Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 106 (2008) 905–913
Food Chemistry
www.elsevier.com/locate/foodchem

Flavouring of imitation cheese with enzyme-modified cheeses (EMCs): Sensory impact and measurement of aroma active short chain fatty acids (SCFAs)

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Received 6 February 2007; received in revised form 10 May 2007; accepted 28 June 2007

Abstract

Enzyme-modified cheeses (EMCs) are used to impart flavour to imitation cheese products. Cheeses (pH 6 or 5.5) were formulated with 5% w/w EMC, having low, medium or high levels of lipolysis and were examined by a sensory panel. Free fatty acid analyses were performed using SPME/GC. The flavour profile of the flavoured cheeses was affected by EMC composition and pH of the cheese base. Cheeses at a pH of 6.0, flavoured with low lipolysis EMCs, were described as 'bland'. Lowering the pH of the cheese matrix to 5.5 appeared to increase the flavour intensity of the cheese flavoured with low lipolysis EMC and panellists ranked this cheese the highest, describing its flavour as 'well-balanced and 'cheesy'. This study shows that the flavours of imitation cheeses are influenced by the level of lipolysis of the EMCs used to flavour them and also by the pH of the cheese base.

Keywords: Imitation cheese; Enzyme-modified cheese; Lipolysis; SPME/GC; Sensory

1. Introduction

Imitation cheese is used by food manufacturers due to its cost-effectiveness, which is attributable to the simplicity of its manufacture and the replacement of selected milk ingredients by cheaper vegetable products (Eymery & Pangborn, 1988). Imitation cheese is known to be bland and lacking in flavour. To overcome this problem, flavour delivery systems are used to help increase the resemblance of imitation cheeses to their natural counterparts. These flavour systems can be either artificial or natural in origin (Shaw, 1984).

Enzyme-modified cheeses (EMCs) are defined as concentrated cheese flavours that are natural in origin and are produced enzymatically from cheeses of various ages (Kilcawley, Wilkinson, & Fox, 1998). EMCs are available in a range of flavours, differing in purported character and intensity (Kilcawley et al., 1998; Moskowitz & Noelck, 1987; Sutherland, 1991). Free fatty acids (FFA) are major contributors to the flavour of EMCs (Kilcawley, Wilkinson, & Fox, 2006). The latter are released upon lipolysis and the short and intermediate-chain FFAs contribute directly to cheese flavour (Bills & Day, 1964). These short and intermediate-chain fatty acids (C_4–C_{12}) have relatively low perception thresholds and each gives a characteristic flavour note. Butanoic acid (C_4) contributes "rancid" and "cheesy" flavours while hexanoic acid (C_6) has a "pungent", "blue cheese" flavour note (Collins, McSweeney, & Wilkinson, 2003). Depending on their concentration and perception threshold, volatile fatty acids can either contribute positively to the aroma of the imitation cheese or to a rancid defect.

Solid phase micro-extraction (SPME) is a powerful modern solvent-free extraction method which permits volatile components present in food or non-food matrices to be very effectively concentrated by exposing a suitably chosen SPME fibre to the headspace vapour of the matrix in a

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doi:10.1016/j.foodchem.2007.06.059

sealed system. Thermal desorption of the volatiles in the heated injection block of a gas chromatograph and separation of individual compounds on high performance GC columns can then allow for either qualitative profiling of the volatiles or for quantitative measurement of selected compounds, with the aid of internal standards if necessary. This technique can be applied to the analysis of volatiles of a wide range of foods including cheese (Chin, Bernhard, & Rosenberg, 1996).

An important objective of the present study was to attempt to relate the sensory properties of imitation cheeses prepared containing EMCs to the levels of key flavour active ingredients, such as short chain fatty acids (SCFAs) derived from the added EMCs. Another aim was to examine how the level of hydrolysis in EMCs, which produced different levels of aromatic SCFAs, impacted on the cheese-like sensory character of imitation cheeses flavoured with these products. To do this, it was necessary to develop a method for the determination of free fatty acid levels in both EMCs and flavoured imitation cheese products. The importance of pH to the concentration of the aroma active SCFAs emerged during the course of the method development work and, as a result of this, the objectives of the study were extended to include an examination of the influence of pH on the flavour intensity/mouthfeel properties of imitation cheese products.

2. Materials and methods

2.1. Imitation cheese ingredients

Three commercial Cheddar EMC products were obtained as gifts from Kerry Ingredients Ltd. (Listowel, Co. Kerry, Ireland), labelled B, D, H, respectively. All EMC products were in paste form and were stored at 4 °C prior to analysis.

