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Flavouring reduced fat high fibre cheese products with enzyme modified cheeses (EMCs)

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ABSTRACT

Medium (13%) and low (2%) fat imitation cheeses (pH 6 or 5.5) were flavoured with 5% w/w EMC containing 16%, 28% or 47% total free fatty acids (low to high levels of hydrolysis, respectively) and were examined by a sensory panel. Aroma active short-chain free fatty acids were monitored using gas chromatographic techniques. Regardless of cheese pH or EMC composition, panellists ranked all medium-fat cheeses similarly. Low-fat cheeses flavoured (pH 6 or 5.5) with low or medium lipolysis EMC were described as 'well-balanced' and 'cheesy' and were significantly more preferred to cheeses containing high hydrolysis EMCs. Low-fat cheeses were least preferred of all cheeses because of 'very intense' bursts of off-flavours. Lower pH cheeses were softer and less melting. Higher fat levels in imitation cheese modulated a greater retention of fat-based flavour compounds and improved their release during consumption more than did lower fat levels.

1. Introduction

With better understanding by consumers of the link between diet and health, there is increasing pressure on the food industry to facilitate a reduction in the amount of fat and sugar and an increase the amount of fibre consumed by the population (COMA, 1991). Food manufacturers have responded by introducing, to the market, a number of low-fat and high fibre food products. However, it is well established that reducing the fat content of foods modifies the pattern of flavour release in the mouth on chewing, and significantly impairs the perception of this key sensory attribute (Taylor & Linforth, 1996).

Enzyme-modified cheeses (EMCs) are concentrated cheese flavours produced enzymatically from dairy substrates and are designed to provide a concentrated source of cheese flavour. Most EMCs are produced from cheese pastes made from immature cheese (West, 1996). The production of EMCs has increased, in part due to the inclusion of EMCs in zero and low-fat foods, replacing the flavour of full-fat cheese in such products (Freund, 1995).

Previous work from this research group indicated that the flavour of imitation cheese could be improved by the inclusion of EMCs with medium levels (28% total free fatty acids) of hydrolysis. Our studies showed that the levels of butanoic acid, a strongly aromatic short-chain fatty acid (SCFA), were fundamental in determining the intensity and acceptability of flavoured imitation cheese products (Noronha, Cronin, O'Riordan, & O'Sullivan, 2008).

It has been proposed that fat in natural cheese provides a fat–water–protein interface for flavour forming reactions to occur (Collins, McSweeney, & Wilkinson, 2003). In addition, fat acts as a solvent for fat-soluble flavour compounds, helping to modulate their retention in cheese (Olson & Johnson, 1990; Wijesundera & Drury, 1999).

One of the main objectives of this study was to attempt to produce acceptably flavoured high fibre, reduced and low-fat imitation cheese products. Resistant starch was used to replace the fat component of imitation cheese, as recent work from this laboratory has shown that reduced and low-fat imitation cheeses with acceptable mouthfeel and texture could be produced when resistant starch was used to substitute for the fat (Noronha, O'Riordan, & O'Sullivan, 2007). However, these fat-reduced cheeses lacked flavour and it was thought that the incorporation of EMCs might improve the flavour of such products, especially since EMCs are used regularly in the industry to confer cheese flavour on a number of processed foods, including cheese sauces and pies.

The strategy proposed for the present study was to examine the influence of fat (13% or 2%) and moisture levels (52% or 60%) on the flavour and textural properties of imitation cheeses containing EMCs of different levels of lipolysis. Previous work from this research group (Noronha et al., 2008) suggested that the pH of the cheese base influenced flavour perception in imitation cheeses flavoured with EMCs. Therefore, another objective of this study was to examine the influence of pH on flavour intensity and acceptance,

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as well as cheese functionality, in reduced and low-fat imitation cheese products.

