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Fat-soluble vitamin contents and fatty acid composition in organic and conventional Italian dairy products

Paolo Bergamo[a,*], Elena Fedele[a], Luigi Iannibelli[b], Giovanni Marzillo[b]

[a]*National Research Council, ISPAAM, Via Argine 1085, Naples, Italy*
[b]*Regional Agency for Environmental Protection, Naples, Italy*

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Abstract

Fatty acid composition and fat-soluble vitamin concentrations were measured to compare the milk fat composition in organic certified milk and dairy products with those produced by conventional systems. Significantly higher *cis*-9 *trans*-11 $C_{18:2}$ (CLA), linolenic acid (LNA), *trans*-11 $C_{18:1}$ (TVA) and α-tocopherol (TH) concentrations were measured in organic buffalo milk and mozzarella cheese. Similar results were obtained from the analysis of heat-treated cows milk and dairy products where all organic samples contained significantly higher CLA, TVA, LNA, TH and β-carotene concentrations than did conventional dairy foods. A negligible influence of milk processing on CLA and TVA yield was seen. Among the different parameters, the CLA/LA ratio value better characterised organic versus conventional milk fat and its use as a marker for the identification of organic dairy products is suggested. The influence of animal diet, and potential implications of milk fat composition, on nutritional quality of organic dairy products is considered.

Keywords: Organic; Dairy products; Fat-soluble vitamins; Fatty acid composition; Conjugated linoleic acid

1. Introduction

Dietary milk fats, on account of their higher content of saturated fatty acids, have long been associated with a variety of human diseases; however, recent studies have focussed on the healthy components of milk fats, including conjugated linoleic acids (CLA) (Parodi, 1997). This is a group of positional and geometric fatty acid isomers derived from linoleic acid (LA) that has attracted considerable attention because of its potential beneficial role for human health (Whigham, Cook, & Atkinson, 2000). Milk fat is the richest dietary source of CLA (Parodi, 1999) in which the *cis*-9 *trans*-11 isomer accounts for 90% of total CLA isomers (Chin, Liu, Storkson, Ha, & Pariza, 1992) and a considerable research effort has been made to increase *cis*-9 *trans*-11 content in milk to enhance its nutritional quality. In this context, the improvement in milk CLA content was achieved by selective milk fat fractionation (O'Shea, Devery, Lawless, Keogh, & Stanton, 2001) and by animal diet manipulation; indeed grazing pasture (Dhiman, Anand, Satter, & Pariza, 1999) oil supplements (Baer et al., 2001; Kelly et al., 1999) and correct forage concentrate ratio (Jiang, Bjoerck, Fondén, & Emanuelson, 1996) have increased milk CLA content.

Milk fat represents a good dietary source of retinol, TH and β-carotene (Panfili, Manzi, & Pizzoferrato, 1994), which exert their antioxidant activity in biological tissues as well as in foods. β-Carotene and retinol scavenge both singlet oxygen and lipoperoxides, thus preventing or limiting the oxidation of fatty acid (Donnelly & Robinson, 1995).

One of the major objectives of the European Regulation is the production of high quality food (EC Regulation 2092/91 and 1804/99). Organic products increasingly attract consumers because they are persuaded that organic food has higher nutritional quality, and is safer and more wholesome than conventional products. However the term "organic" refers to the process not the final product and the improvement of food quality features is mostly associated with the production system rather than any intrinsic quantifiable

* Corresponding author. Tel. +39-081-569-6006; fax: +39-081-596-5291.
E-mail address: p.bergamo@iabbam.na.cnr.it (P. Bergamo).

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feature (FAO, 2000) and contradictory results are reported in the literature about quality differences between organic and conventional foods. In particular, insignificant differences were reported in a German review (Woese, Lange, Boess, & Bögl, 1997); by contrast, higher concentrations of nutrients (minerals and vitamin C) were reported in organically produced foods by Smith (1993) and by Worthington (2001). However, the lack of reliable comparative investigations of organic versus conventionally produced food is the main reason for the lack of stringent conclusion about the quality of organically produced foods (Häring, Dabbert, Offerman, & Nieberg, 2001).

In the absence of reliable data on compositional differences between organic and conventional dairy products, the aim of the present study was to determine fatty acid and fat-soluble vitamin contents in buffalo and cow's milk dairy products and to compare the composition of organic milk fat with that obtained by a conventional management system (rather than between individual products).