Rennet casein was also 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). 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. Materials for analytical measurements

The solvents methanol, ethanol, hexanol, propanol and dichloromethane were all obtained from Sigma Aldrich Chemical Co. (Dublin, Ireland), as were the fatty acid standards, butanoic acid (C_4), iso-butanoic acid (iso-C_4), hexanoic acid (C_6) and 4-methyl-pentanoic 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 KOH in 100 ml of 95% ethanol. Standard methanolic KOH (0.02 M) for determination of FFAs in EMC samples was prepared by dilution of standard 1 M KOH in methanol (BDH, Dublin, Ireland) with anhydrous methanol.

Solid phase micro-extraction (SPME) fibres (Carboxen/PDMS, 75 μm thickness) were obtained from Supelco (Supelco-Aldrich, Dublin, 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 cheese

Imitation cheeses, with a moisture content of 52% (w/w) 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). The formulation, expressed on a dry weight basis (% w/w), of the imitation cheese was as follows: 49.8% rennet casein, 29.2% hydrogenated palm oil, 14.4% vegetable oil, 1.8% trisodium citrate, 0.8% disodium phosphate, 2.8% NaCl, 0.2% sorbic acid and 1% (pH 6 cheese) or 2% (pH 5.5 cheese) citric acid.

Batches (4 kg) of imitation cheese were manufactured by agitating the vegetable oil and water in the cooker at 50 °C for 1 min. Trisodium citrate, disodium phosphate, sodium chloride and sorbic acid were then added and mixed for a further minute. The rennet casein was subsequently added and, following 1 min of mixing, the temperature was increased to 80 °C by direct steam injection. The mixture was maintained at 80 °C until a uniform mass was obtained. The EMC paste (200 g) was mixed into the molten cheese mass, along with the citric acid at the end of the manufacture process, for one final minute of mixing at 80 °C. The flavoured cheese was discharged into a plastic lined cardboard box which was placed in a freezer at −18 °C, until the temperature decreased to ~20 °C. The temperature of the cheese was determined by inserting a hand-held digital thermometer (Digitron, Devon, England) into the centre of the cheese block for 2 min. The cheese was then transferred to a refrigerator at 4 °C for 24 h, where the cheese was divided into sections, vacuum-packed (Model C10H, Webomatic®, Bochum, Germany) and stored at 4 °C until required.

2.4. Compositional analysis of EMCs and flavoured 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 the protein content was determined 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).

2.5. Sensory analysis of flavoured cheeses

2.5.1. 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, 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.

2.5.2. 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 ($n = 16$) were presented with cheese samples (pH 6 or 5.5) containing 5% w/w of EMCs B, D or H. In both 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 of their observations of the sensory characteristics for the cheese samples.

2.5.3. Paired preference test

Panellists were asked to choose which sample they preferred using the method of Meilgaard et al. (1991) based on the same sample preparation of 2.5. A paired preference test was used to compare the cheese samples containing EMC D (pH 6 *vs.* 5.5).

2.6. Determination of FFA in EMCs by titration

The free fatty acid content of EMCs was determined using a modification of the procedure of Thomas, Nielson, and Olson (1955) to extract a representative fat sample from the EMCs. A sample of EMC (10 g) was mixed with anhydrous sodium sulphate (~20–50 g) to convert the mixture to a dry powder. The latter was then macerated with dichloromethane (50–70 ml), using a mortar and pestle to extract the fat. The dichloromethane extract was filtered through filter paper (Whatman no. 542) and the extraction step was repeated twice more so as to ensure a good recovery of fat from the EMC. Most (90%) of the solvent from the combined extracts solvent was recovered by distillation and the residual solvent was then removed using a water bath in a fume cupboard. Following evaporation, a precise weight of fat (200–400 mg) was placed in a 50 ml conical flask and dissolved in 5 ml of titration solvent (hexane:propanol, 4:1). The resultant solution was titrated with 0.02 M methanolic KOH using phenolphthalein as an indicator (5–6 drops). A blank titration was also carried out on the titration solvent without the fat.

In order to express the results in terms of FFAs as a percentage of total fatty acids, the assay was calibrated for butterfat free fatty acids as follows: a sample of pure butterfat (2 g) contained in a 150 ml conical flask fitted with an air-condenser, was saponified by heating for 15 min on a water bath (80 °C) with 20 ml of 0.5 M ethanolic KOH. After cooling, the free fatty acids were subsequently liberated from their soaps by the addition of 1 M phosphoric acid (20 ml), filtered under vacuum using Whatman no. 1 filter paper, washed with distilled water and dried in a convection oven at 100 °C for 2 h. Replicate samples (50 mg) of the butterfat free fatty acids were titrated as described above and it was found that 1 ml of titrant was required to titrate 4.86 mg of butterfat free fatty acids. The percentages of free fatty acids of the EMC samples were expressed as a percentage of butter fat free fatty acids as follows:

$$\%\ \text{Free Fatty Acids} = \frac{[(\text{net titre} \times 4.86) \times 100]}{\text{weight of EMC fat (mg)}} \quad (1)$$

