Lait 83 (2003) 167–173
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DOI: 10.1051/lait:2003007

Note

Fatty acid composition of Piedmont "Ossolano" cheese

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(Received 19 March 2002; accepted 13 November 2002)

Abstract – Fatty acid composition of 66 samples of "Ossolano", a typical semi-hard cheese produced from raw cow milk in the Ossola valley (North Piedmont, West Italy) was determined. The survey was carried out on 24 summer cheeses produced in mountain farms (1500–2200 m) with milk from pasture-fed cows and 42 winter cheeses produced in valley farms (500–800 m) with milk from hay- and concentrate-fed cows. Seasonal variation in feeding condition was responsible for the observed variations in fatty acid composition of the samples. Long-chain mono- and poly-unsaturated fatty acids were more abundant in the summer cheeses while short- and medium-chain saturated fatty acids were higher in winter products. The ratio of saturated to unsaturated fatty acids was thus lower in summer cheeses compared with winter cheeses. Conjugated linoleic acids, n-3 and n-6 acids were instead higher in summer cheeses according to the results of many authors. The n-6/n-3 ratio is lower in the summer products with an interesting nutritional effect due to the essential role of n-3 polyunsaturated fatty acid in human health.

Fatty acid / mountain cheese / Ossolano cheese / conjugated linoleic acid

Résumé – Composition en acides gras du fromage « Ossolano » du Piémont. La composition en acides gras a été déterminée dans 66 échantillons d'« Ossolano », un fromage à pâte semi-dure qui est produit à partir du lait cru dans la vallée de l'Ossola (Piémont du Nord, Italie). Vingt-quatre de ces fromages ont été fabriqués en été à partir de laits produits sur des pâturages de montagne (1500–2200 m) et quarante-deux en hiver à partir de laits produits dans la vallée (500–800 m) avec une alimentation à base de foin et de céréales. Le changement dans l'alimentation a déterminé le changement de la composition en acides gras des fromages. La proportion en acides gras à chaîne longue mono- et poly-insaturés était significativement plus élevée dans les fromages d'été, alors que celle des acides gras saturés à chaîne courte et moyenne était plus élevée dans les fromages d'hiver. Par conséquent le rapport entre les acides saturés et acides insaturés était plus petit dans les fromages d'été que dans les fromages d'hiver. Les CLA et les acides n-3 et n-6 sont plus élevés dans les fromages produits en été comme de nombreux auteurs l'ont déjà souligné. Le rapport n-6/n-3 est plus petit pour les fromages produits en été, ce qui entraîne un effet nutritionnel intéressant grâce au rôle essentiel des acides gras poly-insaturés n-3 dans l'alimentation humaine.

Acide gras / fromage de montagne / fromage « Ossolano » / acide linoléique conjugué

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1. INTRODUCTION

Lipids are very important components of the human diet for their indispensable vital functions. Modern guidelines for a healthy diet recommend, however, reducing animal fat consumption, especially if rich in saturated fatty acids, due to their by now well-demonstrated hypercholesterolemic effect. But it is important to remember that there are also unsaturated fatty acids present in animal fat which may not only reduce the cholesterol content in the blood, but in some cases even have anticarcinogenic effects and develop a protection to cardiovascular diseases [1, 12, 14, 25, 28]. Linoleic ($C_{18:2}$ n-6) and linolenic ($C_{18:3}$ n-3) acids are even known as "indispensable fatty acids" which cannot be synthesized by men or animals.

Fatty acids of milk are derived from two major sources. Acetate and 3-OH-butyrate originating from ruminal fermentation are the carbon sources for $C_{4:0}$ to $C_{14:0}$ and one half of $C_{16:0}$ fatty acids. The remaining $C_{16:0}$ and all of the longer-chain fatty acids are derived from blood lipids produced during the digestion and absorption of dietary fat or from mobilization of fatty acids from adipose tissue [10, 18, 34]. So diet could modify the chemical composition of milk and thus influence the composition of milk products [2, 5, 6, 8, 9, 11, 19, 27, 31]. Recent studies have shown that pasturing or feeding cows with green forage determines an increase in the unsaturated long-chain fatty acids and a reduction in the short-chain saturated ones [2, 7, 13, 15, 20, 33, 35]. This effect is particularly evident in the traditional mountain cheeses due to prolonged grazing on a variety of vegetal essences even though not many studies on these particular products have yet been done [3, 4, 6]. The aim of this work was to verify if for "Ossolano" cheese, one of the best-known Piedmont cheeses, it was also possible to observe a fatty acid difference between the summer production (cows fed with green forage in mountain pasture) and the winter production (cows fed with hay and concentrate in valley farms).

