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Model System for the Production of Enzyme Modified Cheese (EMC) Flavours

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A model system was developed and successfully used to generate a range of Cheddar-type enzyme-modified cheese (EMC) flavours from base dairy ingredients using selected commercial enzymes and lactic acid bacteria.
This process can be used at laboratory scale to screen enzymes and lactic acid bacteria for their flavour generating capacity, or scaled up to produce EMC products. The process is easily modified to create a myriad of natural cheese-type flavours.

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Optimising Flavour Formation in Enzyme Mediated Dairy Flavour Ingredient Systems

(Model System for the Production of Enzyme Modified Cheese [EMC] Flavours)

Armis No. 4771

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Summary and Conclusions

Natural cheese flavour ingredients, in the form of enzyme modified cheeses (EMCs), are widely used in the convenience food industry and can provide high volume added opportunities for the cheese industry.

Many EMCs are produced using commercial enzyme preparations and previous studies have indicated that they contain side activities in addition to their stated main activity (see DPRC Report No.10). Therefore, it is critical that the exact enzyme complement of these preparations are known before they can be used to produce EMC of specific requirements on a consistent basis.

The scientific basis of rapid enzyme mediated flavour formation in the production of EMCs is not fully understood. Consequently this knowledge gap is a major obstacle in the development of high value cheese flavour ingredients.

Hence, a major objective of this project was to deepen the scientific understanding of flavour formation with a view to the production of natural enzyme-mediated dairy flavour ingredients with commercial potential.

The ultimate aim was to develop the technology to produce customised high value dairy flavour ingredients in an optimised process.

Main Conclusions and Achievements

* *A model system was developed to evaluate the potential of commercial enzymes and lactic acid bacteria to produce customised Cheddar flavour EMC products in an optimised environment.*

The importance of selected hydrolysis, compositional and production parameters in the production of EMC were identified.

* *The model EMC system can be used to produce a range of differing cheese flavoured EMC products from base dairy ingredients comparable to commercial products utilising combinations of commercial enzymes and/or lactic acid bacteria.*

* *Manipulation of compositional, proteolytic and lipolytic parameters, and the inclusion of glutamic acid can be used to develop distinctly different products for specific applications.*

Research and Results

Commercial Enzyme Study

A comprehensive study on the enzyme activities of a range of food grade commercial enzymes was completed and reported in *DPRC Report No.10*. The study highlighted the necessity for more detailed information on the specificity of the main enzyme component and for information relating to side enzyme activities from suppliers.

Development of an EMC Model System

In this study the main component of the substrate used for EMC production consisted of a high moisture, high pH, rennet cheese curd, produced from full fat milk without starter culture. This rennet curd was blended with anhydrous butterfat, de-ionised water, salt and emulsifying salts in a Stephan cooker until homogeneous and subsequently heat-treated at 80…C for 20 min. Rennet curd was chosen as it is cheap to produce and any flavour subsequently developed in the production processes could be directly attributed to added enzymes rather than indigenous enzymes in the cheese curd.

Anhydrous butterfat was added to provide more substrate for lipolysis as previous studies have shown that the level of lipolysis in EMCs is linked to flavour intensity.

In the process, enzymes and/or lactic acid bacteria are added to the substrate in a reaction vessel with constant agitation under controlled temperatures for a fixed period, after which the product is batch heat-treated to at least 80…C for 20 min. This system can be used at laboratory scale (1 - 2 kg) or production scale (1000 kg). The choice of processing conditions depends upon the enzymes or starter cultures added, and the flavour required.

A temperature of 45…C and a processing time of 24 h period was used.

Investigating the Flavour Potential of Combinations of Commercial Enzymes in a Model System

The model system developed was used to investigate the potential of a selected number of commercial enzyme preparations to produce

good Cheddar-type EMC flavour in a two step process: Step 1 consisting of hydrolysis using only proteolytic enzymes, followed by Step 2 which involves treatment with lipase preparations.

The products produced were compared to a commercial EMC product previously shown to have good Chedder-type flavours by a trained descriptive sensory panel. All products were assessed for compositional, proteolytic, lipolytic and sensory properties; trials were carried out in triplicate.

Four EMC products (EMC 1 - 4) were produced using only proteolytic enzymes, and in the second step one of these products was subsequently used as the substrate to produce four EMC final products (EMC 5 - 8) using lipase preparations. These final products were compared to the target commercial EMC product.