2.7. SPME/GC headspace analysis of short chain fatty acids (SCFAs)

GC analysis was performed on a ATI Unicam Model 6100 gas chromatograph fitted with a flame ionisation detector (FID) and interfaced to a Spectra-Physics SP4290 computing integrator. The column used was a 15 m FFAP (Quadrex Inc.) fused silica column (0.53 mm i.d., film thickness, 1 μm). Hydrogen was used as a carrier gas at a flow rate of 8 ml/min. The column temperature was 140 °C and the injection block was set at 300 °C.

The volatile SCFAs, butanoic (C_4) and hexanoic (C_6), in the EMC samples were measured by SPME headspace analysis using iso-butanoic (iso-C_4) and 4-methyl-pentanoic acids (4-Me-C_5) as internal standards (IS), respectively. Samples (2 g) of EMC pastes were ground using a mortar and pestle with exactly 10 ml of water, containing each of the internal standards at a concentration of 0.4 g/l. An aliquot (5 ml) of the resultant slurry was transferred to a 10 ml screw thread glass vial, fitted with a magnetic stirring bar. After sealing the vial with a polytetrafluoroethylene (PTFE) silicone rubber septum, the SPME needle was inserted through the latter so as to position the fibre 15 mm above the surface of the slurry. The slurry was heated with stirring by placing the vial in a thermostatically controlled water bath at 50 °C. After equilibration for 10 min, the headspace was sampled by exposing the fibre for 10 min. The concentration of the volatile fatty acids (VFAs) was determined using the following equation:

$$\%\ \text{analyte}\ (C_4\ \text{or}\ C_6) = \frac{\text{peak area of analyte} \times \text{concentration of IS} \times 100}{\text{peak area of IS} \times \text{response factor} \times \text{wt of sample(g)}} \quad (2)$$

The response factors for the two analytes were established by carrying out SPME headspace analysis of an aqueous standard (0.2 g/l each of C_4, iso-C_4, C_6 and 4-Me-C_5 acids) under exactly the same conditions as described above for the EMC samples.

$$\text{Response factor}\ (C_4\ \text{or}\ C_6) = \frac{\text{peak area } C_4}{\text{peak area iso-}C_4} \quad \text{or} \quad \frac{\text{peak area } C_6}{\text{peak area 4-Me-}C_5} \quad (3)$$

Response factors obtained were 0.97 ± 0.03 for C_4 and 1.02 ± 0.02 for C_6. GC retention times of the SCFAs under the conditions used were 2.2, 2.8, 5.6 and 6.6 min for iso-C_4, C_4, 4-Me-C_5 and C_6, respectively. A typical chromatogram obtained for an EMC sample is presented in Fig. 1.

For the measurement of SCFAs in the imitation cheeses flavoured using different EMC formulations, it was intended to employ the above internal standard method with minor modifications to take account of the considerably lower SCFA levels in these products. However, the limitations of this approach were soon evident when it was shown that, for reasons directly related to the chemical composition of the imitation cheese matrix, high proportions of the SCFAs, including the added internal standards, were irreversibly absorbed by the cheese matrix during preparation of samples for SPME analysis. Notwithstanding this difficulty, reasons why the internal standard approach may still be suitable for measuring SCFAs in the imitation cheeses are examined in the Results and discussion section.

Because of the matrix problems described above, it was decided that, in spite of potential limitations, it would also be of interest to examine the use of a simple external standard calibration method for measuring SCFAs in the imitation cheeses. Calibration curves (peak area *vs.* concentration) were prepared using aqueous standards of C_4 and C_6 SCFAs (4–40 μg/ml) and the SPME headspace conditions described earlier (50 °C, 10 min each equilibration and sampling). Good linearity in the curves for both acids was obtained with R^2 values of 0.99 and 0.97 for butanoic and hexanoic acids, respectively.

2.8. Texture profile analysis (TPA)

Textural properties of the flavoured imitation cheeses were measured with an Instron Universal Testing Machine (Instron Model 5540, Instron Corp., Canton, Mass., USA) fitted with a 500 N load cell and 35 mm diameter plates. Cylinders of cheese 20 mm high and 25 mm diameter were cut with a cheese borer, wrapped in cellophane to prevent dehydration, and allowed to equilibrate to 22 °C by placing the samples in a temperature control room (25 °C) until the desired temperature was obtained. Samples were compressed by 80% of their initial height using a 35 mm diameter plate at a crosshead speed of 50 mm/min. The uniaxial compression test was performed in two successive cycles, and the textural parameters, hardness and cohesiveness, were calculated. Hardness is derived as the force necessary to attain a given deformation and the cohesiveness is the strength of the internal bonds making up the body of the product (Szczesniak, 1963).

