Lait 81 (2001) 757-773

Original article

Relationships between flavour and chemical composition of Abondance cheese derived from different types of pastures

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(Received 12 February 2001; accepted 24 April 2001)

Abstract — The relationships between the flavour and composition of half-cooked pressed Abondance cheese (Haute-Savoie, France) were studied under natural cheese production conditions on the basis of different pastures exploited by 3 producers. Cheeses manufactured from milk produced from mountain pastures (M, n = 5, 1500–1850 m) were deemed to be more "fruity", "animal", "boiled milk" and "hazelnut" and less pungent and "propionic acid" than cheeses made from milk produced from valley pastures (V, n = 5, 850–1100 m). It was possible to partly attribute these differences in flavour to the presence of protein-based volatile compounds in the cheeses. The V cheeses had a greater variety of flavours than the M cheeses. Cheeses from gramineae-rich pastures had the most intense "cooked cabbage" odours, related to the greater amounts of sulphur compounds in these cheeses. Terpenes, which are more abundant in cheeses produced from dicotyledon-rich pastures, did not contribute directly to cheese aroma. The differences in flavour between the cheeses manufactured by the 3 producers were of the same magnitude as those observed between different pastures used by a same producer. The origin of the volatile compounds in the cheeses – whether of microbial origin or from the feed – was discussed.

cheese / flavour / volatile compound / proteolysis / pasture

Résumé — **Relations entre la flaveur et la composition chimique de fromages d'Abondance fabriqués à partir de laits produits sur différents types de pâturages.** Les relations entre la flaveur et la composition des fromages à pâte pressée demi-cuite de type Abondance (Haute-Savoie, France), ont été étudiées dans des conditions naturelles de production du fromage à partir de pâturages différents exploités par 3 producteurs. Les fromages fabriqués à partir de laits produits sur des pâturages de montagne (M, n = 5, 1500–1850 m) ont été jugés plus « fruité », « animal », « lait cuit » et « noisette »

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et moins piquant et « propionique » que les fromages issus de laits produits sur des pâturages de vallée (V, n = 5, 850–1100 m). Ces différences de flaveur ont pu être attribuées en partie à la présence dans les fromages de composés volatils issus du catabolisme des acides aminés. Les fromages V présentaient une plus grande diversité de flaveur que les fromages M. Les fromages issus de pâturages riches en graminées étaient les plus intenses en odeur « chou cuit », flaveur liée aux quantités plus importantes de composés soufrés dans ces fromages. Les terpènes, plus abondants dans les fromages issus de pâturages riches en dicotylédones, n'ont pas contribué directement à l'arôme des fromages. Les différences de flaveur des fromages entre les 3 producteurs ont été du même ordre de grandeur que celles observées chez un même producteur entre différents pâturages. Les origines microbienne et alimentaire des composés volatils des fromages ont été discutées.

fromage / flaveur / composé volatil / protéolyse / pâturage

1. INTRODUCTION

Although it is empirically accepted that the type of pasture influences the sensory properties of cheese [27] and its flavour in particular, few experimental studies on the topic have yet been undertaken. It is nevertheless particularly important to identify the relationship between forage type and cheese properties in the case of Protected Designation of Origin (PDO) cheeses, since it constitutes one of the bases for their relationship with the "*terroir*" [28].

Bosset et al. [9], Buchin et al. [13] and Verdier-Metz et al. [38], have demonstrated significant differences in cheese flavour depending on the altitude of the pastures and botanical composition of the forage consumed by the animals, whether in the form of pasture or preserved forage. Nevertheless, many gaps remain in the existing knowledge of the mechanisms involved in the influence of forage type on cheese flavour. The above-mentioned authors observed differences in volatile compound composition in cheeses – whether these compounds were of microbial origin or originated from the feed – that may be linked to differences in flavour. On the one hand, there may be a specific type of microflora in each production zone, as has been demonstrated [18, 21], which would lead to significant variations in the sensory quality of the cheese [7, 12, 20]. On the other hand, certain compounds present in forage, such as terpenes, may be found later in the dairy products [40] and thereby contribute to cheese aroma [8, 9, 22, 24, 39]. Buchin et al. [13] also considered the possibility that there could be mechanisms involving milk proteolytic enzymes such as plasmin.

These initial studies show that the type of pasture does influence cheese flavour, but the relationships established between the raw material (grass) and the final product (cheese) remain incomplete.

The aim of our study was to explore the relationships between the composition and flavour of cheeses produced from different types of pasture. This study is part of a project aimed at understanding the influence of pastures on the sensory properties of cheese, of which the first results have already been presented [14–16]. The study was undertaken under real conditions of Abondance cheese production (Haute-Savoie, France), in order to benefit from a wide variety of pasture types and be able to monitor the entire production chain from grass to cheese via milk.

2. MATERIALS AND METHODS

2.1. Experimental conditions

The experiment was undertaken during the spring and summer of 1998 in 3 farms (X, Y, Z) producing Abondance cheese,