Step 1: Preliminary proteolysed EMC products (EMC 1 - 4)

Four commercial proteinase preparations were used which were derived from *Bacillus subtilis*, *Aspergillus oryzae* or *Aspergillus niger*. These enzymes were selected on the basis of their differing activity towards sodium caseinate. One of the preparations, Glutaminase F contained glutaminase activity, which produces free glutamic acid, a natural flavour enhancer, known to be useful in EMC production.

A peptidase preparation derived from *Aspergillus oryzae* and *Lactococcus lactis* was added to each EMC product (1 - 4) to aid flavour development and control bitterness.

A description of each enzyme preparation and the dose rates used are given in *Table 1*.

Table 1: Description and dose rates of commercial proteolytic enzymes used in the production of preliminary EMC products in Step 1.

	Proteinase Preparation (Conc. w/w)	+	Peptidase Preparation (Conc. w/w)
EMC 1	Glutaminase F* (0.026% w/w)	+	Debritase DBP20** (0.3% w/w)
EMC 2	Protease A Amano 6* (0.005% w/w)	+	Debritase DBP20** (0.3% w/w)
EMC 3	Bioprotease A Conc*** (0.50% w/w)	+	Debritase DBP20** (0.3% w/w)
EMC 4	Neutrase 0.5L**** (0.01%w/w)	+	Debritase DBP20** (0.3% w/w)

* *Amano Enzyme Europe Ltd, Chipping Norton, Oxfordshire OX7 5SR, UK*
** *Rhodia-Foods UK, Stockport, Cheshire SK6 1PQ, UK*
*** *Quest International, Carrigaline, Co. Cork, Ireland*
**** *Novozymes A/S, Bagsvaerd, Denmark*

Table 2: Primary and secondary proteolytic levels in EMC products 1 - 4 and the commercial target EMC.

Product	WSN (% TN) Av	WSN (% TN) sd	PTA-N (% TN) Av	PTA-N (% TN) sd	Total FAA (ug/g) Av	Total FAA (ug/g) sd
EMC 1	58.90	0.13	12.20	0.30	10165	85
EMC 2	53.10	0.19	11.30	0.12	10908	1067
EMC 3	54.80	5.08	7.30	0.00	9449	2723
EMC 4	55.40	0.40	6.80	0.19	7548	263
Commercial EMC	52.07	1.90	21.39	1.40	23496	308

WSN = pH 4.6 water soluble nitrogen
PTA-N = 5% Phosphotungstic acid soluble nitrogen
FAA = Free amino acids

Only slight differences were found in terms of composition between EMCs 1 - 4, each having similar levels of primary and secondary proteolysis as measured by WSN (%TN) and PTA - N (%TN) respectively *(Table 2)*. However, differences in free amino acid contents *(Table 2 and Fig. 1)* were apparent between these products and can be directly attributed to differences in the specificity of the proteinases used in their preparation. EMC 1 had the highest level of

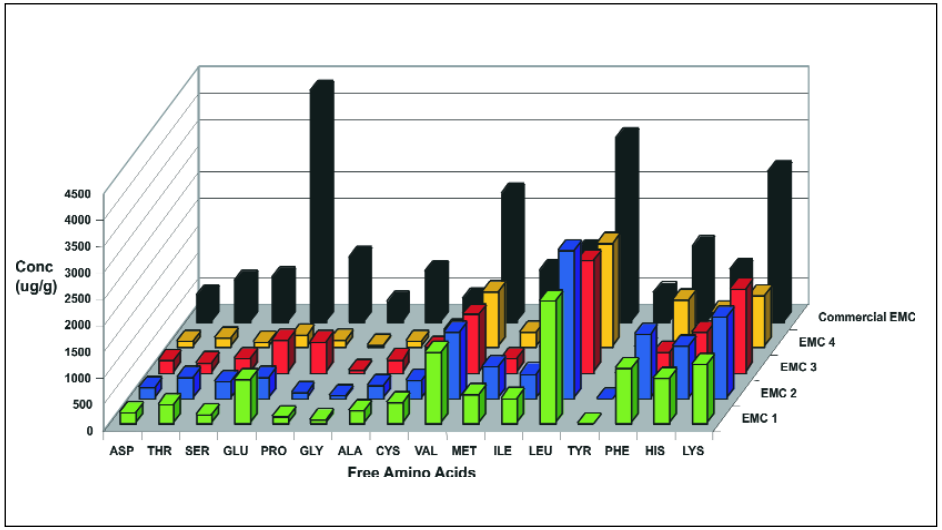

Fig. 1: Free Amino Acid Profiles of EMCs 1 - 4 and the commercial product.

free glutamic acid, confirming the presence of glutaminase activity in the enzyme preparation used in its production. High levels of certain free amino acids (leucine, lysine, valine, phenylalanine and histidine) in EMCs 1 - 4 were due to the action of the peptidase preparation, which had been previously shown to have high levels of general aminopeptidase activity.

