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Relationships between sensory attributes and the volatile compounds, non-volatile and gross compositional constituents of six blue-type cheeses

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Abstract

Relationships between odour and flavour attributes of six blue-type cheeses and their volatile compounds, free amino acids (FAA), free fatty acids (FFA) and gross compositional constituents were determined. Relationships were also determined between texture attributes and gross compositional constituents. Fifteen assessors described the odour, flavour, appearance and texture profile of cheeses. Volatile compounds were isolated using a model-mouth apparatus. FAA, FFA and gross compositional constituents were determined using standard methods. Using Partial Least Squares Regression two odour and five flavour attributes were found to correlate with subsets of volatile compounds, FAA, FFA and gross compositional constituents. For example, "mouldy" flavour was positively correlated with the concentrations of pH 4.6-soluble nitrogen and 2-pentanone, 2-heptanone, 2-octanone and 2-nonanone. Three texture attributes were found to correlate with subsets of gross compositional constituents. For example, "crumbly" texture was positively correlated with concentration of fat and protein and negatively correlated with levels of moisture in the non-fat substance and moisture.

Keywords: Sensory attributes; Blue cheese; Volatile; Free fatty acid; Free amino acid; Gross compositions

1. Introduction

Blue-type cheeses, characterised by the growth of *Penicillium roqueforti* in fissures throughout the cheese matrix, are one of the most easily identifiable cheese-types. During ripening, blue-type cheeses undergo extensive proteolysis and lipolysis resulting in odour, flavour, appearance and texture development (Coghill, 1979; Le Bars & Gripon, 1981; Hewedi & Fox, 1984; Zarmpoutis, McSweeney, & Fox, 1997; Fox, Guinee, Cogan, & McSweeney, 2000). Proteolysis results in a gradual softening of the cheese body and the production of the water-soluble nitrogenous components, whose contribution to cheese flavour, both directly, through formation of peptides and amino acids, and indirectly, by acting as precursors of volatile compounds, has been demonstrated (McGugan, Emmons, & Larmond, 1979; Aston & Creamer, 1986; Engels, Dekker, deJong, Neeter, & Visser, 1997; Molina, Ramos, Alonso, & López-Fandiño, 1999). Lipolysis leads to the liberation of free fatty acids (FFA), which subsequently undergo β-oxidation to form n-methyl ketones (Molimard & Spinnler, 1996; McSweeney & Sousa, 2000).

To determine relationships between volatile compounds, non-volatile compounds and/or gross compositional constituents of cheeses and their odour and/or flavour attributes a number of statistical methods have been used. These include principal component analysis (PCA), factor analysis and Partial Least Squares Regression (PLSR) (Roth, Engst, & Erhardt, 1982; Vangtal & Hammond, 1986; Virgili et al., 1994; Lawlor, Delahunty, Wilkinson, & Sheehan, 2001). Determination of such relationships can identify the volatile compounds, non-volatile compounds and gross compositional constituents related to sensory attributes, thus increasing the understanding of such attributes.

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However, in order for such statistical relationships between cheese sensory attributes and compositional constituents to be meaningful, the methods used to determine compositions must be chosen with care. The method used for preparation of a compositional extract determines its content (Delahunty & Piggott, 1995). Measurement of non-volatile constituents can be done by standard analytical methods as no reliable methods to measure the non-volatile constituents available for perception currently exist. For volatile compounds, the method of isolation must represent, as closely as possible, in vivo consumption conditions if such measurements are to be regressed against sensory attributes. Using regression analysis Virgili et al. (1994) reported positive correlation's between the concentration of volatile and non-volatile compounds and odour and flavour attributes. However, volatile compounds were isolated using simultaneous distillation extraction and dynamic headspace. The latter method and others, such as standard static and dynamic headspace, do not provide realistic measures of the volatile compounds available for perception during consumption. To simulate consumption conditions, a number of ''model mouth'' devices have been developed to account for mouth mastication, salivation and temperature effects (Piggott & Schaschke, 2001).

Cheese texture has been investigated by relating rheological and/or gross compositional measurements to perceived textural attributes (Casiraghi, Lucisano, & Pompei, 1989; Hough et al., 1996; Hort, Grys, & Woodman, 1997; Noël et al., 1998; Antoniou, Petridis, Raphaelides, Omar, & Kesteloot, 2000; Lawlor et al., 2001, 2002). Noël et al. (1998) used partial least squares regression (PLSR) to relate measurements including rheological and gross compositional constituents to the texture attributes of ten different samples of Swiss Appenzeller and Italian Parmigiano Reggiano. These authors also reported a number of relationships and recommended PLSR for similar investigations. In addition, PLSR is now supplemented with a jack-knife method in order to improve the reliability of the final PLSR models (Martens & Martens, 2000, 2001). The jack-knife method has been designed to avoid misinterpreting spurious effects, to identify the dominating sources of instability in modelling, and to allow more or less automatic optimisation of the model (Martens & Martens, 2000).

The objectives of the present study were to determine relationships between the flavour attributes of a number of blue-type cheeses and their gross compositional constituents, free amino acids (FAA), FFA and volatile compounds using PLSR. In addition, a further objective was to determine relationships were between cheese texture attributes and gross compositional constituents. Volatile compounds extracts were isolated using a novel model-mouth method developed to obtain extracts similar in composition to that produced by a consumer during eating (van Ruth, Roozen, & Cozijnsen, 1994). The relationships determined will improve understanding of blue cheese sensory character.

2. Materials and methods

2.1. Samples

Six blue-type cheeses, selected to represent a range of different blue cheese-types, were purchased from a local cheese supplier (Horgan's Delicatessen, Mitchelstown, Co. Cork, Ireland). Cheeses included the following: Bleu d'Auvergne (France), Blue Shropshire (England), Blue Stilton (England), Cashel Blue (Ireland), Danish Blue (Denmark) and Huntsman (England). Samples for sensory and gross compositional analysis were stored at 4°C prior to analysis. Samples for analysis of volatile compounds, FAA and FFA were vacuum-packed (Webomatic type D463; Werner Bonk, Bochum, Germany) and stored at −20°C prior to analysis. The vacuum packaging material consisted of Cryovac® film (45 cm^3/24 h at STP; W.R. Grace Europe Inc., Lausanne, Switzerland).