2. Materials and methods

2.1. Imitation cheese ingredients

Three commercial EMC products were obtained as gifts from Kerry Ingredients Ltd. (Listowel, Co., Kerry, Ireland). All EMCs were prepared from the same starting substrate and enzymes but had been incubated for various times during manufacture, resulting in different levels of hydrolysis. All EMC products were in paste form and were stored at 4 °C prior to analysis.

Rennet casein was obtained from Kerry Ingredients Ltd. (Listowel Co., Kerry, Ireland). Rapeseed oil and hydrogenated palm oil were obtained from Trilby Trading Ltd. (Drogheda, Co., Louth, Ireland). Resistant starch, Novelose 240, was obtained from National Starch Ltd. (Manchester, England). All chemicals, including anhydrous disodium phosphate (Albright and Wilson Ltd., Cheshire, England), trisodium citrate and anhydrous citric acid (Jungbunzlauer GmbH., Pernhofen, Austria), sodium chloride (Salt Union, Cheshire, England) and sorbic acid (Hoechst Ireland Ltd., Dublin, Ireland), were of food grade quality.

2.2. Reagents for analytical measurements

The solvents, methanol, ethanol, hexane, propane and dichloromethane, were all obtained from Sigma–Aldrich Chemical Co. (Dublin, Ireland) as also were the fatty acid standards butanoic acid (C_4), iso-butanoic acid (iso-C_4), hexanoic acid (C_6) and 4-methylpentanoic acid (4-Me-C_5). Stock solutions (400 μg/ml) of these acids were prepared in distilled water, either singly or as appropriate mixtures when required.

Ethanolic KOH (0.5 M) for fat saponification was prepared by dissolving 2.8 g of KOH in 100 ml of 95% ethanol. Standard methanolic KOH (0.02 M) for determination of free fatty acids (FFAs) in EMC samples was prepared by dilution of standard 1 M KOH in methanol (BDH, Dublin, Ireland) with anhydrous methanol.

Solid phase microextraction (SPME) fibres (Carboxen/PDMS, 75 μm thickness) were obtained from Supelco (Supelco-Aldrich, Ireland) and were conditioned under a flow of nitrogen (10 ml/min) at 300 °C for 2.5 h prior to use.

2.3. Manufacture of flavoured imitation cheeses

Series of cheeses (4 kg batches) with 52% or 60% w/w moisture, 13% or 2% fat and pH values of 6.0 or 5.5 were manufactured in a Blentech twin-screw cooker (model CC-0010, Blentech Corporation, Rhonert Park, CA, USA), using the manufacturing procedure of Noronha et al. (2008). The formulations of flavoured imitation cheeses, expressed on a wet weight basis (% w/w), are shown in Table 1.

2.4. Compositional analysis of EMCs and flavoured imitation cheeses

The fat content of EMCs and flavoured imitation cheeses was determined by the Gerber method (National Standards Authority of Ireland, 1955), moisture by the oven drying method (IDF, 1958) and protein content by macro-Kjeldhal (IDF, 1993). The ash content was determined by the AOAC official method (AOAC, 2002). A glass/Ag/AgCl pH electrode attached to a Unicam 9450 pH meter (Unicam Ltd., Cambridge, UK) was used to measure the pH directly. The salt content of EMCs and imitation cheeses was determined using the potentiometric method of Fox (1963).

Table 1
Ingredient formulation (% w/w) for imitation cheese bases

Ingredients (% w/w)	13% fat, pH 6	13% fat, pH 5.5	2% fat, pH 6	2% fat, pH 5.5
Water	52.000	52.000	60.000	60.000
Rennet casein	23.194	23.194	19.701	19.701
Novelose 240	10.205	10.205	15.609	15.609
Hydrogenated palm oil	3.765	3.765	0	0
Rapeseed oil	7.424	7.424	1.792	1.792
Trisodium citrate	0.922	0.922	0.783	0.783
Disodium phosphate	0.426	0.426	0.362	0.362
NaCl	1.450	1.450	1.232	1.232
Sorbic acid	0.085	0.085	0.072	0.072
Citric acid	0.529	1.058	0.449	0.898

