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Cheddar enzyme modified cheese:
Influence of protease and lipase on flavour

by

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Submitted in partial fulfilment of the requirements
for the degree MInst Agrar (Food Processing)

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I declare the dissertation herewith submitted for the MInst Agrar (Food Processing) degree at the University of Pretoria, has not been previously submitted by me for a degree at any other University.

ABSTRACT

Cheddar enzyme modified cheese:

Influence of protease and lipase on flavour

by

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Cheddar enzyme modified cheese (EMC) is a highly accelerated ripened cheese product made by the addition of enzymes and other additives to a slurry of curd or cheese and then ripened at elevated temperatures before heat treatment to make the product shelf stable. EMC is used as a cheese replacer in processed food products since it has many times the flavour of normal cheese for the same amount of substrate (curd).

Most EMCs are imported into South Africa since only one company produces EMC locally. No published work exists on producing an EMC in South Africa using local ingredients. To test the quality of an EMC it must be tested with sensory methods but there is no published literature of sensory work done on EMC since it is assumed that the studies that apply to cheese can also be applied to EMCs.

The major objective of this study was to successfully produce an EMC using local dairy ingredients and combinations of commercial lipase and protease preparations and to determine whether any modifications to the internationally established production methods are required. During the incubation, the free fatty acids (FFA) and free amino nitrogen (FAN) were measured as well as the microbiological safety. The EMCs were taste tested in a savoury soup using a taste panel and a standard set of flavour descriptors normally used for cheese characterisation.

The microbiological testing detected *Bacillus cereus* (a known pathogen) that would need to be eliminated before commercial production since hygiene legislature prohibits its occurrence in food.

The FAN increased for those EMCs made with added protease and the FFA increased for those EMCs made with added lipase. The FAN levels were about 10 lower than published values, and when diluted for taste testing the FAN levels were below the flavour threshold and so had no sensory effect. The FFA levels were at the right levels as compared to literature.

Sensory profiles of all EMCs, controls and standards were successfully carried out. Flavour wheel analysis and Principal Component Analysis of the sensory results confirmed that the FAN levels were too low and therefore only the FFA effects were evaluated during taste testing. The low FAN levels were caused by the recommended dosage of protease being too low. The development of good cheese flavour with no protease effects confirms that lipase effects are the most important in EMCs in contrast to cheese where protease effects are the most important.

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UITTREKSEL

Cheddar ensiem gemodifiseerde kaas:

Die invloed van protease en lipase op geur

deur

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Cheddar ensiem gemodifiseerde kaas (EGK) is 'n kaas produk waar die ryp-maak proses versnel word deur die byvoeging van ensieme en ander bymiddels by die wrongel-smeersel. Die produk is dan ryp gemaak onder verhoogde temperature. Die produk ondergaan hitte verwerking om dit rak stabiel te maak. EGK is gebruik as 'n kaas vervanger in vervaardiging van voedsel produkte omdat dit meer geur het as normale kaas vir dieselfde hoeveelheid substraat. In ander woorde, dit is meer gekonsentreerd as gewone kaas.

Die meeste EGK's word ingevoer omdat daar net een plaaslike vervaardiger is. Daar is geen gepubliseerde werk oor die vervaardiging van EGK's in Suid-Afrika met die gebruik van plaaslike bestanddele nie. Die kwaliteit van 'n EGK word getoets deur middel van sensoriese toetse. Daar is geen rekord van spesifieke sensoriese werk uitgevoer op EGK, want dit word aangeneem dat die studies wat op kaas toegepas word kan ook op EGK's toegepas word.

Die hoofsaaklike doel van hierdie projek was om suksesvol 'n EGK te vervaardig deur gebruik te maak van plaaslike suiwel bestanddele en kommersieel beskikbare lipase en protease en om dan te bepaal of die internasionale produksie metode aangepas moet word. Gedurende die ontwikkeling proses is die vry vettige suur (VVS) en vry amino stikstof (VAS) hoeveelhede gemeet. Die

mikrobiologiese veiligheid van die produk is ook getoets. Die EGK's is sensories gevalueer in 'n sop basis deur 'n paneel opgelei mense wat 'n standaard reeks van beskrywende woorde gebruik om die produkte te beskryf. Die reeks beskrywende woorde is dieselfde as wat gewoonlik gebruik word om kaas te beskryf.

Die mikrobiologiese toetse het die teenwoordigheid van "Bacillus cereus", 'n bekende patogenese organisme, ontdek. Volgens die gesondheids wet mag hierdie organisme glad nie teenwoordig wees in voedsel nie. Die kommersiele vervaardigings proses van EGK's sal aangepas moet word om te verseker dat die bogenoemde organisme verwyder word.

Die VAS het vermeerder in die EGK's wat met bygevoegde protease vervaardig is en die VVS het vermeerder vir die EGK's vervaardig met bygevoegde lipase. Die VAS vlakke aangeteken gedurende hierdie projek, is tien keer laer as die gepubliseerde VAS waardes. Die VAS vlakke aangeteken was lae as die geur drumpel met die gevolgtrekking dat hulle nie 'n sensories effek gehad het nie. Die VVS vlakke wat aangeteken is het goed vergelyk met die gepubliseerde waardes.

Die sensoriese profiel van alle EGK's, kontrole en standaarde is suksesvol uitgevoer en aangeteken . Analise van die sensoriese uitslae het bevestig dat die VAS vlak te laag was en daarom was net die VVS beoordeel gedurende die sensoriese analise. Die lae vlak van VAS is waarskynlik as gevolg van die feit dat die aanbeveelde dosis van protease te laag was. Die vervaardiging van 'n goeie kaas geur met geen protease bevestig dat lipase baie belangrik is in EGK vervaardiging in vergelyking met die vervaardiging van kaas waar protease belangrik is.

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CHAPTER 1 - INTRODUCTION

Cheddar cheese is a popular savoury food flavourant, though its high cost makes it an expensive food ingredient. Cheddar cheese flavour is produced by fatty acids and amino acids, and their reaction products, present in small but specific concentrations. This delicate mix of flavour chemicals is not easily replaced by a blend of pure chemical flavour compounds, unlike some blue cheeses for instance, in which the main flavour can be approximated by a single ketone (Moskowitz & La Belle, 1981). Enzyme modified cheese (EMC) overcomes this problem to a large extent. It is a highly accelerated ripened cheese product, in which the same cheese flavour compounds are created as in normally ripened cheese. EMC is added to foodstuffs as a cheese flavour, and since it is many times stronger than regular cheese, it is added in far lower quantities, and becomes a cheap but effective alternative. EMC is used in a variety of food products from maize snacks to instant sauces.

EMC is manufactured from curd or cheese, and normally ripened in less than 24 h by adding extra enzymes and using elevated ripening temperatures. It is produced either in the form of a slurry or a spray dried powder. The flavour of the EMC is derived from the breakdown products of proteins and lipids and their interactions. The decompositions and reactions occurring in naturally ripened cheese, are not all accelerated at the same rate, so that the end product has a flavour similar to normal Cheddar but is not balanced. This is not a problem, as long as it is only used to add a cheesy flavour to a food product.

In South Africa, EMC is used in products such as snack dustings for extruded maize snacks though it is often omitted from formulations due to its high price (pers. comm. - R. Ponquett, CEO FlavourCraft, 1999). EMC has a high price locally because it is imported from countries such as the United Kingdom, with whom South Africa has a poor exchange rate. There is a need in South Africa for cheaper EMC, made locally and using local ingredients. Milk and cheese curd are readily available, so is the relatively simple technology to produce EMC. However, the enzymes required to produce EMC, still have to be imported. So far, Cerevac is the only South African company that produces EMC commercially, but its recipes and methods are secret. Other companies wanting to produce an EMC would need to learn the technology for themselves. As such, no scientific literature exists on the feasibility of producing an EMC in South Africa.

The chemistry of EMC flavour production is complex and is based primarily on the enzymes and additives used during production. From the large body of literature the best additives can be ascertained, though which and how much enzyme to use is not so clear. Takafuji (1993) isolated, extracted and purified his own enzymes for EMC production but specialised enzyme preparations are available that eliminates these steps. The enzyme preparations normally come with recommended usage levels but this means that typically a number of trials will be needed to find the optimum usage levels. These levels can be determined using parameters such as free fatty acids (FFA) and free amino nitrogen (FAN). FFA and FAN are commonly used since they give a measurement of the degree of protein and lipid breakdown which, together with their reaction products, are the basis for cheese flavour.

Much work has been done on what contributes to flavour in cheese (e.g. Kristoffersen, Mikolajcik & Gould, 1967; Law, 1984; Fox, Law, McSweeney & Wallace, 1993). However, little or no sensory work has been done on EMC. This may be due to the assumption that since EMC is a cheese replacer the sensory studies used for cheese would apply to an EMC. This is not necessarily the case. Firstly, EMC may not have a balanced flavour which means that sensory studies on cheese may not be applicable to EMC. Further, while sensory testing can be done on cheese, EMC must be evaluated in a food base, since that is how it will be ultimately consumed. Also, it appears that protein breakdown products are the most important in Cheddar cheese flavour (Fox, O'Connor, McSweeney, Guinee & O'Brien, 1996), whereas in a Cheddar EMC the lipid breakdown products are the most important (BioCatalysts, 1996).

1.1 STATEMENT OF THE PROBLEM

EMC is an expensive imported material that should be cheaper to produce using local ingredients yet no published work exists on producing an EMC in South Africa. EMCs also need to be evaluated using taste panels but there are no published studies of sensory work done on EMC.

1.2 OBJECTIVES

1. To produce an EMC using local dairy ingredients and commercial lipase and protease preparations.
2. To measure the change in FFA and FAN content as a function of incubation time during EMC production. To evaluate whether the manufacturer recommended enzyme concentrations and incubation times are optimum for the available substrate and conditions.
3. To evaluate the sensory properties of different batches of EMC made with combinations of lipase and protease.
4. To evaluate whether either proteolysis or lipolysis is the major contributor to flavour production in an EMC.

CHAPTER 2 - LITERATURE REVIEW

2.1 INTRODUCTION

Enzyme-modified cheese (EMC) is a powder or paste derived from cheese or curd using enzymes, and is used as a cheese replacer in certain foods such as processed cheese, cheese sauces, spaghetti sauces, soups and dips (Sutherland, 1991). EMC may replace 20 to 50 times their weight of natural cheese in a food product and are cheaper to produce than cheese (Fox, 1998). Hence their primary reason for production is economy, since cheese is an expensive food ingredient (Main, 1991). EMC provides the food manufacturer with a product that is cost effective, has a natural flavour and is also nutritious as compared to non-nutritive pure chemical-based flavours (Moskowitz & Noelck, 1987). In the United States, EMC has GRAS status (generally regarded as safe) and as such can be added to a variety of foods (Freund, 1995).

From Table 1 it can be seen how Cheddar EMC fits into the variety of Cheddar cheese products available and that EMC is valued for its flavour rather than its texture and flavour as in other cheese products. EMC is a form of accelerated ripened cheese, ripened to the point where the enzymatic breakdown becomes excessive and texture quality is lost (Fox, 1998). Due to this loss of texture quality EMC is produced either as a paste or a spray-dried powder (Moskowitz & Noelck, 1987). Manufacturing the EMC as a paste also increases the available area for enzymolysis.

Henceforth, unless mentioned to the contrary, any reference to cheese or EMC should be taken as referring to Cheddar cheese and Cheddar EMC respectively.

2.2 CHEDDAR CHEESE, IT'S HISTORY AND RELEVANCE TO THE FOOD INDUSTRY

Cheese has been known since antiquity, is mentioned in The Bible (2 Samuel 17:29, Job 10:10) and is believed to have originated in the "Fertile Crescent" between the Tigris and Euphrates rivers in Iraq, some 8000 years ago. There are over 500 varieties of cheeses available world-wide with new varieties being discovered/created every year (Fox *et al.*, 1996). No other cheese is as well known as Cheddar cheese, which originated in the town of Cheddar in Somerset, England. In Elizabethan

Table 1 Comparison of different types of Cheddar cheese products

Product	Process used	Importance
Cheese	Traditional cheese production.	Good as a savoury flavour, used "as is" or in a variety of products (Sutherland, 1991).
Accelerated ripened cheese	Traditional cheese production with added enzymes or elevated maturation temperatures (Fox *et al.*, 1996).	Reduced maturation time, which reduces inventory costs (Kilara, 1985). Must taste the same as traditional cheese.
Processed cheese	Cheese processed by addition of emulsifiers to make a smooth "plastic" paste.	Good shelf life and easily incorporated into food products (Mann, 1993).
Cheesebase	Highly accelerated production of liquid cheese products from ultrafiltration concentrated milk in 12-24 hours, then pasteurised.	Simulates flavour of a young cheese. Can be spray dried but low flavour strength (Sutherland, 1991).
Enzyme-Modified Cheese	Curd slurry technique (Kristoffersen *et al.*, 1967; Singh & Kristoffersen, 1971a).	High strength, low-cost product simulates mature cheese flavour with mouthfeel and texture not important. Taste, aroma and flavour important (Moskowitz & Noelck, 1987; West & Pawlett, 1996).

times, Thomas Fuller described Cheddar cheese as "the best and biggest in England" with his one regret being "they were so few and so dear, hardly to be met with save at some great man's table" (Anon, 1991). Cheddar cheese is in high demand for its good savoury flavour (Sutherland, 1991).

Of the estimated 480 million tonnes of milk produced world-wide in 1988, 33% went into cheese resulting in 13.25 million tonnes of cheese with 25% of the cheese being Cheddar or its different varieties (Anon, 1991). In 1991, South Africa's cheese consumption was estimated at 1.8 kg per person per annum, which is far less than the largest consumer, France, where consumption is 22.8 kg per person per annum (International Dairy Federation, 1993).

The Australian Dairy Corporation in 1990 (according to Sutherland, 1991) stated that of the 126 600 tonnes of cheese produced in Australia, 49% was sold "as is" to consumers, 21% was used for making processed cheese and 30% was used in industry and food service sectors for further processing. Hard cheese is used in products like pizzas, cheese sauces, bread topping, sausages, pasta dishes and microwave meals while soft cheese is used in cheesecakes, flans and chilled desserts. This use of cheese in the food processing industry has also resulted in the formulation of

new dairy products e.g. cheese flavour (from enzyme-modified cheese), processed cheese and cream flavour (enzyme-modified butterfat) (Main, 1991).

Cheese has special legislation over and above the standard laws applying to the production of food, which cover amongst other factors, the starter bacteria and enzymes permissible in cheesemaking. The International Dairy Foundation (1990) published a report that summarises the legislation covering enzyme and starter use in many countries. As of the report date, South Africa had no regulations controlling the use of enzymes or starters in cheesemaking.

2.3 CHEDDAR CHEESE FLAVOUR FORMATION

The flavour of cheese has been studied since the beginning of the 20th century but it wasn't until gas chromatography (GC) was invented in the 1950s that the many different volatile chemicals in cheese could be detected (Fox *et al.*, 1996). Once the volatile and non-volatile chemicals that were responsible for flavour and taste could be detected, it needed to be ascertained which of the many chemicals were important for cheese flavour. From this information the key chemicals for some cheese flavours were discovered. Moskowitz & La Belle (1981) stated that the key flavour chemicals for Roquefort cheese are 2-heptanone, 2-nonanone and 2-undecanone. This easy characterisation of blue cheese flavour compounds in general is due to the fact that the main flavour compounds come from the specific fungi used in their production (Vafiadis, 1996). Cheddar cheese on the other hand does not have any characteristic chemical flavour but rather has a flavour profile or fingerprint and Mulder (1952) (according to Aston & Dulley, 1982) called this phenomenon the component balance theory.

Much work has been done on Cheddar cheese flavour with some authors observing that certain chemicals or classes of chemicals such as FFA were of key importance to cheese flavour since they increased with cheese flavour (Singh & Kristoffersen, 1970). Other authors found little or no relationship between FFA and flavour (Reiter, Fryer, Sharpe & Lawrence, 1967) implying that the relationship between FFA and flavour is more complex than a simple proportional one. Fox *et al.* (1996) report that many recent authors have concluded that "the flavour of cheese depends on a weighted concentration ratio of all components present". This statement is similar to the component

balance theory proposed by Mulder 40 years ago, and so we are no closer to finding what the key chemicals are (if any) that cause Cheddar cheese flavour (Delahunty, Crowe & Morrisey, 1996).