2.2. Descriptive sensory analysis

A panel of 15 assessors, with an average age of 33 years and experienced in the evaluation of many cheese types, participated in the present sensory analysis. Prior to analysis, assessors attended a number of round table discussions of 1 h in duration. Using a previously agreed vocabulary (Lawlor & Delahunty, 2000), the odour, flavour, appearance and texture attributes of the present blue-type cheeses were discussed by sampling a number of 5 g cubes of each cheese. A revised vocabulary was selected and defined to describe the sensory characteristics of the present blue-type cheeses. Descriptive sensory analysis was carried out following the method of Lawlor et al. (2002) using a final agreed vocabulary of nine odour, fifteen flavour, six appearance and nine texture attributes (Table 1). All cheeses were analysed in duplicate by each assessor. Cheese evaluation took place over 4 consecutive days to prevent panel fatigue, with three cheeses evaluated on each day. Data were recorded using the Compusense five V.3.8 sensory data acquisition programme (Guelph, ON, Canada). All assessments were conducted in individual booths at the sensory laboratory at University College, Cork, which complies with international standards for the design of test rooms (ISO, 1988).

Table 1
List of attributes and their definitions used by assessors to describe blue-type cheeses

Attribute	Definition
Odour	
Pungent	Physically penetrating sensation in the nasal cavity. Sharp smelling or tasting, irritant.
Caramel	Dairy caramel, toffee that has been made with sugar or melted further.
Mushroom	Organic. The aromatics associated with raw mushrooms.
Sweaty/sour	The aromatics reminiscent of perspiration generated foot odour. Sour, stale, slightly cheesy, moist, stained or odorous with sweat.
Fruity	The aromatic blend of different fruity identities.
Mouldy	The combination of aromatics associated with moulds. They are usually earthy, dirty, stale, musty and slightly sour.
Cheddar	The odour of which Cheddar cheese is typical.
Sweet	Blend of sweet aromas.
Creamy	The smell associated with creamy/milky products.
Flavour	
Buttery	Of the nature of, or containing butter.
Caramel	Dairy caramel, toffee that has been made with sugar or melted further.
Rancid	Sour milk, fatty, oxidised, having a rank, unpleasant taste or smell characteristic of oils and fats when no longer fresh.
Mushroom	Organic. The aromatics associated with raw mushrooms.
Oily	Oily, fatty, greasy taste of any kind.
Mouldy	The combination of aromatics associated with moulds. They are usually earthy, dirty, stale, musty and slightly sour.
Processed	Tastes of plastic, packaging, shallow. To taste artificial. Made by melting, blending and frequently emulsifying other cheeses.
Sweet	Fundamental taste sensation of which sucrose is typical.
Salty	Fundamental taste sensation of which sodium chloride is typical.
Acidic	Sour, tangy, citrus-like, the fundamental taste sensations of which lactic acids and citric acids are typical.
Bitter	Chemical-like, disprin, asprin. Taste sensations of which caffeine and quinine are typical.
Burnt-aftertaste	Flavour similar to burnt beef, Bovril-aftertaste. Flavour similar to burnt beef, Bovril.
Astringent	Mouth drying, harsh. The complex of drying, puckering and shrinking sensations in the lower cavity causing contraction of the body tissue.
Strength	Intensity or concentration of flavour, ranging from bland or tasteless to a concentrated intense flavour.
Balanced	Mellow, smooth, clean. In equilibrium, well arranged or disposed. With no constituent lacking or in excess, nothing standing out.
Appearance	
Colour intensity	The colour of cheese ranging from white to orange (excluding the mould).
Crumbly	The extent to which the cheese structure breaks up in the mouth, in the first 2–3 chews.
Mouldy	The degree of mouldiness/visible mould growth in the cheese structure.
Softness	Yielding easily to pressure, easily moulded, pliable, easily spreadable.
Openness	The extent to which the interior of the cheese (that is the cut surface) is open, this encompasses cracks, pinholes, irregular shaped holes and any other openings (including the mould).
Shiny	The extent to which the surface of the cheese is shiny, glossy, moist or sweaty looking, as opposed to looking matt or dull.
Texture	
Firmness	Ranging from soft to firm. The extent of resistance offered by the cheese, judged in the first half of chewing using the front teeth.
Crumbly	The extent to which the cheese structure breaks up in the mouth, in the first 2–3 chews.
Smooth	The smoothness of the cheese against the palate as it breaks up during mastication.
Moist	The perceived moisture content of the cheese. Ranging from dry to moist.
Oily	Oily, fatty, greasy mouth-feel of any kind.
Chewy	Requiring a good deal of mastication, toffee like texture. Degree of chewing needed to break up the cheese.
Slimy	Of the nature of slime, soft, glutinous or viscous substance, soft, moist and sticky.
Grainy	The extent to which granular structures are formed as the sample breaks down, perceived in the second half of chewing.
Mouth-coating	The extent to which the cheese coats the palate and the teeth during mastication.

2.3. Non-volatile and gross compositional constituents

Gross composition was analysed using standard methods: moisture (IDF, 1982), salt (IDF, 1988), fat (IDF, 1986), protein (IDF, 1993), ash (Kindstedt & Kosikowski, 1985) and calcium (IDF, 1984). pH was measured using an Orion Model 720A pH meter (Orion Research inc., Boston, USA) (BSI, 1976).The level of pH 4.6-soluble nitrogen (pH 4.6-SN) was measured using a modification of the method of Kuchroo and Fox (1982) as described by Fenelon, Guinee, Delahunty, Murray, and Crowe (2000). FAA concentrations were determined using the method of Fenelon et al. (2000). The concentrations of FFA ($C_{2:0}$, $C_{3:0}$, $C_{4:0}$, $C_{6:0}$) were determined using the method described by Kilcawley, Wilkinson, and Fox (2001).

