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Characterization of Maltese goat milk cheese flavour using SPME-GC/MS

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Abstract

The effect of lactation on the flavour of cheese made with raw milk of Maltese goats bred in Sicily (Italy) was studied. The goat cheese flavour was analyzed for the first time by Solid Phase Microextraction (SPME) coupled with Gas chromatography/Mass Spectrometry (GC/MS). The suitability of the analytical method was determined by calculating the coefficients of variation (CV%). Data were processed using ANOVA in relation to the stage of lactation. Forty four components (methyl ketones, free fatty acids, aldehydes, alcohols, esters, terpenes and lactones) were identified and quantified. The most representative fatty acids were butyric, baproic, baprylic and bapric (from 30 to 75% of the total identified fraction). Many other important cheese odorants were determined giving a substantial contribution to the aroma profile. The highest percentages were, for alcohols: isoamyl and phenethyl alcohols at the 2nd month of lactation (6.82% and 2.81%, respectively); as regards ketones, at the 5th and 6th months of lactation: 2-heptanone (7.88% and 10.10%, respectively) and 2-nonanone (18.52% and 14.81%, respectively). The highest percentage of esters (2.5%) was found in the 7th month of lactation. The lactation significantly influenced the flavour components. Good CV percentages were obtained (less than 5.0). This easy and fast method permits a quali-quantitative description of the goat's cheese flavour in relation to the lactation. The results testify the difficulty in attributing the variations to a single effect so as to standardize this product, even if this analytical method could help to give some indications, before marketing, regarding the consumer's perception and acceptance.

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Introduction

Goat milk has played an important role in human nutrition for a long time because of its nutritional and dietetic characteristics (Morand-Fehr *et al.*, 2000). It is often processed into cheese. Goat cheese is of a hard-texture, made with full-cream raw milk. Curdling is carried out in woody tubs at 35 °C for 45 min by adding lamb's rennet. The ripening ranges from a few days to some months (Rubino, 1996). Its sensorial profile is particularly appreciated among cheese tasters. Cheese flavours originate from bio-degradation processes occurring during ripening, and lipolysis and proteolysis being the most important ones. Flavouring molecules, such as methyl ketones (e.g. 2-nonanone: fruity) or secondary alcohols (e.g. 2-pentanol: mild green) are often distinctive character-impact flavour compounds, even at trace levels (Urbach, 1973). Dairy farmers are today more concerned about the knowledge of the chemistry of flavours, because this can help the assessment of the quality and authenticity of a cheese. Since the flavour somewhat reflects what is in the cheese itself, the aroma screening can provide more information about the manufacturing, the diet of the animals and the ripening time (Wilson, 1993). Substantially, it can satisfy the increasing demand for food safety and the food products' traceability.

The flavour chemistry is a quite recent development of food chemistry, mostly due to the introduction of gas chromatography and mass spectrometry (Fennema, 1985). The analysis of flavours requires initial isolation of volatiles from the sample matrix and their simultaneous concentration; this should be carried out without distorting the flavour chemical composition. In this perspective, a useful tool has proved to be Solid Phase Microextraction (SPME), a solventless extraction technique introduced in the early 1990s (Pawliszyn, 1997). It mainly consists of a special holder (that features as a syringe for GC) provided with a silica support coated with a stationary phase (fibre). Chemical compounds are absorbed onto the fibre, which is exposed to the sample headspace, and then desorbed into the hot GC injector.

Since the composition of milk, and consequently of the cheese manufactured from it, is influenced by many variables, such as stage of lactation, feeding regimen, age and breeding system, this paper represents a first step towards a more extensive evaluation of the cheese aroma composition. The purpose of this study was to investigate the variations of the flavour of goat cheese during lactation, through the application, for the first time, of an easy, fast and reliable analytical technique.