Ney (1981), Fox (1989) and Wilkinson (1993) stated that cheese flavour comes from the glycolysis, lipolysis and proteolysis of the carbohydrates, lipids and proteins and in milk with the importance of each process varying between the different varieties of cheeses. Flavour compounds are also formed from inter-reactions between the substrates and products of glycolysis, lipolysis and proteolysis (Sharpe, 1979). Proteolysis is perhaps the most important biochemical event that contributes to Cheddar cheese flavour in contrast to some of the blue cheeses where lipolysis is the key event (Fox & Wallace, 1997). A summary of the main events that take place in Cheddar flavour formation is shown in Figure 1. Each of the different processes will now be discussed individually.

2.3.1 Glycolysis

The glycolytic breakdown of lactose in milk to lactic acid is important for the making of cheese and this is done by starter lactic acid bacteria (LAB) and non-starter LAB (NSLAB) (Fox *et al.*, 1996). The low pH created by the lactic acid, as well as the action of the rennet (typically chymosin) on the caseins, leads to the formation of curd due to the breaking of the colloidal suspension (see the section on proteolysis for more details). Cows milk typically has 5% lactose (Ney, 1981) and during Cheddar curd formation 98% of the lactose (either as lactose or lactic acid) is lost during whey draining resulting in a lactose/lactic acid level in the cheese of 1-1.5% (Fox & Wallace, 1997).

Lactose and lactic acid are the only active flavour compounds in fresh cheese curd since the casein and fats are bland (Ney, 1981). In the glycolytic pathway of lactose metabolism the intermediate compound is pyruvic acid (Ney, 1981) which also gets converted by the LAB and NSLAB to acetic acid, diacetyl, acetaldehyde, ethanol and CO_2 (Ney, 1981; Law, 1984). Lactose is also broken down into its constituent products of galactose and glucose but the levels of these sugars are below the flavour threshold (Ney, 1981). Lactic acid has taste in its own right (Fox, McSweeney & Singh, 1995a), and Kielwiein & Daum (1979) (according to Ney, 1981) stated that there must be more L(+)-lactic acid than D(-)-lactic acid for taste. Fox *et al.* (1995a) disagree with Kielwiein & Daum and stated that the racemization of lactic acid is unimportant from a taste point of view.

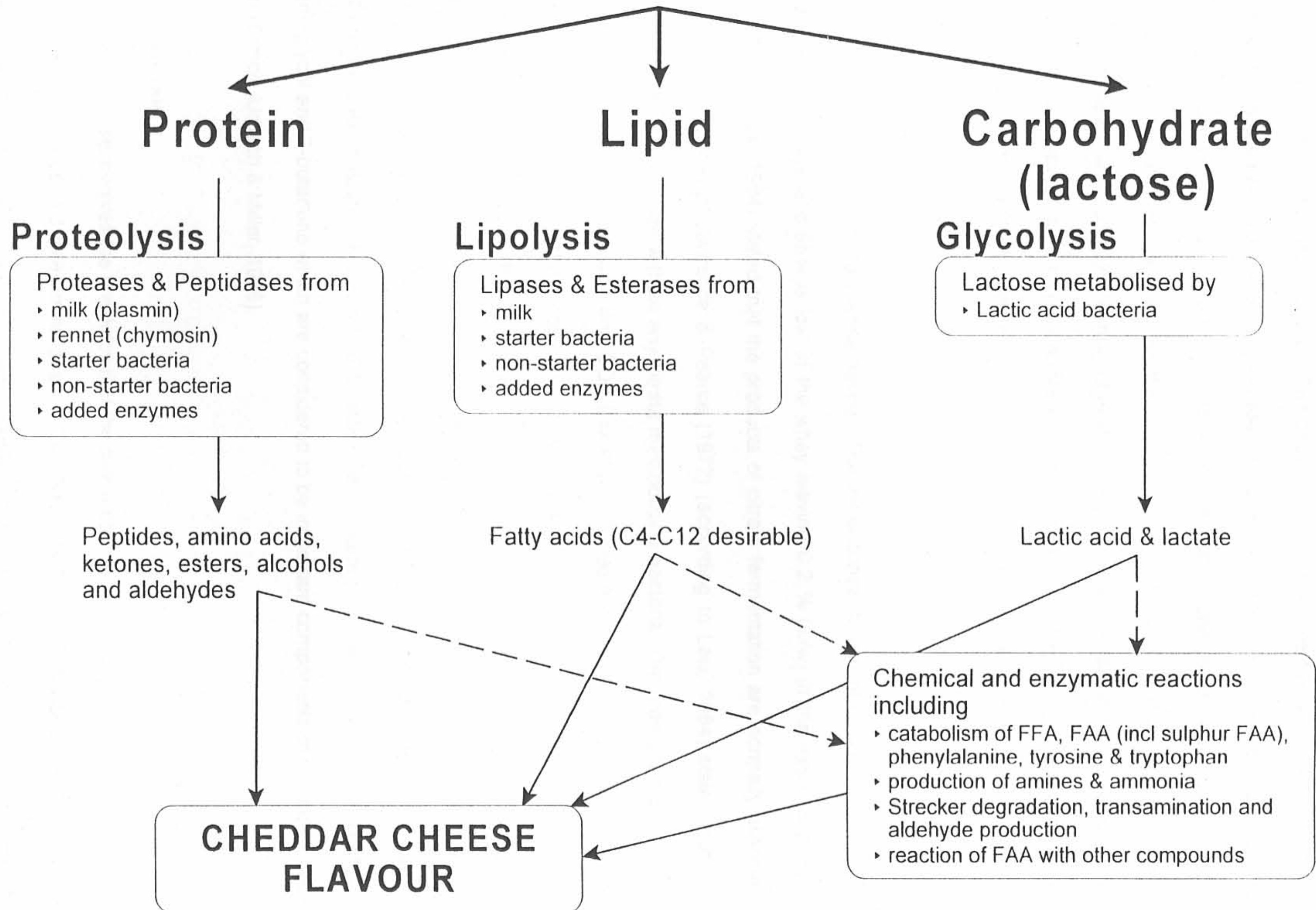

Figure 1 Formation of flavour compounds in Cheddar cheese (adapted from Fox *et al.*, 1996; Fox & Wallace, 1997)

A very important effect of the lactic acid production is the lowering of the pH with Fox *et al.* (1995a) stating that this is the key parameter for good flavour development in cheese since it has follow-on effects. The pH has an effect on essential and non-essential microorganism growth, enzyme function as well as the various biochemical reactions that occur during cheese ripening (Fox & Wallace, 1997). Artificially increased lactose levels during cheese production lead to faster flavour development though the resultant flavour is harsh (Fox & Wallace, 1997). Fox *et al.* (1993) stated that good cheese flavour is a result of rapid and complete metabolism of any residual lactose in the cheese.

2.3.2 Citrate metabolism

Citrate metabolism is important to cheese flavour. The initial concentration of citrate in cows milk is approximately 1.8 g/litre and 95% is lost in the whey leaving 0.2 % (w/w) in the fresh curd (Fox & Wallace, 1997). Law (1984) stated that the products of citrate fermentation are normally associated with cheese aroma though Lawrence & Pearce (1972) (according to Law, 1984) stated that good Cheddar cheese can be made without any citrate metabolising bacteria. The main products of citrate fermentation are diacetyl, acetic acid and CO_2 (Fox *et al.*, 1995a) and formate (Fryer, 1970). Excess CO_2 leads to bad quality Cheddar cheese (called blowing) but a limited amount is required in Dutch-type cheese for eye production (Fox & Wallace, 1997). Acetic acid is important for Cheddar cheese flavour though excess will cause off-flavours (Ney, 1981; Aston & Dulley, 1982). Diacetyl gives a creamy flavour, is essential in butter and yoghurt (Moskowitz & La Belle, 1981) and is important for good Cheddar cheese flavour (Moskowitz & Noelck, 1987). Diacetyl is also converted to acetoin, 2,3-butylene glycol and 2-butanone which are considered to be important components of Cheddar cheese flavour (Dimos, Urbach & Miller, 1996).

2.3.3 Proteolysis

Proteolysis in cheese involves the breakdown of the milk proteins (mostly casein) sequentially to large peptides, small peptides and free amino acids (FAA) (Ney, 1981; Fox & Wallace, 1997). The other non-casein proteins that occur in milk (α-lactalbumin, β-lactoglobulin, serum albumin, immunoglobulins and proteose-peptone components) are left in the whey during curd formation and as such are called whey proteins. Whey proteins are only involved in cheese production when the cheese is made from ultra-filtered milk, where the milk is concentrated before the production of the

curd. For this review, only cheese that has not been made from ultra-filtered milk will be considered for discussion so the contribution of whey proteins to flavour will not be discussed. Proteolysis is the primary and most complex of the biochemical reactions contributing to Cheddar cheese flavour (Fox *et al.*, 1996).

Casein is a phospho-protein complex composed of α, β, κ and γ-caseins associated in micelles (Fox, 1981) and Nagodawithana (1995) stated that κ-casein is well known for its ability to stabilise the micelles in suspension. Rennet is added to cheese together with the LAB to form curds. Rennet consists mostly of chymosin (EC 3.4.23.4) and it cleaves the $\text{Phenylalanine}_{105}$-$\text{Methionine}_{106}$ bond of κ-casein (Delfour *et al.* 1965 according to Fox *et al.*, 1996). The cleaving of this bond in casein combined with the low pH from the lactic acid, causes the colloidal suspension of casein to collapse to form a coagulum or gel (Fox, 1981). A low pH is also needed for the proteolytic action of chymosin to be highly effective with the optimum activity of chymosin near pH 4 (Fox *et al.*, 1996). The coagulum is then cut, heated and the whey drained off and the resultant gel is called curd. For Cheddar cheese there is an extra process called Cheddaring that involves stacking the cut blocks of curd to express more of the whey as well as to complete lactic acid production. After Cheddaring the curd is milled and salt added before pressing into moulds and then storing for ripening (Scott, 1986). Fox *et al.* (1995a) mentioned that 6% of the rennet is retained in the curd after the curd is pressed and this plays a major role in the initial proteolysis of caseins in many cheese varieties yielding mostly macropeptides (O'Keeffe, Fox & Daly, 1978)

Chymosin is rather weakly proteolytic (Fox, 1989) yet it cleaves the $\text{Phenylalanine}_{105}$-$\text{Methionine}_{106}$ bond of κ-casein because it has been found to be many times more susceptible to hydrolysis than any other casein peptide bond (Fox *et al.*. 1996). Why this bond in particular is so susceptible to hydrolysis is not well understood (Fox, 1981) though it appears as if the amino acid sequence surrounding the bond causes its susceptibility (Fox *et al.*, 1996). The primary task of chymosin is to clot the milk yet many other proteases that clot milk cause a higher degree of proteolysis relative to their clotting ability, leading to reduced cheese yields and/or inferior quality cheese (Fox *et al.*, 1996). The secondary task of chymosin is to continue proteolysis during ripening which contributes to the flavour of the cheese.

Rennet is an animal product derived from the stomachs of suckling calves. Increased demand for cheese as well as a lowering of the cull rate of calves has lead to a shortage of rennet (Fox, 1981). Rennet from other sources has been investigated with the following rennets being found suitable: bovine, porcine and chicken pepsins and the acid proteases of *Rhizomucor miehei*, *R. pusillus* and *Cryphonectria parasitica* (Fox *et al.*, 1996). Companies like Gist Brocades, Pfizer and Hansen's have also cloned the gene for calf chymosin into microorganisms and the chymosin produced from these microorganisms is steadily gaining acceptance in several countries (Fox *et al.*, 1996). Historically, non-animal enzymes (including those from cloned bacteria) have not been as effective as animal enzymes such as rennet and pre-gastric esterases in producing high quality cheese since their enzyme action is different (Coulson, Pawlett & Wivell, 1992).

Plasmin and cathepsin-D are proteases that naturally occur in milk. Plasmin (fibrinolysin, EC 3.4.21.7) is an alkaline enzyme and probably plays little part in the ripening of Cheddar cheese due to the low pH of cheese of about pH 5 (Fox *et al.*, 1996). Cathepsin-D has a similar specificity to that of chymosin yet it coagulates milk very slowly (McSweeney *et al.*, 1995 according to Fox *et al.*, 1996). Since cathepsin-D is a weak protease (as compared to chymosin) yet similar to chymosin, its effects can either be ignored or grouped together with that of chymosin. Another protease has been found by Kaminogawa & Yamauchi (1972) which is an acid protease similar to cathepsin-D with an optimum at pH 4 and with an enzyme action that is similar to that of chymosin.

The LAB are weakly proteolytic though they have the ability to break down the casein all the way to FAA (Moskowitz & La Belle, 1981; Law, 1984). Fox *et al.* (1996) stated that the starter LAB bacteria are considered crucial for cheese flavour development and according to Fox *et al.* (1995a), LAB are the major source of proteases and peptidases in cheese apart from chymosin. NSLAB and secondary starter microorganisms have a range of proteases and peptidases that are also involved in the proteolytic ripening of cheese (Fox *et al.*, 1996). The bacteria in the cheese lyse after death during the ripening of cheese, releasing aminopeptides, dipeptides and tripeptides which contribute to the level of FAA in the cheese (Law, Sharpe & Reiter, 1974; Fox & Wallace, 1997). Law, Andrews, Cliffe, Sharpe & Chapman (1979) in contrast found that no significant change in cheese quality, yield or

proteolysis could be detected if they added up to 10^7 cfu/ml of psychrotrophic non-starter bacteria to milk prior to cheesemaking.

The caseins in cheese curd are bland and do not add directly to cheese flavour since they are too high in molecular weight, but the peptides, FAA and their breakdown products do contribute to cheese flavour (Ney, 1981). Some peptides are bitter and much research has been done to reduce the bitterness caused by these. It has been shown that if a peptide has a value of Q>5862 J/mol and a molecular weight less than 6000 Da it will be perceived as being bitter, where Q is defined as the free energy of formation of the constituent amino acids with glycine being assigned a heat of formation of 0 J/mol (Ney, 1981; Fox *et al.*, 1996). Some bitterness is required in cheese since that is the nature of the product but excess bitterness will create an undesirable product. A number of authors (Aston & Creamer, 1986; Cliffe, Marks & Mulholland, 1993; Engels & Visser, 1994) have shown that peptides <500 Da make a significant contribution to Cheddar flavour and McGugan, Emmons & Larmond (1979) stated that peptides are also important since they can bind onto flavour compounds. Any excess bitterness can be eliminated either by reducing/controlling the degree of proteolysis or by the addition of peptidases. The bitterness rule mentioned above can also be applied to individual amino acids to show that some of them are bitter, as can be seen in Table 2 (Ney, 1981).

Approximately 3% of the total nitrogen content of cheese is present as FAA nitrogen (Law, Castañón & Sharpe, 1976). FAA on their own contribute directly to cheese flavour, though their contribution is probably small (Fox & Wallace, 1997). There has been disagreement over what the most important FAA in Cheddar flavour are: Marsili (1985) gives Glu, Met and Leu; Wood, Aston & Douglas (1985) give Leu, Glu, Asp, Lys and Phe; Engels & Visser (1994) give Glu, Leu and Phe and Aston & Creamer (1996) give Leu and Met.

FAA also play a part by serving as a substrate for the formation of flavour compounds (Fox & Wallace, 1997). Catabolism of FAA by microorganisms can result in a number of flavour compounds, as can be seen in Figure 2. Manning (1979) has shown that certain sulphur-containing compounds (methanethiol and hydrogen sulphide) could be produced in cheese with no starter, by lowering the redox potential of cheese, implying that they are produced chemically rather than by microbial

Table 2 Heat of formation of amino acids (Fox & Wallace, 1997 adapted from O'Callaghan, 1994)

Amino acid	*Q (J/mol)	Amino acid	*Q (J/mol)
Serine	-1256	Lysine	6280
Aspartic acid	0	Valine	6280
Glutamic acid	0	Leucine	7536
Glycine	0	Tyrosine	9630
Threonine	1675	Phenylalanine	10467
Alanine	2093	Proline	10886
Histidine	2093	Isoleucine	12351
Arginine	3140	Tryptophan	14235
Methione	5443		

*Q=free energy of formation with glycine defined as 0 J/mol

metabolism. Lowering the pH artificially without starter resulted in no flavour formation so Manning (1979) concluded that the low redox potential is required for flavour compound formation and/or preservation.