2.4. Volatile composition

Sample preparation and subsequent isolation using a model-mouth apparatus (van Ruth et al., 1994) were described in a previous paper (Lawlor et al., 2002) Volatile compounds were trapped on Tenax-TA (Sigma-Aldrich Ireland Ltd., Airlon Road, Tallagh, Dublin 24, Ireland). Tenax-TA tubes were thermally desorbed using a Tekmar 3000 concentrator (JVA Analytical Ltd. Unit 1, Longmile Business Centre, Longmile Road, Dublin 12, Ireland) for 5 min at 220°C, focused at −120°C using a Tekmar Cryofocusing Module (JVA Analytical Ltd.). Volatile compounds desorbed were injected into the Gas Chromatograph over 2 min at 235°C. Volatiles were analysed using a Saturn GC-3400cx Gas Chromatograph-Saturn 3 Mass Spectrometer (GC-MS) (JVA Analytical Ltd.). The GC column used was a capillary column BPX5 (60 m length, 0.32 mm internal diameter and 1.0 μm film thickness; SGE, UK). The carrier gas was helium at a flow rate of 3 mL min^{-1}. The GC oven temperature was raised from 40°C (4 min) to 90°C at 2°C min^{-1} (25 min), increased to 130°C at 4°C min^{-1} (10 min), increased to 250°C at 8°C min^{-1} (15 min) and baked at 250°C for 10 min to give a total run time of 64 min. All analyses were performed in triplicate.

2.5. Data treatment

Duplicate scores from each assessor were analysed by one-way analysis of variance (ANOVA) using SPSS v 10.0 (SPSS Inc. Chicago, IL 60611, USA) using a significance level of $P<0.05$. A graph of the P-value versus the mean square error (MSE) showed the ability of individual assessors to discriminate between samples and reproduce a score for duplicate samples, respectively. Spearman's rank correlation values were calculated to compare the ability of individual assessors to rank samples compared with the whole panel. Kendall's coefficient of concordance was calculated to measure the agreement of assessors as a whole panel. A Spearman value greater than 0.35 and a Kendal value greater than 0.30 were considered as satisfactory abilities of individuals and the panel as a whole, respectively, to rank in a descriptive analysis test (McDonnell, Hulin-Bertaud, Sheehan, & Delahunty, 2001). Attributes with more than five assessors with Spearman values below 0.35 (representing a third of the overall panel) were considered unreliable and consequently excluded from further analysis. For the remaining attributes, with more than ten reliable assessors, assessors with Spearman values below the set threshold were removed and the Kendall value recalculated. Attributes with Kendal values of below 0.30 were subsequently discarded. Duplicate scores of the final list of attributes used (Table 2) were then analysed using one-way ANOVA (SPSS v 10.0 SPSS Inc.).

Sensory duplicates were standardised (1/standard deviation) and analysed by means of PCA; (Piggott & Sharman, 1986) using Guideline +7.5 (CAMO ASA, Oslo Norway). ANOVA was performed on duplicate scores to determine the principal components (PC) that gave significant ($P<0.05$) between cheese effects. Duplicate scores were then averaged and a PCA was performed. The final number of components to use was based on the discriminating ability ($P<0.05$) of each PC and a visual inspection of explained validation variance, to indicate whether additional PCs were modelling information or noise.

Volatile compounds were identified and quantified following the method of Lawlor et al. (2002). A PCA was performed on a matrix consisting of the volatile compounds, FAA, FFA and gross compositional constituents. All data were standardised (1/standard deviation) prior to analysis.

Relationships between sensory attributes and volatile compounds, FAA, FFA and gross compositional constituents were determined by PLSR type 1 (PLSR1), with jack-knife estimation of parameter uncertainty (Martens & Martens, 1986, 2000) using the method outlined by Lawlor et al. (2002). Selection of the final regression model for each attribute was based on both parameter reliability (jack-knife) and predictive ability, measured using the root mean square error of prediction (RMSEP). The RMSEP is an estimate of the average prediction error, expressed in the same units as the original response variables (i.e., sensory scores on a scale of 1–100). The RMSEP was also used to determine the optimum number of components (A_{Opt}) for the model. Both predictive ability (testing reproducibility) and parameter reliability (jack-knife) were tested by full cross-validation on the averaged data of the six blue cheese samples.

3. Results and discussion

3.1. Descriptive sensory analysis

Results of one-way ANOVA showed that assessors varied in their abilities to discriminate between samples and to reproduce a score (data not shown). Thybo and Martens (2000) also reported different levels of reliability between attributes and assessors. With regards to reproducibility of duplicate scores, blue cheese is an extremely heterogeneous food and differences such as extent of mould proliferation throughout the cheese matrix may lead to subtle differences in sensory character throughout the cheese body. In the present work, samples presented to assessors for sensory evaluation were taken from all areas of the cheese body to average out these differences. Therefore, although duplicate samples of blue cheese may not be as similar as

Table 2
Result of descriptive sensory analysis on six blue-type cheeses showing the averaged attribute scores (1–100) and one-way analysis of variance (ANOVA)

Attribute	Cheese						ANOVA			Kendall's W test
	Bleu d'Auvergne	Blue Shropshire	Blue Stilton	Cashel blue	Danish blue	Huntsman	*F*-ratio	*P*-value	LSD	
Odour										
Pungent	44	31	24	33	59	26	10.81	0.006	10	0.49
Mushroom	24	25	22	23	36	14	2.92	0.112	11	0.39
Fruity	16	21	13	12	35	30	14.35	0.003	7	0.44
Mouldy	34	35	25	30	49	14	19.94	0.001	6	0.46
Cheddar	5	16	16	8	5	42	154.94	0.000	3	0.60
Sweet	15	24	23	24	31	35	12.18	0.004	5	0.39
Flavour										
Oily	44	21	37	35	35	17	1.85	0.237	19	0.55
Mouldy	40	48	34	33	47	26	6.02	0.025	9	0.46
Processed	21	12	14	12	9	37	14.94	0.002	7	0.47
Salty	74	51	60	48	54	36	8.13	0.012	11	0.49
Acidic	39	30	41	29	52	32	6.31	0.022	9	0.43
Strength	62	57	63	45	73	46	5.92	0.026	11	0.56
Balanced	25	45	33	49	27	49	10.21	0.007	9	0.55
Appearance										
Colour intensity	10	79	24	18	9	30	37.28	0.000	11	0.73
Crumbly	21	68	38	17	53	32	15.70	0.002	12	0.68
Mouldy	56	53	40	41	69	38	12.79	0.004	9	0.49
Openness	52	50	36	41	57	29	3.31	0.080	15	0.68
Shiny	43	19	45	55	35	29	3.64	0.073	17	0.50
Softness	52	26	47	54	42	31	1.92	0.225	21	0.68
Texture										
Firmness	36	40	33	27	40	50	4.69	0.043	9	0.45
Crumbly	12	57	24	21	41	26	18.76	0.001	10	0.58
Smooth	57	42	67	66	52	54	3.08	0.102	13	0.52
Moist	52	39	59	54	50	35	4.39	0.050	11	0.58
Slimy	23	7	18	25	28	7	14.95	0.002	6	0.48
Grainy	8	30	12	20	16	14	9.43	0.008	6	0.44

