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Effects of season and management on fatty acid profile, ACE-inhibitory activity and anti-oxidant properties of Italian Alpine cheeses

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ABSTRACT

Mountain dairy products are recognised as high-quality food but there are still few studies concerning the effects of seasonality and herd management on the profile of bioactive compounds in cheeses. This study was planned to assess the effect of season (summer versus winter) and feeding management (pasture versus integration) on fatty acids (FAs) profile, anti-hypertensive (ACE-IA) and anti-oxidant properties (ABTS-SA, FRAP), total thiol (SH) and phenolic (TP) contents of cheeses from two dairy cow farms (Farm A and Farm B) located in Piedmontese Alps (Italy). Cheese samples collected in the farms were submitted to an integrated analytical approach and the results were processed by full factorial ANOVA and PCA. The trends observed from the FAs profile confirmed the beneficial influence of supplying fresh forage to lactating cows. The ACE-IA was higher in summer than in winter cheese but depended upon the farm factor. Among the indicators of antioxidant activity, only the ABTS-SA was affected by the season, even though with significant differences between the farms. The TP content did not show any clear pattern, but it was higher than the values described in the literature. The PCA of all the data showed that several FAs and the ABTS-SA gave relevant contributions to clearly group the cheese samples according to the production season or farm. In conclusion, alpine cheese exhibited high nutritional quality under the consumers' health standpoint, and the identification of the healthier summer cheeses for traceability or labelling purposes, can be obtained.

HIGHLIGHTS

- Seasonality and herd management practices affected the nutritional quality of cheese produced in two dairy farms in Piedemontese Alps.
- Lipid quality was affected by the feeding strategy, with the greater improvements obtained by pasture grazing or fresh grass consumption in the barn.
- ACE-inhibitory activity and anti-oxidant properties were influenced by both seasonality and farm of origin, with the best values measured in summer cheese.

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Mountain cheese; bioactive compounds; dairy product quality; cheese traceability; antihypertensive activity

Introduction

Livestock farming in mountain areas is strongly affected by seasonal availability of pastures and by the possibility to produce hay and silages to be used outside the grazing period. Compared to the intensive and specialised dairy cattle farming, such traditional semi-extensive livestock system, is based on reproductive and productive events, regularly distributed along the year, with different feeding management according to the season. In the alpine regions for the dairy livestock system classified as 'original traditional' (Sturaro et al. 2013), the grazing period is concentrated during the summer, lasting from 90 to 100 days, when animals go transhumance from the valley to the medium-high mountain (Bergamaschi et al. 2016).

During last years, popular belief has been directed towards the consideration of dairy fats as detrimental for arterial health and the development of cardiovascular diseases. However, recently, scientists are doing efforts in order to demonstrate that dairy consumption is part of a healthy diet (Mozaffarian 2019; Nestel and Mori 2022). Moreover, it is known that fresh grass feeding and pasture grazing contribute on a favourable milk fatty acid (FA) profile, involving the relative

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composition in several FAs associate to positive effects on consumers health (Hanus et al. 2018) such as *cis* and *trans* FAs, odd and branched chain FAs (OBCFA) and conjugated linoleic acid (CLA).

In addition to healthful FAs, the bioactive compounds contained in cheese include bioactive peptides and anti-oxidants. Bioactive peptides are responsible for several health benefits, such as antihypertensive, immune-modulatory, antimicrobial, antioxidant, and anti-inflammatory activities (Vargas-Bello-Pérez et al. 2019). Among bioactive peptides, those with antihypertensive activity have particular importance due to the significant increase in hypertension in industrialised Countries, closely associated with the risk for stroke and cardiovascular disease (Danaei et al. 2014). Milk proteins are the main precursors of peptides with antihypertensive properties. Most of the biopeptides investigated for their antihypertensive effect have shown to possess Angiotensin-I-converting enzyme (ACE)-inhibitory activity *in vitro* (Sieber et al. 2010). The ACE is one of the key enzymes in the regulation of blood pressure (BP) as it converts Angiotensin-I to Angiotensin-II resulting in vasoconstriction and facilitating degeneration of bradykinin, which has vasodilatory properties (Marcone et al. 2017). Different cheeses of Italian, Spanish, Dutch and Swiss origin (Santiago-López et al. 2018) have been characterised for the presence of effective ACE-inhibitory peptides. In cheese, it has been observed that the release of these naturally formed biopeptides from the native protein and their bioactivity depend on milk-type, starter microorganisms, fermentation conditions, manufacturing processes and ripening environments and age (Santiago-López et al. 2018).

The antioxidant activity of dairy products is of great interest both from the technological and nutraceutical standpoints. The oxidative stability of milk and dairy products is of concern to the dairy industry because oxidation processes can result in strong off-flavours and a reduction of nutritional quality (Dimick and Kilara 1983). On the other hand, the dietary intake of compounds with marked anti-oxidant properties is of great benefit to counteract and to prevent metabolic diseases (Lin and Yen 1999; Wang et al. 2006). For these reasons, notable efforts have been recently spent to try increasing the antioxidant activity of food, and, particularly, milk and dairy products (Branciari et al. 2015). In dairy products, the antioxidant activity is mainly related to the intrinsic milk constituents such as caseins, carotenoids, vitamins, and other low molecular weight substances (Khan et al. 2019), and it can also depend on its microbiological profile (Morandi et al. 2016) and on the cheese making procedures, including ripening conditions (Perna et al. 2015).

To date, little research was addressed to an integrated characterisation of the nutritional quality of Italian mountain cheeses. Therefore, the present work aimed at characterising cheese samples from two small-scale farms from the Alpine arch of Piedmont (Italy) for the FA profile, the ACE-inhibitory and the anti-oxidant activity, in relationship with the herds' feeding strategies and the seasonality effect (summer and winter production).

