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Short communication

Flavour characterisation and classification of cheeses by gas chromatographic–mass spectrometric profiling

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Abstract

As part of a study concerning the biotechnical production of enzyme modified cheese flavours, gas chromatography–mass spectrometry was used for objective measurement of cheese aroma characteristics. In order to obtain the necessary reference information for steering the biotechnical process towards typical cheese flavour characters, such as Gouda and Emmental, the volatile fractions of three Gouda cheeses from different producers and three Emmental cheeses from different origins (Swiss, French and Austrian) were studied. The volatile fractions of the different cheeses were composed of fatty acids, methylketones, lactones, aldehydes and alkenes. Principal component analysis was used for interpretation of the complex semi-quantitative data matrix and allowed classification of the different cheeses. It was observed that the Gouda cheeses from different producers had similar aroma patterns, while more differences were observed between the Emmental cheeses. Austrian Emmental was clearly differentiated from the French and Swiss products.

Keywords: Cheese; Food analysis; Principal component analysis; Ketones; Organic acids; Lactones; Alkenes; Aldehydes

1. Introduction

Enzyme modified cheese flavours are natural flavour ingredients, which are produced by adding proteolytic and lipolytic enzymes to emulsions of natural cheese substrates. The fat and/or protein portions of the substrates are then modified by the added enzymes and, after inactivation of the enzymatic and biological activity by heat treatment, intensely flavoured preparations can be obtained. In order to steer these biotechnical processes towards the desired flavour character, GC–MS analyses can be used for objective monitoring of the flavour (aroma) profiles. This study discusses the reference profiles for the Gouda and Emmental character, which were determined by GC–MS analyses of extracts, obtained by simultaneous steam distillation–extraction (SDE, Likens–Nickerson), from three Gouda cheeses from different producers and three Emmental cheeses from different origins (Swiss, French and Austrian).

The volatile composition of several types of cheese has already been studied by several authors and some interesting reviews have been published [1–4]. Badings and Neeter also give a survey of some earlier work performed on different cheeses like Cheddar, blue-veined, Camembert, Swiss and Gouda [5]. Vandeweghe and Reineccius compared different techniques for isolation of volatiles from Cheddar cheese: distillation, dialysis and solvent extraction [6]. Characterisation of six artisanal Spanish cheeses by GC–MS of their medium volatile (SDE) fraction was performed by de Frutos et al. [7]. Moio et al. compared the neutral volatile compounds

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in Mozzarella cheese made from bovine and water buffalo milk and used extract dilution GC–sniffing to determine the powerful odorants in cheeses with milk from both origins [8–10].

From these studies it was clear that cheese flavour is not linked to a small number of character impact compounds, but is the result of a delicate balance of a complex blend of components. An effective approach for instrumental characterisation of cheese aroma and flavour should be a combination of a suitable aroma isolation procedure, GC or GC–MS profiling and processing of the semi-quantitative data by multivariate (pattern recognition) statistics. Aishima and Nakai used GC patterns for classification of Cheddar, Gouda, Edam, Swiss and Parmesan cheeses [11]. In our study we combined SDE isolation with GC–MS profiling for determination of the semi-quantitative composition and principal component analysis for interpretation of the complex data matrix.

2. Materials and methods

2.1. Materials

Gouda cheeses (code: Gouda A, B and C) from three different producers (company A, B and C) and Emmental cheeses (code: Emmen S, F and A) from Swiss, French and Austrian origin were obtained through the courtesy of a food ingredient manufacturer. The samples were cut from a larger block, wrapped in tin aluminium foil and then vacuum packed. The samples were stored in a refrigerator at 4°C until they were needed for analysis. Representative samples were taken by cutting the samples from the centre to outer part just prior to analysis.

2.2. Isolation of volatiles

A sample of 50 g of blended cheese was suspended in 600 ml of water in a 1-l flask. The sample flask was attached to a modified Likens–Nickerson apparatus. In a 100 ml round-bottomed flask, 50 ml of chromatographic grade dichloromethane and 60 μg (in the case of Gouda cheeses) or 120 μg (for isolation of volatiles from Emmental cheeses) of dodecane in dichloromethane as internal standard were added. Since the concentrations of aroma components were higher in the Emmental cheeses, twice the amount of internal standard was used. Additionally, 10 ml of dichloromethane was added to the Likens–Nickerson solvent return loop. Both solvent and sample mixture were heated to boiling with heating mantles and allowed to reflux for 4 h. After cooling to ambient temperatures the dichloromethane fractions of the solvent flask and the return loop were collected. Concentration of the combined fractions to 0.5 ml (Gouda samples) or 2 ml (Emmental samples) was performed in a Kuderna Danish concentrator (Alltech, USA). For each cheese sample triplicate isolations of volatiles were performed.