Materials and Methods

The research was carried out on six batches of cheese processed using milk from the same goats of the Maltese race at different stages of lactation. The animals of different ages (from 2 to 5 years) and order of kidding, were bred in extensive conditions on mixed pasture in the province of Agrigento (Italy). During the winter they received 400 g/head/d of concentrate (91% dry matter (DM)) and 205 g crude protein (CP)/kg DM, 23 g ether extract (EE)/kg DM, 56 g crude fibre (CF)/kg DM and 1 kg/head/d of meadow hay (DM = 94%; CP = 102 g/kg DM, EE = 10 g/kg DM, CF = 328 g/kg DM). The chemical composition of the feed was determined according to the AOAC (2000) official methods. Every month from the 2^{nd} to the 7^{th} month of lactation (from November to April), individual milk samples were taken from the same animals by hand milking and processed using the traditional method of cheese making (Rubino, 1996). The batches of cheese, weighing 500 g each, were ripened for one month. At the end of ripening each batch of cheese was divided into three samples. All the samples were put under vacuum and kept at –18 °C until analysis. For the analyses, each sample of cheese was defrosted at room temperature and freed of the rind before grinding. Samples of approximately 3 g each were placed in a 10 mL vial and then sealed.

For the SPME process an AOC-5000 (Shimadzu) autosampler was used, equipped with a fibre, provided by Supelco (Milan, Italy) coated with the following sorbent material: Divinylbenzene/Carboxen/Polydimethylsiloxane. The sample extraction was as follows: the vial containing the cheese was heated at 60 °C for 10 min and agitated (clockwise-anticlockwise alternate rotation) at 500 rpm. Subsequently, the SPME fibre was exposed to the cheese headspace for 50 min at 60 °C under the aforementioned agitation parameters. The fibre was then inserted in the GC injector and held for 5 min. The GC analyses were performed on a Shimadzu gas chromatograph GC-2010 (Shimadzu, Milan, Italy), equipped with a FID detector and a GC Solution software for data acquisition (Shimadzu). The capillary column was an RTX-1301 (Restek, PA, USA) 30 m x 0.25 mm x 0.25 μm and temperature programmed as follows: 40 °C (5 min) to 230 °C at 5 °C/min. The GC was equipped with a split/splitless injector (250 °C), operated in a splitless mode for 5 min, then with a split ratio of 20 : 1. The carrier gas was helium at a linear velocity of 35 cm/sec. The FID temperature was 280 °C, detector gases were H_2 at 50 mL/min and air at 400 mL/min; makeup at 50 mL/min (Air/N_2). The GC/MS analyses were carried out on a Shimadzu GCMS 2010 (Shimadzu, Milan, Italy) in order to identify cheese aroma compounds by comparing their MS spectra with those present in reference libraries of the mass spectrometer. The gas chromatographic conditions were the same as for GC-FID analyses, except for column pressure (44.5 kPa).

The suitability of the analytical method was determined only in one batch of cheese (3^{rd} month of lactation) by calculating the coefficient of variation (CV% = (mean value of three percentage areas/standard deviation of three percentage areas) x 100) for each chemical component relative to the three (one for each sample) repeated measurements. Data were processed using ANOVA (GLM proc. – SAS, 2001) considering the variable: month of lactation.

Results and Discussion

Tables 1, 2, 3, 4, 5 show the mean values of the 44 components expressed as percentages of the total identified fraction, the root standard error of the means (s.e.m.), the statistical significance of differences (P) in relation to lactation and the coefficient of variation (CV %) values referred to the 3^{rd} month of lactation. Among the flavour components the acids (ranging from C_4 to C_{12}) were the most abundant (37-79%), followed by ketones (4-32%), alcohols (2-10%), esters (1-2%), terpenes (0.8-1.7%) and aldehydes (0.06-0.6%). The higher levels of caprylic, capric and lauric acids at the 2^{nd}, 3^{rd}, and 4^{th} month of lactation (Table 1) could be correlated to the integration of concentrate and hay in the feeding regimen of the goats during the winter months (November, December and January), as reported by Urbach (1979).

As regards alcohols (Table 2), isoamyl alcohol and phenethyl alcohol showed the highest level in the 2nd month of lactation; ketones (Table 3) were more concentrated in the 5th and the 6th months of lactation; esters (Table 4) account for about 2.5% in the 7th month of lactation.

