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CHEMICAL, NUTRITIONAL AND MICROBIOLOGICAL CHARACTERIZATION OF ORGANIC FONTINA PDO CHEESE

CARATTERIZZAZIONE CHIMICA, NUTRIZIONALE E MICROBIOLOGICA DEL FORMAGGIO FONTINA DOP BIOLOGICO

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ABSTRACT

Chemical, microbiological and nutritional characteristics of organic Fontina, an Italian Protected Designation of Origin (PDO) raw cow's milk cheese, and of bulk milk used for its production, were studied. Organic winter-indoor and summer-pasture productions were compared; organic summer samples were compared to conventional summer ones. The organic and conventional summer samples from fresh grass-fed cows showed better lipid-

RIASSUNTO

È stato condotto uno studio comparativo sulle caratteristiche chimiche e nutrizionali del formaggio Fontina DOP biologico prodotto durante i mesi di stabulazione e di alpeggio. Come conseguenza dell'alimentazione al pascolo delle bovine, i campioni di Fontina prodotti durante l'estate (sia biologici che convenzionali) così come i corrispondenti campioni di latte, hanno mostrato migliori caratteristiche nutrizionali: quantitativi più bassi di acidi gras-

- Key words: conjugated linoleic acid, fatty acid composition, Fontina PDO cheese, microbiological quality, organic dairy products, seasonal variation -

ic compositions than the winter ones. The hypercholesterolemic saturated fatty acids content was lower while the unsaturated fatty acids and rumenic acid contents were higher. No significant chemical and microbiological differences were observed between the organic and conventional summer milk and the cheese samples.

si saturi ipercolesterolemici e contemporaneamente valori più elevati di acidi grassi insaturi totali e di acido linoleico coniugato. Una seconda indagine è stata poi realizzata per verificare l'eventuale esistenza di differenze a livello chimico-microbiologico e nutrizionale tra i campioni di Fontina biologica e convenzionale prodotti durante l'estate. A tale riguardo non sono state rilevate differenze significative tra le due tipologie.

INTRODUCTION

Fontina cheese is a full fat semicooked cheese made with raw whole milk from a single milking exclusively from dual-purpose autochthonous Aosta cows (Red Pied, Black Pied and Chestnut). It is only produced locally in the mountain territory of the Autonomous Region of Aosta Valley (northwestern Italian Alps) on a small-medium scale, but is well known and appreciated throughout Italy and in other parts of Europe. Since 1996 Fontina cheese has had the Protected Designation of Origin (PDO) denomination conferred by the European Union Commission (COMMISSION REGULATION, 1996). In 2003, the Manufacturing Rules were updated by the Region of Aosta Valley (in MATHIOU, 2007) and specific government financing fostered a new marketing phase with the production of organic Fontina PDO cheese at the Fromagerie Haute Val d'Ayas (Brusson, Aosta, Italy). This new product came about as a consequence of ethical concerns of consumers regarding both animal welfare and environmental issues such as soil acidification and eutrophication and greenhouse gas emissions. In particular, consumers are aware that organic foods have healthier and safer properties than traditional foods, and there has been increased interest and a growing demand for organic products, even though they are generally more expensive (MAGKOS *et al.*, 2006; WILLIAMS, 2002; ROSATI and AUMAITRE, 2004; HÄRING, 2003). Currently the Fromagerie Haute Val d'Ayas is the only cheese house that is allowed to produce organic Fontina PDO cheese in the entire Aosta Valley. About 1.3 million litres of cow milk are processed into organic Fontina cheese annually. Since 2003 about 13,000 forms have been produced according to the regulations for organic farming systems (COUNCIL REGULATION, 1991; COUNCIL REGULATION, 1999).

While organic Fontina is highly prized and consumed in great quantities, little is known about its chemical, microbiological and nutritional/nutraceutical features. Moreover, as the characteristics of traditional Fontina PDO cheese are known to vary widely between winter (hay/concentrate-fed cows) and summer (alpine pasture-fed cows) production (CHATEL *et al.*, 1995; CHATEL *et al.*, 1997), similar variations would be expected to occur in organic Fontina, but no studies have been conducted to date to determine these variations.

Dietary factors are known to be the cause of the greatest variation in the amount of some important, biologically active fatty acids such as rumenic acid (*c*9*t*11 C18:2, the most abundant isomer of conjugated linoleic acid, to which the acronym CLA is referred in this study), in milk and dairy products (DHIMAN *et al.*, 2005). Milk fat obtained from cows grazing on alpine pastures has very high levels of CLA and omega-3 fatty acids (VAN DORLAND *et al.*, 2006). CLA has been shown to have biological activities that protect against carcinogenesis, obesity, diabetes, arteriosclerosis and other inflammatory diseases (COLLOMB *et al.*, 2006). Omega-3 fatty acids ensure brain structure and function and are negatively correlated with coronary heart diseases and psychiatric disorders (KRIS-ETHERTON *et al.*, 2003; BUCHER *et al.*, 2002; BOURRE, 2005).

Differences between winter- and summer-produced cheeses are mainly due to the chemical and microbiological characteristics of the milk. In the case of Fontina, better chemical characteristics of summer milk are combined with lower microbial contamination (CHATEL *et al.*, 1997), due to the particular conditions in which the cows are fed and milked in Aosta Valley (BARMAZ, 1992; CHATEL and BASSIGNANA, 2004). The lactic acid bacteria in summer milk frequently have a greater capacity to multiply and colonize than winter milk (AMBROSOLI and PISU, 1996). The summer milk also has a better aptitude for overcoming coliforms which therefore have less impact on cheese quality (CHATEL *et al.*, 1993-94; BARMAZ, 1995; CHATEL *et al.*, 1997). This factor is very important in the production of Fontina cheese that is typically characterized by a "low" acidifying activity of the lactic microflora involved (BATTISTOTTI *et al.*, 1976, 1977; MATHIOU, 2007), which has particular genetic features (BATTISTOTTI *et al.*, 1993; ANDRIGHETTO *et al.*, 2002) and is critical for attaining the typical sensorial characteristics of the final product.