2.3.4 Lipolysis

Lipolysis in curd/cheese involves the breakdown of the milk lipids into diglycerides, monoglycerides and FFA. Some FFA are also synthesised by LAB, NSLAB and other bacteria from carbohydrate and protein in cheese but the bulk of evidence indicates that lipolysis is the principal contributor of FFA greater than C4 (Aston & Dulley, 1982). This is also borne out by the fact that skim milk cheese fails to develop cheese flavour (Harper, Kristoffersen & Wang, 1978). West & Pawlett (1996) differentiated between esterases and lipases by defining esterases as those enzymes which hydrolyse water-soluble esters and lipases as those enzymes which hydrolyse the ester bonds in oils and fats. For this discussion on lipolysis no distinction will be made between esterases and lipases.

Lipids can be degraded either by lipolysis or by oxidation but the possible contribution of oxidation to good cheese flavour has been largely ignored since it only occurs to a small degree (Fox & Wallace, 1997). Typical concentrations of total FFA are 1000 mg/kg for Cheddar and 30 000 mg/kg for Roquefort cheese (Fox & Wallace, 1997) which is well above the typical aroma and flavour threshold of 0.3 to 100 mg/kg for the range of fatty acids (Baldwin, Cloniger & Lindsay, 1973). Even though lipolysis may not be the major event in Cheddar cheese flavour (see section on proteolysis) it is still

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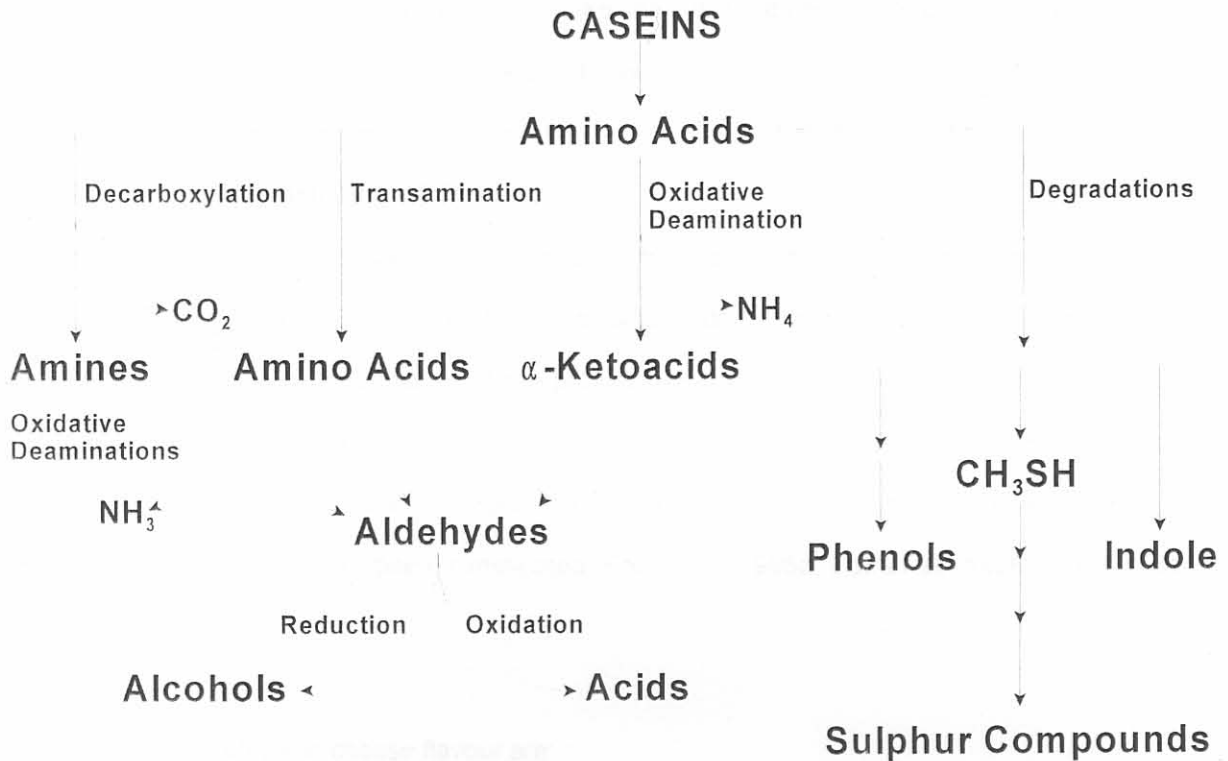

Figure 2 General pathways of microbial catabolism of amino acids during cheese ripening (Fox *et al.*, 1995a adapted from Hemme *et al.*, 1982)

important since cheese made with skim milk fails to develop flavour and low-fat Cheddar cheese has poor flavour (Ohren & Tuckey, 1969 according to Harper *et al.*, 1978).

Milk contains a well-characterised lipoprotein lipase (Fox & Wallace, 1997) which causes significant lipolysis in raw milk cheese but is mostly deactivated during pasteurisation. In Cheddar production the rennet is virtually lipase free (Fox & Wallace, 1997) and so the lipases must come from other sources. Lipases from psychrotrophic bacteria contribute significantly since they are heat stable and are concentrated in the curd during curd formation since they adsorb onto the fat globules (Foda, Hammond, Reinbold & Hotchkiss, 1974; Fox & Wallace, 1997).

Much work has been done on the importance of FFA to Cheddar cheese, there is not much agreement on which and how much FFA is important except that an excess causes rancidity. Kristoffersen (1967) and Singh & Kristoffersen (1970) stated that FFA are of key importance since FFA increase with flavour but Reiter *et al.* (1967) found no relationship between flavour and FFA. Milk fat is also important as a solvent for sapid compounds produced from other constituents (Fox &

Wallace, 1997). The FFA are broken down into ketones, lactones and other compounds, though these are of more importance in blue cheese flavour development (Fox & Wallace, 1997).

2.3.5 Other factors, including off-flavours

As mentioned previously, physical parameters such as pH affect the formation of cheese flavour. These physical parameters affect the microorganisms, added and indigenous enzymes as well as the inter-reactions between the products of the previous processes. These physical parameters include Eh, pH, water activity and salt content. Salt is also a taste compound in its own right but its action is relatively well known. Many other reactions involving the products from lipid, protein and carbohydrate metabolism/breakdown have been investigated (Fox *et al.*, 1995a; Fox *et al.*, 1996; Fox & Wallace, 1997).

Some common defects in cheese flavour are:

Bitterness - probably the principal defect in cheese, which is caused mainly by the accumulation of small peptides which are relatively rich in hydrophobic residues (Fox *et al.*, 1995a). FAA and their breakdown products can also contribute to bitterness (Fox *et al.*, 1996).

Astringency - a result of reactions between tannins and proteins in saliva (Lindsay, 1985).

Fruitiness - caused by reactions between ethanol and FFA to form esters (e.g. ethyl hexanoate and ethyl octanoate) (Ney, 1981; Fox & Wallace, 1997).

"Unclean" off-flavours - linked to Strecker-type products including phenylacetaldehyde, phenethanol, 3-methyl butanol, 2-methyl propanol, phenol and *p*-cresol (Dunn & Lindsay, 1985).

Rancidity - caused by excessive or unbalanced lipolysis.

Higher cleanliness in cheese manufacture today, as compared to traditional cheesemaking, has reduced the non-starter, citrate fermenting and other bacteria normally found in milk, with the result of a more consistent product but a milder flavoured cheese (Fox *et al.*, 1996). Work is being done to selectively add some of these bacteria in addition to the standard LAB starter but this is not yet common practice and is also legislated against in certain countries (International Dairy Federation, 1990). There is hope that with the selective addition of these indigenous bacteria a higher flavoured product will result, without any loss in quality.

2.4 ENZYME-MODIFIED CHEESE

As seen in the previous section the chemistry of cheese flavour formation is complex. If the multitude of known volatile Cheddar flavour profile chemicals (Table 3) are added to a bland base, the resultant product will not taste like Cheddar cheese due to as yet undetected, below threshold compounds (Nagodawithana, 1995). Even if the full reaction chemistry of Cheddar cheese flavour were known, the cost of producing it synthetically would probably be prohibitive (Kilcawley, Wilkinson & Fox, 1998). Therefore, enhancement of the major flavour pathways that occur in natural cheese presently provides the most viable route to the production of intense cheese flavours. This is best achieved by the enzymatic modification of cheese to produce EMC (Kilcawley *et al.*, 1998). Fox (1998) stated that the flavour from EMC does not approximate natural cheese flavour but rather has the ability to potentiate cheese flavour in various foods.

2.4.1 Enzyme modified Cheddar cheese chemistry

Proteolysis is the major event in Cheddar cheese flavour development (see section on Cheddar flavour development) but lipolysis is the major event in the production of Cheddar EMC (BioCatalysts, 1996). Godfrey & Hawkins (1991) reported that lipases can eliminate the need for the extensive protein hydrolysis, which is needed for strong flavour but which can also result in bitterness. For EMC production Kilara (1985) gave the activity ratios of protease to lipase as 1:2 to 1:3. As mentioned previously, EMC is a highly accelerated ripened cheese product and at present there are four ways that cheese can be ripened more quickly than normal (Table 4). As will be seen in the next section, EMC is produced using a combination of the first, second and fourth methods.

Kristoffersen *et al.* (1967) are quoted by many authors as the inventors of EMC through their investigations of the curd slurry technique (Sutherland, 1991; Thakar & Upadhyay, 1992; Kilcawley *et al.*, 1998). In their landmark study, curd was blended with saline solution (same salinity as in the curd, i.e. 5% NaCl in the moisture) and 100 mg/kg glutathione (GSH) and then stored at 30°C for 4 to 5 days with routine sampling. The resulting paste had the flavour of a mild Cheddar cheese and the authors commented that the product was ideally suited for use in processed foods. These techniques form the basis of EMC production today, though as the name implies enzymes are now also added.

Table 3 Volatile compounds important for Cheddar cheese flavour (Fox & Wallace, 1997)

Acetaldehyde	Dimethyl disulphide	2-Methylbutanol
Acetoin	Dimethyl trisulfide	3-Methybutanol
Acetone	δ-Dodecalactone	3-methyl-2-butanone
Acetophenone	Ethanol	3-Methylbutyric acid
β-Angelicalactone	Ethyl acetate	2-Nonanone
1,2-Butanediol	2-Ethyl butanol	δ-Octalactone
n-Butanol	Ethyl butyrate	n-Octanoic acid
2-Butanol	Ethyl hexanoate	2-Octanol
Butanone	2-Heptanone	2,4-Pentanediol
n-Butyl acetate	n-Hexanoic acid	2-Pentanol
n-Butyric acid	n-Hexanol	Pentan-2-one
Carbon Dioxide	2-Hexanone	n-Propanol
p-Cresol	Hexanethiol	Propanal
γ-Decalactone	2-Hexenal	Propenal
δ-Decalactone	Isobutanol	n-Propyl butyrate
n-Decanoic acid	Isohexanal	Tetrahydrofuran
Diacetyl	Methanethiol	Thiophene-2-aldehyde
Diethyl ether	Methional	2-Tridecanone
Dimethyl sulphide	Methyl acetate	2-Undecanone

Table 4 Major categories of methods for accelerating cheese ripening (adapted from Law & Goodenough, 1995; Fox *et al*., 1996)

Method	Advantages	Disadvantages
1. Elevated temperature	No legal barriers, technical simplicity	Non-specific action, increased microbial spoilage potential
2. Enzyme addition	Low cost, specific action, choice of flavour options	Limited source of useful enzymes, danger of over-ripening, difficult to incorporate
3. Modified starter	Probably no legal barriers, natural enzyme balance retained, easy to incorporate	Technical complexity, uneconomical at present
4. Cheese/curd slurries	Very rapid flavour development, used to study new enzymes	Cannot be used to accelerate flavour in solid cheese

EMCs are produced using curd slurries since this is the quickest procedure and texture is not important (Fox *et al*., 1996). Thakar & Upadhyay (1992) have reviewed the curd slurry technique and conclude that the major biochemical pathways of flavour formation in curd slurries and cheese are

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similar. Curd slurries have been used as a method of testing new cheesemaking techniques and additives and EMC evolved from this model system (Kilcawley *et al.*, 1998).

EMC is made from cheese, cheese scraps or fresh curd (West & Pawlett, 1996). Some authors have added additional starter culture during the production process (Jang & Lee, 1985; Takafuji, 1993) but others have not and still produced acceptable product (West & Pawlett, 1996). Many authors have tried different additives in curd slurries to enhance flavour e.g. Singh & Kristoffersen (1970) added $CoCl_2$, sodium citrate, $MnSO_4$ and riboflavin and noticed a slight improvement in flavour. Singh & Kristoffersen (1971a) and Singh & Kristoffersen (1971b) reported that if the curd was made using direct acidification then starter culture, GSH, cobalt solution, riboflavin and diacetyl were needed as well as daily pH adjustment for development of Cheddar-like flavour in 8 days.

The one additive in EMC/curd slurries to enhance flavour that is reported more than any other is GSH (Kristoffersen *et al.*, 1967; Harper & Kristoffersen, 1970; Singh & Kristoffersen, 1971a; Manning, 1979; Kilara, 1985; Thakar & Upadhyay, 1992). Moskowitz & Noelck (1987) stated that GSH is important for flavour development by dissociating proteins, protecting esterases from degradation and maintaining flavour compounds in a reduced state. They also stated that GSH does not alter the redox potential but rather enhances production of H_2S and methanethiol and thereby enhances cheese flavour. Cysteine can be used as a substitute for GSH with comparable results (Samples, Dill, Richter & Dill, 1986).

Buhler (1995) in a trade publication, stated that Cheese Buds (similar to Cheesebase in Table 1) is better than EMC since EMC leaves a bitter aftertaste. Vafiadis (1996) and West & Pawlett (1996) confirmed that EMC originally had problems with bitterness. This has been solved by better understanding and control of proteolysis and the addition of peptidases from *Bacillus subtilis* and *Aspergillus oryzae* or preparations containing enzymes similar to these such as Debitrase® (West & Pawlett, 1996).

2.4.2 EMC technology

The production of EMC remains as much an art as a science (Moskowitz & Noelck, 1987). A number of different methods can be used to produce EMC, depending on the EMC type, manufacturer's preference, product application and appearance of the end product (Kilcawley *et al.*, 1998). A major variation over the curd slurry technique invented by Kristoffersen *et al.* (1967) (apart from the addition of enzymes) is the use of cheese instead of curd as a starting substrate by some authors. A more authentic flavour is produced with curd though EMC can be made from waste/defective cheese scraps since texture is not important (West & Pawlett, 1996).

Takafuji (1993) detailed the design and investigations required to commercially produce a Gouda EMC. Natural, mature Gouda is shredded and then mixed with additives (water, LAB, salt, protease from *Penicillium camemberti* and kid/lamb pregastric esterase) before going into a holding tank. The paste is then stirred with a recirculating pump for 10 days before pasteurisation at 80°C to deactivate the added enzymes. The product is then homogenised and packaged into plastic bags.

Bush Boake Allen (according to Vafiadis, 1996) manufacture EMC using fresh curd direct from their sister company on the same site, add enzymes and then incubate for a set period of time. Heat is used to deactivate the enzymes with care being taken not to destroy the cheese flavour during the heating process. The time and temperature relationships during flavour production and pasteurisation are critical for a good quality product. In a trade article, Freund (1995) gave the temperature and incubation parameters for Bush Boake Allen's process as 30°C or above for a minimum of 24 hours.

Sutherland (1975) made curd slurries by macerating unpressed salted curd with added water and salt to give a finely ground slurry with 40% total solids and 3.2% salt. The curd slurry was then incubated at 25°C after addition of rennet, GSH and/or lipase. Sutherland (1991) commented that the dairy industry uses techniques similar to the curd slurry technique to produce highly flavoured cheese slurries.