Materials and methods

Farming conditions

Two Piedmont (Italy) alpine cattle dairy farms were considered in the study. The investigation covered two seasons: summer (August–September 2018, 50 days) and winter (January–March 2019, 77 days). The two farms (namely, Farm A and Farm B) were somewhat different as far as the management and the feeding strategies adopted. Farm A was mainly composed by Italian Brown (IB) and Pezzata Rossa (PR) cattle breeds. During summer, Farm A exclusively adopted alpine pastures grazing (2000 m altitude) as feeding strategy. During winter, the cows were fed a total mixed ration (TMR) composed of grass hay, alfalfa hay and concentrates. Farm B was a mono-breed of Italian Brown cows. During summer, cows in Farm B were supplied daily with cut fresh grass harvested at 1300–1400 m above sea level and, during the night, a TMR integration including alfalfa hay and concentrates was supplied in the barn. During winter, cows were fed a TMR composed of grass hay and concentrates. Moreover, in both seasons, a further integration of concentrates was supplied to the cows directly in the milking parlour. More detailed information about the characteristics of the farms and the composition of feedstuffs have been recently published by Lopez et al. (2022).

Cheese characteristics and sampling

A total of 35 *Ossolano*-like cheese samples have been included in the study, of which 19 (11 in Farm A and 8 in Farm B) were sampled during the winter and 16 (8 in Farm A and 8 in Farm B) during summer. In both farms, cheese manufacture was performed with raw milk, salt, and rennet as follows: (i) milk was curdled at 30–34 °C; (ii) the clotting time ranged between 35 and 45 min; (iii) the curd was finely broken and then cooked (44–46 °C) for 20 min; and (iv) the mass was extracted from the whey and placed in moulds

subjected to pressing for about 4–8 h. The ripening time was 60 days at 10 °C and 14 °C ambient. All the samples were collected directly in the farms and stored under vacuum at −20 °C, then transported to the laboratories under controlled temperature condition and stored at −20 °C until analysis.

Fatty acid profile

Fatty acid profile was analysed by gas chromatography (GC) and flame ionisation detection (FID) according to the method employed in Lopez et al. (2022). Briefly, total lipids were extracted by a cold mixture of $CHCl_3$/MeOH (2:1); about 30 mg of extracted lipids were used for fatty acids methyl esters (FAMEs) preparation performed using sodium methoxide in methanol 1 M. The analytical equipment employed for FAMEs identification consisted in a TRACE™ 1300 gas chromatograph equipped with a TR-FAME column (120 m, 0.250 mm id, 0.25 μm film thickness) by Thermo Fisher Scientific (Waltham, MA), and connected to an FID detector. FAMEs peaks were identified by comparing retention times with those of FAMEs standard mixtures and pure standard purchased by Sigma-Aldrich (St. Louis, MO) and Larodan (Solna, Sweden).

ACE-inhibitory activity (ACE-IA)

The water-soluble extract (WSE) of cheeses was prepared according to the procedure described by Basiricò et al. (2015). Five grams of cheese sample were homogenised using an Ultra-Turrax-T25 basic homogeniser (IKA Works Inc., Wilmington, NC), incubated at 40 °C for 1 h, and then centrifuged at 3000×*g* for 30 min at 4 °C. Cheese suspension was acidified to pH 4.4 with 1 M HCl to precipitate CN. The supernatant was filtered (Whatman grade 2, GE Healthcare, Little Chalfont, UK), centrifuged at 3000 × *g* for 30 min at 4 °C. The 3-kDa permeate of WSE was obtained using an Amicon Ultra 3 kDa UF system (Amicon, Millipore, Bedford, MA). The WSE was stored at −80 °C and used for the ACE-inhibitory activity test.

The concentration of peptides in the WSE of cheese samples and their ACE-IA were measured as previously described (Basiricò et al. 2015). The concentration of peptides in WSE was determined by a colorimetric method (BCA TM Protein Assay Kit, Pierce, Rockford, IL). The absorbance was measured at 595 nm using a Sunrise Plate Reader (Tecan Trading AG, Mannedorf, Switzerland). All WSE samples were performed in triplicate. The ACE-IA of WSE was measured by a colorimetric method (ACE kit-WST, Dojindo Inc., Kumamoto, Japan) using a commercial kit according to the manufacturer's instructions. Absorbance was measured at 450 nm using a Sunrise Plate Reader (Tecan Trading AG, Mannedorf, Switzerland) and the IC_{50} values were calculate as the concentration required to inhibit 50% of the ACE activity *in vitro* and was expressed as micrograms of peptides per millilitre of 3 kDa WSE.

Anti-oxidant properties

The thiol content (SH), the ABTS-scavenging activity (ABTS-SA), and the ferric-reducing antioxidant power (FRAP) were performed on water soluble extract prepared as follows. The cheese samples were warmed at room temperature, then 10 g of finely grated cheese and 30 mL of NIST Type I water (0.055 μS cm) was added in a 50 mL screw cap tube. The mixture was submitted to a homogenisation step through Cole Palmer/Branson B220 Ultrasonic Cleaner ultra-sounds bath (Branson, UK) for 10 min. Samples were then centrifuged at 5000 × g for 20 min at 4 °C and the water-soluble layers (WSE) were then filtered through 597 ½ Ø 150 mm folded filters (Schleicher & Schulle Italia S.r.L., Italy). After a further filtration step through 0.45 μm membrane filters (Corning Incorporation, Corning, NY, NAT), several aliquots of WSE per cheese sample were stored at −80 °C until analyses. The SH assay was performed in triple according to Ellman (1959) with the modifications proposed by Perna et al. (2015). The absorbance measurements were performed at 412 nm against air by using a Lambda 20 UV–VIS spectrophotometer (Perkin Elmer, Waltham, MA). As the blank, 125 μL of sodium phosphate buffers was used in the place of WSE. The number of free thiols per gram of cheese was obtained by the following equation:

