Food Chemistry 121 (2010) 393–399

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Variation of fatty acid and terpene profiles in mountain milk and "Toma piemontese" cheese as affected by diet composition in different seasons

Andrea Revello Chion [a,*], Ernesto Tabacco [a], Daniele Giaccone [a], Pier Giorgio Peiretti [b], Giovanna Battelli [c], Giorgio Borreani [a]

[a] Dipartimento di Agronomia, Selvicoltura e Gestione del Territorio, University of Turin, via L. da Vinci 44, 10095 Grugliasco (Turin), Italy
[b] Institute of Sciences of Food Production, National Research Council, via L. da Vinci 44, 10095 Grugliasco (Turin), Italy
[c] Institute of Sciences of Food Production, National Research Council, via G. Celoria 2, 20133 Milan, Italy

ARTICLE INFO

Article history:
Received 23 June 2009
Received in revised form 23 October 2009
Accepted 11 December 2009

Keywords:
"Toma piemontese" cheese
Milk
Fatty acid profile
Terpenes
Pasture
Conserved forage

ABSTRACT

The influence of seasonal variation of diets (conserved forage and pasture) on fatty acid (FA) and terpenoid profiles of bovine milk and "Toma piemontese" cheese was studied in a Piedmont mountain farm, under the usual conditions of farming and management of herds in the highlands. The dairy products obtained in summer from pasture-based diets presented a more favourable FA profile. Compared with winter, summer milk had lower contents of saturated FA (SFA) (−15.8%) and higher contents of monounsaturated FA (MUFA) (+33.0%), polyunsaturated FA (PUFA) (+68.2%), conjugated linoleic acid (CLA) (+161%) and vaccenic acid (+148%). The milk from pasture-based diets presented higher contents of terpenes than did that from winter diets based on hay. Processing milk into ripened cheese had no effect on the FA composition and terpene profile of dairy products. These molecules can confer specific organoleptic and nutritional properties to the dairy products that provide an added value to the product and justify its higher price.

1. Introduction

In the Italian mountain regions, most of the milk produced is transformed into traditional cheeses, and often they are granted Protected Designation of Origin (PDO) status. Among them is the "Toma piemontese" cheese, typical semi-cooked, semi-hard cheese, cylindrically shaped (15–35 cm × 6–12 cm) with weight of 2–8 kg, produced in Piedmont from raw whole milk. The total production per year is 1216 tonnes of cheese derived from 11,700 tonnes of milk, 30% of which is produced directly from mountain dairy farms. The producing mountain farming system is based on local forage resources, with a combination of fresh pasture, in the summer period, and local conserved forages, in the rest of the year (Borreani, Giaccone, Mimosi, & Tabacco, 2007). The local forage-based diets are part of the basic link between dairy products and their original 'terroir', a notion at the basis of the PDO labelling and image of the product quality from sensory, nutritional, or safety points of view (Engel et al., 2007). The forages are known to confer specific organoleptic and nutritional qualities on the milk products (Martin, Verdier-Metz, Buchin, Hurtaud, & Coulon, 2005) and to provide an added value to the product that could justify its higher price and offer the consumers a healthy image of the mountain environment. This healthy image is confirmed by several studies that have revealed high contents of beneficial functional fatty acids (FA) in dairy products derived from Alpine grazing systems (e.g. Hauswirth, Scheeder, & Beer, 2004; Leiber, Kreuzer, Nigg, Wettstein, & Scheeder, 2005). Natural products, and specifically dairy fats, reportedly contain over 25 conjugated linoleic acid (CLA) isomers to which studies have attributed a wide range of healthy effects, e.g. anticarcinogenic, immunomodulating, and antiatherosclerotic effects (Belury, 2002; Parodi, 2003). The biological activities attributed to CLAs have to be confirmed, if such attributes are due to a single isomer or to the mixture. The predominant isomers, *cis*-9, *trans*-11-CLA (9-CLA, the rumenic acid) and *trans*-10, *cis*-12-CLA (10-CLA) are the primary focus of most of the studies evaluating the biological activities of CLA (Sailas & Spener, 2009). Furthermore, epidemiological and clinical studies have established that the C18:2n−6 fatty acid (linoleic acid) and n−3 fatty acids (α-linolenic acid, eicosapentaenoic acid and docosahexaenoic acid) collectively protect against coronary heart disease and they are dietary essential components because they are required for normal physiological functions linked to membrane integrity and regulatory cell signals (Wijendran & Hayes, 2004). The CLA of milk and dairy products arises both directly from incomplete microbial hydrogenation of polyunsaturated FA of the diet in the rumen and indirectly from the activity of Δ^9 desaturase in mammary tissue that dehydrogenates the vac-

