ASPECTS OF PROTEOLYSIS IN CHEESE

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Christina June Coker

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ABSTRACT

The purpose of the present study was to elaborate methods for the detailed examination of proteolysis pathways in cheese (reviewed in Chapter 1) and to demonstrate their usefulness. Many techniques, including solvent fractionation, chromatographic separation and electrophoresis have been used previously and were revisited in this study.

Gel electrophoresis can be a powerful technique and was examined in detail. The methods investigated were: 1) a slab gel system using the apparatus of the E-C Apparatus Corporation and a polyacrylamide gel in a Tris-EDTA-borate buffer at alkaline pH and containing urea; 2) a mini-slab gel system using the Bio-Rad mini-Protean II apparatus, a polyacrylamide stacking and resolving gel with a discontinuous (Tris-chloride/Tris-EDTA-borate) buffer system that contained urea; 3) a mini-slab gel system using the Bio-Rad mini-Protean II apparatus, a polyacrylamide stacking and resolving gel and acetic acid-ammonium acetate buffers at acidic pH that contained urea; 4) a mini-slab gel system using the Bio-Rad mini-Protean II apparatus, a polyacrylamide gel with a stacking and resolving gel in Tris-HCl buffers containing sodium dodecyl sulphate (SDS) and a Tris-chloride-glycine electrode buffer.

The mini-slab alkaline urea polyacrylamide gel electrophoresis (PAGE) method was considered to be the most suitable for monitoring the loss of intact casein during cheese ripening. However, SDS-PAGE gave good resolution of para-k-casein, b-lactoglobulin and a-lactalbumin and it could therefore be used for the analysis of cheese in which whey proteins have been incorporated or for monitoring the breakdown of para-k-casein (Chapter 4) in cheese. Two-dimensional PAGE revealed the presence of more bands than were visible using any single method of electrophoresis. Traces of protein were found to lie beneath the a11-casein band and this explained why, even after considerable proteolysis, some a11-casein appeared to remain.

Storing cheese samples in such a way that there is a minimum of further change was examined using several different storage methods and temperatures, including storage as: freeze-dried powder at 4°C in the dark, frozen at -9, -16, -35, -75 and -100°C, and dissolved in 6 M urea solution and stored at 4 and -16°C. The trial ran for 6 months and involved the multiple sampling and detailed analysis of three Cheddar cheeses by reversed phase fast protein liquid chromatography (RP-FPLC) for the water-soluble fraction (WSF) and alkaline urea-PAGE for the protein fraction.
None of the methods used to store the cheese samples was completely satisfactory. Cheese stored at temperatures of -9 and -16°C was unstable, with proteolysis discernible after 66 days. Storage of cheese samples at these temperatures is, therefore, not recommended. Cheese stored at temperatures of -35, -75 and -100°C was unstable after 94 days, although the samples stored at -100°C were more stable. This lack of stability probably arose during thawing as well as during storage of the frozen cheese samples. Storage of freeze-dried samples at 4°C in the dark was equivalent to storing the frozen cheese at -100°C. Storage of samples in alkaline urea sample buffer was better at -16°C than at 4°C but should be for no longer than 1 month.

An indication of the differences to be expected within the normal range of Cheddar cheese was determined using three very similar Cheddar cheeses ripened at 5 and 13°C (Chapter 3, part II). Cheeses ripened at 5°C for 6 months were similar to those ripened at 13°C for 2 months and the proteolytic pathways appeared to the same at both temperatures.

The proteolytic pathways in Cheddar and Mozzarella cheeses, manufactured according to standard protocols, ripened at 13°C and sampled at regular intervals over a six month period were examined using a variety of techniques: total nitrogen (TN), non-protein nitrogen (NPN), water-soluble nitrogen (WSN), alkaline urea-PAGE, low molecular weight (LMW) SDS-PAGE, RP-FPLC and size exclusion high performance liquid chromatography (SE-HPLC). The TN and NPN analyses were done at the time of sampling whereas the other assays were done on samples that had been stored at <-75°C so that they could be analysed simultaneously.

The increase in WSN and NPN was greater in Cheddar cheese than in Mozzarella cheese and reflected the greater microbial enzyme activity in this cheese type.

Alkaline urea-PAGE revealed that there was more $\alpha_{s1}$-casein hydrolysis (with the formation of $\alpha_{s1}$-casein-I) in Cheddar cheese than in Mozzarella cheese, indicating that rennet activity was greater in Cheddar cheese. The presence of peptides believed to be $\beta$-I- ($\beta$-casein f1-189/192) and $\beta$-II-casein ($\beta$-casein f1-165) indicated that rennet may have hydrolysed $\beta$-casein. The amount of $\beta$-casein hydrolysis (and $\gamma$-casein formation) was greater in Mozzarella cheese, reflecting the greater plasmin activity in this cheese type. Both LMW SDS-PAGE and SE-HPLC of the whole cheese provided little additional information.

Examination of the WSF of each cheese by PAGE analysis showed that many of the
larger peptides may have been present in both cheese types. The different concentrations of these peptides in each cheese type were consistent with different rennet and plasmin activities and suggested that they may have been products of these enzymes. RP-FPLC and SE-HPLC analysis of the WSF of Cheddar cheese revealed that, although the larger peptides continued to accumulate during ripening, there was also a large increase in the amount of small peptides and amino acids in the cheese. In the Mozzarella cheese, the larger peptides accumulated and there was little evidence of their further hydrolysis to small peptides and amino acids.

The present studies indicate that SE-HPLC using a Toyo-Soda SW 2000 column and a 36% acetonitrile/0.1% trifluoroacetic acid solvent system is a promising new technique that may be useful in determining cheese type and maturity and in relating changes in the molecular weight distribution of the peptides to changes in the textural, functional and flavour characteristics of cheese.

It was concluded that the results are consistent with the concept that differences in the manufacture of Cheddar and Mozzarella cheeses result in the formation of two cheeses, each with different amounts of similar enzymes (rennet, plasmin, and the enzymes of the starter and non-starter lactic acid bacteria), and that these differences in enzyme concentration, combined with the modifying effect of pH, temperature, moisture content and S/M, result in different enzyme activities and patterns of proteolysis in the two types of cheese and these, in turn, result in cheeses with different functional properties.
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