Levels of primary proteolysis in EMCs 1 - 4 were similar to the commercial product, but levels of secondary proteolysis were considerably lower. This difference in secondary proteolysis was

most likely due to a number of factors including higher levels of protein in the commercial product, the use of higher levels of peptidase in its manufacture, the use of pre-hydrolysed substrate (mature cheese) or from high levels of free glutamic acid which could have been added exogenously.

Statistical analysis of the sensory results by ranked preference found no significant difference between EMCs 1 - 4; however as EMC 4 had the highest preferred score, it was thus used as a source of substrate for the production of the final EMC products in Step 2 of the process.

Step 2: Final lipolysed EMC products (EMC 5 - 8)

Lipase preparations were selected based on their specificity and were derived from *Penicillium roqueforti*, *Mucor miehei*, *Candida rugosa* or pancreatic tissue. A description of each enzyme preparation and the dose rates used are given in *Table 3*.

The gross compositions of the resulting products were similar.

Levels of proteolysis only increased in EMC 6, which was probably due to the presence of a trypsin-like side proteinase activity, previously found in the lipase preparation used in its production *(Table 4)*. Fatty acid profiles of EMCs 5 - 8 and the commercial product are shown in *Fig. 2*. The amount of free fatty acids in these products appear quite similar despite the fact that lipases of differing specificity were used in their production.

This appears to indicate that lipase specificity is not an issue in systems where very high levels of lipolysis are attained.

Table 3: Description and dose rates of commercial lipolytic enzymes used in the production of final EMC products in Step 2.

EMC 5	Lipomod 338* (0.50% w/w)
EMC 6	Lipomod 299* (0.90% w/w)
EMC 7	Palatase 20,000L** (5.50% w/w)
EMC 8	Lipase AY30 Amano***(0.50% w/w)

* Biocatalysts Ltd, Main Avenue, Pontypridd CF37 5UT, UK
** Novozymes A/S, Bagsvaerd, Denmark
*** Amano Enzyme Europe Ltd, Chipping Norton, Oxfordshire OX7 5SR, UK

Table 4: Proteolytic and lipolytic indices of EMCs.

Product	WSN (% TN)		PTA-N (% TN)		Total FAA (ug/g)		Acid Degree Value		Total FFA (ug/g)	
	Av	sd	Av	sd	Av	sd	Av	sd	Av	sd
EMC 5	50.40	1.44	9.90	0.60	6968	383	20.7	0.7	63290	4416
EMC 6	78.30	3.60	36.10	10.76	9756	578	15.3	0.4	26993	4979
EMC 7	51.20	1.47	10.33	0.15	7292	218	22.6	2.1	66445	4002
EMC 8	50.33	0.47	9.33	0.31	7816	359	22.8	0.5	65843	7171
Commercial EMC	52.07	1.90	21.39	1.40	23496	308	20.4	0.1	57978	5749

WSN = pH 4.6 water soluble nitrogen
PTA-N = 5% Phosphotungstic acid soluble nitrogen
FAA = Free amino acids
FFA = Free fatty acids ($C_{4:0}$ - $C_{18:3}$)

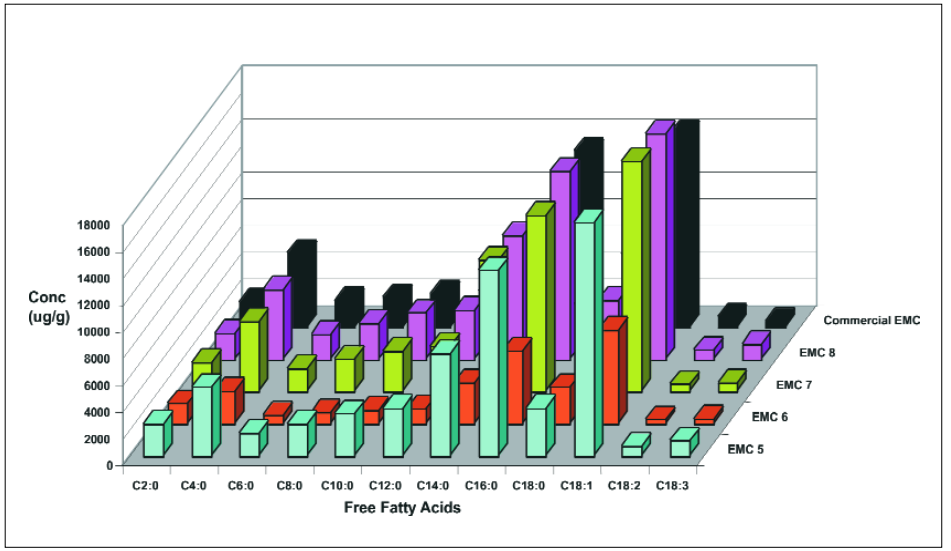

Fig. 2: Free Fatty Acid Profiles of EMCs 5 - 8 and the commercial product.

