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.