* Corresponding author. Tel.: +39 011 6708907; fax: +39 011 6708798.
E-mail address: andrea.revellochion@unito.it (A. Revello Chion).

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doi:10.1016/j.foodchem.2009.12.048

cenic acid (*trans*-11-18:1) produced in the rumen (Griinari et al., 2000). The diet is the most important factor influencing milk CLA content (Bauman & Griinari, 2003; Chilliard, Ferlay, & Doreau, 2001; Collomb, Bütikofer, Sieber, Jeangros, & Bosset, 2002a). In particular, the CLA concentration is higher in milk from animals fed pasture than those fed dry diets (Dhiman, Helmink, McMahon, Fife & Pariza, 1999; Ferlay, Martin, Pradel, Coulon, & Chilliard, 2006), and decreases with increasing forage maturity stage (Elgersma, Tamminga, & Ellen, 2006). Minor variations in CLA content in milk are ascribed to the stage of lactation, parity (Kelly, Kolver, Bauman, VanAmburgh, & Muller, 1998), and breed (White et al., 2001). Furthermore, animal diet can influence the composition of volatile compounds in raw milk by direct transfer (Buchin, Martin, Dupont, Bornard, & Achilleos, 1999). Dumont and Adda (1978) and Mariaca et al. (1997) found a lot of volatile components in cheeses derived from natural highland pastures. Among these components, the terpenes, that are plant secondary metabolites biosynthetically derived from isoprene units, pass through the animal into the milk with minor alterations and occur in dairy products at levels highly dependent on dietary supply (Agabriel et al., 2007). The terpenes have been studied by several authors because they can be utilised as potential biomarkers in milk and cheese of the presence of diversified forages in dairy cow diet (Martin et al., 2005; Viallon et al., 1999), especially for mountain cheeses (Tornambé et al., 2006). Terpene content in forage is mainly affected by its botanical composition: *Poaceae* are terpene-poor, whereas many dicotyledons contain higher amounts of terpenes (Mariaca et al., 1997). Furthermore, terpene concentrations increase in mature forage (Cornu et al., 2001) and seem to be partially lost during forage harvesting and storage (Viallon et al., 2000). Terpenes and sesquiterpenes influence the aroma of mountain cheeses (Dumont, Adda, & Rousseaux, 1981). Increasing numbers of terpenes are being found to have anti-bacterial (Calsamiglia, Busquet, Cardozo, Castillejos, & Ferret, 2007), and anti-cancer properties: the relatively low toxicity and high bioavailability of dietary monoterpenes make these compounds attractive as potential therapeutic agents (Crowell, 1999).

The aim of the present study was to evaluate the seasonal variation of fatty acid and terpenoid profile in milk and "Toma piemontese" cheese produced from a Piedmont mountain farm, as affected by the usual conditions of farming and management of the herd.