$$SH(\mu moles/100g) = 100 \times \frac{V}{W} \times \frac{(A - A_0)}{\varepsilon} \quad (1)$$

where *V/W* is the water volume/cheese weight ratio, *A* and A_0 are the absorbances of the sample and the blank, respectively, and ε is the molar extinction coefficient (14.15 $M^{-1}cm^{-1}$). The ABTS radical cation scavenging assay was performed according to the improved method of Re et al. (1999). The absorbance was monitored for 6 min at 30 s intervals by using an UV-1601 spectrophotometer (Shimadzu Italia S.r.L., Italy). As anti-oxidant standard, the ascorbic acid (Carlo Erba, Italy) in ultrapure water was used. Five points calibration curves (3.48–56.63 mg/L, $R^2 \geq 0.989$) were obtained daily, against which the ABTS-SA of the sample, expressed in milligrams of ascorbic acid equivalent (mgAAE), was extrapolated as follows:

$$ABTS - SA\ \ (mgAAE/100g) = 100 \times \frac{V}{W} \times \frac{(A_{734} - b)}{m} \quad (2)$$

where *V/W* is the water volume/cheese weight ratio in the WSE, A_{734} is the absorbance of the sample, *b* and *m* are the intercept and the slope of the calibration curve, respectively. To take into account that the behaviour of the anti-oxidants versus ABTS reaction can be more than a simple first order kinetic, the area under the curve (AUC) approach was used (Re et al., 1999).

The FRAP assay was performed according to Benzie and Strain (1996). The absorbance was measured at 593 nm through a Lambda 20, UV–VIS spectrometer (Perkin Elmer, Waltham, MA) against acetate buffer (pH 3.6). The ascorbic acid was used as standard. Four points calibration curves (0.05–0.44 mg/L, $R^2 \geq 0.996$) were obtained daily, against which the FRAP of the sample, was computed as follows:

$$FRAP\ \left(\frac{mgAAE}{100g}\right) = \ 100 \times \frac{V}{W} \times \frac{(A - b)}{m} \quad (3)$$

where *V/W* is the water volume/cheese weight ratio (mL/g) in the WSE, *A* is the absorbance of the sample, *b* and *m* are the intercept and the slope of the calibration curve, respectively. To proportionate the SH, ABTS-SA and FRAP to a unit of weight of water-soluble protein, the protein content of the WSE was measured by using the Pierce BCA Assay kit (23225) (Thermo Scientific, Rodano, Italia) according to the manufacturer's instruction.

The total polyphenols content of the cheese samples was assayed by the Folin–Ciocalteau method on the methanolic extracts proposed by Shaiban et al. (2006) and modified by Rashidinejad et al. (2013). The absorbance measurements were performed at 750 nm against air with an UV-1601 UV-visible spectrophotometer (Shimadzu Italia, S.r.L.). The total polyphenolic (TP) content was quantified against 5-points linear calibration curves (range 12.5–400 μg/mL; $R^2 \geq 0.990$) constructed by using the gallic acid (Sigma-Aldrich, St. Louis, MO) as a standard, according to the following equation:

$$TP\left(mg\frac{GA}{100g}\right) = \ 0.1 \times \frac{V}{W} \times \frac{(A - b)}{m} \quad (4)$$

where *V/W* is the water volume/cheese weight ratio (mL/g) in the methanolic extracts, *A* is the absorbance of the sample, *b* and *m* are the intercept and the slope of the calibration curve, respectively. For the aforementioned assays, the measurement was performed in triple, or in double (TP), and the mean values were used in the statistical analysis.

Statistics

All data were submitted to a statistical evaluation performed through the GLM procedure (Statistica 10, StatSoft Inc., College Station, TX) according to the following factorial design:

$$y_{ijk} = \mu + F_i + S_j + (F \times S)_{ij} + \ \varepsilon_{ijk}$$

where *y* is the cheese characteristic (FA, ACE-IA, ABTS-SA, FRAP, SH, TP), μ is the overall mean, *F* is the case study effect (*i* = Farm A, Farm B), *S* is the season effect (j = summer, winter), $F \times S$ is the first order interaction and ε is the error term. For the ABTS-SA and FRAP assay data, both the total soluble proteins extracted in water (WSP) and the total polyphenols (TP) were entered in the model as covariates. The significance of the differences in the comparison between means was assayed through the Tuckey's *post hoc* test. Significance was declared at $p < .05$. To get insights about which were the main factors able to potentially discriminate the cheeses between seasons and/or farms, a Principal Component Analysis (PCA) was performed (JMP Pro16, SAS, Cary, NC). All the measured variables (individual FAs, ACE-IA, SH, ABTS-SA, FRAP and TP) were fused in a 35 (samples) × 51 (variables) data matrix and data were auto-scaled before the analysis. For data interpretation, only the original variables that accounted for more than 50% of the variance explained by a combination of PC1 and PC2 were retained.

Results

Fatty acid profile

Fatty acids (FAs) composition of cheese samples is reported in Table 1. Significant differences dependent on evaluated factors, *F*, *S* and their interaction $F \times S$, were detected. Particularly, the feeding system adopted in Farm A during summer minimised the amount of short and medium chain saturated fatty acids (SC- and MC-SFAs) from C6 to C16; on the contrary, it maximised the content of the long chain SFAs (LC-SFAs, >C18). Actually, mean values for these FAs detected in cheese from Farm A were always significantly higher ($p < .001$) than in the other all groups of samples. Within Farm B, the profile for SFAs was similar in both the seasons, with the only exception for a significant difference for stearic acid (18:0) that resulted higher ($p < .001$) in winter (10.34%) than in summer (8.76%).

Odd and branched chain FAs (OBCFAs) showed a different tendency. The odd-chain FAs (OCFAs) showed significant ($p < .01$) differences due to the

Table 1. Fatty acids composition (mean values, g/100 g of total FAs) of winter and summer cheese samples collected in the two farms.