Results of Kendall's coefficient of concordance showing the degree of agreement among the panel as a whole for each attribute can also be seen.

foods with a more homogeneous nature, the data obtained from the sensory analysis of blue cheese may in fact be more relevant as it represents an averaged sensory response over a heterogeneous cheese body. After examination of Spearman's and Kendall's values, the final attribute list included six odour, seven flavour, six appearance and six texture attributes (Table 2). Previously, a Spearman coefficient and a Kendall's value of 0.35 and 0.30, respectively, were recommended as satisfactory ability, for individual assessors and for the panel as a whole, to rank in a descriptive analysis using this attribute (McDonnell et al., 2001). All Spearman coefficients and Kendall values met the latter criteria in the present work.

One-way ANOVA, on the reduced set of attributes, found that all attributes, apart from "mushroom" odour, "oily" flavour, "openness", "shiny" and "softness" appearance and "smooth" texture, discriminated significantly ($P<0.05$) between cheeses (Table 2). One-way ANOVA showed that three PCs (PCs 1, 2 and 3), accounting for 89% of the experimental variance, discriminated significantly ($P<0.05$) between cheeses. The scores and loadings for each PC, the percentage variance accounted for by each PC, and results of ANOVA are shown in Tables 3 and 4.

PC1 distinguished the sensory character of Danish Blue from Huntsman (Table 3). Danish Blue had a "pungent", "mushroom" and "mouldy" odour, an "oily", "salty", "acidic" and "strength" flavour, a "mouldy", "openness" and "softness" appearance and a "moist" and "slimy" texture (Tables 3 and 4). Huntsman (a double Gloucester with Blue Stilton) had a "cheddar" odour and a "processed" and "balanced" flavour. PC2 distinguished both Danish Blue and Blue Shropshire, from Cashel Blue and Blue Stilton. Blue Shropshire and Danish Blue cheeses had a "mushroom", "fruity" and "mouldy" odour, a "mouldy" flavour, a "crumbly", "mouldy" and "open" appearance and a "crumbly" texture. Cashel Blue and Blue Stilton had a "shiny" and "softness" appearance and

Table 3
Result of principal component analysis on the descriptive sensory data of six blue-type cheeses, showing the scores for each cheese on significant principal components

Cheese	Principal component		
	1	2	3
Bleu d'Auvergne	2.91	−1.33	0.42
Blue Shropshire	−2.13	3.81	−2.45
Blue Stilton	0.74	−2.15	−0.39
Cashel blue	0.52	−2.88	−1.57
Danish blue	3.34	3.30	1.86
Huntsman	−5.39	−0.75	2.14
% Variance	43	33	13
ANOVA			
P-value	0.00	0.00	0.00
F-ratio	13.11	25.78	21.74

The percentage variance accounted for by each component as well as results of one-way analysis of variance (ANOVA) are also shown.

Table 4
Result of principal component analysis on descriptive sensory data of six blue-type cheeses, showing the loadings of each variable on the first three principal components

Attribute	Principal component		
	1	2	3
Odour			
Pungent	0.21	0.16	0.21
Mushroom	0.24	0.20	0.00
Fruity	−0.06	0.23	0.39
Mouldy	0.23	0.21	−0.03
Cheddar	−0.28	−0.03	0.18
Sweet	−0.17	0.10	0.30
Flavour			
Oily	0.27	−0.14	−0.01
Mouldy	0.16	0.28	−0.16
Processed	-0.21	−0.10	0.29
Salty	0.24	−0.04	−0.07
Acidic	0.20	0.11	0.32
Strength	0.22	0.18	0.13
Balanced	−0.25	−0.05	−0.19
Appearance			
Colour Intensity	−0.17	0.18	−0.34
Crumbly	−0.04	0.32	−0.10
Mouldy	0.20	0.24	0.11
Openness	0.22	0.21	−0.06
Shiny	0.15	−0.28	−0.03
Softness	0.22	−0.24	−0.01
Texture			
Firmness	−0.19	0.16	0.32
Crumbly	−0.07	0.31	−0.16
Smooth	0.08	−0.32	0.01
Moist	0.24	−0.17	−0.09
Slimy	0.27	−0.07	0.08
Grainy	−0.10	0.21	−0.37

"smooth" texture. PC3 separated the "fruity" and "sweet" odour, "processed" and "acidic" flavour and "firmness" texture of Huntsman and Danish Blue from the "colour" appearance and "grainy" texture of Blue Shropshire.

3.2. Volatile, FAA, FFA and gross compositional analysis of cheese

The concentrations of volatile compounds, FAA, FFA and gross compositional constituents, in six blue-type cheeses are shown in Tables 5–8, respectively. PCA of the data showed that four PCs (PCs 1, 2, 3 and 4), accounting for 94% of the explained variance, were needed to describe the differences in volatile compounds, FAA, FFA and gross compositional constituents between samples. The first two PCs accounting for 64% of the variation are shown in Fig. 1.

PC1 distinguished Cashel Blue and Bleu d'Auvergne from Blue Shropshire (Fig. 1). Blue Shropshire was characterised by its high concentrations of protein, total amino acids and FAA including, threonine, glutamic acid, prolein, glycine, alanine, valine, methionine, leucine, phenylalanine and lysein. Of the current cheeses investigated, Blue Shropshire had the highest concentrations of protein ($24.41\,g\,100\,g^{-1}$), total amino acids ($52410\,\mu g\,g^{-1}$) and most individual FAA (Tables 6 and 8). Blue Shropshire had a relatively high level of pH 4.6-SN ($50.07\,g\,100\,g^{-1}$) indicating a high rate of overall proteolysis. Considerable proteolysis occurs in blue-type cheese (Coghill, 1979; Hewedi & Fox, 1984; Le Bars & Gripon, 1981) resulting from low cook temperatures and a high rate of acid production in the vat during manufacture, and the action of rennet, plasmin and proteinases from secondary microflora such as *P. roqueforti*.