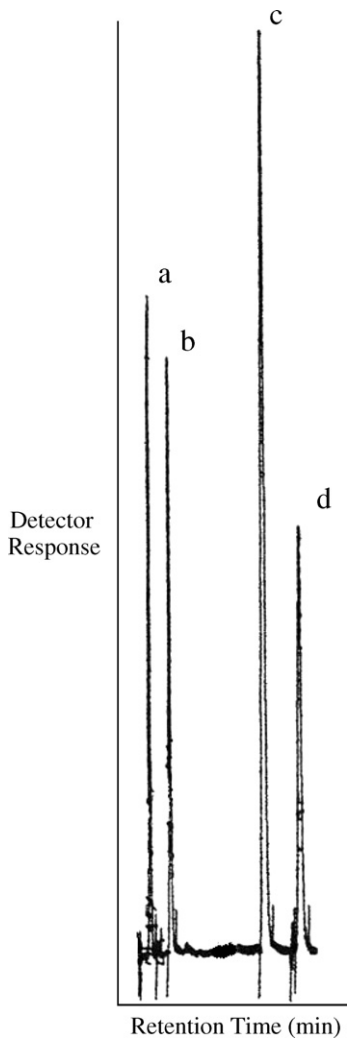

Fig. 1. Chromatogram of headspace volatiles sampled using SPME/GC from an aqueous slurry (20% w/v) of EMC D containing the internal standards iso-C_4 and 4-Me-C_5 SCFAs at a concentration of 0.4 g/l. [Insert: (a) iso-C_4; (b) C_4; (c) 4-Me-C_5 and (d) C_6.]

2.9. Flow test and melting temperature of imitation cheese

The method of Mounsey and O'Riordan (1999) was used to assess flowability. Cylinders (25 mm diameter, 20 mm height, 10 g ± 0.05 g weight) were cut from blocks

of imitation cheese, wrapped in aluminium foil and tempered to 10 °C. The foil was then removed and the cylinders were individually put into one end of a Pyrex glass tube (250 mm length, 30 mm diameter). The end containing the cheese was closed with a solid rubber stopper, and the opposite end was plugged with a stopper, pierced with a hole to allow gas to escape. The tubes were placed horizontally in a conventional oven at 180 °C for 10 min. The tubes were removed from the oven and, after 1 min at room temperature, the horizontal distance flowed (length) from a reference line was measured in mm using a purpose built ruler.

The crossover temperature (T_c) measurements were performed on a controlled stress rheometer (model SR 2000 (air bearing), Rheometrics Inc., Piscataway, NJ) fitted with a 25 mm parallel plate with a 2.2 mm gap according to the method of Mounsey and O'Riordan (1999). Disc-shaped samples of cheese (24 mm diameter, 2.4 mm thick) were cut from the cheese block, using a meat slicer (Chef's Choice International, model 662, Bristol, UK) and cork borer. The sample discs were placed on the lower plate and compressed by 0.2 mm to prevent slippage. All measurements were made in a controlled environmental chamber at a frequency of 1 Hz. The crossover temperature (T_c), i.e. occurring when $G' = G''$, was used as an index of the melting temperature.

2.10. 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 otherwise stated. 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 to evaluate the quality of the fit. Data from the ranking test were evaluated for their statistical significance ($p < 0.05$) using Friedman's test (Minitab version 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 Chi-square analysis.

3. Results and discussion

3.1. Characterisation of EMCs and flavoured imitation cheeses

The compositions of Cheddar EMCs are shown in Table 1. A variation in compositional parameters was found for different Cheddar EMCs especially in respect of the free fatty acid fraction, which confirmed that manipulation of composition by lipolysis is an important part of the EMC manufacturing process. The pH values of EMCs varied from 5.3 to 6.0, indicating the possibility of manipulating pH to confer a specific acidic character, or to enhance the microbial safety of the product. A decrease in EMC pH was associated with an increase in the percentage of total FFA. Levels of NaCl in EMCs varied from 1.2% to 2% and this ingredient could be used as a preservative or to give a specific 'salty' flavour to the final product.