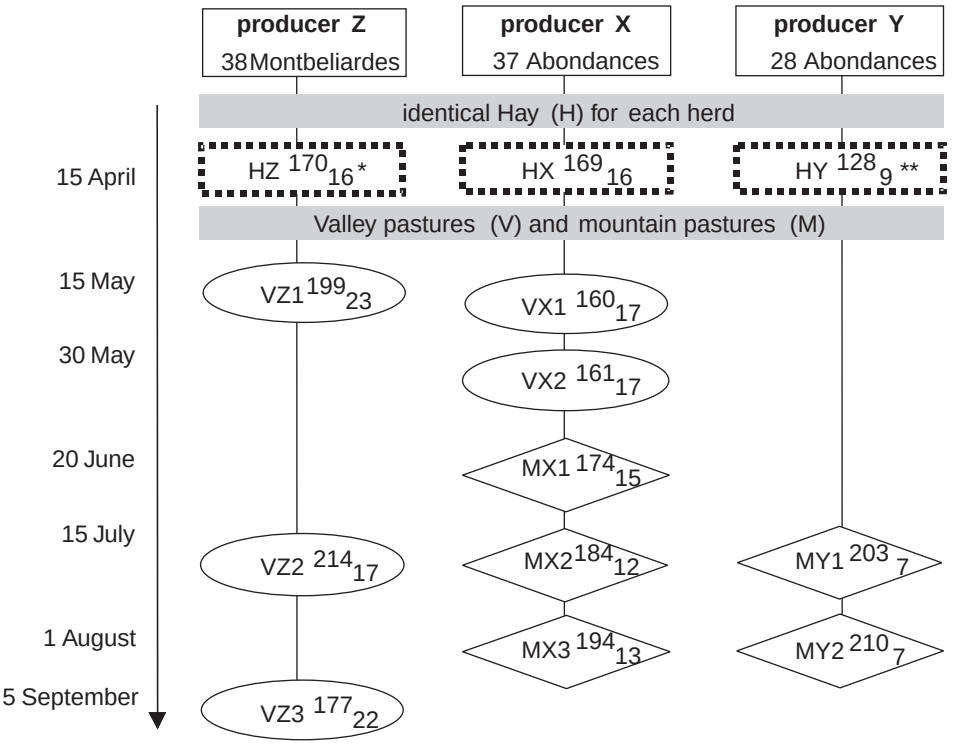

Figure 1. Experimental design.
* HZ^{a}_{b} where ^{a} was the average stage of lactation (d) and $_{b}$ the milk yield ($L \cdot d^{-1}$).
** The Y studied milk provided exclusively from the morning milking.

where each producer manufactured cheese using the milk of his own herd. Ten pastures, described by Bugaud et al. [14], belonging to these producers, were used as the basis of this study: 5 valley pastures (V) located at altitudes of between 850 and 1100 m and 5 mountain pastures (M) located at altitudes of between 1550 and 1850 m. For each pasture, and during the last 3 d of pasture, the cheesemaking process was monitored and a cheese was sampled each day for analysis.

As a preliminary stage, the 3 systems of production (herd + cheesemaking process), were compared by producing cheeses using milk derived from hay-based feeding (H), which was identical for each of the producers. The herds and milks have been described by Bugaud et al. [15]. The manufacture and ripening of Abondance cheese have been described by Bugaud et al. [16]. The experimental design is presented in Figure 1.

2.2. Analyses

All the analyses were carried out at the end of the cheese-ripening period (24 weeks).

2.2.1. Sensory analysis

Two panels of trained judges assessed the flavour of the ripened cheeses. Panel 1 comprised 14 judges trained to assess the intensity of 9 descriptors on a scale of 1 to 7,

using commercial Abondance cheese. The descriptors were the following: intensity of taste and odour, "fruity" and "hazelnut" aroma, "sweet", "salty", "pungent" and "bitter" taste, and "fruity" odour. The descriptor "fruity" designated aromatic richness with positive connotations (a term used by cheese production professionals) rather than fruit aroma as such.

Panel 2 comprised 20 judges trained to recognise the presence of 52 descriptors of odour and aroma for all types of cheese. These descriptors were marked on a presence/absence scale. Those descriptors whose detection frequencies (number of "presence" responses out of the total number of responses) were below 10% on average for all the pastures were not kept for statistical data processing.

2.2.2. Cheese composition

2.2.2.1. Volatile fatty acids

Volatile fatty acids were solvent-extracted under reflux in a Jalade extractor and then saponified as described by Ardö and Polychroniadou [1]. They were then analysed by gas chromatography after release by soap acidification. The chromatograph (GC 8000, Carlo Erba, Thermoquest, Les Ulis, France) was equipped with a split/splitless injector (240 °C, split flow of 120 mL·min^{-1}), a FID detector (245 °C) and a capillary column coated with a FFAP phase (Stabilwax DA, Restek, Evry, France, length 30 m × internal diameter 0.53 mm × phase thickness 1 µm), and preceded by an uncoated retention gap (Supelco, L'Isle d'Abeau, France, length 1 m × internal diameter 0.53 mm). The carrier gas was helium (column head pressure of 40 kPa, flow of 6 mL·min^{-1} at 120 °C), the temperature gradient was 120 °C for 1 min, 120 to 240 °C at 10 °C·min^{-1}, 240 °C for 5 min, 240 to 250 °C at 20 °C·min^{-1}.

2.2.2.2. Volatile compounds

The other volatile compounds were extracted using a dynamic head-space technique (Purge and Trap LSC 3000; Tekmar, Cincinnati, OH), separated by gas phase chromatography (chromatograph 6890, Hewlett Packard Agilent Technologies, Les Ulis, France) and identified by mass spectrometry (MSD 5973, Hewlett Packard Agilent Technologies). The volatile compounds were analysed in two steps: (i) all volatile compounds except sesquiterpenes, in all the cheeses, (ii) sesquiterpenes only, in one cheese per pasture. The corresponding sample treatments were the following: (i) Ten grams of cheese were cut into 7 mm cubes and placed in a cylindrical glass container (140 mm high and 30 mm in diameter) connected to the "Purge and Trap" system. Volatile compounds were trapped for 15 min at 25 °C with a helium flow of 40 mL·min^{-1}. (ii) The cheese was grated, diluted at 10% (p/v) in deodorised milliQ water (boiled for 15 min), and then ultra turrax-homogenised for 1 min. Ten millilitres of the solution were introduced into a U-shaped 25 mL glass vial (Sparger 5182-0849 Hewlett Packard Agilent Technologies) connected to the "Purge and Trap" system. Sesquiterpenes were trapped for 60 min at 55 °C with a helium flow of 40 mL·min^{-1}. In both analyses, the purge was operated with a MCS temperature of 225 °C, a desorb temperature of 225 °C and a desorb time of 2 min. The chromatographic conditions were the same for both analysis methods and are described by Buchin et al. [13]. Ions between 29 and 206 amu (atomic mass units) were collected by the mass detector. The relative amount extracted for each volatile compound was given by the surface area of the peak of its most specific ion (semi-quantitative analysis).

2.2.2.3. Gross chemical composition

The analysis methods of pH, fat in dry matter, moisture of skim cheese, sodium

chloride in the moisture, nitrogen soluble in tri-sodium citrate 0.5 mol·L^{-1} at pH 4.4 normalised to total nitrogen (pH 4.4-SN/TN), nitrogen soluble in phosphotungstic acid normalised to total nitrogen (PTA-SN/TN), and casein ratios α_{S1}-I/α_{S1+S2}, $\alpha S_{deg}/\alpha_{S1+S2}$, γ/β, have been described by Bugaud et al. [16].