Table 1 Mean (% of the total identified fraction), s.e.m. and probability values of the acids in the flavour of goat cheese processed using milk from the 2nd to the 7th month of lactation

Compound (%)	2nd	3rd	4th	5th	6th	7th	P	s.e.m.	CV%*
Acetic acid	3.909	1.961	2.333	5.458	2.726	4.288	0.005	0.944	2.69
Isobutyric acid	0.098	0.1	0.104	0.402	0.074	0.078	< 0.0001	0.012	0.21
Butyric acid	5.118	6.561	8.666	5.347	8.6	9.164	< 0.0001	0.317	1.79
Isovaleric acid	0.511	0.577	0.548	0.670	0.619	0.288	< 0.0001	0.022	2.24
2-Methyl butyric acid	0.103	0.166	0.124	0.165	0.13	0.084	< 0.0001	0.008	3.20
Caproic acid	26.18	26.715	19.603	11.968	21.016	31.352	< 0.0001	4.351	0.63
Heptanoic acid	0.19	0.297	0.217	0.107	0.175	0.307	< 0.0001	0.074	2.69
Caprylic acid	18.254	23.566	28.615	6.335	11.207	17.847	< 0.0001	0.949	3.09
Benzoic acid	0.18	0.067	0.038	0.029	0.045	0.32	< 0.0001	0.019	0.85
4-Methyl octanoic acid	0.017	0.038	0.06	0.041	0.133	0.041	< 0.0001	0.015	2.55
Nonanoic acid	0.124	0.192	0.141	0.084	0.106	0.136	< 0.0001	0.019	2.01
Capric acid	14.542	17.859	16.991	6.195	7.573	11.224	< 0.0001	1.101	5.73
Lauric acid	0.776	1.118	1.199	0.401	0.217	0.444	0.0544	0.417	1.09

* values refer to the 3rd month of lactation

Table 2 Mean (% of the total identified fraction), s.e.m. and probability values of the alcohols in the flavour of goat cheese processed using milk from the 2nd to the 7th month of lactation

Compound (%)	2nd	3rd	4th	5th	6th	7th	P	s.e.m.	CV%*
Ethyl alcohol	0.419	4.942	1.591	3.986	3.526	5.427	< 0.0001	0.303	4.59
2-Pentanol	0.057	0.4	0.091	0.421	0.696	0.188	< 0.0001	0.020	3.11
Isoamyl alcohol	6.824	0.835	0.191	0.242	0.456	1.73	< 0.0001	0.195	3.20
2-Heptanol	0.182	1.545	0.123	0.363	1.14	0.302	< 0.0001	0.043	4.75
2-Decanol	0.045	0.44	0.171	0.266	0.739	0.129	< 0.0001	0.062	4.47
Phenethyl alcohol	2.806	0.304	0.105	0.186	0.38	0.768	< 0.0001	0.017	2.26

* values refer to the 3rd month of lactation

Table 3 Mean (% of the total identified fraction), s.e.m. and probability values of the ketones in the flavour of goat cheese processed using milk from the 2nd to the 7th month of lactation

Compound (%)	2nd	3rd	4th	5th	6th	7th	P	s.e.m.	CV%*
Acetone	1.025	1.294	1.114	0.54	1.338	1.167	< 0.0001	0.119	3.44
2-Pentanone	0.354	0.108	0.577	4.056	5.122	0.27	0 0002	1.101	4.18
2-Heptanone	1.143	1.104	1.705	7.877	10.104	1.381	< 0.0001	0.439	4.98
2-Octanone	0.013	0.023	0.017	0.201	0.156	0.007	< 0.0001	0.022	3.92
8-Nonen-2-one	0.024	0.075	0.15	0.348	0.466	0.085	< 0.0001	0.019	2.35
2-Nonanone	1.772	1.604	2.674	18.521	14.812	3.48	< 0.0001	1.045	3.62
2-Undecanone	0.024	0.013	0.015	0.392	0.006	0.065	< 0.0001	0.456	3.94

* values refer to the 3rd month of lactation

In addition, the presence of two terpenes (Table 5), β-pinene and limonene, must be highlighted because they are responsible for the green/grassy flavour of the milk fat, both being typical constituents of the volatile fraction in citrus essential oils. The presence of these terpenes can be noticed more during certain seasons of the year (Wilson, 1989) and is strictly correlated to the diet of the goats (e.g. administration of citrus by-product, Chiofalo *et al*., 2004). Flavour components (Tables 1, 2, 3, 4, 5) were influenced (P < 0.0001 and P < 0.001) by the lactation, except the lauric acid which showed no differences (P > 0.05) in relation to the month of lactation (Table 1).