The level of ash in the EMCs ranged from 4.5% to 5%, with higher levels corresponding to higher salt values. The high levels of ash indicate the addition of emulsifying agents, such as orthophosphates or pyrophosphates to the products. With the aid of heat and shear, these emulsifiers initiate the physico-chemical conversion of cheese protein into a homogeneous emulsified substrate (Guinee, O'Brien, & Rawle, 1994; Shimp, 1985). The percentage FFA across the EMCs ranged from 16% to 47% (Table 1) and, as would be expected, the longer the incubation time, the greater was the level of lipolysis. The internal standard method was used to quantify the amounts of C_4 and C_6 acids in EMCs (Table 1). The levels of both butyric and hexanoic acid in EMCs B, D and H increased linearly with hydrolysis time ($R^2 = 0.99$ for each acid). The EMC containing the lowest levels of both acids (EMC B) also had the lowest total free fatty acid percentage (Table 1) and increase in the latter was well correlated with increase in C_4 and C_6 levels ($R^2 = 0.98$). The C_4:C_6 ratio in all EMCs was ~2:1, which is similar to that in pure butterfat.

To prepare the experimental imitation cheeses, the EMCs were incorporated into two separate unflavoured cheese bases (50 g EMC/kg cheese), one having a pH of 5.5 and the other a pH of 6.0. The cheeses that were flavoured with EMCs labelled B, D or H (16%, 28% or 47% total free fatty acids, respectively) had sensory characters ranging from mild to medium to strongly cheese-like, respectively, when the pH 6.0 cheese base was used. The same order of cheese-like characters was observed when

Table 1
Compositions of a series of commercial Cheddar EMCs

EMC type	Moisture (%)	Protein (%)	Fat (%)	Ash (%)	Salt (%)	pH	C_4 (%)	C_6 (%)	Total FFA (%)	Incubation time (h)
B	52.11^{c}	11.2^{a}	27.2^{a}	4.51^{a}	1.22^{a}	6.00^{a}	0.29^{a}	0.13^{a}	16.2^{a}	5
D	51.07^{b}	11.9^{a}	27.5^{a}	4.72^{a,b}	1.31^{b}	5.45^{b}	0.43^{b}	0.23^{b}	28.5^{b}	10
H	49.39^{a}	13.9^{b}	27.7^{a}	5.02^{b}	1.98^{c}	5.32^{c}	0.61^{c}	0.30^{c}	46.8^{c}	18

Values for each EMC represent the mean of triplicate measurements. For each column, means with the same letter a, b, or c do not differ significantly at $P \leqslant 0.05$.

the EMCs were incorporated into the pH 5.5 cheese base, but the overall strengths of the aromas were noticeably more intense for all three cheeses compared to their pH 6.0 counterparts. This aspect is discussed in more detail in Section 3.3.

Since the only difference in formulation of the cheese bases was an increase in the amount of citric acid from 1% to 2%, to alter the pH 6.0 and 5.5 cheeses, respectively, it was expected that very little difference in the gross compositions of the two imitation cheeses would be observed. This was in fact the case, as indicated by the following data: the average moisture content for the pH 6.0 cheeses containing EMC B, D or H was $52.18\% \pm 0.12$ while the respective protein and fat contents were $19.8\% \pm 0.5$ and $23.9\% \pm 0.3$ and the mean cheese pH was 5.99 ± 0.04. Lowering the pH to 5.5 did not markedly affect the composition of imitation cheeses, with the latter having an average moisture content of $51.85\% \pm 0.07$, protein levels of $20.9\% \pm 0.19$ and fat contents of $23.9\% \pm 0.05$. The mean pH of the cheeses containing EMC B, D or H was 5.46 ± 0.02. The imitation cheese products are designed for molten cheese applications that are suitable for cutting and slicing, and can be used either as a pizza topping or be included in a sauce, with these cheese products being in line with typical imitation cheese formulations (moisture 48–52%, protein 18–22% and fat 22–25%).

3.2. Measurement of SCFAs in flavoured imitation cheeses

An important objective of the present study was to attempt to relate the sensory properties of imitation cheeses prepared from selected EMCs to the levels of key flavour active ingredients, such as the SCFAs derived from the added EMCs. While C_4 and C_6 acid concentrations were obviously much lower in the prepared imitation cheeses than in the EMCs, it was expected that these acids could be quantified with relatively little difficulty using the internal standard SPME method already described. However, this did not prove to be the case, since headspace GC analysis of slurries of the different cheeses yielded SCFA peaks which were very much smaller than expected on the basis of the 5% level of incorporation of EMCs into these cheeses. In fact, in the case of the cheese flavoured with the EMC having the lowest level of lipolysis (B), measurement of butanoic acid was quite close to the limit of detection of the method. Compositional features of the unflavoured imitation cheeses which could impact significantly on both the perceived aroma intensity of the EMC flavoured products, as well on the measurement of the concentrations of the aromatic SCFAs therein, were suspected of being responsible for the above phenomena. For example, it was possible to demonstrate that the emulsifying salts present in the unflavoured cheese matrix had the ability to 'mop up' or neutralise a considerable proportion of the free fatty acids in the added EMC, including the C_4 and C_6 SCFAs. SPME headspace analysis of slurries (20%) of EMC D flavoured imitation cheeses, prepared using 0.1 M orthophosphoric acid (pH 3), gave peak areas for the SCFAs that were several-fold higher than those prepared using distilled water and which had a pH of 6.0.