	Farm A		Farm B					
Farm	Summer	Winter	Summer	Winter	*F*	*S*	*F* × *S*	RMSE
4:0	4.75	4.8	4.70	4.77	0.057	0.571	0.247	0.308
6:0	1.98 b	2.61 a	2.65 a	2.66 a	<.001	<.001	<.001	0.210
8:0	1.01 b	1.57 a	1.65 a	1.67 a	<.001	<.001	<.001	0.113
10:0	1.79 c	3.26 b	3.60 a	3.55 a	<.001	<.001	<.001	0.202
12:0	1.94 c	3.58	4.03 a	3.95 a	<.001	<.001	<.001	0.207
14:0	7.62 c	11.21 b	12.16 a	11.74 a	<.001	<.001	<.001	0.317
16:0	24.66 b	30.02 a	29.6 a	28.74 a	<.001	<.001	<.001	1.095
18:0	13.44 a	10.71 b	8.76 c	10.34 b	<.001	<.01	<.001	0.693
20:0	0.25 a	0.20 b	0.14 c	0.15 c	<.001	<.001	<.001	0.020
22:0	0.12 a	0.09 b	0.06 c	0.06 c	<.001	<.001	<.001	0.012
24:0	0.08 a	0.06 b	0.03 c	0.04 bc	<.001	0.206	<.001	0.015
SFAs	57.64 b	68.16 a	67.47 a	67.67 a	<.001	<.001	<.001	1.306
13:0	0.06 c	0.09 b	0.10 a	0.10 ab	<.001	<.001	<.001	0.009
15:0	1.14 b	1.28 a	1.12 bc	1.04 c	<.001	0.134	<.001	0.062
17:0	0.87 a	0.72 b	0.53 c	0.51 c	<.001	<.001	<.001	0.023
17:1	0.09 c	0.26 a	0.03 d	0.17 b	<.001	<.001	<.01	0.016
21:0	0.04 a	0.03 ab	0.03 bc	0.02 c	<.001	0.086	0.506	0.006
OCFAs	2.20 b	2.39 a	1.81 c	1.84 c	<.001	<.001	<.01	0.080
*iso*13	0.05 a	0.04 b	0.05 a	0.03 b	0.983	<.001	0.072	0.006
*iso*14	0.17 a	0.18 a	0.14 b	0.17 a	<.01	<.001	<.01	0.013
*iso*15	0.37 a	0.32 b	0.24 c	0.24 c	<.001	<.001	<.01	0.019
*anteiso*15	0.67 a	0.67 a	0.54 b	0.53 b	<.001	0.503	0.561	0.031
*iso*16	0.32 b	0.40 a	0.28 c	0.30 bc	<.001	<.001	<.001	0.020
*iso*17	0.01 b	0.14 a	0.02 b	0.21 a	0.069	<.001	0.186	0.056
*anteiso*17	0.61 a	0.61 a	0.45 b	0.40 b	<.001	0.398	0.322	0.081
BCFAs	2.20 b	2.36 a	1.71 d	1.87 c	<.001	<.001	0.871	0.112
OBCFAs	4.4 b	4.76 a	3.53 c	3.71 c	<.001	<.001	0.134	0.162
14:1	0.53 d	0.87 c	1.14 a	0.97 b	<.001	<.001	<.001	0.060
t-16:1	0.20 a	0.04 d	0.13 b	0.06 c	<.001	<.001	<.001	0.009
c-16:1	1.57 a	1.38 a	0.33 b	1.25 a	<.001	<.001	<.001	0.333
*t*9-18:1	0.35 b	0.29 b	0.44 a	0.28 b	<.05	<.001	<.05	0.062
*t*11-18:1	3.74 a	0.99 d	2.17 b	1.32 c	<.001	<.001	<.001	0.238
*c*9-18:1	27.00 a	19.53 b	18.95 b	19.29 b	<.001	<.001	<.001	0.809
*c*11-18:1	0.60 a	0.41 c	0.56 b	0.41 c	0.054	<.001	0.051	0.032
20:1 n-9	0.05 b	0.06 ab	0.06 a	0.05 b	0.418	0.504	<.001	0.010
22:1 n-9	0.03	0.03	0.02	0.03	0.122	0.655	0.111	0.009
MUFAs	34.05 a	23.61 b	23.74 b	23.66 b	<.001	<.001	<.001	0.919
t9t12-18:2 n-6	0.03 c	0.18 b	0.01 d	0.21 a	0.363	<.001	<.001	0.012
c9t12-18:2 n-6	0.02 b	0.03 ab	0.03 ab	0.04 a	<.01	<.01	0.923	0.012
t9c12-18:2 n-6	0.04 a	0.02 b	0.03 ab	0.03 ab	0.888	<.01	0.151	0.011
c9c12-18:2 n-6	1.27 c	1.82 b	2.81 a	2.77 a	<.001	<.001	<.001	0.140
18:3 n-6	0.02 ab	0.02 b	0.03 a	0.03 ab	<.01	<.05	0.929	0.008
18:3 n-3	0.75 a	0.48 b	0.72 a	0.69 a	<.001	<.001	<.001	0.044
c9t11-18:2	1.49 a	0.53 d	1.26 b	0.71 c	<.05	<.001	<.001	0.078
20:2	0.02 c	0.03 b	0.03 a	0.03 a	<.001	<.01	<.01	0.004
20:3 n-6	0.04 d	0.08 c	0.11 a	0.09 b	<.001	<.001	<.001	0.007
20:4 n-6	0.10 d	0.12 c	0.16 a	0.14 b	<.001	0.793	<.001	0.010
20:3 n-3	0.01	0.01	0.01	0.01	0.441	0.362	0.884	0.008
20:5 n-3	0.05	0.05	0.05	0.05	0.460	0.707	0.751	0.010
22:5 n-3	0.07	0.09	0.09	0.08	0.930	0.699	0.07	0.021
PUFAs	3.91 c	3.46 d	5.34 a	4.88 b	<.001	<.001	0.189	0.190
Total n-3	0.88 a	0.87 a	0.63 b	0.83 a	<.001	<.001	<.001	0.041
Total n-6	1.52 c	3.18 a	2.27 b	3.31 a	<.001	<.001	<.001	0.160
n-6/n-3	1.73 c	3.59 b	3.65 b	4.00 a	<.001	<.001	<.001	0.188