Tocopherolquinone yield reflects the consumption of TH, resulting from antioxidant activity (Faustman, Liebler, & Burr, 1999). In this study the levels of α-tocopherolquinone/α-tocopherol (TQ) were used as an index of oxidative stress and to examine the influence of processing on fatty acid composition.

Finally, the relationship between these parameters for dairy products obtained by different management system was tested and such differences were used as markers to distinguish organic from conventional milk fat.

2. Materials and methods

2.1. Materials

Fatty acid methyl esters (FAMEs) of $C_{14:0}$– $C_{18:3}$, *trans*-11 octadecenoic acid (TVA), CLA, retinol, β-carotene and TH standards (HPLC grade) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). d-α-Tocopherolquinone from Acros-Organics (Carlo Erba, Milan, Italy) was used. High purity solvents from Romil (Cambridge, UK) were used and other chemicals were of analytical grade.

2.2. Sample collection

"Organic" labelled food denotes products that have been produced in accordance with organic standards throughout production, handling, processing and marketing stages and a certification body is responsible for verifying that the product sold or labelled as organic is produced or prepared according to these guidelines. In particular, certification requirements, for animal food products to be labelled as "organic", entails husbandry practices intended to ensure quality rather than maximising production (EEC Regulation No 1804/99).

Buffalo mozzarella cheese is a typical cheese obtained by manual manufacturing steps (Mucchetti, Carminati, & Addeo, 1997) and with regional market distribution. Buffalo samples used in this study represented the total organic population (there are only three organic certified buffalo farms in Italy). Conventional samples were representative of a greater number of farms; indeed, buffalo milks processed in each conventional dairy were supplied by different farms (almost five) in each area. Duplicate raw buffalo milk and mozzarella cheese samples, produced with the same milk batch, were obtained from Italian certified organic farm dairies ($n=3$) or conventional dairies ($n=8$) in the province of Salerno, Caserta and Latina. Buffalo samples were collected during a 3-month period (from April to June), to minimise the effect of seasonal variations on milk fat composition, cooled to 4 °C and transported to the laboratory within 2 h.

Cow's milk dairy products, were industrially processed with different thermal treatments (Cappelli & Vannucchi, 1990). Four different brands ($n=2$ organic certified and $n=2$ conventional) of widely distributed dairy products (pasteurised and UHT-treated cow's milk, parmigiano cheese, mozzarella, butter, ricotta, crescenza and fontina) were purchased from supermarkets linked to the Europe-wide network of distribution.

2.3. Lipid extraction and fatty acid analysis

Lipids were extracted from samples with chloroform and methanol, as described by Bligh and Dyer (1959) and the fat amount was determined from dried lipid weights. Twenty milligrammes of extracted fat were derivatized to methyl ester as described by Chin et al. (1992). Gas-liquid chromatography analysis of fatty acid methyl esters (FAME) was carried out on a Carlo Erba gas chromatograph (model 5300) equipped with a flame-ionisation detector and a digital integrator. Aliquots (1 ml) of FAME in hexane were fractionated by a SP-2340 fused silica capillary column (60 m×0.25 mm i.d.×0.2 mm film thickness; Supelco Inc., Bellefonte PA) using He as carrier gas at a pressure of 3 bar. The injector and detector temperatures were 230 and 260 °C, respectively. The oven temperature was held at 170 °C for 26 min, then increased to 220 °C at 16 °C per min and held for 13 min. Fatty acids were identified by comparing the retention times with methylated standards and the amounts of individual fatty acids were calculated by dividing the area under the fatty acid peak by the sum of the total reported FAME peaks and expressed as mg/g of fat.

2.4. Fat-soluble vitamins and tocopherolquinone analysis

Concentrations of retinol, TH and α-tocopherolquinone were determined by HPLC according to a published procedure (Fedele & Bergamo, 2001). β-Carotene elution was monitored at 450 nm, by using a spectrophotometric detector (SPD-10Avp, Shimadzu, Kyoto, Japan) and its concentration was calculated on the basis of standard curves prepared with pure standard. Vitamin content was expressed as μg/g of fat and the TQ value (TQ = tocopherolquinone/TH × 100) was used to evaluate the extent of lipid oxidation.

