckii subsp. lactis, Lb. casei and Lb. plantarum. These authors indicate that the cells subjected to 2 cycles of freezing and thawing autolysed faster than those subjected to 1 or 3 cycles. In the same study (4) the authors also demonstrated that a heat treatment of 65°C/16 s led to a lag phase of 3 h where no autolysis could be observed. This was then followed by little autolysis varying from 2 to 10% during the 120 h incubation time of the experiment.

Our results are also consistent to the work of BIE and SJOSTROM (3) who indicated a 100% increase in DNA release from the cells subjected to freezing and thawing. OHMIYA and SATO (16) also obtained higher rates of autolysis from cells stored at -20°C when compared to cells stored at 3°C. Contrary to our findings, BIE and SJOSTROM (3) measured higher autolysis rates in cells of Lb. helveticus subjected to the following subsequent heat treatments 54, 59, 64°C for 20 s. The difference could be attributed to the methods used to follow autolysis. In fact, BIE and SJOSTROM (3) used DNA release as an index for cell autolysis; their data show very high autolysis rate at zero time. However, our results are comparable to the work of THIBOUTOT et al (14) and our previous study (6).

3.3 Influence of the attenuation method on intracellular enzyme activity

Both heat-shocking and freeze-shocking resulted in a reduction in aminopeptidase, dipeptidylaminopeptidase and esterase activity (Fig. 3). However, loss in enzyme activity was greatest after the heat-shocking treatment. Less activity could be measured after 2 cycles of freezing and thawing when compared to cells subjected to 1 freeze/thaw cycle. High enzyme inhibition after heat-shocking was previously reported (5). Evidence for little enzyme activity after freeze-shocking has also been documented (12).

3.4 Influence of the attenuation method on acid production

In Fig. 4 we report the effect of freeze-shocking and heat-shocking on acid production by Lb. helveticus and Lb. casei cultures. The results clearly indicate that heat-shocking lead to a considerable delay in acid production compared to not attenuated cells. In fact the pH of the milk inoculated with not attenuated Lb. casei T cells

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reached a pH of 4.0 after 24 h of incubation at 36°C. The corresponding pH for the heat-shocked cells was 6.3 while it was 5.3 for the freeze-shocked cells. A similar trend was also noticed in Lb. casei A and Lb. helveticus I. Not attenuated Lb. helveticus can, however, be distinguished from the 2 Lb. casei cells by its lower rate of acid production in milk and its higher final pH, which is a desirable characteristic in adjunct cultures.

Fig. 4: Acid production of not attenuated (☐) or one cycle freeze-shocked (★), two cycle freeze-shocked (▲); and heat-shocked (●) attenuated cultures of lactobacilli in buffer system.

3.5 Impact of cell attenuation on enzyme release and extent of proteolysis in cheese slurries

The levels of aminopeptidase and dipeptidylaminopeptidase release from the Lactobacillus cells are shown in Table 1. Both enzymatic activities were markedly affected by the method used for cell attenuation. Very little enzymatic activity could be measured in the cheese slurries manufactured with heat-shocked lactobacilli. The highest levels of aminopeptidase and dipeptidylaminopeptidase activity were detected in the slurries made with the freeze-shocked cells followed by the not attenuated cells. These results are consistent with our data on enzymatic activity and autolysis. Accordingly, the slurries made with freeze-shocked cells showed the highest values of water-soluble nitrogen and free amino acids followed by the not attenuated cells (Table 1). Slurries made with Lb. helveticus I contained the highest levels of water-soluble nitrogen and free amino acids.

3.6 Organoleptic evaluation of cheese slurries

The organoleptic evaluation of the slurries (results not shown) showed a preference to the slurries made with Lb. helveticus. The Cheddar flavor was described as strong, cultured and sulphury in the case of the slurries made with not attenuated Lb. helveticus cells while it was described as strong, cheesy, sharp and sulphury for the slurries made with freeze-shocked Lb. helveticus cells. The slurries made with no adjuncts were reported to lack Cheddar flavor while a mild Cheddar flavor was attributed to the slurries made with Lb. casei cells.

4. Conclusion

This study revealed that freeze-shocking has considerable positive effect on the % autolysis of adjunct lactobacilli and had little or no effect on their intracellular enzyme activities. Heat-shocking leads to a dramatic reduction of enzymatic activities and a considerable delay in enzyme release. Cheese slurry results indicate that freeze-shocked Lb. helveticus I would improve cheese flavor. Based on our previous work (6, 7, 14, 15) and the present study on attenuation methods of various Lactobacillus cultures we conclude that freeze-shocked Lb. helveticus I provides the most desirable adjunct culture for Cheddar cheese ripening improvement. Further work will evaluate the impact of both viable (not attenuated) and attenuated adjunct lactobacilli on the rate of flavor and texture changes in actual cheese-making trial. An economical evaluation of both systems is also needed to determine the best form of adjunct to be used for commercial applications.

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Quantitative contribution of rennet and bacterial proteolytic enzymes to the primary proteolysis in sodium caseinate solution

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In this study the contribution of different proteolytic agents to primary proteolysis in a sodium caseinate solution under cheese-like conditions was quantified. The sodium caseinate solutions were treated with rennet, sonicated cell suspension of Lactococcus lactis subsp. cremoris SK11 and heat inactivated sonicated cell suspension. Proteolysis was assessed after 3, 10 and 17 days incubation at 8°C by urea-PAGE gels of the pH 4.6-insoluble fraction. Principal component analysis of the gels estimated rennet and incubation time to contribute to >90% and ~3%, respectively, of the total variation in primary proteolysis between the assessed samples. Sonicated cell suspension was estimated to contribute to ~8% of the total variation in primary proteolysis between samples incubated for 17 days. Bacterial proteolytic enzymes showed an interactive effect with rennet by degrading β-I-CN (β-CN f1-192) in the rennet-treated samples. A heat-inactivated sonicated cell suspension had no detectable influence on proteolysis.

58 Caseinate (primary proteolysis)

1. Introduction

Primary proteolysis of casein in cheese is caused mainly by residual rennet and to a lesser extent by plasmin and perhaps cathepsin D, resulting in large and intermediate-sized peptides which are subsequently degraded by proteolytic enzymes from the starter and non-starter flora (1, 2). It is of interest to study possible interaction effects between rennet or plasmin and bacterial proteinases during primary proteolysis and to quantify the contribution of the different proteolytic agents. It has been found that primary proteolysis is strongly influenced by environmental conditions (3, 4, 5). Therefore, it should be of interest to establish if treatment of a sodium caseinate solution with a sonicated cell suspension containing heat-inactivated proteolytic enzymes might change the environmental conditions and thereby proteolysis.

Data analysis techniques make it possible to quantify the contribution of different proteolytic agents on primary proteolysis. Principal component analysis (PCA) is a data exploratory technique concerned with explaining the variance-covariance structure through a few linear combinations of the original variables. Geometrically speaking, the first principal component (PC) constructs a vector through the multidimensional space that extracts maximum variance in the data set. The second and higher PCs extract maximum remaining variance uncorrelated with previously extracted PCs. Each PC explains a given amount of the variation in the data set and most of the total variation in the data set is often explained by the first few PCs. By studying score plots and how samples are separated along the PCs, it is possible to identify sources of variation in the data set explained by each of the PCs. In this way, it is possible to estimate how much specific sources of variation contribute to the total variation in the data set (6, 7).

The objective of this study was to quantify the contribution of different proteolytic agents to primary proteolysis.