a, b, c, d = values in the same row with different letters were significantly different ($p < .05$, $p < .01$, $p < .001$).
BCFAs: branched chain fatty acids; MUFAs: monounsaturated fatty acids; OCFAs: odd chain fatty acids; PUFAs: polyunsaturated fatty acids; SFAs: saturated fatty acids.

$F \times S$ interaction, with the highest levels (2.39%) found in winter cheese from Farm A, followed by summer cheese from Farm A (2.20%) and both summer and winter cheese from farm B (1.81–1.84%). The branched-chain FAs (BCFAs) did not show differences due to the $F \times S$ interaction, but a significant effect of seasonality ($p < .001$) and Farm ($p < .001$) was found, with the highest amounts (2.36%) detected in winter cheese from Farm A and the lowest (1.71%) in summer cheese from Farm B.

The feeding system affected the amounts of some *cis* and *trans* monounsaturated FAs (MUFAs), that are primary constituents of dairy products, in cheese collected in the two farms. In details, *t*-16:1, *t11*-18:1

(vaccenic acid) and c9-18:1 (oleic acid) showed significantly higher amounts ($p < .001$) in cheese samples collected in Farm A during summer (0.20%, 3.74% and 27.00%, respectively). With the only exception for oleic acid, samples collected in Farm B during summer showed intermediate mean values and samples collected in winter the lowest values. The *cis* isomer of vaccenic acid (*cis*-vaccenic acid, *c11*-18:1) showed a significant difference ($p < .001$) due to the effect of seasonality, with the highest values recorded during summer (0.56–0.60%) and the lowest during winter (0.41%).

Among the polyunsaturated fatty acids (PUFAs), the main conjugated of linoleic acid (CLA) rumenic acid (*c9t11*-18:2) showed the highest ($p < .001$) mean value in cheese collected in summer cheese from Farm A (1.49%), followed by summer cheese from Farm B (1.26%) and winter cheese from Farm B (0.71%) and Farm A (0.53%), respectively. At the same time, cheese from Farm B was characterised by the highest ($p < .001$) amounts of linoleic acid (*c9c12*-18:2) in both summer (2.81%) and winter (2.77%) compared to Farm A (1.27% in summer and 1.82% in winter).

ACE-inhibiting activity

Water-soluble extracts obtained from cheese samples produced in Farm A showed IC_{50} values ranging between 2.21 and 2.72 μg of peptides/mL and a mean IC_{50} value of 2.51 ± 0.16 μg of peptides/mL. In Farm A, cheese samples manufactured in summer season (IC_{50} 2.21 ± 0.17 μg of peptides/mL) presented a lower ($p < .05$) IC_{50} than cheeses produced in winter season (IC_{50} 2.72 ± 0.23 μg of peptides/mL) (Figure 1). Cheese samples of Farm A exhibited different ACE-IA in relation to the production season, greater in summer cheeses. In Farm B, the IC_{50} values of cheese samples ranged between 1.99 and 2.18 μg of peptides/mL and showed a mean IC_{50} value of 2.09 ± 0.07 μg of peptides/mL. Cheese samples produced in the summer season by Farm B showed an IC_{50} value of 1.99 ± 0.11 peptides/mL μg and in winter season an IC_{50} value of 2.18 ± 0.08 μg of peptides/mL (Figure 1). In Farm B, the mean IC_{50} value of summer cheeses tended to be lower than the winter cheeses, but no significant differences were observed between the two production seasons. At the same time, cheese samples from Farm B showed lower ($p < .05$) IC_{50} value (1.99 ± 0.11 μg of peptides/mL) than cheese samples produced by Farm A (2.72 ± 0.23 μg of peptides/mL), above all in winter season (Figure 1).

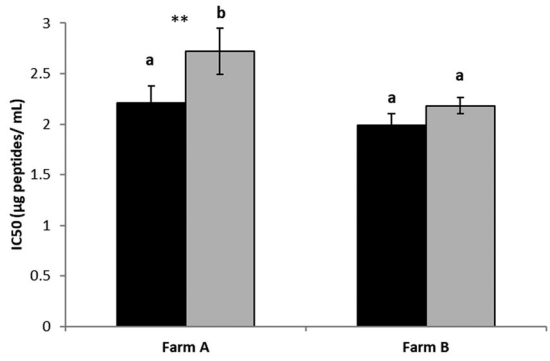

Figure 1. Effects of the factors farm (Farm A and Farm B) and season of production (summer, black bars; winter grey bars) on the IC50 [inhibit 50% of the ACE activity in vitro] values (mean ± SE) of fractions collected from water-soluble extracts (WSE) of mountain cheeses. Lowercase letters indicate differences at $p<.05$ between farms within the same production season. Asterisks (**) highlight significant differences at $p<.05$ between seasons within the same farm.

