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Flavour precursor development in Cheddar cheese due to lactococcal starters and the presence and lysis of *Lactobacillus helveticus*.

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Abstract

The rapid release of intracellular enzymes due to autolysis of lactic acid bacteria in the cheese matrix has been shown to accelerate cheese ripening. The objective of this work was to investigate the evolution of the flavour precursors, individual free amino acids (FAAs), free fatty acids (FFAs) and volatile compounds that contribute to the sensory profiles of cheeses at 2, 6 and 8 months of ripening in Cheddar cheese manufactured using starter systems which varied with respect to their autolytic properties. Starter system A contained a blend of two commercial *Lactococcus lactis* strains (223 and 227) which had a low level of autolysis. System B was identical to A but also included a highly autolytic strain of *Lactobacillus helveticus* (DPC4571). System C contained only strain DPC4571. Levels of all individual FAAs were elevated in cheeses B and C relative to A after 2 months of ripening. By 8 months of ripening the main FAA were glutamate, leucine, lysine, serine, proline and valine. Levels of $C_{6:0}$, $C_{8:0}$, $C_{12:0}$ and $C_{18:0}$ fatty acids did not vary greatly over ripening, while levels of $C_{4:0}$, $C_{10:0}$, $C_{14:0}$, $C_{16:0}$ and $C_{18:1}$ were elevated in cheeses B and C. Principal component analysis of the headspace volatiles separated cheese A from cheeses B and C. Cheeses B and C had highest levels of dimethyl disulphide, carbon sulphide, heptanal, dimethyl sulphide, ethyl butanoate, 2-butanone, and 2-methyl butanal and were described as having a 'caramel' odour and 'sweet', 'acidic' and 'musty' flavour. Cheese A had highest levels of 2-butanol, 2-pentanone, 2-heptanone, 1-hexanol and heptanal and was described as having a 'sweaty/ sour' odour and 'soapy', 'bitter' and 'mouldy' flavour. The results highlight the impact of starter lactococci on flavour precursor development and the positive effect of *Lb. helveticus* and the lysis of this strain on enhancing levels of substrate and flavour precursors early during ripening resulting in early flavour development.

Keywords: *Lb. helveticus*; Autolysis; Free amino acids; Lipolysis; Volatile analysis; Acceleration; Flavour improvement.

1. Introduction

Flavour development in cheese is the result of a complex series of microbiological, biochemical and chemical processes that occur during ripening (Fox & Wallace, 1997). Flavour compounds are formed by various processes acting in a concerted and/or sequential manner, i.e., conversion of lactose and citrate (glycolysis and pyruvate metabolism), fat (lipolysis), and caseins (proteolysis) (McSweeney & Sousa, 2000). Lactic acid bacteria (LAB), milk and rennet provide the enzymes involved in the biochemical conversions that give rise to volatile and non-volatile compounds which contribute to cheese flavour. However, the ripening of some cheeses can take a considerable length of time and a better understanding of the processes involved could enable reduction in the ripening time required to produce mature flavours.

Selection of starter systems with high levels of peptidase activity and high autolytic abilities results in an increase in the levels of flavour forming substrates such as free amino acids (FAAs). However, wide differences in the expression of proteinases, peptidases, amino acid converting enzymes, lipases and esterases exist between strains of LAB

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(Pritchard & Coolbear, 1993; Amárita, Requena, Taborda, Amigo, & Peláez, 2001) as well as their autolytic abilities (O'Sullivan, Ross, Fitzgerald & Coffey, 2000; Valence, Deutsch, Richoux, Gagnaire, & Lortal, 2000; Madkor, El Soda & Tong, 1999, 2000; Hannon, Wilkinson, Delahunty, Wallace, Morrissey, & Beresford., 2003). *Lactobacillus helveticus* has previously been shown to have a potent proteolytic system (Hickey, Hillier, & Jago, 1983; Sasaki, Bosman, & Tan, 1995; Christensen, Dudley, Pederson, & Steele, 1999, Kenny, FitzGerald, O'Cuinn, Beresford, & Jordan, 2003) and has been shown to enhance early flavour development in Cheddar cheese (Hannon et al., 2003).

Starter lysis has been shown to result in an increase in FAAs and flavour development by releasing the enzyme complement directly into the cheese matrix, thus favouring access of enzymes to their substrates (Bie & Sjöström, 1975 a, b; Chapot-Chartier, Deniel, Rousseau, Vassal, & Grippon, 1994; Wilkinson, Guinee, O'Callaghan, & Fox, 1994; O'Donovan, Wilkinson, Guinee, & Fox, 1996, Kawabata et al., 1997). Studies on the influence of starter autolysis on lipolysis are limited. Collins, McSweeney, and Wilkinson (2003a) reported increases in a number of FFAs in Cheddar cheese by autolysis of *Lactococcus lactis* subsp. *cremoris* AM2, suggesting that the influence of autolysis of starter bacteria on cheese enzymology and biochemistry may not be confined to proteolysis but also lipolysis and other enzyme-mediated flavour reactions. However, the role of lysis on the production of flavour compounds is not fully understood (Lortal & Chapot-Chartier, 2005).

Although FAA degradation due to the action of microbial enzymes appears to be rate limiting in terms of flavour development, the increased formation of these precursors early in ripening is a necessary step to enhance flavour development (McSweeney & Sousa, 2000; Smit et al., 2000). Both the FAAs and FFAs are considered not only to contribute to the background flavour of cheese but also to act as flavour precursors, which are catabolised to volatile compounds by the enzymes present in the cheese matrix (Urbach, 1997). In view of the fact that the release and turnover of flavour pre-cursors by lactococci and lactobacilli (and their autolytic ability), has implications for flavour development in cheese during ripening, this study was undertaken to investigate the evolution of FAAs, FFAs and volatile compounds in Cheddar cheese manufactured with starter systems which contained only lactococci, lactococci with an adjunct of *Lb. helveticus* and only *Lb. helveticus*. Each starter system varied with respect to the level of lysis.

The work reported here was performed on cheeses previously described in Hannon et al. (2003). The starter systems used were: A, a blend of two *Lactococcus lactis* strains (223 and 227) that had a low level of autolysis; B, identical to A but included an adjunct of a highly autolytic strain of *Lb. helveticus* (DPC4571); and C, consisted only of strain DPC4571 as starter. Compositional analysis revealed no significant differences in moisture, fat or protein, but did reveal differences in the salt-in-moisture (5.35, 4.78 and 4.61 g $100\,g^{-1}$) and pH (5.32, 5.17, 5.07) for cheeses A, B and C, respectively. NSLAB were detected at $\sim 10^1$ cfu g^{-1} of cheese in all cheeses the day following manufacture. However, in cheeses B and C which contained *Lb. helveticus* NSLAB numbers increased to only to 10^3 to 10^4 cfu g^{-1} of cheese over the entire ripening period, while attaining $\sim 10^8$ cfu g^{-1} cheese in cheese A, suggesting that their growth was inhibited due to the presence of *Lb. helveticus*. Primary proteolysis (as measured by changes in pH 4.6-soluble nitrogen) was not affected by lysis whereas secondary proteolysis (as measured by changes in phosphotungstic acid soluble nitrogen and total amino acids) was significantly enhanced. Descriptive sensory analysis showed an acceleration of ripening and flavour development in cheeses containing *Lb. helveticus* and higher levels of lysis.

2. Materials and methods

2.1. Cheesemaking strains and cheese manufacture

Cheeses were manufactured according to Hannon et al. (2003).

