

Aroma characterisation of Gouda-type cheeses

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Abstract

The aim of this study was to characterise flavour differences between short-ripened Gouda-type cheeses produced from raw and pasteurised milk and to follow, in the case of pasteurised milk cheeses, flavour evolution during ripening. The volatile composition of the cheeses was studied using a combination of simultaneous steam distillation–extraction and gas chromatography–mass spectrometry. Visualisation of the analytical results was performed by principal component analysis. A clear differentiation between raw milk cheese and pasteurised milk cheese was observed and the evolution of the volatile composition of the pasteurised cheese samples during ripening was clearly demonstrated. Partial least squares regression was used to examine possible relationships between the chemical–analytical and sensory descriptive data.

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1. Introduction

Volatiles play an important role in flavour perception of cheese. Typical cheese aroma is the result of volatiles formed by lipolysis, proteolysis and metabolism of lactose, lactate and citrate (Marilley & Casey, 2004; McSweeney & Sousa, 2000; Smit, van Hylekama Vlieg, Smit, Ayad, & Engels, 2002; Urbach, 1997). The fat-derived flavour volatiles in Gouda cheese were clearly described by Alewijn, Sliwinski, and Wouters (2005). Fatty acids are the main precursors of the secondary fat-derived compounds (methyl ketones, free fatty acids (FFAs), aldehydes, lactones and ethyl esters). Alewijn, Smit, Sliwinski, and Wouters (2007) also illustrated that the formation of lactones in Gouda cheese is a one-step intramolecular *trans*-esterification reaction. δ -Lactones are produced more rapidly than γ -lactones during ripening (Alewijn et al., 2005). Ketones, especially methyl ketones, are produced slightly and do not contribute substantially to the flavour of Gouda-type cheeses (Alewijn et al., 2005). On the contrary, methyl ketones are important flavour compounds in Blue-mould-type cheeses (Molimard & Spinnler, 1996). Moulds are responsible for the conversion of FFAs into methyl ketones (Gehrig & Knight, 1963). The

contribution of (ethyl) esters to cheese flavour is concentration-dependent (Liu, Holland, & Crow, 2004). An overview of mechanisms of ester biosynthesis in dairy and non-dairy micro-organisms is given by Liu et al. (2004).

During ripening of cheese, the enzymatic degradation of amino acids leads to the formation of flavour-impact volatiles (Marilley & Casey, 2004; McSweeney & Sousa, 2000; Visser, 1993). Caseins are degraded into peptides and amino acids. Methionine, the aromatic and the branched-chain amino acids are the precursors for sulphur components (methional, dimethyl disulphide, dimethyl trisulphide) (Engels et al., 2002), for aromatic and branched-chain aldehydes (benzaldehyde, phenylacetaldehyde, 3-methylbutanal, 2-methylbutanal, 2-methylpropanal) and for branched-chain volatile acids and alcohols (3-methylbutyric acid, 2-methylbutyric acid, isobutyric acid, 3-methylbutanol, 2-methylbutanol).

Besides lipids and proteins, lactose is also a major milk constituent for the formation of cheese aroma (Marilley & Casey, 2004; McSweeney & Sousa, 2000). Lactose, lactate and citrate contribute to the formation of diacetyl, acetoin, ethanol and acetate (Cogan & Hill, 1993; Escamilla-Hurtado, Tomasini-Campocoso, Valdés-Martínez, & Soriano-Santos, 1996; Henriksen & Nilsson, 2001; Melchiorsen, Jokumsen, Villadsen, Ismelsen, & Arnau, 2002; Syu, 2001).

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Several parameters determine flavour formation in cheese, e.g., starter cultures (Kieronczyk, Skeie, Langsrud, Le Bars, & Yvon, 2004; Smit, Smit, & Engels, 2005), non-starter lactic acid bacteria or NSLAB flora in the cheese plant (Beresford & Cogan, 2000; Østlie, Eliassen, Florvaag, & Skeie, 2005), cheesemaking technology, salt and fat content (Banks, Hunter, & Muir, 1993; Mistry & Kasperson, 1998). Flavour formation in cheese is also importantly influenced by the type of milk (raw vs. pasteurised) and the ripening period. As a result of the destruction of some desirable non-starter lactic acid bacteria during pasteurisation, cheese made from pasteurised milk ripens more slowly and develops a less intense flavour than raw milk cheese (Fox & McSweeney, 1998; Grappin & Beuvier, 1997; Johnson, Nelson, & Johnson, 1990; Scifu, Buys, & Donkin, 2004).

In this work, the volatile composition of some commercial Gouda-type cheeses produced from raw and pasteurised milk were studied using a combination of simultaneous steam distillation–extraction (SDE) and gas chromatography–mass spectrometry (GC–MS). In the case of the pasteurised milk cheeses also, the evolution of the volatile composition as a function of ripening was followed. Principal component analysis (PCA) was used to evaluate the semi-quantitative SDE–GC–MS results and to visualise the changes in flavour profile of the cheeses during ripening. Additionally, the cheeses were submitted to a descriptive sensory test for some selected attributes. The relationship between sensory data and analytical GC–MS data was demonstrated by means of partial least square regression (PLS2).

2. Materials and methods

2.1. Materials and sampling

Two commercial Gouda-type cheeses were provided by a Belgian raw milk cheese maker and by a Belgian pasteurised milk cheese maker. All cheese samples were produced from cows' milk.

The milk used for the raw milk cheese samples was only heated to 37 °C. The starters consisted of a mixture of different strains of *Lactococcus lactis* subsp. *lactis* biovar *dlacetylactis* and subsp. *cremoris* (MPM De Block, Edegem, Belgium). The raw milk cheese was ripened for 6 weeks at 85–90% relative humidity and 13 °C. The final round-shaped cheeses produced from raw milk had a weight of 10.0 kg and an initial moisture, fat and salt content of 36.6% (w/w), 37.1% (w/w) and 2.0% (w/w), respectively.