2.2.3. Statistical analysis of the results

The differences in flavour and the differences in volatile fatty acid and volatile compound composition between the cheeses were studied by analysis of variance (Version 6.12, SAS Institute INC., 1996) in two stages: (i) comparison between H, V and M cheeses, (ii) comparison between H cheeses, between V cheeses and between M cheeses. The analysis of variance carried out on the H cheeses allowed us to determine the combined effect of cheese technology and herd. In the analysis of the sensory descriptors used by Panel 1, the forage type, the judges and the forage type x judge interactions were considered as factors.

To describe synthetically the volatile compound composition of the cheeses, a principal components analysis (PCA) was performed on the volatile compounds (except volatile fatty acids) that exhibited significant differences at the 5% threshold in at least one of the analysis of variance models.

The relationships between cheese flavour and composition were assessed using Partial Least-Squares (PLS) regression [36] carried out using Splus 3.4® software. The sensory variables were explained using the following variables: volatile fatty acids, volatile compounds and gross chemical composition of the cheeses. Bitter and salty tastes were analysed by linear correlation between: (i) salty taste and sodium chloride in moisture, (ii) bitter taste and gross chemical composition, because both descriptors were supposed not to be influenced by the volatile compound composition.

Due to technological problems, four cheeses were withdrawn from the result processing procedure. One cheese for period VX1 and for period MX1 (the fermenting agent had very little acidification effect resulting in the cheeses being acidified only very little) and two cheeses for MY2 (cooling occurred too rapidly during cheese pressing leading to insufficiently drained cheeses) were not taken into consideration in the statistical analyses.

3. RESULTS

3.1. Cheese flavour

The differences in flavour between the cheeses are presented in Tables I and II. 8 descriptors of Panel 1 and 10 of Panel 2 made it possible to significantly discriminate the cheese groups ($P < 0.05$).

Of the H, V and M cheeses, M cheeses were the least pungent, the most "animal" ($P < 0.001$), "boiled milk", "hazelnut" ($P < 0.01$) and "fresh cream" ($P < 0.05$). The V cheeses had the least "fruity" and the most "propionic acid" aromas ($P < 0.05$). The H cheeses had the least "bread crust" aroma ($P < 0.05$).

Numerous descriptors made it possible to distinguish between the V cheeses. The VZ1 cheeses had the most intense odour and aroma ($P < 0.001$), they were the most "fruity", "cooked cabbage" ($P < 0.001$) and "rancid" ($P < 0.01$). The VZ2 cheeses were the most pungent and salty ($P < 0.001$) and were similar to the VZ1 cheeses for "cooked cabbage" odour and "rancid" aroma. The VZ3 cheeses were the most bitter and "fresh cream" ($P < 0.001$).

The variations in sensory properties between M cheeses were just as great as between V cheeses. Nevertheless, due to greater variability between the replicates, there were significantly fewer differences between the M cheeses than between the V cheeses. The MY1 cheeses were distinctly

Table I. Sensory characteristics of cheeses assessed by Panel 1 (score average / 7).

			intensity		odour	aroma		taste		
	cheeses	n	odour	aroma	fruity	fruity	hazelnut	salty	pungent	bitter
	HZ	3	3.2 (1.4)	4.7^{a} (1.3)	2.6^{b} (1.5)	3.3 (1.7)	1.4 (1.0)	4.3^{a} (1.6)	3.5^{a} (1.4)	2.2^{a} (1.3)
Hay	HX	3	3.8 (1.3)	4.4^{ab} (1.0)	3.5^{a} (1.2)	3.7 (1.4)	1.4 (0.7)	4.3^{a} (1.3)	3.1^{a} (1.4)	1.6^{b} (0.8)
	HY	3	3.3 (1.2)	4.0^{b} (1.1)	2.6^{b} (1.5)	3.2 (1.4)	1.3 (0.7)	3.5^{b} (1.4)	2.1^{b} (1.4)	1.8^{b} (1.1)
analysis of variance			ns	**	***	ns	ns	**	***	**
	VZ1	3	4.5^{a} (1.2)	4.7^{a} (1.3)	3.5^{a} (1.3)	3.2^{a} (1.7)	1.3^{b} (0.6)	3.8^{bc} (1.8)	2.8^{c} (1.7)	2.2^{ab} (1.6)
Valley pastures	VZ2	3	3.1^{b} (1.2)	4.9^{a} (1.4)	2.8^{b} (1.6)	2.6^{bc} (1.4)	1.5^{ab} (1.0)	4.4^{a} (1.5)	4.1^{a} (1.4)	2.0^{bc} (1.4)
	VZ3	3	3.1^{b} (1.1)	3.7^{c} (1.2)	2.6^{b} (1.2)	2.4^{c} (1.2)	1.6^{a} (1.0)	2.9^{d} (1.6)	1.9^{d} (1.3)	2.6^{a} (1.9)
	VX1	2	4.0^{a} (1.0)	4.5^{a} (1.0)	3.4^{a} (1.3)	3.2^{a} (1.5)	1.4^{ab} (0.8)	4.1^{ab} (1.8)	3.3^{b} (1.6)	1.6^{c} (0.8)
	VX2	3	3.3^{b} (1.2)	4.1^{b} (1.1)	2.5^{b} (1.2)	2.9^{ab} (1.4)	1.3^{ab} (0.6)	3.4^{c} (1.7)	2.7^{c} (1.5)	1.7^{bc} (1.2)
analysis of variance			***	***	***	***	*	***	***	***
	MX1	2	3.4^{b} (1.1)	4.1 (1.2)	3.0^{b} (1.5)	3.0^{a} (1.3)	1.5 (0.9)	3.4^{ab} (1.3)	2.7^{a} (1.7)	1.7^{ab} (1.0)
Mountain pastures	MX2	3	3.7^{ab} (1.2)	4.4 (1.0)	3.3^{ab} (1.3)	3.5^{a} (1.3)	1.6 (1.0)	3.4^{ab} (1.6)	2.3^{abc} (1.6)	1.4^{b} (1.0)
	MX3	3	3.6^{b} (1.3)	4.6 (1.2)	2.8^{b} (1.6)	3.0^{a} (1.6)	1.5 (1.1)	4.0^{a} (1.4)	2.6^{ab} (1.4)	2.3^{a} (1.7)
	MY1	3	4.5^{a} (1.0)	4.5 (1.0)	4.0^{a} (1.1)	3.5^{a} (1.4)	1.9 (1.1)	3.3^{b} (1.3)	1.8^{c} (0.9)	1.6^{ab} (1.0)
	MY2	1	3.0^{b} (0.8)	3.9 (1.0)	2.4^{b} (1.2)	2.3^{b} (1.2)	1.3 (0.7)	2.3^{c} (0.8)	1.9^{bc} (0.9)	1.9^{ab} (1.4)
analysis of variance			**	ns	**	*	ns	**	**	*
analysis of variance between H, V, M			ns	ns	ns	* V < H + M	** H < M	ns	*** M < H + V	ns