To assess the magnitude of the buffering effect of the emulsifying salts on the status of fatty acids used as internal standards in the measurement of SCFAs in imitation cheeses, slurries (20% w/w, pH 6.0 or 5.5) of EMC D flavoured cheese, were prepared with 20 μg/g of iso-C_4 and 4-Me-C_5 acid internal standards in either (a) distilled water or (b) 0.1 M orthophosphoric acid. SPME headspace analysis of replicate samples of both slurries, showed the recoveries of internal standards and also of the cheese C_4 and C_6 SCFAs to be only $14 \pm 0.32\%$ (pH 6.0) or $30 \pm 0.12\%$ (pH 5.5) of those obtained in the acidified (pH 3.0) samples (data not shown). Reducing the pH further with stronger acid did not improve the recoveries of the SCFAs. These data suggested that, since all the short chain acids seemed to be affected to the same extent by the cheese matrix, the use of the internal standard method might still be acceptable for measuring SCFAs in EMC flavoured imitation cheeses containing emulsifying salts.

Support for this hypothesis was provided in an experiment where a sample of unflavoured imitation cheese was slurried in a solution containing 0.2% each of C_4, iso-C_4, 4-Me-C_5 and C_6 acids. In spite of the large reduction in the peak areas compared to those in water, the peak area response factors (C_4/iso-C_4 and C_6/4-Me-C_5) were still close to the values of 1.0 established in aqueous solution. Table 2 gives concentrations of C_4 and C_6 acid in three EMC flavoured imitation cheese samples (pH 6.0) which were calculated using the internal standard method, as well as the external standard procedure employing an aqueous standard calibration graph, as described in the experimental section (2.7). Values obtained from the graph assumed recoveries of only 14% for both acids and were adjusted accordingly to take account of this. The Table shows that the level of agreement between the two methods was reasonably good.

Table 2
Levels of SCFAs in imitation cheeses, flavoured using 5% w/w of EMCs B, D or H (pH 6.0), as measured by headspace GC, using the external standard and internal standard (ratio) methods

pH 6	External standard method		Ratio method	
EMC type	C_4 (μg/g)	C_6 (μg/g)	C_4 (μg/g)	C_6 (μg/g)
B	21^{as}	14^{ax}	21^{as}	14^{ax}
D	40^{bt}	21^{by}	38^{bt}	18^{by}
H	83^{cu}	43^{cz}	72^{cu}	48^{cz}

Values represent the means of three replicate measurements. For each column, means having the same superscript letter a, b, or c did not differ significantly. For each fatty acid, means having the same superscript letter s, t, u or x, y, z did not differ significantly between external standard or ratio method.

3.3. Relationship between chemical properties and sensory characteristics of EMC flavoured imitation cheese

An important aim of the present work was to examine how the level of hydrolysis in selected EMCs which produced different levels of aromatic SCFAs impacted on the cheese-like sensory character of imitation cheeses flavoured with these products at the same level of incorporation (5% w/w). As a result of the observed ability of the imitation cheese matrix to neutralise significant amounts of the SCFAs in the added EMC, as observed in the analytical studies, an additional aim of this work was to investigate the feasibility of modifying the composition of the cheese base with a view to improving the quality of the EMC flavoured products. Essentially, the strategy was to adjust the formulation using 1% or 2% citric acid to give products having pH values of 6.0 and 5.5, respectively (Section 2.3). After adding an EMC to each cheese base, the flavour/aroma of the resulting cheeses, and the concentration therein of free butanoic and hexanoic acids, would be of considerable interest.

3.3.1. Effect of EMC composition

The imitation cheese flavoured with EMC D was the most preferred of all the pH 6.0 cheeses when consumed at 60 °C. It was described as being 'well-rounded' in overall character and 'cheesy' in flavour and aroma and for these reasons was ranked first (Table 3). The cheese that was flavoured with EMC H received the second highest ranking scores with panellists describing the cheese as 'strong' with a 'mature cheese' flavour. By contrast, the imitation cheese containing EMC B was ranked the lowest and was characterised as 'insipid' and 'bland'. EMC B was the mildest of the three flavours (16% total FFA) and therefore it would be expected that flavour intensity would be quite low in the final end-product. Overall, there was a good correlation between flavour intensity and degree of lipolysis of the EMC used in the imitation cheese. However, optimum flavour quality appeared to be associated with the product made using the EMC with the intermediate level of total FFAs and SCFAs.