2.5. Measurement of total free acids and SCFAs

Total free fatty acids in EMCs and SCFAs in both EMCs and imitation cheeses were determined as described by Noronha et al. (2008). Anhydrous fat samples were isolated from the EMCs and the extent of lipolysis therein was evaluated using a titrimetric method to measure their free fatty acid contents. The aromatic SCFAs butanoic (C_4) and hexanoic (C_6) acids were measured by a GC headspace method using an internal standard method for EMCs and a modification of this, using external standards (iso-butanoic (iso-C_4) and 4-methyl pentanoic acids (4-Me-C_5)) for the imitation cheese samples.

2.6. Sensory analysis of flavoured imitation cheeses

2.6.1. General

A sensory evaluation of the imitation cheeses was conducted by an untrained 16-member panel. The panel of assessors comprised 10 males and 6 females aged between 25 and 65 years, selected from the University College, Dublin, School of Agriculture, Food Science and Veterinary Medicine. All panellists were seated in separate booths and samples were presented under a red/green light to avoid visual bias. Prior to assessment, each cheese was cut into 10 g cubes and equilibrated to room temperature (21 °C). Panellists were presented with samples that had been heated by placing aluminium foil-covered cheese cubes (1 cm) in a pre-heated fan oven at 200 °C for 4 min and subsequently cooled on the bench for 1 min to 60 °C. The panellists were instructed to taste and assess the hot samples immediately after unwrapping.

2.6.2. Ranking test

Panellists were asked to rank the products based on preference for flavour and mouthfeel using the method of Meilgaard, Civille, and Carr (1991). Panellists were presented with cheese samples containing either 52% moisture and 13% fat or 60% moisture and 2% fat at pH levels of 6.0 or 5.5. Samples were formulated using 5% w/w of EMCs A, B or C. In all cases, the assessors were instructed to evaluate samples based on overall flavour and mouthfeel using a score from 1 (most preferred sample) to 3 (least preferred sample). Panellists were also instructed to report any descriptors arising from their observations of the sensory characteristics of the cheese samples.

2.6.3. Paired preference test

Panellists were presented with two cheese samples and asked to choose which they preferred using the method of Meilgaard et al. (1991).

2.7. Texture profile analysis (TPA) of flavoured imitation cheese

Texture profile analysis was performed on cheese samples using the Instron Universal testing machine model 5540 (Instron corp., Canton, MA), fitted with a 100 N load cell and 35 mm diameter plates using the method of Mounsey and O'Riordan (1999).

2.8. Flowability of imitation cheese

The flowability was determined using a modification of the method of Mounsey and O'Riordan (1999). Cylindrical samples (25 mm diameter, 20 mm height) were bored from the block of cheese and, after 10 min at 180 °C, the distance flowed by the melted cheese was recorded. Flow tests were performed in triplicate.

2.9. Statistical analysis

Three separate batches of each imitation cheese were manufactured in a block design. PROC GLM of SAS (SAS© Institute, 1985, Cary, NC, USA) was used to determine the analysis of variance (ANOVA). Treatment means were considered significantly different at $p < 0.05$ unless stated differently. When significant differences were indicated by ANOVA, Tukey pair-wise comparisons were performed to indicate where the differences between properties existed. Linear regression analysis was performed and the correlation coefficient, R^2, was used as an indicator of the quality of the fit. Data from the ranking test were evaluated for their statistical significance ($p < 0.05$) using Friedman's test (Minitab 12, Pennsylvania, USA) and the multiple comparison procedure to determine which products differed from each other (Meilgaard et al., 1991). Data from the paired preference test were evaluated for their statistical significance ($p < 0.05$) using χ^2-analysis.

