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Conjugated linoleic acid (CLA) and fatty acid composition of milk, curd and Grana Padano cheese in conventional and organic farming systems

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CLA levels and fatty acid composition were measured to compare the fat composition in organic bulk milk, destined to the production of Grana Padano cheese, with those produced by conventional system. The curds and Grana Padano cheeses were also analysed to evaluate the effects of the production technology on the CLA content. All analysed organic samples were characterized by higher annual means of CLA, vaccenic acid (TVA) and linolenic acid (LNA) in comparison with conventional samples (with $P<0{\cdot}05$). Nevertheless, no particular effect of the production technology was seen on the CLA content. The animal diet appears to be the factor which has the highest effect on the CLA concentration in milk and milk products and an organic diet based on fresh or dried forage, that is rich in CLA precursory fatty acids, may improve the yield of fatty acids with beneficial effects on health.

Keywords: CLA, milk, curd, Grana Padano, cheese, organic, conventional.

In this study, samples of organic and conventional bulk milk, destined for the production of Grana Padano cheese, were analyzed at different times between summer 2004 and summer 2005 for CLA level and fatty acid composition. The respective organic and conventional curds and Grana Padano cheeses were also analyzed. The aims of the present survey were to compare the CLA contents of organic milk and milk products with those obtained by a conventional management system and to evaluate effects of the Grana Padano production technology on CLA content.

Conjugated linoleic acids (CLA), a group of conjugated linoleic acid isomers, have been reported to have a wide range of beneficial effects, including: anticarcinogenic, antiatherogenic, antidiabetic and immune stimulatory (Nagao & Yanagita, 2005; Collomb et al. 2006). They have also been shown to alter nutrient partitioning and lipid metabolism, and reduce body fat in a number of different animal species (DeLany & West, 2000; Dugan et al. 2004).

Milk fat, cheese and ruminant meat are an important source of potential anticarcinogens from the naturally occurring CLA. *Cis*-9, *trans*-11-octadecadienoic acid is the major isomer (80–90%) (Sehat et al. 1999). CLA in ruminant milk arises both directly and indirectly from incomplete microbial hydrogenation of polyunsaturated fatty acids in the rumen. It is formed in the rumen by anaerobic bacteria as an intermediate in the biohydrogenation of linoleic acid (LA) and from desaturation of vaccenic acid (*trans*-11 $C_{18:1}$, TVA) in the mammary gland via Δ^9-desaturase (Bauman et al. 2001).

The concentration of CLA in milk has been reported to vary considerably through the animal diet; high CLA values were found with pasture feeding, organic diet and diet supplemented with plant or marine oils (Bergamo et al. 2003; Lock & Garnsworthy, 2003; Zheng et al. 2005; Shingfield et al. 2006; AbuGhazaleh & Holmes, 2007).

In cheese the CLA content is certainly affected by specific features of the milk used in manufacturing, with special reference to the species and the CLA content of the milk (Prandini et al. 2001, 2007), whereas the role of processing technology on CLA content of cheese is still controversial (Werner et al. 1992; Shanta & Decker, 1993; Gnädig et al. 2004; Bisig et al. 2007).

Materials and Methods

Animal feeding and sample collection

Grana Padano is an important PDO (Protected Designation of Origin) Italian cheese produced throughout Northern

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Italy and details of manufacturing technology are available at website of Dairy Science and Food Technology http://www.dairyscience.info/htm/grana.asp.

The samples of bulk milk, curds and cheese (both conventional and organic) were supplied by a dairy industry adhering the Consorzio per la Tutela del Formaggio Grana Padano (S. Vittoria Soc. Coop. a r.l.) situated in the plain of the province of Piacenza. On the basis of known animal dietary rations, cow daily intakes of CLA precursor fatty acids (α-linolenic and linoleic acids) were estimated using literature data of feed fatty acid composition: corn silage and concentrate (Elgersma et al. 2004), fresh alfalfa and alfalfa hay (Ribeiro et al. 2005), corn (White et al. 2007) and barley (Wang et al. 1993).

In conventional farming system the cows (Italian Holstein) were fed during all year with a mixed ration consisting of corn silage (20–26 kg/head/day), alfalfa hay (4–6 kg/head/day) and concentrates (9–12 kg/head/day). The daily intakes of α-linolenic and linoleic acids were an average of 81 and 217 g, respectively (total CLA precursor fatty acids = 298 g).

In organic farming system the cows' feeding was based on alfalfa pasture in late spring-summer and on alfalfa hay in late autumn-winter. The remaining diet consisted of concentrates (3 kg/head/day) and cereals (2 kg/head/day; barley : corn = 50 : 50). The daily intakes of α-linolenic and linoleic acids in the spring-summer periods were an average of 279 and 134 g, respectively (total = 413 g), whereas in the autumn-winter periods were an average of 141 and 106 g, respectively (total = 247 g).

