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Influence of milk quality and cheese-making procedure on functional fatty acid transfer in three Italian dairy products: Mozzarella, Raveggiolo and Ricotta

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ABSTRACT

Cheese quality traits are affected firstly by milk quality and secondly by processing protocols. The cheese-making procedure plays an important role in the transfer of functional fat components from milk to cheese with important implications on the nutritive value of dairy products.

To evaluate the influence of the cheese-making procedure, three fresh Italian cheeses from the same milk bulk, Mozzarella, Raveggiolo and Ricotta were processed and analyzed in terms of fatty acid transfer from milk to dairy products. The fatty acids were transferred with several differences. In particular, C14:0 ($P = 0.0011$) and C14:1 ($P = 0.0007$) showed the highest value in Raveggiolo while C16:0 was higher in Raveggiolo and Ricotta ($P = 0.0002$). *Trans*-monoene fatty acids have a detrimental effect on human health, however trans C18:1 isomers, from 6 to 10, and trans12 showed no significant differences in transfer from milk to dairy products. In contrast, vaccenic and linoleic acids, which are beneficial fatty acids for human health, were recovered in higher percentages in Raveggiolo than in Mozzarella and Ricotta ($P = 0.0188$ and $P < 0.0001$, respectively). The recovery of oleic acid, an antiatherogenic fatty acid, was higher in Ricotta ($P < 0.0001$). No effect on conjugated linoleic acid was found.

1. Introduction

The daily consumption of milk and derived products is recommended by nutritionists in the Mediterranean diet as a source of important nutrients such as essential amino acids, fatty acids (FAs) and calcium. With regard to the lipid fraction, cheese is a good source of functional FAs in the human diet (Minieri et al., 2018, 2020). Several functional FAs, such as conjugated linoleic acid (CLA) and conjugated linolenic acid (CALNA) or vaccenic acid (VA), are present in products derived from ruminant livestock as a consequence of rumen microbial activities in dietary FAs (Buccioni, Decandia, et al., 2012). Cheese also contains α-linolenic acid (α-LNA) derived from the dietary regimen of the animals, and oleic acid (OA) derived from the activity of the mammary tissue or from the endogenous energy reserve mobilized by the animals in energy deficit (Cabiddu et al., 2005; Correddu et al., 2016; Cosentino et al., 2021; Nudda et al., 2020; Toral et al., 2020; Uken et al., 2021).

The healthy properties of all these FAs are reported in literature (Banni et al., 2001; Belury, 2002; McGuire & McGuire, 1999; Pintus et al., 2012; Sofi et al., 2008; Song et al., 2019; Wang et al., 2020) and for OA and α-LNA, the European Food Safety Association (EFSA) published scientific opinions for their health claims (2009b, 2011a, 2011b). Extrapolating data from animal model studies, the dose of CLA that may provide bioactive effects for human health has been estimated to be 700–800 mg *per* day for an individual weighing 70 kg (Bauman & Lock, 2006). Several authors showed that functional FAs content in cheeses collected from large-scale distributions is highly variable, affecting the daily intake of them by consumers (Cicognini et al., 2014).

There are many kinds of cow cheeses, produced around the world with cheese-making procedures that differ in terms of milk quality and treatments, starter cultures, breaking and processing of the curd, and microbial activity during cheese ripening.

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Abbreviations

AA	arachidonic acid
ADF	acid detergent fiber
BCFA	branched chain fatty acid
CALNA	conjugated linolenic acid
CLA	conjugated linoleic acid
CF	crude fat
CP	crude protein
FA	fatty acid
FAME	fatty acid methyl ester
LA	linoleic acid
α-LNA	α-linolenic acid
MOZ	mozzarella
NDF	neutral detergent fiber
OA	oleic acid
OCFA	odd chain fatty acid
RAV	raveggiolo
RIC	ricotta
SA	stearic acid
SCC	somatic cell count
SD	standard deviation
SFA	saturated fatty acid
UFA	unsaturated fatty acid
VA	vaccenic acid

There two key strategies for increasing the concentration of nutraceuticals in dairy products. Dairy product can be enriched in functional components by directly adding bioactive molecules to the milk before making the cheese. In this case their transfer is quantitative and provides novel or nutraceutical foods (Nzekoue et al., 2021; Turek & Wszołek, 2021; Villamil et al., 2021) exploiting the fact that they are naturally present in milk thanks to the animal's physiology.

The FA profile of cheese is strongly affected by milk FAs and beneficial molecules are transferred from milk into the and products (Mele et al., 2011; Nudda et al., 2021; Santillo et al., 2016). Hence, the cheese-making procedure plays an important role in the transfer of functional fat components from milk to dairy product and increases their nutritive value. Cattani et al. (2014) and Formaggioni et al. (2020), studied the recovery of FAs in cow cheeses in the north of Italy and found a quantitative transfer of functional FAs.