Table 4 Mean (% of the total identified fraction), s.e.m. and probability values of the esters in the flavour of goat cheese processed using milk from the 2nd to the 7th month of lactation

Compound (%)	2nd	3rd	4th	5th	6th	7th	P	s.e.m.	CV%*
Ethyl butyrate	0.744	0.162	0.116	0.449	0.205	0.322	< 0.0001	0.609	2.75
Ethyl caproate	0.055	0.449	0.392	0.820	0.62	1.07	< 0.0001	0.036	3.95
Isoamyl butyrate	0.427	0.039	0.043	0.031	0.037	0.265	< 0.0001	0.019	4.41
Ethyl caprylate	0.063	0.506	0.298	0.285	0.417	0.476	< 0.0001	0.017	2.89
Ethyl caprate	0.071	0.497	0.256	0.154	0.142	0.333	< 0.0001	0.014	3.91
Ethyl laurate	0.007	0.023	0.011	0.019	0.013	0.015	< 0.0001	0.002	2.10

* values refer to the 3rd month of lactation

Table 5 Mean (% of the total identified fraction), s.e.m. and probability values of the miscellaneous compounds in the flavour of goat cheese processed using milk from the 2nd to the 7th month of lactation

Compound (%)	2nd	3rd	4th	5th	6th	7th	P	s.e.m.	CV%*
Hexanal	0.155	0.366	0.058	0.133	0.07	0.084	< 0.0001	0.018	1.89
Nonanal	0.037	0.117	0.057	0.397	0.076	0.108	< 0.0001	0.415	0.01
Decanal	0.009	0.022	0.006	0.082	0.006	0.021	< 0.0001	0.005	3.59
-Pinene	0.144	0.06	0.01	0.117	0.21	0.06	0 0005	0.054	4.44
Limonene	1.032	0.623	1.038	0.693	1.536	0.255	< 0.0001	0.079	0.69
Acetonitrile	0.266	0.756	0.201	0.573	0.343	0.195	< 0.0001	0.099	2.75
Hexane	0.155	0.366	0.058	0.133	0.07	0.084	< 0.0001	0.018	1.89
1,3-Dimethoxy Benzene	0.008	0.193	0.089	0.059	0.043	0.063	< 0.0001	0.028	4.31
Tetradecane	0.113	0.016	0.011	0.033	0.007	0.018	< 0.0001	0.010	3.18
-Decalactone	0.039	0.039	0.028	0.066	0.028	0.024	< 0.0001	0.005	3.27
Heptadecane	0.01	0.011	0.022	0.02	0.008	0.052	< 0.0001	0.457	2.41

* values refer to the 3rd month of lactation

All the data obtained in this research are in agreement with those provided in the literature (Larràyoz *et al*., 2001), as many components identified in the samples analyzed are considered typical of the flavour of goat cheese. Among these, caproic, caprylic and capric acids and their corresponding esters have already been reported in other works, where the extraction of the cheese aroma was carried out not only by SPME, but also by means of conventional techniques such as SDE (Simultaneous Distillation Extraction) solvent extraction, dynamic and static headspace (Sablè & Cottenceau, 1999).

The analytical method developed in the present study is based on the use of an automated SPME system that permitted to draw from the analyses CV% values slightly higher than 5.0 (capric acid = 5.73%), an astonishing result, considering that when operating SPME by hand it is often difficult to obtain CV values of <10%. In addition, the use of an SPME autosampler permits to avoid artefact formation (ghost peaks in the chromatogram) which are often due to the several steps needed for sample preparation. It could be combined with the sensorial (Rouel *et al*., 2002), GC/sniffing and GC/olfactometer (GC/O) analyses (Moio, 1998) to evaluate in detail the effects of changes within the sensory profile of a dairy product (Wilson & Ken, 1998).

Conclusions

For the first time solid phase microextraction hyphenated with gas chromatography/mass spectrometry (SPME-GC/MS) has been applied to the analysis of the aroma of goat cheese. In relation to the influence of lactation, the results show remarkable quantitative fluctuations obtained for each component of the cheese flavour. They suggest, for these products, the difficulty in evaluating separately the different variables (stage of lactation, season, availability of pasture, breeding system and so on) which influence the sensorial profile of the milk and the cheese manufactured from it, where, moreover, various biochemical events occur. Nevertheless, in Sicily it is very difficult to standardize the breeding conditions of the goat as well as its productions. This is due to the typical semi-extensive production system of these animals which allows the exploitation of marginal areas, greatly represented in this region.

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