3.3.2. Effect of cheese base pH

The flavour preference order changed when the pH was lowered from 6.0 to 5.5 in the flavoured imitation cheeses. The product flavoured with EMC B had the highest ranking scores (Table 3), while the cheese flavoured with EMC H had the lowest. Thus, the result of decreasing the pH of imitation cheeses to 5.5 had the positive effect of improving the overall acceptability of the lowest lipolysis cheese (from EMC B) which, as noted above, was weakly flavoured when manufactured to a pH of 6.0.

Lowering the pH of all imitation cheeses to 5.5 had the effect of not only increasing the intensity of the flavour but of also changing the perception of the mouthfeel character of the product. Panellists described all the lower pH cheeses as 'smoother', 'creamier' in the mouth and in addition were 'more cheesy' with more 'balanced cheesy notes', than the higher pH cheeses. There was very little difference between ranking scores of the medium and high lipolysis cheeses (pH 5.5), with both cheeses being described by panellists as having 'strong' and 'overpowering' flavours. Thus, while decreasing cheese pH from 6.0 to 5.5 positively influenced the aroma and flavour of the EMC B cheese, the excessively intense flavour induced in cheeses prepared from EMCs D and H was highly negative from a sensory point of view.

Table 4 presents comparative data on the apparent concentrations of SCFAs in pH 6.0 and 5.5 imitation cheeses, which help to provide some explanation for the observed sensory difference between these products, as discussed above. In particular, the concentration of butanoic acid (C_4), the most potent of the SCFAs from a sensory viewpoint, was 65%, 33% and 30% higher at pH 5.5 compared to 6.0 in imitation cheese prepared with EMC types B, D and H, respectively. It is also of interest to note that the C_4 level (35 μg/g) in the preferred cheese 'B' at pH 5.5 was quite similar to that in cheese 'D' (40 μg/g) which was the highest ranked cheese in the pH 6.0 group. It is possible that, for imitation cheeses of the type examined in the present study, C_4 levels of ~40 μg/g may represent a desirable optimal concentration, below which the product is excessively bland and

Table 3
Ranking preference scores of panellists for hot (60 °C) imitation cheese (52% moisture) samples flavoured with 5% (w/w) EMCs B, D or H (pH 6 or 5.5)

Samples	pH 6[a]	pH 5.5[a]
Cheese B	44[y]	22[x]
Cheese D	24[x]	34[y]
Cheese H	28[x]	40[y]
T[b]	14	10.50
Upper – 5% probability of χ^2 – distribution	5.99	5.99
LSD rank[c]	11.09	11.09
P value	0.001	0.005

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

Table 4
Comparative headspace levels of butanoic (C_4) and hexanoic (C_6) acids in pH 6.0 and 5.5 imitation cheeses flavoured using 5% (w/w) EMCs B, D and H

	pH 6.0 cheese		pH 5.5 cheese	
EMC type	C_4 (μg/g)*	C_6 (μg/g)*	C_4 (μg/g)*	C_6 (μg/g)*
B	21[as]	14[ax]	35[aw]	12[ax]
D	40[bt]	21[by]	54[bt]	25[by]
H	83[cu]	43[cz]	108[cv]	54[cz]

Values represent the mean of 3 replicate trials. For each column, means having the same superscript letter a, b, or c and x, y or z did not differ significantly. For each fatty acid, means having the same superscript letter s, t, u, v, w or x, y, z did not differ significantly between pH 6.0 or 5.5 cheeses.
* External standard headspace method with data corrected for 14% and 30% recoveries for pH 6.0 and 5.5 samples, respectively.

Table 5
Hardness, cohesiveness, flowability and crossover temperature values of imitation cheese (52% moisture) products flavoured with 5% (w/w) EMC B, D or H at a pH level of 6 or 5.5, respectively

EMC type	pH	Hardness (N)	Cohesiveness	Flow (mm)	T_c (°C)
B	6	274.46 ± 34.20^{ax}	0.39 ± 0.02^{ax}	128.17 ± 7.21^{ax}	46.09 ± 0.68^{ax}
D	6	272.01 ± 37.95^{ax}	0.39 ± 0.08^{ax}	122.50 ± 8.12^{ax}	46.72 ± 1.86^{ax}
H	6	262.16 ± 39.01^{ax}	0.40 ± 0.01^{bx}	129.17 ± 7.58^{ax}	43.16 ± 1.42^{bx}
B	5.5	201.54 ± 20.59^{ay}	0.38 ± 0.01^{axy}	115.00 ± 5.39^{ay}	54.44 ± 0.56^{az}
D	5.5	192.46 ± 28.62^{ay}	0.38 ± 0.02^{axy}	106.67 ± 4.89^{by}	54.19 ± 1.34^{az}
H	5.5	194.73 ± 43.93^{ay}	0.37 ± 0.02^{ay}	119.17 ± 5.12^{ay}	48.45 ± 0.37^{by}