The cheese-making technology is key to the physicochemical characteristics, FA profile, proteolysis evolution and sensory properties of cheeses. However, many of the studies that investigated the recovery and the transfer of functional FAs from milk to dairy products, do not compare how the cheese making typologies have a different impact on the FA profile of the end products derived from the same milk batch (Agradi et al., 2020; Bonanno et al., 2013; Cattani et al., 2014; Innosa et al., 2020; Luna et al., 2007; Santillo et al., 2016).

The aim of the present study was to evaluate and to compare the influence of the cheese-making procedure on functional FA transfer from milk to three Italian fresh dairy products using the same milk batch: Mozzarella (MOZ), Raveggiolo (RAV) and Ricotta (RIC).

2. Material and method

2.1. Experimental design

The trial was carried out at the farm of the Centro di Ricerche Agro-Ambientali "Enrico Avanzi" of Pisa University. After a 4-week monitoring period of the animal feeding strategy, on days 30 and 37, milk was collected for cheese-making. Milk was used to produce three different kinds of Italian dairy products: Mozzarella (MOZ), Raveggiolo (RAV) and Ricotta (RIC). The cheese-making was repeated twice. Each lot of raw bulk milk used for the cheese making was sampled in triplicate for chemical analysis and to determine the hygienic parameters. Three molds of each dairy product per cheese making trial were randomly sampled and analyzed for proximate analysis and FA profile characterization. The procedures and methods are described in the following subsections.

2.2. Animal feeding and milk production

Fifty Italian Friesian cows, average body weight 587 ± 63.9 kg (mean ± standard deviation, SD) and 200 ± 20 days of lactation, were housed in free stall systems with access to pasture during the morning after the milking. Animals were fed a diet composed of 5 kg/head and day of a commercial concentrate, administered during the morning and the evening milking (06:00 and 18:00) and hay *ad libitum*. During the morning cows grazed for 4 h.

The botanical profile of the pasture was characterized by a mix of oats, red clover, alfalfa and Italian ryegrass. Feed samples were collected weekly and stored at −80 °C until analysis. Samples were freeze-dried and then ground for chemical analysis with a Brabender mill (GmbH & Co, KG, Germany), using a mesh size of 1 mm. Crude protein (CP), crude fat (CF), and ash were determined according to the AOAC methods (1995; 976.06, 920.39 and 942.05, respectively). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin were determined according to van Soest et al. (1991), using heat stable amylase and sodium sulfite, and were expressed inclusive of residual ash. Feed FAs were extracted according to Folch et al. (1957), esterified according to Sukhija and Palmquist (1988) with C19:0 as the internal standard, and identified using the same procedure described below for FAs of milk samples.

The nutritional profiles of hay, concentrate and pasture are shown in Table 1.

2.3. Milk analysis

Before cheese-making was begun, samples of raw bulk milk were collected in triplicates and each sample was divided into two aliquots for analysis: the first aliquot was processed immediately after sampling in order to assess fat, lactose, protein and casein content, using the Milkoscan 6000 FT (Foss Electric, Hillerød Denmark), and total somatic cell count (SCC) according to ISO 13366-2lIDF 148-2 (2006), using a Fossomatic 5000 (Foss Electric, Hillerød Denmark). Data are shown in Table 2.

The second aliquot of milk samples was stored at −80 °C until FA extraction and analysis, according to Buccioni et al. (2010). Fatty acid

Table 1
Nutritional profiles of diet ingredients.

Compunds[a]	Hay	Pasture	Concentrate
	g of fresh matter		
DM	94.74	90.59	87.64
	g/100g DM		
CP	18.38	19.63	19.86
CF	1.06	1.98	3.15
NDF	58.88	46.01	17.62
ADF	30.21	28.9	7.61
ADL	4.99	4.4	2.09
Ash	5.72	7.63	8.95

[a] DM, dry matter; CP, crude protein; CF, crude fat; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, lignin.

Table 2
Chemical and hygienic profile of milk.

Compunds[a]		SD[b]	Max[c]	Min[d]
CP (g/100 g of milk)	3.26	0.01	3.28	3.25
CF (g/100 g of milk)	3.38	0.04	3.43	3.33
Lactose (g/100 g of milk)	4.78	0.02	4.79	4.74
SNF (g/100 g of milk)	8.95	0.03	9.00	8.90
TBC (CFU x 1000/ml)	10.54	0.08	10.66	10.47
SCC x 10^3/ml	194	2.25	197	192
Freezing point (°C)	0.52	0.00	0.52	0.52
Urea (mg/ml of milk)	22.02	0.14	22.22	21.85

[a] CP, crude protein; CF, crude fat; SNF, solid not fat residual; TBC, total bacterial count; SCC somatic cell count.
[b] SD, standard deviation.
[c] Max, maximum value detected.
[d] Min, minimum value detected.

methyl esters (FAMEs) were prepared with a base-catalyzed transesterification according to Christie (1982), and separated on a GC equipped with a capillary column (CP-Select CB for FAMEs Varian, Middelburg, the Netherlands: 100 m × 0.25 mm i.d., film thickness 0.20 mm).