Another area of interest involves the variation in quality between organic and conventional Fontina PDO cheese. To date there have been a limited number of studies to compare the chemical, nutritional and microbiological features of milk and dairy products obtained from organic and conventional farming systems. Moreover, the approach used in most of these studies (foods generally purchased from retail markets) could be affected by uncontrolled confounding factors (WILLIAMSON, 2007; BOURN and PRESCOTT, 2002). In general, organic products have a better fatty acid profile than conventional ones (more CLA and n3 fatty acids), but the results obtained by different authors are not always in agreement (BISIG *et al.*, 2007). BERGAMO *et al.* (2003) reported a marked difference between the fatty acid composition in organic and conventional Fontina cheese. This result could be quite misleading, because the difference could be related to winter production only. The differences in the chemical composition between organic and conventional Fontina cheeses should be greatly influenced by the season during which the milk is produced. Organic and conventional Fontina cheeses made from summer milk (cows have an almost identical pasture-based feeding regimen) are expected to have very similar gross composition and fatty acid profiles. From the microbiological point of view, mainly the safety aspects of organic *versus* conventional food have been evaluated; organic farming methods, while potentially liable to increase microbial contamination, do not have any higher risk of foodborne pathogens than conventional ones (MAGKOS *et al.*, 2003a; CENCIČ and BOREC, 2004; CHEN, 2005; MACHADO *et al.*, 2006). No conclusive information is available about microorganisms of technological interest in organic food.

The aims of this study were to: 1) determine if there are any differences in the chemical and nutritional attributes of organic Fontina PDO cheese made from winter- or summer-milk; 2) investigate whether organic and conventional Fontina PDO cheeses produced from summer milk can be distinguished based on their fatty acid composition and/or microbiological features; and 3) verify if the same trends observed in organic and conventional Fontina PDO cheeses also occur in the corresponding raw milk used to produce them. The influence that Fontina cheese processing has on the fatty acid profile was also evaluated, because no previous studies are available in the literature on this subject.

MATERIALS AND METHODS

Animal feeding and management

According to the latest Manufacturing Rules for Fontina PDO cheese (MATHIOU, 2007), cows were only fed with forage grown in the Aosta Valley territory during the entire experimental period (December 2006-August 2007).

From December to May, the cows (according to the organic farming system) were kept indoors on the farms located in the valley bottoms (800 to 1,000 m a.s.l.) and were fed the typical dietary regimen used for milk destined for the production of organic Fontina PDO cheese. The base diet consisted of an average of 70% hay and 30% concentrates; all feed was produced by organic agricultural methods, no chemical fertilizers or pesticides were used.

In June and July, cows (under conventional and organic farming systems) were allowed to pasture at medium altitudes (1,500-2,000 m a.s.l.), and then moved to higher altitudes (>2,000 m a.s.l.) where they stayed until the end of August. The traditional daily grazing regime allowed the cows to pasture for a total of six-seven h/day; they were moved indoors twice a day for milking (at 4:00 a.m. and 4:00 p.m.). For both organic and conventional systems, pasture feeding was characterized by *ad libitum* fresh grass plus a daily supplement of 1.5-2 kg of concentrate per head in order to meet nutritional requirements. The concentrate composition was in accordance with organic farming rules. The conventional system used a similar pasture-feeding regime but application of chemical fertilizers may have been used in the pasture lands.

Cheese-making

All Fontina PDO cheese samples were produced according to the Fontina PDO cheese Manufacturing Rules (MATHIOU, 2007). Briefly, milk from a single milking was collected in steel boilers, starters were added and the milk was coagulated with bovine rennet at 36°C (coagulation time 40 min). Starters were made up of a mixture of autochthonous strains of *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *lactis* and *Lactococcus lactis* produced under the control of the Aosta Valley government. The curd was cut into maize grain-sized pieces and the curd-whey mixture was heated to 48°C under stirring. After 10 min rest in the whey, the curd was extracted and moulded in typical Fontina forms. Fontina PDO cheese has a typical flattened cylindrical form (35-45 cm diameter, 7-10 cm height and 7.5-12 kg weight).

The forms were subjected to moderate pressing for about 12 h and then transferred to the maturing site. The cheese matured for 3 months in natural caves (5°-12°C at >90% relative humidity). Cheeses were turned daily and the surfaces were manually salted and washed (*frottages*) with brine. During such period the typical red-brown rind smear is obtained.

Sampling

Milk and cheese samples were collected from the Fromagerie Haut Val d'Ayas (Brusson, Aosta, Italy). The organic bulk milk samples were collected monthly from December 2006 to August 2007; this time period covered the seasonal variation and was almost the entire lactation cycle of the dairy cows. In Aosta Valley calving commonly occurs in the autumn and winter months so that the cows are put out to pasture in the late lactation period when they have fewer nutritional requirements (CHATEL *et al.*, 1995; CHATEL *et al.*, 1997; MATHIOU, 2007). From December to May (stabled period) six samples (one sample/month) were taken and in June-August (summer pasture) six samples were collected (two samples/month, on consecutive days in order to have the same number of samples for all the groups for the statistical analysis). From June to August conventional bulk milk samples were also collected monthly (six samples), following the same schedule as that for summer organic samples. All milk samples were collected from the steel boilers just before the starters were added.

The batches of Fontina PDO cheeses corresponding to each of the collected milk samples were marked and ripened. A total of 18 forms were produced from the above-described milk batches (6 winter organic, 6 summer organic and 6 summer conventional). At the end of the three-month ripening period, a representative sample of each cheese batch was taken for the analyses. Cheese samples for microbiological analysis were only taken in the summer period.

Chemical analysis

Chemical and fatty acid composition of milk

One aliquot of each milk sample was stored in a portable refrigerator at 4°C and immediately transported to the laboratory to analyse the fat, protein, lactose and urea contents, and to determine the somatic cell count (SCC). A second aliquot to be used for the fatty acid analysis was stored for 4-5 h at 4°C before freezing at -20°C.