2. Materials and methods

2.1. Experimental design

The study was conducted over the 2002–2003 period at the "Stazione Sperimentale Alpina – Vittorino Vezzani" experimental farm in Sauze d'Oulx (1850 m above sea level, 45°02′N, 6°53′E, mean annual rainfall 640 mm, mean annual temperature 4.5 °C, Italy) on a herd of 40 multiparous Aosta Red Pied dairy cows, producing a mean annual yield of 3565 ± 238 kg of milk. The cows were managed under the usual conditions of farming and management of herds in the Piedmont mountain environment: in winter (from October to late May), the cows were housed indoors, maintained in stanchion-type housing and fed hay (70% of dry matter intake, DMI) from native grassland located at 1500–1600 m a.s.l and concentrates, whereas, in summer, they were fed natural pasture, grazed from 1800 to 2000 m a.s.l. (90% of DMI) with low amounts of concentrates. Parturition of the herd took place on average at the end of November. In the Alpine environments, the breeding commonly emphasizes calving in November–December in order to have the peak of lactation over the winter–spring period and to avoid fresh cows with high nutritional requirements during the summer grazing season. From December to September, four experimental periods of two months were identified: two in winter (W1–W2) and two in summer (S1–S2). During October and November the samplings were not performed because almost all cows produced no milk. The milk was sampled 4 times during each experimental period (each every 15 d). The same milk was used for the production of "Toma piemontese" cheese according to the PDO specifications. The corresponding cheese was sampled after 90 days of ripening.

2.2. Hays and pastures

The hays were produced during year 2002 on native grassland meadows. The hay meadows were cut on the first 15 days of July and field-cured. The hays were baled in round bales after 3–4 days of wilting, and stored in the hayloft for 6–10 months. During the summer season the cows grazed on a native highland pasture at different moments of the morphological development of the pasture, from June to September.

The botanical composition of the hay meadows, where hays were produced, and of pastures, was determined from clipped samples cut from 10 randomly selected $0.1 \times 10\ m^2$ areas. Forage samples were cut using 10 cm-wide battery-powered grass shears and hand-separated into *Poaceae*, *Fabaceae*, *Lamiaceae*, *Asteraceae* and other botanical families as a whole. Sub-samples were dried in an oven at 80 °C to a constant weight in order to calculate the ratios between the classes.

The FA profiles of fresh and conserved forages, and of the concentrate (expressed as g $100\ g^{-1}$ FA) and the composition of the diets fed during the four experimental periods (expressed as $100\ g^{-1}$ DM) are shown in Table 1. The contributions of forages to the dietary DM were 68.3%, 67.1%, 98.3% and 84.7% for W1, W2, S1, and S2, respectively. The concentrate fed to animals was the same throughout the experimental period and had the following composition (%): corn grain 29.7, soybean meal (44% CP) 27.0, corn gluten feed 9.5, wheat bran 9.5, sunflower meal 7.8, rice hull 6.5, calcium carbonate 2.8, sugarcane molasses 2.0, sodium bicarbonate 1.0, mineral-vitamin supplement 1.0, vegetable fat 0.8, dicalcium phosphate 0.8, bentonite 0.8, and salt 0.8.

Table 1
Composition of the diets fed in the four experimental periods and fatty acid (FA) composition of concentrate and forages.

	Component (g $100\ g^{-1}$ of DM)		
Experimental period	Concentrate	Pasture	Hay
W1	31.7	-	68.3
W2	32.9	-	67.1
S1	1.7	98.3	
S2	15.3	84.7	

	Fatty acid (g $100\ g^{-1}$ of FA)				
	Concentrate	S1	S2	W1	W2
C12:0	0.21	0.31	0.64	0.33	0.39
C14:0	0.43	1.02	1.12	0.35	0.34
C16:0	20.5	10.3	13.0	13.5	13.2
cis-9 C16:1 c-9	0.14	0.10	1.03	0.34	0.26
C18:0	3.72	1.51	2.10	1.39	1.56
cis-9 C18:1 c-9	32.1	6.01	13.5	5.54	4.53
cis-9, *cis*-12 C18:2 n-6	38.8	18.1	23.9	19.0	16.3
cis-6, *cis*-9, *cis*-12 C18:3 n-3	0.06	0.10	<0.10	3.61	2.70
cis-9, *cis*-12, *cis*-15 C18:3 n-3	2.18	46.6	29.7	34.1	38.7
cis-6, *cis*-9, *cis*-12, *cis*-15 C18:4 n-3	<0.10	0.69	0.76	1.39	1.25
C20:0	0.38	0.49	0.88	0.28	0.29
Unidentified[a]	1.40	14.8	13.4	20.2	20.5