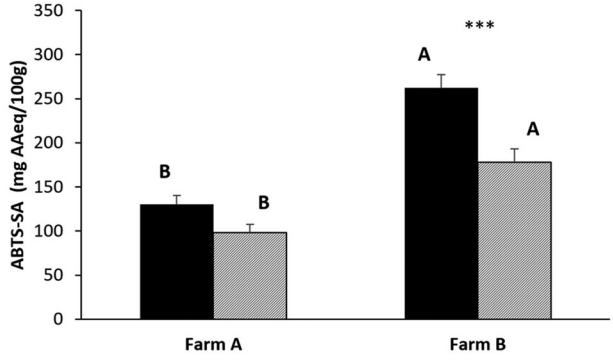

Figure 2. Effects of the factors farm (*F*: Farm A and Farm B) and season of production (*S*: summer, black bars; winter grey bars) on the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) scavenging activity (mean ± SE) of mountain cheeses. Letters in capital denotes differences at $p<.01$ between farms within the same production season. Asterisks (***) highlight significant differences at $p<.001$ between seasons within the same farm. The $F \times S$ interaction was not significant.

Antioxidant activity and polyphenols

As expected, the season of production had a highly significant effect ($p < .001$) on the ABTS-SA expressed as mg AAE per 100 g of product. Summer cheeses showed a + 48.7% average scavenging potential than the winter ones, though only for Farm B the significance was ascertained (Figure 2). In addition, differences ($p < .001$) were observed as far as the ABTS-SA comparing the farms, with Farm B cheeses that showed about a double activity in summer and +181% activity in winter, if compared with the cheese produced by the Farm A. No significance was

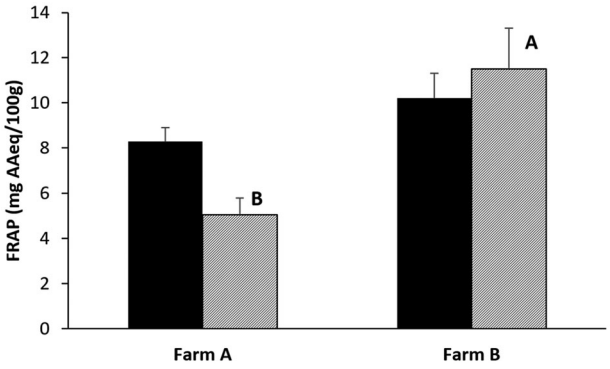

Figure 3. Effects of the factors farm (*F:* Farm A and Farm B) and season of production (*S*: summer, black bars; winter grey bars) on the ferric reducing potential (FRAP) (mean ± SE) of mountain cheeses. Letter in capital denotes differences at $p<.01$ between farms within the same season of cheese production. The $F \times S$ interaction was not significant.

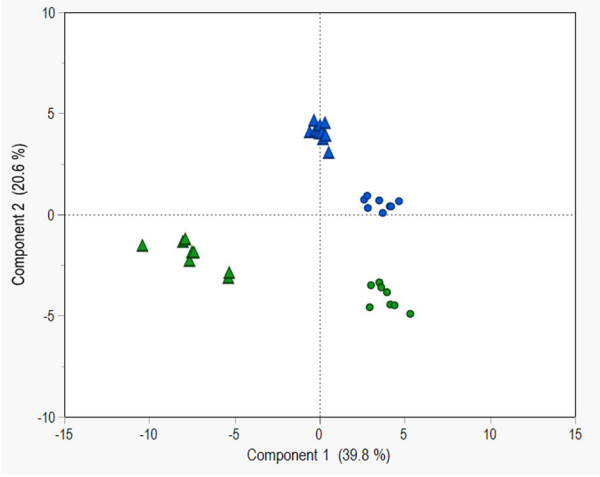

Figure 4. Principal Component Analysis (PCA) scores plot. The variance explained by PC1 and PC2 is reported within brackets.

observed as far as the $F \times S$ interaction. Neither the water-soluble proteins (WSP), nor the total polyphenols (TP), if introduced as covariates in the statistical model (data not shown), showed significant effects.

As far as the reducing capacity evaluated through the FRAP assay, only the case study affected the measured values ($p < .001$), but only for the winter cheeses it was observed a difference ($p < .01$) between the farms (5.06 ± 0.74 and 11.51 ± 1.79 mg AAE/100 g for Farm A and Farm B, respectively) (Figure 3). As for the ABTS-SA, no significance was observed as far as the $F \times S$ interaction. Neither the WSP nor the TP, if introduced as covariates in the statistical model, had significant effects on the total reducing capacity of cheeses assessed through the FRAP test.

The total thiols content in the cheese samples extracts was affected ($p < .01$) by the farm only, with no significant $F \times S$ interaction. In particular, the SH content of the cheeses from Farm A was about a half of the value observed for the arm B (7.80 ± 1.04 and 14.67 ± 1.54 μmol/100 g, respectively). As observed for the previous assays, the WSP, if introduced as covariate in the statistical model, was not significant. The TP content did not differ between the case studies (300.1 ± 14.5 and 313.1 ± 27.7 mg GA/100 g for Farm A and Farm B, respectively) or between the seasons (326.9 ± 22.7 and 288.5 ± 14.5 mg GA/100 g for the summer and winter cheeses, respectively), but a significant $F \times S$ interaction ($p < .01$) was observed. Such interaction was the result of a different tendency showed by the TP content that in Farm A surprisingly increased (+11.6% on average) passing from the summer to the winter cheeses, but in Farm B, cheeses an opposite behaviour (-31.9% on average) was observed. However, the *post hoc* Tuckey test did not statistically support such numerical differences (data not shown).

Multivariate analysis

With the purpose to investigate if any information lying in a systematic correlation structure underlying the data undetectable by univariate statistics, a principal component analysis was performed (Figure 4 and Table 2). The two first principal components explained 60.4% of the variance; the third component explained the 7.5% and, as it did not allow the separation of any group, it was not considered further. In the plane defined by PC1 and PC2, cheeses samples clustered separately depending on the farm-to-season combination, and no partial overlapping was observed (Figure 4). Particularly, along the direction of the maximum variance explained by PC1, samples clustered in three main groups. Cheese produced in Farm A during summer characterised by negative scores, while cheese produced in Farm B during both seasons were characterised by positive PC1 scores (Table 2). Finally, cheese produced in Farm A during winter was characterised by intermediate PC1 scores. Along the direction of maximum variance explained by PC2, samples clustered in different groups. Cheese produced in Farm A during winter was characterised by the highest positive scores, while cheese produced in Farm B during summer was characterised by the highest negative scores. Finally, intermediate PC2 scores characterised cheese produced in Farm A during summer and in Farm B during winter. The matrix of loadings on PC1 and PC2 is reported in Table 2.