$^{a, b, c, d}$ Mean value comparison by Newman-Keuls' test. Analysis of variance: ns non significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
n: number of replicates; ( ): deviation standard calculated with cheeses and judges replicates.

Table II. Detection frequencies of sensory descriptors of cheeses assessed by Panel 2.

			odour				aroma					
	cheeses	n	cooked cabbage	boiled milk	fresh milk	fresh cream	fresh cream	bread crust	animal	propioni acid	butyric acid	rancid
	HZ	3	5 (5)	19 (11)	21 (9)	26^{b} (10)	35 (17)	3^{ab} (3)	3 (3)	19 (8)	15 (5)	21 (5)
Hay	HX	3	3 (3)	14 (10)	26 (16)	31^{b} (6)	40 (12)	0^{b} (0)	6 (5)	17 (19)	17 (6)	31 (8)
	HY	3	5 (9)	21 (5)	31 (9)	52^{a} (2)	48 (16)	7^{a} (3)	4 (4)	19 (8)	10 (3)	17 (3)
analysis of variance			ns	ns	ns	**	ns	*	ns	ns	ns	ns
	VZ1	3	19^{a} (6)	9 (3)	7^{b} (3)	33^{b} (8)	32^{c} (5)	16 (16)	17 (8)	26 (5)	22 (8)	25^{a} (3)
Valley	VZ2	3	17^{a} (3)	16 (0)	33^{a} (7)	50^{ab} (3)	45^{b} (3)	9 (6)	9 (6)	38 (17)	21 (8)	26^{a} (8)
pastures	VZ3	3	4^{b} (3)	18 (6)	24^{ab} (10)	59^{a} (10)	59^{a} (6)	8 (3)	5 (2)	25 (3)	12 (3)	6^{b} (6)
	VX1	2	5^{b} (0)	13 (11)	16^{ab} (0)	39^{b} (4)	50^{ab} (4)	13 (11)	5 (4)	29 (4)	9 (6)	18^{ab} (4)
	VX2	3	2^{b} (3)	18 (3)	23^{ab} (8)	47^{ab} (5)	42^{b} (5)	5 (5)	2 (3)	19 (8)	12 (2)	18^{ab} (3)
analysis of variance			***	ns	*	*	***	ns	ns	ns	ns	**
	MX1	2	15 (0)	28 (4)	23^{b} (4)	50 (7)	48 (11)	5 (7)	10 (4)	20 (0)	16^{ab} (5)	23 (4)
Mountain	MX2	3	7 (3)	27 (10)	25^{b} (5)	45 (10)	52 (10)	13 (3)	18 (10)	13 (8)	22^{a} (6)	18 (3)
pastures	MX3	3	4 (3)	20 (3)	24^{b} (6)	47 (10)	47 (6)	8 (3)	18 (3)	27 (3)	12^{ab} (3)	20 (9)
	MY1	3	4 (6)	24 (7)	40^{a} (5)	55 (17)	55 (14)	21 (11)	14 (7)	14 (11)	8^{b} (2)	17 (8)
	MY2	1	11 (-)	16 (-)	32^{ab} (-)	68 (-)	58 (-)	16 (-)	32 (-)	21 (-)	16^{ab} (-)	11 (-)
analysis of variance			ns	ns	*	ns	ns	ns	ns	ns	*	ns
analysis of variance between H, V, M			ns	** V < M	ns	* H < M	ns	* H<V+M	*** H+V<M	* H+M<V	ns	ns

[a, b, c, d] Mean value comparison by Newman-Keuls' test. Analysis of variance: ns non significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
n: number of replicates; ( ): deviation standard.

different to the others in terms of their more intense and fruitier odour, their less pungent taste ($P < 0.01$), their more "fresh milk" odour and their less "butyric acid" aroma ($P < 0.05$). The MX3 cheeses were the most bitter and the most salty ($P < 0.01$). The MX2 cheeses resembled the MY1 cheeses in terms of the intensity of their odour and "fruitiness" and they were the most "butyric acid" ($P < 0.05$).

On the basis of identical feeding (hay period), the HY cheeses were the least salty ($P < 0.001$) and pungent ($P < 0.01$) with a more intense "fresh cream" odour ($P < 0.01$) and "bread crust" aroma ($P < 0.05$). The HZ cheeses were the most bitter and had the most intense aroma ($P < 0.01$). The HX cheeses had the fruitiest odour ($P < 0.001$).