PC2 distinguished Danish Blue and Huntsman from Bleu d'Auvergne, Blue Stilton and Cashel Blue. Danish Bleu and Huntsman were characterised by the their high levels of alcohols (2-hexanol and 2-methyl-1-butanol), esters (ethyl butyrate and ethyl hexanoate) and the alkan-2-one, 2-pentanone. Apart from the branched-chain primary alcohol, 2-methyl-1-butanol, produced by catabolism of isoleucine (Engels et al., 1997), the latter volatile compounds result from fatty acid metabolism, and thus, may reflect the important contribution of lipolysis to the production of volatile compounds in these cheese types.

Danish Blue and Huntsman were also characterised by their low pH values on PC2. The pH of blue-type cheeses usually rises dramatically during ripening with the increase in the centre being greater than at the surface. Bleu d'Auvergne, Blue Stilton and Cashel Blue cheeses were characterised by the presence of 2-pentanol, 2-heptanol, 3-methyl-2-pentanone, pentanal and octanal. Gallois and Langlois (1990) reported the

Table 5
Results of volatile compositional analysis on six blue-type cheeses showing the averaged peak areas (in arbitrary units) of triplicate determinations and mean percentage coefficient of variation (%CV)

Compounds	Cheese						
	Bleu d'Auvergne	Blue Shropshire	Blue Stilton	Cashel blue	Danish blue	Huntsman	%CV
Alcohols							
Ethanol	671	669	203	1058	415	922	66.48
2-Butanol	ND	396	ND	73	ND	ND	63.45
2-Pentanol	128	2589	259	230	ND	381	57.70
2-Hexanol	ND	50	110	ND	1431	604	71.40
2-Heptanol	710	3362	3671	173	ND	1769	58.09
2-Methyl-1-butanol	426	1784	2728	2510	4230	4044	48.43
Aldehydes							
Butanal	ND	ND	17263	ND	ND	535	67.19
Pentanal	742	ND	3262	217	ND	ND	56.46
Hexanal	567	267	356	339	359	506	37.83
Heptanal	610	280	472	368	362	527	45.75
Octanal	266	149	256	206	ND	233	29.17
Benzaldehyde	276	178	406	256	350	474	64.94
Esters							
Methyl butyrate	ND	2967	531	96	3879	810	61.02
Methyl hexanoate	60	2087	835	104	5566	1006	57.23
Ethyl acetate	32	68	ND	205	109	523	40.71
Ethyl butyrate	420	1383	304	403	1534	7262	60.66
Ethyl hexanoate	204	250	76	71	3922	1379	54.56
Propyl butyrate	ND	88	26	10	112	168	41.44
Ketones							
2-Butanone	327	1267	21725	297	5556	2880	55.88
2-Pentanone	9980	30102	13511	420	253362	15681	52.42
2-Hexanone	261	1555	761	ND	8536	1042	43.54
2-Heptanone	12690	95895	22337	3862	185053	29868	55.46
2-Octanone	3041	1342	706	180	5985	465	42.73
2-Nonanone	1478	1878	856	339	17353	527	51.30
3-Methyl-2-pentanone	86	700	1013	57	ND	ND	57.48
8-Nonen-2-one	94	209	78	ND	ND	ND	42.75
Sulphur compounds							
Dimethylsulphide	40	109	343	55	78	76	58.74
Dimethyldisulphide	334	ND	ND	950	916	1375	33.93
Terpenes							
α-Pinene	220	461	156	33	221	154	56.18
Limonene	51	212	461	114	78	92	58.98

ND = not detected.

presence of the branched ketone, 3-methyl-2-pentanone, and the secondary alcohols, 2-pentanol and 2-heptanol, in French blue cheeses. Straight chain aldehydes have been reported in a number of different cheeses (Bosset & Gauch, 1993; Engels et al., 1997; Lawlor et al., 2001).

PCs 3 and 4 highlighted other important differences between cheeses. PC3 (not shown) highlighted that, within the current range of cheeses, Bleu d'Auvergne had the highest content of salt (4.18 g 100 g^{-1}), salt in moisture (S/M) (8.84 g 100 g^{-1}) and pH 4.6-SN (50.46 g 100 g^{-1}).

3.3. Relationships between odour and flavour attributes and the concentrations of volatile compounds, FAA, FFA and gross compositional constituents

Relationships were found between two odour and five flavour attributes and subsets of volatile compounds, FAA, FFA and gross compositional constituents. Calibration and validation coefficients for the models ranged between 0.92 and 0.99, and 0.71 to 0.97, respectively. RMSEP values ranged from 2 to 7 (on a scale of 1–100) indicating that all models had good predictive ability (Table 9).

Table 6
Result of free amino acid analysis on six blue-type cheeses. Results are from a single analysis

Free amino acid[a]	Cheese					
	Bleu d'Auvergne	Blue Shropshire	Blue Stilton	Cashel blue	Danish blue	Huntsman
Asp	1548	1968	2072	712	1303	800
Thr	878	1565	1240	532	962	781
Ser	2158	2653	1907	1951	3589	2030
Glu	4589	9310	7256	3734	6435	4733
Pro	1174	4106	2369	858	1851	1102
Gly	476	894	728	397	615	457
Ala	873	2732	1789	659	1155	720
Cys	498	492	532	398	500	282
Val	2327	4070	3256	1920	2354	2210
Met	1082	1973	1614	1007	1693	1431
Ile	1609	2656	2008	974	1376	1016
Leu	3698	6077	5284	3354	4784	4215
Tyr	1907	1333	1768	1505	1629	1372
Phe	2006	3262	2609	1550	2129	2203
His	1666	2170	1736	773	2296	1242
Lys	4400	7150	5331	2850	4833	4680
Total	30892	52410	41499	23127	37516	29274

[a] Values expressed as $\mu g\, g^{-1}$ cheese.

Table 7
Results of free fatty analysis of six blue-type cheeses. Results are means of duplicate analyses

Cheese	Free fatty acid			
	Acetic acid[a]	Propionic acid[a]	Butyric acid[a]	Hexanoic acid[a]
Bleu d'Auvergne	807	51.5	15088	10956
Blue Shropshire	1837	62	12218	7919
Blue Stilton	1742	111	6924	3171
Cashel blue	138	ND	1258	341
Danish blue	1073	ND	5070	2518
Huntsman	1620	140.5	77	20

ND = not detected.
[a] Values expressed as $\mu g\, g^{-1}$ cheese.