2. MATERIALS AND METHODS

2.1. Samples

"Ossolano" cheese is a round semi-hard cheese weighing 8–9 kg and made using raw milk exclusively from Bruna Alpina cows. This cheese is produced in the north of Piedmont, in the alpine zone bordering Switzerland. For the cheesemaking, raw whole (in summer) or raw partially skimmed (in winter) cow milk is coagulated without starter at 34–37 °C by adding bovine rennet. The curd is cut with a wire cutter into 3–4 mm curd particles. The curd-whey mixture is heated to 37–45 °C over a period of 15–48 min and then stirred without heating for a further 40 min. The curd is finally removed and placed into moulds, drained and pressed for 24 h. The cheeses are then removed from the moulds and salted dry for 48 h after which they are ripened at 8–12 °C in curing rooms at high humidity (80–90%).

The survey was carried out on 66 cheese samples, of which 24 were from the summer production of mountain farms located between 1500 m and 2200 m (S: 7 producers) and 42 from the winter production of valley farms (W: 6 producers). Summer feeding was exclusively green forage in mountain pastures whereas hay and concentrate were used for winter feeding.

Each sample was collected from a whole cheese after 60 d of ripening and immediately frozen at –18 °C. Analyses were performed within one week.

2.2. Chemical analysis

Dry matter, fat and protein determinations in sample cheeses were performed according to "Official Methods of Cheese Analysis" [26].

2.3. Preparation of fatty acid methyl esters

The fatty acid methyl esters were prepared by trans-esterification with potassium hydroxide according to ISO 5509:2000E [16].

Ten grams of cheese were homogenized, then an aliquot (0.15 g) was collected in a screw-capped test tube and sodium sulfate anhydrous (1 g), isooctane (4 mL) and methanolic potassium hydroxide solution (300 μL; 2 mol·L^{-1}) were added. The test tube was closed and shaken vigorously for about 1 min. After adding 1.5 g of sodium hydrogen sulfate monohydrate the tube was shaken again for 30 s to neutralize the excess of potassium hydroxide. The organic upper layer containing methyl esters was then transferred into a vial and immediately analyzed.

2.4. Gas chromatographic analysis

Fatty acid methyl esters were analyzed on a Varian gas chromatograph (Model 3400; Varian Assoc. Inc., Walnut Creek, CA, USA) using a DB-WAX capillary column (30 m length, 0.25 mm internal diameter, 0.25 μm phase thickness; J&W Scientific Inc., Folsom, CA, USA), a split-splitless injector and a flame ionization detector. The injected volume was 2 μL. The temperature program and operating conditions were as follow: injector and detector temperature 250 °C; carrier gas helium at 1 mL·min^{-1}; injection in splitless mode for 0.30 min and then split 1:60; the column was maintained at 35 °C for 5 min then ramped at 2 °C·min^{-1} to 220 °C and finally 5 min at 220 °C. Each sample was analyzed twice.

Data integration was performed with the EZCHROM™ data system (Scientific Software Inc., San Ramon, CA, USA).

Fatty acid methyl esters were identified by comparing their retention times with those of the reference standard (FAME mix C_8–C_{24}, Supelco Park, Bellefonte, PA, USA) and by a Shimadzu GC-17A gas chromatograph coupled with a Shimadzu QP-5000 quadrupole mass spectrometer (Shimadzu, Tokyo, Japan) operating under the same chromatographic conditions. Mass spectra were recorded in electron impact mode at ionization voltage of 70 eV in the 29–350 amu mass range. The ion source and interface were maintained at 220 °C. Compound identification was carried out by NIST 12 and NIST 62 mass spectral database.

Table I. Major component concentrations of summer cheeses produced from mountain farms (24 samples) and winter cheeses produced from valley farms (42 samples).

	Winter cheeses		Summer cheeses	
	x	σ	x	σ
Dry matter (%)	52.92	4.75	55.39	2.54
Fat (% dm)	19.53	5.71	27.89	3.49
Protein (% dm)	28.45	2.53	23.96	1.98

x: mean value; σ: standard deviation; dm: dry matter.