The injector and flame ionization detector temperatures were 270 °C and 300 °C, respectively. The programmed temperature was 40 °C for 4 min, increased to 120 °C at a rate of 10 °C min^{-1}, maintained at 120 °C for 1 min, increased to 180 °C at a rate of 5 °C min^{-1}, maintained at 180 °C for 18 min, increased to 200 °C at a rate of 2 °C min^{-1}, maintained at 200 °C for 1 min, increased to 230 °C at a rate of 2 °C min^{-1} and maintained at this last temperature for 19 min. The split ratio was 1:100 and helium was the carrier gas with a flux of 1 mL min^{-1}. Individual FAMEs were quantified using nonadecanoic acid (C19:0) and valeric acid (C5:0) methyl esters (Sigma Chemical Co., St. Louis, MO) as internal standards and identified by comparison of the relative retention times of FAME peaks from samples, with those of the standard mixture 37 Component FAME Mix (18919-1AMP, Supelco, Bellefonte, PA, USA), individual C18:1 *trans*9 and C18:1 *trans*11 (46903 and v1381 respectively, Sigma-Aldrich, St. Louis, Missouri, USA), individual C18:2 *cis*9, *trans*11 (1255, Matreya Inc Pleasant GAP, PA, USA), CLA mix standard (05632, Sigma-Aldrich, St. Louis, Missouri, USA) and published isomeric profile (Cruz-Hernandez et al., 2006; Kramer et al., 1997, 2004).

The C18:1 isomer elution sequence was performed according to Kramer et al. (2008). In addition, a standard mix of α-LNA isomers (47792, Supelco, Chemical Co., St. Louis, MO) and LA isomers (47791, Supelco, Chemical Co., St. Louis, MO) and published isomeric profiles (Destaillats et al., 2005) were used to identify the isomers of interest. Two bacterial acid methyl ester mixes (47080-U Supelco, Chemical Co., St. Louis, MO and GLC110, Matreya, Pleasant Gap, PA) and one individual standard for methyl ester of C14:0 *iso*, C14:0 *anteiso*, C15:0 *iso* and C17:0 *anteiso* (21-1211-11, 21-1210-11, 21-1312-11 and 21-1415-11, Larodan Malmo, SW) were used to identify the branched chain FA (BCFA) profile. Inter and intra-assay coefficients of variation were calculated using a reference standard butter (CRM 164, Community Boureau of Reference, Bruxelles, Belgium). The detection threshold of FAs was 0.01 g/100g of FA (Contarini et al., 2013). All FA composition results are expressed as g/100g of fat. The FA profile is shown in Table 3.

Table 3
Fatty acid profile of milk (g/100g of fat).

Fatty acid	Mean	SD[a]	Max[b]	Min[c]
C4:0	1.98	0.033	2.05	1.95
C6:0	1.21	0.161	1.40	1.05
C8:0	1.18	0.033	1.22	1.14
C10:0	2.72	0.082	2.98	2.57
C10:1	0.267	0.048	0.320	0.207
C11:0	0.030	0.007	0.040	0.020
C12:0	3.44	0.265	3.86	2.94
C12:1	0.061	0.008	0.070	0.050
C13 iso	0.040	0.009	0.050	0.030
C13 ante	0.060	0.007	0.069	0.050
C13:0	0.072	0.006	0.093	0.056
C14 iso	0.124	0.027	0.162	0.076
C14:0	12.3	0.917	13.6	11.0
C15 iso	0.284	0.033	0.324	0.248
C14:1	0.951	0.094	1.08	0.830
C15 ante	0.595	0.097	0.670	0.452
C15:0	1.16	0.151	1.28	0.971
C16 iso	0.285	0.073	0.364	0.203
C16:0	31.8	2.99	34.3	27.8
C16:1	0.908	0.612	1.73	0.302
C17 iso	0.402	0.017	0.509	0.326
C17:0	0.551	0.084	0.644	0.444
C17:1	0.221	0.042	0.290	0.176
C18:0 (SA)	9.88	0.680	10.69	8.71
C18:1t5	0.016	0.006	0.024	0.000
C18:1t6-8	0.206	0.007	0.244	0.070
C18:1 t9	0.272	0.042	0.336	0.233
C18:1 t10	0.372	0.171	0.502	0.150
C18:1 t11 (VA)	0.971	0.228	1.23	0.708
C18:1 t12	0.321	0.016	0.341	0.304
C18:1 c9 (OA)	20.2	1.59	21.5	18.0
C18:1 c11	0.134	0.048	0.187	0.080
C18:1 c12	0.413	0.030	0.503	0.371
C18:2 c9 c12 (LA)	2.30	0.360	2.69	1.98
C18:3 c9 c12 c15 (α-LNA)	0.342	0.113	0.460	0.206
C20:0	0.176	0.065	0.257	0.098
C18:2 c9 t11 (CLA)	0.498	0.076	0.630	0.401
C20:1	0.070	0.021	0.092	0.050
C21:0	0.017	0.023	0.040	0.000
C20:2	0.020	0.007	0.034	0.010
C20:4 n6	0.086	0.060	0.143	0.005
C20:3 n3	0.143	0.064	0.199	0.051
C22:0	0.069	0.061	0.146	0.000
C22:1	0.013	0.012	0.026	0.000
C20:5	0.011	0.010	0.022	0.000
C23:0	0.024	0.013	0.042	0.010
C24:0	0.028	0.022	0.057	0.004

[a] SD, standard deviation.
[b] Max, maximum value detected.
[c] Min, minimum value detected.