3.2. Cheese composition

3.2.1. Volatile fatty acids

The volatile fatty acid concentrations of the Abondance cheeses are given in Table III. Propionic acid content (C3) was found to vary greatly between the cheeses:

Table III. Concentrations of volatile fatty acids in cheeses (mg·100 g^{-1} cheese).

	cheeses	n	C2	C3	C4	iC4	iC5	C6
	HZ	3	130^{a} (18)	175^{a} (43)	9.5 (1.4)	1.4 (0.3)	3.7 (0.4)	2.9 (0.7)
Hay	HX	3	79^{c} (5)	27^{b} (12)	10.5 (1.0)	1.0 (0.7)	2.9 (1.3)	3.1 (0.6)
	HY	3	103^{b} (4)	112^{a} (54)	9.4 (1.0)	1.1 (0.4)	2.4 (0.8)	2.5 (0.2)
analysis of variance			**	*	ns	ns	ns	ns
	VZ1	3	88 (19)	39^{b} (11)	10.1^{b} (1.0)	3.4^{a} (1.1)	7.6^{a} (2.1)	3.8 (0.3)
Valley pastures	VZ2	3	55 (20)	33^{b} (25)	9.3^{bc} (0.4)	1.9^{b} (1.0)	5.2^{ab} (3.3)	3.6 (0.2)
	VZ3	3	94 (28)	138^{a} (63)	8.1^{c} (0.7)	1.3^{b} (0.1)	3.1^{ab} (0.2)	3.6 (0.8)
	VX1	2	74 (3)	15^{b} (9)	12.2^{a} (0.3)	1.6^{b} (0.5)	3.5^{ab} (0.7)	4.0 (0.1)
	VX2	3	71 (11)	8^{b} (3)	10.4^{b} (0.8)	1.0^{b} (0.2)	2.4^{b} (0.3)	3.4 (0.2)
analysis of variance			ns	**	**	*	*	ns
	MX1	2	102^{a} (26)	5^{ab} (2)	7.7^{b} (1.0)	2.0^{b} (0.8)	3.6^{b} (1.0)	3.3 (1.7)
Mountain	MX2	3	96^{a} (10)	8^{a} (1)	9.9^{b} (1.1)	4.3^{a} (0.8)	8.6^{a} (1.5)	3.0 (0.5)
pastures	MX3	3	99^{a} (6)	4^{b} (1)	8.9^{b} (0.5)	1.7^{b} (1.1)	3.7^{b} (1.7)	2.5 (0.1)
	MY1	3	64^{b} (9)	2^{b} (2)	9.2^{b} (0.1)	0.6^{b} (0.3)	1.5^{b} (0.3)	3.1 (0.3)
	MY2	1	87^{ab} (-)	6^{ab} (-)	14.1^{a} (-)	0.4^{b} (-)	1.2^{b} (-)	2.1 (-)
analysis of variance			*	*	**	**	**	ns
analysis of variance between H, V, M			* V<H	*** M<V<H	ns	ns	ns	** M+H<V

[a, b, c, d] Mean value comparison by Newman-Keuls' test. Analysis of variance: ns non significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, n: number of replicates, C2: acetic acid, C3: propionic acid, C4: butyric acid, iC4: isobutyric acid, iC5: isovaleric acid, C6: caproic acid.

from 2 to 175 mg·100 g^{-1} of cheese. Acetic acid (C2), butyric acid (C4), caproic acid (C6) and branched chain fatty acids, isobutyric acid (iC4) and isovaleric acid (iC5) respectively varied between 55–130, 8.1–14.1, 2.1–4.0, 0.4–4.3, and 1.2–8.6 mg·100 g^{-1} of cheese.

Of the H, V and M cheeses, H cheeses had the highest C2 ($P < 0.05$) and C3 concentrations ($P < 0.001$). The V cheeses had the highest C6 concentration ($P < 0.01$).

Of the V cheeses, VZ3 cheeses differed from the others by their higher C3 concentrations and lower C4 concentrations ($P < 0.01$). The VZ1 cheeses had the highest iC4 and iC5 concentrations ($P < 0.05$).

Of the M cheeses, MX2 cheeses had the highest iC4 and iC5 concentrations ($P < 0.01$). The MY2 cheese had the highest C4 concentration ($P < 0.01$).

On the basis of identical feeding, HX cheeses had the lowest C2 ($P < 0.01$) and C3 concentrations ($P < 0.05$).

3.2.2. Other volatile compounds

In all, 217 volatile compounds belonging to 13 chemical families were detected in the cheeses (Tab. IV).

Although they were analysed using an enhanced extraction method, sesquiterpenes were detected only in M and VX cheeses (Tab. V). They were the most abundant in MX3 and MY2 cheeses.

All the volatile compounds but the fatty acids and sesquiterpenes are represented in a PCA (Fig. 2). The first two axes represent 54% of total variability.

The first axis PC1 (36%) made a distinction between monoterpenes, furans and unsaturated hydrocarbons on the one hand and esters, alcohols and above all sulphur-containing compounds on the other. The second axis PC2 (16%) made a distinction between ketones, aldehydes, saturated hydrocarbons and the precursors or derivatives of 2 and 3-methyl butanol (aldehydes, alcohols, esters) on the one hand, and the precursors or derivatives of hexanol and butanol on the other.

Table IV. Repartition on the basis of chemical families of volatile compounds detected in the whole of the cheeses.

chemical family	number
monoterpenes	29
sesquiterpenes[1]	32
saturated hydrocarbons	21
unsaturated hydrocarbons	17
benzene derivatives	16
furans	8
ketones	16
aldehydes	14
esters	29
alcohols	15
ether	1
sulphur-containing compounds	11
chloride derivatives	8
Total	**217**

[1] Sesquiterpenes were extracted from cheeses by a different method with respect to other volatile compounds (cf. Sect. 2.2.2.).

Table V. Repartition of sesquiterpenes in cheeses.

cheeses	number
HX, HY, HZ, VZ1, VZ2, VZ3	0
VX1	1
VX2	2
MX1	3
MX2	6
MX3	31
MY1	11
MY2	32