Table 2. PC1 and PC2 loadings matrix; only variables that accounted for > 50% of the explained variance were retained.

	PC-1	PC-2
12:0	0.973	0.136
10:0	0.968	0.157
14:0	0.954	0.149
14:1	0.940	−0.118
20:3 n6	0.939	−0.019
8:0	0.936	0.203
c9c12, 18:2 n6	0.909	−0.282
13:0	0.855	0.067
6:0	0.840	0.214
20:2	0.840	−0.069
20:4 n6	0.833	−0.306
16:0	0.759	0.352
ABTS	0.531	−0.738
17:1	0.037	0.977
iso16	−0.276	0.842
t9t12, 18:2 n6	0.321	0.826
iso17	0.400	0.627
cis, 16:1	−0.566	0.515
anteiso15	−0.741	0.464
iso15	−0.904	0.289
20:0	−0.924	0.189
15:0	−0.230	0.571
17:0	−0.950	0.184
cis11, 18:1	−0.477	−0.799
24:0	−0.830	0.177
trans11, 18:1	−0.726	−0.665
c9t11-18:2	−0.472	−0.860
22:0	−0.917	0.087
18:0	−0.919	0.117
cis9, 18:1	−0.922	−0.267
trans,16:1	−0.646	−0.751
18:3 n3	−0.095	−0.865

Discussion

The trend observed from the main classes of FAs (SFAs, MUFAs, PUFAs) was in agreement with literature data on milk fat quality from grass-fed cows, establishing as the consumption of fresh grass and alpine pastures generally decrease SFAs and increase MUFAs and PUFAs (Chilliard et al. 2007). Particularly, cheese samples collected in Farm A during summer showed the lowest amounts of de-novo synthesis FAs (< C16) and the highest amounts of LCFAs. Recently, Formaggioni et al. (2020) obtained similar results, detecting lower MCFAs and higher LCFAs in cheese obtained from cows led to valley or mountain pastures. These result could be explained by the fact that high PUFAs intake from pasture could have competed with de novo FAs esterification in cows mammary gland, leading to a decrease in the synthesis of SC- and MC-SFAs, as suggested by Esposito et al. (2014). It has been recently demonstrated that, despite the high content in SFAs, dairy fats does not promote atherogenesis; moreover, it has been reported that LCFAs ingestion could even decrease the risk of development of many cardiovascular diseases (Astrup et al. 2020; Kang et al. 2020). In light of this, the higher amounts of LCFAs, together with lower amounts of SCFAs and MCFAs, in summer cheese from Farm A could be considered of interest in the characterisation of the nutritional quality of cheese analysed in this study. Particularly, myristic acid (14:0), considered as the most powerful raising LDL-cholesterol in dairy products, was detected at significantly lower amounts in summer cheese from Farm A, according to previous findings for cheese from mountain pastures (Formaggioni et al. 2020).

In this study, odd and branched chain FAs (OBCFAs) showed different trends depending on both the seasonality and the farm where cheese were collected. Mainly, the differences in the content of OBCFAs reflect variations in the bacterial ruminal population of animals; moreover, many of the OBCFAs can also derive from mammary gland de novo synthesis (Vlaeminck et al. 2006). The determination of the amounts of branched FAs (BCFAs) in dairy products, including cheese, could have a pivotal role for the characterisation of nutritional quality. Actually, even if constituting a lesser component of cow milk (about 3% of total FAs), BCFAs, *iso* and *anteiso* forms, are important bioactive components in ruminant products, associated to positive effects on consumers health, including the health of gut microbiota and the regulation of inflammatory processes in human cells (Gómez-Cortés et al. 2018; Hanus et al. 2018).

Cheese from Farm B showed the higher amounts of linoleic acid, related to the feeding strategy adopted in the farm, characterised by higher amount of concentrates, well known as natural sources of linoleic acid. On the contrary, pasture grass used as feed ingredient or directly grazed by cows in the farms involved in this study was characterised by 50–75% of α-linolenic acid (18:3n-3) (Lopez et al. 2022). Most of linoleic acid and α-linolenic acid introduced with the diet by ruminants undergo to rumen biohydrogenation (RBH) processes (Chilliard et al., 2007; Dewhurst et al., 2006). In the RBH pathway, linoleic and α-linoleic acids are saturated to stearic acid. Many intermediates of the process accumulate in the rumen, becoming available for absorption and transport to the mammary gland. Among them, vaccenic acid, that is in the mammary gland, is transformed in rumenic acid by the Δ9-desaturase (Chilliard et al. 2007;). In this study, both vaccenic acid and rumenic acid resulted higher in cheese produced during summer by grazing cows (Farm A), followed by cheese samples produced in Farm B, where high levels of fresh grass were fed even if directly in the barn. These outcomes agree with the knowledge that the pasture practice is

strictly related to higher PUFAs intake, higher RBH and higher amount of the intermediates leading to the mammary gland for the CLA synthesis (Chilliard et al. 2007). According to this, Formaggioni et al. (2020) found the highest amounts of vaccenic and rumenic acid in cheese from grazing cows. Similar to what has been reported for SFAs, in the last years, there has been an increasing interest towards the role of *trans* fatty acids in human nutrition. Generally, it is thought that the consumption of *trans* FAs is related to adverse effects on human health (pro-inflammatory, dysfunctions, insulin resistance, etc.). However, scientific opinions specified that *trans* FAs naturally present in ruminant products, appear quantitatively insufficient to increase LDL-cholesterol levels (Mozaffarian 2019; Nestel and Mori 2022).