2.2. Individual free amino acid analysis

Individual free amino acids were determined on the 12% trichloroacetic acid (TCA)-soluble fraction using a Beckman model 6300 Amino Acid Analyser (Beckman Instruments Ltd., High Wycombe, UK) equipped with a Beckman model P-N 338052 Na^+ cation exchange column (12 cm × 0.4 cm). Samples for individual free amino acids were deproteinisied by mixing equal volumes of 24% (w/v) TCA, allowed to stand for 10 min before centrifuging (Microcentaur, MSE, UK) at 14,400 × *g* for 10 min. The samples were diluted (1:2) with internal standard (Norleucine, Sigma Chemical Co., St. Louis, MO, USA) to give 125 nmol of each amino acid residue per injection volume, and filtered through 0.2 μm filter (Gelman Ltd., Dunlaoghaire, Dublin, Ireland). Filtrate (50 μL) was loaded onto the analyser. The amino acids were separated using ion exchange chromatography with post-column derivatisation and visible colorimetric detection; amino acids were detected at 570 nm with the exception of proline, which was detected at 400 nm. Results were recorded using a Minichrom© data handling system (VG Data Systems, Altrincham, Cheshire, UK). All samples were analysed in duplicate at 2, 6 and 8 months of ripening.

2.3. Individual free fatty acid analysis

Individual free fatty acids were extracted from the cheese and quantified according to the method of De Jong and Badings (1990) with the following modifications. An internal standard of 0.5 mg mL^{-1} consisting of valeric acid ($C_{5:0}$), nonanoic acid ($C_{9:0}$) and heptadecanoic acid ($C_{17:0}$) was used. Recoveries of individual FFAs were based on the

recoveries of these internal standards. Solid phase extraction (SPE) was carried out using 500 mg, 3 mL Strata aminopropyl columns (Phenomenex, Macclesfield, Cheshire, UK). These SPE columns were conditioned with 10 mL of heptane and neutral lipids were eluted using 10 mL of hexane/2-propanol (3:2 (v/v)). FFAs were eluted in 5 mL ether containing 2 g $100\,g^{-1}$ (v/v) formic acid. Individual FFAs ($C_{4:0}$, $C_{6:0}$, $C_{8:0}$, $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$) were quantified by gas chromatography (GC) using a Varian 3800 Gas Chromatograph with flame ionisation detection, a Varian 1079 Universal capillary injector, a Varian 8410 liquid autosampler and Varian Star operating software (Varian Analytical Instruments, Harbor City, California, USA). The column used was J & W Scientific WCOT fused silica capillary column DP-FFAP, 30 m × 0.32 mm ID, 1 μm DF (Agilent, Carl Stuart Ltd, Dublin 24, Ireland) with direct on column injection. Injector temperature was 65 °C (held for 0.1 min), then increased to 250 °C at 200 °C min^{-1} (held for 1 min), followed by cooling to 65 °C at 200 °C min^{-1} (held for 20 min). Carrier gas was helium at a flow rate of 4 mL min^{-1}. Oven temperature was 65 °C (held for 1.5 min), then heated to 240 °C at 10 °C min^{-1}, for a total run time of 44 min. Detector temperature was 300 °C. All samples were analysed in triplicate at 2, 6 and 8 months of ripening.

2.4. Volatile analysis

The headspace volatile compounds of the cheeses were isolated by a dynamic headspace analyser Tekmar 3000 concentrator (JVA Analytical Ltd. Unit 1, Longmile Business Centre, Longmile Road, Dublin 12, Ireland). Prior to analysis, frozen samples (−20 °C) were thawed at 4 °C overnight. The outer layer (2 cm) of each cheese was removed to minimise the possibility of compounds from the packaging which may have migrated into the cheese being detected. Cheese internal sections were grated and mixed before taking a 20 g sample. This was diluted with 40 g Pestiscan ultra pure water (Sigma-Aldrich Ireland Ltd., Dublin 24, Ireland) and homogenised using a benchtop waring blender on high for 3 × 20 s intervals. This mixture (20 g) was weighed into a 35 mL fritted glass purge sampler and heated to 37 °C and purged for 15 min with ultrapure helium at a flow rate of 40 mL min^{-1} to isolate headspace volatiles. The volatiles emitted under these conditions were trapped on a Tenax-TA trap (Sigma-Aldrich Ireland Ltd., Dublin 24, Ireland). The trapped compounds were thermally desorbed at 235 °C for 4 min and injected at 200 °C onto the column of a Saturn GC-3400cx Gas Chromatograph (Varian Analytical Instruments, Harbour City, California, USA) equipped with a J & W DB-5 capillary column (60 m × 0.32 mm × 1.0 μm film thickness); (Phenomenex, Macclesfield, Cheshire SK10 2BN, UK). Volatile compounds were separated under the following conditions: carrier gas: helium 1 mL min^{-1}, initial column temperature was −60 °C held for 4 min, heated to 20 °C at 250 °C min^{-1}, followed by heating to 40 °C at 3 °C min^{-1}, heating to 110 °C at 5° C min^{-1}, heating to 250 °C at 40 °C min^{-1} and holding for 10 min for a total run time of 39.76 min. The GC column was connected without splitting to the ion source of a Varian Saturn 2000 mass spectrometer (Varian Analytical Instruments, Harbour City, California, USA), operating in the scan mode within a mass range of m/z 25–300 at 2.5 scan s^{-1}. Ionisation was performed by electronic impact at 70 eV; calibration was performed by autotuning. Quantification was performed by integrating the peak areas of total ion chromatograms (TIC) by the Saturn GC/MS software. Compounds were tentatively identified by computer matching of mass spectral data with those of Nist 98 Mass Spectral Database and by comparing their mass spectra and retention times to those of authentic standard compounds under identical operating conditions, and comparing the Kovats indices obtained after running a series of *n*-alkanes with those in published literature. Standard compounds were purchased from Sigma-Aldrich (Sigma-Aldrich Ireland Ltd., Dublin 24, Ireland), at a purity of 99.9%. Analyses were repeated in triplicate on freshly prepared homogenate for all samples for all trials at 2, 6 and 8 months of ripening.

2.5. Descriptive sensory analysis

Descriptive sensory analysis was carried out as described in Hannon et al. (2003) at 2, 6 and 8 months of ripening.

2.6. Statistical analysis

One-way analysis of variance (ANOVA) was used to test the ability of (1) the descriptive vocabulary attributes (2) peak area of volatile compounds (3) levels of FAAs and (4) levels of FFAs to discriminate between cheeses. Duncan's multiple comparison test was used for pair comparisons of the treatment means. Differences among treatments that are described subsequently as being significant, were determined at $P<0.05$. The data were further analysed by ANOVA using a general linear model. A statistical model was constructed and fitted with terms to account for variations due to treatment (starter system), age (ripening time) and the interaction of the treatment and age. ANOVA was carried out using SPSS v 8.0 (SPSS Inc., Chicago, IL 60611, USA.

All sensory attribute scores, volatile compounds and FFAs were subsequently averaged across replicates, standardized (1/Standard Deviation of the mean score for each attribute) and analysed using principal component analysis (PCA) (Piggott & Sharman, 1986). ANOVA was used to investigate how each principle component (PC) discriminated between the cheese scores for each PCA. PCA was carried out using Unscrambler v. 6.1 (CAMO AS, N-7041 Trondheim, Norway). A further PCA was performed on a matrix consisting of the FAAs, FFAs, volatile compounds and sensory scores.

3. Results and discussion

Although differences in the pH and salt-in-moisture content of these cheeses were reported in Hannon et al. (2003), both of which have an effect on enzyme activities as well as flavour perception, these differences were within the range identified as acceptable for Cheddar cheese (Lawrence et al., 2004) and considered to have little potential to result in significantly different ripening patterns.