For confidentiality reasons, the process parameters and the ripening conditions of the pasteurised milk cheeses were not completely revealed. Round-shaped cheeses produced from milk of different seasons and ripened for 6 weeks, 4 months and 10 months were delivered at the same moment to the laboratory. The cheeses had a weight of 13.0, 12.5 and 11.5 kg, respectively. The initial moisture, fat and salt

content of the pasteurised milk cheese ranged from 42.0% to 44.0% (w/w), 28.0% to 29.0% (w/w) and 1.80% (w/w), respectively. The fat and salt content increased during ripening (after 10 months: 32.0% (w/w) and 2.20% (w/w)), respectively, while the moisture content decreased (after 10 months: 37.0% (w/w)).

Cheese samples were taken in duplicate by cutting 250 g of sample from the centre to the outer part just before analysis. The cheese samples were stored in a refrigerator at 4 °C until they were needed for analysis. For sensory analysis, the samples were held at ambient temperature for about 1 h before evaluation.

2.2. Simultaneous steam distillation–extraction (SDE)

For the isolation of the cheese volatiles, a sample of 50 g of blended cheese was suspended in 600 mL of water and was extracted in a simultaneous SDE apparatus (Alltech Associates, Inc., Lokeren, Belgium) using 60 mL of dichloromethane. For determination of the semi-quantitative volatile composition, undecane was used as internal standard and was spiked into the solvent. Undecane was not detected in the samples and was well separated from the other cheese volatiles. After cooling to ambient temperature, the combined dichloromethane fractions were concentrated to a final volume of 0.5 mL in a Kuderna–Danish concentrator (Alltech Associated Inc., Deerfield, IL, USA). For each cheese sample, duplicate isolations of volatiles were performed.

2.3. Gas chromatography–mass spectrometry (GC–MS)

At the inlet of a HP 5890 gas chromatograph coupled to a HP 5971A MSD mass spectrometer (Agilent Technologies, Santa Clara, CA, USA), 1 µL of a cheese extract was injected. The GC system was equipped with a DB-1MS capillary column (100% dimethylpolysiloxane, L 60 m × I.D. 0.25 mm × 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA). Helium was used as carrier gas with a flow rate of 1 mL min⁻¹ and the column temperature was held at 40 °C for 5 min, then programmed at a rate of 5 °C min⁻¹ to increase to 250 °C, which was maintained for 13 min. A split ratio of 1/10 was used and the injector and detector were maintained at 250 and 280 °C, respectively. The mass spectra were obtained by electron impact of 70 eV and the total ion current (TIC) chromatograms were recorded by monitoring the TIC in a scan range of 40–260 amu (atomic mass units). A solvent delay of 6.0 min was used to avoid excessive solvent disturbing the MS source. Identification of the cheese volatiles was made by comparing the mass spectra of the different volatiles with the mass spectra of a commercial Mass Spectral Library, Wiley275 (Wiley, Somerset, NJ, USA). Semi-quantitative data of the isolated aroma compounds were calculated by relating the peak area of the cheese volatile to the peak area of undecane. The concentrations of the volatiles were expressed as ng g⁻¹ of cheese.

2.4. Descriptive sensory analysis

Descriptive sensory analyses were performed in a separate panel room using a trained panel of eight assessors (three male, five female, aged between 20 and 60 years). During training tests, the panellists developed a vocabulary of six flavour attributes and five taste attributes (Table 1). The selected attributes were based on the sensory vocabulary used in the experiment of Muir, Hunter, Banks, and Horne (1995). The assessors were presented cubes of randomly coded cheese samples, equilibrated to room temperature. They assessed the intensity of the 11 attributes using an unstructured 100-mm-score line. After sensory analysis, the scores were converted to numerical values. For data analysis of the sensory profiles of the cheese samples, mean scores for each attribute were calculated. The obtained sensory data of the samples were visualised by spider web diagrams.

2.5. Statistical analysis

PCA was performed on the semi-quantitative aroma compositions of the cheese samples using the Unscrambler 6.1 (Camo, Oslo, Norway). Partial least squares regression (PLS2) (Unscrambler 9.7, Camo) was used to examine possible interrelationships between sensory descriptions and the aroma compounds. All data were weighted with $1/SD$ (standardisation) and a leverage correction was used to validate the models.

3. Results and discussion

3.1. Descriptive sensory analysis

Descriptive sensory analyses for six flavour attributes and five taste attributes were performed on the raw and pasteurised milk cheese of the same ripening time (6 weeks) and on the pasteurised milk cheese of different ripening times (6 weeks, 4 months, 10 months). Fig. 1 illustrates the average sensory scores for raw and pasteurised milk cheese of 6 weeks of ripening. The raw milk cheese had higher scores for the attributes 'sweet', 'bitter', 'fruity, flowery', 'nutty, chocolate-like' and 'animal'. While the pasteurised milk cheese had higher scores for the attributes 'flavour intensity', 'sour' and 'pungent'. For the attributes 'creamy,

Table 1
Descriptive vocabulary of 11 attributes for descriptive sensory analysis of Gouda-type cheeses

Flavour attributes	Taste attributes
Flavour intensity	Taste intensity
Creamy, buttery	Sweet
Fruity, flowery	Sour
Nutty, chocolate-like	Salt
Pungent	Bitter
Animal	

buttery', 'taste intensity' and 'salt', no differences in scores by panellists were measured. Although both cheeses had similar ripening times, some characteristic differences were observed between cheeses produced from raw and processed milk. The sensory differences between the two types of cheeses probably originate from the used milk (milk pre-treatment) and from the different fat content of the cheeses.