The "fruity" odour of Danish blue and Huntsman cheeses (Table 2) was positively correlated with the levels of the esters, propyl butyrate, ethyl acetate and methyl hexanoate. "Fruity" odour was negatively correlated with pH value and level of fat in the dry matter (FDM) (Table 9). Danish Blue and Huntsman had relatively high concentrations of most ester compounds, relatively low pH values and low concentrations of FDM (Tables 5 and 8). Ester compounds have been reported to have "fruity", "floral" odour notes (Molimard & Spinnler, 1996). The "sweet" odour of Huntsman and Danish Blue cheeses was also correlated with the levels of ethyl butyrate and methyl hexanoate.

The "mouldy" flavour, that characterised all the blue-type cheeses in the current study, especially Blue Shropshire and Danish Blue cheeses (Tables 3 and 4), was positively correlated with the concentrations of: histidine and serine, butanoic acid and hexanoic acid, 2-pentanone, 2-heptanone, 2-octanone and 2-nonanone and pH 4.6-SN. In contrast, it was negatively correlated with the levels of the aldehydes, butanal, heptanal, hexanal and benzaldehyde.

Blue-type cheeses undergo extensive proteolysis and lipolysis during ripening. In the present study, the correlations of "mouldy" flavour with the concentrations of pH 4.6-SN, histidine and serine highlights the importance of proteolysis to the development of "mouldy" flavour in blue cheese. The extensive proteolysis that occurs in blue-type cheeses is caused, primarily, by the proteolytic system of the secondary microflora (*P. camemberti* and *P. roqueforti*) (McSweeney & Sousa, 2000). Factors affecting the degree of proteolysis include the mould strain and the ripening time (Hewedi & Fox, 1984; Larsen, Kristiansen, & Hansen, 1998). In addition to their proteolytic activity, *P. roqueforti* also excretes two lipases with different substrate specificities that are optimally active at different pH values (McSweeney & Sousa, 2000). The activity of these lipases may account for the presence of butanoic acid and hexanoic acid, the concentrations of which were correlated with "mouldy" flavour. The flavour of blue-type cheeses is thought to be due to the accumulation of fatty acids and their subsequent degradation to alkan-2-ones (Coghill, 1979; Gallois & Langlois, 1990; Molimard & Spinnler, 1996; Lawlor et al., 2001). Thus, the positive correlation between "mouldy" flavour and the concentrations of

Table 8
Results of gross compositional analysis of blue-type cheeses. Values for pH were the result of a single analysis. Values for all other compositional constituents were the result of duplicate analyses

Cheese	pH	Moisture[a]	Salt[a]	Fat[a]	Protein[a]	Ash[a]	Calcium[b]	FDM[a,c]	S/M[a,d]	MNFS[a,e]	pH 4.6-SN[a,f]
Bleu d'Auvergne	6.3	47.3	4.2	26.6	20.4	5.6	533	50.5	8.8	64.5	50.5
Blue Shropshire	6.8	38.3	1.8	35.3	24.4	2.2	239	57.1	4.7	59.1	50.1
Blue Stilton	6.6	39.8	1.9	34.1	22.7	2.8	355	56.7	4.8	60.5	48.1
Cashel blue	5.9	45.7	2.4	29.7	19.7	3.4	428	54.8	5.3	65.1	40.2
Danish blue	5.3	41.6	3.0	30.2	21.8	4.4	570	51.8	7.1	59.6	48.7
Huntsman	5.3	39.2	2.3	32.4	22.6	3.8	612	53.3	5.7	58.0	38.7

[a] Values expressed as $g\,100\,g^{-1}$ cheese.
[b] Values expressed as $mg\,100\,g^{-1}$ cheese.
[c] FDM = fat in the dry matter.
[d] S/M = salt in moisture.
[e] MNFS = moisture in the non-fat substance.
[f] pH 4.6-SN = pH 4.6-soluble nitrogen.

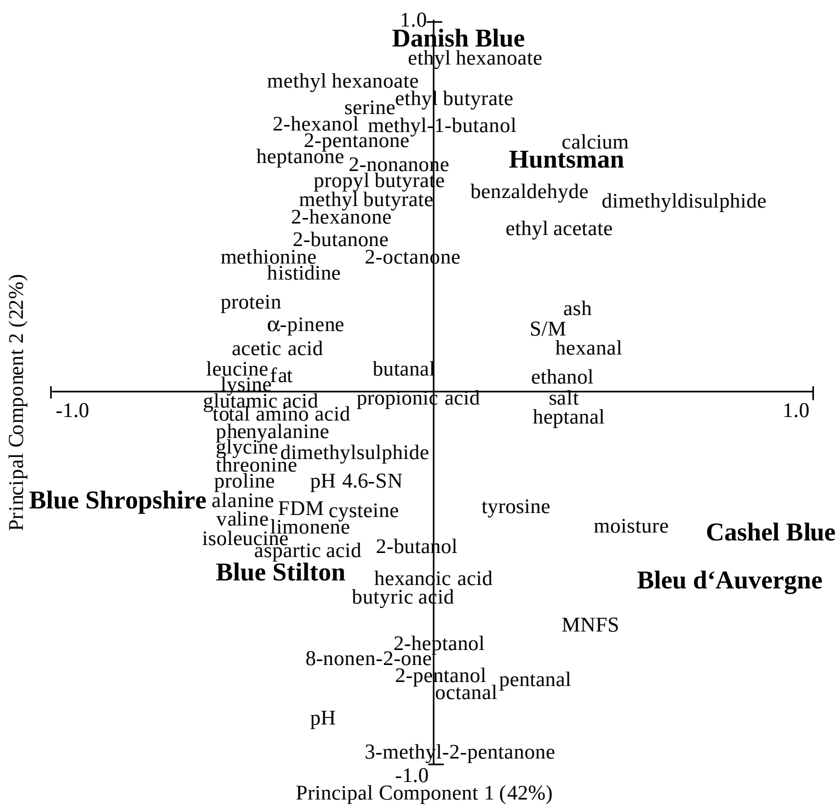

Fig. 1. Result of principal component analysis on the volatile compounds, free amino acid, free fatty acid and gross compositional constituents of six blue-type cheeses (in bold font) showing the first two significant principal components. FDM = fat in the dry matter, MNFS = moisture in the non-fat substance, pH 4.6-SN = pH 4.6 soluble nitrogen, S/M = salt in moisture.