3.1. Individual free amino acid analysis

The results of the individual FAAs are shown in Fig. 1 at 2, 6 and 8 months of ripening. ANOVA of the FAAs revealed significant differences ($P<0.05$) existed due to the starter system used (df = 2, $F = 55.70$, $P \leqslant 0.000$), ripening time (df = 2, $F = 30.17$, $P \leqslant 0.000$), and the interaction of starter system and ripening time (df = 4, $F = 39.36$, $P \leqslant 0.000$). In agreement with previous findings (Fenelon, Ryan, Rea, Guinee, & Ross, 1999; O'Donovan, Wilkinson, Guinee, & Fox, 1996; Wilkinson, Guinee, O'Callaghan, & Fox, 1994) the principal FAAs in Cheddar cheeses were glutamic acid, leucine, phenylalanine, valine, lysine, serine and proline. At all ripening times, levels of all individual FAAs were elevated in cheeses containing the highly autolytic strain *Lb. helveticus* DPC4571 (cheese B and C) compared to cheese A, which only contained lactococci in the starter mix. Levels detected were also higher in cheese C

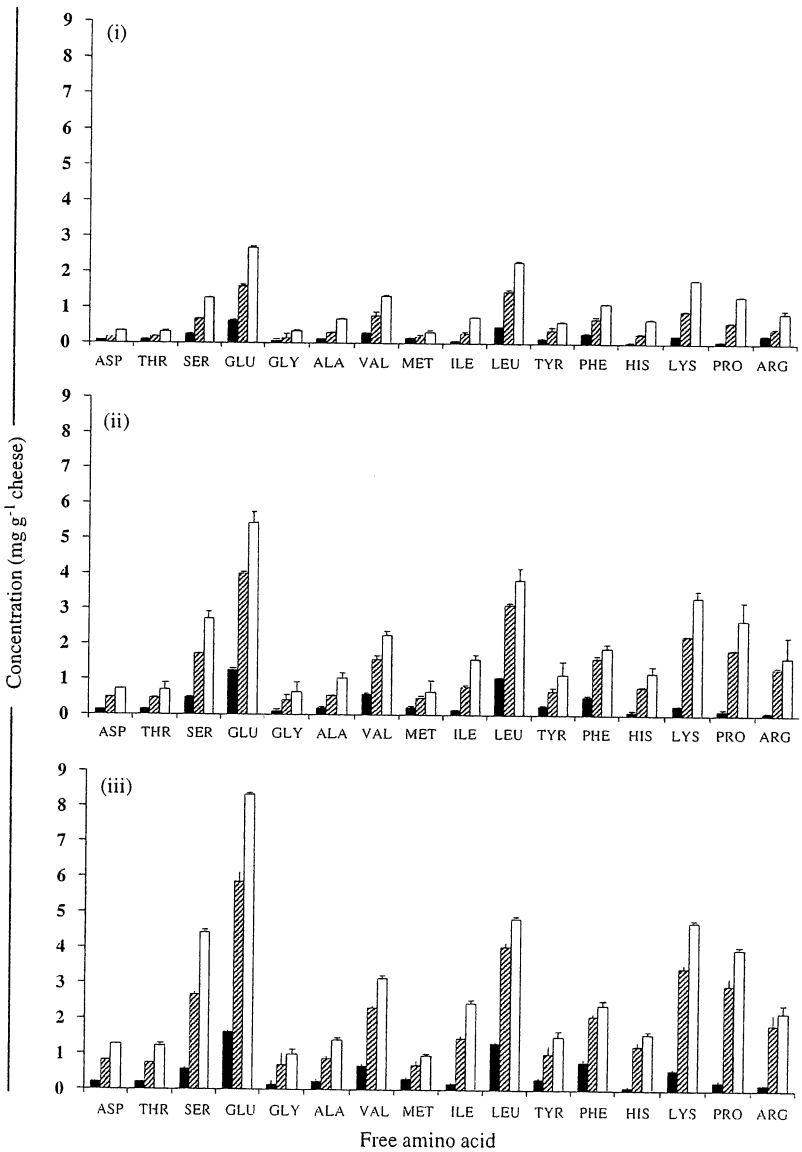

Fig. 1. Levels of individual free amino acids (FAAs) detected in cheeses made with starter system A (■), starter system B (▨) or starter system C (□) at (i) 2, (ii) 6 and (iii) 8 months of ripening. The starter systems used were: A, a blend of two *Lactococcus lactis* strains (223 and 227) that had a low level of autolysis; B, identical to A but included an adjunct of a highly autolytic strain of *Lb. helveticus* (DPC4571); and C, consisted only of strain DPC4571 as starter. The values presented are the means of three replicate trials. ANOVA revealed that significant differences ($P<0.05$) existed for all individual FAAs at each time point. Error bars indicate standard deviations obtained across the three trials.

compared with cheese B that had a higher initial population of *Lb. helveticus*.

Many reports in the literature have correlated a positive relationship between the FAA release in cheese and autolysis of the starter (Chapot-Chartier, Deniel, Rousseau, Vassal, & Gripon, 1994; Wilkinson et al., 1994; Crow et al., 1995; O'Donovan et al., 1996; Morgan, Ross, & Hill, 1997; Kawabata et al., 1997). According to Ardö, Thage, and Madsen (2002) the concentration of FAAs detected in cheese only partly reflects the composition of casein. The level of individual FAAs in cheese is the result of microbial metabolic activities in which some FAAs are used and others may be produced and excreted into the cheese matrix (Sousa, Ardö, & McSweeney, 2001). According to Fox (1989), the greatest source of FAAs is via the proteolytic activities of starters. In the early period of ripening, utilisation of FAAs is mainly due to starter cultures, whereas during the latter stages of ripening, NSLAB contribute to both the appearance and disappearance of amino acids (Crow, Coolbear, Holland, Pritchard, & Martley, 1993). In cheeses B and C, NSLAB numbers remained very low throughout ripening (Hannon et al., 2003) and hence are not considered to be either producing or consuming significant amounts of FAAs. Similar levels of lactococci were detected in cheeses A and B throughout ripening, hence differences in the levels of FAAs detected by 2 months were considered to be mainly due to the presence and lysis of *Lb. helveticus* DPC4571 adjunct. Initial levels of *Lb. helveticus* DPC4571 were 1 log higher in cheese C on day 1 and resulted in higher levels of lysis and the subsequent detection of much higher levels of FAAs due to a greater release of intracellular enzymes directly into the cheese matrix.

Recently, Kenny, FitzGerald, O'Cuinn, Beresford and Jordan. (2003) reported that *Lb. helveticus* DPC4571 contains a broad range of intracellular peptidase activities including aminopeptidase, dipeptidase, tripeptidase, endopeptidase and proline specific peptidase activities. This represents a significant potential source of enzymes when released into the cheese matrix on lysis with direct access to their substrates. The elevated levels of individual FAAs detected in cheeses B and C are indicative of the high level and activity of these enzymes in the cheese matrix.

3.2. Individual free fatty acid analysis

Fig. 2 shows the results of individual FFAs detected at 2, 6 and 8 months of ripening. ANOVA of the FFAs revealed significant differences ($P<0.05$) existed due to the starter system used ($df = 2$, $F = 450.15$, $P \leqslant 0.000$), ripening time ($df = 2$, $F = 73.78$, $P \leqslant 0.000$), and the interaction of starter system and ripening time ($df = 4$, $F = 27.12$, $P \leqslant 0.000$). Levels of FFAs were numerically higher in cheeses containing *Lb. helveticus* and were highest in cheese C which had the highest levels of lysis, suggesting a relationship between the lysis and lipolysis as suggested by Collins et al. (2003a). In cheese, FFAs released as a result of lipolysis, especially short- and medium-chain even numbered fatty acids are thought to directly contribute to cheese flavour due to their low perception thresholds. Long-chain FFAs (>12 carbon atoms) are considered only to play a minor role (Mollimard & Spinnler, 1996). Each short-chain FFA gives a characteristic flavour note. For example $C_{4:0}$ contributes to a 'rancid' and 'cheesy' flavour while $C_{6:0}$ has a 'pungent' and $C_{8:0}$ a 'soapy', 'musty', 'rancid' or 'fruity' flavour note (Collins, McSweeney, & Wilkinson, 2003b). Depending on their concentration and flavour threshold values, volatile fatty acids can either contribute positively to the aroma of the cheese or to a rancidity effect. Long-chain FFAs accumulated with ripening time in all the cheeses, but especially in cheeses B and C. As all other sources of lipases are similar in all the cheeses, the differences observed between the cheeses are attributed to the differences in starter type and lysis of *Lb. helveticus* DPC4571.