2.4. Cheese making procedure

The cheese-making trial was repeated twice in the plant laboratories of Florence University. In each experiment, the three dairy products (MOZ, RAV and RIC) were produced from the same lot of milk to eliminate any effects of variability of the raw material. The milk was pasteurized before cheese making and the procedures are described below:

MOZ. The milk was heated to a temperature of 35–38 °C and coagulated with the addition of liquid rennet (Caglificio Clerici, SACCO srl, Como, Italy) and of microbial starters (*Streptococcus termophilus* ST051, SACCO srl, Como, Italy) up to a pH of 5.8–5.6. After the coagulation, the curd was cut into large cubes and then into walnut-size pieces. At this point, the curd was placed on a drainage chamber to mature at temperatures of about 30 °C. It was, then cut again into large slices and left to acidify until it reached a pH of about 5.2–5.1. At this point, the slices were placed in a hot water bath at 75–85 °C (with 10 g/100 mL of NaCl) and stretched manually. The dough was then manipulated to form round shapes, immediately cooled by immersion in cold water, at temperatures of about 8–10 °C. This was followed by packaging in preserving liquid (0.5 g/100 mL NaCl) and subsequent storage at refrigeration temperatures, until sampling and analysis.

RAV. Firstly, lactic ferments (*Lactobacillus helveticus* and *S. termophilus* LH91, SACCO srl, Como, Italy), small amounts of salt and liquid rennet (Caglificio Clerici, SACCO srl, Como, Italy) were added to the milk, which was then heated to 30–36 °C. The acidification of the milk was slow (1 h) until complete coagulation. The curd was then very gently broken manually using a ladleand placed in a drainage chamber

(20 °C). After purging, when the cheese reached the right consistency, the curd was transferred into plastic mold shapers and stored at refrigeration temperatures until sampling and analysis.

RIC. Ricotta is traditionally produced at the end of the cheese-making, from the residual whey and the process involves the hot coagulation of lactalbumin in an acid environment. In order to reproduce this industrial procedure, firstly, the residual whey from MOZ and RAV cheese makings was heated in a boiler until a temperature of 60–70 °C. Then, a 10% of milk was added to ensure sufficient fat and the heating proceeded up to temperatures of around 85–90 °C. Next, a dilute solution of citric acid (E330, 10 g/L, 1 g per L of whey) was added. The heat and the acid environment flocculated the whey proteins into a white gelatinous mass with low consistency, which floated up to the surface. The RIC was collected with perforated ladles, placed into baskets, and left in the drainage chamber. It was then stored at refrigeration temperatures, until sampling and analysis.

Sample collection, in triplicate, took place the day after each cheese-making procedure. All samples were separated into two aliquots for analysis: the first one was freeze-dried and processed to assess proximate composition (CP, CF, ash) according to AOAC (1995; methods 976.06, 920.39 and 942.05, respectively); the second one was stored at −80 °C until analysis for FA extraction and profile characterization, according to Buccioni et al. (2010).

The transfer of individual FAs ($TRAN_{FA}$) from milk to cheese was calculated as the ratio between cheese and milk individual FA content, expressed as grammes of individual FA per 100 g of fat:

$$TRAN_{FA} = Cheese\ FA/Milk\ FA. \quad (1)$$

The recovery (REC) of protein (REC_{CP}) and fat (REC_{FAT}) was calculated according to the following formula:

$$REC_{CP} = Cheese_{CP}\ (g)/Milk_{CP}\ (g) \quad (2)$$

$$REC_{FAT} = Cheese_{FAT}\ (g)/Milk_{FAT}\ (g) \quad (3)$$

2.5. Statistical analysis

Descriptive statistics were performed for milk proximate analysis and FA profile (Mean ± SD; minimum and maximum values).

Proximate data and FA profile of dairy products and the transfer of FAs from milk to cheeses were analyzed using the following mixed model:

$$y_{ij} = \mu + C_i + R_j + e_{ij}$$

where the kind of dairy product was considered as the fixed factor, and the cheese-making replicates was the random factor (SAS Institute, 2008):

y_{ij} is the observation; μ is the overall mean; C_i is the fixed effect of kind of dairy product (i = 1 to 3); R_j is the random effect of cheese making replicate (j = 1 to 2); and e_{ij} is the residual error. The covariance structure was compound symmetry, which was selected based on Akaike's information criterion of the mixed model of SAS. Statistical significance of the diet effect was tested against variance of sheep nested within diet according to repeated measures design theory (Littell et al., 1998).