West & Pawlett (1996) gave two methods of producing EMC based cheese flavourings, namely the component approach and the one-step process. The component approach involves the production of

several different components which are then mixed into a final product. As an example, hydrolysed cheese curd (protein derived component) could be mixed with lipolysed cream (lipid derived component) to get an EMC. The component approach has a better control of product through variation of the blend. The one step process has an advantage in that certain flavour forming reactions occur synergistically (see section on Cheddar flavour formation for more details) and a more authentic flavour can be produced (Fox & Stepaniak, 1993). West & Pawlett (1996) demonstrated this synergism by showing the increase in lipolytic action of a lipase when used in conjunction with a protease (Savorase®) as compared to the lipase on its own. They gave a method of EMC production as:

1. Make a slurry (40-55% dry solids) using curd or cheese off-cuts
2. Heat treatment at 72°C for 10 min
3. Cool to 40-55°C and add lipase and/or protease
4. Incubate at 40-55°C for 8-36 h
5. Heat treatment at 72°C for 25-35 min

As can be seen from the method, EMC development now can take between a few hours and a few days (Vafiadis, 1996) while traditional production of cheese flavour used to require several months or more (Kilara, 1985).

Takafuji (1993) went through the long process of extracting, selecting and determining the correct concentration of enzymes to be used for EMC production. This takes a long time and though large companies may have the resources for such investigations, small companies do not. Many companies including Amano Enzymes, BioCatalysts, Imperial Biotechnology, Novo Nordisk and Röhm market purified enzyme preparations extracted from organisms such as *Aspergillus niger*, the recipes of which are not in the public domain (Kilara, 1985; Sutherland, 1991).

BioCatalysts produce a range of enzyme preparations for EMC production (BioCatalysts, 1996). The method of EMC production given by West & Pawlett (1996) is identical to that given by BioCatalysts since West works for BioCatalysts. The most commonly used enzymes from BioCatalysts for EMC production are Lipomod 187 (lipase) and Promod 215 (protease), both which are of microbial origin

and as such have Kosher and vegetarian status (pers. comm. - S. West, Director of BioCatalysts, Pontypridd, Wales, 1998).

2.4.3 Factors affecting EMC quality

For a high quality product, Talbott & McCord (1981) stated that temperature, pH and agitation must be tightly controlled and the progress of the reactions monitored such that the reaction is stopped when the product is at optimum quality. The time and temperature control during product pasteurisation is crucial to preserve the flavour while removing residual enzyme activity which would spoil any product that the EMC was used in (Vafiadis, 1996).

As mentioned previously, fresh salted curd must be used for the most authentic flavour and GSH addition is recommended for good Cheddar flavour. Since there will be many peptides produced in the curd-slurry, bitterness may be a problem but this can be eliminated by the addition of debittering enzymes e.g. Debitrase® (West & Pawlett, 1996). Horwood, Shanley & Sutherland (1994) mentioned that headspace oxygen increases the chance of fruity off-flavours in curd slurries, implying that either vacuum packaging or a nitrogen blanket must be used for EMC production. Spoilage by bacteria and yeast is a major problem due to the relatively high processing temperatures. Potassium sorbate (Dulley, 1976) and nitrates, sorbic acid and nisin (Mann, 1993) can be used to reduce microbial growth depending on legislation governing their use.

Kilcawley *et al.* (1998) published a comprehensive review on EMC. In their review they gave the key factors involved in EMC production as: the type of cheese required which is directly related to the starting material, type and specificity of enzymes or cultures used, their concentration, processing parameters (pH, temperature, agitation, aeration and incubation time) and use of processing aids (emulsifiers, bacteriocins, flavour compounds and precursors). The dosage of enzyme and/or starter culture used is dependent on the intensity of flavour required, processing time and temperature and the quality of the substrate. Kilara (1985) gave a table of factors that affect the enzymatic hydrolysis of proteins (Table 5). Most of these factors can also applied to lipases since they are parameters that pertain to enzymes in general.

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Table 5 Factors affecting the enzymatic hydrolysis of proteins (Kilara, 1985)

Factors	Comments
Enzyme specificity	No single protease completely hydrolyses a protein. Mixtures of proteases are used.
Extent of protein denaturation	Denatured proteins are more susceptible to hydrolysis.
Substrate and enzyme concentration	Should be controlled.
pH	Optimum varies with enzyme.
Temperature	Preferably >45°C.
Ionic strength	Critical but neglected parameter.
Inhibitory substance	Should be absent.

2.5 NON-SENSORY METHODS FOR DETERMINING CHEESE FLAVOUR QUALITY

As previously discussed in the section on Cheddar cheese flavour, there is no chemical or class of chemicals that can be considered as the basis or fundamental for Cheddar cheese flavour. Fox & Wallace (1997) gave a list of volatile compounds that are known to contribute to Cheddar cheese flavour (Table 3) though if these chemicals were all added together they would not equate with good cheese flavour (Nagodawithana, 1995). This implies that any test for these chemicals or these classes of chemicals will be unable to determine whether a cheese is of high or low quality (Manning, Ridout & Price, 1984). This must be done subjectively by taste panels (Fox & Wallace, 1997). The ideal instrument would be a "cheese quality meter" but this is not available at present. A more realistic goal would be to produce low-cost and simple methods for the determination of the key parameter(s) in cheese that would be helpful as a guide in the evaluating cheese quality.

The development of gas chromatography in the 1950s and its interfacing with mass spectrometry has given scientists the tools to track the chemical changes in cheese by measuring the volatiles given off by cheese (Fox & Wallace, 1997). The non-volatile components like peptides can be analysed by Reverse Phase - High Performance Liquid Chromatography (RP-HPLC). Most of these techniques are far too time consuming for routine analysis and much work has been done to find the key parameter(s) that will give an indication of the quality of the cheese being tested. Manning *et al.* (1984) reviewed non-sensory methods for predicting cheese flavour quality including methods such as compositional analysis (salt, moisture and fat), volatile composition, redox potential and statistical

methodology. Few of the methods they mentioned are used commercially for grading cheese except compositional analysis, which has been successfully used in New Zealand since 1977.

Since the primary events in cheese and EMC flavour formation are proteolysis and lipolysis, measuring the degree of both of these will give some degree of understanding as to what is occurring during flavour formation.

2.5.1 Proteolysis

Proteolysis is the breakdown of proteins to peptides and FAA and methods for determining these compounds should be able to estimate the degree of proteolysis. Fox, McSweeney & Singh (1995b) described non-specific methods that involve measuring the fraction of protein soluble in solvents such as urea solution, water, acid, $CaCl_2$ solution, NaCl solution, methanol, ethanol, trichloroacetic acid, ethanol, phosphotungstic acid (PTA) and others. The soluble fraction will contain varying amounts of peptides and FAA depending on the method e.g. PTA precipitates all peptides over 600 Da (Jarrett, Aston & Dulley, 1982). The resulting liquor after centrifuging is then analysed for nitrogen content using the Kjeldahl or other method. Fox *et al.* (1995b) described direct methods for proteolysis determination including the measurement of ammoniacal nitrogen (using Nesslerization), soluble tyrosine/tryptophan, buffering capacity (which increases with proteolysis) and spectrophotometric determination of dyes which bond selectively to amino acids and/or amines e.g. ninhydrin. They also stated that commercial amino acid analysers based on ion-exchange chromatography combined with ninhydrin are used to quantify individual amino acids concentrations.

Techniques that can separate individual peptides are available such as electrophoresis or chromatography. Chromatography has been done in varying forms including paper, silica gel, metal chelate, Sephadex gel, ion exchange and RP-HPLC though HPLC techniques are still confined to the research laboratory (Fox *et al.*, 1995b).

2.5.2 Lipolysis

Deeth, Fitzgerald & Snow (1983) described a GC technique for the quantitative determination of FFA. A hexane-diethyl ether extract of cheese is prepared in the presence of anhydrous sodium sulphate

and passed through a column of neutral, deactivated alumina to separate fat. The FFA retained on the alumina are eluted with a small volume of 6% formic acid in di-isopropyl ether which is then evaluated using a GC. Shanley *et al.* (1979) (according to Horwood *et al.*, 1994) gave a similar but more complex method. Other methods that have been used historically (e.g. conversion of FFA to methyl esters followed by evaluation with a GC) can cause hydrolysis of the lipids during sample preparation which can lead to erroneous results (Deeth *et al.*, 1983).

There is a standard method for measuring total FFA in fat, which involves the titration of the product in hot neutral ethyl alcohol (Skoog, West & Holler, 1988).

2.5.3 Off-flavours

Of the factors listed in the section on off-flavours in cheese (bitterness, astringency, fruitiness, unclean flavour and rancidity), fruitiness (esters from ethanol and FFA reactions) and rancidity (lipolysis) are relatively easy to determine. Ethanol production is the rate-limiting step in ester formation so measuring this will give a guide to fruitiness in the ripened cheese (Fox & Wallace, 1997). Lipolytic rancidity is caused by the FFA levels being too high and so this can be measured by the tests mentioned in the section on lipolysis.

Peptide determination for bitterness using a chromatographic method is a long process and is not suitable for routine analysis. Cliffe & Law (1990) used RP-HPLC to study enzyme-treated Cheddar cheese slurries and confirmed that bitter peptides are relatively rich in hydrophobic residues. The bitter peptides exhibited higher retention on the reverse phase column.

Astringency and unclean flavours are harder to detect since the chemistry of formation is not well known and therefore any analytical test would not be able to guarantee an absence of the defect.

2.6 SENSORY METHODS FOR DETERMINING CHEESE FLAVOUR QUALITY

Cheese used to be assessed for flavour at least twice between manufacture and marketing to determine the most suitable marketing stage for the cheese, with only the best quality cheeses being ripened for up to 12 months. In the modern dairy industry, mature cheese is specifically produced but

still must be taste tested before being released for sale. There are three types of taste testing, namely expert panels, consumer panels and descriptive analysis taste panels. Since cheese flavour is similar to EMC flavour the same methods that apply to cheese can be applied to EMC.

2.6.1 Expert panels

The least scientific of the three types of taste testing is the expert panel since normally it is done by three to five people and little (if any) statistical analysis is done on the results. Expert panels are used for the 'day to day' quality determination of cheese and are commonly called cheese graders. Grading for cheese is normally done under three classifications: flavour and aroma, body and texture, and colour. Manning *et al.* (1984) commented that by and large they do an effective job. McBride & Hall (1979) found no correlation between consumer preferences and expert grading though this is to be expected since the two methods used would yield different results. Non-sensory (analytical) methods are being investigated as ways to replace expert graders though at present this is not possible since as discussed in the section on Cheddar cheese flavour the exact compounds that lead to good flavour are not known.

2.6.2 Consumer panels

Consumer panels involve getting the public to taste samples of product to see whether they like the product, prefer it over another product or just find the product acceptable (Heymann, 1995a). Since this is not a descriptive technique the only result would be ranking of products or an indication of product acceptability while no key is given as to why the product is acceptable. Consumer panels are normally only done towards the end of product development and after descriptive analysis had been done (Heymann, 1995a).

2.6.3 Descriptive analysis taste panels

Descriptive analysis is the most scientific of the three methods mentioned. Techniques such as quantitative descriptive analysis (QDA), quantitative flavour profiling (QFP) and sensory spectrum are all descriptive analysis methods with various adaptations (Heymann, 1995b). Descriptive analysis involves describing the flavour using descriptors and there are two variations of the technique. In the first technique the panel generate the descriptors using reference standards and other products in the

category chosen. In the second technique the descriptors and references are provided. Heymann (1995b) used a combination of the two techniques where the panel generates the descriptors but the panel leader may modify the list by combining, adding or deleting terms based on prior experience with the product type. Stampanoni (1993) stated that the objective of descriptive analysis is to find a minimum number of descriptors that will convey a maximum amount of information regarding the sensory characteristics of the product. The use of commonly available flavour/aroma references will allow the comparison of different studies, which is not possible using either consumer or expert panels.

Samples for descriptive analysis are initially done in triplicate to determine judge repeatability and thereafter the product must be tested in duplicate but preferably in triplicate. All the standard sensory practices including sample coding, randomised serving sequences and use of individual booths should be employed during the judging (Heymann, 1995b). The use of non-numerical scales is recommended by Stone & Sidel (1985) (according to Stampanoni, 1993).

2.7 CONCLUSION

Cheddar cheese is used as an ingredient in many foodstuffs since it has a good savoury flavour. In Australia for example, 50% of cheese is either processed or used in factory produced foods. EMC has many times the flavour strength of cheese and is used by industry to reduce the cost of adding Cheddar cheese flavour to a product. The EMC is a slurry or paste and therefore has poor texture but this is unimportant in products such as sauces where only a good imparted flavour of Cheddar cheese is important. This means that the EMC does not necessarily need to have a balanced flavour in itself but rather needs to taste balanced once incorporated into a foodstuff.

The production of Cheddar cheese flavour is a complex process, starting with the simple breakdown of proteins, lipids and lactose by added enzymes such as chymosin and those enzymes from starter and non-starter bacteria and ending with inter-reactions of the reaction products and substrates. The keys to defining Cheddar cheese flavour appear to still be beyond our grasp. Therefore Cheddar cheese flavour still cannot be made artificially, directly from pure chemicals, and hence the need for an accelerated ripened cheese product such as EMC. The chemistry of off-flavour development is

easier to define since it is normally the result of a few compounds or a variation in an easily measured parameter such as pH.

The chemistry of Cheddar EMC flavour formation is similar to that of Cheddar cheese though lipolysis is reported to be more important during flavour formation than proteolysis as compared to Cheddar where proteolysis is dominant. The technology used for EMC production has evolved from the early work done by Kristoffersen (1967) which involved incubating curd slurries at elevated temperatures for a few days. The modifications to the curd slurry technique allow for the addition of enzymes, the option of using cheese instead of curd as the substrate and the addition of other compounds such as preservatives. To make matters more complex, most EMC production is carried out using commercial enzyme preparations of unknown recipes since the art of producing and extracting enzymes would probably not be feasible for an EMC producer. The technology of commercial EMC production is complex and as with cheese production the variations in recipes and technologies are numerous and are largely dependent on what product is required.

EMC is heat treated after production to make a shelf stable product by denaturing the added and endogenous enzymes. This heat treatment is vital to make a stable product but since EMC is composed of many flavour compounds of varying heat stability, heat damage must be limited by good control of the heating process.

Non-sensory based tests are unable to guarantee good quality Cheddar cheese due the complexity of Cheddar cheese flavour. Tests that analyse for the progress of proteolysis, lipolysis and glycolysis will give some understanding as to the expected flavour quality but non-sensory based tests appear to only be good for detecting gross imbalances in flavour and the presence of off-flavour compounds.

The ultimate tester of good quality Cheddar flavour is the consumer so sensory testing will give the best indication of flavour quality. During the research and development phase of a Cheddar cheese product, descriptive analysis is most suitable to find out where the strengths and weakness of a product are. Once a variety of test products have been made they can be tested again with descriptive analysis or with difference tests that are normally carried out on people not associated with

the production of the product. Finally, expert graders are used for the daily quality control and grading of flavour once the production phase has been reached.

CHAPTER 3 - MATERIALS AND METHODS

The project was designed in three phases linked to the desired objectives, namely:

Phase 1 - EMC production

Phase 2 - Chemical analyses

Phase 3 - Sensory analyses

The experimental work was carried out at a company called FlavourCraft in New Germany, KwaZulu Natal, South Africa. FlavourCraft produces flavours and foods including top notes, soups, maize snack dustings and soya minces. Research and development of their products is carried out in their laboratories. The company is locally owned and has been in business since 1993. FlavourCraft commissioned this study due to the high import cost of EMC, which they use as an ingredient in their products.

3.1 PHASE 1 - EMC PRODUCTION

The key parameters in the production of an EMC are the type of enzyme, the substrate and the production method.

3.1.1 Enzymes and other additives

3.1.1.1 *Enzyme Selection*

As discussed, EMC is made with a lipase, protease or a combination of the two. A few companies such as Novo Nordisk, Christian Hansen and BioCatalysts produce EMC enzymes but FlavourCraft has a relationship with BioCatalysts so their enzymes were used. FlavourCraft specified that the enzymes should have kosher status so that the final EMC could obtain kosher status if so required, to make the product acceptable to the largest market possible. BioCatalysts only produce one kosher lipase (Lipomod 187) and one kosher protease (Promod 215) both of which are typically used for Cheddar EMC (CEMC) production (BioCatalysts, 1996). Their specific characteristics are shown in Table 6.