2.5. Statistics

Samples were analysed at least in duplicate; the data were presented as averages ± standard deviation (SD). Data were subjected to analysis of variance. The significance of the differences between means was evaluated by Student's t test taking $P<0.05$ as significant. Trend curves, regression analysis and correlation test were done by the programme "Graph Pad Prism 3″ (Graph Pad Software, San Diego, CA). Significance was determined according to the test of Sokal & Rohlf (1994).

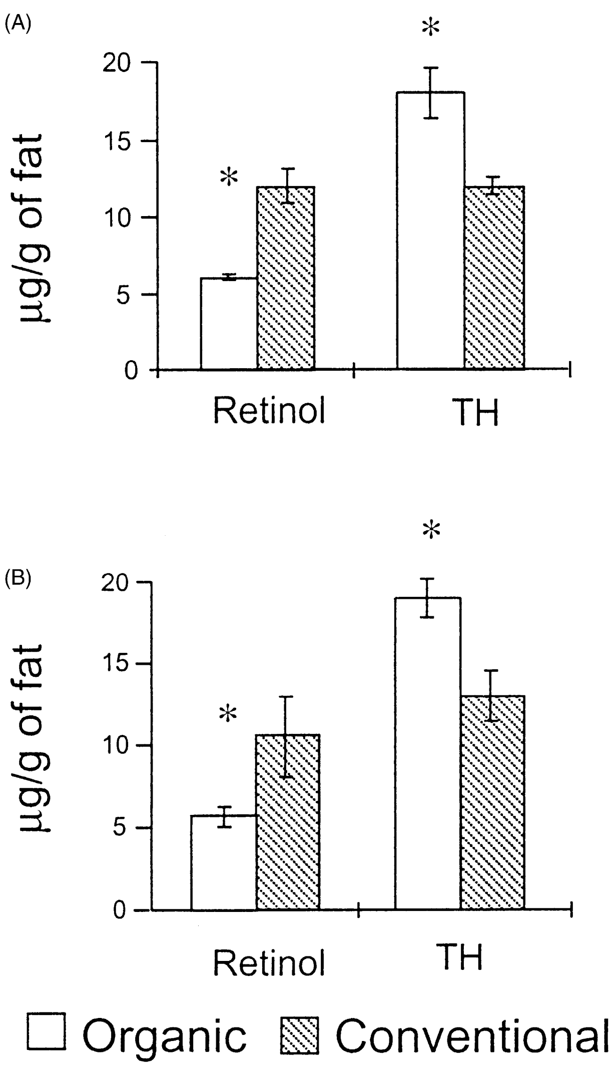

Fig. 1. Fat-soluble vitamin content and TQ level in buffalo milk and mozzarella cheese. TH and retinol concentration were measured in buffalo milk (panel A) and mozzarella cheese (panel B) obtained by organic (white) or by conventional dairies (grey). TH and retinol concentrations are expressed as μg/g of fat. Each sample was analysed in duplicate and values are the means ± S.D. Significant differences are shown as * ($P<0.05$).

3. Results

3.1. Fat-soluble antioxidants

TH and retinol concentration were measured in organic and conventional buffalo milk and mozzarella cheese samples. Significantly higher TH ($P<0.05$) and lower retinol concentration ($P<0.01$) were found in organic than in conventional milk (Fig. 1A). Similarly, higher levels of TH ($P<0.05$) and lower retinol concentration were measured in organic than in conventional mozzarella samples ($P<0.05$) (Fig. 1B).

Results obtained from the analysis of cow's milk products were comparable with those obtained in buffalo samples; indeed, marked differences in the concentrations of TH, retinol and β-carotene, between organic and conventional samples, were seen (Fig. 2). In particular, TH concentration in organic cows milk fat (21.9 ± 2.5; range 19.0–26.2 μg/g of fat) was significantly higher than that measured in conventional milk samples (15.0 ± 2.0; range 13.9–18.0 μg/g of fat; $P=0.00004$). Moreover, organic milk fat contained significantly higher amount of β-carotene (3.2 ± 0.7, range 2.4–4.8 μg/