2.3. Gas chromatography–mass spectrometry

The Likens–Nickerson extracts were analysed by injection of 1 μl of aroma concentrate on a HP 5890 gas chromatograph coupled to a HP 5971A MSD mass spectrometer (Hewlett-Packard, USA). The GC system was equipped with a fused-silica column (HP PONA cross-linked methyl silicone, 50 m×0.21 mm I.D., 0.5 μm film thickness). Carrier gas was helium (1 ml min^{-1} flow-rate) and the column temperature was initially maintained at 40°C for 5 min and subsequently programmed from 40 to 250°C at a rate of 5°C min^{-1}, where it was held for 13 min. Split injection (1:5 split ratio) was used and the injector and transfer lines were respectively maintained at 250 and 280°C. The mass spectra were obtained by electron impact at 70 eV. The chromatograms were recorded by monitoring the total ion current in the 40–260 mass range using a solvent delay of 6.5 min. Identification of the volatiles was based on comparison of the spectra with those of the NBS49K library and a self-made library and was assisted by Kovats indices. Semi-quantitative determinations of the volatile constituents were calculated by relating the peak intensities to the intensity of dodecane (internal standard) and were expressed as ng g^{-1} of cheese. Mean values for the individual constituents were calculated from triplicate analyses of each cheese.

2.4. Statistical analyses

To visualize the complex data matrix and the relationships between the different cheeses and their volatile composition, a principal component analysis was performed using Unscrambler 6.1 (Camo, Norway) statistical software.

3. Results and discussion

In Figs. 1 and 2, typical GC–MS profiles of Gouda C and Emmental S cheeses, respectively, are presented. The components identified by MS are listed in Table 1, which presents the semi-quantitative composition (mean of triplicate analyses, dodecane as internal standard) of the six cheeses, classified according to chemical classes. The volatiles of the different cheeses were ketones, acids, lactones, alkenes and aldehydes.

2-Methylketones (C_5 to C_{15}) with odd carbon number were the main constituents in all cheeses. These compounds are lipid degradation products and are formed by β-oxidation and decarboxylation of fatty acids. A major bifunctional ketone, 3-hydroxy-2-butanone (acetoine) was present in larger amounts in the Gouda cheeses compared to the Emmental cheeses and showed also a large variation within the group of Gouda cheeses. Acetoine is probably related to carbohydrate metabolism and can be formed by enzymatic condensation of two acetaldehyde molecules or from diacetyl reduction. Diacetyl, with a strong buttery odour, was not measured as it eluted very close to the solvent.

Free fatty acids were another series of important components in the Gouda and Emmental cheeses. They were found in much larger concentrations in the Emmental cheeses compared to the Gouda cheeses. Propionic acid, formed by the action of propionibacteria from pyruvic acid, and butanoic acid were typical of Emmental and were not detected in Gouda. Also the branched acids, 3-and 2-methylbutanoic acid, showed higher levels in the Emmental cheeses. Both acids should be related to proteolysis and degradation of the corresponding branched amino acids. High levels of medium chain fatty acids (C_6 to C_{10}) with even carbon number were present in the Swiss and French Emmental. Fatty acids with even carbon numbers (C_6 to C_{16}) are formed by lipid degradation. There is a decreasing amount going from French to Swiss to Austrian