The samples of conventional and organic bulk milk were collected at monthly intervals between August 2004 and July 2005 (excluding January and March 2005) and were analyzed for CLA content and fatty acid composition. The respective conventional and organic curds, obtained from the coagulation of raw cows' milk by the addition of bovine liquid rennet, were also analyzed both before and after cooking at 53–56 °C and as well as the Grana Padano cheeses aged 12 months.

The samples were frozen at −18 °C after sampling and defrosted before analysis. All analysis were effected in duplicate and carried out on samples of finely ground cheese and curd.

Chemical analysis

Lipid extraction was performed according to the 'Röse-Gottlieb method' (FIL-IDF 1A:1969) for milk samples and in cold conditions in accordance with the modified Folch's technique (Christie, 1989) for cheese and curd samples. The lipids were then esterified according to the method described by Bannon et al. (1985) with modifications.

CLA and fatty acid methyl esters were quantified using a GC (Varian 3350) equipped with a flame ionization detector and a CP-Select CB capillary column for FAME (100 m × 0·25 mm i.d.; 0·25 μm film thickness; Chrompack, Varian, Inc., CA). GC oven parameters, gas variables and fatty acid peak identification were as previously described (Prandini et al. 2007).

Milk, curds and cheese CLA content and fatty acids were expressed as g/100 g of total fatty acids, calculated with peak areas corrected by factors according to AOAC 963.22 method (AOAC, 2000).

Statistical analysis

The data were presented as annual averages. The simple analysis of variance according to the SAS® technique (SAS/STAT, version 8, 2000, SAS Inc., Cary, NC) was effected for fatty acid composition found in the different milk and milk products. The significance of the differences between means was evaluate taking $P<0·05$ as significant.

Results and Discussion

CLA level in bulk milk, curds, Grana Padano cheese

Gas-chromatographic analysis of FAME considered only the *cis*-9,*trans*-11 CLA isomer among all isomers of CLA. The *cis*-9,*trans*-11 CLA levels were expressed in g/100 g total fatty acids. Table 1 reports the annual average content of CLA in bulk milk, curds before and after cooking to 53–56 °C and Grana Padano cheese, produced by conventional and organic system. In the different sampling dates all the organic samples were characterized by higher *cis*-9,*trans*-11 CLA levels than those measured in the respective conventional dairy products (data not shown) with statistically significant differences ($P<0·05$) between the annual means.

No particular variation of CLA content was detected in the conventional and organic curds after the cooking. Furthermore, similar annual means of CLA content were found in the bulk milk and respective curds; only the conventional curds before heat treatment were characterized by an annual average of 18·76% significantly higher ($P<0·05$) compared with that of conventional bulk milk (statistical analysis not shown).

The annual averages measured in conventional and organic Grana Padano cheese were similar to those of the conventional and organic curds after cooking.

The CLA percentages found in the conventional and organic samples of bulk milk, curds and Grana Padano cheese show negligible effects of cheese cultures, processing conditions and aging period on the CLA level in accordance with Werner et al. (1992), Jiang et al. (1997), Gnädig et al. (2004), Nudda et al. (2005) and Ryhänen et al. (2005). The major contents of CLA measured in milk and milk products obtained from an organic management system, characterized by higher cows' intakes of CLA precursory fatty acids than a conventional management system, were in accordance with Jahreis et al. (1997) and Bergamo et al. (2003).

These results suggest that the CLA concentration in dairy products depends mainly on CLA content of the milk

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Table 1. Partial fatty acid composition (annual mean) and CLA level of conventional and organic bulk milk, conventional and organic curds before and after cooking to 53–56 °C, and conventional and organic Grana Padano cheese. Fatty acids are expressed as g/100 g total fatty acids

Fatty acids % (*annual mean*)	Bulk milk		Conventional curds		Organic curds		Grana Padano cheese	
	Conventional	Organic	Before cooking	After cooking	Before cooking	After cooking	Conventional	Organic
*Trans*11-18 : 1 (TVA)	1·00 ^{A}	1·62 ^{B}	1·08 ^{A}	0·99 ^{A}	1·56 ^{B}	1·58 ^{B}	1·07 ^{A}	1·71 ^{B}
18 : 2 (LA)	2·43 ^{A}	1·89 ^{B}	2·35 ^{A}	2·44 ^{A}	1·88 ^{B}	1·88 ^{B}	2·41 ^{A}	2·02 ^{B}
18 : 3 *omega*3 (LNA)	0·38 ^{A}	0·68 ^{B}	0·43 ^{A}	0·39 ^{A}	0·65 ^{B}	0·67 ^{B}	0·40 ^{A}	0·67 ^{B}
*cis*9, *trans*11-18 : 2†	0·52 ^{A}	0·82 ^{B}	0·57 ^{A}	0·52 ^{A}	0·76 ^{B}	0·79 ^{B}	0·55 ^{A}	0·86 ^{B}
Short chain‡	6·99	7·59	6·77 ^{A}	6·86 ^{AB}	7·59 ^{B}	7·16 ^{AB}	7·09	7·13
Medium chain‡	20·01	20·57	20·19	20·14	20·69	20·48	19·78	19·57
Long chain‡	68·28	67·57	68·21	68·20	67·23	67·72	68·40	68·29
Saturated	64·06	64·36	64·02	64·15	64·63	64·15	64·01 ^{A}	62·87 ^{B}
Monounsaturated	27·02	27·06	26·93	26·87	26·77	27·05	27·03	27·74
Polyunsaturated	4·21	4·31	4·20	4·19	4·11	4·16	4·23	4·38