Fig. 2 visualises the average sensory scores for pasteurised milk cheese of different ripening times. The panel scores of most attributes increased as function of ripening, with exception of the attributes 'sweet' and 'creamy, buttery', which decreased during ripening. As expected, ripening seems to have a clear effect on the flavour characteristics of cheese.

3.2. SDE-GC-MS profiling of cheese

3.2.1. Volatile composition

SDE-GC-MS profiling of the Gouda-type cheeses resulted in the identification of 63 volatiles. In accordance with previous work (Dirinck & De Winne, 1999), 63 volatiles were classified in chemical classes (Table 2) and were related to their origin and to their sensory characteristics.

Branched-chain aldehydes (3- and 2-methylbutanal), branched-chain acids (2-methylpropanoic acid, 3- and 2-methylbutanoic acid) and branched-chain alcohols (3-methylbutanol) are formed by the catabolism of branched-chain amino acids initiated by an aminotransferase (Atiles, Dudley, & Steele, 2000; Marilley & Casey, 2004). 3-Methylbutanal and 2-methylbutanal are characterised by a malty flavour (Friedrich & Acree, 1998; Griffith & Hammond, 1989; Kubickova & Grosch, 1997; Rychlik & Bosset, 2001b; Thierry & Maillard, 2002). The corresponding alcohols, 3-methylbutanol and 2-methylbutanol, have impact on alcoholic and fruity odours (Thierry & Maillard, 2002). The branched-chain acids (2-methylpropanoic acid, 3-methylbutanoic acid and 2-methylbutanoic acid) are responsible for sweaty, rancid, faecal, putrid, ester and rotten fruit-like flavours (Brennand, Ha, & Lindsay, 1989; Karagül-Yüceer, Cadwallader, & Drake, 2002; Marilley & Casey, 2004; Moio, Piombino, & Addeo, 2000; Rychlik & Bosset, 2001a; Thierry & Maillard, 2002).

From the catabolism of aromatic amino acids, compounds such as benzaldehyde, phenylacetaldehyde, 2-phenylethanol, benzenemethanol and indole are formed (Marilley & Casey, 2004; McSweeney & Sousa, 2000). Depending on their concentration, those volatiles have an impact on the cheese aroma. Benzaldehyde is characterised by a bitter almond; phenylacetaldehyde by a honey-like, floral, rosy and violet-like; 2-phenylethanol by a rosy, violet-like and floral; indole by a faecal, putrid, musty odour (Curioni & Bosset, 2002; Friedrich & Acree, 1998; Kubickova & Grosch, 1997; Marilley & Casey, 2004; Moio et al., 2000; Rychlik & Bosset, 2001a; Thierry & Maillard, 2002).

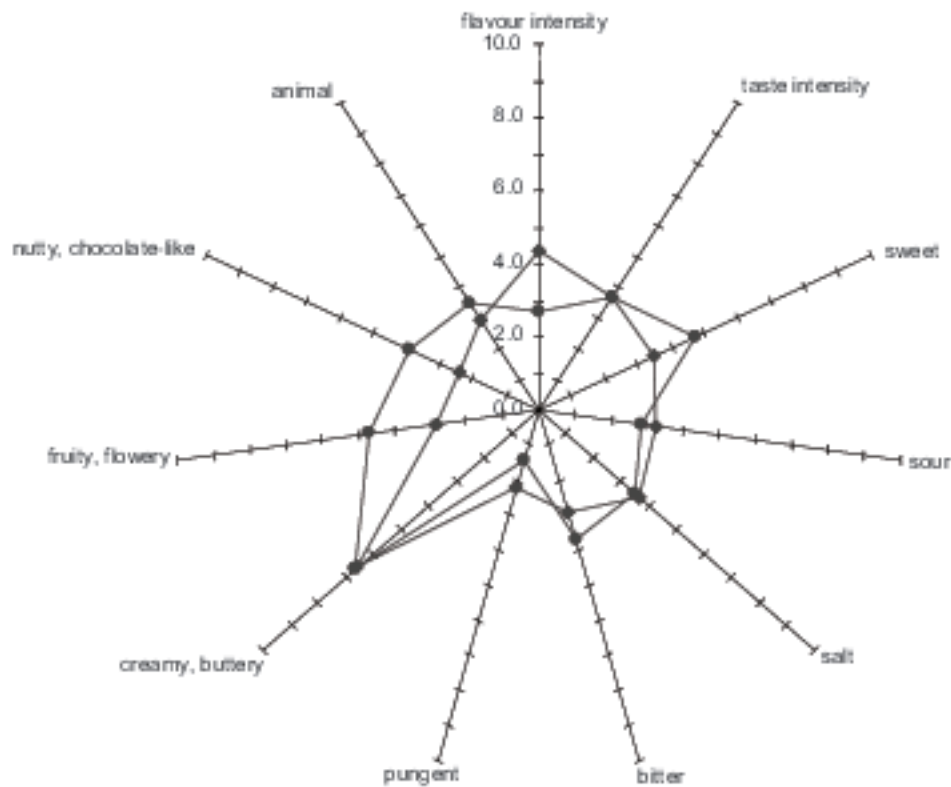


Fig. 1. Spider web diagram of the average sensorial scores for raw milk (●) and pasteurised milk (◆) cheeses of 6-weeks ripening.