Milk fat, protein, lactose and urea contents were determined by infrared spectroscopy (MilkoScan FT6000, Foss Electric, Hillerød, Denmark) and the somatic cell count was carried out using an automatic cell counter (Fossomatic 5000, Foss Electric, Hillerød, Denmark).

For the fatty acid analysis, milk fat was extracted by centrifugation according to LUNA *et al.* (2005). The fatty acid methyl esters (FAME) were prepared by esterification with boron trifluoride as catalyst (AOAC, 2000) and determined by a gas chromatograph equipped with a HP88 capillary column (100 m x 0.25 mm ID, 0.2 m film thickness; J&W Scientific). The column temperature was held at 60°C for 1 min and then increased 20°C min^{-1} to a final temperature of 190°C, where it was held for 40 min. Temperatures of the injector and flame-ionization detector were maintained at 250° and 280°C, respectively; the injection volume was 0.1 μL; the nitrogen constant linear flow rate was set at 40 mL min^{-1}. Peaks were identified by comparing the retention times with pure CLA isomers (Matreya Inc., Pleasant Gap, PA, USA) and FAME (Restek Corporation, Bellefonte, PA, USA) standards. The fatty acid composition of the milk is expressed as the percentage of each fatty acid methyl ester per total FAMEs detected.

Chemical and fatty acid composition of cheese

Cheeses were cut into slices, the rind was removed and then the samples were frozen at -20°C until analysed. Dry matter (DM), ash, fat (EE) and protein determinations were performed according to AOAC (2000).

For the fatty acid analysis, after acid

hydrolysis of the sample, total lipids were extracted as reported by FOLCH *et al.* (1957). Esterification and fatty acid methyl esters separation, identification and quantification were carried out as described for milk.

All analyses were performed twice.

Microbiological analysis

Milk samples were collected in sterile 20-mL plastic screw-top containers, stored at 4°C in a portable refrigerator and immediately transported to the laboratory for the following analyses:

total aerobic mesophilic bacterial count on Petrifilm (3M) "Aerobic Count Plate" (72 h incubation at 30°C);

Coliforms and *Escherichia coli* on Petrifilm (3M) "*E. coli*/Coliform Count Plate" (24-48 h incubation at 37°C);

Proteolytics on Milk agar (Oxoid) (72 h incubation at 30°C).

For cheese analyses, the interior of each form was aseptically sampled in different points to obtain a final sample of approximately 100 g, which was submitted to the following determinations:

total aerobic mesophilic bacterial count on Petrifilm (3M) "Aerobic Count Plate" (72 h incubation at 30°C);

Coliforms and *Escherichia coli* on Petrifilm (3M) "*E. coli*/Coliform Count Plate" (24-48 h incubation at 37°C);

Yeasts on YGC agar (Oxoid) (5 days incubation at 25°C):

Streptococcus thermophilus on M17 agar (Oxoid) (24-48 h incubation at 45°C);

Lactobacillus spp. (thermophilic) on MRS agar (Oxoid) (48-72 h anaerobic incubation at 45°C);

Lactococcus spp. on M17 agar (Oxoid) (24 h incubation at 22°C).

All analyses were performed twice.

Statistical analysis

Based on the functional effects of individual fatty acids on cholesterol metabolism, the hypocholesterolaemic to hypercholesterolaemic fatty acids ratio (HH) was also assessed. Some changes were made in the mathematical equation reported by SANTOS-SILVA *et al.* (2002): 1. C20:4n6, C20:5n3, C22:5n3 and C22:6n3 were not considered in this study because they were not detected; 2. lauric acid was introduced in the denominator as a potential cholesterol-increasing agent (GERMAN and DILLARD, 2004); 3. myristic acid was multiplied by four because it has a greater influence as a hypercholesterolemic fatty acid than lauric and palmitic acids (GERMAN and DILLARD, 2004). The final formula was:

$$HH = (C18{:}1n9 + C18{:}2n6 + C18{:}3n3) / (C12{:}0 + 4*C14{:}0 + C16{:}0).$$

The statistical analysis of the data was performed using the SPSS software (2004). The data (for both milk and cheese) were submitted to one-way analyses of variance (ANOVA) according to the following model:

$$X_{ij} = \mu + \alpha_i + \varepsilon_{ij},$$

where: X_{ij} = observation; μ = overall mean; α_i = effect of type of dairy product (1 = winter organic; 2 = summer organic; 3 = summer conventional); ε_{ij} = residual error.

The assumption of equal variances was assessed by Levene's homogeneity of variance test. If such an assumption did not hold, the Brown-Forsythe statistic was performed to test for the equality of group means instead of the F one (SPSS, 2004). Pairwise multiple comparisons were performed to test the difference between each pair of means (Tukey's HSD test and Tamhane's T2 in the cases of equal variances assumed or not assumed, respectively).

The effect of the cheese-making process on the fatty acid profile was assessed by performing a paired sample t-test. A correlation analysis between fatty acids determined in Fontina cheese samples and in the corresponding unprocessed milk samples was also carried out.

Raw microbiological data were submitted to the independent sample t-test, in order to detect possible differences between organic and conventional samples.

Significance was declared at $P<0.05$. All data are expressed as mean ± standard deviation (SD).

RESULTS AND DISCUSSION

Chemical characteristics

Gross composition and somatic cell count

The gross composition of Fontina PDO cheese produced under organic (indoor and outdoor production) and conventional (outdoor production) farming systems is shown in Table 1. No statistically significant differences were found in dry matter, ash, protein and fat contents between organic cheese from winter/spring and summer productions. The organic and conventional samples from summer production also had very similar values. All cheese samples had >45% fat content (on the dry matter), as required by the Fontina Valle d'Aosta PDO Manufacturing Rules (in MATHIOU, 2007).