3. Results

3.1. Milk proximate and FA profile

The physicochemical and hygienic characteristics of the milk were appropriate for cheese making (Table 2). Milk FA composition was similar to commercially available milks (Web page Crea, n.d.) and to those described in the literature, with high values of C14:0 and C16:0 (Jensen et al., 1991). A good content of OA was found. The data are reported in Table 3.

3.2. Dairy products proximate and FA profile

Crude protein and CF of dairy products were consistent with commercial ones (Web page Crea, n.d.).

Significant differences between the three dairy products were found for DM, CP and ash content. Dry matter was higher in RIC and lower in RAV, while ash content was higher in RAV and lower in MOZ. The CP content was the highest in RIC.

The data are reported in Table 4.

Table 5 highlights the differences in the FA profile of cheeses. C14:0 and C14:1 were the highest in RAV and thus lower in MOZ and RIC. C16:0 was similar in RAV and RIC with higher values than in MOZ. In contrast, C16:1 had a lower content in RAV and MOZ with respect to RIC. In RIC, the stearic acid (C18:0; SA) concentration was higher, while VA was lower than in the other two dairy products. No differences were found in amount of C18:1 *trans*10 in the three cheeses. However, several variations were found for OA and C18:1 *cis*12 content which were the highest in RIC, while the C18:1 *cis*11 content was the highest in MOZ and the lowest in RAV. Linoleic acid (C18:2 *cis*9 *cis*12; LA) concentration was the lowest in MOZ and the highest in RAV.

3.3. FA transfer from milk to dairy products

Table 6 reports the recovery of CP and fat for each dairy product. Several differences in FA transfer from milk to dairy products were found - see Table 7. The C10:0, C12:0, C14:0, C14:1, C16:0, C17:1 transfer extents were significantly different among the three cheeses and the amount was <1. In contrast, C14:0 *iso*, C15:0, C16:1, C17:0, C18:0, C18:1 *trans*11, C18:1 *cis*9, C18:1 *cis*11, C18:1 *cis*12 and LA transfer extents were significantly different but with a value > 1. C20:1 showed a varying trend with a transfer <1 in MOZ and >1 in RAV and RIC. Although not significantly different between the three cheeses, a transfer extents >1 were shown for C12:1, C13:0 *iso*, C13:0, C15:0 *iso*, C15:0 *ante*, C16:0 *iso*, C18:1 *trans*6-8, C18:1 *trans*9, C20:0, C20:4 and C20:3, however <1 for C10:1, C13:0 *ante*, C17:0 *iso*, C18:1 *trans*10, C18:1 *trans*12, CLA. All these latter FAs are normally linked to microbial activities.

4. Discussion

The dairy products from cow milk are generally part of balanced and healthy diet plans because their FA profile is associated with beneficial functions for human health (Minieri et al., 2018; 2020b). Thus, dairy products can be defined as functional foods because they match the definition "any food or food ingredient that could provide a health benefit over the traditional nutrients it contains" (Das et al., 2012).

There have recently been two reviews of the pathogenesis of modern diseases related to diet (Minieri et al., 2018; 2020b). Researchers have

Table 4
Chemical profile of dairy products.

Compunds[a]	MOZ	RAV	RIC	SEM[b]	P[c]
	g of fresh matter				
DM	37.7 b	34.5 c	38.3 a	0.429	0.0016
	g/100 DM				
CP	60.5 a	60.6 a	46.5 b	0.451	<0.0001
CF	41.0	40.3	47.3	1.23	0.1260
Ash	4.28 c	5.32 a	4.74 b	0.046	<0.0001

[a] DM, dry matter; CP, crude protein; CF, crude fat.
[b] SEM, standard error mean.
[c] P, probability of significant effect due to experimental factors; a, b, within a row, means with different Latin letters are significantly different (P < 0.05).

Table 5
Fatty acid profile of dairy products (g/100g of fat).

