PERFORMANCE OF THREE COMMERCIAL LIPASES IN A MODEL ENZYME MODIFIED CHEESE SYSTEM

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ABSTRACT

The effects of Amano 'R' (from *Penicillium roqueforti*), Palatase (from *Mucor miehei*) and kid lipase (from kid goat) activity on hydrolysis of triglycerides in a constant enzyme modified cheese (EMC) base have been investigated. The effects of incubation time, temperature, enzyme concentration, pH, water activity (a_w) and salt-in-moisture content on enzyme activity were studied. Under the same conditions (0.15% enzyme, 30°C, 24 h), Palatase and Amano 'R' showed a greater extent of hydrolysis (total free fatty acids) than Kid lipase. The total free fatty acids (FFAs) released by Palatase, Amano 'R' and Kid lipase were 224, 188 and 20.5 mmol FFA. kg EMC.$^{-1}$, respectively. The optimum temperature for hydrolysis by Amano 'R', Palatase and Kid lipase was around 30°C, 55°C and 45°C respectively. Amano 'R' was very heat sensitive, compared to the other two enzymes. Hydrolysis increased with increasing initial pH. The optimum pH's determined for Amano 'R', Palatase and Kid lipase were 7.5, 8.0 and 5.5 respectively. Enzyme activity decreased slightly as water activity decreased and salt-in-moisture content increased, for all enzymes. The incubation time and enzyme concentration showed the expected trend.

At all process conditions, a high percentage (about 55%) of the fatty acids released by kid lipase was butyric acid. Both Palatase and Amano 'R' were relatively non selective and released large amounts of all fatty acids. Compared to Palatase, Amano 'R' selectively released a higher percentage of butyric acid (about 15% compared to 10%). Generally, the rate of release of butyric acid was greater at lower incubation temperatures for all enzymes. Also, the percentage of butyric acid release decreased with increasing initial pH for Palatase lipase.

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CHAPTER 1

INTRODUCTION

The use of cheese flavour in various food products is commonly accepted. As a consequence, the cheese industry faces an increasing demand for cheese with high flavour intensity from the prepared food industries. Many prepared food products require sources of cheese such as Cheddar, Swiss, Blue, Romano and others which impart typical flavour characteristics. The main alternatives to the use of natural cheese flavour are high-intensity cheese flavour concentrates, such as enzyme modified cheese (EMCs).

A number of compounds have been identified as being characteristic flavour components of certain natural cheese varieties. These flavours can be produced by procedures such as use of specific enzyme systems. To obtain pure compounds a detailed knowledge of the reaction systems is vital (Grueb and Gatfield, 1989) and is probably cost-prohibitive. Therefore, enhancement of the major flavour pathways that occur in natural cheese presently provides the most economic route to the production of intense cheese flavours.

Free fatty acids in cheese can contribute directly to flavour, but threshold values of the individual fatty acids in cheese are not known and are difficult to estimate. Further breakdown of fatty acids and reactions with other components of the maturing cheese are likely to occur, and may contribute to the formation of additional flavour components (Siezen and Van den Berg, 1994). An increase in the amount of free fatty acids in cheese is possible

by inducing lipolysis in the milk or dairy system. This technique is also used during the manufacture of enzyme modified cheese (EMC).

The degree and contribution of lipolysis to cheese flavour varies considerably between cheese varieties (Fox, 1993, Kilcawley, *et al.*, 1998). It is also concluded that for some EMC types, the flavour profile or intensity are proportional to the degree of lipolysis and release of low molecular weight free fatty acids (FFAs), as with Romano or Provolone type EMCs.

The effect of lipase on cheese flavour formation has been evaluated by a number of researchers (Dziezak, 1986). It is reported that various lipases could be selected to give the intensity of flavour required in the final product. A wide range of lipases are commercially available from a number of sources, mainly animal and microbial. The correct choice of lipase is extremely important since the FFA and flavour profiles generated vary significantly with the type of lipase used (Kilara, 1985). The flavour compounds, and their relative concentrations may also vary, depending on the conditions used to manufacture the product (Moskowitz and Noelck, 1987).

The objective of this study is to develop and collect information on the technology of controlled lipolysis in EMC production using commercially available food grade lipases. The plan is to characterise Amano 'R', Palatase and Kid lipase effects on a constant cheese substrate (EMC base) in terms of the amounts of fatty acid (FA) released by hydrolysis and the percentages of FAs released, expressed as a percentage of the total of that particular FA bound initially in the milk triglyceride, and as a percentage of the total free fatty acids (FFAs).

Total free fatty acids, expressed as mmoles per kg of EMC base, indicates the concentration of free fatty acids accumulating in the model EMC system. Total free fatty acid concentration is an indicator of EMC flavour intensity. The percentage of fatty acid released expressed as a percentage of the total amount of that particular fatty acid originally esterified in the milk fat triglyceride, indicates the "extent" of the lipase reaction (degree of hydrolysis). The percentage of fatty acid released, expressed as a percentage of the total free fatty acids released, indicates the specificity of a particular lipase. Lipase specificity impacts on the flavour profile of an EMC.

The enzyme activities will be investigated at different processing variables, namely the incubation time, temperature, enzyme concentration, pH, water activity and salt-in-moisture to find out their impact on hydrolysis of triglycerides in EMC base. Where reaction conditions are described as being optimal, this refers to the conditions required to achieve maximum enzyme activity.

The EMC base to be used is an immature processed cheese, which has undergone proteolysis but not lipolysis. A better understanding of the influence of process variables on the lipase activity and the resultant free fatty acid (FFA) profiles will provide useful information on EMC manufacture and suggest ways to produce new varieties of enzyme modified cheese.