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Understanding and exploiting the biogenesis of cheese flavour



Key external stakeholders:

Cheese producers, dairy industry, food manufacturers

Practical implications for stakeholders:

The project investigated mechanisms to control and accelerate Cheddar cheese flavour and the information generated within this project has significantly enhanced the understanding of flavour generation in Cheddar cheese which can also be applied to many other cheese varieties.

- This research has provided invaluable information on a range of factors that influence cheese quality and rate of cheese ripening.
- Factors which impact on the activity of chymosin were elucidated.
- Mechanisms to enhance lipolysis in Cheddar cheese were identified.
- The performance of commercial accelerating ripening agents in Cheddar cheese were evaluated.
- Microfluidization was identified as a practical method to create specific populations of attenuated lactic acid bacteria for use as adjuncts in cheese production.
- Microfluidization was identified as a suitable method to create food grade liposomes which can be used to deliver exogenous enzymes in cheese curd, with minimum losses to the whey
- Factors governing the encapsulation efficiency of enzymes and cell free extracts in liposomes were determined.

Main results:

This project investigated a range of factors that influence the ripening of Cheddar cheese. The major areas of focus were enhancing lipolysis and proteolysis through addition of exogenous enzymes, use of adjunct cultures and process manipulation of cheesemilk to control and accelerate cheese ripening.

Opportunity / Benefit:

The capacity and expertise generated within this project is readily available and can be utilized for specific cheese applications by contacting the relevant researchers involved.

Collaborating Institutions:

University College Cork; University of Limerick; Institute of Chemical Technology Prague; McGill University

Teagasc project team:

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1. Project background:

Cheese ripening has been the object of considerable research because of its commercial significance. However, key aspects of the biochemical factors involved in cheese ripening and the mechanisms of incorporation, distribution and release of enzymes in curd that influence flavour development have not been fully elucidated. In this project the impact of a range of factors and process variables impacting on the flavour development of Cheddar cheese were investigated.

2. Questions addressed by the project:

- What are the factors influencing the incorporation, retention and distribution of enzymes in cheese and how this affects the subsequent ripening?
- What are the factors impacting on chymosin retention in cheese curd?
- Can lipolysis be increased in Cheddar cheese to enhance flavour development, without adversely impacting on the overall cheese quality?
- How effective are commercially available Accelerating Ripening agents for Cheddar cheese?
- Is microfluidization a practical method to attenuate lactic acid bacteria and how effective is it?
- How is bacterial cell lysis and enzyme release influenced by attenuation using microfluidization?
- Can microfluidization be used to encapsulate exogenous enzymes in liposomes?
- Has encapsulation of enzymes or cell free extracts in liposomes potential in accelerating cheese ripening?

3. The experimental studies:

Two separate methodologies were assessed to enhance the level of lipolysis in Cheddar cheese to alter its sensory character without adversely impacting on quality: Low pressure homogenization (<100 bar) of the raw milk to activate the indigenous lipoprotein lipase (LPL) and the addition of phospholipases.

Chymosin has a significantly role in cheese manufacture and factors that influence the retention of chymosin in curd were assessed; casein concentration of the milk, pH at renneting, pH at whey drainage, ionic strength and the quantity chymosin added. A novel coagulant, camel chymosin with higher milk coagulating properties than bovine chymosin was also assessed in Cheddar production.

Mechanisms to accelerate cheese ripening without adversely impacting on quality or shelf life remain a major objective for large scale Cheddar producers. The performance of three commercial Cheddar cheese accelerated ripening systems (Accelase AM317 & Accelase AHC50, Danisco, France, & Accelerzyme, CPG, DSM, The Netherlands) were independently assessed.

Adjunct cultures are widely utilized in large scale cheese production to enhance, modulate or control flavour. In this study the performance of a novel autolytic culture was assessed as an adjunct culture in Cheddar cheese.

The potential of microfluidization, a high pressure processing technique was evaluated as a method to attenuate lactic acid bacteria for the production of adjunct cultures. These adjunct cultures were then assessed as mechanisms to accelerate Cheddar cheese ripening.