Since enzymes have varying activities, the BioCatalysts suggested dosages listed in Table 6 were used for the experimental work. Cheddar cheese curd is approximately pH 5.5 (Rosenthal, 1991), so

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Table 6 Parameters for a lipase and protease which typically are used to produce EMCs (BioCatalysts, 1996)

Enzyme name	Lipomod 187	Promod 215
Enzyme type	Lipase	Protease
Typical activity	Esterase 11000 units/g (Tributyrin substrate)	Endo-proteinase (caseinase) 120 units/g
pH optimum	5.0 - 7.0	7.0
Temperature range for maximum activity	40 - 50°C	45 - 55°C
Maximum temperature for long incubation (≥16 h)	45°C	45°C
Enzyme source	Fungal	*Aspergillus* sp.
Suggested dosage	0.1% (w/w) curd solids	0.1% (w/w) curd solids

the pH was not adjusted since it is within the optimum pH range for the lipase and near the optimum pH for the protease. An incubation temperature of 45°C was used since it is the maximum suitable for long incubations.

3.1.1.2 *Other additives*

Many additives other than enzymes have been used in the production of EMC and as can be seen from the Literature Review the most often mentioned additive is GSH. Researchers have reported that the addition of GSH greatly enhances flavour if used at a level of 0.01% (w/w) curd (Kristoffersen, 1967; Sutherland, 1991) so GSH was added at that level.

The other additive commonly mentioned in commercial preparations of EMC is an anti-microbial agent such as potassium sorbate, which is needed because of the high digestion temperatures which also would promote microbial growth (Cliffe & Law, 1990). South African food law (Act 965 of 1977, page 907) limits addition of potassium sorbate to 1 g/kg (0.1% w/w) so this rate was used to increase the safety of the product for the sensory evaluation in Phase 3.

3.1.2 Substrate selection

As discussed, the starting substrate for EMC can be milk, fresh cheese curd or young cheese. BioCatalysts (1996) recommended the use of fresh curd as the substrate for EMC production and so did Kristoffersen *et al.* (1967), the inventors of EMC.

The curd was sourced from a small-scale cheese farm that produces curd using non-animal rennets so that the curd could be certified as kosher if so required. The farm was run by Francis and Alan Webster and is in Weenen in the KwaZulu Natal Midlands in South Africa. The farm details are as follows:

Name: Foundation Jersey Farm

Telephone: (03635) 41714

Postal Address: P.O. Box 219, Weenen, 3325, South Africa.

3.1.2.1 *Curd production*

The EMC was made using unpasteurised, full cream Jersey milk curd sourced from a small-scale cheese farm in KwaZulu Natal, South Africa. The milk was added to a 250 l tank during milking and the starter (type O - R704 ex. Christian Hansen, Hørsholm, Denmark) added to the tank shortly after the first milk entered. Once the starter had been in the tank for 1 h with constant stirring the rennet (Chymogen 900 ex. Christian Hansen, Hørsholm, Denmark) was added at the recommended dose. After one hour the curd was cut and stirred while the temperature was increased to 37°C. Once the titration reached 0.15% titratable acidity, the whey was drained and the curds stacked. The curd was left until the runoff whey reached 0.7% titratable acidity. The curd was milled into approximately 1 cm^3 cubes and approximately 2% salt by weight was mixed into it by hand. The curd was left overnight at room temperature to complete the lactic acid production as recommended by BioCatalysts (1996) before storage in a freezer (Plate 1).

3.1.3 Production method

The EMC production method of West & Pawlett (1996) was used. This method is based on the original method of Kristoffersen *et al.* (1967).

3.1.3.1 *Incubation method*

Heat-sealed plastic bags were used for the EMC incubation (Takafuji, 1993; Horwood *et al.*, 1994) since this would reduce problems with contamination during sampling and would also simplify incubation and heat treatment. Each batch of curd slurry was divided up with approximately 45 g in four bags for the 0, 4, 8 and 12 h samples and the remainder into a fifth bag for the 16 h sample and sensory testing. The division of the batch into separate pouches allowed sampling at 4 h intervals

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without contaminating the rest of the batch. The 45 g of curd slurry was calculated as being sufficient for analytical testing. The main disadvantage was that the batch could not be stirred continuously but the flexibility of the bags allowed them to be agitated at frequent intervals.

Initially, sterile stomacher bags were used for incubation but after incubation and subsequent heat treatment it was discovered that the bags were permeable as evidenced by the fact that they smelled very strongly of butyric acid and had a greasy feel. A second attempt with laminated foil bags, used for packaging products such as potato crisps, was successful. They are "vapour-tight" at ambient temperature, whereas stomacher bags are not. However, they were not sterile and since they cannot withstand temperatures greater than 100°C they cannot be autoclaved. To reduce contamination, the bags were heat-treated empty at 80°C for 2 h.

3.1.3.2 *Enzyme incubation*

The curd was turned into a smooth paste by mixing 150 g sterilised 5% saline solution with 400 g curd in a liquidiser to get a total moisture content of 45%, which was within the recommended moisture content limits of 40-55% (BioCatalysts, 1996). The total batch size of 550 g was the capacity limit of the liquidiser and provided sufficient material for subsequent chemical and sensory testing. The liquidising was done at 45°C since at lower temperatures the curd was too firm to liquidise. The slurry was heat-treated in a sealed bag for 10 min at 80°C and allowed to cool to below 50°C (to reduce enzyme denaturation) before mixing the enzymes and other additives into the curd slurry in a liquidiser (Plate 2). It was crucial that the slurry was liquidised immediately after the heat treatment to ensure a slurry with a small particle size since the high temperatures caused the macerated curd to amalgamate into a solid mass. The solid mass of curd was also easier to liquidise at elevated temperatures. The additives were 0.01% GSH (w/w), 0.1% potassium sorbate (w/w) and 0.1% enzymes (w/w), all added by weight of dry curd solids. Nitrogen was bubbled into the curd slurry for 5 min while liquidising since Horwood *et al.* (1994) observed that dissolved and headspace oxygen increased off-flavours during EMC incubation.

The samples were incubated at 45°C for 16 h with sampling every 4 h including zero time (Plate 3). Zero time was taken as when the slurry was introduced into the incubator. The finished product and 4 h samples were heat-treated at 80°C for 30 min as soon as they were taken from the incubator. This

heat treatment was done to denature the added enzymes and therefore halt the proteolysis and/or lipolysis.

3.1.3.3 *Microbiological safety*

During the initial experimentation the EMC (after 16 h incubation and subsequent heat treatment) was tested for common pathogens and a pathogen indicator, since the EMC would need to be of a good microbiological standard for human consumption. Swift microbiological laboratory, Durban, South Africa (part of CSIR Foodtek) carried out the testing. A total plate count was done as well as testing for the following pathogens: Coliforms, *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens*, and salmonella and *Escherichia coli*, which is an indicator of faecal contamination. All the tests were carried out using conventional plate count methods, except for salmonella where the Malthus method was used.

3.2 PHASE 2 - CHEMICAL ANALYSES

3.2.1 Measurement of FAN to assess degree of proteolysis

A useful way to measure the progress of flavour formation in Cheddar cheese is to measure the breakdown products of the proteins and lipids, since they and their reaction products are the main contributors to flavour formation. Protein breakdown is normally measured using FAN and the lipid breakdown is measured using FFA (Fox & Wallace, 1997).

The FAN was measured using a combination of methods by Jarrett *et al.* (1982) and the European Brewery Convention (1975). There was an error in Jarrett *et al.* (1982) where the concentration of sulphuric acid should be 3.95 M and not 16.8 M (Aston, Durward & Dulley, 1983a).

The EMC was blended into a sodium citrate solution (emulsifier) and then the solids were removed by centrifuging. The supernatant was then acidified with sulphuric acid and phosphotungstic acid (PTA) which caused the soluble proteins to precipitate leaving only the single amino acids and peptides smaller than 600 Da in solution. The dibasic amino acids and proline are also precipitated out of solution. The solution was filtered and the remaining PTA and sulphuric acid was neutralised with sodium hydroxide. An aliquot was reacted with trinitrobenzene sulphonic acid (TNBS) in a phosphate

buffer medium at a constant temperature. Since TNBS is a chromophoric agent it reacted with the amino acids, and any peptides remaining in solution, to form compounds that absorb light at 340 nm (Plate 4). Quartz cuvettes were used instead of glass since glass cuvettes start to absorb light near 325 nm.

The method was calibrated using a standard glycine-sodium citrate solution together with a pre-precipitated sulphuric acid-PTA mixture as specified by Jarrett *et al.* (1982). When sulphuric acid and PTA are mixed at the levels used in the method then PTA precipitates out of solution creating a solution saturated in PTA, which is filtered before use in calibrating the method using the spectrophotometer. This step is crucial since PTA also absorbs at 340 nm therefore the level of PTA must be the same in both the standards and samples so that only the different concentrations of the TNBS-amino acid complex are detected. The glycine was also dissolved in sodium citrate solution so that the levels of sodium citrate were the same in both the sample and standard.

3.2.1.1 *Spectrophotometer calibration*

The spectrophotometer was calibrated each time it was used for FAN analysis. The straight lines that were fitted to the calibration data (glycine concentration versus absorbance) had a mean r^2 of 0.990 across all the calibrations. The good curve fit confirmed that the spectrophotometer was precise.

3.2.2 Measurement of FFA to assess degree of lipolysis

The general analytical method for FFA such as that described by the American Association of Cereal Chemists (1983) (originally based on a method from the American Oil Chemists' Society) was used. The sample of EMC was titrated in hot neutral ethanol to an endpoint detected by phenolphthalein (Plate 5). The sodium hydroxide used in the method was standardised using potassium hydrogen phthalate as detailed by Skoog *et al.* (1988). All the free fatty acids are titrated by the base and the results are reported as if only a single FFA was in the sample. The American Association of Cereal Chemists method reports the FFA as '% FFA as oleic acid' but in cheese butyric acid is commonly used as the basis for FFA (e.g. Fox *et al.*, 1996). Therefore, the molecular weight of butyric acid was used in the formula and the results are reported as % FFA expressed as butyric acid.

3.2.3 Statistics

The means of the values for the three replicates were plotted against their incubation time. On the same plot the error bars were shown, which represent the standard error of the mean.

3.3 PHASE 3 - SENSORY ANALYSIS

The quantitative flavour profiling (QFP) technique (Stampanoni, 1993) was used for taste panel tasting. A variation of the technique described by Heymann (1995b) was used which allows the panellists to generate the descriptors but the panel leader may modify the list by combining, adding or deleting terms. This adjustment of terms was done to reduce the descriptor list to approximately 10 descriptors, which is the maximum that a panellist can evaluate at a single sitting (pers. comm. - Mrs Henrietta L. de Kock, Department of Food Science, University of Pretoria).

3.3.1 Base for taste testing

The EMC was taste-tested in an umami base/savoury soup based on a method supplied by Kilcawley (EMC PhD student, Dairy Products Research Centre, Fermoy, Ireland, 1998 - pers. comm.). One major change was the addition of food colouring to offset any visible colour differences between the different EMCs caused by different degrees of breakdown of the lipids and proteins since the fat is yellow and the protein is white. The savoury soup recipe is given in Table 7.

The soup powder (10 g) was blended with 200 ml water and then the EMC was homogenised into the soup at a 1.75% (w/v) level. The soup was heated to approximately 40°C before tasting. The level of 1.75% (w/v) was based on a simple pre-trial where the EMC was diluted using a serial dilution and tasted until an acceptable level was found. The combination of EMC and the savoury soup was called EMC soup.

3.3.2 Taste Testing

3.3.2.1 *Panellists*

The panellists chosen for taste testing were staff at FlavourCraft. Most of the panellists had been on sensory training courses and are involved in research and development work on products mostly of a

Table 7 Recipe of 'umami' soup for sensory analysis of EMC

Ingredient	Quantity	Supplier
Creamer	25 g	Bordens econo creamer
Quicksperse	15 g	Universal Starch
Salt	15 g	Cerebos
Mono-sodium glutamate	1.5 g	Ajinomoto
Yeast extract	1.5 g	Gistex
Citric acid	0.25 g	Savannah Fine Chemicals
Sunset yellow supra	0.03615 g	Pointings

savoury nature, such as cheese flavoured maize snack dustings, soya minces, instant sauces and soups. The details of the panellists appear in Table 8.

Table 8 Sex and age details of taste panellists

Initials	Sex	Age
BW	Female	22
BS	Male	26
DO	Female	21
LT	Female	22
MC	Female	27
NS	Female	21

3.3.2.2 *Developing descriptors*

During an initial sensory session, the panellists each provided a list of terms whereby they described the flavours of the different EMC soups including both a control (processed curd and soup) and blank (soup only). After comparing this to a lexicon developed by Heisserer & Chambers (1993) for cheese taste testing, it was decided to modify their lexicon slightly rather than developing a new lexicon, since this would allow this study to be compared with other works which may use a similar lexicon.

Through training and elimination the 30 terms originally identified were reduced to those listed in Table 9 as being the best terms to describe the EMC. Some generic terms such as 'cheesy' and 'salty' had to be excluded even though all panellists were able to taste them since the list of

Table 9 Final EMC sensory descriptors with their respective standards and dilutions (adapted from Heisserer & Chambers, 1993)

Flavour Category	Flavour Term	Reference standard	Reference standard dilution
Dairy	Buttery	†	†
Dairy	Cooked Milk	†	†
Dairy	Dairy Fat	†	†
Dairy	Dairy Sweet	†	†
Fatty-acid/animal	Butyric Acid	†	†
Fatty-acid/animal	Goaty	Hexanoic acid	1% (w/w) in propylene glycol
Fatty-acid/animal	Sweaty	Isovaleric acid	5% (w/w) in Vema 45‡
Fatty-acid/animal	Waxy	Undecanoic acid	10% (w/w) in propylene glycol
Other Aromatics	Fruity	Trans-2-hexanal	10% (w/w) in propylene glycol
Other Aromatics	Pineapple	Ethyl Hexanoate*	1% (w/w) in propylene glycol
Fungal	Mouldy	2-ethylfenchol*	0.1% (w/w) in propylene glycol
Fungal	Mushroom	1-octen-3-ol*	0.1% (w/w) in propylene glycol
Mouthfeelings	Sharp	Propionic acid	1% (w/w) in propylene glycol
Fundamental tastes	Bitter	†	†
Fundamental tastes	Sweet	†	†

*Reference standard altered from that of Heisserer & Chambers (1993)

†Due to panellists' experience, no standards were required

‡Vema 45 is a fat manufactured by Hudson and Knight that melts at 45°C

descriptors was too long for panellists to evaluate at a single sitting. Standards were only used for those terms where panellists had difficulty identifying the descriptor. The standards for a few descriptors were changed due to lack of availability and these were done on the advice of a flavourist at FlavourCraft. All the terms and standards appear in Table 9 and where the standard has been changed the chemical is marked with an asterisk. The pure chemicals are too overpowering to be smelled in pure form so they were diluted before their use as a reference standard.

3.3.2.3 *Sensory sheet*

The copy of the final marking sheet used in the sensory evaluation appears in Figure 3. The use of non-numerical scales, recommended by Stone & Sidel (1985) (according to Stampanoni, 1993), was used. Random 5 digit numbers were used for each sample which were changed after each batch of taste testing and also the samples were taste tested in random order at each sitting. The layout for sensory evaluation can be seen in Plate 6.

Name:
Sample No.:

Dairy

Buttery	Low		High
Cooked Milk	Low		High
Dairy Fat	Low		High
Diary Sweet	Low		High

Fatty-acid/animal

Butyric Acid	Low		High
Goaty	Low		High
Sweaty	Low		High
Waxy	Low		High

Other Aromatics

Fruity	Low		High
Pineapple	Low		High

Fungal

Mouldy	Low		High
Mushroom	Low		High

Mouthfeelings

Sharp	Low		High

Fundamental tastes

Bitter	Low		High
Sweet	Low		High

Figure 3 The panellist sensory marking sheet

3.3.3 Calculations and statistics

A large quantity of data points was collected. The sensory profiles were measured in millimetres from the marking sheets (Figure 3) and converted to a dimensionless number by dividing by the total length of the line, which was 186 mm. Each data point had the following format:

Samples:	Control and EMCs made with protease only, lipase only, lipase and protease, 1:1 blend of lipase only and protease only and soup.
Panellist:	BW, BS, DO, LT, MC or NS
Run:	Run 1, Run 2 or Run 3.
Replicate:	Replicate 1, Replicate 2 or Replicate 3
Descriptor:	Buttery, Cooked Milk, Dairy Fat, Dairy Sweet, Butyric Acid, Goaty, Sweaty, Waxy, Fruity, Pineapple, Mouldy, Mushroom, Sharp, Bitter or Sweet.