Table 2
Levels of butanoic (C_4) acid for imitation cheeses (13% or 2% fat), flavoured with 5% (w/w) EMCs A, B and C at pH levels of 6.0 and 5.5

Cheese code*	Imitation cheese (pH 6.0) C_4 (μg/g)		Imitation cheese (pH 5.5) C_4 (μg/g)	
	13% fat	2% fat	13% fat	2% fat
Cheese 'A'	24^{xa}	21^{xa}	31^{xb}	37^{xb}
Cheese 'B'	38^{ya}	39^{ya}	61^{yb}	69^{yb}
Cheese 'C'	96^{zab}	70^{za}	113^{zb}	119^{zb}

* Flavoured with EMCs A, B or C, respectively. For each column, means with the same letter x, y or z, do not differ significantly at $p \leqslant 0.05$. For each row, means of cheeses containing the same fat content with the same letter a, b or c, do not differ significantly at $p \leqslant 0.05$.

Table 3
Levels of hexanoic (C_6) acid for imitation cheeses (13% or 2% fat), flavoured with 5% (w/w) EMCs A, B and C at pH levels of 6.0 and 5.5

Cheese code*	Imitation cheese (pH 6.0) C_6 (μg/g)		Imitation cheese (pH 5.5) C_6 (μg/g)	
	13% fat	2% fat	13% fat	2% fat
Cheese 'A'	16^{xa}	14^{xa}	19^{xa}	29^{xb}
Cheese 'B'	22^{ya}	22^{ya}	28^{ya}	43^{yb}
Cheese 'C'	52^{za}	47^{za}	59^{za}	61^{zb}

* Flavoured with EMCs A, B or C, respectively. For each column, means with the same letter x, y or z, do not differ significantly at $p \leqslant 0.05$. For each row, means of cheeses containing the same fat content with the same letter a, b or c, do not differ significantly at $p \leqslant 0.05$.

3. Results and discussion

3.1. Compositional features of EMCs and flavoured imitation cheeses

Food flavour, which results from the combination of taste and aroma, is one of its most important quality attributes. Volatile components of the food which interact with receptors on the olfactory bulb in the upper nasal cavity are responsible for aroma, while the sensation of taste is initiated by the interaction of non-volatile compounds with receptors on taste buds in the mouth. Proteolysis during manufacture of EMCs gives rise to taste-active small peptides which are important for the taste of foods flavoured with EMCs (Kilcawley, Wilkinson, & Fox, 1998), while the dominant contributors to the potent aromas of the latter are the SCFAs arising from lipolysis.

The mean moisture, protein, fat, ash and salt contents of cheddar EMCs A, B and C were 50.86 ± 0.36%, 12.3 ± 0.13%, 27.46 ± 0.28%, 4.75 ± 0.43% and 1.50 ± 0.38%, respectively. Their respective pH values were 6.00, 5.45 and 5.32. The latter values were related to the length of the incubation times used to manufacture the EMCs which also determined the concentration of total free fatty acids present, with high levels of the latter being associated with the longer incubation times. EMCs A, B and C, which were made from the same batch of curd, had been incubated for 5, 10 and 18 h during manufacture, and the extracted fat fraction from these contained 16.2%, 28.4% and 46.8% total free fatty acids, respectively. The SCFA levels in the EMCs, as measured by GC headspace analysis were also found to increase in line with the total free fatty acids. For EMCs A, B and C, the respective levels of butanoic acid (C_4) were 0.29, 0.43 and 0.61%, while those of hexanoic acid (C_6) were 0.13%, 0.23% and 0.30%.

The ingredient formulations for the imitation cheeses (2% and 13% fat, pH 5.5 and 6.0) examined in the present study are given in Table 1. Based on these and on a 5% w/w inclusion of the EMC component, the mean moisture, protein and fat contents of the imitation cheeses containing 13% fat and 10.2% resistant starch were 52.40 ± 0.22%, 21.0 ± 0.35% and 12.81 ± 0.21%, respectively. The pH values were either 5.96 or 5.45 ± 0.01. Cheeses containing 2% fat and 15.6% w/w starch had mean moisture, protein and fat contents of 59.68 ± 0.36%, 18.2 ± 0.29% and 2.01 ± 0.09%, respectively, and the pH of these products was either 6.01 or 5.44 ± 0.01.