† Conjugated linoleic acid
‡ Short chain fatty acids (C 4 : 0 – C 9 : 0); Medium chain fatty acids (C 10 : 0 – C 15 : 1); Long fatty acids (C 16 : 0 – C 22 : 6 *omega3*)
Different letters in the same line correspond to statistically significant differences ($P<0·05$) between conventional and organic samples of the same type (Bulk milk, Curds and Grana Padano cheese)

used in manufacturing. Variation in CLA content in milk has been associated with several factors such as stage of lactation, parity (Kelly et al. 1998), and breed (Secchiari et al. 2001; White et al. 2001). However diet is the most important factor influencing milk CLA concentrations (Collomb et al. 2002). Some authors reported higher CLA concentrations in the milk of cattle fed with hay or grass (e.g. Lock & Garnsworthy, 2003; Chilliard & Ferlay, 2004) and an increase of CLA content was found in cows' milk receiving fibre-rich diets by Dhiman et al. (1999). Therefore, 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 CLA compared with a conventional diet.

Fatty acid composition of bulk milk, curds, Grana Padano cheese

Also reported in Table 1 are the partial fatty acid compositions (annual means) of bulk milk, curds before and after cooking to 53–56 °C and Grana Padano cheese, produced by conventional and organic system.

Bulk milk. Similar contents of short, medium and long chain fatty acids and of saturated, monounsaturated and polyunsaturated fatty acids were detected in conventional and organic bulk milk. The organic bulk milk showed higher percentages of vaccenic acid (TVA = 1·62%) and α-linolenic acid (LNA = 0·68%) and a lower percentage of linoleic acid (LA = 1·89%) with statistically significant differences ($P<0·05$) versus conventional bulk milk.

Curds. The conventional and organic curds were characterized by a fatty acid composition similar to that of the corresponding bulk milk. A statistically significant difference ($P<0·05$) was found between mean content of short chain fatty acids in conventional curds (6·77%) and that in organic curds (7·59%) both before cooking. The organic curds showed higher levels of TVA and LNA before and after heat treatment and lower levels of LA in comparison with conventional curds with statistically significant differences ($P<0·05$). Particular variations of fatty acid composition were not detected in the curds after cooking to 53–56 °C.

Grana Padano cheese. No particular variation of fatty acid composition was seen among curds after cooking and respective Grana Padano cheeses except a statistically significant decrease ($P<0·05$) in the percentage of saturated fatty acids in organic Grana Padano cheese (62·87%) compared with organic curds (64·15%). Furthermore, organic Grana Padano cheese showed a lower content of saturated fatty acids (62·87%) than that of conventional Grana Padano cheese (64·01% with $P<0·05$). In organic Grana Padano higher percentages of TVA and LNA and lower LA content were detected compared with those found in conventional Grana Padano ($P<0·05$).

A negligible influence of the milk processing on the fatty acid composition of organic and conventional dairy products was seen. The major contents of TVA and LNA and the minor content of LA found in organic milk and milk products were in accordance with Bergamo et al. (2003). In their study, carried out on fatty acid composition and fat-soluble vitamin concentrations in organic and conventional Italian dairy products, organic management resulted in the production of milk containing improved CLA, TVA and LNA concentrations. Therefore, considering the positive effects of these fatty acids on the human health (Nagao & Yanagita,

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2005), the nutritional value of dairy foods from cows fed diet with fresh forage and/or hay in an organic system seem to be higher than that of conventional products.

Conclusions

The current study shows that organic milk and milk products are characterized by higher amounts of CLA, TVA and LNA in comparison with conventional milk and milk products. The lactic acid bacteria added to the milk in the production Grana Padano cheese, the processing technology, particularly heat treatment, and the aging do not appear to influence the CLA content in dairy products. Therefore, the animal diet is the factor which most affects the CLA content in milk and the CLA concentration in dairy products depend on the milk used in manufacturing. An organic diet based on fresh or dried forage, that is rich in polyunsaturated fatty acids, may remarkably improve the CLA yield in milk fat and so increase the nutritional quality of organic dairy products.

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