Total data points = No. Samples x No. Tasters x No. Runs x No. Replicates x No. Descriptors

= 6 x 6 x 3 x 3 x 15

= 4860 points

3.3.3.1 *Panellist consistency*

The panellist's consistency was tested i.e. whether their three tasting replicates were similar. The standard error was calculated for each panellist for each sample and descriptor. So if for example BW taste tested three replicates of Run 1 of the lipase only sample for the Buttery descriptor and got scores of 0.156, 0.081 and 0.124 then the standard error would be 0.022. If all these standard errors were averaged for each panellist then they would give an indication of panellist consistency.

3.3.3.2 *Flavour wheel of flavour descriptor*

The dimensionless means of the descriptors were sorted according to the standard deviation of the means for each descriptor to aid the visualisation of the data. The data were then plotted clockwise on a flavour wheel so that the descriptor with the least score variation appears at the 12 o' clock position with increasing score variability through the revolution.

3.3.3.3 *Plot of descriptor error bars*

The mean dimensionless scores for each descriptor and sample were plotted together with standard error bars.

3.3.3.4 *Principal Components Analysis*

Principal components analysis (PCA) was carried out on the data using XLSTAT v4.3 which is an add-in for Excel 97 for Windows. The software used the standard method of calculating the correlation coefficients and from these the independent factors. From this the two eigenvectors that account for the maximum variance were plotted against each other. Two plots were created, one each for the samples and the descriptors.

3.4 COLOUR PLATES OF EXPERIMENTAL WORK

Plate 1 Frozen curd

Plate 2 Curd slurry in liquidiser together with enzymes and other additives

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Plate 3 Batches of EMC in incubator

Plate 4 TNBS-PTA-FAN solutions and spectrophotometer

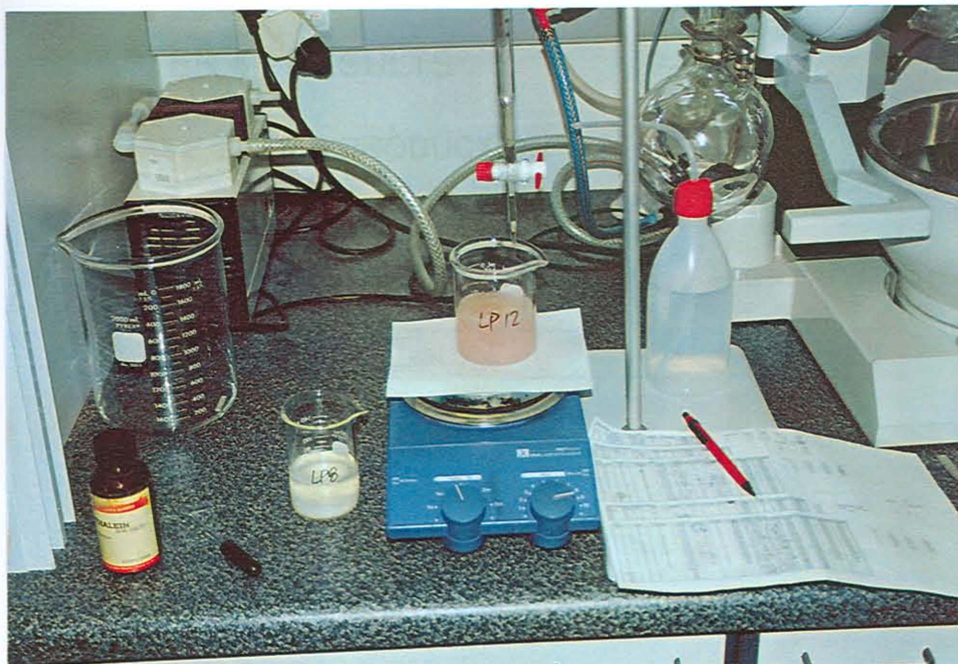

Plate 5 FFA titrations

Plate 6 Taste testing samples, carrot, apple and sensory reference standards

CHAPTER 4 - RESULTS

4.1 PHASE 1 - EMC PRODUCTION

4.1.1 Microbiological safety

Table 10 Pathogen and total plate counts for a CEMC sample

Test type	Count* (cfu/g)
Total plate count	1000
Coliforms	<10
Escherichia coli	0
Staphylococcus aureus	0
Bacillus cereus	100
Clostridium perfringens	0
Salmonella	0

*Detection limit is 10 cfu/g

Table 10 shows that negative pathogen results were obtained for all but *Bacillus cereus*, which accounted for 10% of the total plate count.

4.2 PHASE 2 - CHEMICAL ANALYSIS

4.2.1 FAN plot

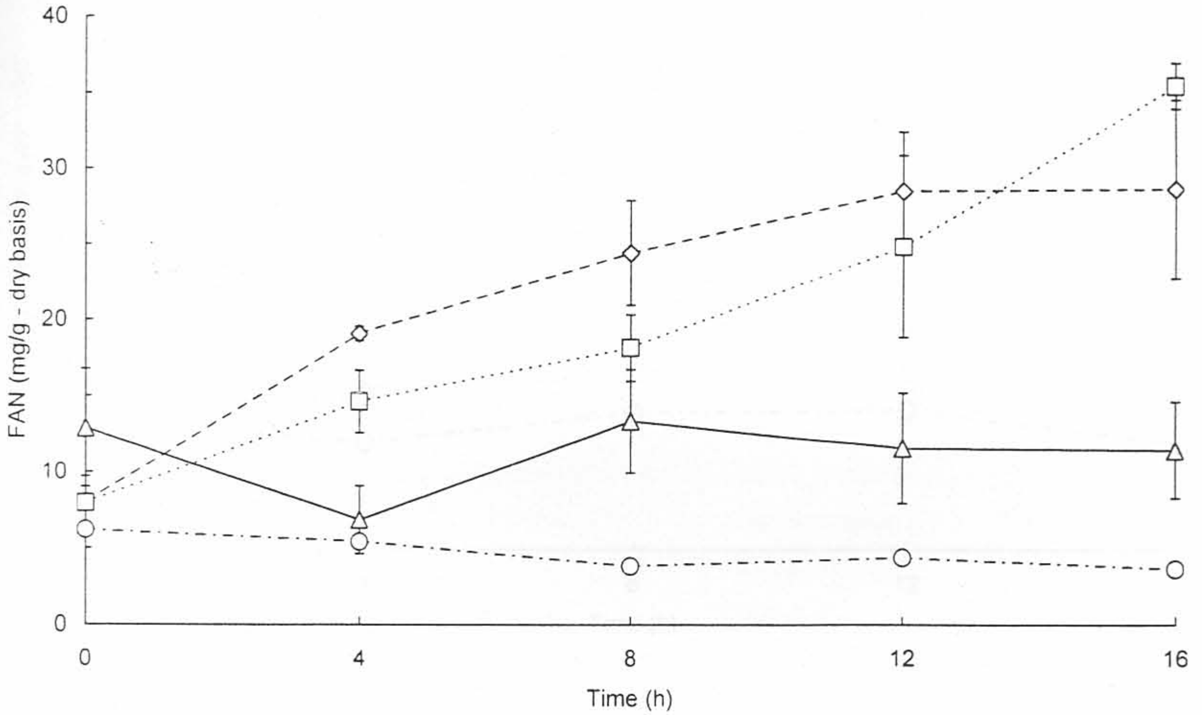

Figure 4 Effect of lipase and protease addition on FAN production from Cheddar curd. Mean FAN concentrations including standard error bars are plotted against time: control (○), protease only (□), lipase only (Δ) and lipase and protease (◊).

When protease was added to the curd there was an increase in FAN with incubation time. No increase in FAN occurred in the control (no enzyme added) or with lipase addition, although FAN levels were generally higher when lipase was added together with protease.

4.2.2 FFA plot

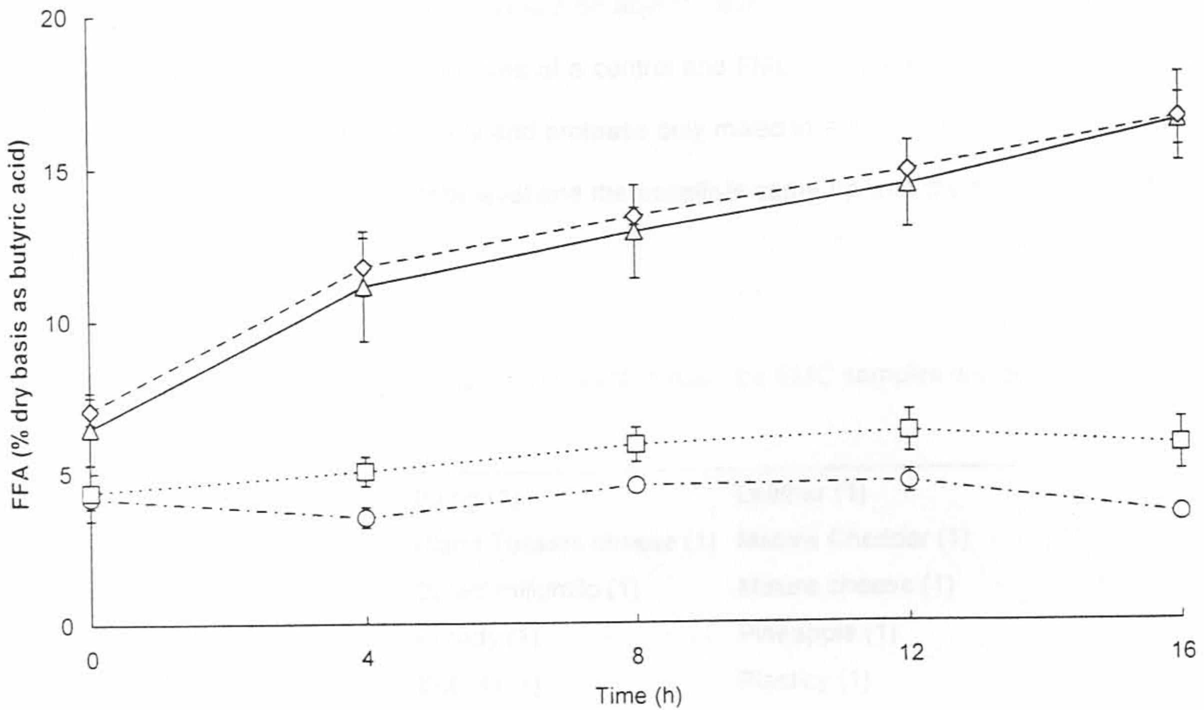

Figure 5 Effect of lipase and protease addition on FFA production from Cheddar curd. Mean FFA concentrations including standard error bars are plotted against time: control (○), protease only (□), lipase only (Δ) and lipase and protease (◊).

When lipase was added to the curd, there was an increase in FFA with incubation time. No increase in FFA occurred in the control (no enzyme added) or with protease addition, although FFA levels were generally higher when protease was added together with lipase.

4.3 PHASE 3 - SENSORY ANALYSIS

4.3.1 Lexicon determination

Initially it was thought that the panellists would be able to develop their own lexicon. As part of their training they were given unlabeled samples of a control and EMC made with lipase only, lipase and protease, protease only, and lipase only and protease only mixed in a 1:1 ratio. All the samples were diluted in an umami soup at the 1.75% level and the panellists came up with the terms listed in Table 11.

Table 11 Flavour descriptors used by taste panellists to describe EMC samples diluted in a savoury base

Butyric acid/vomit/sicky (5)*	Biting (1)	Leather (1)
Bitter (4)	Bland Tussers cheese (1)	Mature Cheddar (1)
Creamy (4)	Boiled milk/milo (1)	Mature cheese (1)
Fruity/pineapple (4)	Bready (1)	Pineapple (1)
Cheesy (3)	Buttery (1)	Plasticy (1)
Cooked/cooked milk (3)	Cereal (1)	Rancid milk (1)
Salty (3)	Cheddary (1)	Raw floury pasta (1)
Sweet milk (3)	Chicken soup (1)	Ripe (1)
Blue (2)	Choking (1)	Rooty (1)
Goaty (2)	Croutons (1)	Sharp (1)
Mouldy / mouldy cheese (2)	Decayed veg (1)	Smelly feet (1)
Mushroom / mushroom soup (2)	Earthy (1)	Spinach (1)
Processed (2)	Eggy (1)	Umami/rounded (1)
Rounded (2)	Fatty (1)	Wet dog smell (1)
Sweet (2)	Fermented vomit (1)	
Waxy (2)	Hydrolysed Veg. Protein(1)	

*Number of panellists who selected the item

Most of the terms can be consolidated into Heisserer & Chambers's lexicon (Heisserer & Chambers, 1993) as was done in this study. Their comprehensive study on what descriptors and their relevant references were suitable for taste testing cheeses was used.

4.3.2 Panellists consistency

Table 12 Mean standard error of panellist scores across all samples and descriptors

Panellist	Mean standard error
BW	0.0273
DO	0.0289
MC	0.0321
BS	0.0391
NS	0.0438
LT	0.0545

The mean standard errors for the panellists were of similar magnitude, with a range of 0.027-0.055. BW was the most consistent taste panellist while LT was the least consistent.

4.3.3 Sensory profile

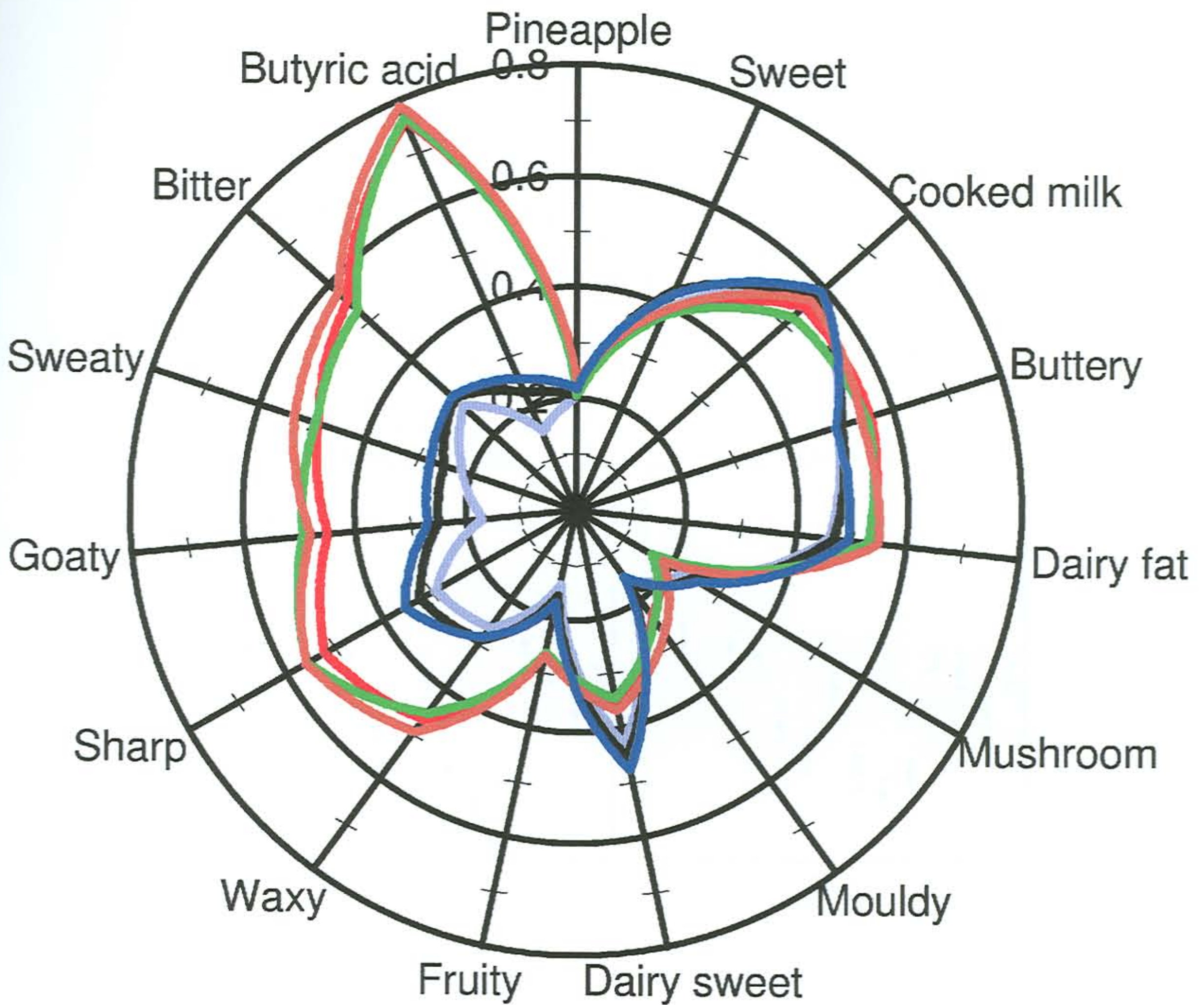

Figure 6 Comparison of sensory profiles of EMCs made with added protease and lipase, diluted in an umami soup: curd control (—), protease only (—), lipase only (—), lipase and protease (—), 1:1 blend of lipase only & protease only (—) and sensory control - soup only (—). The mean dimensionless flavour scores for each descriptor and product were plotted clockwise with increasing standard error between the samples.