To date, lipases and esterases that have been identified and characterised in lactic acid bacteria appear to be exclusively intracellular (Castillo, Requena, Fernandez de Palencia, Fontecha, & Gobetti, 1999; Collins et al., 2003b). Lee and Lee (1990) reported esterolytic and lipolytic enzyme activity in cheese due to cell lysis of *Lb. casei* subsp. *casei* LLG. Recently, Collins et al. (2003a), while studying the influence of starter autolysis on lipolysis in Cheddar cheese made with *L. lactis* AM2 or HP, reported significantly higher levels of a number of FFA ($C_{8:0}$, $C_{14:0}$, $C_{16:0}$ and $C_{18:0}$). These increases were attributed to the lysis of the highly autolytic strain *L. lactis* AM2, and the accumulation of the long chain FFAs were similar to the pattern observed in this study.

PCA was performed on the individual FFA data, to illustrate how patterns of lipolysis differed between the cheeses made with the different starter systems. Fig. 3 shows the biplot of the scores of the samples and the loadings of the individual FFAs. PC1 and PC2 explained a cumulative variation of 90% and separated the cheeses according to the starter system used. Cheese A, at all ripening times, was grouped with cheese B at 2 months, and well separated from the other cheeses indicating a very different profile of FFAs in these cheeses. The grouping of cheese B at 2 months with cheese A may be indicative of the lactococcal component of the starter system used in its manufacture. Cheese B at 6 and 8 months of ripening grouped with Cheese C at 2 months, reflecting that cheese C at 2 months had a similar level of FFAs to cheese B at 6 or 8 months. This suggests that lysis of *Lb. helveticus* DPC4571 results in increased lipase/esterase activity in the cheese matrix. Cheese B, however, was associated with higher levels of $C_{6:0}$, $C_{8:0}$ and $C_{12:0}$ indicating that greater catabolism of these FFAs may have occurred in cheese C and hence contribute to altering the flavour profile. Finally, cheese C was grouped at 6 and 8 months of ripening and showed that these cheeses contained higher levels of $C_{4:0}$, $C_{10:0}$, $C_{14:0}$, $C_{16:0}$, $C_{18:0}$ and $C_{18:1}$. As the cheeses that

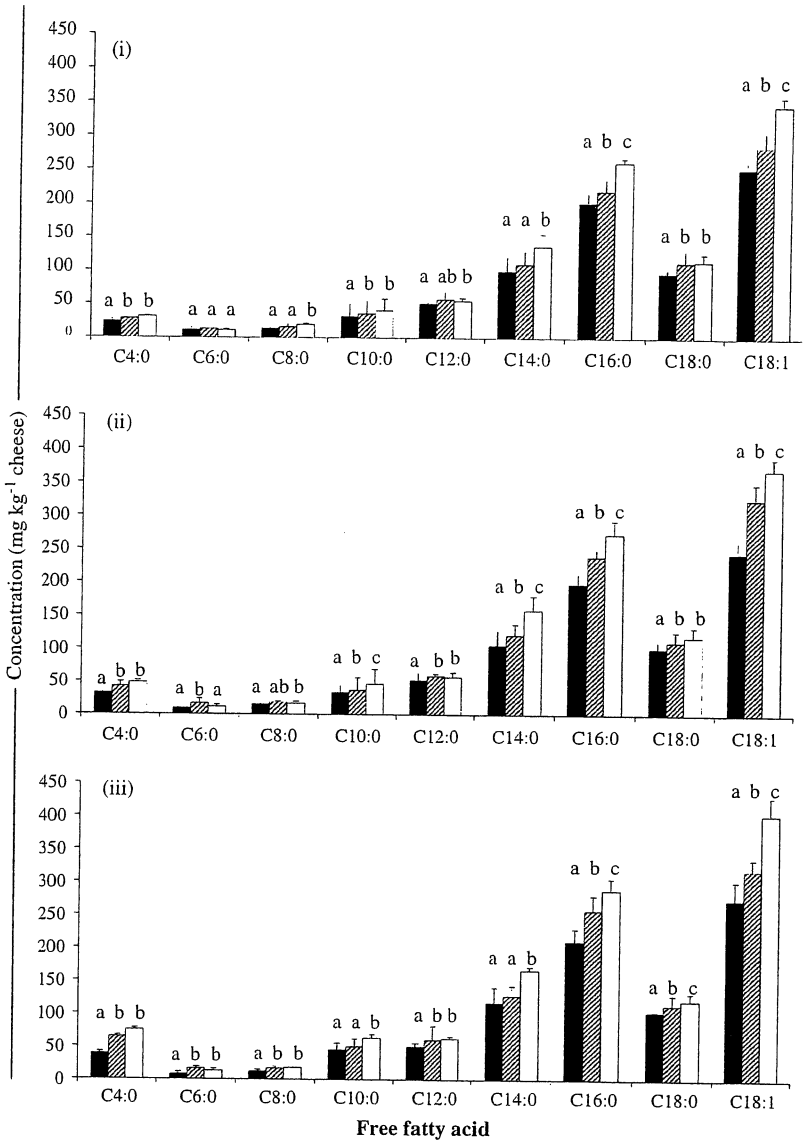

Fig. 2. Levels of individual free fatty acids (FFAs) detected in cheeses made with starter system A (■), starter system B (▨) or starter system C (□) at (i) 2, (ii) 6 and (iii) 8 months of ripening. The starter systems used were: A, a blend of two *Lactococcus lactis* strains (223 and 227) that had a low level of autolysis; B, identical to A but included an adjunct of a highly autolytic strain of *Lb. helveticus* (DPC4571); and C, consisted only of strain DPC4571 as starter. The values presented are the means of three replicate trials. Letters a, b, c indicate results of Duncans post hoc test on individual FFAs. Bars not having a common letter indicate significant differences ($P<0.05$). Error bars indicate standard deviations obtained across the three trials.

contained *Lb. helveticus* DPC4571 were well separated from cheeses that only contained lactococci as starter (cheese A), it could be concluded that the difference in FFA profiles is due to the presence and lysis of this strain, favoring access of esterase/lipase enzymes to their substrates and enhancing lipolysis in these cheeses.

3.3. Assessment of volatile compounds

Production of volatile compounds in cheese results from the catabolism of FAAs, FFAs, lactose and citrate, and is mediated by the complex and intricate enzyme complement of the starters, NSLAB and the prevailing conditions of the cheese matrix. The volatile compounds detected by Purge and Trap, which are likely to contribute to the aroma of the cheeses at 2, 6 and 8 months of ripening are given in Table 1. Twenty-four compounds consisting of 4 alcohols, 8 aldehydes, 2 esters, 6 ketones and 4 sulphur compounds were identified in all the cheeses at each ripening time. All of these compounds have previously been identified in Cheddar cheese (Maarse, Visscher, Willemsens, Nijssen, & Boelens, 1994). PCA of the volatile compounds was performed to demonstrate how volatile compounds discriminated between the cheeses. ANOVA found that the first two PCs significantly discriminated between the cheeses (PC1: df = 2, $F = 4.318$, $P = 0.029$; PC2: df = 2, $F = 6.90$, $P = 0.035$) and accounted for a cumulative explained variance of 59%. A biplot of the scores of the

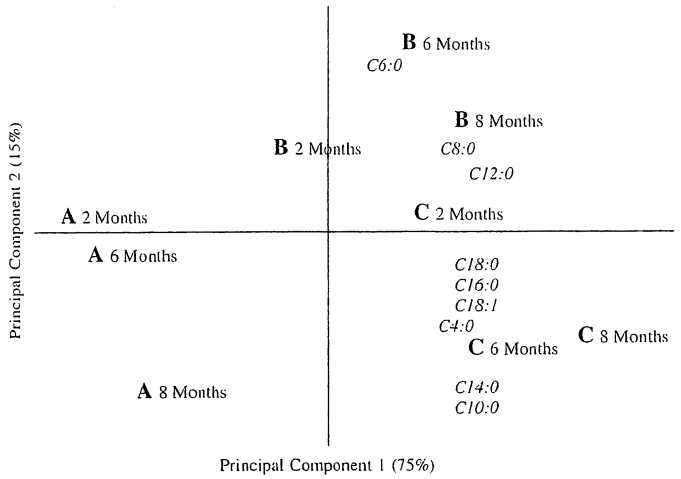

Fig. 3. PCA showing the first two Principal Components of individual FFA analysis of Cheddar cheeses made with starter system A, B or C at 2, 6 and 8 months of ripening. The starter systems used were: A, a blend of two *Lactococcus lactis* strains (223 and 227) that had a low level of autolysis; B, identical to A but included an adjunct of a highly autolytic strain of *Lb. helveticus* (DPC4571); and C, consisted only of strain DPC4571 as starter. The values presented are the means of three replicate trials.

cheeses and the loadings of the attributes is presented in Fig. 4.