2-pentanone, 2-heptanone, 2-octanone and 2-nonanone was expected. Of the cheeses evaluated in the current study, Danish Blue and Blue Shropshire had the highest concentrations of all alkan-2-ones apart from 2-octanone.

The "salty" flavour of Bleu d'Auvergne cheese (Table 2) was positively correlated with pH value and with the contents of moisture, salt, S/M, and pH 4.6-SN. In contrast, it was negatively correlated with fat content. Bleu d'Auvergne, a French blue-type manufactured using cow's milk (typically raw), had a pH of 6.28, and the highest concentrations of S/M ($8.84\,g\,100\,g^{-1}$), pH 4.6-SN ($50.46\,g\,100\,g^{-1}$), moisture ($47.31\,g\,100\,g^{-1}$) and salt ($4.18\,g\,100\,g^{-1}$) of all cheeses investigated. Bleu d'Auvergne had the lowest fat content ($26.60\,g\,100\,g^{-1}$) of all the cheeses studied. Previous studies (Lawlor et al.,

Table 9
Result of partial least squares regression analysis between individual odour and flavour attributes (Y-matrix) and volatile compounds, free amino acids (FAA), free fatty acids (FFA) and gross compositional constituents (composition) (X-matrix) of cheeses. The fit of the current model, calibration coefficient, (calibration), its ability to predict, validation coefficient, (validation), the root mean square error of prediction (RMSEP) and the optimum number of components (A_{Opt}) in each model are shown

Attribute	Composition	Positive correlation	Negative correlation	Calibration	Validation	RMSEP	A_{Opt}
Odour							
Fruity			pH*, FDM[a]	0.98	0.97	2	1
	FAA						
	FFA						
	Volatile	Propyl butyrate, ethyl acetate*, methyl hexanoate*	Pentanal*, 3-methyl-2-pentanone*				
Sweet			pH*, moisture, MNFS*,[b], pH 4.6-SN[c]	0.99	0.96	2	2
	FAA						
	FFA		Butyric acid*				
	Volatile	Ethyl butyrate*, methyl hexanoate*					
Flavour							
Mouldy		pH 4.6-SN		0.99	0.92	3	2
	FAA	Histidine*, serine*					
	FFA	Butyric acid, hexanoic acid*					
	Volatile	2-Pentanone, 2-heptanone, 2-octanone, 2-nonanone	Butanal*, heptanal*, hexanal*, benzaldehyde*				
Salty		S/M[d], salt, moisture, pH*, pH 4.6-SN*	Fat	0.96	0.81	7	2
	FAA						
	FFA						
	Volatile						
Oily		Moisture, MNFS	Fat, protein	0.97	0.84	5	2
	FAA	Tyrosine*					
	FFA						
	Volatile						
Strength		Salt, S/M, pH 4.6-SN*		0.92	0.71	7	1
	FAA	Threonine, glutamic acid*, tyrosine, aspartic acid*, histidine*, cysteine					
	FFA	Butyric acid, hexanoic acid					
	Volatile	2-Pentanone, 2-hexanone, 2-heptanone, 2-octanone*, 2-nonanone	Ethanol*, 2-butanol				
Balanced		FDM, fat, protein	Moisture, ash, salt, S/M, pH 4.6-SN	0.97	0.77	7	2
	FAA		Tyrosine, cysteine, aspartic acid, histidine*				
	FFA						
	Volatile		2-Pentanone, 2-hexanone, 2-octanone*, 2-nonanone, 2-octanone, 2-heptanol, 2-butanol*, ethyl hexanoate*				

Significant variables are marked (*).
[a] FDM = fat in the dry matter.
[b] MNFS = moisture in the non-fat solids.
[c] pH 4.6-SN = pH 4.6-soluble nitrogen.
[d] S/M = salt in moisture.

2001, 2002) also indicated a positive relationship between "salty" flavour and the concentrations of S/M, salt and moisture. The positive correlation between "salty" flavour and the level of pH 4.6-SN suggests that increased breakdown of the protein matrix may have contributed to "salty" perception through an increased rate of salt release, making it available to receptors.

The "oily" flavour (defined by the panel as "oily, fatty, greasy taste of any kind") that described Bleu d'Auvergne cheese was positively correlated with the levels of moisture, moisture in the non-fat substance (MNFS) and tyrosine. "Oily" flavour was negatively correlated with concentrations of fat and protein. Bleu d'Auvergne had a relatively high level of moisture, a low concentration of protein ($20.39\,g\,100\,g^{-1}$) and a high level of MNFS ($64.06\,g\,100\,g^{-1}$) (Table 8). Bleu d'Auvergne had the lowest fat content ($26.60\,g\,100\,g^{-1}$) of all cheeses investigated (Table 8). Bleu d'Auvergne had the highest concentration of tyrosine ($1907\,\mu g\,g^{-1}$) of all cheeses investigated. Bleu d'Auvergne also had a relatively high content of butanoic acid and hexanoic acid, indicating a high degree of lipolysis during ripening (Table 7). Thus, the combined effects of the high moisture content, and high levels of proteolysis, and lipolysis may have resulted in the "oily" flavour of Bleu d'Auvergne.

The "strength" flavour of Danish Blue cheese (Tables 2) was positively correlated with concentrations of salt, S/M, pH 4.6-SN, FAA (glutamic acid, aspartic acid, histidine), FFA (butanoic acid and hexanoic acid) and alkan-2-ones (2-pentanone, 2-hexanone, 2-heptanone, 2-octanone and 2-nonanone) (Table 9). This cheese is manufactured from unpasteurised cows milk, which may have contributed to its "strength" flavour (Grappin & Beuvier, 1997). In addition, the cheese body was extensively pierced encouraging prolific growth of *P. roqueforti*, and hence, an increase in proteolytic activity. The sensory panel rated this cheese highest for mouldy appearance (Table 2). Finally, prior to manufacture, the milk was homogenised, exposing milk fat to the action of lipases, and increasing lipolytic activity. This may explain the high concentrations of FFA, butanoic acid, hexanoic acid, 2-pentanone, 2-hexanone, 2-heptanone, 2-octanone and 2-nonanone found. Robinson (1995) previously stated that these manufacturing procedures lead to an "intense" flavoured cheese.

