

2001 MAFMA Final Report

Project Title: **Use of Microbial Proteases to Increase Plasmin Activity and Enhance Ripening Rate of Cheese**

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Background:

Cheese manufacturers are very interested in shortening the time required for cheese ripening, due to its high cost. Reducing the ripening time by 2 months of just the Cheddar cheese produced per year in the U.S. would save almost \$450 million/year. Such a reduction in ripening time has been achieved by a 3-4 fold increase in the native milk enzyme, plasmin. This enzyme activity is important in the ripening of numerous cheeses, including Cheddar. However, methods tested previously to increase plasmin levels in cheese are not practical or legally allowed. Our preliminary data in model systems show that such an increase in plasmin activity is possible using certain microbial proteases to help convert inactive plasminogen to active plasmin in milk. Specifically, a protease isolated from *Pseudomonas fluorescens* M3/6 (a dairy source culture) enhanced the activity of plasminogen activator, which converts plasminogen to plasmin.

Objective Summary:

The objective of this study was to determine if proteases produced by select dairy-related microbial cultures (ones consistent with cheese manufacturing) can increase levels of plasmin in milk, to enhance the ripening rate of Cheddar cheese. Acceleration of the ripening time would represent a significant cost savings to industry.

Objective Accomplishments:

Cheese was manufactured on a pilot-plant scale using a traditional Cheddar cheese process and starter culture. In a set of preliminary experiments to ensure reproducibility, plasmin level was measured in the curd and whey fractions at each step of production. The plasmin level, on a wet or dry weight basis, increased in the curd fraction during the cheesemaking process. While the majority of the plasmin was retained in the curd fraction, some plasmin was lost into the whey at each step.

To produce the experimental cheeses, partially purified proteases from *Pseudomonas fluorescens* M3/6 were added to cheese milk. As expected, the *P. fluorescens* M3/6 protease isolated for use in these studies acted as a plasminogen activator enhancer. Experimental cheeses were produced with two levels of protease addition, with level two being twice the amount of level one. Control cheeses without protease addition were made at the same time from the same milk. Post production, the cheeses were vacuum-sealed and stored under refrigerated conditions. This experiment was done in duplicate. The produced cheeses and their whey fractions were analyzed for chemical, physical and sensory properties over a 28-day ripening period.

The control cheeses were reproducible and similar to other pilot plant cheeses in appearance and chemical properties (salt, fat, and protein contents; plasmin levels). However, the experimental cheeses were not, and the plasmin levels did not increase as expected (see unexpected findings below).

Unexpected Findings:

Consistent with the action of the microbial protease on plasminogen activators, the experimental cheeses made with the protease preparation had significantly higher plasminogen activator activity than the control cheese at 0, 14 and 28 days post cheese production. However, this increased plasminogen activator activity did not translate into increased plasminogen and plasmin activity as expected. Levels of plasmin and its zymogen precursor, plasminogen, were significantly less in the experimental cheeses than in the control cheese throughout ripening. These results suggested that the microbial proteases may have been so active as to degrade the plasmin and plasminogen, but experiments to test this hypothesis showed that plasmin activity remained constant in the presence of the microbial protease preparation. Recent results from other experiments in our laboratory suggest that polypeptides produced by microbial protease action on milk proteins can cause inhibition of enzymes in the plasmin enzyme system. This may at least partially explain the results obtained in the current experiment.

The incorporation of the microbial proteases had a detrimental effect on curd production. Due to proteolysis by the microbial proteases, the protease curds were very fragile and broken down. Increased loss of solids to the whey fraction was noted. Experimental curds retained whey and had slightly higher moisture contents than the control cheese. Microbial protease addition caused a significant drop in the pH of the cheese.

Sensory evaluation of the cheeses after 28 days of ripening revealed that the control cheese was preferred. The protease cheeses were extremely bitter, with an aspirin-like after taste. Texture also was affected by the protease incorporation. With the higher moisture content, the experimental ripened cheese was very soft and similar to a cheese spread. Odor from the experimental cheeses was described as “barn-like” and unclean.

Although the proteases had a dramatic effect on the cheese curd, further analysis showed that the proteases were not retained in the curd. General proteolytic activity in the whey fraction was up to ten times greater than in the curd for the protease cheeses.

Practical Impacts of Research Efforts:

The increase in plasmin activity that was observed with protease addition to a model system was not seen in an actual cheese system. The economic advantage of accelerated cheese ripening still exists. Further research could include efforts to incorporate a more purified protease or the partially purified protease at much lower concentrations and also ripen cheese for a shorter time.

It was interesting to note that the microbial proteases were associated with the whey fraction. Since the microbial proteases are extremely heat stable and survive heat processing, this could be important to whey quality. Whey is no longer a discarded by-product of cheesemaking and is now considered a valuable food ingredient. Whey from milk with high microbial counts could present serious quality issues to many finished products.