PC1 (31% of explained variance) separated the cheeses on the basis of age with the younger cheeses scoring negatively and the older cheeses scoring positively on PC1. PC 2 (28% of explained variance) separated the cheeses on the basis of starter system used with cheese A scoring negatively at all time points and cheeses B and C scoring positively on PC2. The biplot reflects the development of volatile aroma compounds in the different cheeses. All cheeses at 2 months of ripening were grouped with cheese A at 6 months, suggesting that the volatile profile of these cheeses detected by Purge and Trap are similar at this stage of ripening. The second grouping included both cheeses B and C at 6 and 8 months and suggests the influence of *Lb. helveticus* and possibly its lysis to the development of a different volatile profile to the control. The separation of cheese A at 8 months is possibly due to the absence of *Lb. helveticus* from the starter used and to the higher levels of NSLAB detected in this cheese by the end of ripening. The positioning of the cheeses on the biplot clearly illustrates the effect of the different enzyme complements present in each of these cheeses due to the presence of lactococcal starter system, *Lb. helveticus*, levels of lysis and levels of NSLAB. The relative positions of Cheese C at 6 months and Cheeses A and B at 8 months across PC1 highlights the intense aroma that has developed in cheese C by 6 months of ripening.

Although all the compounds listed in Table 1 were identified in all the cheeses at all ripening times, it is the balance in the concentrations of compounds that change with time and is contributing to the discrimination of the volatile aroma profile between the cheeses. Cheeses containing *Lb. helveticus* and higher levels of lysis (cheeses B and C) were characterised by relatively high levels of sulphur compounds (formed from catabolism of methionine), esters, branched chain aldehyde 2-methyl-butanal (formed from catabolism of isoleucine), straight chain aldehydes (originating from lipolytic pathways), some ketones and 2-butanone. These results suggest that perhaps the catabolism of the flavour precursors (FAAs and FFAs) is also enhanced due to the presence and lysis of *Lb. helveticus*.

3.4. Descriptive sensory analysis

Individual results, averaged across assessors and replicates are given in Table 2 for the 7 odour and 15 flavour terms used. ANOVA found that the first two PCs discriminated significantly between the cheeses (PC1: $df = 2$, $F = 4.549$, $P = 0.033$; PC2: $df = 2$, $F = 2.903$, $P = 0.046$) and accounted for a cumulative explained variance of 75%. A biplot of the scores of the cheeses and the loadings of the attributes are presented in Fig. 5.

The biplot shows the effect on flavour development due to the different starter systems used as well as differences in levels of NSLAB, and comparison of the relative positions of the cheeses reflects the development of flavour in each cheese over the entire ripening period. PC1 (44% of explained variance) distinguished the cheeses on the basis of age or intensity of flavour with the younger less intensely flavoured cheeses scoring positively and the mature intensely flavoured cheeses scoring negatively on PC1. PC 2 (31% of explained variance) distinguished the cheeses on the basis of starter system used.

The flavour profiles of cheeses A and B at 2 months were similar and reflect the influence of the lactococcal starter used in these cheeses. However, cheese B which contained an adjunct of *Lb. helveticus*, attributes such as 'caramel', 'creamy', 'sweet' odour and 'balanced' flavour were more enhanced, suggesting the influence that the presence of this strain and its lysis has on altering the flavour profile of the cheese. Cheese C at 2 months of ripening was grouped between cheese B at 6 and 8 months of ripening, and highlights the intensity of flavour that had developed in this cheese at such a young age and correlates well with the higher levels of substrate (FAAs, FFAs) detected in this cheese early in ripening. The flavour profile that developed in cheese A at 6 and 8 months of ripening was completely different to that which developed in cheeses containing *Lb. helveticus*. Cheeses containing *Lb. helveticus* DPC4571, either as a single starter or as a component in the starter blend, were associated with 'strength', 'acidic', 'sweet', 'caramel', and 'mushroom' flavour notes and 'musty', 'fruity', 'sweet', 'creamy', 'caramel' odour, while cheeses containing only lactococcal starters developed a 'mouldy', 'bitter', 'rancid', 'soapy' flavour and a 'sweaty-sour', 'pungent' odour.

Table 1
Volatile compositional analysis of Cheddar cheeses made with different starter systems showing the averaged peak areas (in arbitrary units ÷ 1000) of triplicate determinations, coefficient of variation[a] and ANOVA

Compound	Cheese									ANOVA[c]		
	A			B			C					
	2M[b]	6M[b]	8M[b]	2M[b]	6M[b]	8M[b]	2M[b]	6M[b]	8M[b]	Treat[d]	Age[e]	Treat*age
Alcohols												
2-Butanol	117±25	121±29	154±28	123±23	165±26	127±21	17±42	38±16	11±28	*	*	*
2-Hexanol	211±18	111±14	398±19	42±15	197±14	140±19	63±15	190±17	105±19	*	*	*
2-Heptanol	6±17	134±14	191±12	9±18	232±21	360±18	32±17	252±21	267±24	*	*	*
2/3-Me-1-Butanol	26±25	43±28	75±19	31±18	25±24	28±26	22±21	71±27	26±24	*	*	*
Aldehydes												
Pentanal	105±16	105±18	80±20	455±22	305±28	420±21	314±23	434±28	339±18	*	*	*
Hexanal	992±10	491±15	933±21	341±17	687±12	839±16	12±15	931±16	972±27	*	*	*
Heptanal	1467±23	214±33	5217±29	211±25	1309±33	1544±25	6±17	1273±26	4227±26	*	*	*
Octanal	116±28	870±27	1314±21	116±20	1389±24	2839±31	199±30	1611±27	3805±28	*	*	*
Nonanal	194±34	80±25	3608±33	343±21	202±17	575±32	5±30	1263±26	100±34	*	*	*
2-Me-Butanal	21±10	22±14	55±13	70±13	59±13	79±19	53±15	177±18	131±11	*	*	*
3-Me-Butanol	336±17	118±19	1691±15	95±11	480±14	685±13	84±12	417±19	380±14	*	*	*
Benzaldehyde	151±21	221±26	188±24	205±15	222±23	585±24	81±18	235±22	401±20	*	*	*
Esters												
Ethyl Acetate	13±17	370±13	103±19	17±12	125±25	213±15	21±16	103±23	609±24	*	*	*
Ethyl Butanoate	53±11	94±12	25±10	54±12	133±15	17±11	25±11	129±16	110±16	*	*	*
Ketones												
Acetone	1369±27	5533±33	3087±30	1333±28	4034±30	3044±27	1998±34	3723 ±28	9171±27	*	*	*
2-Butanone	279±14	35±12	41±16	218±17	571±15	359±12	153±11	456±20	252±16	*	*	*
2-Pentanone	462±11	190±12	258±16	172±13	37±12	104±89	6±15	26±11	72±12	*	*	*
2-Heptanone	216±12	77±14	514±10	145±12	204±10	204±14	9±13	254±12	266±15	*	*	*
2-Nanone	1±21	203±18	123±16	2±15	119±19	281±21	16±23	131±22	506±28	*	*	*
3-Me-2-Hexanone	370±34	8084±24	5248±31	5082±29	2689±24	2869±24	1113±25	413±25	911±27	*	*	*
Sulphur compounds												
Methanethiol	25±22	54±24	106±25	29±32	80±30	474±29	46±27	163±32	334±35	*	*	*
Carbon disulphide	227±11	27±18	135±12	502±15	160±15	203±11	943±13	597±12	862±18	*	*	*
Dimethyl sulphide	84±14	1±12	2±11	9±10	287±14	71±16	4±15	355±10	87±17	*	*	*
Dimethyl disulphide	19±12	35±14	64±15	97±12	71±14	84±11	83±13	52±12	71±15	*	*	*

[a]Coefficient of variation obtained from a total of nine measurements.
[b]M = months of ripening.
[c]ANOVA performed using general linear model. * = $P<0.05$.
[d]Treat = treatment (Starter system used).
[e]Age = Ripening time.

