Milk-Clotting Enzymes. 1. Proteolysis During Cheese Making in Relation to Estimated Losses of Yield[1]

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ABSTRACT

Cheddar cheese was made in beakers (by the process for aged cheese) to compare milk-clotting enzymes for their effect on proteolysis during coagulation, cooking, and cheddaring. All enzymes released more N into whey during cooking and cheddaring than calf rennet except the equal mixture of calf rennet and swine pepsin during cheddaring. Levels of true protein were the same in whey during cooking and during cheddaring with all enzymes, except those from *Bacillus polymyxa* showed proteolysis of whey proteins. Hydrolysis of casein occurred after cooking and during cheddaring with all enzymes including calf rennet. Data were discussed with respect to protein recoveries in cheese from ultrafiltered milk.

Probable reductions in yield were estimated using a factor of 27.06 for converting estimated casein reductions in milk to percentage of yield losses. This factor assumes: the prices of milk fat and cheese are equal; cheddar cheese of the same composition is made of .37 moisture and .017 salt; whey contained .065 fat-free, curd-free solids; milk contained .036 fat and .02464 casein or was standardized to the same casein:fat ratio, considering casein losses; and yield of cheese was 9.834 kg/100 kg milk. Yield losses (± least significant differences), compared with losses of calf rennet, were estimated as: bovine pepsin, −.14 (± .08); equal mixture of calf rennet and swine pepsin, −.07 (± .08); *Mucor pusillus*, −.49 (± .11); *M. miehei* (preparation I), −.63 (± .11); *M. miehei*, (preparation II), −.68 (± .12); *Endothia parasitica*, −1.24 (± .32); *B. polymyxa*, −4.79% (± .32%). These would be economically important losses of concern to cheese makers.

Special attention was given to replications and statistical analyses to reduce least significant differences: quadruplicate Kjeldahl analyses, 6 to 12 replicate trials per enzyme, with comparison among enzymes within days.

Comparison of levels of N in whey during simulated cheese making in beakers is suggested for comparing milk-clotting enzymes for their effect on yield of different varieties of cheese. This could be considered a basic loss of yield; losses of curd and fat in whey would be additional losses.

(Key words: milk-clotting enzymes, cheese yield, proteolysis in whey)

INTRODUCTION

Milk-clotting enzymes are proteolytic. The clotting mechanism includes splitting the Phe-Met bond in κ-casein and release of the macropeptide. Other peptide bonds are split but at a much slower rate (9, 11). Various milk-clotting enzymes vary widely in their proteolytic activity, some being much too proteolytic to be seriously considered for cheese making (9).

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One criterion for assessing enzymes is the clotting:proteolysis ratio; a high ratio would be favorable, chymosin [calf rennet (CR)] being the highest tested to date (11).

Enzymes considered for commercial use, e.g., those derived from *Mucor miehei*, *Mucor pusillus*, *Endothia parasitica*, *Bacillus polymyxa*, swine pepsin (SP) and bovine pepsin (BP), are more proteolytic than chymosin on artificial substrates, such as fractionated casein (11, 19). It is natural to hypothesize that higher proteolytic activity on such substrates would mean higher proteolytic activity in skim milk or in cheese making. Results with such artificial substrates (1, 16) were not indicative of how the enzyme from *M. miehei* hydrolyzed milk in other studies, so such studies must be viewed with some caution in how enzymes act during cheese making.

Information in the literature is not consistent on the proteolytic activity of milk-clotting enzymes, vis-à-vis cheese making. Most have found higher activity on casein with most enzymes than with chymosin (6, 11). Few have found significantly ($P<.05$) lower cheese yields with the enzymes compared with calf rennet (6, 8, 13, 15). One of the possible reasons for this apparent diversity of results is that the design or statistical analysis of some experiments was not such that differences of possible economic importance could be detected (6).

The testing of enzymes for suitability in cheese making is difficult, expensive, and time-consuming. A protocol was developed by Zwaginga and Naudts (20). Rapid screening tests are useful; the one developed by Raadsveld and Klomp (17) evaluates quickly the effect of an enzyme on flavor. A rapid test would be useful to evaluate enzymes for proteolysis and, indirectly, for their effects on yield. It would be desirable if effects on yield of as little as .1% could be detected, because a small difference in yield can equate to relatively large differences in cost of enzyme; this latter point is an important economic consideration.

The purposes of this study were: 1) to detect whether these enzymes affect N (protein) losses in whey during the making of Cheddar cheese, and 2) to estimate the relation between such protein losses in whey and yield losses of cheese. This is a complete report of data given, in part, elsewhere (3, 4).

MATERIALS AND METHODS

Enzymes

The BP and calf rennet (CR) were those used in a previous study (8) and were supplied by Chr. Hansen's, Inc., Milwaukee, WI and Marschall Division of Miles Laboratories, Inc., Madison, WI. The BP had been specially prepared to contain only 9% of chymosin (8). Other enzymes were an equal mixture (CR:SP) of CR and SP (Marschall Division), Fromase (*M. miehei*, Chr. Hansen's Inc.), Marzyme (*M. miehei*, Marschall Division), Emporase (*M. pusillus*, Dairyland Food Laboratories, Inc., Waukesha, WI), Sur-Curd (*E. parasitica*, Chas. Pfizer, Inc., Milwaukee, WI), and *B. polymyxa* (Dairyland Food Laboratories).

Cheese Manufacture In Beakers

Whole milk from the herds at the Central Experimental Farm or from the Animal Research Centre was pasteurized at 63 to 65°C for 30 min and stored overnight at 5°C. To a 1-L beaker was added 600 ml of milk; it was heated to 31°C and transferred to a water bath at 31.5°C, which maintained a temperature of 31°C in the milk. The starter was an equal mixture of strains of *Lactococcus cremoris* ML_1 and R_6 (H. R. Whitehead, New Zealand Dairy Research Institute), grown separately and added at a rate of 2.5%. One hour later, sufficient enzyme was added to give first signs of coagulation in 10 to 12 min. Curd was cut at 2.5 times the coagulation time, e.g., a coagulation of 10 min after adding enzyme resulted in a time from adding to cutting of 25 min; it was first cut horizontally into 6-mm thick plates with a special cutter and then vertically into 6-mm cubes with a thin spatula. Twenty minutes after cutting, the beaker was transferred to a water bath at 39°C, which resulted in the temperature rising to 38°C in about 30 min and maintaining that temperature. Curd was stirred gently at 3-min intervals with a glass rod. pH was measured at room temperature with standard small calomel and glass electrodes (Beckman Instruments, Fullerton, CA)and an expanded-scale pH meter (Model 26) (Radiometer A/S, Copenhagen). To measure pH, curd was macerated in a mortar and pestle, then packed into a 3-cm plastic sleeve (1 cm i.d.), and gently pushed over the glass electrode. A small beaker of saturated KCl formed