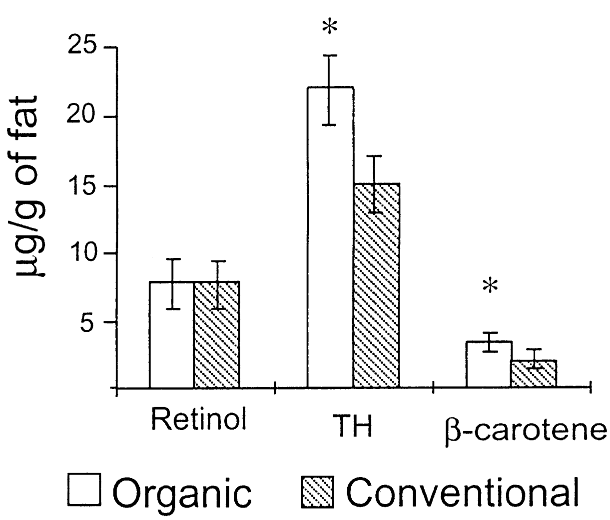

Fig. 2. Comparison between fat-soluble vitamin contents in certified organic and conventional cow milk and dairy products. Average values of fat-soluble vitamin content in organic and in conventional cow's milk products are reported (pasteurised and UHT) and in dairy product samples (parmigiano, mozzarella, butter, ricotta, crescenza and fontina) from organic (white) conventional dairy products (grey). Fat-soluble vitamin concentrations are expressed as μg/g. Each sample was analysed in duplicate and the values are the means ± S.D. Significant differences are shown as *($P<0.05$).

Table 1
Fatty acid composition of organic and conventional buffalo milk and mozzarella cheese

	Organic	Conventional
Milk		
Fat (%)	8.0±0.5 a	7.9±0.7 a
Fatty acids (mg/g of fat) (mg/g)		
$C_{14:0}$	117±3.5 a	120±6.5 a
$C_{16:0}$	344±7.8 a	357±8.9 b
$C_{16:1}$	21.6±1.0 a	19.5±0.8 a
$C_{18:0}$	126±1.5 a	130±2.6 a
TVA	26.2±1.8 a	13.3±0.9 b
$C_{18:1}$	217±2.2 a	233±3.5 b
LA	18.0±0.9 a	24.2±1.4 b
CLA	7.3±0.8 c	5.5±0.5 d
LNA	4.6±0.1 a	3.5±0.2 b
CLA/LA	0.41±0.02 c	0.23±0.01 d
Mozzarella		
Fat (%)	25.3±1.2 a	24.6±1.2 a
Fatty acids (mg/g of fat)		
$C_{14:0}$	112±2.5 a	119±6.9 a
$C_{16:0}$	334±6.8 a	330±9.8 a
$C_{16:1}$	22.0±1.2 a	19.8±1.9 a
$C_{18:0}$	134±2.5 a	132.0±2.9 a
TVA	30.5±2.0 a	14.9±1.0 b
$C_{18:1}$	229±3.1 a	245±4.6 b
LA	16.8±1.5 a	24.8±1.1 b
CLA	9.0±0.6 c	6.2±0.8 d
LNA	4.5±0.2 a	3.2±0.1 b
CLA/LA	0.54±0.03 c	0.25±0.01 d

Fat content and fatty acid composition were measured in buffalo milk and mozzarella obtained from organic and conventional dairies. Average values ± S.D. from duplicate analysis are reported. Fat content is expressed as g/100 g of product (%). Fatty acid concentration is expressed as mg/g of fat and means within the same row followed by different letters are statistically different (a–b, $P<0.05$, c–d, $P<0.01$). The value of the CLA/LA ratio was also calculated.

g of fat) than the conventional one (2.0±0.7, range 1.0–3.0 μg/g of fat; $P=0.004$). Finally, retinol content in organic milk fat (7.7±1.8, range 5.5–11.0 μg/g of fat) was not significantly different from that found in conventional products (7.8±1.8, range 6.2–12.0 μg/g of fat).

3.2. Fat content and fatty acid composition

The total fat content and fatty acid composition were determined in organic and in conventional buffalo milk and mozzarella samples. Fat content in organic samples was comparable with that found in conventional types, whereas significantly higher CLA, TVA and LNA concentrations ($P<0.05$, 0.01 and 0.05, respectively) were measured in organic buffalo milk fat. Moreover, lower LA concentration was measured in organic buffalo samples ($P<0.05$) and the value of the CLA/LA ratio was almost twofold higher in organic than that determined in conventional samples (0.5 and 0.2, respectively; $P<0.01$) (Table 1).