The flavour wheel shows that Pineapple had the least variation across the samples whereas butyric acid had the greatest variation. The lipase-treated samples clearly followed the same trend, which was distinctly contrasted from the non-lipase-treated samples. For most descriptors the lipase-treated products scored higher than did those not treated with lipase. Little difference was observed between the products containing no added lipase, but Soup did tend to have lower scores than P and Control for Sharp, Goaty, Sweaty and Butyric acid.

4.3.4 Plot of descriptor error bars

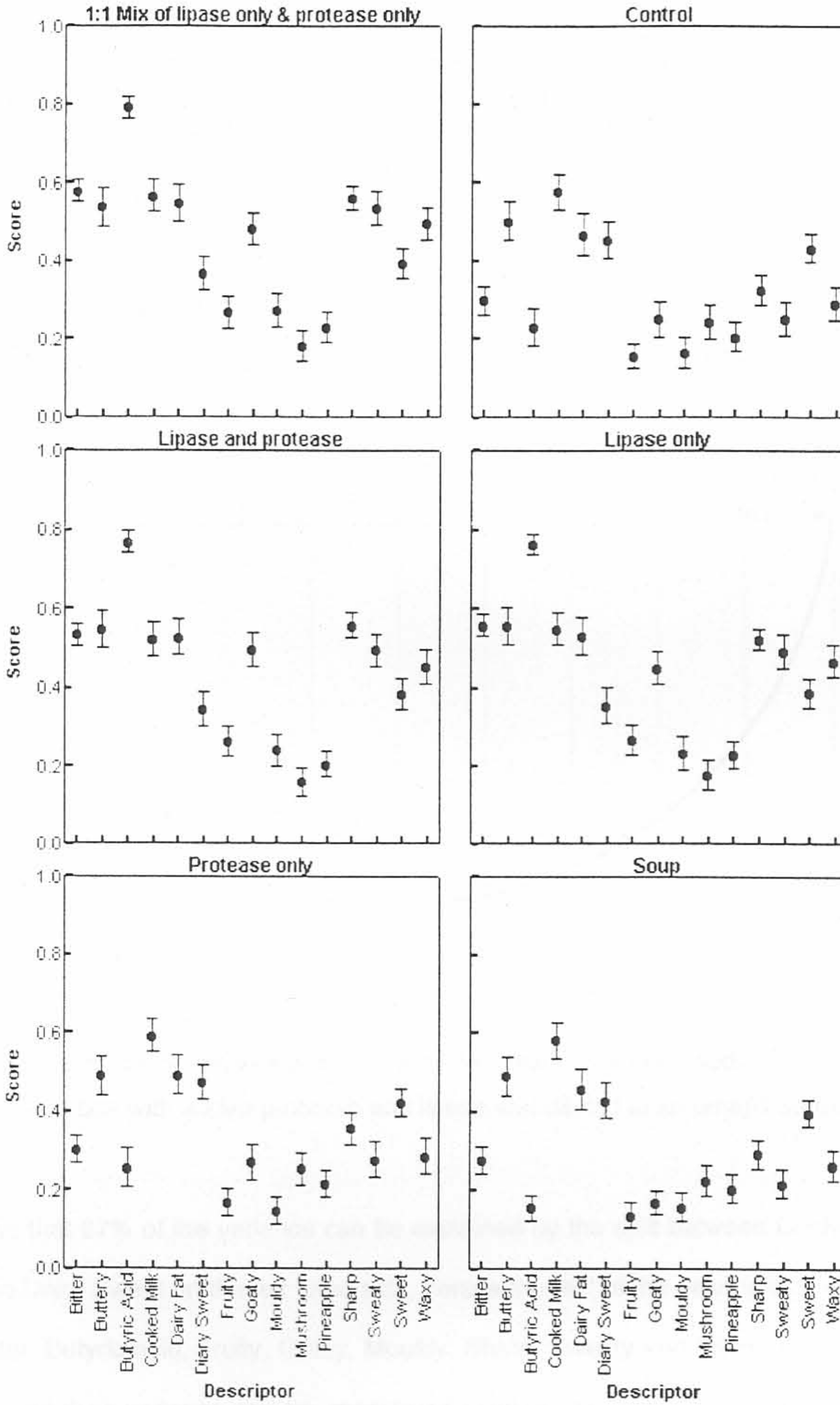

Figure 7 Comparison of the standard errors of the flavour descriptors used in the sensory profiling of EMCs made with added protease and lipase and diluted in an umami soup.

The majority of the error bars for the descriptors were of the same magnitude and the error bars for some descriptors overlap for all samples, e.g. Goaty and Sweaty.

4.3.5 Principal component analysis

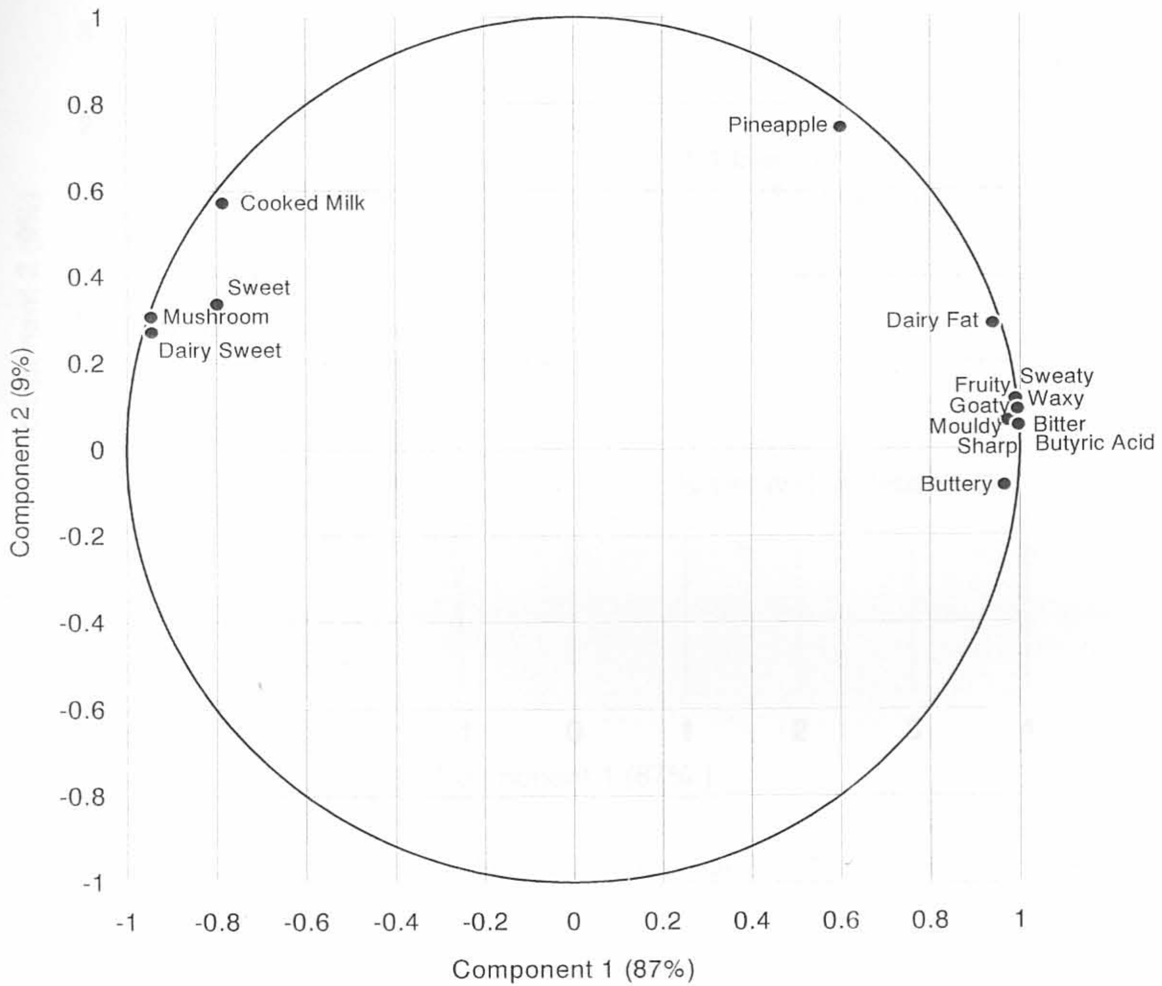

Figure 8 Principal component analysis plot of flavour descriptor variance used in the sensory profiling of EMCs made with added protease and lipase and diluted in an umami soup.

Figure 8 shows that 87% of the variance can be explained by the split between Cooked Milk, Sweet, Mushroom and Dairy Sweet on the left hand side, versus the rest of the descriptors on the other side. The terms Bitter, Butyric acid, Fruity, Goaty, Mouldy, Sharp, Sweaty and Waxy are all grouped close to each other and their variance can be considered identical as compared to the other descriptors. The differentiation on the second axis that explains 9% of the variance is less marked with Buttery showing a negative correlation as compared to the other descriptors that show a positive correlation.

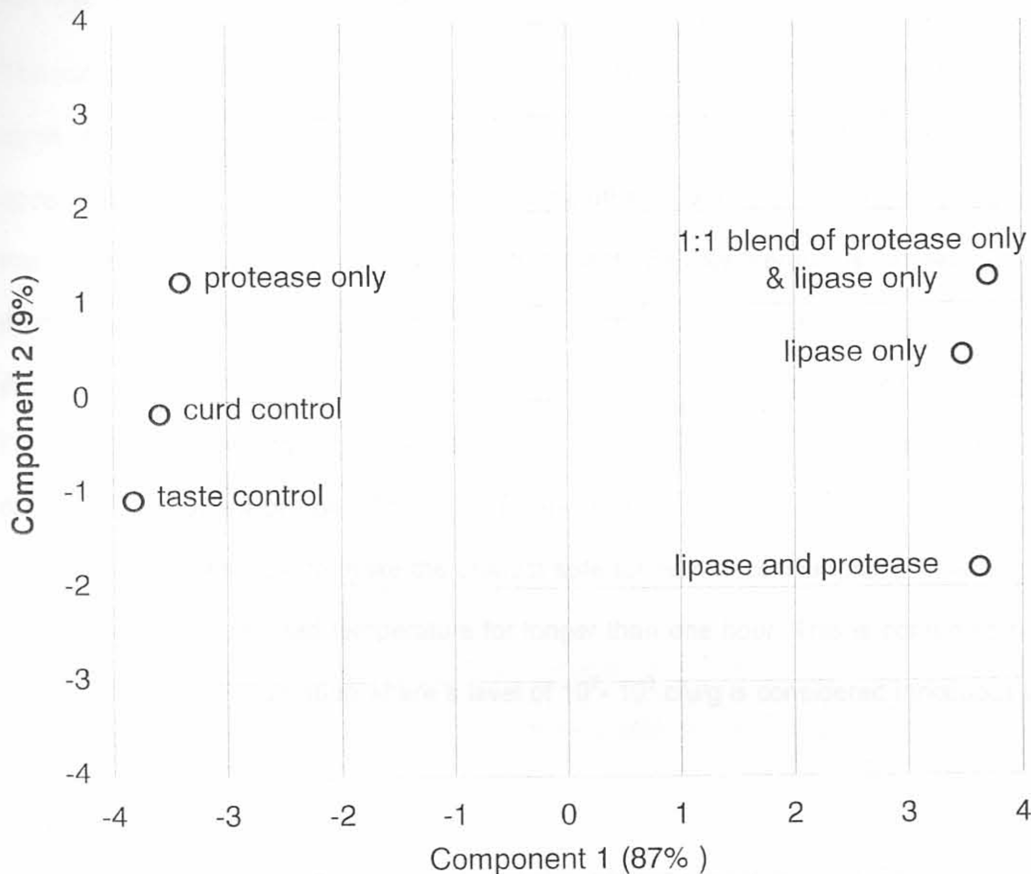

Figure 9 Principal component analysis plot of the flavour variance of EMCs made with added protease and lipase and diluted in an umami soup.

EMCs can be divided into two groups: those with protease only, curd control and taste control on the left (with a negative effect of component 1) and 1:1 blend of lipase only and protease only, lipase only and lipase and protease on the right (positive effect of component 1) and this division explains 87% of the variance. The 9% of variance accounted for by component 2 is less marked but is acting most strongly on lipase and protease.

CHAPTER 5 - DISCUSSION

The pathogen *Bacillus cereus* was found during microbiological testing of the EMC and it must have come either from the substrate, ingredients or from the equipment used during processing. It accounted for 10% of the total plate count so the other 90% must have been non-pathogenic bacteria that would have survived the mild heat treatment. *Bacillus cereus* is an aerobic mesophile that produces detectable toxin levels at 10^7 cfu/ml which can cause diarrhoea and vomiting (Jay, 1996). *Bacillus cereus* contamination is not allowed in food as regulated by South Africa law (Foodstuffs, Cosmetics and Disinfectants Act 54/1972 - GNR 692/1997). According to Professor B.H. Bester (pers. comm. - Department of Food Science, University of Pretoria, South Africa, 1999) the count of 100 cfu/g was sufficiently low to make the product safe for human consumption, if the EMC soup product was not left at an elevated temperature for longer than one hour. This is confirmed by the proposed amendments to the legislation where a level of 10^2- 10^3 cfu/g is considered innocuous (Department of Health, 2000).

More severe thermal processing would probably not reduce the level of *Bacillus cereus* since it is a spore former and the spores will survive 80°C (Rosenthal, 1991). Increasing the temperature of the heat treatment may also reduce the quality of the flavour. As the principles of HACCP state (Jay, 1996), it is better to first use raw materials of good quality (i.e. with low microbial counts) and if this is not possible then the process must be modified to guarantee that the microbial count is reduced to acceptable levels. Since no bacterial pathogen counts were done on the curd it was not known whether the contamination came from the curd or the processing. The potassium sorbate is primarily effective against moulds though it is known to be effective against some bacteria also (Jay, 1996). Potassium sorbate does not appear to be the correct preservative to keep the levels of *Bacillus cereus* down though the preservative may have had a bacteriostatic effect and kept the contamination at a constant level.

The FAN increased over the course of the incubation in those samples that contained added protease, which was expected, though there were significant variations between the replicates. In the EMCs with no added protease, the FAN remained constant which means that any proteases, either endogenous to milk or from lactic acid bacteria, were denatured during the pre-heat treatment

process. There was no difference between EMCs made with protease only and those made with lipase and protease. This is to be expected since the lipase preparation should not have contained any protease or FAN.