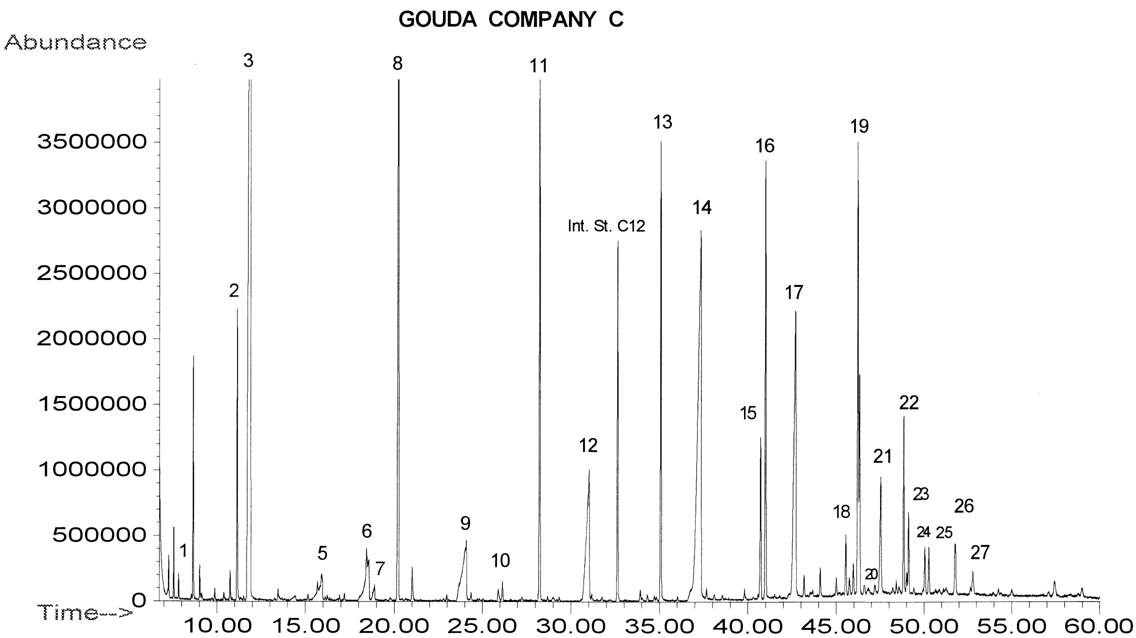

Fig. 1. GC–MS analysis of the Likens–Nickerson extract of Gouda cheese from company C. Peak numbering is in accordance with the numbering in Table 1. Time scale in min.

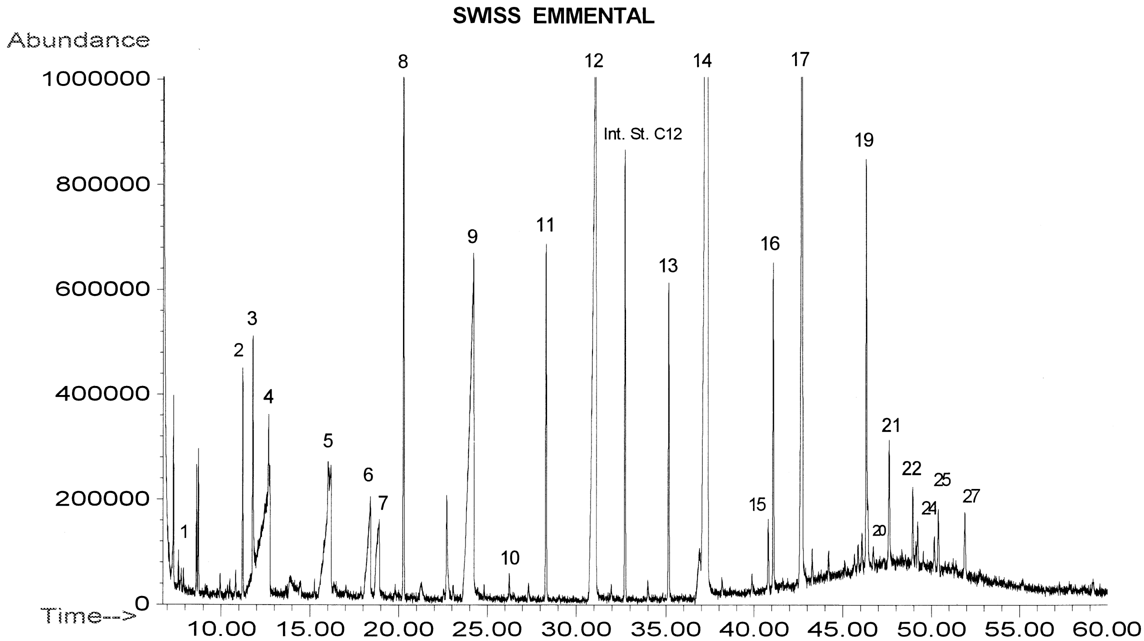

Fig. 2. GC–MS analysis of the Likens–Nickerson extract of Swiss Emmental cheese. Peak numbering is in accordance with the numbering in Table 1. Time scale in min.

Emmental and even lower concentrations were found in Gouda.

Four lactones were identified in this study. Lactones are also related to lipid degradation and are formed by cyclisation of γ- and δ-hydroxyacids. δ-Decalactone and δ-dodecalactone were relatively more abundant in the Gouda cheeses compared to the Emmental cheeses. γ-Dodecalactone was found in all types of Gouda and in Austrian Emmental but not present in either the Swiss or the French Emmental. Lactones are known to contribute to a buttery character in cheese.