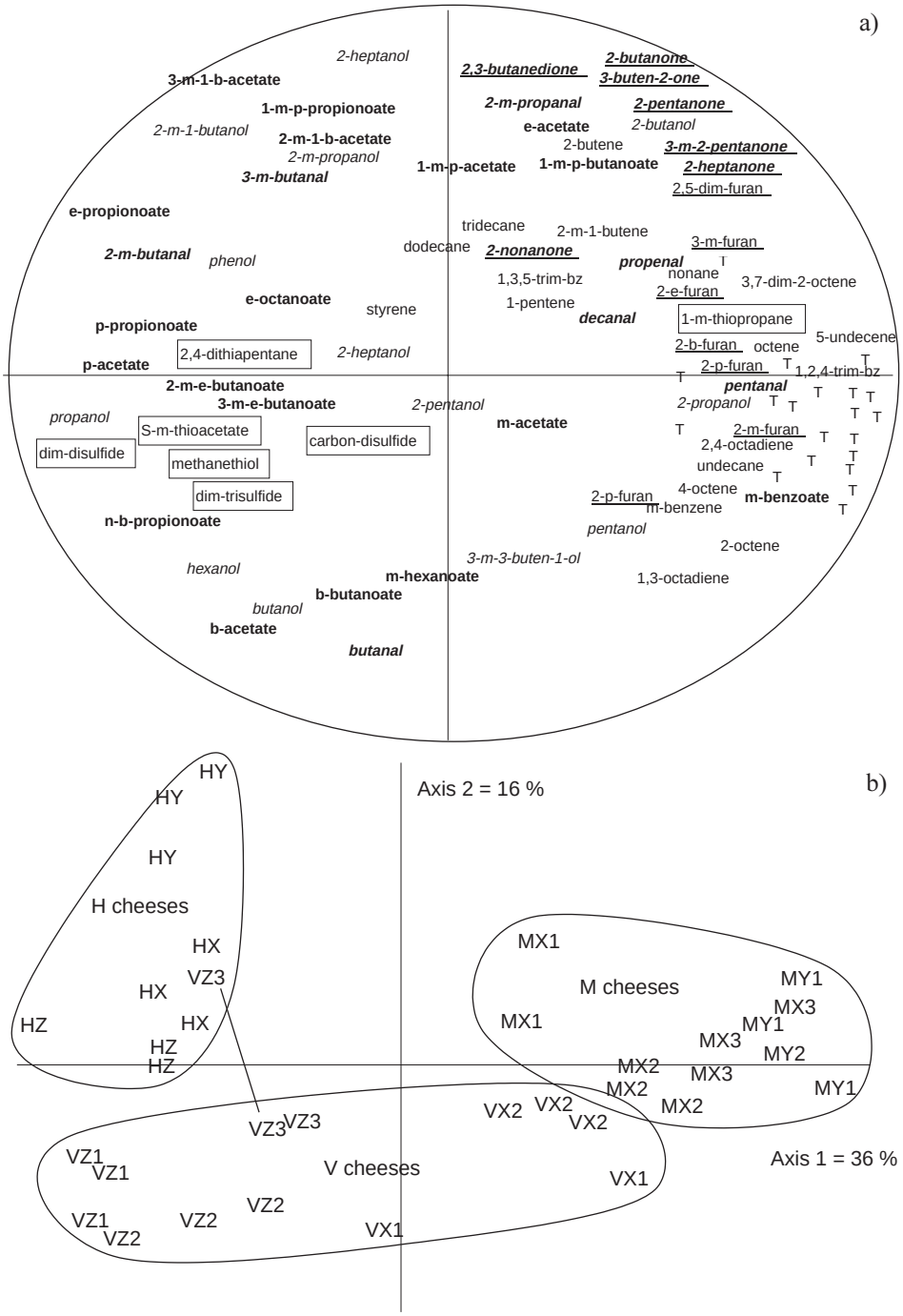

Figure 2. Principal component analysis of the volatile compounds of the cheeses: plot of principal axes 1 and 2.
a) Correlation circle.
T: monoterpenes, m: methyl, e: ethyl, p: propyl, b: butyl, bz: benzene
In bold: esters, ***italic bold***: aldehydes, ***italic bold underline***: ketones, *italic*: alcohols, boxed: sulphur-containing compounds, underline: furans, normal: hydrocarbons, benzene derivatives and terpenes.
b) Representation of cheeses.

Cheeses H, V and M were separated on axes PC1 and PC2. The M cheeses were the most rich in monoterpenes, furans and unsaturated hydrocarbons and the least rich in esters, sulphur-containing compounds and alcohols. The V cheeses were the least rich in ketones, aldehydes and saturated hydrocarbons. The H cheeses were the most rich in 2- and 3-methyl butanol precursor or derivative compounds.

Of the V cheeses, VX cheeses were the most rich in monoterpenes and VZ cheeses were the most rich in sulphur-containing compounds.

Of the M cheeses, MX1 and MX2 cheeses were less rich in monoterpenes than MX3, MY1 and MY2 cheeses. On the PC3 axis which is not shown (7% of total variability), MX cheeses contrasted with MY cheeses in terms of their greater amounts of ethyl esters, 2- and 3-methylbutanol and secondary alcohols such as 2-pentanol and 2-heptanol, and their smaller amounts of decanal.

On the basis of identical feeding, HY cheeses were distinct from HX and HZ cheeses on the PC2 axis. The HY cheeses were richer in ketones, aldehydes and precursors or derivatives of 2- and 3-methyl butanol.

3.3. Relationships between cheese flavour and cheese composition

The relationships between the sensory descriptors and the chemical composition variables of the ripened cheeses were studied using PLS regression. The first 25 variables, predicting each of the descriptors, were taken into account for the interpretation. Only the interpretable descriptor/ variable relationships are given (Tab.VI). With the exception of "hazelnut" aroma and "fresh milk" odour, the predictions of the descriptors explained over 53% of variance.

The odour, aroma and "fruity" intensities were positively associated with nitrogen fractions PTA-SN/TN and pH 4.4-SN/TN, branched chain volatile fatty acids (iC4 and iC5) and acetic acid esters. These descriptors were associated negatively with 2-methyl-1-butanol and its derivatives and with propionic acid esters. Moreover, the intense and fruity odours were positively associated with decanal and negatively associated with fat in dry matter and C3.

The "butyric acid" aroma was positively associated with branched chain volatile fatty acids (iC4 and iC5), nitrogen fractions PTA-SN/TN and pH 4.4-SN/TN and sulphur compounds, and negatively associated with ketones of 4 and 5 carbons.

The descriptor "fresh milk", with low prediction accuracy (explaining 35% of variance), was positively associated with ketones and negatively associated with esters, sulphur compounds and branched chain volatile fatty acids. "Fresh cream" aroma was positively associated with propionic acid esters, whereas "fresh cream" odour was associated with saturated and unsaturated hydrocarbons and with pentanal derivatives. These two descriptors were negatively associated with nitrogen fraction PTA-SN/TN and with salt content in the moisture.