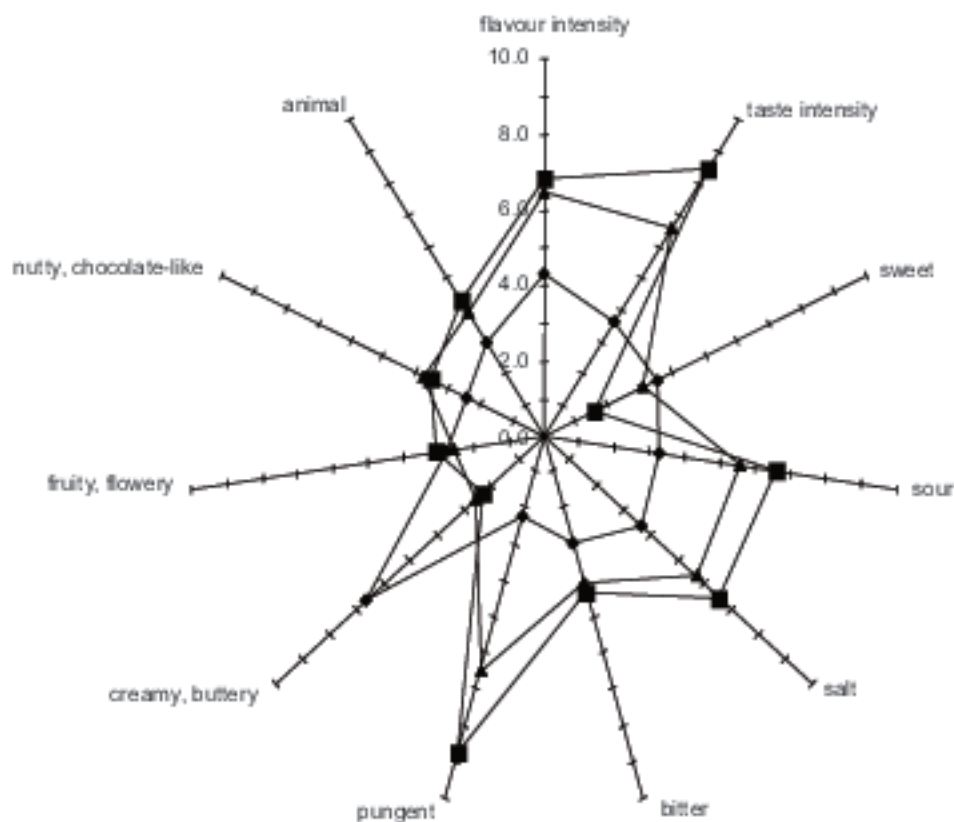


Fig. 2. Spider web diagram of the average sensorial scores for 6-weeks (◆), 4-months (▲) and 10-months (■) ripened pasteurised milk cheeses.

Table 2

Volatile composition of Gouda-type cheeses, obtained by SDE GC MS (steam distillation extraction and gas chromatography mass spectrometry) and expressed in ng g⁻¹

Flavour volatiles	Average (S.D.) ^a			
	Raw 6 weeks	Pasteurised 6 weeks	Pasteurised 4 months	Pasteurised 10 months
Branched-chain and aromatic aldehydes				
3-Methylbutanal	12.0 (2.0)	9.32 (2.74)	37.4 (12.0)	45.5 (3.6)
2-Methylbutanal	n.q.	n.q.	5.11 (0.63)	7.00 (0.37)
Benzaldehyde	12.1 (5.0)	11.4 (0.4)	15.4 (1.1)	18.6 (0.8)
Phenylacetaldehyde	39.4 (3.0)	36.6 (1.8)	118 (17)	138 (9)
Linear aldehydes				
Pentanal	5.54 (1.00)	4.00 (0.01)	3.25 (0.34)	2.20 (0.00)
Hexanal	5.00 (0.60)	4.01 (1.22)	3.94 (1.03)	4.48 (1.71)
Heptanal	4.80 (0.42)	4.18 (0.03)	n.q.	6.28 (0.47)
Nonanal	5.20 (0.00)	12.4 (2.2)	6.54 (1.71)	7.77 (1.86)
Dodecanal	24.0 (3.0)	23.3 (4.2)	16.0 (1.0)	13.7 (3.1)
Tetradecanal	50.1 (3.6)	47.2 (7.2)	34.0 (4.0)	25.8 (5.3)
Hexadecanal	183 (34)	200 (39)	144 (3)	127 (1)
2-Nonenal	0.00	4.53 (1.00)	n.q.	3.46 (1.12)
2-Decenal	0.00	5.17 (0.17)	n.q.	n.q.
2,4-Heptadienal	12.4 (4.3)	0.00	0.00	0.00
2,4-Decadienal	6.10 (2.00)	0.00	0.00	0.00
Ketones				
2-Pentanone	487 (59)	417 (15)	447 (22)	593 (64)
2-Hexanone	17.3 (1.3)	14.5 (8.0)	21.3 (9.0)	32.2 (4.2)
2-Heptanone	2281 (75)	1613 (188)	1770 (53)	2330 (312)
2-Octanone	24.1 (0.1)	17.7 (3.0)	17.1 (2.2)	25.6 (2.3)
2-Nonanone	1833 (1)	1395 (140)	1254 (170)	1878 (16)
2-Decanone	22.4 (0.6)	n.q.	11.9 (2.0)	18.0 (4.0)
2-Undecanone	2159 (42)	1465 (221)	1133 (207)	1440 (42)
2-Dodecanone	30.8 (2.0)	n.q.	n.q.	16.7 (0.0)
2-Tridecanone	2163 (91)	1604 (274)	1182 (196)	1264 (231)
2-Pentadecanone	1745 (113)	1509 (289)	1106 (120)	1405 (234)
3-Hydroxy-2-butanone	1927 (164)	3281 (264)	1395 (511)	355 (177)
Branched-chain volatile acids				
2-Methylpropanoic acid	0.00	163 (26)	0.00	0.00
3-Methylbutanoic acid	0.00	1141 (55)	0.00	0.00
2-Methylbutanoic acid	0.00	60.8 (0.0)	0.00	0.00
Free fatty acids				
Butanoic acid	n.q.	168 (16)	n.q.	n.q.
Hexanoic acid	n.q.	454 (10)	18.0 (0.0)	n.q.
Octanoic acid	33.9 (7.3)	1864 (133)	303 (188)	9.13 (0.00)
Nonanoic acid	n.q.	35.0 (1.3)	n.q.	n.q.
Decanoic acid	1524 (39)	6855 (616)	3319 (1174)	720 (343)
Undecanoic acid	21.6 (6.3)	43.0 (4.0)	20.5 (7.0)	7.67 (3.44)
Dodecanoic acid	1384 (110)	3273 (581)	1733 (611)	833 (423)
Tetradecanoic acid	724 (196)	1097 (281)	468 (55)	387 (250)
Hexadecanoic acid	152 (65)	133 (59)	34.0 (6.2)	63.0 (48.0)
Ethyl esters				
Ethyl hexanoate	0.00	0.00	10.0 (2.1)	17.3 (0.1)
Ethyl octanoate	0.00	0.00	4.90 (2.50)	20.2 (0.0)
Ethyl decanoate	0.00	0.00	n.q.	16.4 (3.0)
Ethyl dodecanoate	0.00	0.00	12.8 (3.0)	14.6 (0.2)
Ethyl tetradecanoate	0.00	0.00	n.q.	53.0 (14.0)
Ethyl hexadecanoate	0.00	0.00	8.48 (5.10)	22.1 (10.0)
Lactones				
γ -Nonalactone	n.q.	n.q.	2.59 (1.10)	9.21 (4.77)
γ -Undecalactone	12.0 (0.3)	17.4 (3.6)	8.58 (1.50)	6.83 (0.30)
α,γ -6-dodecenoic acid lactone	136 (7)	101 (22)	101 (17)	133 (30)
γ -Dodecalactone	468 (26)	401 (56)	340 (47)	395 (40)
δ -Octalactone	21.0 (0.4)	22.9 (1.9)	9.24 (3.50)	2.42 (0.00)
δ -Decalactone	525 (16)	654 (78)	432 (66)	331 (45)