Alkenes were detected in higher amounts in Gouda types. Their presence in cheese is probably not related to the ripening process but to the cow's diet. Phytadiene was not found in French Emmental.

Visualisation of the complex data matrix was performed by principal component analysis (PCA), with the six cheeses as objects and all 27 volatile compounds as variables. However, instead of the mean values in Table 1, the individual values of the triplicate analyses (18 objects) were used, in order to allow visual evaluation of the reproducibility of the analytical method. Fig. 3 shows the results in a two-dimensional scatter plot with objects and variables presented in the same plane. A plot of the two first principal components explained 81% of the total variance (58% for PC1 and 23% for PC2). PCA analysis mainly showed three clusters: Gouda cheeses, Austrian Emmental, and a cluster of the Swiss and French products. This was in good accordance with sensory analyses by descriptive profiling and triangle tests, a full discussion of which is beyond the scope of this publication. However, from the sensory analyses it was clear that the Gouda and Emmental cheeses had quite different flavour characters: Gouda was described as more buttery, while an important descriptor for Emmental was yoghurt-like. In the triangle tests the Austrian Emmental was significantly different from the French and Swiss products, which could not be differentiated. No significant differences were found among the Gouda cheeses from different producers.

Examination of the PCA of the instrumental data in Fig. 3 also shows close relationships (similar GC–MS patterns) between the Gouda cheeses.

Table 1
Semi-quantitative data of volatiles, isolated by Likens–Nickerson extraction of Gouda and Emmental cheeses[a]

No.	Volatile compounds	Gouda A	Gouda B	Gouda C	Emmen. S	Emmen. F	Emmen. A
	Ketones						
1	2-Butanone	54±14	64±3	63±1	124±14	337±13	207±15
2	2-Pentanone	676±106	767±35	762±73	1015±57	989±48	936±35
3	3-Hydroxy-2-butanone	4146±430	12476±869	7495±323	1214±188	2500±177	2063±488
8	2-Heptanone	2903±158	3210±147	3137±163	3332±179	3251±69	3598±83
11	2-Nonanone	1841±24	1893±70	1960±53	1889±41	1768±46	1986±82
13	2-Undecanone	1437±43	1427±6	1639±90	1817±80	1702±59	1793±60
16	2-Tridecanone	1378±105	1360±33	1695±150	1928±39	1806±116	1860±144
19	2-Pentadecanone	1387±87	1443±110	1762±155	2224±364	2436±161	2116±170
	Acids						
4	Propanoic acid	n.q.	n.q.	n.q.	5922±876	14284±1405	2500±761
5	Butanoic acid	n.q.	n.q.	n.q.	4125±553	4565±872	1701±184
6	3-Methylbutanoic acid	696±107	516±47	670±142	1898±296	1107±116	383±34
7	2-Methylbutanoic acid	n.q.	n.q.	n.q.	1342±229	1398±176	1795±181
9	Hexanoic acid	1140±88	1554±143	1061±114	8998±805	12939±1275	3740±480
12	Octanoic acid	2266±110	2726±22	1067±130	12338±254	16128±1130	5633±431
14	Decanoic acid	6632±56	7585±615	5103±422	25444±1762	30159±2550	11947±787
17	Dodecanoic acid	2718±458	3291±413	2292±195	9037±1534	14311±5250	2900±318
21	Tetradecanoic acid	811±5	929±76	517±130	2695±273	2844±699	667±122
26	Hexadecanoic acid	149±4	195±4	134±1	345±4	380±32	n.q.
	Lactones						
15	δ-Decalactone	671±81	676±54	691±62	449±105	381±73	230±13
18	γ-Dodecalactone	86±15	88±3	236±19	n.q.	n.q.	968±49
20	δ-Dodecalactone	845±112	845±112	896±78	372±79	362±15	n.q.
27	δ-Tetradecalactone	351±48	366±26	379±15	471±82	440±43	188±23
	Alkenes						
22	Phytene A	1063±87	1177±96	747±71	499±42	303±48	747±47
24	Phytadiene	496±23	497±29	214±26	207±21	n.q.	104±19
25	Phytene B	227±15	256±23	207±24	391±24	334±27	277±23
	Aldehydes						
10	Phenylacetaldehyde	n.q.	n.q.	n.q.	168±11	n.q.	339±40
23	Hexadecanal	142±64	162±17	288±29	n.q.	n.q.	n.q.
	n.q.: not quantified (very small peak)						

[a] Mean of triplicate analysis, expressed as ng g^{-1} of cheese±standard deviation.

