

# Influence of Alpine highland pasture on the fatty acid and terpene composition of milk and Plaisentif cheese from various Piedmont farms

Gai F.<sup>1\*</sup>, Battelli G.<sup>1</sup>, Bottero M.T.<sup>2</sup>, Civera T.<sup>2</sup>, Dalmasso A.<sup>2</sup>, Pattono D.<sup>2</sup>, Peiretti P.G.<sup>1</sup>

<sup>1</sup>Institute of Sciences of Food Production, National Research Council, Italy

\*e-mail: francesco.gai@ispa.cnr.it

<sup>2</sup>Department of Veterinary Sciences, University of Torino, Italy

**Abstract.** The aim was to characterize, monitor and certify Plaisentif, known as the "cheese of the violets", produced with raw milk from cows fed on pastures in the Chisone and Susa valleys (NW Italy) when violets are in bloom. A preliminary study was conducted over the summer of 2013 on five Alpine dairy farms to determine the fatty acid (FA) composition and terpenoid profile for the first growth cycle in permanent meadows, and for milk and ripened cheese. The pastures showed a wide diversity both in FA and terpenoid profiles. Among FAs, the most abundant were  $\alpha$ -linolenic acid (from 40 to 61% of total FA) and linoleic acid (from 13 to 31% of total FA), while p-Cymene and  $\alpha$ -Pinene were the most important terpenoids. As far as milk and cheese FA content are concerned, palmitic and oleic acid accounted on average for 47.2 and 47.7% of total FA, respectively. Healthy FAs, such as  $\alpha$ -linolenic acid and conjugated linoleic acid, showed mean values of 1.4-2.1% of total FA, respectively. Terpenoid profiles of milk and cheese were very similar, even though a slight concentration of some terpenes in cheese was observed compared to the corresponding milk. Moreover, Alpine highland pasture modified the FA and terpenoid profile of Plaisentif cheese, while processing milk into ripened cheese did not affect its FA composition and terpene profile with the exception of eicosapentaenoic, butyric and caproic acids among FA, and limonene, p-Cymene and allo-Ocimene among terpenes.

**Keywords.** Fatty acid – Terpene – Mountain – Milk.

## *Influence du pâturage alpine sur la composition du lait et du fromage" Plaisentif "des différentes fermes du Piémont*

**Résumé.** Le but était de caractériser le Plaisentif, le "fromage des violettes", produit avec du lait cru de vaches nourries sur les pâturages des vallées de Chisone et Suse (NO, Italie) pendant la période de floraison des violettes. Une étude a été réalisée pendant l'été 2013 dans cinq fermes laitières alpines pour déterminer les profils des acides gras (AG) et des terpènes de prairies lors du premier cycle de pâturage, du lait et du fromage affiné. Les pâturages ont montré une grande diversité dans la composition en AG et en terpènes. Les AG les plus abondants étaient l'acide  $\alpha$ -linoléique (de 40 à 61% du total AG) et l'acide linoléique (de 13 à 31% du total AG), tandis que les terpénoïdes les plus importants étaient le p-Cymène et  $\alpha$ -Pinène. En ce qui concerne le profil des AG des laits et des fromages, les acides palmitique et oléique représentaient respectivement de 47,2 à 47,7% du total des AG. L'acide  $\alpha$ -linoléique et l'acide linoléique conjugué, ont montré des valeurs moyennes respectivement de 1,4 à 2,1% du total des AG. Les profils terpénoïdes des laits et des fromages sont très semblables, même si une légère concentration de les terpènes dans le fromage a été observé par rapport au lait correspondant. En outre, le pâturage des prairies alpines a affecté le profil en AG et en terpènes du fromage Plaisentif, alors que la transformation du lait en fromage affiné n'a pas modifié la composition et le profil en AG et en terpènes à l'exception de l'acide eicopentaénoïque, acide butyrique et acide caproïque parmi AG, et le limonène, le p-Cymène et l'allo-Ocimène parmi les terpènes.

**Mots-clés.** Acides gras – Terpènes – Montagne – Lait.

## I – Introduction

In Italian mountain regions, most of the milk produced are transformed into traditional cheeses. One of them, Plaisentif, also known as the ancient "cheese of the violets", is produced in the Piedmont Region during the flowering period of violets (June-July) using raw cow's milk. It has been shown how grazing quality can greatly affect the organoleptic characteristics of raw milk, with particular reference to fatty acid (FA) composition and volatile aromatic compounds such as terpenes, alcohols and ketones (Revello Chion *et al.*, 2010). Several aspects of terpenes have been studied, in particular as potential biomarkers of diet, used to trace milk and mountain cheeses (Martin *et al.*, 2005; Tornambé *et al.*, 2006). In dairy products, particular attention concerns the content of n-3 polyunsaturated FAs and conjugated linoleic acid (CLA). Many studies have shown that these FA have beneficial effects on human health (Parodi, 2003). The presence of these compounds in milk is due to the rumen biohydrogenation of polyunsaturated FAs in animals fed with fresh forage. For this reason, their content tends to increase in milk produced from cows reared in the wild or fed with fresh grasses (Leiber *et al.*, 2005; De Noni and Battelli, 2008; Revello Chion *et al.*, 2011). The aim of the present study was to characterize the FA and terpenoid profile in pasture, milk, and Plaisentif cheese produced on different Piedmont mountain farms.