2.5. Statistical analyses

Differences between S and W cheeses were investigated using analysis of variance (Statistica ver. 6.0; StatSoft Inc., Tulsa, OK, USA).

3. RESULTS AND DISCUSSION

Dry matter, fat and protein concentrations for S and W cheeses are reported in Table I. The difference for fat content is due to the different milk used in cheese-making. Summer cheeses are produced with whole milk while winter cheeses are produced with partially skimmed milk.

In Table II the composition in FAMEs with 4 to 20 carbon atoms of S and W cheeses is reported.

Due to different diet compositions, short- (C_4, C_6, C_8) and medium-chain (C_{10}, C_{12}, C_{14} and C_{16}) saturated fatty

Table II. FAMEs composition (C4 to C20) of summer (24 samples) and winter (42 samples) cheeses and significance value of analysis of variance (*P*).

	Winter cheeses		Summer cheeses		
	x	σ	x	σ	*P*
$C_{4:0}$	3.51	0.37	3.11	0.22	<0.01
$C_{6:0}$	2.34	0.21	1.73	0.19	<0.01
$C_{8:0}$	1.47	0.18	0.99	0.19	<0.01
$\Sigma(C_{4:0}+C_{6:0}+C_{8:0})$	7.32	0.67	5.84	0.54	<0.01
$C_{10:0}$	3.01	0.47	1.92	0.44	<0.01
$C_{10:1}$	0.37	0.10	0.23	0.05	<0.01
$C_{12:0}$	3.25	0.85	2.07	0.39	<0.01
$C_{13:0}$	0.11	0.04	0.12	0.05	0.555
$C_{14:0}$	11.92	0.85	8.32	0.82	<0.01
$C_{14:0\ branched}$	0.20	0.07	0.22	0.04	0.353
$C_{14:1}$	1.04	0.14	0.67	0.11	<0.01
$C_{15:0}$	1.24	0.14	1.48	0.23	<0.01
$C_{15:0\ branched}$	1.03	0.15	1.19	0.11	<0.01
$C_{16:0}$	30.74	1.89	24.26	1.00	<0.01
$C_{16:0\ branched}$	0.41	0.08	0.38	0.08	0.112
$C_{16:1}$	1.48	0.14	1.65	0.19	<0.01
$\Sigma(C_{10:0}+C_{12:0}+C_{14:0}+C_{16:0})$	48.93	3.37	36.57	2.14	<0.01
$C_{17:0}$	0.74	0.20	0.93	0.13	<0.01
$\Sigma(C_{17:0\ branched})$	1.22	0.15	1.30	0.26	0.101
$C_{17:1}$	0.34	0.09	0.41	0.12	<0.01
$C_{18:0}$	10.17	0.95	12.72	1.06	<0.01
$C_{18:1}$	21.48	1.74	29.37	1.75	<0.01
cis-9, *trans*-11-$C_{18:2}$	0.89	0.16	2.23	0.42	<0.01
$\Sigma(C_{18:2})$*	2.48	0.55	2.95	0.48	<0.01
$C_{18:3}$	0.75	0.15	1.30	0.25	<0.01
$\Sigma(C_{18:0}+C_{18:1}$+c-9,t-11-$C_{18:2}+\Sigma C_{18:2}+C_{18:3})$	34.89	2.67	46.33	2.36	<0.01
$\Sigma(C_{18:1}$+ c-9,t-11-$C_{18:2}+\Sigma C_{18:2}+C_{18:3})$	24.71	1.90	33.62	1.71	<0.01
$C_{20:0}$	0.15	0.06	0.28	0.10	<0.01
$C_{20:1}$	0.14	0.06	0.19	0.06	<0.01
Σ saturated fatty acids	71.51	3.33	61.02	1.75	<0.01
Σ unsaturated fatty acids	28.08	1.92	39.00	1.66	<0.01
$C_{16:0}$ / ($C_{18:0}+C_{18:1}$+ c-9,t-11-$C_{18:2}$ $+\Sigma C_{18:2}+C_{18:3}$)	0.89	0.10	0.53	0.05	<0.01
$C_{16:0}$ / ($C_{18:1}$+ c-9,t-11-$C_{18:2}$ $+\Sigma C_{18:2}+C_{18:3}$)	1.25	0.15	0.72	0.06	<0.01

The results are shown for each FAME as a percentage of total FAMEs. x: mean value; σ: standard deviation; *: sum of all conjugated geometric and positional isomers of linoleic acid except for cis-9,trans-11-$C_{18:2}$ isomer.

acids were significantly more abundant in W cheeses whereas stearic ($C_{18:0}$) and arachidic ($C_{20:0}$) acids were greater in the S products, confirming the results of other authors [2, 3, 6, 13]. Among mono-unsaturated short- and medium-chain fatty acids, $C_{10:1}$ and $C_{14:1}$ were more abundant in W products while $C_{16:1}$ was more abundant in S cheeses, as already shown by Collomb et al. [6].