Gouda cheeses were dominated by lactones (δ-decalactone and δ-dodecalactone) and acetoine, which corresponded to the higher buttery note in these cheeses compared to the Emmental types. The Swiss and French Emmental were also closely related and were characterised by high levels of acids and of methylketones. Some highly volatile acids were typical of Emmental: propionic, butanoic and 2-methylbutanoic acid. The Austrian Emmental was clearly differentiated from the two other Emmental products and characterised by high levels of γ-dodecalactone and phenylacetaldehyde.

4. Conclusion

The developed analysis method, based on Likens–Nickerson extraction, GC–MS profiling and principal component analysis can be used for characterisation of Gouda and Emmental cheese flavour. In a

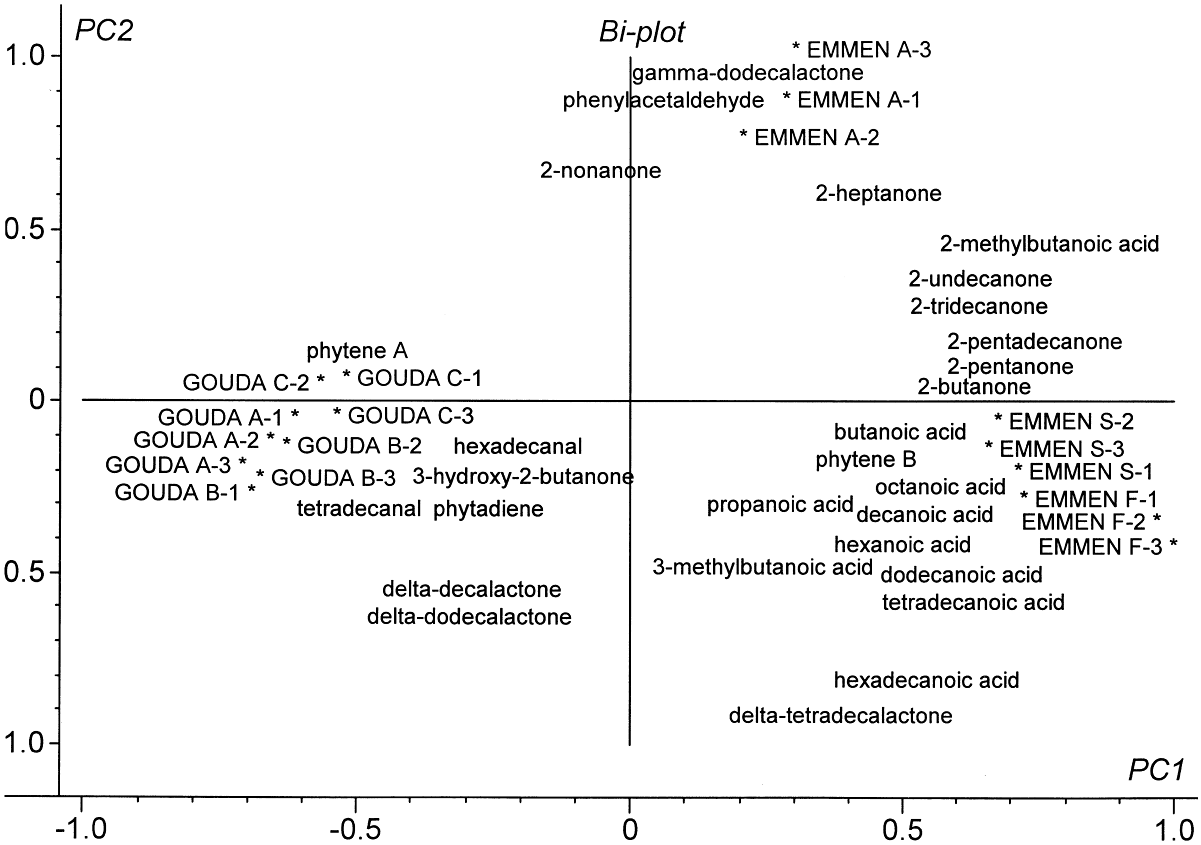

Fig. 3. Principal component analysis of the volatile composition of Gouda and Emmental cheeses.

later stage it could also be used successfully for steering the biotechnical process for production of enzyme modified cheese flavours towards typical flavour characters.

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