Fatty acid	Dairy product			SEM[a]	P[b]
	MOZ	RAV	RIC		
C10:0	2.53	2.55	2.05	0.012	0.1177
C10:1	0.259	0.249	0.241	0.003	0.1980
C12:0	3.07	3.12	3.09	0.014	0.1208
C12:1	0.666	0.063	0.063	0.004	0.0938
C13:0	0.090	0.085	0.090	0.004	0.6931
C13 iso	0.039	0.037	0.036	0.002	0.6132
C13 ante	0.046	0.045	0.047	0.002	0.8279
C14:0	11.1 c	11.3 a	11.1 b	0.021	0.0018
C14 iso	0.315	0.299	0.297	0.007	0.2353
C14:1	0.958 b	0.980 a	0.945 b	0.006	0.0181
C15:0	1.21	1.19	1.21	0.009	0.2297
C15 iso	0.315	0.299	0.296	0.007	0.2353
C15 ante	0.642	0.658	0.641	0.004	0.0536
C16:0	29.4 b	30.2 a	30.2 a	0.104	0.0029
C16 iso	0.338	0.325	0.325	0.005	0.2355
C16:1	1.20 b	1.20 b	1.25 a	0.006	0.0030
C17:0	0.623	0.589	0.625	0.009	0.0616
C17 iso	0.357	0.367	0.358	0.004	0.2603
C17:1	0.204	0.181	0.214	0.006	0.0720
C18:0 (SA)	9.88 c	9.97 b	10.1 a	0.026	0.0011
C18:1t6-8	0.220	0.251	0.202	0.018	0.2433
C18:1 t9	0.368	0.383	0.367	0.017	0.7629
C18:1 t10	0.373	0.365	0.367	0.011	0.8811
C18:1 t11 (VA)	0.990 a	0.997 a	0.927 b	0.011	0.0091
C18:1 t12	0.268	0.261	0.250	0.004	0.1170
C18:1 c9 (OA)	19.1 c	19.1 b	19.5 a	0.024	<0.0001
C18:1 c11	0.138 a	0.117 c	0.128 b	0.002	0.0047
C18:1 c12	0.416 b	0.395 c	0.452 a	0.004	0.0003
C18:2 c9 c12 (LA)	2.38 c	2.59 a	2.46 b	0.008	<0.0001
C18:3 c9 c12 c15 (α-LNA)	0.330	0.323	0.336	0.007	0.2756
C20:0	0.174	0.190	0.187	0.005	0.1077
C18:2 c9 t11 (CLA)	0.425	0.444	0.428	0.007	0.2327
C20:1	0.035 c	0.085 b	0.133 a	0.007	0.0002
C20:4 n6	0.092	0.095	0.099	0.004	0.5805
C20:3 n3	0.150	0.157	0.163	0.005	0.2830

[a] SEM, standard error mean.
[b] P, probability of significant effect due to experimental factors; a, b, within a row, means with different letters are significantly different ($P < 0.05$).

Table 6
Protein and fat recovery in dairy products.

	Dairy product			SEM[b]	P[c]
Item[a]	MOZ	RAV	RIC		
CP	6.97 a	6.39 b	5.44 c	0.02	<0.0001
CF	12.25 b	12.02 b	14.13 a	0.41	0.0121

[a] CP, crude protein; CF, crude fat.
[b] SEM, standard error mean.
[c] P, probability of significant effect due to experimental factors; a, b, within a row, means with different letters are significantly different ($P < 0.05$).

thus focused on genetics, nutrigenomics and on the link between gene expression and nutritional metabolites originating from different food regimens. The cheese-making technology is the main factor affecting the physicochemical characteristics, FA profile, proteolysis evolution and sensory properties of dairy products.

As expected, in this trial, the CP content was found to be lower in RIC than in the other products. In fact, the RIC making procedure, which uses the whey from previous cheese making, is poor in casein and the protein fraction is mainly composed of only lactalbumins. Also the recovery of CP and CF was different among the three cheeses, highlighting the influence of the cheesemaking procedure.

In fresh cheeses, where ripening is irrelevant, the quality of the FA content is fundamental for the sensory and nutritional properties. It is thus important to improve the knowledge on the effect of cheese-making procedures on the functional molecule transfer from milk to the derived

Table 7
Fatty acid transfer in dairy products (g/100g of fat).