Table 2 (continued)

Flavour volatiles	Average (S.D.) ^a			
	Raw 6 weeks	Pasteurised 6 weeks	Pasteurised 4 months	Pasteurised 10 months
δ -Undecalactone	n.q.	n.q.	n.q.	11.15 (1.61)
δ -Dodecalactone	657 (11)	851 (170)	588 (119)	590 (56)
δ -Tetradecalactone	248 (11)	353 (75)	246 (26)	234 (8)
δ -Hexadecalactone	52.3 (3.1)	68.0 (15.0)	39.1 (1.0)	36.5 (3.5)
Sulphur compounds				
Dimethyl disulphide	n.q.	3.24 (0.62)	4.78 (0.31)	7.40 (0.39)
Methional	4.30 (2.01)	n.q.	10.6 (0.9)	12.1 (2.0)
Dimethyl trisulphide	n.q.	1.61 (0.45)	1.83 (0.63)	2.24 (0.19)
Alcohols				
1-Butanol	n.q.	8.03 (2.55)	18.4 (4.3)	18.9 (1.7)
3-Methyl-1-butanol	6.36 (0.15)	27.4 (0.3)	51.2 (5.8)	27.3 (10.4)
Benzemethanol	3.86 (1.77)	6.89 (0.47)	5.11 (1.92)	9.52 (0.47)
2-Phenylethanol	13.6 (0.7)	10.9 (1.8)	29.1 (6.0)	59.2 (4.2)
2,3-Butanediol	17.4 (3.2)	11.6 (1.1)	12.7 (0.0)	n.q.
Indole	4.28 (0.01)	6.83 (1.22)	3.07 (0.88)	36.6 (2.4)

^a Average of analyses of two different samples (S.D.); n.q.: not quantified.

Sulphur compounds (dimethyl disulphide, dimethyl trisulphide, methional) originating from methionine (McSweeney & Sousa, 2000; Yvon & Rijnen, 2001) generally have low odour threshold values (Maarse, 1991) and have powerful odours described as sulphurous or cabbage-, onion-, or garlic-like (Maarse, 1991).

Free fatty acids (even numbered C_4 – C_{16} , nonanoic and undecanoic acid), 2-methyl ketones (C_5 – C_{15} , major compounds have odd carbon numbers), δ -lactones (even numbered C_8 – C_{16} and δ -undecalactone) and γ -lactones (C_9 , C_{11} , C_{12} and unsaturated *cis*- γ -6-dodecenoic acid lactone), saturated and unsaturated aldehydes (saturated C_5 , C_6 , C_7 , C_9 , C_{12} , C_{14} , C_{16} , 2,4-heptadienal and 2,4-decadienal) and ethyl esters (even numbered C_6 – C_{16}) are fat-derived flavour volatiles that play a role in the overall flavour of cheese (Alewijn et al., 2005). FFAs are formed by lipid degradation and have a pungent to rancid odour (Gómez-Ruiz, Ballesteros, Gonzalez Vinas, Cabezas, & Martinez-Castro, 2002). Methyl ketones are present in most cheeses, but in lower levels than in mould-ripened cheeses (Molimard & Spinnler, 1996). These compounds are lipid degradation products and are formed by β -oxidation and decarboxylation of fatty acids (Bills & Day, 1964; Marilley & Casey, 2004). Methyl ketones probably contribute to the background aroma of cheese. Lactones are also related to lipid degradation (Alewijn et al., 2007; Collins, McSweeney, & Wilkinson, 2003; Marilley & Casey, 2004; McSweeney & Sousa, 2000; Molimard & Spinnler, 1996). The sensory characteristics of lactones are different: buttery, fruity and coconut-like (Keeney & Patton, 1956; Maarse, 1991). The saturated aldehydes can originate from fresh milk but they can also be formed, together with the unsaturated aldehydes, by autooxidation of unsaturated fatty acids (Ho & Chen, 1993). Saturated aldehydes have a green-like, hay-like, paper-like

odour, while unsaturated aldehydes have a fatty, oily, frying odour (Maarse, 1991). Ethyl esters, originated from the enzymatic or chemical esterification of fatty acids with ethanol, possess pleasant sweet and fruity notes (Bosset & Liardon, 1984; Fernández-García, Mariaca, & Nuñez, 1998; Moio & Addeo, 1998; Molimard & Spinnler, 1996).