Concerning the corresponding milk samples, organic milk fat and protein percentages were significantly higher while lactose content was significantly lower in summer with respect to winter/spring months (Table 2). The stage of lactation is one of the main parameters that influences the gross composition of milk in ruminants. In the advanced lactation period, fat and protein concentrations normally increase, while lactose, even if it is the least variable component of milk, decreases. From June to August Aosta cows are normally in late lactation; so the results of this study reflect the trends that are generally observed as lactation progresses (AULDIST *et al.*, 1998). In summer, the gross composition of the organic and conventional milk samples was very similar, as already observed for the Fontina cheese samples. Such results are in agreement with the previous findings of PIRISI *et al.* (2002) and TOLEDO *et al.* (2002) on ewe and cow milk, respectively. TOLEDO *et al.* (2002) reported that their results were coherent because the gross composition of raw milk is mainly determined by a combination of the breed and diet (in their study as in this one organic and conventional milk samples were derived from the same cow breeds and the same diet). TOLEDO *et al.* (2002) also reported that the milk urea contents were ap-

Table 1 - Chemical composition of Fontina PDO cheese from organic (winter/spring and summer production) and conventional (summer production) farming systems[1].

	Organic (winter/spring) n = 6	Organic (summer) n = 6	Conventional (summer) n = 6	P value
DM (%)	60.90 ± 1.70	60.30 ± 0.36	61.17 ± 0.45	0.698
Ash (%DM)	7.45 ± 0.21	7.00 ± 0.76	6.57 ± 0.35	0.281
Protein (%DM)	38.90 ± 1.84	39.60 ± 0.70	38.63 ± 0.38	0.688
Ether extract (%DM)	46.40 ± 0.71	45.80 ± 1.30	47.20 ± 1.01	0.364

[1]Values are expressed as mean ± standard deviation of the mean.
DM = Dry Matter.

Table 2 - Chemical composition and somatic cell count of bulk milk used for the production of Fontina PDO cheese from organic (winter/spring and summer production) and conventional (summer production) farming systems[1].

	Organic (winter/spring) n = 6	Organic (summer) n = 6	Conventional (summer) n = 6	P value
Fat (%)	3.47 ± 0.14 b	3.70 ± 0.06 a	3.83 ± 0.09 a	0.004
Protein (%)	3.17 ± 0.08 b	3.36 ± 0.09 a	3.39 ± 0.04 a	0.004
Lactose (%)	4.89 ± 0.03 a	4.82 ± 0.02 b	4.78 ± 0.03 b	0.001
Urea (mg dL^{-1})	20.65 ± 2.06	21.35 ± 3.22	21.45 ± 4.66	0.916
SCC (n° 10^3 mL^{-1})	162 ± 34 b	265 ± 45 a	318 ± 56 a	0.001

[1]Values are expressed as mean ± standard deviation of the mean.
Different letters within rows indicate statistically significant difference among milk types.
SCC = Somatic Cell Count.

preciably lower in organic samples with respect to conventional samples. This was attributed to a difference in production intensity (forage/concentrate ratio). In the current study no statistically significant difference was found in the urea content, which reflects no discrepancy in the levels of milk production between the two farming systems in Aosta Valley.

Milk SCC is an important indicator of udder inflammation and mastitis infection in cows. In the organic milk samples, the value increased markedly from 162,000 mL^{-1} in winter to 265,000 mL^{-1} in summer. This increase normally occurs in late lactation (COULON *et al.*, 1996) and while grazing on alpine pastures (LAMARCHE *et al.*, 2000) and was previously observed for the same breeds by BIANCHI *et al.* (2003). Organic samples usually had a lower SCC than the conventional ones as already pointed out by OLIVO *et al.* (2005), TOLEDO *et al.* (2002) and HAMILTON (2000). Conventional summer milk samples exceeded the average value of 300,000 cells mL^{-1}. This value is commonly considered the threshold limit caused by management difficulties and unsanitary conditions of the cows (COULON *et al.*, 1998).

Fatty acid composition

Seasonal variations

The fatty acid profiles of Fontina cheese (winter organic, summer organic and summer conventional samples) are shown in Table 3. Organic Fontina PDO cheese obtained during the outdoor grazing period had a substantially higher nutritional value than organic Fontina produced during the stabled months. Almost all saturated fatty acids, with the exception of caproic (C6:0) and stearic (C18:0) acids, showed significantly lower values during the summer production. The percentage differences ranged from 12% in palmitic (C16:0) acid to 20% in heptadecanoic (C17:0) acid. The caprylic (C8:0), capric (C10:0) and lauric (C12:0) acid contents were approximately 17-20% lower in summer samples, while the variation in myristic (C14:0) acid was less (13%). Such results are important because lauric, myristic and palmitic acids are universally considered detrimental for human health due to their hypercholesterolemic activity, while caproic and stearic acids (the latter is a relatively abundant saturated fatty acid in dairy products) do not exert the same unfavourable effect (GERMAN and DILLARD, 2004).

Among the monounsaturated fatty acids, only the oleic acid (C18:1n9) content was higher in the organic summer samples; the extent of variation was however quite important (17%). Even if the other monounsaturated fatty acids did not show any change due to seasonal variation, it must be considered that oleic acid is the most abundant monounsaturated fatty acid in dairy products and is favorable from a nutritional perspective (HAUG *et al.*, 2007).