The "cooked cabbage" odour and the "animal" aroma were positively associated with sulphur compounds and negatively associated with esters. The secondary alcohols also predicted a "cooked cabbage" odour whereas branched chain fatty acids and furans predicted the "animal" aroma.

The "hazelnut" aroma, with low prediction accuracy (explaining 21% of variance), was positively associated with monoterpenes and unsaturated hydrocarbons of 8 carbons and negatively associated with secondary alcohols and C4.

It was not possible to interpret the other descriptors (pungent taste, "bread crust", "propionic acid" and "rancid" aroma, and "boiled milk" odour) on the basis of the cheese chemical composition variables.

Table VI. Prediction of sensory descriptors on basis of cheese composition by PLS-regression.

descriptor	positive correlations	negative correlations	explained variance
odour intensity	PTA-SN/TN, esters (4), C2, aldehydes (2), iC4	F/DM, M/SC, C3	99%
aroma intensity	PTA-SN/TN, pH 4.4-SN/TN, iC5, iC4, esters (3)	esters (6), ketones (2), alcohols (2)	53%
fruity odour	PTA-SN/TN, iC4, esters (4)	F/DM, esters (3), alcohols (3), C3	81%
fruity aroma	PTA-SN/TN, iC4, iC5, pH 4.4-SN/TN, esters (4)	S compounds (1), esters (3), F/DM	71%
butyric acid aroma	iC5, iC4, S compounds (4), PTA-SN/TN, esters (2), pH 4.4-SN/TN, NaCl/M	esters (2), ketones (4), alcohols (3)	63%
fresh milk odour	furan (1), ketones (6)	S compounds (3), esters (6), alcohols (3), iC4, iC5	35%
fresh cream aroma	esters (6), ketones (1)	S compounds (1), NaCl/M, PTA-SN/TN	80%
fresh cream odour	unsaturated hydrocarbons (4)	NaCl/M, esters (2), PTA-SN/TN, C6	78%
cooked cabbage odour	S compounds (4), alcohols (4)	esters (2), M/SC, C3, pH	75%
animal aroma	S compounds (4), iC4, iC5, furans (3), esters (2)	F/DM, esters (3)	76%
hazelnut aroma	unsaturated hydrocarbons (3), terpenes (8)	alcohols (2), esters (2), C4	21%

Between ( ): number of compounds of the chemical family, S compounds: sulphur-containing compounds, C2: acetic acid, C3: propionic acid, C4: butyric acid, C6: caproic acid, iC4: iso-butyric acid, iC5: iso-valeric acid, PTA-SN/TN: nitrogen soluble in phosphotungstic acid in total nitrogen, pH 4.4-SN/TN: nitrogen soluble in a solution of tri-sodium citrate at pH 4.4 in total nitrogen, F/DM: fat in dry matter, M/SC: moisture of skim cheese, NaCl/M: sodium chloride content in the moisture.

The salty taste could not be correlated with the salt content in the moisture, nor the bitter taste with the primary and secondary proteolysis variables.

4. DISCUSSION

In order to understand the relationships that may exist between cheese flavour and types of pasture, it is necessary to identify the possible origin of the compounds responsible for cheese flavour and particularly the origin of volatile fatty acid and other volatile compounds.

4.1. Volatile compounds in cheese

Volatile compounds in cheese can originate from two different sources. Most of them result from the metabolism of cheese

microorganisms, endogenous microflora of raw milk or starters. In some cases they come directly from the feed.

Our results confirm that terpenes (mono- and sesquiterpenes) detected in cheese were of plant origin [8, 9, 22, 24, 39]. Cheeses M and VX, which were the most rich in terpenes, were made from the most terpene-rich milks [15]. According to Bugaud et al. [15] and Viallon et al. [40], the terpene composition of milk depends on that of the forage consumed, which in turn depends on the botanical composition of the forage [14, 33]. Most notably, M and VX pastures where dicotyledons – in particular umbelliferae, composites and plantaginaceae – were predominant, were the most terpene-rich, both in terms of diversity and quantity [14]. By contrast, VZ pastures, which were rich in gramineae, were poor in terpenes and therefore resulted in terpene-poor cheeses.

On the contrary, the unsaturated hydrocarbons, which were found more abundantly in M cheeses because they were distributed uniformly in milk, were unlikely to have originated directly from plants. According to Frankel [25], some of these compounds, such as 2- and 4-octene, may result from the oxidation of unsaturated fatty acids which were found in greater proportions in mountain milks [15].

According to Dumont and Adda [23], the furans present in dairy products may be the result of a Maillard reaction between an amino acid and a sugar. There is however another possible pathway for the production of furans, which has never been suggested in dairy products, i.e. the oxidation of polyunsaturated fatty acids [17]. In our study, the total amount of furans in cheese was correlated with the proportion of polyunsaturated fatty acids in the milk ($R^2 = 0.62$, $P < 0.001$). The higher polyunsaturated fatty acid contents in mountain milk [15] may thereby explain the greater amounts of furans in the corresponding cheeses.

The other volatile compounds identified in Abondance cheeses were mainly the products of microbial metabolism.

The sulphur-containing compounds present in cheese mainly originate from the degradation of sulphur-containing amino acids, notably methionin, by the bacteria present in the cheese [11]. The richness in sulphur-containing compounds of VZ cheeses can therefore have been linked to the composition of this microbial flora, but also to the physico-chemical conditions of cheese. Thereby, the higher pH value of these cheeses may have promoted the production of sulphur-containing compounds [11]. Conversely, the greater amounts of terpenes in VX and M cheeses may have inhibited the production of sulphur-containing compounds, which have been found less abundantly in these cheeses. Indeed, the hypothesis that terpenes have an inhibitory action on the production of sulphur-containing compounds, and possibly of other compounds, has already been suggested [35], but to date no study has demonstrated it.

Propionic acid fermentation has been shown to occur to a lesser extent in Abondance cheeses than in Swiss type cheeses (Comté, [3], Emmental [5]), in particular due to ripening in a colder cellar (10–11 °C). The high degree of propionic acid fermentation of VZ3 cheeses was certainly related to the higher pH value of these cheeses at the end of pressing [26], and a possible relationship with green maize-based feed is difficult to explain. It is also possible that there was an inhibitory effect of long chain unsaturated fatty acids, found in greater proportions in M milks, on propionic acid bacteria [10].