In order to confirm results obtained for buffalo milk samples, widely distributed organic and conventional dairy foods were analysed. Significant differences in total fat content between the two groups of samples were not found (data not shown) and insignificant differences in fatty acid composition were measured among duplicate samples. As shown in Table 2, CLA concentration in organic milk fat (8.8±2.1 mg/g of fat, range 6.2–11.8) was significantly higher than that measured in conventional samples (5.6±0.5 mg/g of fat; range 5.0–6.2; $P=0.004$). Moreover, significantly higher TVA content was found in organic than in conventional samples (22.5±4.1 versus 15.2±1.8 mg/g of fat; $P=0.001$), and LNA concentration in organic milk fat was higher than that measured in conventional milk fat

Table 2
Fatty acid composition in organic and conventional cow dairy products

	Past	UHT	Parmigiano	Mozzarella	Butter	Ricotta	Crescenza	Fontina
Conventional								
TVA	15.5±1.0	15.8±1.2	16.9±0.4	12.9±0.7	16.6±1.0	14.5±1.0	18.9±1.5	13.9±1.0
LA	30.2±2.2	28.9±1.5	25.3±2.1	24.6±1.8	22.0±1.9	24.5±1.8	27.1±2.3	29.6±2.2
CLA	5.1±0.4	6.2±0.3	6.1±0.2	5.0±0.3	5.7±0.2	5.0±0.3	5.4±0.4	6.2±0.3
LNA	5.2±0.3	5.6±0.2	6.9±0.2	5.5±0.1	5.5±0.6	4.5±0.4	4.7±0.3	6.4±0.5
CLA/LA	0.17±0.01	0.21±0.01	0.24±0.02	0.20±0.01	0.26±0.01	0.20±0.01	0.20±0.02	0.21±0.02
Organic								
TVA	23.3±2.0	16.2±1.5	20.1±1.8	17.5±1.7	23.5±2.0	23.9±2.1	23.4±2.0	23.9±2.1
LA	14.5±0.8	20.4 ±1.5	19.8±1.5	17.2±0.9	16.1±1.3	18.5±0.9	20.7±1.6	17.4±1.0
CLA	6.3±0.4	11.2±0.9	9.7±0.6	5.8±0.3	9.8±0.6	7.0±0.5	11.8±0.9	10.3±0.8
LNA	6.0±0.5	11.0±0.8	11.6±1.0	6.9±0.4	10.5±0.8	6.1±0.3	8.1±0.7	9.6±0.6
CLA/LA	0.43–0.02	0.55–0.02	0.49–0.03	0.34–0.02	0.61–0.03	0.38–0.03	0.57–0.03	0.59–0.03

TVA, LA, CLA and LNA contents in different brands of organic or conventional dairy products are reported. Fatty acid concentration is expressed as mg/g of fat. Average values ± S.D. from duplicate analysis are reported. Deviation over 5% from measured values was not found. The value of the CLA/LA ratio was also calculated.

(7.9±2.7 vs 5.1±1.2 mg/g of fat; $P<0.01$). By contrast, organic milk fat had a lower LA concentration (17.9±2.0 mg/g of fat; range 14.5–20.7) than conventional (26.1±2.7 mg/g of fat; range 22.0–30.2; $P=0.01$). Moreover, the CLA/LA ratios calculated in organic samples were approximately twofold higher (0.46±0.12) than those found in the corresponding conventional products (0.20±0.001; $P<0.0004$). Finally, CLA concentration was positively correlated with LNA ($r=0.72$; $P<0.01$) (Fig. 3A) and with TVA amount ($r=0.70$; $P<0.01$) (Fig. 3B). A correlation between CLA and LA content was also found ($r=0.54$; $P<0.05$) (Fig. 3C).

3.3. Fat oxidation extent

TQ level was calculated in order to evaluate the extent of milk fat oxidation and to examine the influence of lipid oxidation extent on the CLA and TVA yields. As expected, undetectable amounts of α-tocopherolquinone were found in buffalo raw milk whereas its amount was noticeably increased in mozzarella cheese. The fact that TQ levels in organic mozzarella cheese (7.3±2.1) were comparable with those obtained in conventional cheese (6.8±1.1) was consistent with the negligible influence of milk processing on CLA and TVA yields.

TQ values in organic cow milk fat varied from 2.2 to 10.2 (5.7±4.5) and from 2.0 to 11.0 (5.8±4.5) in conventional products. This wide range of variation might be explained by the different processing methods. However, the lack of correlation between TQ and CLA concentrations (data not shown) supported the negligible influence of thermal treatment intensity on CLA and TVA yields.