Along with the polyunsaturated fatty acids, linolenic acid (LNA) values were significantly higher in the organic summer samples compared to the organic indoor samples. The CLA levels were higher in the organic summer products (both milk and cheese), but in cheese samples the differences with respect to the indoor production were not statistically significant. Such results are in agreement with previous findings on the seasonal variation in the fatty acid profile of dairy products (REGO *et al.*, 2008; MOLKENTIN and GIESEMANN, 2007; LOCK and GARNSWORTHY, 2003; PRECHT and MOLKENTIN, 2000). The results are certainly due to the different feeding strategies in the two periods of analysis. In fact, pasture-fed ruminants were found to provide an improvement in the quality of the fatty acid composition of milk in several studies (D'URSO *et al.*, 2008; ATTI *et al.*, 2006; FERLAY *et al.*, 2006; WHITE *et al.*, 2001). Furthermore, alpine pasture-fed cows produce milk that has an extraordinarily relevant dietetic value for human health due to its high CLA and n3 fatty acid content (LEIBER *et al.*, 2005; HAUSWIRTH *et al.*, 2004). These positive qualities are probably due to the species-rich alpine meadows (with a high proportion of secondary plant ingredients) and to the animal energy deficiencies which lead to body fat mobilization. No significant difference related to seasonal variation was found in the linoleic acid (C18:2n6, LA) content of the cheese.

In general, the summer organic Fontina had a lower total SFA, and higher total MUFA and total PUFA contents and a higher HH ratio with respect to winter production.

Organic *versus* conventional (summer)

There were no significant statistical differences between the fatty acid profiles of summer organic and summer conventional Fontina cheese samples, or for the milk used for its manufacture, with respect to all the parameters determined (Tables 3 and 4). This is in contrast with the previous findings by BERGAMO *et al.* (2003) who reported higher LNA (more than 50%) and lower LA (about -31%) contents in organic Italian products (Fontina cheese included) with respect to conventional ones.

Concerning conjugated linoleic acid, earlier studies are in contrast with each other. Moreover, in the study by MOLKENTIN and GIESEMANN (2007) a seasonal influence on the variation of the CLA content in organic and conventional milk was observed where organic milk had a higher CLA content in the summer (but not the winter) months. In the current study, the CLA levels did not differ statistically between organic and conventional samples. These findings are in agreement with the results of ELLIS *et al.* (2006), TOLEDO *et al.* (2002) and BUTLER *et al.* (2008), but are in contrast with the results obtained by JAHREIS *et al.* (1996), BERGAMO *et al.* (2003) and COLLOMB *et al.* (2008).

It has been suggested that some parameters related to the fat composition could be used as markers for distinguishing organic dairy products from conventional ones (BERGAMO *et al.*, 2003; ELLIS *et al.*, 2006; MOLKENTIN and GIESEMANN, 2007). They are the CLA/LA ratio, the PUFA/MUFA ratio, the n3 fatty acids content (which were found to be significantly higher in organic products) and the n6/n3 fatty acids ratio (which in contrast was higher in the conventional ones). Our results show that the fatty

Table 3 - Mean values and standard deviations of fatty acid methyl esters (% of total FAMEs) in organic (winter/spring and summer production) and conventional (summer production) Fontina PDO cheese[1].

	Organic (winter/spring) n = 6	Organic (summer) n = 6	Conventional (summer) n = 6	P value
C6:0	1.25 ± 0.29	1.25 ± 0.13	0.97 ± 0.04	0.106
C8:0	0.93 ± 0.08 a	0.77 ± 0.03 b	0.63 ± 0.11 b	0.001
C10:0	2.46 ± 0.27 a	1.96 ± 0.22 b	1.62 ± 0.09 b	0.002
C10:1	0.20 ± 0.06	0.16 ± 0.01	0.15 ± 0.01	0.251
C12:0	3.28 ± 0.39 a	2.63 ± 0.23 b	2.22 ± 0.12 b	0.003
C12:1	0.23 ± 0.04	0.21 ± 0.02	0.20 ± 0.03	0.467
C14:0	12.84 ± 0.72 a	11.13 ± 0.19 b	9.86 ± 0.35 b	0.000
C14:1	0.43 ± 0.11	0.41 ± 0.04	0.44 ± 0.09	0.925
C15:0	0.75 ± 0.11	0.44 ± 0.04	0.58 ± 0.25	0.170
C15:1	1.32 ± 0.03	1.29 ± 0.16	1.26 ± 0.13	0.821
C16:0	33.95 ± 2.55 a	29.83 ± 0.65 b	28.20 ± 0.42 b	0.005
C16:1	0.36 ± 0.07	0.28 ± 0.09	0.31 ± 0.07	0.338
C17:0	1.71 ± 0.09 a	1.36 ± 0.16 b	1.46 ± 0.14 b	0.005
C17:1	0.66 ± 0.06	0.63 ± 0.03	0.61 ± 0.04	0.373
C18:0	11.10 ± 1.00	12.13 ± 0.35	11.97 ± 0.46	0.169
C18:1*c*9	25.11 ± 2.95 b	30.34 ± 0.47 a	33.89 ± 0.87 a	0.001
C18:2*c*9*c*12 (LA)	2.41 ± 0.38	2.52 ± 0.33	2.60 ± 0.47	0.785
C18:3*c*9*c*12*c*15 (LNA)	0.18 ± 0.03 b	0.26 ± 0.05 a	0.22 ± 0.02 ab	0.023
C18:2*c*9*t*11 (CLA)	1.25 ± 0.04 b	2.39 ± 0.70 ab	2.82 ± 0.23 a	0.046
CLA/LA	0.53 ± 0.13 b	0.98 ± 0.38 ab	1.12 ± 0.29 a	0.015
n6/n3	13.78 ± 3.78	9.96 ± 2.52	11.96 ± 2.90	0.312
SFA	67.89 ± 2.78 a	61.51 ± 1.12 b	57.52 ± 0.95 b	0.000
MUFA	28.30 ± 3.05 b	33.33 ± 0.60 a	36.86 ± 0.72 a	0.001
PUFA	3.84 ± 0.34 b	5.17 ± 0.53 a	5.64 ± 0.27 a	0.000
SFA/UFA	2.13 ± 0.25 a	1.60 ± 0.08 b	1.35 ± 0.05 b	0.001
PUFA/MUFA	0.14 ± 0.02	0.16 ± 0.01	0.15 ± 0.01	0.324
HH	0.32 ± 0.05 b	0.43 ± 0.10 a	0.52 ± 0.03 a	0.000