Isobutyric acid and isovaleric acid result from the degradation of amino acids, valine and leucine respectively [23, 37]. In MX2, VZ1 and VZ2 cheeses, the formation of branched chain volatile fatty acids appeared to be related to the amount of free amino acid precursors, the presence of

which was indicated by the PTA-SN/TN fraction. The catabolism of amino acids also leads to the formation of aldehydes, alcohols and branched chain esters, such as the derivatives and precursors of 2,3-methyl butanol and 2-methyl propanol [30]. The fact that these compounds were present in greater quantities in H cheeses may be due to a higher enzyme potential and/or a physico-chemical environment that was more favourable to their formation and preservation in cheese.

Long chain methyl-ketones and secondary alcohols (of over 4 carbons) result from fatty acid catabolism whereas the shorter ones result from fermentation. In particular, 2,3-butanedione, 2-butanone and 2-butanol result from citrate fermentation [23]. The use of a particular acidifying agent (calf rennet macerated in boiled acidified whey) by producer Y may be the reason explaining the higher concentrations of these compounds observed in Y cheeses.

4.2. Cheese flavour

In spite of the fact that some compounds that are potentially tasteful or aromatic (amino acids, peptides, pyrazines, furanones, amines, lactones) were not taken into consideration in this study, it was possible to identify relationships between cheese composition and cheese flavour.

The descriptors intensity and aromatic richness (fruity), both in terms of aroma and odour, had very clear links to the secondary proteolysis indicators (PTA-SN/TN). These results, which confirm those found on cooked pressed cheeses [4, 6] or on Cheddar cheese [2], show that free amino acids and small peptides play an important role in the flavour of Abondance cheese by contributing either directly to cheese taste or indirectly as precursors of flavour compounds. Thereby, isobutyric acid and isovaleric acid, originating from amino acids, and which have been associated with the "butyric acid" aroma in cheese, also contribute to the intensity and aromatic richness of odour and aroma. This is consistent with the results of Berdagué et al. [4] who showed that the fruitiness of Comté cheeses was correlated with isovaleric acid. This explains how the higher concentrations of small peptides and free amino acids in cheeses MX2, MY1 and VZ1 led to the fruitiest cheeses.

The intense "cooked cabbage" odour of cheeses VZ1 and VZ2 was mainly related to sulphur compounds that was found more abundantly in these cheeses. This is consistent with the studies by Cuer et al. [19] and by Kubickova and Grosch [31] who demonstrated the important role played by sulphur compounds in the "cooked cauliflower", "cabbage" and "garlic" aromas in cheese. Moreover, certain sulphur-containing compounds, associated with isobutyric acid, isovaleric acid and furans, may contribute to the "animal" aroma of cheeses. Given the distribution of these different molecules between cheeses, it would appear that the "animal" note of VZ1 cheeses was related to the presence of sulphur-containing compounds and volatile fatty acids, whereas the "animal" note of M cheeses was related rather to the presence of furans. The latter compounds, which are not very aromatic, may be labelling compounds of other molecules that are aromatic and characteristic of the "animal" note. Bosset et al. [9] observed that mountain Gruyère cheeses had a more intense "animal" odour than cheeses from plains, although they were unable to relate this odour to volatile compounds in cheese.

Milky aromas have been preferentially perceived in the absence of compounds with strong aromatic notes, such as sulphur-containing compounds or branched chain fatty acids. Therefore, the more intense "fresh milk" and "fresh cream" odours of Y cheeses may be related to molecules that are not very odorous, such as 2-butanone and 2-butanol [37] or characteristic of milky aroma such as 2,3-butanedione [31].

The only aroma that seems to be related to terpenes was "hazelnut" aroma, which was more intense in the M cheeses. However this note was only weakly perceived in the cheeses (maximal note below 1.9 on a 7 point scale) and its prediction rates by terpenes was low (only 21% of variance explained). So, although Guichard [29] in cooked pressed cheeses, and Moio et al. [34] in Gorgonzola cheese, identified a fruit note from limonene by olfactometry, the direct role played by terpenes in the the overall appreciation of cheese flavour appears to be limited.

The retention of volatile compounds by fat could explain why odour intensity and "fruity" odour were negatively correlated to the fat in dry matter content of the cheeses.

Our study did not enable us to relate chemical composition to salty and bitter tastes in cheese and would suggest that the latter were linked rather to the peptide and amino acid composition [6, 32] which were not analysed. The more intense bitterness of cheeses VZ3 and MX3 may also be explained by a delay in acidification observed during the manufacture [16]. These delays in acidification, probably related to the presence of natural starter inhibitors in the milk, may have led to defects in drainage and the occurrence of off-tastes such as bitterness [41].

5. CONCLUSION

This study was undertaken under natural conditions of cheese production and so made it possible to demonstrate considerable sensory differences between the cheeses depending on cow feeding (valley pastures, mountain pastures or hay). Most of the volatile compounds were microbially produced and depended on both the composition of the microflora present (endogenous microflora in the raw milk and/or from starters) and on the physico-chemical parameters in the milk and cheese determining the way in which microorganisms develop and their enzyme activity.

This work completes three previous studies [14–16], and made it possible to link the botanical composition of pastures and the terpene composition of cheeses. However, the role played by these terpenes as well as fatty acids, which were also related to pasture properties, seems to contribute indirectly to cheese flavour.

It would be worthwhile to validate these results by carrying out targeted studies. However, these results should make it possible to improve our understanding of the origin of the diversity in cheese flavours as well as provide additional information and objectives for further thought concerning the evolution of the PDO cheese sector.

ACKNOWLEDGEMENTS

This study was co-financed by the Northern Alps Scientific Interest Grouping and the French National Institute for Agricultural Research. We would like to thank J.C. Salmon (INRA, Poligny) for his help in analysing and identifying volatile compounds, L. Tessier (INRA, Poligny) for carrying out the PLS regressions, T. Métais (Les Maisons du Goût, La Roche-sur-Foron) for undertaking the sensory assessments and B. Martin (INRA, Theix) and Y. Noël (INRA, Poligny) for critically reviewing the manuscript.

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