3.4. Organic milk fat characterisation

The percent difference (Organic value—Conventional value/Conventional value×100) was computed (Worthington, 2001) to compare the average content of a nutrient in organic to the corresponding conventional food and to establish which, among the used parameters, better distinguished organic from conventional milk fat. Results obtained on fat-soluble vitamins, fatty acid concentrations and CLA/LA level are shown in Fig. 4. The retinol and LA content in organic were 6 and 31% lower, respectively, than in conventional milk fat. Organic samples contained almost 50% more TH, TVA, LNA and CLA and 76% more β-carotene than conventional samples. Interestingly CLA/LA average value in organic milk fat was 131% higher than in conventional milk fat.

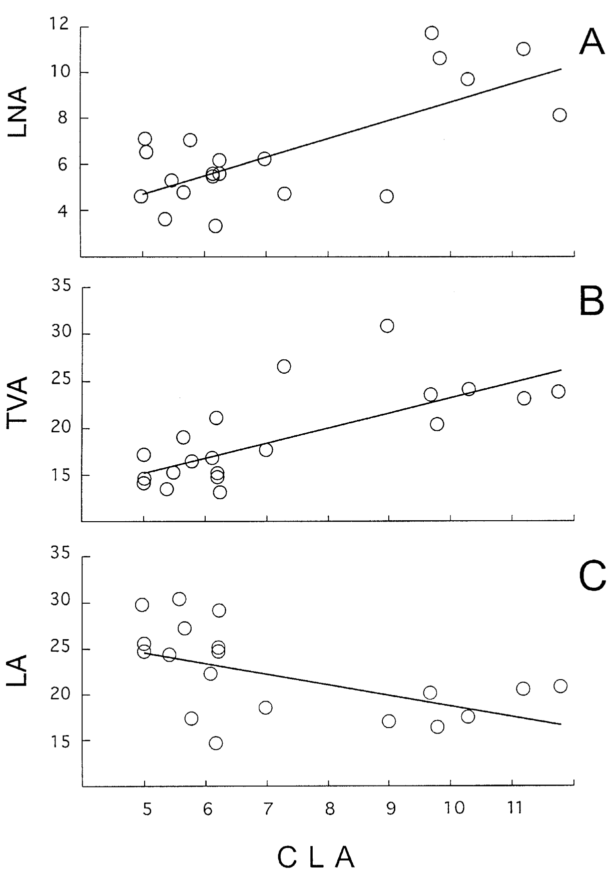

Fig. 3. Correlation between concentrations of CLA and TVA or LNA in the milk fat of buffalo and cow samples. The contents of different fatty acids are expressed as mg/g of fat and mean values are reported. **Panel A**: correlation between CLA and LNA ($r=0.072$; $P<0.01$) contents. **Panel B**: correlation between CLA and TVA contents ($r=0.070$; $P<0.01$). **Panel C**: correlation between CLA and LA contents ($r=0.054$; $P<0.01$).

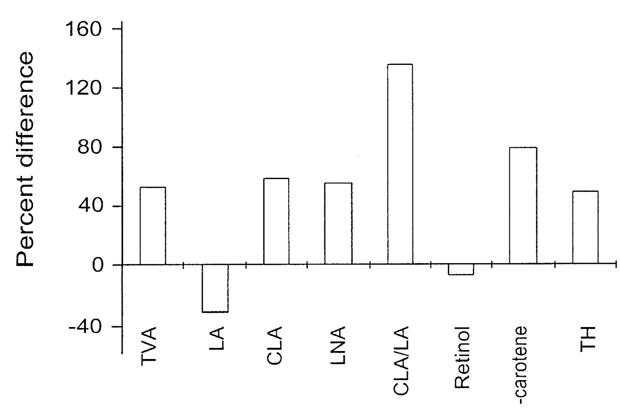

Fig. 4. Mean percent of fatty acid, CLA/LA ratio and fat-soluble vitamin content in organic compared to conventional products. The percent differences of individual fatty acids (TVA, LA, CLA, $C_{18:3}$), fat-soluble vitamin concentrations (TH, retinol, β-carotene) and CLA/LA level between organic and conventional products were computed (organic value−conventional value/conventional value×100) and used to evaluate which of the measured parameters best characterised organic milk fat.