## II – Materials and methods

A preliminary study was conducted over the summer of 2013 in five Alpine dairy farms located at 1400-1800 m a.s.l. in the Chisone and Susa valleys (NW Italy). Samples of pasture, pooled milk and 60-day ripened cheese were analysed to determine the FA and terpenoid profiles. Samples of pasture and milk were immediately frozen, freeze-dried and then stored at -20°C until the FA extraction phase. After ripening, the cheeses were cut into wedges and then frozen at -20°C. FA analyses were performed according to the method described by Revello Chion *et al.* (2010). The FA methyl esters in hexane were then injected into a gas chromatograph (Dani Instruments S.P.A. GC 1000 DPC; Cologno Monzese, Italy) equipped with a flame ionisation detector, a PTV injection port and a Supelcowax-10 fused silica column (60 m × 0.32 mm, 0.25 µm). The peak area was measured using a Dani DDS 1000 Data Station. Each peak was identified according to pure methyl ester standards (Supelco and Restek Corporation, Bellefonte, PA) and the data were expressed as relative values. The FA composition was expressed as g/100 g of FA. Terpene analysis was carried out on freeze-dried pasture and on the milk and ripened cheese fat (200 mg) extracted without the use of solvents. Terpene analysis was performed according to the method described by De Noni and Battelli (2008) by means of dynamic headspace extraction (Dani Instruments S.p.A.), gas chromatography-mass spectrometry (Agilent Technology; 500 ml He<sub>2</sub> for 18 min at 65°C). Data were expressed as arbitrary units, as log<sub>10</sub> of the peak area of the corresponding selected ion. Data were analysed according to ANOVA in order to evaluate the effect of transformation of milk into cheese.

## III – Results and discussion

The three dominant FAs detected in the pasture were α-linolenic acid (ALA, C18:3n-3), linoleic acid (LA, C18:2n-6) and palmitic acid (PA, C16:0) that accounted on average for more than 81% of total FA. Concerning milk and cheese FA composition, five FAs were the most abundant: PA, oleic acid (C18:1n-9), stearic acid (C18:0), myristic acid (C14:0) and vaccenic acid (C18:1n-7), that accounted for an average of more than 74% of total FA, while the sum of ALA and LA contents resulted lower than 4% of total FA both in milk and cheese. Conjugated linoleic acid (CLA, C18:2 cis-9,trans-11) content ranged from 1.6 to 2.4 % of total FA in milk and from 1.7 to 2.5 % of total FA in cheese, respectively (Table 1). The FA content of Plaisentif cheese was similar to that reported for the Toma Piemontese and Bitto cheeses produced in the Italian alpine area (Revello Chion *et al.*, 2010; De Noni and Battelli, 2008). The terpenes found

in all samples of pasture, milk and cheese were as follows:  $\alpha$ -Pinene, Camphene,  $\beta$ -Pinene,  $\delta$  3-Carene, Limonene, p-Cymene and allo-Ocimene. The most important terpenoids in the pasture were p-Cymene and  $\alpha$ -Pinene, while  $\alpha$ - and  $\beta$ -Pinene were the most abundant in milk and cheese. The latter two were also the main terpenes found in other cheeses produced in Italian alpine regions (De Noni and Battelli, 2008; Berard *et al.*, 2007). The terpene profile of pasture differed strongly from that of milk and cheese, due to the comparatively high amount of allo-Ocimene, and low amount of  $\delta$ -3-carene (Table 2). The terpenoid profiles of milk and cheese were very similar, even though a slight concentration of limonene, p-Cymene and allo-Ocimene in cheese was observed compared to the corresponding milk.

**Table 1. Fatty acid (FA) content (g/100 g of total FA) of pasture (n=9), milk (n=10), and Plaisentif cheese (n=10) from five Piedmont farms.**