From metabolism of lactose, lactate and citrate 2,3-butanediol (diacetyl) and its reduction products 3-hydroxy-2-butanone (acetoin) and 2,3-butanediol are formed (Cogan & Hill, 1993; Marilley & Casey, 2004; McSweeney & Sousa, 2000). Unfortunately, because of the high volatility, diacetyl (with a 'buttery' note) was not measured in this method, as the peak was covered by the solvent.

3.2.2. Principal component analysis of the semi-quantitative SDE–GC–MS data

The concentrations of the 63 identified volatile compounds in the Gouda-type cheeses, listed in Table 2, varied during ripening and were different between the raw and the pasteurised cheese samples. To visualise the SDE–GC–MS results of the cheese samples, PCA was performed on the complex data (63 volatiles, 4 × 2 cheese samples). Individual values of the duplicate analyses were used in order to allow visual evaluation of the reproducibility of the SDE–GC–MS technique.

Fig. 3 illustrates a 2D-PCA biplot of the SDE–GC–MS results of raw and pasteurised milk cheese of 6 weeks of ripening. The biplot explained 64% (PC1: 53%; PC2: 11%) of the total chemical information. A good classification between raw and pasteurised milk cheese was obtained. The cheese samples produced from raw milk were located on the negative PC1 axis. Also phenylacetaldehyde and 2-phenylethanol with a 'flowery' flavour character

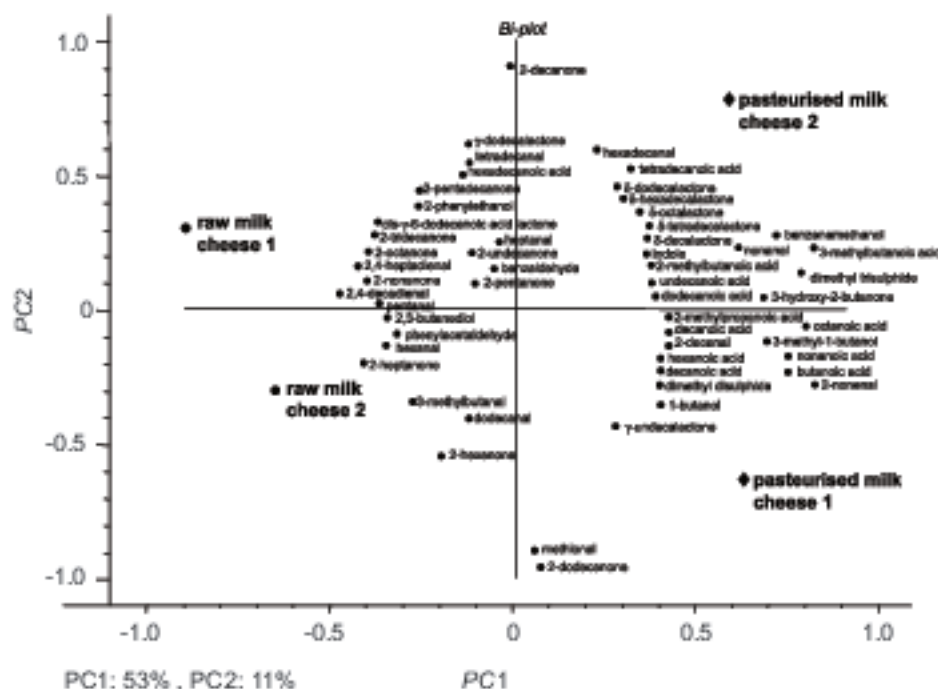


Fig. 3. Principal component analysis (PCA) of the volatile compositions (SDE GC MS) of raw milk (●) and pasteurised milk (◆) cheeses of 6-weeks ripening.