4. Discussion

Milk fat is a good source of excellent quality proteins (Rosenthal, 1991) and fat-soluble vitamins, such as TH, retinol and β-carotene, that play a beneficial role in protecting milk lipids from oxidative degradation. TH, retinol and β-carotene concentrations in milk fat, measured in conventionally produced dairy foods, were similar to earlier findings (Panfili et al., 1994). To our knowledge, this is the first study that reported differences in fat-soluble vitamin concentrations between organic and conventional dairy products. Dietary intake of TH may be protective against cardiovascular disease (Meydani, 2000), mutagenic alteration (Claycombe & Maydani, 2001) and cancer (Negri et al., 1996) whereas carotenoids appear to have cancer preventive properties (Van Poppel, 1993). The finding of higher levels of TH and β-carotene in organic milk fat might have positive implications in human nutrition as these substances, besides the specifically protecting polyunsaturated fatty acids, reduce cholesterol oxidation and, therefore, its cytotoxicity and atherogenicity (Kumar & Singhal, 1991).

As expected, organic management resulted in the production of milk containing improved CLA, TVA and LNA concentrations (Jahreis, Fritsche, & Steinhart, 1996). In particular, CLA content in cow and buffalo milk fat was comparable with that reported in the literature (Chin et al., 1992; Lin, Boylston, Chang, Luedke & Shultz, 1995; Prandini, Geromin, Conti, Masoero, Piva & Piva, 2001). It is noteworthy that the finding of higher CLA, TVA and LNA amounts, in all organic samples analysed, strongly suggests a high nutritional value of organic milk fat. In particular, on account of the LNA importance for human nutrition and health (Connor, 2000) and, on the basis of the data reporting the cancer preventive effect of TVA and CLA-rich foods (Ip et al., 1999), the nutritional quality of organic dairy foods seems to be higher than that of conventional products.

Several factors have been shown to affect CLA concentration in milk fat, including animal diet (Parodi, 1999), seasonal variation (Parodi, 1977), endogenous synthesis from TVA (Griinari, Corl, Lacy, Chouinard, Nurmela, & Bauman, 2000) or free-radical oxidation of LA during processing (Ha, Grimm, & Pariza, 1989).

The influence of milk processing on CLA yield is controversial. In particular, CLA instability during processing and storage was reported by other authors (Garcia-Lopez, Echeverria, Tsui, & Balch, 1994; Shantha, Decker, & Ustunol, 1992). Heating conditions used in products examined ranged from no treatment (raw milk) to 142–145 °C for 2–5 min (UHT milk); TQ values were found to fluctuate over a wide range. TQ values in organic samples were comparable with those measured in their corresponding conventional products. This result together with the lack of correlation between TQ values and CLA concentration, indicated that the concentration of these fatty acids in cheese is mainly affected by milk fat content rather than by the intensity of heat treatment (Dhiman, Helmink, McMahon, Fife, & Pariza, 1999; Shantha, Ram, O'Leary, Hicks, & Decker, 1995).

Animal diets have a major influence on milk CLA content. Kepler and Tove (1966) identified CLA and TVA as intermediate products of the incomplete biohydrogenation of dietary LA by *Butyrivibrio fibrisolvens*, a hemicellulose-digesting bacterium, found in high numbers when cattle are fed hay or grass (Bryant & Small, 1956). Indeed, as increased CLA concentration was found in the milk of cattle fed with fibre-rich diets (Dhiman, Helmink et al., 1999), it is conceivable that an organic diet, containing at least 60% of the dry matter of roughage, fresh or dried fodder (EC Reg. 2092/91 and 1804/99), may well improve microbial biohydrogenation, yielding higher concentrations of these fatty acids. In addition, the higher fat-soluble vitamin content in organic dairy products is well attributable to the animal diet. In particular, the finding of a negative effect of forage intake on milk yield (Hullar & Brand, 1993), together with data reporting the lack of relationship between milk yield, TH and β-carotene milk contents (Jensen, Johansen & Hermansen, 1999) suggests that fibre-rich organic diets may improve fat-soluble vitamin concentration in milk by decreasing milk yield.

Interestingly, the CLA/LA percent difference between organic and conventional samples was noticeably higher (130%) than that measured by any individual indicator considered in this study. This result clearly indicates that the CLA/LA value better characterised organic milk fat than any other parameter.

A more extensive sampling is currently in progress to confirm the higher nutritive quality of organic milk fat and the value of CLA/LA as a specific indicator of organic versus conventional milk fat.

Acknowledgements

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