3.5. Principal Component analysis of FAAs, FFAs, volatile and sensory data

PCA was performed on a matrix consisting of the FAAs, FFAs, volatile compounds and sensory data in an attempt to relate the flavour profiles with flavour precursors and volatile compounds. A biplot explaining a cumulative variation of 72% of the variation is presented in Fig. 6. Cheeses containing *Lb. helveticus* and higher levels of lysis (cheeses B and C) were characterised by high levels of both FAAs as well as FFAs, especially by the end of ripening. The FAAs and FFAs not only act as flavour precursors but also contribute to the background flavour of the cheeses (Urbach, 1997) and probably contribute to the 'strength' and 'astringent' flavours associated with cheeses B and C. These cheeses were also characterised by a 'musty' odour possibly originating from the high levels of sulphur compounds (carbon disulphide, dimethyl disulphide, dimethyl sulphide and methionine) associated with these cheeses. The odour attribute 'fruity' was grouped with the esters, ethyl acetate and ethyl butyrate, and the ketones, acetone and 2-nonanone (all of which have a fruity flavour note), suggesting that these compounds were responsible for the fruity/sweet odour associated with these cheeses. Cheese A, containing only the lactococcal starter and higher levels of NSLAB, was characterised as having a more 'sweaty/sour' odour, possibly associated with the higher levels of 3-methyl-butanal (harsh green flavour note), 2-heptanone (blue cheese flavour note) and aldehydes, nonanal, hexanal and heptanal (green, fatty flavour note). However, as all compounds were detected in all cheeses, it is the relative balance of compounds and flavour

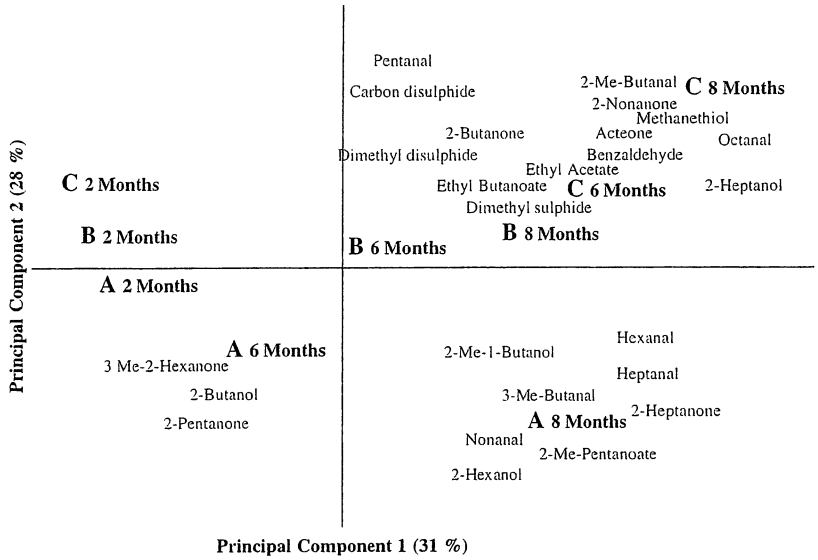

Fig. 4. PCA showing the first two Principal Components of volatile compound data of Cheddar cheeses made with starter system A, B or C at 2, 6 and 8 months of ripening. The starter systems used were: A, a blend of two *Lactococcus lactis* strains (223 and 227) that had a low level of autolysis; B, identical to A but included an adjunct of a highly autolytic strain of *Lb. helveticus* (DPC4571); and C, consisted only of strain DPC4571 as starter. The values presented are the means of three replicate trials.

Table 2
Quantitative descriptive sensory analysis of Cheddar cheeses made with different starter systems A, B and C at 2, 6 and 8 months of ripening showing the averaged attribute scores (1–100), standard deviation[a] and ANOVA

Attribute	Cheese											
	A			B			C			ANOVA[c]		
	2M[b]	6M[b]	8M[b]	2M[b]	6M[b]	8M[b]	2M[b]	6M[b]	8M[b]	Treat[d]	Age[e]	Treat*age
Odour												
Pungent	20±3	25±5	33±9	19±2	27±4	27±6	23±4	28±6	28±7	NS	**	NS
Sweaty/Sour	20±4	27±5	31±6	19±4	22±2	25±4	22±3	22±4	24±4	*	*	NS
Caramel	21±4	21±3	18±4	23±7	24±2	21±3	19±7	18±2	18±4	*	NS	NS
Creamy	28±3	26±3	24±3	27±3	27±5	24±4	28±3	25±2	24±4	NS	*	NS
Sweet	25±5	24±3	22±3	24±7	25±4	25±3	25±5	23±4	23±3	NS	NS	NS
Fruity	12±3	11±4	13±3	10±3	16±4	14±3	14±5	12±3	13±3	NS	NS	NS
Musty	12±4	14±5	14±4	13±5	12±2	16±5	18±6	21±8	22±9	**	NS	NS
Flavour												
Buttery	31±5	33±5	29±3	32±3	33±7	30±4	29±4	29±5	29±3	NS	NS	NS
Sweet	22±3	19±4	17±3	23±5	28±2	28±3	21±2	29±2	34±3	***	**	***
Salty	33±6	35±4	41±3	34±2	36±4	38±5	36±1	38±5	39±3	*	**	NS
Mushroom	15±2	15±4	16±2	19±4	14±3	16±4	17±4	18±3	18±4	NS	NS	NS
Nutty	14±2	17±2	15±3	15±2	16±2	17±3	15±1	16±3	15±3	NS	NS	NS
Rancid	10±3	14±5	18±3	9±3	8±3	10±3	12±3	14±2	11±3	**	*	*
Mouldy	8±2	14±4	15±3	8±3	7±2	10±4	7±2	10±4	7±3	**	*	*
Smokey	8±2	11±4	11±3	8±4	10±2	10±2	10±1	7±3	7±4	NS	NS	NS
Soapy	24±6	26±4	25±6	17±6	16±2	15±3	16±2	16±3	13±3	***	NS	NS
Processed	37±6	30±6	29±6	25±5	19±5	15±5	19±4	11±4	9±3	***	***	NS
Acidic	21±2	23±4	29±6	25±5	32±5	40±5	39±6	44±5	49±4	***	***	NS
Bitter	25±4	41±9	47±7	23±4	20±5	23±3	23±4	22±5	19±5	***	*	
Astringent	11±2	16±4	20±5	13±2	14±4	18±4	19±3	23±8	23±2	**	**	NS
Balanced	41±7	29±8	27±5	43±5	39±3	33±5	34±6	30±8	30±2	*	**	NS
Strength	31±5	43±4	48±3	35±5	43±4	47±4	43±4	51±3	53±3	***	***	NS

[a]Standard deviation obtained from a total of nine measurements.
[b]M = months of ripening.
[c]ANOVA performed using general linear model. NS = non-significant; $P>0.05$; * = $P<0.05$; ** = $P<0.01$; *** = $P<0.001$.
[d]Treat = treatment (Standard system used).
[e]Age = Ripening time.