Using a small (three member) panel a preliminary assessment of the flavour properties of the three EMC formulations used in the present study was carried out by incorporating them into a bland white sauce. The sauces could be readily distinguished from one another as their sensory properties were found to be primarily associated with the potent aroma properties of the SCFAs and increased in the sauces as a function of the increasing levels of hydrolysis in EMCs A to C referred to above. Thus, because of the dominant contribution of SCFAs to flavour in foods flavoured using the present EMC formulations, it was decided to focus on the SCFA status of the four different imitation cheeses (13% and 2% fat, each at pH 6.0 and 5.5) investigated in the present study. Data, obtained by GC headspace analysis of concentrated aqueous slurries of the cheeses, are presented in Tables 2 and 3 and show the following:

(a) SCFA levels in all the cheese samples, while much lower than those in the EMCs, followed the same order as the latter, increasing with degree of lipolysis in the cheeses flavoured with EMCs A to C.
(b) In general, reducing the fat content of the imitation cheese from 13% to 2% did not significantly affect levels of volatile SCFAs detectable in the cheeses.
(c) Decreasing the pH of the cheese base from 6.0 to 5.5 significantly increased the headspace concentration of the SCFA volatiles. For the 13% fat (52% moisture, 10.2% starch) cheeses, the mean increase in C_4 and C_6 levels for the cheese samples containing EMCs A, B and C were ~36% and 20%, respectively. For the 2% fat (60% moisture, 15.6% starch) imitation cheeses, the increases in the SCFAs on decreasing pH from 6.0 to 5.5 were significantly higher, amounting to ~77% for C_4 and 65% for the C_6 acids.

3.2. Influence of fat content and pH on sensory aspects of EMC flavoured imitation cheeses

Recent studies by the present authors (Noronha et al., 2008) on the flavouring of high fat (24%) imitation cheese products with EMCs showed that levels of lipolysis in the latter correlated well with the concentrations of SCFAs in the cheeses, as well as with intensity of 'cheesy' flavour. In addition, reduction in the pH of the cheese base increased headspace concentrations of SCFA, while increasing perceived cheese flavour intensity. The major aim of the present work was to extend the above studies to include an examination of the effects of moisture (52% or 60%) and fat (13% or 2%) levels, as well as variation in pH (6.0 and 5.5), in cheese bases on the sensory quality of cheeses flavoured with EMCs of different levels of hydrolysis.

3.2.1. 13% fat imitation cheeses

The flavour and aroma of cheeses (13% fat, pH 6.0) containing EMCs with increasing levels of hydrolysis (A, B and C) were described as 'cheesy'. All samples had similar ranking scores, as shown in Table 4. Lowering the cheese pH from 6.0 to 5.5 did not significantly alter these scores. Overall, there was a correlation between perceived flavour quality and the degree of hydrolysis of the EMC used in flavouring the 13% fat imitation cheeses. The level of butanoic acid, the most potent of the SCFAs, was $\sim$ 4 fold higher in cheeses (pH 6.0 or 5.5) flavoured with EMC C than in those flavoured with EMC A, with panellists describing the latter as 'mild' and those flavoured with EMC C as 'mature' in flavour (Table 4). However despite being able to distinguish the flavour associated with each cheese, panellists failed to record a definite preference for one particular cheese. The apparent inability of the panel to rank a preference for cheeses prepared with EMCs having different levels of hydrolysis is not easy to explain and can possibly be attributed to panellists' personal preferences for a particular cheese flavour; for example some panellists prefer milder cheeses while others prefer stronger more mature cheese flavours. The difficulty in expressing a preference for cheeses could also possibly be related to the partial replacement of fat with resistant starch in these cheeses. For example, the formation of a complex between the starch and lipid ingredients might lead to increased binding of the SCFAs by the cheese matrix, resulting in a slower release during chewing, an effect which might then serve to mask variations in headspace SCFA concentrations and aroma intensities of the different cheeses.