Transition from the hay/concentrate diet to the grazing diet was also followed by a great increase in the proportion of saturated, mono- and poly-unsaturated long-chain fatty acids ($C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$).

Very important for its nutritional effect is the increment in the S cheeses of conjugated linoleic acids, also known as CLA, that have been recently recognized as anticarcinogenics. In Table II the percentage of the most important isomer, the cis-9, trans-11 linoleic acid or rumenic acid and the sum of the other five geometric and positioned isomers are reported separately for these compounds.

Rumenic acid is reported separately because it is considered to be the most important CLA in terms of anticarcinogenic activity. In fact, it is the only isomer incorporated into the phospholipid fraction of tissues, it modulates the activity of cytochrome P450, reduces the induction of ornithine decarboxylase and protein kinase C, known as tumour production indicators, and probably inhibits protein and nucleotide biosynthesis [28].

CLA are the intermediate stage of full biohydrogenation of linoleic acid to stearic acid [21, 22, 29], so their higher amount in S cheeses can be linked to the higher amount of $C_{18:1}$ and $C_{18:0}$ in these cheeses.

The high proportion of c-9, t-11-$C_{18:2}$ found in the S cheeses can be due to pasturing, as reported by other authors [12, 23, 25, 32] but also to the milk-contaminating microflora activity [19, 30] and the prolonged cooking of the curd (45–48 min at 40–45 °C in the summer production; 15–20 min at 37–40 °C in the winter production).

As reported by Lin et al. [24], the agitation of cheese curd during cooking facilitates CLA formation by incorporating air, enhancing the initiation of lipid oxidation and the production of linoleic radicals. Moreover, the interaction between proteins and fat globules increases and enables proteins to donate hydrogen to convert linoleic acid radicals to CLA.

The great increase in the S cheeses of saturated, mono- and poly-unsaturated long-chain fatty acids ($C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$) leads to a reduction in $C_{16:0}/(C_{18:1} + C_{18:2} + C_{18:3})$ (1.20 for W cheeses; 0.67 for S cheeses) and $C_{16:0}/(C_{18:0} + C_{18:1} + C_{18:2} + C_{18:3})$ (0.86 for W cheeses; 0.50 for S cheeses) ratios, confirming the major influence of pasturing and particularly of mountain vegetation on milk fat composition [6, 31, 33, 35].

Compared with W cheeses, in the S cheeses there is also an increase in n-3 fatty acids and a decrease in the n-6/n-3 ratio due to cows grazing in mountain pastures, with a very important nutritional effect since omega-3 polyunsaturated fatty acids are recognized as playing an essential role in human health and are particularly important for the proper functioning of the brain, heart and the retina of the eye [17].

4. CONCLUSION

This preliminary work has confirmed that, for "Ossolano" cheese, different feeding can significantly influence the cheese fat composition. Saturated short- and medium-chain fatty acids were thus more abundant in winter cheeses produced with the milk of cows fed on hay and concentrate. On the contrary, saturated, mono- and poly-unsaturated long-chain fatty acids were more abundant in summer cheeses produced with the milk of cows fed in mountain pastures. Very important

for its nutritional effect is the increment in CLA and n-3 fatty acids and the decrease in the n-6/n-3 ratio in these latter cheeses.

These differences in such factors of good quality and "typicality", along with other parameters such as terpenes, sesquiterpenes and aromatic polycyclic hydrocarbons could be used to indicate the mountain origin of the cheese with the prospect of an application for "Protected Denomination of Origin" (PDO).

ACKNOWLEDGEMENT

This research was supported by the Regione Piemonte – Assessorato Agricoltura – Project Interreg II "Caratterizzazione del formaggio Nostrano Ossolano" – Italy.

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