	Pasture				Milk				Cheese				Prob.
	Min	Max	Mean	SD <sup>†</sup>	Min	Max	Mean	SD <sup>†</sup>	Min	Max	Mean	SD <sup>†</sup>	
C4:0					2.8	3.6	3.1	0.25	2.6	2.9	2.7	0.11	0.001
C6:0					1.4	1.7	1.5	0.10	1.2	1.6	1.3	0.11	0.001
C8:0					0.58	0.87	0.76	0.10	0.52	0.77	0.67	0.09	0.061
C10:0					1.3	2.2	2.0	0.30	1.3	2.0	1.7	0.24	0.083
C12:0					1.8	2.7	2.4	0.33	1.8	2.7	2.3	0.27	0.486
C14:0					8.8	11.6	10.2	0.96	8.6	11.0	9.6	0.90	0.190
C14:1					0.49	0.90	0.63	0.15	0.48	0.82	0.64	0.12	0.808
C15:0					1.1	1.4	1.3	0.07	1.1	1.4	1.3	0.09	0.480
C16:0	8.2	10.9	9.5	0.79	23.6	27.9	26.4	1.49	23.4	26.9	25.5	1.25	0.150
C16:1n-7					1.3	1.5	1.4	0.08	1.3	1.6	1.5	0.09	0.089
C17:0					0.80	1.1	0.96	0.08	0.72	1.2	0.90	0.16	0.328
C17:1					0.17	0.34	0.22	0.05	0.21	0.47	0.28	0.09	0.105
C18:0	1.3	2.5	1.8	0.42	9.5	12.4	11.0	1.09	10.2	12.6	11.3	1.01	0.552
C18:1n-9	2.6	8.5	4.5	1.88	19.0	23.9	20.8	1.59	20.0	24.4	22.2	1.59	0.070
C18:1n-7					4.4	7.0	5.8	1.00	4.8	7.0	6.0	0.85	0.602
C18:2n-6	13.0	30.8	17.2	5.07	1.9	2.4	2.2	0.16	2.1	2.5	2.3	0.15	0.171
C18:3n-6	0.73	4.3	2.5	1.09	0.05	0.07	0.06	0.01	0.05	0.07	0.06	0.01	1.000
C18:3n-4					0.12	0.19	0.14	0.02	0.12	0.16	0.14	0.01	0.892
C18.3n-3	39.7	60.6	54.7	5.72	1.2	1.5	1.4	0.12	1.3	1.5	1.4	0.11	0.635
CLA1 <sup>‡</sup>					1.6	2.4	2.0	0.30	1.7	2.5	2.2	0.34	0.262
CLA2 <sup>§</sup>					0.09	0.17	0.13	0.03	0.09	0.15	0.13	0.03	0.684
C20:0					0.22	0.36	0.27	0.04	0.24	0.28	0.26	0.01	0.457
C20:1n-9					0.07	0.26	0.14	0.08	0.10	0.27	0.18	0.06	0.234
C20:3n-6					0.00	0.06	0.04	0.02	0.04	0.05	0.04	0.00	0.362
C20:4n-6					0.08	0.15	0.10	0.02	0.08	0.11	0.10	0.01	1.000
C20:4n-3					0.05	0.09	0.07	0.01	0.06	0.08	0.07	0.01	1.000
C20:5n-3					0.07	0.08	0.08	0.00	0.08	0.10	0.09	0.01	0.015
C22:0					0.09	0.11	0.10	0.01	0.08	0.15	0.11	0.02	0.291
C22:5n-3					0.12	0.40	0.17	0.08	0.11	0.19	0.14	0.03	0.327
Unknown	6.6	13.7	9.8	1.72	4.2	5.2	4.6	0.32	4.4	5.3	4.8	0.31	0.255

<sup>†</sup> SD: standard deviation. <sup>‡</sup> cis-9,trans-11 conjugated linoleic acid. <sup>§</sup> trans-10,cis-12 conjugated linoleic acid.

**Table 2. Terpene composition of pasture (n=9), milk (n=10), and Plaisentif cheese (n=10) from five Piedmont farms.**

	Pasture				Milk				Cheese				Prob.
	Min	Max	Mean	SD <sup>†</sup>	Min	Max	Mean	SD <sup>†</sup>	Min	Max	Mean	SD <sup>†</sup>	
α-Pinene <sup>§</sup>	6.48	7.60	7.07	0.42	5.88	6.44	6.15	0.18	5.91	6.52	6.24	0.24	0.376
Camphene	5.49	6.78	6.02	0.40	4.85	5.49	5.18	0.22	4.95	5.92	5.32	0.29	0.237
β-Pinene	6.05	7.61	6.86	0.53	5.48	5.89	5.64	0.14	5.46	6.00	5.73	0.20	0.240
δ 3-Carene	4.85	5.64	5.26	0.26	4.71	5.88	5.21	0.38	4.91	5.91	5.41	0.37	0.250
Limonene	4.79	6.93	5.88	0.72	5.08	5.61	5.43	0.15	5.45	6.41	5.65	0.28	0.046
p-Cymene	6.36	7.76	7.17	0.55	5.05	5.57	5.27	0.15	5.25	5.89	5.51	0.20	0.009
allo-Ocimene	5.26	6.81	6.10	0.59	4.01	4.51	4.23	0.20	4.10	4.70	4.43	0.24	0.050

<sup>†</sup> SD: standard deviation. <sup>§</sup> Data expressed as arbitrary units of log<sub>10</sub> of the peak area of the corresponding selected ion.

## IV – Conclusions

Grazing Alpine highland pasture affected the FA and terpenoid profiles of dairy products, while processing milk into ripened cheese did not substantially modify the FA composition and terpene profile of Plaisentif cheese with the exception of eicosapentaenoic, butyric and caproic acids among FA, and limonene, p-Cymene and allo-Ocimene among terpenes. These results confirm the importance of Alpine highland pasture in obtaining milk and cheese that have a favourable FA profile, potential health benefits and an aromatic profile with molecules that confer specific organoleptic and nutritional properties to the Plaisentif cheese.

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