Fatty acid	Dairy product			SEM[a]	P[b]
	MOZ	RAV	RIC		
C10:0	0.915 a	0.919 a	0.904 b	0.002	0.0095
C10:1	0.991	0.950	0.921	0.016	0.0617
C12:1	1.04	0.995	1.00	0.048	0.7848
C12:0	0.892 c	0.907 a	0.898 b	0.001	0.0005
C13 iso	1.01	0.968	0.932	0.051	0.5995
C13 ante	0.712	0.694	0.725	0.038	0.8511
C13:0	1.17	1.11	1.17	0.038	0.4211
C14:0	0.876 c	0.891 a	0.881 b	0.001	0.0011
C14 iso	1.45 a	1.34 b	1.21 c	0.040	0.0156
C14:1	0.988 b	1.01 a	0.975 c	0.003	0.0007
C15:0	1.07 a	1.05 b	1.07 a	0.003	0.012
C15 iso	1.15	1.09	1.08	0.022	0.1535
C15 ante	1.12	1.14	1.11	0.010	0.1706
C16:0	0.873 b	0.895 a	0.897 a	0.001	0.0002
C16 iso	1.33	1.28	1.28	0.014	0.0813
C16:1	1.51 b	1.50 b	1.57 a	0.006	0.0006
C17:0	1.21 a	1.14 b	1.21 a	0.015	0.0262
C17 iso	0.815	0.837	0.817	0.010	0.3224
C17:1	0.912 a	0.814 b	0.959 a	0.024	0.0141
C18:0 (SA)	1.02 c	1.03 b	1.04 a	0.002	0.0005
C18:1t6-8	1.22	1.39	1.12	0.083	0.1443
C18:1 t9	1.35	1.40	1.34	0.063	0.7720
C18:1 t10	0.964	0.944	0.951	0.286	0.8825
C18:1 t11 (VA)	1.08 b	1.09 a	1.01 c	0.014	0.0188
C18:1 t12	0.820	0.798	0.766	0.013	0.0755
C18:1 c9 (OA)	1.00 c	1.01 b	1.02 a	0.001	<0.0001
C18:1 c11	1.19 a	1.01 b	1.10 b	0.024	0.0051
C18:1 c12	1.02 b	0.965 c	1.10 a	0.007	<0.0001
C18:2 c9 c12 (LA)	1.07 b	1.17 a	1.11 b	0.004	<0.0001
C18:3 c9 c12 c15 (α-LNA)	1.08	1.02	1.09	0.053	0.8523
C20:0	1.20	1.30	1.29	0.037	0.1708
C18:2 c9 t11 (CLA)	0.858	0.895	0.864	0.011	0.1233
C20:1	0.450 c	1.098 b	1.715 a	0.125	0.0011
C20:4 n6	1.17	1.21	1.26	0.024	0.1009
C20:3 n3	1.21	1.27	1.32	0.035	0.2021

[a] SEM, standard error mean.
[b] P, probability of significant effect due to experimental factors; a, b, within a row, means with different letters are significantly different ($P < 0.05$).

products (Nzekoue et al., 2021). Mozzarella, RAV and RIC are valued by consumers for their moderate fat content, the presence of proteins with a high nutritional value, and their high digestibility. In particular, MOZ and RIC are recommanded in hypocaloric diets.

Mozzarella, RAV and RIC are fresh dairy products with an ageing of less than three days. This makes them suitable for studying the FA transfer from the milk to the final products, because it is not influenced by the microbial activities developed during the ripening. The FA profile of the three cheeses reveals several interesting differences. The data reported in Tables 5 and 7 on C14:0, C14:1 and C16:0 seem to show the selective transfer of these FAs, probably due to their position in the triglyceride structure and consequently to the interaction with the casein mesh (Gresti et al., 1993). On the other hand, the significant differences in C16:1, SA and VA content in the dairy products could be related to the microbial activities of the starter cultures. In fact, the lowest content of C16:1 in RAV and in MOZ, the highest concentration of SA and the lower value of VA in RIC than in the other products is probably due to the microbial biohydrogenation of the mono-unsaturated FAs to their saturated forms.

In this study, starter cultures containing strains of *Lactobacillus helveticus* and *Streptococcus thermophilus* were used. Several strains of lactobacilli and streptococci produce CLA from LA in a growth medium or in milk (Hsiao & Siebert, 1999). An effect of the starter culture on FA biohydrogenation has been previously hypothesized also during the ripening of Tuscan pecorino cheese obtained from pasteurized (Buccioni et al., 2010) and raw (Buccioni, Minieri, et al., 2012) milk, both with the addition of a starter culture composed of *L. helveticus* or *S. thermophilus*.

The BCFAs comprise mainly saturated fatty acids (SFAs) from C5:0 to

C17:0, characterized by the presence of one or more methyl groups in the *iso* or *ante-iso* positions. They are synthetized by microorganisms to ensure the plasticity of cell membranes, and their presence in milk is due to rumen microbial activity.

In this trial, no significant differences in the BCFA contents were found among dairy products and milk, indicating that the cheese-making processes did not affect their concentration in the end products. Data from animal models demonstrate that BCFAs may alter the ecology of the gut microbiota and reduce the damage caused by enterocolitis, thanks to their anti-inflammatory properties (Ran-Ressler et al., 2011). Considering the interesting role in human nutrition of these FAs, their quantitative transfer from milk to dairy products may thus be positive.

Our data are in accordance with Nudda et al. (2021), who reported that the BCFA concentration in fresh cheese fat was strongly related to the FA content in the unprocessed raw milk from sheep and goats.

The human metabolism is not able to produce odd chain FAs (OCFAs) and dairy products represent an important source of these functional FAs. In fact, a higher dietary intake and circulating levels of OCFAs have been associated with lower risks of adiposity, chronic inflammation, cardiovascular diseases, metabolic syndromes, type 2 diabetes, nonalcoholic steatohepatitis, chronic obstructive pulmonary disease, and pancreatic cancer (Imamura et al., 2018; Khaw et al., 2012; Sun et al., 2007; Venn-Watson et al., 2020; Warensjö et al., 2010).