These results differ somewhat from our recent sensory observations on high (24%) fat imitation cheeses which showed a significantly lower preference for cheeses containing low hydrolysis EMCs (described as 'bland and insipid'), compared to cheeses containing high hydrolysis EMCs. However, when a paired preference test was used on cheese flavoured with EMC B (medium level of lipolysis) having pH values of 6.0 or 5.5, panellists significantly ($p < 0.05$) preferred the higher pH cheeses, which had lower free SCFA levels (Table 2) than the pH 5.5 cheese, describing these preferred cheeses as 'less intense' and 'more rounded' in cheese-like flavour. The preference of the panel for the pH 6.0 cheese over its pH 5.5 counterpart in the case of the cheese flavoured using EMC B is in agreement with the results of Noronha et al. (2008), who found that good cheese flavour was obtained in high fat imitation cheeses at pH 5.5, only when a low hydrolysis flavouring such as EMC A was used, and butanoic acid levels of around 40 μg/g, similar to those in the pH 6.0 cheese prepared from EMC B (Table 2), were present.

3.2.2. 2% fat imitation cheese

Table 5 shows the ranking sum scores for low-fat (2%) flavoured imitation cheeses. There was a significant ($p = 0.028$) preference for cheeses flavoured with EMCs A and B (pH 6.0). These cheeses were described as 'cheesy' and 'smooth in flavour'. The cheese that was flavoured with EMC C was least preferred, as panellists thought it excessively 'strong/mature'. Lowering the pH from 6.0 to 5.5 did not change the overall preference of the panel which still significantly ($p = 0.047$) preferred the cheeses containing low and medium hydrolysis EMCs. Panellists described the pH 5.5 product prepared with high free fatty acids (EMC C) in even more negative terms than its pH 6.0 counterpart. A paired preference test was performed on cheeses prepared with the medium hydrolysis EMC B at pH 6.0 and 5.5 and panellists reported a preference for the higher pH cheeses ($p < 0.05$). Panel members noted that there was a pronounced 'short burst of flavour' that they considered undesirable and that off-notes were perceptible on continued chewing of the pH 5.5 cheese.

3.2.3. 13% vs 2% fat imitation cheeses

Cheeses with fat levels of 13% or 2% (pH 6 and 5.5) containing EMC B were also directly compared using a paired preference test, to see whether reducing the fat level or pH affected the overall sensory properties of reduced fat imitation cheeses. The results were quite clear-cut for the pH 6.0 cheese, in that a total of 90% of panellists preferred the 13% fat cheeses and reported that the lower fat (2%) products were initially too strong in aroma and lost flavour very quickly on chewing. In the case of the pH 5.5 cheeses, panellists were unanimous in their preference for the 13% fat product, describing the low-fat pH 5.5 cheeses as being 'too intense' and 'sharp' in flavour and as producing a 'short burst of off-flavours' during consumption. Overall, the 13% fat cheeses (pH 6.0 or 5.5) had a superior aromatic character and the flavour release during chewing was better than that for the lower fat (2%) cheeses. The 13% fat cheeses were described as 'more rounded and cheesy' in aroma and flavour than the 2% fat samples (pH 6.0 or 5.5).

Table 4
Ranking preference scores and descriptors of panellists for hot (60 °C) imitation cheese (13% fat) samples flavoured with 5% (w/w) EMC A, B and C (pH 6 or 5.5)

Samples	Sum of ranks (pH 6)[a]	Descriptors of pH 6 cheeses	Sum of ranks (pH 5.5)[a]	Descriptors of pH 5.5 cheeses
Cheese 'A'	32[x]	Mild, cheesy flavour	32[x]	Mild, cheesy flavour
Cheese 'B'	31[x]	Cheesy flavour	29[x]	Good cheesy flavour
Cheese 'C'	33[x]	Mature cheesy flavour	35[x]	Well-rounded cheese flavour
T[b]	0.13		1.13	
Upper – 5% probability of χ^2-distribution	5.99		5.99	
LSD rank[c]	11.09		11.09	
p-Value	0.939		0.570	

[a] Number of panellists were 16.
[b] Test statistic.
[c] Least significant difference for ranked preference sensory analysis.
[x] Significantly different.