The FAN levels in this study ranged from 5-40 mg α-amino nitrogen/g curd (dry basis). Since the EMC has approximately 45% moisture this equates to approximately 1.6-12.8 μmoles/g FAN (wet basis) using a conversion factor derived from Jarrett *et al.* (1982). The upper FAN value fits into Table 13 at about the 1 month old level of Cheddar cheese. This shows that an EMC should have up to 10 times more FAN than the EMCs from this study. The very low levels of FAN compared to other EMCs and Cheddar cheese show that if more protease had been added then perhaps the relative system errors would have been lessened. If the FAN levels had been 10 times higher, the errors for the samples containing no added protease would have appeared less in relation to the maximum FAN values. As resolution is increased, the larger the amount of random errors will be since the level is closer to the natural background FAN values.

It is difficult to say from the rate of FAN production what the level of activity of the protease was at the end of the incubation. The errors in the FAN data make this kind of interpretation difficult so more research would be required to determine the stability of proteases in the production of EMCs. All the FAN data also show that the FAN curves appear to intersect before 0 h, which is probably due to a delay between when the enzymes were added to the curd slurry, and putting the curd slurry bags into the incubator. Zero time was only recorded when the bags were introduced into the incubator.

The low levels of FAN obtained with the recommended dosage of protease are a cause for concern. Two potential causes are:

1. At the normal curd pH of 5.5 (Rosenthal, 1991) the endo-proteinase activity is 50% of maximum and Leucine amino-peptidase activity was 30% of maximum (BioCatalysts, 1996). The optimum is pH 7. (Table 6).
2. BioCatalyst's catalogue suggest 1-2% protease on casein for pure protein-based flavours. Approximately 40% of the dry solids in Cheddar cheese are protein and 80% of the proteins are caseins (Rosenthal, 1991). Since 100g of cheese solids would contain 32 g of casein,

Table 13 Published free amino nitrogen values for Cheddar cheese and Cheddar enzyme modified cheese (wet basis)

Cheddar product	Age	FAN (µmoles/g)	Method‡	Reference
Cheddar cheese	1 d	14	PTA-TNBS	Jarrett *et al.* (1982)
Cheddar cheese	1 mo	14	PTA-TNBS	Aston, Grieve, Durward & Dulley (1983b)
Cheddar cheese	1 mo	16	PTA-TNBS	Aston *et al.* (1983a)
Cheddar cheese	1 mo	54	PTA-TNBS	Jarrett *et al.* (1982)
Cheddar cheese	2 mo	21	AAA	Law & Wigmore (1982)
Cheddar cheese	2-3 mo	49†	AAA	Moskowitz & Noelck (1987)
Cheddar cheese	3 mo	33	PTA-TNBS	Aston *et al.* (1983b)
Cheddar cheese	3 mo	34	PTA-TNBS	Aston *et al.* (1983a)
Cheddar cheese	3 mo	94	PTA-TNBS	Jarrett *et al.* (1982)
Cheddar cheese	4 mo	64	AAA	Law & Wigmore (1982)
Cheddar cheese	6 mo	65	PTA-TNBS	Aston *et al.* (1983a)
Cheddar cheese	6 mo	71	PTA-TNBS	Aston *et al.* (1983b)
Cheddar cheese	6 mo	169	PTA-TNBS	Jarrett *et al.* (1982)
Cheddar cheese	8 mo	118†	AAA	Fox & Wallace (1997)
Cheddar cheese	9 mo	98	PTA-TNBS	Aston *et al.* (1983a)
Cheddar cheese	9 mo	114	PTA-TNBS	Aston *et al.* (1983b)
Cheddar cheese	unknown	184†	AAA	Fox *et al.* (1996)
Cheddar EMC	unknown	127†	AAA	Moskowitz & Noelck (1987)

† Original data in mg/g FAN and converted to µmoles/g FAN using the molecular weight for Leucine.

‡ PTA-TNBS - FAN measured using a spectrophotometer, phosphotungstic acid (PTA) and trinitrobenzene sulphonic acid (TNBS)

‡ AAA - FAN calculated by addition of individual amino acid levels measured with an Amino Acid Analyser (AAA).

1-2% enzyme on casein would require a multiplier of 32/100 and therefore the protease usage should be 0.32-0.64% enzyme addition on a dry basis and not 0.1% as suggested.

If one combines the two effects i.e. increase from 50% efficiency to 100% efficiency by using pH 7 and 3.2 to 6.4 times more Promod the FAN levels would have been 6 to 13 times higher. As discussed, the FAN levels only need to be 10 times higher to be comparable to the published EMC values. The optimum pHs for the lipase and protease are different (Table 6) so perhaps it might be better to create lipase only and protease only EMCs both of which had their pH adjusted to match the optimum pH of their respective enzymes. As mentioned previously, this is the component approach to EMC manufacture but its main drawback is that any flavour compounds created by reactions between

the FAN and FFA and their breakdown products is lost. Fox & Stepaniak (1993) consider this synergy important for cheese flavour.

The EMC was taste tested at the 1.75% level in an soup and from Table 13 it can be seen that the FAN levels in the soup would be approximately 500 times lower than that of a 6 month old cheese. The flavour thresholds of individual amino acids in solution have a range of 3-300 mg / 100 ml (Fox & Wallace, 1997) and since the highest total FAN would have been 1.7 mg / 100 ml in the soup, it is not possible for any of the free amino acids to have had an impact on the flavour of the EMC in solution.

The FFA error bars for EMC made with lipase only and the product made with lipase and protease indicate that there was no difference between them. There is a difference between the FFA values of the EMC made with protease only and the control that shouldn't exist since they both contained no added lipase, but this difference is probably not significant. There is a possibility of the protease preparation containing a small amount of lipase but since there is no difference between the EMCs made with lipase only and lipase and protease this is probably not the case. Another possibility is that since the FFA method is an acid/base titration the FAN would have been detected as FFA since they are also acids. Again this is probably not the case since no such difference was detected between the EMCs made with lipase only and protease only.

Errors exist in the FFA results, especially for the EMC containing lipase only. The FFA data range was 4-17% butyric acid on dry basis, which with approximately 45% moisture equates to about 2-8% FFA as butyric acid on wet basis. Some published FFA values are shown in Table 14 for comparison. It was assumed that the authors reported the FFA on a wet basis where it was not specifically mentioned. The upper value of FFA for the products equates to that of a 20x strength EMC. A 20x strength EMC appears to have over 70 times the amount of FFA than a normal Cheddar but yet is only 20x in strength so there is not a direct proportional relationship between FFA levels and EMC strength. As discussed in the literature review, the strength of an EMC is not directly dependant solely on one component such as FFA, but is a combination of factors including some not yet known.

Table 14 Published free fatty acid values for Cheddar cheese and Cheddar enzyme modified cheese

Product	Age/strength	FFA (% as butyric)	Reference
Cheddar cheese	2-3 mo	0.2	Moskowitz & Noelck (1987)
Cheddar cheese	3 mo	0.143	Jeon (1994)
Cheddar cheese	4 mo	0.231	Deeth *et al.* (1983)
Cheddar cheese	4-6 mo	0.109	Jeon (1994)
Cheddar cheese	8 mo	0.1038	Fox & Wallace (1997)
Cheddar cheese	7-12 mo	0.146	Jeon (1994)
Cheddar cheese	unknown	0.075	Law & Goodenough (1995)
Cheddar cheese	unknown	0.09	Godfrey & Hawkins (1991)
Cheddar EMC	5x	1.26	Talbott & McCord (1981)
Cheddar EMC	5x	4.22	Talbott & McCord (1981)
Cheddar EMC	20x	7.39	Talbott & McCord (1981)
Cheddar EMC	unknown	5.9	Moskowitz & Noelck (1987)

The lipase enzymes were active at the end of the 16 h, as can be seen by the upward trend over the course of the incubation and the lack of any plateau. Decreasing the enzyme concentration and either increasing the incubation temperature or time to get the same end FFA concentrations could reduce the EMC production costs. This potential decrease in production costs must, however, be balanced against a potential increase in contamination caused by a longer incubation time (BioCatalysts, 1996). BioCatalyst's catalogue also stated that temperatures of 50°C cannot be used for long incubation times. Contamination studies over 8 h, 16 h, 24 h, 32 h should be done to see whether BioCatalysts' concerns about increased contamination are valid. Our samples were contaminated with *Bacillus cereus* and it would be interesting to see if a shorter incubation with higher amounts of enzymes would reduce this contamination to below detectable levels.

The FFA measurement method was by acid/base titration and since curd is a buffer (Fox *et al.*, 1996) this could have affected the results. Fox *et al.* (1996) reported that curd is a stronger buffer at pH 4.5-5.5 than at pH 5.5-6.5 and since Cheddar cheese typically has a pH of 5.0-5.5 (Rosenthal, 1991), errors in the results are to be expected. More comprehensive methods are available to eliminate the buffering effect of the milk protein, in which the FFA are turned into esters which are then measured using a GC. However, these methods are more time consuming and expensive than a titration (Deeth

et al., 1983). The buffering action should have affected all the FFA results to the same degree so it does not explain the errors.

Taste testing was done at the 1.75% level of EMC in an umami soup so the maximum FFA of 8% would be diluted to the 0.15% level in the soup, which would be approximately the same as that of a 6 month old cheese (Table 14). Talbott & McCord (1981) recommended that their 20x EMC be used at the 0.5-1% level in a product formulation. Since the EMCs from this study had roughly the same levels of FFA as the 20x EMC had, taste testing at the 1.75% level means the taste testing was done at about twice Talbott & McCord's recommended FFA level.

From the chemical analysis and results quoted in the literature it can be seen that the FFA levels are correct but the FAN levels are almost 450 times too low when diluted in the soup. This means that the FFA levels (which were at the right level) would override any flavour contributed by FAN. This also means that no synergy would be observed since the FAN levels are too low and so essentially the results can be reduced to lipase and non lipase-treated EMCs. It appears as if the suggested enzyme usage levels given by BioCatalysts were meant to be starting figures that were to be iterated upon until the right flavour was found. The suggested FFA levels appear to be correct but the FAN levels were too low to have any sensory effect so the next iteration must be an increase in protease activity by 10 times.

The taste panellists were similar in their taste testing consistency and very little assistance was required in selecting terms since they were all experienced in sensory work as part of their jobs. The standard error of 0.04 equates to an error of about 4% which, though high for chemical testing, is normal for sensory testing (3% - Aston *et al.*, 1983a; 4% - Law & Wigmore, 1982).

The primary sensory differentiating factor between the EMCs is the absence or presence of lipase since the flavour wheel shows that the sensory results follow two different trends, with the lipase-treated EMCs following one trend and the non-lipase-treated EMCs another different trend. A secondary factor on the flavour wheel affected the flavour of the non-lipase EMCs in the following decreasing order: protease only > Control > Soup, though there was no real difference between the

EMCs when the error bar chart is studied. The flavour of the lipase-treated EMCs was still cheesy and reasonably balanced according to the panellists, which confirms what the literature survey showed, namely that FFA are important for good EMC flavour.

The errors for all the descriptors across all products were of the same magnitude, which shows that the panellists found the terms equally easy or difficult to quantify. The error bars show that the minor differences observed on the flavour wheel between lipase-treated EMCs are not significant. Likewise for the non-lipase-treated EMCs, confirming once again that protease might as well have not been used since the Control scored the same as the EMC made with protease only.

The error bars for some of the terms overlapped e.g. Goaty and Sweaty and so potentially they could have been amalgamated. This would have reduced the time for sensory evaluation and satisfied the objective of descriptive analysis which is to find a minimum number of descriptors that will convey a maximum amount of information regarding the sensory characteristics of a product (Stampanoni, 1993). Pineapple could be eliminated since it scored the same for all samples. However this reduction in terms might only hold true for this study since Heisserer & Chambers (1993) found that all the descriptors used in this study were separate descriptive terms when used in the sensory evaluation of cheese.

On the PCA plot for descriptors, Component 1 clearly represents the presence/absence of lipase, which explained 87% of the variance since all the terms on the right (positive effect) were the descriptors that received high scores for lipase-treated EMCs. Those on the left (negative effect) were the descriptors in which lipase-treated products scored lower than the non-lipase-treated products. The point for Pineapple is not grouped with the rest of the descriptors but then it consistently got low scores and thus did not contribute to the product differentiation. Component 2 accounted for another 9% of the variation but it is difficult to identify what this represents. It could be the absence or presence of protease but there is little evidence to support this. The terms Mouldy, Fruity, Waxy, Sharp, Goaty, Sweaty, Bitter and Butyric acid appear so close together on the PCA plot that they could be considered identical in their variance. These descriptors also score high on the flavour wheel for lipase-treated EMCs and so they appear to be good indicators of lipase based flavours. If one was

looking for sensory differentiation between lipase and non-lipase-treated EMCs rather than sensory description then some of these terms could be eliminated (e.g. Sharp and Waxy) since they have similar scores for both lipase and non lipase-treated EMCs.

The PCA plot of EMC samples confirms that 87% of the variance (Component 1) could be explained by the absence (negative effect) or presence of lipase (positive effect). Component 2 accounted for 9% of the variance and might represent flavour strength since the strength of FFA was strongest in the 1:1 mix of lipase only and protease only and less but equivalent in lipase only and lipase and protease and the strength of FAN in protease only>Control>Soup. The reason why the 1:1 mix of EMCs made with lipase only and protease only may have been stronger than either lipase and protease or lipase only is that lipase only and protease only were mixed in a 1:1 ratio and then tasted at a double concentration. This means that since protease only, contained a base level of FFA and lipase only, contained a base level of FAN (and their resultant reaction and breakdown products) the resultant mix would have a higher concentration of FFA or FAN when it was tasted at twice the concentration as compared to lipase only, protease only or lipase and protease.

The sensory work confirms the results of the chemical analysis. The results can be reduced to two sets i.e. lipase-treated and non-lipase-treated EMCs since the FAN levels were too low to affect the sensory data.

CHAPTER 6 - CONCLUSIONS & RECOMMENDATIONS

It was shown that an EMC could be successfully produced using local curd and equipment. Even with the skewed results from low FAN levels a good EMC flavour in those EMCs made with added lipase was obtained. This flavour should be further improved with further iterations in enzyme concentrations.

The EMCs were contaminated by a pathogen (*Bacillus cereus*) that is innocuous at the levels detected. This contamination must be monitored in any future trials since higher levels than those detected would make the product unfit for human consumption. Perhaps an additional preservative (e.g. Nisin) could also be used that is known for its effectiveness against bacteria.

As expected, FAN only increased where protease was added and the FFA only increased where lipase was added. In absolute terms the FAN results were not consistent with those expected and were about 10 times too low as compared to published values. The low FAN values appear to be due to the enzyme concentration recommended verbally by BioCatalysts being 6 times lower than that recommended by their catalogue. An approximate 50% reduction in the protease activity from the pH not being at the optimum also contributed to the iow FAN values. The FFA results were consistent with those recorded in literature. It is recommended that this study be repeated with 10 times higher levels of protease and the same amount of lipase.

The lipase was still active at the end of the incubation so it would be interesting to see whether reducing the enzyme concentrations and increasing the incubation time would get the same end result. Any increase in incubation time would have to be balanced against decreased enzyme stability and increased microbial counts.

Sensory profiling was carried out successfully in this study and produced good results with the inevitable variances caused by differences in personal sensory perception. The use of the standard lexicon from Heisserer & Chambers (1993) for the sensory profiling of cheese reduced the initial work required to set up descriptors and will also allow this work to be compared to that of others. Some of

their descriptors could have been amalgamated for this study since they gave similar sensory results for all EMCs but whether this was due to the low FAN values is unknown.

The low contribution of FAN to the flavour of the EMCs was detected by the flavour wheel and statistically by the principal component analysis since the results for the EMC made exclusively with protease wasn't that different from the control. This confirmed the analytical results, which shows that that certain key indicators such as FFA can be used to predict sensory quality to a limited degree.

The FAN levels were below the flavour threshold during sensory profiling yet the EMCs were still considered cheese like which confirms the findings of some authors that FFA are the major contributor to EMC flavours.

People in developed countries are eating more pre-prepared meals than decades ago, and a trade journal reported that in some countries like the United States, more than half of the under-25 population has never cooked a meal (Convenience Food Systems, 2000). This appears to be a trend that is likely to increase use of EMC in pre-prepared meals, snacks and instant sauces since cheese is a popular flavour which can readily be emulated by EMCs.

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