C15:0 and C17:0 are the most representative of OCFAs in milk because they are synthetized by rumen microorganisms as components of their cell membrane (Vlaeminck et al., 2006). C15:0 seems to be the most bioactive. It is an agonist of peroxisome proliferator-activated receptors and repairs mitochondrial function, reduces pro-inflammatory state, and lowers the glucose and cholesterol content. Anemia, liver iron overload and nonalcoholic steatohepatitis *in vivo* are also attenuated by this FA.

In addition, C15:0 appears to be an essential FA (Venn-Watson et al., 2020). In this trial, both C15:0 and C17:0 concentrations were similar among the three cheeses, however a different behavior was shown for their transfer from milk into cheeses. Although the differences was small, in MOZ and RIC the transfer was higher than in RAV indicating that the cheese-making procedure affect the recovery of these FAs in dairy products. A possible explanation could be in the microbial activity of starter cultures in line with the role of these FAs in microbial metabolism and resilience to environmental condition changes.

Werner et al. (1992) suggested that different milk processing conditions and the addition of different starter cultures may also affect the unsaturated FA (UFA) profile in cheeses. Unsaturated fatty acids are sensitive to moderate heating and the cheese-making conditions may favor the migration of double bonds along the carbon chain (Ha et al., 1989; Shantha et al., 1992). In our trial, CLA was transferred from milk to all the cheeses but without significant differences among them. This finding is consistent with Luna et al. (2004, 2005, 2007) who showed that different thermal treatments did not significantly alter the total CLA content or the CLA isomer profile, during yoghurt or cheese production, using raw or pasteurized milk. Similar results were also observed by Gnädig et al. (2004) during the production of Emmental cheese.

Considering arachidonic acid (AA), the cheese making procedure did not affect the transfer from milk to dairy products. Arachidonic acid has an important role in mammal physiology, and is a fundamental component of cell membranes. Arachidonic acid is now no longer considered by the scientific community a "harm-generating molecule" but rather as being essential for the metabolism. This FA is important for infant brain development and cognitive functions, for the skeletal muscle and immune systems. Additionally, AA promotes and regulates type 2 immune responses against intestinal and blood flukes, and is an invaluable endoschistsomicide and endotumoricide (European Food Safety Authority, 2009a; Salem & Van Dael, 2020; Tallima & El Ridi, 2018; Wang et al., 2020). In cow milk AA is not abundant and its quantitative transfer from raw material to dairy products assumes a fundamental role. This thus confirms that the milk FA profile is an important determinant for cheese nutritional quality (Nudda et al., 2021).

No differences were found in C18:1 *trans*10 concentration among the three cheeses. Considering the detrimental effect of this isomer on consumer health, this result highlights the importance of the milk fat profile and the role of cheese-making in conserving the functional components of milk. In contrast, several variations were noted for OA, C18:1 *cis*12, and C18:1 *cis*11. In particular, the first two FAs were higher in RIC than in the others, while the third was higher in MOZ than in the others. This thus confirms that the microbial activities of the starter coltures play a fundamental role in C18:1 isomerization and, consequently, in the nutritious profile of dairy product fat (Buccioni et al., 2010).

Linoleic acid was transferred from milk into the three dairy products with significant differences. In RAV, the LA transfer was higher than in the others, due to the cheese-making effect. It is likely that the starter cultures affect the cheese FA profile (Buccioni et al., 2010) or, alternatively, the profile may be affected by an affinity between LA with the milk protein fraction, such as casein (Duerasch et al., 2020).

5. Conclusions

The results of this trial confirmed that the cheese-making process plays an important role in the quantitative transfer of functional FAs from milk into cheese. The milk nutritional value associated with the FA profile was similarly transferred to the cheeses however, the kind of cheese-making process affected the transfer of several FAs in a selective manner. The differences found among the cheeses, considered in this study, could also be due to the microorganism activities of starter cultures. Further investigations are thus needed to improve the knowledge on the role of cheese-making on the FA profile modulation to improve the nutritious value of the end products.

Author contributions

Arianna Buccioni and Sara Minieri conceptualization; Arianna Buccioni and Sara Minieri investigation; Arianna Buccioni, Sara Minieri and Federica Mannelli methodology; Matteo Daghio and Federica Scicutella data curation; Matteo Daghio and Federica Scicutella formal analysis and software; Arianna Buccioni and Federica Mannelli writing and original draft preparation; Stefano Rapaccini resources, funding acquisition and supervision; Federica Scicutella and Matteo Daghio writing, review and editing; Arianna Buccioni and Sara Minieri project administration.

Ethical statement

- This material is the authors' own original work, which has not been previously published elsewhere.
- The paper is not currently being considered for publication elsewhere.
- The paper reflects the authors' own research and analysis in a truthful and complete manner.
- The paper properly credits the meaningful contributions of co-authors and co-researchers.
- The results are appropriately placed in the context of prior and existing research.
- All sources used are properly disclosed (correct citation). Literally copying of text must be indicated as such by using quotation marks and giving proper reference.
- All authors have been personally and actively involved in substantial work leading to the paper, and will take public responsibility for its content.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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