Table 5
Ranking preference scores of panellists for hot (60 °C) imitation cheese (2% fat) samples flavoured with 5% (w/w) EMC A, B and C (pH 6 or 5.5)

Samples	Sum of ranks (pH 6)[a]	Descriptors of pH 6 cheeses	Sum of ranks (pH 5.5)[a]	Descriptors of pH 5.5 cheeses
Cheese 'A'	25[x]	Good cheesy flavour	25[x]	Well-rounded cheese flavour
Cheese 'B'	31[x]	Smooth cheese flavour	32[x]	Good cheese flavour
Cheese 'C'	40[y]	Very mature, strong cheesy flavour	39[y]	Too strong, bitter, mature flavour
T[b]	7.13		6.13	
Upper – 5% probability of χ^2-distribution	5.99		5.99	
LSD rank[c]	11.09		11.09	
p-Value	0.028		0.047	

[a] Number of panellists were 16.
[b] Test statistic.
[c] Least significant difference for ranked preference sensory analysis.
[x,y] Significantly different.

Development in technologies for the real-time monitoring of flavour release during eating (Roberts & Taylor, 2000), has allowed for detailed study of the effect of fat reduction on flavour perception during food consumption. In general, fat reduction has been found to impact negatively on flavour, giving rise to the 'flavour burst' phenomenon of a rapid initial increase in flavour which then declines rapidly to produce a rather flat sensation. This has been shown in a number of foods, including, for example, cheeses where higher rates of release of certain volatile methyl ketones have been demonstrated in reduced fat products (Delahunty, Piggot, Conner, & Paterson, 1996). The downgrading of the low-fat imitation cheese compared to its medium fat counterpart, as discussed above, would appear to be due to this type of effect, where a more rapid release of the aromatic SCFAs during consumption gave rise to the unbalanced intense flavour described by the panel.

In summary, the present study has shown that the 13% fat imitation cheeses containing resistant starch (pH 5.5 or 6.0), and with comparably ranked positive sensory properties, could be prepared using 5% w/w inclusions of commercial EMC flavourings with low to high levels of hydrolysis and flavour intensities. Reduction of fat to 2% reduced the perceived flavour quality of the products which had undesirably strong flavours when prepared with EMCs having high levels of hydrolysis.

3.3. Influence of cheese composition on physical properties of flavoured imitation cheese

3.3.1. Effecs of pH and fat content

The texture and melting properties of flavoured imitation cheeses were examined to investigate whether or not pH and/or fat content had an effect on the functionality of these products. Data on both 13% and 2% fat imitation cheese products are presented in Table 6. The mean cheese hardness and flowability decreased from ~326 to 312 N and from 124 to 92 mm, respectively, when cheese pH (13% fat) was reduced from 6.0 to 5.5. Cheese cohesiveness (mean, 0.39) was unaffected by pH. The mean hardness, cohesiveness and flowability of cheeses containing 2% of fat decreased from ~245 to 210 N, 0.39 to 0.36 and 126 to 104 mm, respectively, with decreasing pH.

The literature is somewhat conflicting when the effect of pH on cheese functionality is discussed. Paulson, McMahon, and Oberg (1998) and Watkinson et al. (2001) reported that lowering the pH in natural cheeses normally results in decreased hardness. However, Stampanoni and Noble (1991) stated that deceasing the pH of cheese analogues (6.2 to 5.0) resulted in cheeses with increased hardness and elasticity. The hardness of all the cheeses in the present study decreased with decreasing pH. A decrease in cheese pH may solubilise calcium from casein aggregates, causing decreased interactions between proteins and weakening of the protein matrix. Such an outcome could be manifested in an overall reduction in both the hardness and the cohesion of the cheese. While the observed effects of pH on cohesion in the present study are not very marked, they are generally in line with work described by Pastorino, Hansen, and McMahon (2003) and Noronha et al. (2007) where altered protein interactions also affected cheese cohesiveness.