Statistical analysis of the sensory results by ranked preference found no significant difference between preference of these products and the commercial Cheddar-type EMC.

This result appears to indicate that the overall sensory perception of these products was quite similar indicating that they are comparable to the commercial product.

Detailed descriptive sensory analysis found that EMCs 5, 7 and 8 were the most similar and this could be attributed to the fact that their compositional, proteolytic and lipolytic parameters were also the most similar. EMC 6 was distinguished from all other products and was most likely due to its different levels of proteolysis and lipolysis. The commercial product was distinguished from EMCs 5, 7 and 8 which appeared to be due to differences in composition and secondary proteolysis and levels of free glutamic acid as their lipolytic profiles were quite similar.

This work has shown that a model EMC system can be used to produce different Cheddar-type EMCs comparable to commercial products from base dairy substrates. Differences in aroma and flavour identified between these products appears to be related to differences in defined compositional, proteolytic, lipolytic parameters and levels of free glutamic acid.

Assessing the Potential to Develop EMC Utilising Commercial Enzymes and Lactic Acid Bacteria in a Model System

Commercial EMCs have some limitations which preclude their use in some processed consumer foods.

These limitations generally relate to inconsistent flavour development and an inability to mimic natural cheese flavours. To overcome these problems it was decided to investigate the potential of utilising lactic acid starter bacteria cultures used in natural cheese production for EMC production, since *Lactobacilli* are a good source of important flavour producing enzymes.

In this study a strain of *Lactobacillus helveticus* (DPC 4571) was chosen as previous work has shown that it is autolytic and accelerated natural Cheddar cheese ripening. The objective of this study was to investigate the potential of viable and heat-shocked cells (heat shocking causes lysis and inactivates the cells lactose fermenting ability without affecting residual enzyme activity) of *Lactobacillus helveticus* DPC 4571 in the production of Cheddar-flavoured EMC.

Like the previous study a two step approach was taken. Using the model system the substrate was hydrolysed by two commercial

enzyme preparations Savourase RST100 (Rhodia-Foods UK) and Lipomod 338 (Biocatalysts, UK) for 6 h at 45…C and at 0.42% w/v and 0.11% w/v, respectively. After heat treatment at 80…C for 30 min, one batch was inoculated with viable (10^{10} cfu/ml) cells of *Lb. helveticus*, while a second was inoculated with an equivalent amount of heat-shocked cells (optimum 69…C for 25 s) and incubated for a further 24 h at 37…C. The reaction was terminated by heat treatment at 80…C for 30 min. This experiment was carried out in triplicate and samples were taken for analysis at 0, 8, 16 and 24 h.

All samples had essentially similar compositions, except for a more pronounced reduction in pH in the product produced using viable cells. In general, proteolysis increased in both products, with a slightly higher level in the heat-shocked cells, presumably due to early release of intracellular enzymes. Levels of volatile short chain free fatty acids ($C_{4:0}$ and $C_{6:0}$) remained constant in the viable cell product, but increased slightly in the heat-shocked product. Some differences in the volatile flavour profiles as measured by GC/MS were also noted between both products.

Sensory evaluation on the basis of ranked preference showed an increase in acceptability for heat-shocked product over the viable cell product. A decrease in sensory acceptability appeared to correspond with decreasing in pH, which in turn was linked to an increase acetic acid, which became more pronounced in the viable cell product.

The results show that it is possible to produce acceptable intense Cheddar-type EMC flavours using starter cultures, specifically Lb. helveticus *(DPC 4571) in combination with commercial enzymes utilising the model system. This mechanism could be further exploited to produce a range of intensely cheesey-flavoured EMC.*

Publications

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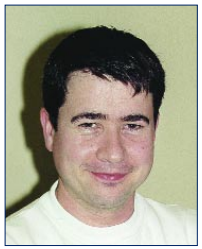
Cover Picture:
Commercial scale LIMITECH in MTL, Moorepark, used in the production of EMCs.



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