INFLUENCE OF COMMERCIAL PROTEASES ON THE PROTEOLYSIS OF ENZYME MODIFIED CHEESE

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Abstract

The influence of four commercial proteases, Protease A, Protease B, Protease C and a two enzyme blend Protease DE, on proteolysis in an enzyme modified cheese (EMC) base has been investigated. Also, a series of preliminary experiments to determine the basic characteristics of the four enzyme preparations in buffer systems has been undertaken.

Generally, the exopeptidase activity of the four enzyme preparations was more stable than the endopeptidase activity of the preparations. The highest enzyme activity for all preparations was given at pH 6.5 and Protease B was found to be sensitive to chelating agents. In addition, Protease B was found to contain at least two exopeptidases.

Residual protease activities in EMC using a 55% moisture cheese base were found to be 0.005%, 0.009%, 0.007% and 0.004% (w/v) for Protease A, Protease B, Protease C and Protease DE, respectively, following inactivation by heating at 95°C heating for 30 minutes.

Under the same incubation conditions (0.15% enzyme at 40°C for 24 h), Protease DE gave greater proteolysis than the three other enzymes and Protease B was the weakest protease. EMC digestion with a combination of proteases was different from that obtained with individual proteases. The combinations of Protease A/Protease C, Protease DE/Protease C, Protease B/Protease C and Protease DE/Protease A showed that the higher the proportion of the former protease in the combinations, the higher the amounts of total amino acids produced in the EMC. The combinations of Protease A/Protease B and Protease B/Protease DE gave greater amounts of total amino acids with the ratio of each enzyme close to 50:50 than with the individual enzymes. With respect to the molecular mass distribution of peptides in the various EMC digestions, Protease DE produced the greatest amount of peptides of 3 or fewer residues and Protease C gave the greatest amount of more medium sized peptides with 11-20 residues. Compared with Protease C, Protease A was more efficient in giving small peptides, while Protease B gave the lowest levels of medium and small peptides, but a high level of free amino acids.

In sensory testing, Protease DE produced EMC with a strong pungent and astringent flavour, Protease C gave bitterness, Protease A gave a sweet flavour at a low concentration but bitter flavours with a high concentration and Protease B produced more savoury flavour without bitterness.

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Chapter 1. Introduction

Today's consumers prefer food full of flavour, especially in low fat food products. Flavour is one of the critical determinants of cheese quality. Cheese flavour is popular and used widely in the food industry. In Cheddar cheese, flavour development usually requires storage for at least 12 months to reach a characteristic full flavour. The cost of storage space and time is a major factor in the overall production cost.

Some investigators indicate that Cheddar cheese has a complicated flavour development process that results from enzymatic reactions (Moskowitz and Noelck, 1987; Fox, 1989a). Nowadays, a number of cheesemakers are using supplementary enzymes to manage Cheddar flavour development to improve targeting of cheese flavour. There are various methods for achieving the extra flavour boost during manufacturing and accelerated ripening, such as adding enzyme to the starter milk or to the curds as whey is drained. However, cheese-flavoured ingredients can be obtained by making enzyme modified cheese (EMC).

Cheese that has been treated enzymatically to improve the flavour or provide a significant portion of the flavour profile is called enzyme modified cheese (Moskowitz and Noelck, 1987). Specific enzymes are added and, as near as possible, conditions are adjusted to optimal for the production of typical cheese flavours. EMC flavour development can take only a few hours or a few days, compared with traditional aging that may require several months or more (Vafiadis, 1996). In addition, the flavour intensity of EMC is approximately 15-30 times that of natural cheese, and it is able to replace up to 75% of the naturally aged cheese used in foods (Anon, 1989). The advantages of enzyme modified cheese therefore include production of a targeted flavour ingredient, avoiding the use of naturally aged cheese with all its inherent costs and production disadvantages, and a reduction of the overall cheese solid content in food products such as low fat, health-orientated products.

Cheese flavour is derived from proteolysis, lipolysis and lactose breakdown. Proteolysis is the most complex and perhaps the most important biochemical event during natural cheese ripening (Fox, 1989a, 1993; Wilkinson, 1993; Kilcawley *et al.*,

1998). Both lipolysis and proteolysis are important in EMC manufacture. The impact of lipases in EMC has been studied (Gunaratne, 1999) and the current project focuses on the influence of proteases on EMC digestion. Proteolysis is the factor that causes softening of the texture of natural cheese during the early stages of ripening and influences cheese flavour development by the formation of amino acids and peptides. The water soluble fraction of cheese contains considerable concentrations of free amino acids that probably contribute to the background flavour of cheese and serve as substrates for various flavour generating reactions. On the other hand, an imbalance of high molecular weight peptide formation may cause bitter flavours. The production of bitterness is the main problem encountered when most commercial proteinases are used to accelerate cheese ripening. Another critical aspect of EMC technology is controlling enzyme activities. Unless they can be inactivated, enzymes may continue to break down the cheese after the desired flavour is developed and thereby produce undesirable flavour. Residual enzyme activity in EMC can also hydrolyse components of the final product in which the EMC is an ingredient.

Proteolytic enzyme activities are influenced by parameters such as incubation time, temperature and enzyme concentration. The purpose of this project is to better understand how these parameters impact on the action of four different proteolytic enzymes, namely Protease A, Protease B, Protease C and the two enzyme blend Protease DE (individually or in combination) in terms of the extent of proteolysis and flavour development of EMC cheese base. High performance liquid chromatography, especially gel filtration chromatography and reversed phase chromatography, were used to profile the molecular mass distribution of peptides and the quantities of amino acids in EMC. These profiles will serve as preliminary maps to aid ongoing efforts to achieve the targeted manipulation of EMC flavour.