[1]Different letters within rows indicate statistically significant difference among Fontina PDO cheese types.
LA = Linoleic Acid; LNA = Linolenic Acid; CLA = Conjugated Linoleic Acid; SFA = Saturated Fatty Acids; MUFA = Mononunsaturated Fatty Acids; PUFA = Polyunsaturated Fatty Acids; UFA = Unsaturated Fatty Acids; HH = Hypocholesterolemic to Hypercholesterolemic Fatty Acids ratio: HH = (C18:1n9 + C18:2n6 + C18:3n3) / (C12:0 + 4*C14:0 + C16:0).

acid analysis does not allow all organic and conventional dairy products to be clearly distinguished. In fact, both organic milk and Fontina cheese samples had the same PUFA/MUFA, CLA/LA and n6/n3 fatty acids ratios, as well as the same n3 fatty acid content, as conventional ones. The different feeding regimens in the two types of farming methods is the explanation (MAGKOS *et al.*, 2003b). In fact, organic farming systems are normally expected to provide larger amounts of fresh grass and less concentrates in the diet with respect to conventional ones, especially in the summer months (CROISSANT *et al.*, 2007). This was also true in Switzerland, even considering the noticeably high level of roughage (87%) normally used in Swiss mountain integrated farms (COLLOMB *et al.*, 2008). For this reason, many proposed analytical techniques for determining organic dairy products are based on parameters linked to pasture consumption. In

Table 4 - Mean values and standard deviations of fatty acid methyl esters (% of total FAMEs) in organic (winter/spring and summer production) and conventional (summer production) bulk milk used for the production of Fontina PDO cheese[1].

	Organic (winter/spring) n = 6	Organic (summer) n = 6	Conventional (summer) n = 6	P value
C6:0	1.16 ± 0.34	0.82 ± 0.54	0.79 ± 0.23	0.312
C8:0	0.89 ± 0.09 a	0.62 ± 0.06 b	0.58 ± 0.04 b	0.000
C10:0	2.34 ± 0.13 a	1.60 ± 0.13 b	1.64 ± 0.12 b	0.000
C10:1	0.27 ± 0.24	0.15 ± 0.03	0.13 ± 0.02	0.494
C12:0	3.00 ± 0.33 a	2.31 ± 0.09 b	2.34 ± 0.19 b	0.005
C12:1	0.22 ± 0.06	0.20 ± 0.05	0.20 ± 0.03	0.818
C14:0	12.43 ± 0.78 a	10.05 ± 0.05 b	10.01 ± 0.46 b	0.000
C14:1	0.37 ± 0.02 b	0.62 ± 0.07 a	0.61 ± 0.07 a	0.000
C15:0	0.58 ± 0.10 b	0.78 ± 0.06 a	0.78 ± 0.05 a	0.006
C15:1	1.24 ± 0.19	1.34 ± 0.16	1.29 ± 0.12	0.707
C16:0	34.12 ± 2.37 a	28.49 ± 0.68 b	28.43 ± 0.96 b	0.002
C16:1	0.36 ± 0.07	0.46 ± 0.08	0.46 ± 0.11	0.178
C17:0	1.83 ± 0.04	1.72 ± 0.46	1.76 ± 0.10	0.868
C17:1	0.69 ± 0.06	0.69 ± 0.09	0.67 ± 0.08	0.915
C18:0	11.88 ± 1.08	13.18 ± 0.60	13.33 ± 0.35	0.065
C18:1*c*9	25.10 ± 2.37 b	32.39 ± 0.43 a	32.44 ± 1.20 a	0.000
C18:2*c*9*c*12 (LA)	2.01 ± 0.10	2.20 ± 0.29	2.34 ± 0.06	0.198
C18:3*c*9*c*12*c*15 (LNA)	0.20 ± 0.04	0.23 ± 0.07	0.22 ± 0.02	0.633
C18:2*c*9*t*11 (CLA)	1.33 ± 0.09 b	2.25 ± 0.23 a	1.98 ± 0.15 a	0.000
CLA/LA	0.66 ± 0.03 b	1.04 ± 0.19 a	0.85 ± 0.04 ab	0.011
n6/n3	10.32 ± 2.32	9.94 ± 1.96	10.54 ± 1.19	0.935
SFA	68.21 ± 2.29 a	59.57 ± 0.89 b	59.67 ± 1.30 b	0.000
MUFA	28.26 ± 2.42 b	35.76 ± 0.50 a	35.80 ± 1.48 a	0.000
PUFA	3.53 ± 0.20 b	4.68 ± 0.38 a	4.54 ± 0.20 a	0.000
SFA/UFA	2.16 ± 0.21 a	1.47 ± 0.05 b	1.48 ± 0.08 b	0.000
PUFA/MUFA	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.861
HH	0.32 ± 0.05 b	0.49 ± 0.02 a	0.49 ± 0.04 a	0.000

[1]Different letters within rows indicate statistically significant difference among milk types.
LA = Linoleic Acid; LNA = Linolenic Acid; CLA = Conjugated Linoleic Acid; SFA = Saturated Fatty Acids; MUFA = Monounsaturated Fatty Acids; PUFA = Polyunsaturated Fatty Acids; UFA = Unsaturated Fatty Acids; HH = Hypocholesterolemic to Hypercholesterolemic Fatty Acids ratio: HH = (C18:1n9 + C18:2n6 + C18:3n3) / (C12:0 + 4*C14:0 + C16:0).

summer, both organic and conventional Fontina PDO cheeses are produced with milk derived from the same cow breeds grazing on very similar alpine pastures (climatic conditions, altitude and local flora) in a limited area of production. In contrast to the findings of COLLOMB *et al.* (2008), no differences were found in the proportion of grasses fed to cows in the two mountain farming systems (fresh grass from pasture *ad libitum* in both cases). The amount of concentrate supplementation was also comparable (about 2 kg *versus* 1.5 kg head^{-1} day^{-1} in organic and conventional farms, respectively). The slightly higher amounts of concentrates commonly used in the organic farms is, however, unusual because the use of concentrates is usually limited in organic farming systems although it can be justified for commercial reasons (added value of organic milk and cheese). The aboe-mentioned similarities in the diet do not allow organic dairy products to be distinguished on the basis of their fat composition.