Values represent the means of 3 replicate measurements. For each column, means having the same superscript letter a, b, or c did not differ significantly. For each physical property, means having the same superscript letter x, y or z did not differ significantly between pH 6 or 5.5 cheeses.

above which the aroma is unpleasantly strong. For imitation cheeses prepared with EMCs D and H at both pH 5.5 and 6.0, the C_4/C_6 ratio was very close to 2.0. However, for cheese B, the ratio was 1.5 at pH 6.0 and 2.9 at pH 5.5, with the latter effect being apparently due to the fact that the pH change did not affect the concentration of the C_6 acid. The reason for this behaviour is not clear. Finally, and in relation to ability of the cheese base to neutralise FFAs in the added EMCs, the recoveries of SCFAs added to the pH 5.5 bases were around 30% compared to 14% from the pH 6.0 product, as discussed earlier.

3.4. Effect of pH reduction on physical properties of flavoured cheese

The texture and melting properties of flavoured imitation cheeses were examined to investigate whether or not pH had an effect on the functionality of these products. Cheese hardness decreased from 270 to 196 N (mean values) as the pH was lowered from 6 to 5.5, respectively, (Table 5). Low pH cheeses have matrices with less structural uniformity and solid-like behaviour (Marchesseau, Gastaldi, Lagaude, & Cuq, 1997; Ramkumar, Campanella, Watkinson, Bennett, & Creamer, 1998) and decreased uniformity does not allow for an even distribution of stress, which would result in low pH cheeses having decreased hardness (Pastorino, Hansen, & McMahon, 2003a; Watkinson et al., 2001). Decreasing the pH could also disrupt calcium-mediated protein interactions, resulting in decreased interactions between proteins, thus weakening the protein matrix.

There was a slight decrease in cheese cohesiveness from 0.39 to 0.38 with a decrease in cheese pH (Table 5). Cohesiveness, by definition, is the strength of the internal bonds making up the body of the product (Szczesniak, 1963) and, if the matrix was to weaken by a reduction in protein interactions, then it may be plausible to suggest that imitation cheese could become less cohesive by the same mechanism. These results are in agreement with work described by Pastorino et al. (2003a) and Pastorino, Ricks, Hansen, and McMahon (2003b), who proposed that cheese cohesiveness is affected by altered protein interactions.

Calcium is a strong promoter of protein-to-protein interactions, and its solubilization would decrease interactions between proteins, thus facilitating the initial flow of cheese. However, in the present study, a slight paradox occurred in that cheeses had lower cheese flow values when cheese pH decreased from 6 to 5.5 (Table 5). While calcium solubilization would initially decrease protein–protein interactions at room temperature, decreasing the pH could also have promoted an increase in the proportion of hydrophobic interactions occurring at higher temperatures, thereby causing reduced cheese flow under the conditions where this property was measured. This could also explain why the crossover temperature increased when the cheese pH decreased from 6 to 5.5. The changes in cheese flow at lower pH values are consistent with increases in structural breakdown and have also been observed in other cheeses, such as natural Mozzarella at pH levels below 5.0 (Kindstedt, Zielinski, Almena-Aliste, & Ge, 2001).

4. Conclusion

The level of hydrolysis in EMCs, which produced different levels of aromatic SCFAs, affected the cheese-like sensory characters of imitation cheeses flavoured using these products. Cheese pH impacted on flavour perception, and decreasing the pH from 6.0 to 5.5 appeared to increase the flavour intensity of the imitation cheese flavoured with low lipolysis EMCs. Cheese hardness and meltability decreased when the pH levels decreased from 6 to 5.5 and panellists preferred the pH 5.5 cheeses, describing them as 'smoother' and 'creamier' in the mouth. The present study has shown that imitation cheese flavours are influenced, not only by the level of lipolysis of the EMCs used to prepare them, but also by the pH of the cheese base, which can be used to modify the concentrations and aroma intensity of the free SCFAs.

Acknowledgements

The authors would like to thank members of the cheese sensory panel at University College Dublin for participating in this study. This work was funded by the Food Institutional Research Measure (FIRM) and is administered by

the Department of Agriculture, Food and Rural Development.

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