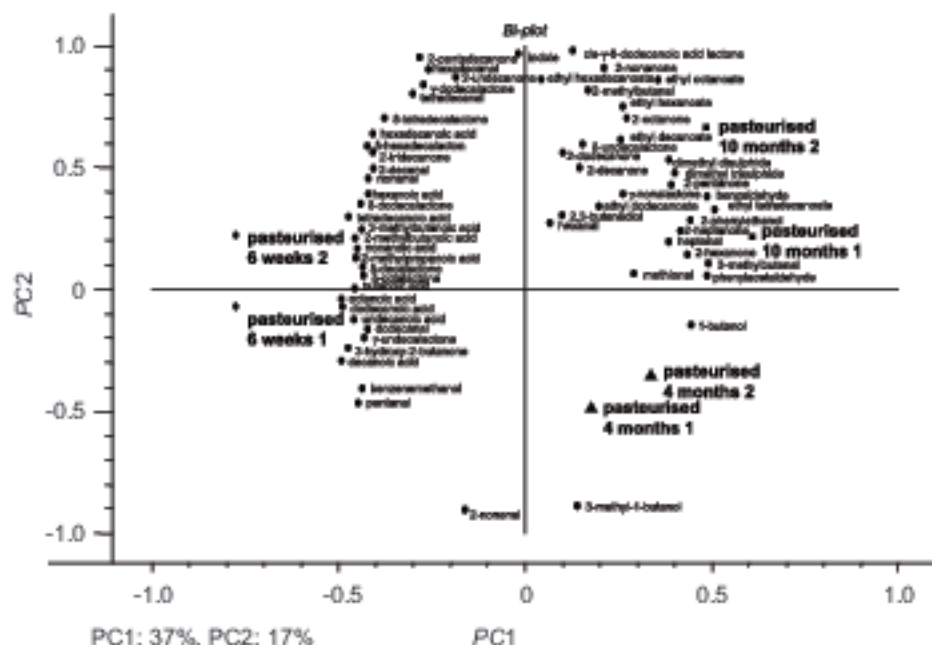


Fig. 4. Principal component analysis (PCA) of the volatile compositions (SDE GC MS) of pasteurised milk cheese of 6-weeks (◆), 4-months (▲) and 10-months (■) ripening.

(Maarse, 1991) and 2,4-heptadienal and 2,4-decadienal with a 'fried' impact on the cheese flavour (Maarse, 1991) were located on the negative PC1 axis. It can be observed that some methyl ketones, linear aldehydes and two γ -lactones (γ -dodecalactone and *cls*- γ -6-dodecenoic acid lactone) were also located near the raw milk cheese

samples. The somewhat higher amount of 3-methylbutanal should be responsible for the higher 'nutty, chocolate-like' character (Maarse, 1991) of the raw milk cheese. It is also clear that the volatile pattern of the pasteurised milk cheese showed higher levels of sulphur compounds (dimethyl disulphide, dimethyl trisulphide) and of the branched-chain

acids, 2-methylpropanoic acid, 3- and 2-methylbutanoic acid. Together with the higher levels of free fatty acids (octanoic acid, decanoic acid, dodecanoic acid, etc.) these compounds (situated on the positive PC1 axis) should be responsible for the higher 'flavour intensity' and the more 'sour' and 'pungent' character (Maarse, 1991) of the pasteurised milk cheese.

The evolution of the volatile composition of pasteurised milk cheese during ripening is illustrated in Fig. 4. The PCA biplot of the semi-quantitative SDE–GC–MS results explained 54% (PC1: 37% and PC2: 17%) of the chemical information. The pasteurised milk cheese of 6 weeks of ripening was situated at the negative PC1 axis of the 2D-PCA biplot. It was clear that after 6 weeks of ripening