Cashel Blue cheese had the lowest concentrations of the compounds 2-pentanone, 2-hexanone, 2-heptanone, 2-octanone and 2-nonanone, whilst Huntsman had the second lowest concentrations of 2-octanone and 2-nonanone. Both Cashel Blue and Huntsman were scored least for "strength" and highest for "balanced" flavour (see Table 2). "Balanced" favour was negatively correlated with contents of moisture, ash and ethyl hexanoate, and positively correlated with the concentrations of FDM, fat and protein.

3.4. Relationship between texture attributes and the gross compositional constituents

The three texture attributes, "firmness", "slimy" and "crumbly", were found to correlate with sub-sets of gross compositional constituents. The calibration and validation coefficients of texture attributes ranged from 0.72 to 0.99, and 0.31 to 0.76, respectively. RMSEP values ranged from 6–15 (Table 10).

The "firmness" that characterised Huntsman cheese, and to a lesser extent Danish Blue and Blue Shropshire (Table 2), was positively correlated with the concentrations of protein and salt and negatively with the levels of MNFS and pH 4.6-SN (Table 10). Previous work (Lawlor et al., 2001, 2002) on different cheese types (including blue and Swiss-types) also demonstrated the existence of correlations between "firmness" texture and the concentrations of moisture, protein and pH 4.6-SN. In the present work, Huntsman, Danish Blue and Blue Shropshire cheeses had relatively high protein contents and low levels of MNFS (Table 8). As the concentration of casein (protein) in the cheese matrix increases, the intra- and inter-strand linkages become more numerous and the matrix is more difficult to deform. In addition, the extent to which the moisture plasticises the protein

Table 10
Result of partial least squares regression analysis between individual texture attributes (Y-matrix) and the gross compositional constituents (X-matrix) of six blue-type cheeses. The fit of the current model, calibration coefficient (calibration), its ability to predict, validation coefficient (validation), the root mean square error prediction (RMSEP) and the optimum number of components (A_{Opt}) in each model are shown

Attribute	Positive correlation	Negative correlation	Calibration	Validation	RMSEP	A_{opt}
Firmness	Protein, salt*	MNFS[a], pH 4.6-SN[b]	0.99	0.76	6	3
Slimy	Moisture, salt*, ash, S/M*[,c], MNFS	Fat, protein, FDM[d]	0.66	0.33	6	1
Crumbly	Fat, protein	Moisture*, MNFS*	0.72	0.31	15	1

Significant variables are marked (*).
[a] MNFS = moisture in the non-fat solids.
[b] pH 4.6-SN = pH 4.6-solublenitrogen.
[c] S/M = salt in moisture.
[d] FDM = fat in the dry matter.

matrix of cheese also determines its "firmness" (Marshall, 1993; Guinard & Mazzucchelli, 1996). In the present study "firmness" was judged in the first half of chewing using the front teeth, and was defined as "the extent of resistance offered by the cheese". "Firmness" was positively correlated with the concentration of protein and negatively correlated with the level of MNFS. In addition, "firmness" was positively correlated with salt concentration and negatively correlated with the concentration of pH 4.6-SN. The level of change in cheese texture during ripening is determined by the level of proteolysis, which in turn is controlled by the proportions of residual rennet and plasmin (Lawrence, Creamer, & Gilles, 1987). In blue-type cheeses, the low pH of the curd at drainage results in a high retention of rennet (depending on the source) and low proportion of plasmin in the curd. However, the increase in pH during ripening leads to an increase in plasmin activity (as it approaches its optimum pH value, pH 7.5) and a decrease in rennet activity (Lawrence, Heap, & Gilles, 1984; Lawrence et al., 1987). The increased plasmin activity coupled with the extensive proteolytic system of the mould, results in extensive breakdown of the protein network in blue cheese, a high pH 4.6-SN, and a high degree of curd softening during ripening. Hence, it is not surprising that the concentration of pH 4.6-SN was negatively correlated with "firmness" texture.

Blue Shropshire scored significantly higher ($P<0.05$) than any other cheese for the attribute "crumbly" (Table 2). This attribute was positively correlated with the contents of fat and protein and negatively with the levels of moisture and MNFS. Of the current cheeses, Blue Shropshire had the highest concentration of fat ($35.27\,g\,100\,g^{-1}$) and protein ($24.41\,g\,100\,g^{-1}$) and the lowest level of moisture ($38.25\,g\,100\,g^{-1}$) and MNFS ($59.09\,g\,100\,g^{-1}$). Clearly, the lower the contents of moisture and MNFS, the more "crumbly" and less "moist" the cheese body is (after Huntsman, Blue Shropshire was scored least for "moist" texture, see Table 2). In addition, Blue Shropshire had the lowest calcium content ($239\,mg\,100\,g^{-1}$) of the cheeses studied. The mineral content of a cheese type is determined by the amount of calcium phosphate lost from the curd, which in turn, is dependant on the level of acidity developed before separation of the curds and whey (Lawrence et al., 1984). In the case of blue cheese types, there is normally a high level of acid production in the vat, which can result in a low mineral content.

4. Conclusion

The present study showed that the individual odour and flavour attributes of blue-type cheeses were correlated with subsets of volatile compounds (isolated in a model-mouth with a mastication device), non-volatile compounds (FFA and FAA) and gross compositional constituents (pH, moisture, salt, fat, protein, ash, calcium, FDM, S/M, MNFS and pH 4.6-SN). Individual texture attributes were correlated with subsets of gross compositional constituents. The use of PLSR with estimation of both predictive ability and parameter reliability (jack-knife method) permitted the identification and elimination of non-contributing variables (volatile compounds, non-volatile and/or gross compositional constituents) leading to flavour and texture models with improved interpretability and reliability. Such an approach is recommended as a "do-it-yourself" method of multivariate analysis for future workers.

However, the concentrations of volatile, non-volatile and gross compositional constituents alone were not enough to explain the flavour and texture attributes perceived in this study. It is concluded that an in-depth knowledge of the effects of manufacturing variables (such as, homogenisation, scald temperature, acid development, mould strain etc.) and subsequent ripening conditions are also required to aid in the understanding of flavour and texture of cheese. In addition, rheological studies are required to improve understanding of perceived texture.

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