A decrease in cheese melt with decreasing pH, which was not dependent on fat content, was observed in the present study. These results are in agreement with previous work from our laboratory (Noronha et al., 2008) and suggest that decreasing the pH could have promoted an increase in the proportion of hydrophobic interactions occurring at higher temperatures (180 °C), causing reduced cheese flow.

Decreasing the fat content affected the overall functionality of flavoured imitation cheeses. Cheese hardness decreased significantly with a decrease in fat content, from 13% to 2% (Table 6), and this decrease can be attributed to the fact that the 2% fat cheeses also had a much higher moisture content (60%) than had

Table 6
Physical properties of imitation cheeses pH 6.0 (a) or 5.5 (b) containing 13% fat [square brackets] or 2% fat (round brackets) flavoured with commercial EMCs A, B and C

EMC type	pH	Hardness (*N*)		Cohesiveness		Melt (mm)	
		13% fat	2% fat	13% fat	2% fat	13% fat	2% fat
(a)							
A	6	[325.5[bx]]	(252.5[cy])	[0.41[bx]]	(0.39[ay])	[118.3[bx]]	(124.5[ax])
B	6	[321.4[bx]]	(239.9[cy])	[0.37[dx]]	(0.39[ay])	[127.5[ax]]	(128.5[ax])
C	6	[331.6[bx]]	(243.8[cy])	[0.38[cdx]]	(0.40[aby])	[127.5[ax]]	(125.8[ax])
(b)							
A	5.5	[312.7[cx]]	(205.8[aby])	[0.39[bx]]	(0.35[ay])	[90.8[cx]]	(102.5[bx])
B	5.5	[314.2[cx]]	(211.0[by])	[0.39[bx]]	(0.37[aby])	[89.2[cx]]	(105.8[ax])
C	5.5	[307.9[cx]]	(211.9[by])	[0.39[bx]]	(0.36[ay])	[96.7[bcx]]	(102.5[ax])

Values represent the means of three replicate trials. For each column, with the same type of brackets used, means with the same letter a, b, c or d, do not differ significantly at $p \leqslant 0.05$. For each row, means with the same letter x, or y, do not differ significantly at $p \leqslant 0.05$.

the 13% fat products (52% moisture). The flowability of imitation cheeses, perhaps a little surprisingly, did not change with a reduction in fat. Again, in this case, any reduction in flow due to the reduction in fat was probably obscured by the effect of the higher moisture content of the 2% fat cheese. This is in agreement with work reported by Noronha et al. (2007), when the fat in a high moisture imitation cheese was partially replaced by resistant starch.

4. Conclusion

The level of hydrolysis in EMCs, which produced different amounts of aromatic SCFAs, affected the cheese-like sensory characters of reduced and low-fat EMC-flavoured imitation products. Fat played an important role in modifying the flavour perception and cheeses with 13% fat displayed superior flavour release properties to those containing 2% fat, irrespective of the degree of hydrolysis of the EMC used. The pH of the cheese base also influenced flavour perception in imitation cheeses, with lower pH (5.5) products having higher levels of free SCFAs and 'stronger' flavours than those formulated with a higher pH (6.0). While the addition of EMCs did not affect the overall functionality of imitation cheeses, decreasing the pH of the cheese base decreased the hardness of the product. The inclusion of low or medium intensity EMCs in reduced and low-fat imitation cheeses may help these imitation cheese products compete with natural dairy cheese on an increasing scale, especially in an age where low-fat foods are more in demand and considered the norm.

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