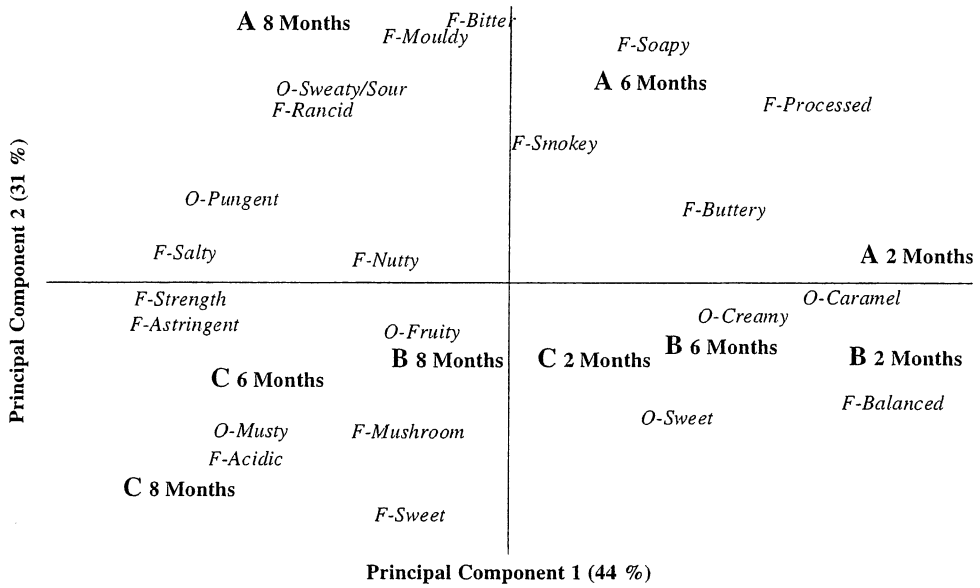

Fig. 5. PCA showing the first two Principal Components of descriptive sensory analysis of Cheddar cheeses made with starter system A, B or C at 2, 6 and 8 months of ripening. The starter systems used were: A, a blend of two *Lactococcus lactis* strains (223 and 227) that had a low level of autolysis; B, identical to A but included an adjunct of a highly autolytic strain of *Lb. helveticus* (DPC4571); and C, consisted only of strain DPC4571 as starter. The values presented are the means of three replicate trials. O = odour; F = flavour.

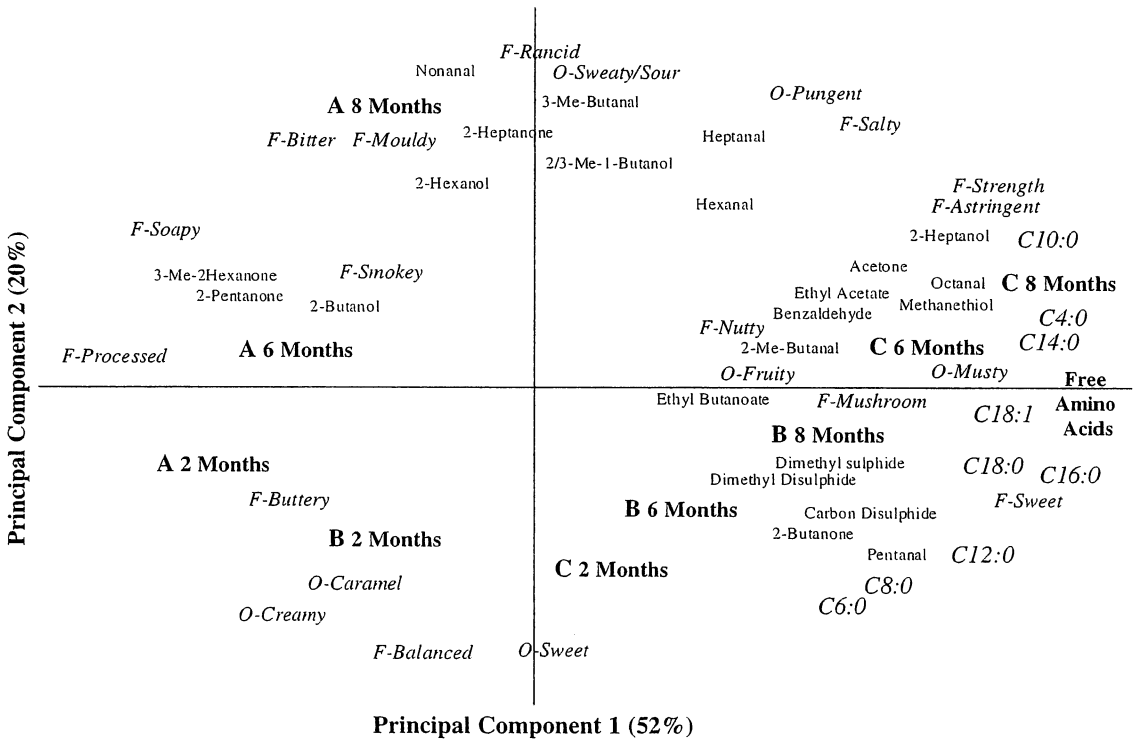

Fig. 6. PCA showing the first two principal components of matrix consisting of the FAA, FFA, volatile compounds and sensory data of Cheddar cheeses made with starter system A, B or C at 2, 6 and 8 months of ripening. The starter systems used were: A, a blend of two *Lactococcus lactis* strains (223 and 227) that had a low level of autolysis; B, identical to A but included an adjunct of a highly autolytic strain of *Lb. helveticus* (DPC4571); and C, consisted only of strain DPC4571 as starter. The values presented are the means of three replicate trials. O = odour; F = flavour.

precursors that contribute to the perception of the final flavour of the cheeses. High levels of FAAs may mask the effect of high levels of FFAs, giving an overall pleasant flavour.

4. Conclusion

The results presented in this study clearly show the evolution of flavour precursors and volatile compounds

due to the use of different starter systems. Addition of low levels of *Lb. helveticus* DPC4571 to a standard lactococcal starter system had a large effect on altering the flavour profile of the final cheeses. The presence of *Lb. helveticus* DPC4571 with its potent proteolytic system as well as extensive lysis resulted in a large increase of flavour precursors and some volatile compounds. Higher levels of lysis were also shown to further enhance the production of flavour precursors. The evolution of FAAs and FFAs, while contributing to some of the background flavour of these cheeses, also represent a high level of potential substrate for further catabolic reactions, mediated by enzymes released through early lysis into the cheese matrix. Enhancement of the catabolism of some branched chain and aromatic amino acids, especially methionine, was observed in the volatile profiles obtained. The enhancement of lipolysis, yielding flavour compounds that also contributed to the volatile profiles was also observed. This study shows that in each of the analyses used it is the *balance* of amino acids, or fatty acids, or aroma compounds that imparts the flavour to the cheeses at each stage of ripening. The use of autolytic starter systems on the early development of flavour was also demonstrated and the data suggest that selection of autolytic starter strains with high levels of peptidase activity can be an efficient method to control specific flavour development.

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References

Amárita, F., Requena, T., Taborda, G., Amigo, L., & Peláez, C. (2001). *Lactobacillus casei* and *Lactobacillus plantarum* initiate catabolism of methionine by transamination. *Journal of Applied Microbiology, 90*, 971–978.

Ardö, Y., Thage, B. V., & Madsen, J. S. (2002). Dynamics of free amino acid composition in cheese ripening. *Australian Journal of Dairy Technology, 57*, 109–115.

Bie, R., & Sjöström, G. (1975a). Autolytic properties of some lactic acid bacteria used in cheese production. Part I: Material and methods. *Milchwissenschaft, 30*(11), 653–657.

Bie, R., & Sjöström, G. (1975b). Autolytic properties of some lactic acid bacteria used in cheese production. Part II: Experiments with fluid substrates and cheese. *Milchwissenschaft, 30*(12), 739–747.