Further investigations will be needed to determine if the above-mentioned markers are suitable for identifying organic and conventional Fontina PDO cheeses produced under indoor-stabled conditions and which markers can be used to differentiate organic from conventional Fontina cheese produced in the outdoor grazing months. Parameters correlated with fertilization (stable nitrogen isotopes) could be appropriate for the latter purpose. In fact, the ^{15}N content is higher in organic manure than in artificial fertilizers, thus affecting the relative $\delta^{15}N$ content in plants. In the organic farming systems the use of artificial fertilizers is prohibited; consequently, as previously pointed out by MOLKENTIN and GIESEMANN (2007), $\delta^{15}N$ may differ between organically and conventionally produced feed. Such differences could be reflected in the milk protein.

Effect of the cheese-making process

The majority of studies dealing with the influence of processing conditions on the fatty acid composition of dairy products have mainly related to variations in the CLA amounts only. Although results obtained from different studies are quite contrasting, the normal processing procedures for dairy products (such as fermentation steps, heat treatments, storage and ripening) have only a negligible effect on the CLA concentrations (BISIG *et al.*, 2007). In the current study, no statistically significant differences were observed in the CLA amounts between Fontina cheese and unprocessed raw milk. A strong correlation between the CLA content of milk and cheese was observed ($P<0.0001$). Such results are consistent with the earlier findings by LUNA *et al.* (2007) on Spanish cow and ewe PDO cheeses, and by NUDDA *et al.* (2005) on sheep Pecorino Romano and Ricotta cheeses.

To the best of our knowledge, only one previous study has dealt with the effect of milk composition on fatty acids other than CLA (LUCAS *et al.*, 2005). In general, Fontina cheese samples had significantly higher levels of lauric, myristic and linoleic acids and significantly lower levels of palmitoleic, heptadecanoic, heptadecenoic and stearic acids with respect to the corresponding milk ones (Table 5). Milk and Fontina cheese samples showed a statistically significant correlation for the majority of parameters determined. Similar to the results reported by LUCAS *et al.* (2005), the results of the present study show that caprylic, decanoic, lauric, myristic, palmitic, stearic and oleic acids, as well as CLA, LNA, total SFA, MUFA and PUFA were significantly correlated between the cheese samples and the corresponding unprocessed milk ones. In contrast to LUCAS *et al.* (2005), no statistically significant correlations were found for caproic and linoleic acids. In our study, none of the other fatty acids (decenoic, myristoleic, pentadecanoic, pentadecenoic, palmitoleic and heptadecanoic acids) were significantly correlated in milk and cheese samples. This latter group of fatty acids was not considered in the study by LUCAS *et al.* (2005). Further investigations will be needed to evaluate the influence of specific processing steps on the variation of individual fatty acids in milk and dairy products.

Microbiological characteristics

Organic *versus* conventional (summer)

Bulk milk had low microbial contamination (data not shown). The mesophilic bacterial counts for both organic and conventional systems were always below the legal limit for raw milk cheeses ($5,00x10^4$ CFU mL^{-1}), and the coliforms and proteolytics never exceeded a few hundred CFU mL^{-1}. No significant differences were found between the two systems and between the winter and summer productions, although

Table 5 - Effect of the Fontina cheese-making process on fatty acid methyl esters (% of total FAMEs)[1].

	Milk n = 18	Cheese n = 18	P value	Pearson's correlation coefficient	Sig.
C6:0	0.98 ± 0.38	1.18 ± 0.24	0.064	0.522	0.082
C8:0	0.74 ± 0.16	0.82 ± 0.15	0.053	0.712	0.009
C10:0	1.98 ± 0.39	2.13 ± 0.43	0.069	0.809	0.001
C10:1	0.21 ± 0.18	0.18 ± 0.05	0.609	-0.399	0.198
C12:0	2.66 ± 0.43	2.85 ± 0.55	0.012	0.933	0.000
C12:1	0.21 ± 0.05	0.21 ± 0.03	0.714	0.666	0.018
C14:0	11.23 ± 1.37	11.68 ± 1.40	0.020	0.919	0.000
C14:1	0.49 ± 0.14	0.43 ± 0.09	0.199	-0.054	0.866
C15:0	0.68 ± 0.13	0.63 ± 0.19	0.538	-0.520	0.083
C15:1	1.28 ± 0.16	1.30 ± 0.09	0.710	0.366	0.242
C16:0	31.29 ± 3.40	31.49 ± 3.18	0.487	0.961	0.000
C16:1	0.41 ± 0.09	0.33 ± 0.08	0.019	0.263	0.408
C17:0	1.79 ± 0.06	1.56 ± 0.06	0.005	0.412	0.183
C17:1	0.68 ± 0.06	0.64 ± 0.05	0.015	0.597	0.040
C18:0	12.57 ± 1.06	11.58 ± 0.88	0.000	0.956	0.000
C18:1*c*9	28.73 ± 4.15	28.61 ± 4.39	0.780	0.939	0.000
C18:2*c*9*c*12 (LA)	2.14 ± 0.21	2.49 ± 0.36	0.009	0.193	0.548
C18:3*c*9*c*12*c*15 (LNA)	0.21 ± 0.04	0.21 ± 0.05	0.682	0.703	0.011
C18:2*c*9*t*11 (CLA)	2.47 ± 0.60	2.65 ± 1.41	0.506	0.929	0.000
CLA/LA	1.15 ± 0.24	1.09 ± 0.60	0.660	0.621	0.031
n6/n3	10.28 ± 1.86	12.37 ± 3.45	0.040	0.445	0.147
SFA	63.92 ± 4.79	63.70 ± 5.02	0.681	0.935	0.000
MUFA	32.02 ± 4.31	31.70 ± 4.32	0.505	0.928	0.000
PUFA	4.07 ± 0.61	4.62 ± 0.90	0.000	0.956	0.000
SFA/UFA	1.82 ± 0.39	1.80 ± 0.39	0.629	0.960	0.000
PUFA/MUFA	0.13 ± 0.01	0.15 ± 0.02	0.000	0.907	0.000
HH	0.40 ± 0.10	0.40 ± 0.10	0.530	0.908	0.000