Microfluidization was evaluated as a mechanism to encapsulate exogenous enzymes in food grade liposomes for use as a mechanism to deliver additional proteolytic activity in cheese production. The factors influencing the encapsulation efficiency were also determined.

4. Main results:

Lipolysis in Cheddar cheese can be subtly enhanced by low pressure homogenization of cheesemilk or addition of phospholipases which subsequently impacts on the sensory character of the cheese.

The addition of phospholipases to cheese milk can also be used to increase lysis of starter bacteria, which can enhance proteolysis and thus accelerate cheese ripening.

The retention of residual chymosin in cheese curd can be enhanced through increasing the ionic strength of milk, decreasing the pH at renneting and reducing the pH at whey drainage below pH 5.7.

Residual chymosin activity increases significantly with decreasing average casein micelle size.

The potential of highly autolytic bacteria as adjunct cultures in Cheddar cheese for accelerated cheese ripening is limited by significant losses of key intracellular enzymes to the whey during production and by instability of these same key enzymes within the curd.

Commercial accelerating ripening agents will accelerate proteolysis and thus reduce ripening times, however in some cases shelf life maybe adversely impacted due to softer/shorter texture.

Microfluidization was identified as an excellent practical food grade technique to attenuate lactic acid bacteria for use as adjunct cultures in cheese making. These adjunct cultures have potential to control flavour development in cheese.

Microfluidization was identified as a useful method to encapsulate enzymes or cell free extracts in liposomes. Encapsulation efficiency in liposomes was dependent upon the enzyme type, its purity, the composition of phospholipids, the microfluidization pressure used, the microfluidization chamber type and the number of passes through the chamber.

Liposomes produced from natural food grade phospholipids were shown to be suitable vectors for the delivery of exogenous enzymes into cheese curd to positively influence cheese ripening.

A recombinant aminopeptidase as a free enzyme or encapsulated was shown to enhance proteolysis in Cheddar cheese and in enzyme-modified cheese.

5. Opportunity/Benefit:

This project has identified a number of mechanisms that can be used to alter or accelerate Cheddar cheese ripening that would also be applicable to many other cheese varieties. The technologies and expertise to achieve this are readily available for utilization by industry.

6. Dissemination:

This research was presented at several conference and Relay workshops; including Enzymes in Food Applications, Cheese Research Highlights 2000-10 and Cheddar Cheese Research: yield, quality and consistency.

Main publications:

- Nongonierma, A.B., Abrlova, M., Fenelon, M.A and Kilcawley, K.N. (2009) Evaluation of three food grade Proliposome preparations to encapsulate a commercial enzyme extract of Debritate DBP20 using microfluidization. *Journal of Agricultural and Food Chemistry* **57**, 3291-3297.
- Azarnia, S., Lee, B.H., St-Gelais, D., Kilcawley, K., and Noroozi, E. (2011). Effect of free or encapsulated recombinant aminopeptidase on proteolytic indices and sensory characteristics of Cheddar cheese. *LWT - Food Science and Technology* **44**: 570-575
- Kilcawley, K., Nongonierma, A.B., Hannon, J.H., Doolan, I.A, and Wilkinson, M.G (2012). Evaluation of commercial enzyme systems to accelerate Cheddar cheese ripening. *International Dairy Journal*. In Press.

Popular publications:

- Kilcawley, K. (2007). Cheese: Unravelling flavour and aroma. *TResearch* **2**: (2), pages 18-20.
- Wilkinson, M.G. and Kilcawley, K. (2007). Carbohydrate metabolism and cheese flavour development. In: *Improving the flavour of cheese*, ed. B.C. Weimer, Woodhead Publishing, Cambridge, UK, CRC Press US. pp. 55-69
- Kilcawley K.N (2009). Determination of lipolysis, in *Handbook of Dairy Food Analysis*, eds, F Toldra and L Nollet, CRC Press USA., pp 432-452.

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