Castillo, I., Requena, T., Fernandez de Palencia, P., Fontecha, J., & Gobetti, M. (1999). Isolation and characterisation of an intracellular esterase from *Lactobacillus casei* subsp. *casei* IFPL731. *Journal of Applied Microbiology, 86*, 653–659.

Chapot-Chartier, M. P., Deniel, C., Rousseau, M., Vassal, L., & Gripon, J. C. (1994). Autolysis of two strains of *Lactococcus lactis* during cheese ripening. *International Dairy Journal, 4*(3), 251–269.

Christensen, J. E., Dudley, E. G., Pederson, J. A., & Steele, J. A. (1999). Peptidases and amino acid catabolism in lactic acid bacteria. *Antonie van Leeuwenhoek, 76*, 217–246.

Collins, Y. F., McSweeney, P. L. H., & Wilkinson, M. G. (2003a). Evidence for a relationship between autolysis of starter bacteria and lipolysis in Cheddar cheese. *Journal of Dairy Research, 70*, 105–113.

Collins, Y. F., McSweeney, P. L. H., & Wilkinson, M. G. (2003b). Lipolysis and free fatty acid catabolism in cheese: a review of current knowledge. *International Dairy Journal, 13*(11), 841–866.

Crow, V. L., Coolbear, T., Gopal, P. K., Martley, F. G., McKay, L. L., & Riepe, H. (1995). The role of autolysis of lactic acid bacteria in the ripening of cheese. *International Dairy Journal, 5*(8), 855–875.

Crow, V. L., Coolbear, T., Holland, T., Pritchard, G. G., & Martley, F. G. (1993). Starters as finishers: Starter properties relevant to cheese ripening. *International Dairy Journal, 3*(4–6), 423–460.

De Jong, C., & Badings, H. T. (1990). Determination of free fatty acids in milk and cheese. Procedures for extraction, clean-up, and capillary gas chromatographic analysis. *Journal of High Resolution Chromatography and Chromatography Communications, 13*, 94–98.

Fenelon, M. A., Ryan, M. P., Rea, M. C., Guinee, T. P., Ross, R. P., Hill, C., & Harrington, D. (1999). Elevated temperature ripening of reduced fat Cheddar made with or without lacticin 3147-producing starter culture. *Journal of Dairy Science, 82*(1), 10–22.

Fox, P. F., & Wallace, J. M. (1997). Formation of flavour compounds in cheese. *Advances in Applied Microbiology, 45*, 17–85.

Fox, P. F. (1989). Proteolysis during cheese manufacture and ripening. *Journal of Dairy Science, 72*, 1379–1400.

Hannon, J. A., Wilkinson, M. G., Delahunty, C. M., Wallace, J. M., Morrissey, P. A., & Beresford, T. P. (2003). Use of autolytic starter systems to accelerate the ripening of Cheddar cheese. *International Dairy Journal, 13*(4), 313–323.

Hickey, M. W., Hillier, A. J., & Jago, J. R. (1983). Peptidase activities in lactobacilli. *The Australian Journal of Dairy Technology, 38*, 118–123.

Kawabata, S., Vassal, L., Le Bars, D., Cesselin, B., Nardi, M., & Gripon, J. C. (1997). Phage-induced lysis of *Lactococcus lactis* during Saint-Paulin cheese ripening and its impact on proteolysis. *Lait, 77*, 229–239.

Kenny, O., FitzGerald, R. J., O'Cuinn, G., Beresford, T., & Jordan, K. (2003). Growth phase and growth medium effects on the peptidase activities of *Lactobacillus helveticus*. *International Dairy Journal, 13*(7), 509–516.

Lawrence, R. C., Gilles, J., Creamer, L. K., Crow, V. L., Heap, H. A., Honoré, C. G., Johnston, K. A., & Samal, P. K. (2004). Cheddar cheese and related dry-salted cheese varieties. In P. F. Fox, P. L. H. McSweeney, T. M. Cogan, & T. P. Guinee (Eds.), *Cheese: Chemistry, Physics and Microbiology*, Vol. 2 (pp. 71–102). London, UK: Elsevier Academic Press.

Lee, S. Y., & Lee, B. H. (1990). Esterolytic and lipolytic activities of *Lactobacillus casei* subsp. casei LLG. *Journal of Food Science, 55*, 119–126.

Lortal, S., & Chapot-Chartier, M. P. (2005). Role, mechanisms and control of lactic acid bacteria lysis in cheese. *International Dairy Journal, 15*, 857–871.

Maarse, H., Visscher, C. A., Willemsens, L. C., Nijssen, L. M., & Boelens, M. H. (1994). *Volatile compounds in foods: Qualitative and quantitative data* (7th ed.). Zeist, The Netherlands: TNO Biotechnology and Chemistry Institute.

Madkor, S. A., El Soda, M., & Tong, P. S. (1999). Evaluation of commercial adjuncts for use in cheese ripening: 2. Ripening aspects and flavour development in cheese slurries prepared with adjunct lactobacilli. *Milchwissenschaft, 54*(3), 133–137.

Madkor, S. A., El Soda, M., & Tong, P. S. (2000). Ripening of Cheddar cheese with added attenuated adjunct cultures of lactobacilli. *Journal of Dairy Science, 83*, 1684–1691.

McSweeney, P. L. H., & Sousa, M. J. (2000). Biochemical pathways for the production of flavour compounds in cheese during ripening: A review. *Lait, 80*, 293–324.

Mollimard, P., & Spinnler, H. E. (1996). Review: Compounds involved in the flavour of surface mould-ripened cheeses: Origins and properties. *Journal of Dairy Science, 79*, 169–184.

Morgan, S., Ross, R. P., & Hill, C. (1997). Increasing starter cell lysis in Cheddar cheese using bacteriocin-producing adjunct. *Journal of Dairy Science, 80*, 1–10.

O'Donovan, C., Wilkinson, M. G., Guinee, T. P., & Fox, P. F. (1996). An investigation into the autolytic properties of three lactococcal strains during cheese ripening. *International Dairy Journal, 6*, 1149–1165.

O'Sullivan, D., Ross, R. P., Fitzgerald, G. F., & Coffey, A. (2000). Investigation of the relationship between lysogeny and lysis of *Lactococcus lactis* in cheese using prophage-targeted PCR. *Applied and Environmental Microbiology, 66*, 2192–2198.

Piggott, J. R., & Sharman, K. (1986). In J. R. Piggott (Ed.), *Statistical procedures in food research* (pp. 181–232). London, UK and New York, USA: Elsevier Applied Science.

Pritchard, G., & Coolbear, T. (1993). The physiology and biochemistry of the proteolytic system of lactic acid bacteria. *FEMS Microbiology Reviews, 12*, 179–206.

Sasaki, M., Bosman, B. W., & Tan, P. S. T. (1995). Comparison of proteolytic activities in various lactobacilli. *Journal of Dairy Research, 62*, 601–610.

Smit, G., Verheul, A., Van Kranenburg, R., Ayad, E., Siezen, R., & Engels, W. (2000). Cheese flavour development by enzymatic conversions of peptides and amino acids. *Food Research International, 33*, 153–160.

Sousa, M. J., Ardö, Y., & McSweeney, P. L. H. (2001). Advances in the study of proteolysis during cheese ripening. *International Dairy Journal, 11*(4–7), 327–345.

Urbach, G. (1997). The flavour of milk and dairy products II. Cheese: Contribution of volatile compounds. *International Journal of Dairy Technology, 50*, 79–89.

Valence, F., Deutsch, S.-M., Richoux, R., Gagnaire, V., & Lortal, S. (2000). Autolysis and related proteolysis in Swiss cheese for two *Lactobacillus helveticus* strains. *Journal of Dairy Research, 67*, 261–271.

Wilkinson, M. G., Guinee, T. P., O'Callaghan, D. M., & Fox, P. F. (1994). Autolysis and proteolysis in different strains of starter bacteria during Cheddar cheese ripening. *Journal of Dairy Research, 61*(2), 249–262.