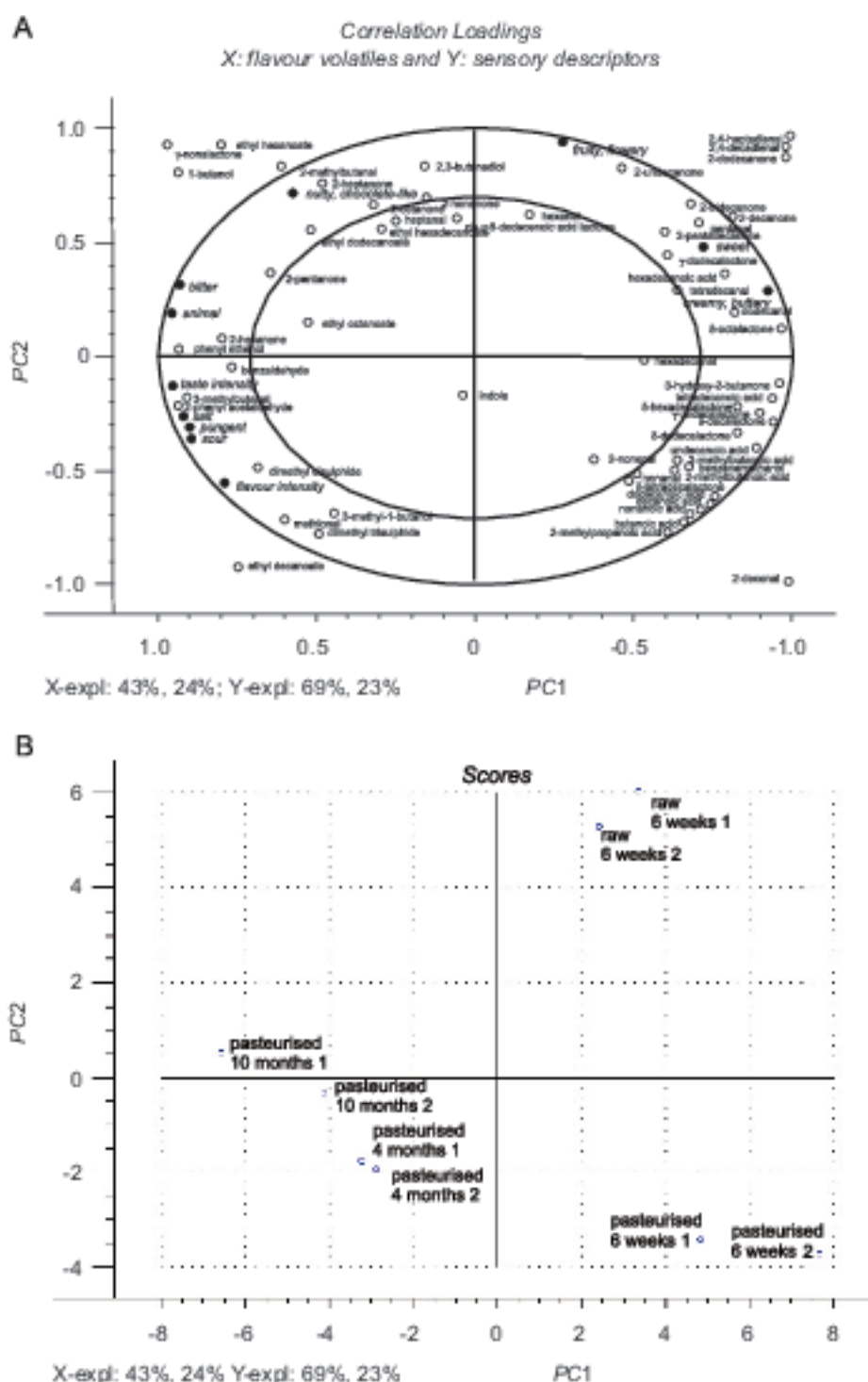


Fig. 5. (A) Partial least squares regression (PLS2) correlation loadings plot (X-variables: flavour volatiles; Y-variables: sensory descriptors) of Gouda-type cheeses (raw milk cheese of 6 weeks, pasteurised milk cheese of 6 weeks, 4 months and 10 months). (B) Partial least squares regression (PLS2) scores plot (raw milk cheese of 6 weeks, pasteurised milk cheese of 6 weeks, 4 months and 10 months) of Gouda-type cheeses, made of raw and pasteurised milk.

higher concentrations of a lot of fat-derived flavour volatiles (FFAs, δ -lactones and linear aldehydes) were determined, but also 3- and 2-methylbutanoic acid and 2-methylpropanoic acid (amino acid-related) had the highest levels in the pasteurised milk cheese of 6 weeks of ripening. Also 3-hydroxy-2-butanone (acetoin), that should contribute to the 'buttery' note (Maarse, 1991), was found at a higher concentration in the 6-weeks-ripened pasteurised milk cheese.

The cheeses ripened for 4 and 10 months were located at the positive PC1 axis and the PC2 axis differentiated the samples of respectively 4 and 10 months of ripening. There seemed to be no clear effect of ripening on the sensory important γ - and δ -lactones which were situated at both sides of the PC1 axis. From the biplot, it was clear that during ripening there was an increase of the concentration of 3- and 2-methylbutanal, sulphur compounds (methional, dimethyl disulphide, dimethyl trisulphide), phenylacetaldehyde, 2-phenylethanol, benzaldehyde and indole. These strong odour active volatiles should have a major impact on the 'aged' cheese flavour. From Fig. 4 and Table 2 it was also clear that ethyl esters (ethyl hexanoate, ethyl octanoate, etc.) were only formed after long ripening periods and increased as a function of ripening time. Ethyl esters contributed to the 'floral' and 'fruity' note (Liu et al., 2004; Maarse, 1991) of cheeses.

3.3. Correlation between sensory and chemical-analytical analysis

Partial least squares regression (PLS2) was performed on the data from raw and pasteurised milk cheeses of different ripening times to examine possible relationships between sensory attributes and chemical aroma compounds. The X-matrix contained the volatile compounds and the Y-matrix contained the sensory scores for each attribute. The ellipses indicate 50% and 100% of explained variance. Fig. 5A shows the correlation between the X and Y variables. The 'fruity, flowery' note can be correlated with some methyl ketones (2-undecanone and 2-tridecanone). Methyl ketones give Blue cheeses a 'fruity', 'spicy' odour (Heath, 1978). The attributes 'sweet' and 'creamy, buttery' have a positive correlation with some lactones (γ -dodecalactone and δ -octalactone) and with 3-hydroxy-2-butanone. Lactones can have 'buttery' and 'fruity' flavour notes, while 3-hydroxy-2-butanone is known for its 'buttery' character. It was also clear that most amino acid-related volatiles (2-phenylethanol, benzaldehyde, 3-methylbutanal, phenylacetaldehyde) correlated with the sensory descriptors 'taste intensity' and 'pungent'. Pungency in cheese should be considered as related to non-volatile compounds, e.g. peptides and amino acids, which are not measured in the analytical approach used in this work. The sulphur compounds (dimethyl disulphide, dimethyl trisulphide and methional) seemed to have a good correlation with the descriptor 'flavour intensity'. It was previously described that those sulphur compounds

have low odour threshold values and can have a great impact on the cheese aroma. It can be observed that most volatile acids (FFAs and branched-chain acids) have negative correlations with most sensory attributes. However, they should be important for the flavour of the pasteurised milk cheese after 6 weeks of ripening. By comparing Fig. 5A (correlation X and Y loadings plot) with Fig. 5B (scores plot), the raw milk cheese seems to have a good correlation with the attributes 'fruity, flowery', 'sweet' and 'creamy, buttery', while the more ripened pasteurised milk cheeses yielded good correlation with the descriptors 'taste intensity', 'pungent' and 'flavour intensity'. The attributes 'animal', 'bitter', 'salt' and 'sour' cannot directly be correlated with flavour volatiles. Those attributes are more related to less volatile high-mass molecules.

4. Conclusions

In this study, the flavour differences between the selected Gouda-type raw and pasteurised milk cheeses and the flavour evolution during ripening of the investigated pasteurised milk cheeses were evaluated. The differences in the composition of volatile flavour compounds between short-ripened raw and pasteurised milk cheeses were demonstrated. During ripening of pasteurised Gouda-type cheeses, there was an increase of more amino acid-related flavour volatiles and of ethyl esters, while the amount of linear aldehydes and volatile acids (FFAs and branched-chain acids) decreased. Correlation analysis suggested that 'sweet' and 'fruity' notes correlated with the raw milk cheese of 6 weeks ripening, while sulphur compounds correlated strongly with the descriptor 'flavour intensity' of the more ripened pasteurised milk cheeses.

In further research, these techniques will be used for objective evaluation of a whole range of flavour influencing parameters (e.g., fat and salt reduction, influence of starter cultures, influence of slicing and packaging). An objective analytical approach can contribute substantially to product and process innovation for the cheese producing companies.

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