[1]Values are expressed as mean ± standard deviation of the mean.
LA = Linoleic Acid; LNA = Linolenic Acid; CLA = Conjugated Linoleic Acid; SFA = Saturated Fatty Acids; MUFA = Monounsaturated Fatty Acids; PUFA = Polyunsaturated Fatty Acids; UFA = Unsaturated Fatty Acids; HH = Hypocholesterolemic to Hypercholesterolemic Fatty Acids ratio: HH = (C18:1n9 + C18:2n6 + C18:3n3) / (C12:0 + 4*C14:0 + C16:0).

the latter usually had slightly higher counts. *Escherichia coli* was never detected. Such favourable hygienic reports are most likely correlated with the rapid processing of the milk (according to Manufacturing Rules) i.e. within 2 h of milking, when it still has natural antibiotic properties.

No significant microbiological differences were found between the summer cheeses from the two systems, due to the great variability among samples, as indicated by the high SD values (Table 6). The total lactic microflora at the end of the maturing period was abundant in both cases, but was slightly higher (about $1.3x10^8$ *versus* $8.0x10^7$ CFU g^{-1}) in conventional Fontina. In this case, *Streptococcus thermophilus* was the prevalent component (almost $1.0x10^8$ CFU g^{-1}), while in the organic system samples *S. thermophilus* was replaced by the mesophilic *Lactococcus* spp. group (where the starter species *Lactococcus lactis* is likely to be found). The thermophilic *Lactobacillus* spp. group

Table 6 - Microbial counts (CFU/g*10^3) in organic and conventional summer Fontina PDO cheeses[1].

Microbial Groups	Organic n = 6	Conventional n = 6	P value
Streptococcus thermophilus	$5.30x10^4 \pm 5.23x10^4$	$9.53x10^5 \pm 1.34x10^3$	0.516
Lactococcus spp.	$2.25x10^3 \pm 2.48x10^3$	450 ± 354	0.489
Thermophilic *Lactobacillus* spp.	$2.55x10^4 \pm 3.46x10^4$	$3.30x10^4 \pm 2.55x10^4$	0.830
Total Coliforms	77.45 ± 103	60.20 ± 84.57	0.872
Escherichia coli	0.51 ± 0.70	0.21 ± 0.28	0.654
Total Mesophilic Aerobic Count	$2.15x10^4 \pm 2.12x10^3$	$2.15x10^4 \pm 3.04x10^4$	1.000
Yeasts	11.50 ± 3.54	177.50 ± 243.95	0.512

[1]Values are expressed as mean ± standard deviation of the mean.

(where the other starter species *Lactobacillus delbrueckii* subsp. *lactis* belongs) was present in similar quantities (2.5-3.0x10^7 CFU g^{-1}) in both cheese types. Considering the very low contamination levels in the bulk milk, such high levels of the lactic microflora could be related to the use of starters at the beginning of processing.

At the end of the maturing period the coliform count was quite low (<1.0x10^5 CFU g^{-1}) in both organic and conventional Fontina. As desired in Fontina manufacturing, the coliforms were surpassed by the lactic acid microflora. *E. coli* were sporadically found, in very low numbers (<1x10^3 CFU g^{-1}).

The uniform microbial situation found in this study for both organic and conventional Fontina could have been due to the low number of cheese forms that were analyzed. It is quite probable that the starter used (the same for conventional and organic cheeses) played an important role in integrating the initially low autochthonous population and standardizing its subsequent evolution. It must be recalled that the starter is a mixture of locally isolated species that have particular genetic features (ANDRIGHETTO *et al.*, 2002) that have been specifically chosen to obtain a correct and consistent maturation.

CONCLUSIONS

This study provides the first characterization of the chemical, microbiological and nutritional quality of organic Fontina PDO cheese comparing the cheeses produced during the indoor period and the summer-pasture period; the organic and conventional Fontina cheeses produced in summer were also compared. As for the cheeses produced in the winter months, those produced from June to August had a higher HH ratio and higher total PUFA content (in both milk and cheese samples); the CLA (milk samples) and LNA (cheese samples) levels were also higher in the summer productions. These findings are consistent with a significant decrease in the SFA/UFA ratio and in the amount of total SFA observed in both the summer milk and cheese samples. Highly significant correlations were found between the main groups of fatty acids (SFA, MUFA and PUFA) as well as between parameters commonly used as nutritional indicators (SFA/UFA and HH) in milk and Fontina cheese. The results clearly show that the Fontina cheese produced in summer has better nutritional characteristics due to the beneficial effect of the pasture-based diet on milk fat composition.

As in previous reports (MAGKOS *et al.*, 2006), the results of this study did not attribute superior safety or nutritional quality to organic foods of animal origin with respect to conventional ones. It seems that the feeding regimen strongly affects the future nutritional value and quality of ruminant-derived products (BUTLER *et al.*, 2008; GIVENS, 2005). This assumption is confirmed by the fact that 1) no variation was found between organic and conventional milk and Fontina cheese samples during the grazing months due to an almost identical diet for the cows and 2) summer conventional milk and cheese samples had a better lipid fraction value than the winter organic ones.

Every country has different regulations for organic production, and each typical PDO cheese is produced according to particular rules that can lead to differences in the organic and conventional systems with respect to chemical and microbiological quality. This variation is also strongly affected by the season of production. Pasture-derived alpine dairy products (and not only the organic ones) often meet the requirements for organic production and have extraordinary nutritional/nutraceutical and physiological properties. For these reasons they should be recognized as "organic foods" regardless of the livestock farming system used.

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