Some healthy indices have been extensively used in order to evaluate the lipid quality of animal products. Even if recently the scientific debate is shifting towards the evaluation of the potential beneficial/detrimental effects of individual FAs, such indices could be considered useful to compare products deriving from different producing methods. Among them, we can list the n-6/n-3 ratio that, in dairy products, essentially reflects the concentrations of linoleic (n-6) and α-linolenic (n-3) acids. This index can be improved by increasing the consumption of fresh grass in ruminants diets, resulting much better in mountain products (Leiber et al. 2005; Elgersma 2015). The n-6/n-3 ratio has already showed to reach values more than two times higher in conventional milk compared to extensive or pasture-based milk (Davis et al. 2020). Accordingly, cheese samples collected in the two farms investigated in this study reached n-6/n-3 values ranging from 1.73 (in summer cheese from Farm A) to 4.00 (in winter cheese from Farm B).

The mountain cheeses WSE of the two farms showed a consistent ACE-inhibitory activity and IC50 value comparable to individual peptides with a strong ACE-inhibitory action isolated from WSE of various cheese categories (Iwaniak and Mogut 2020). These results are indicators of a high quantity of naturally formed biopeptides in mountain cheese and predictive of a potential hypotensive effect in vivo of cheese. Many studies have been carried out to evaluate the ACE-inhibitory activity of cheeses of different manufacture and ripening times (Bütikofer et al. 2008). In the literature, it has been reported that proteolytic process is specific for each type of cheese and might be affected by milk pre-treatment, cultures, and ageing time (Rafiq et al. 2021). Gómez-Ruiz et al. (2002) reported a higher ACE-IA in cheese produced with raw milk compared to cheese obtained from pasteurised milk. The ACE-IA is also strongly influenced by the degree of proteolysis that depends on starter and non-starter lactic acid bacteria involved in the ripening process, since it influences the biopeptide content in the final product (Rafiq et al. 2021). Rafiq et al. (2017) reported that in semi-ripened and ripened cheese a relatively higher bioactive peptides were released in comparison with fully or low ripened cheeses. In the present study, the mountain cheese is artisanal made by farmers on a small scale using traditional practices, without starter culture, and ripened frequently for 60 days. The important ACE-IA observed in this samples may be attributed to the raw milk microbiota and to the short-ripened time. Our results highlighted the influence of the production season on the IC50 value of cheese samples, with IC50 values lower in the cheese produced during summer season compared to winter season, in Farm A. These results suggested that season of production may be an important factor influencing the microbial diversity present in raw milk, also linked to geographical origin. In the winter season, milk for cheese production was obtained from cows reared in barn and fed conserved forages in both farms, while in summer season by grazing in Farm A or fresh-cut grass-fed cows in Farm B, so microbial compositions of raw milk varied greatly above all in grazing cows. This is in accordance with previous work of Franciosi et al. (2004) who observed that in semi-hard cheese obtained from raw milk produced in Trentino the microbial composition of milk samples produced in different areas was rather heterogeneous. Those authors noted differences in distribution of microbial populations in milk samples of the same area in summer and winter periods linked to winter breeding in barn and summer alpine pasture of cows. Thus, indigenous microflora of milk constitutes a link between cheese, environment, and geographical area of production. Therefore, altitude, type of soil, forage essences typical of the geographical area, may contribute to determine a different microbial richness of summer cheeses compared to winter one in Farm A. In farm B, no differences were observed between the two-production season, probably the bacterial ecosystems have been little modified using cut grass in cows feeding.

Differences observed in the IC_{50} mean values of cheese samples between the two farms, as well as being linked to the different feeding management, could be related also to the genetic type of cattle. In Farm A, cheeses were produced using milk of two breeds, IB and PR cows, while in Farm B, exclusively with milk of IB. The genetic variants of milk proteins, responsible for the protein polymorphism, play an important role in the proteolysis during cheese ripening. Perna et al. (2016) observed in Italian Holstein (IH)

cows, that the casein haplotype (αS1, β, and κ) may affect the ACE inhibitory capacities of milk. Moreover, Perna et al. (2015) showed higher protein content and higher proteolysis of β- and para-κ-CN in IB cheese compared with IH cheese.

Milk products, as many other animal food products, contain naturally antioxidant substances, though in variable proportions due to the origin and the technological processes to which the raw material has undergone (Khan et al. 2019) but also due to the different concentrations of plant anti-oxidant substances naturally occurring in fresh forages or pasture compared with conserved forages (e.g. hay) (De La Torre et al. 2018). The measured antioxidant activity depends on the method used (Moon and Shibamoto 2009), and, for this reason, it was decided to use different anti-oxidant tests in this study. The observed effect of the production season (winter versus summer) on the ABTS-SA seems reasonably explained by the different cows' feeding regimes adopted in summer with mowed alpine forages as a relevant component (31.7% SDM) of the daily ration (Farm B) or directly on the mountain meadows (Farm A). The differences between farms can have a more subtle explanations partially rooted in the farm-specific feeding management (Hilario et al. 2010) as well as in the herd genetics. As already reported, the two herds had a different breed assortment (IB/PR versus IB only). In a study on the antioxidant properties of Caciocavallo cheeses ripened from 1 to 150 days, the ABTS-SA of IB cheese was higher than IH cheese (Perna et al. 2015). Being the ABTS-SA related to both lipophilic and hydrophilic anti-oxidants (Re et al. 1999), the differences observed can be explainable upon the different milk proteins of the breeds (Perna et al. 2015). The anti-oxidant properties assayed by the FRAP test showed higher values in the winter cheeses of Farm B that Farm A, and this result sounds in line with what was observed by Perna et al. (2015) who compared ripened Caciocavallo cheeses obtained from IB or IH milk. However, in the present study no differences were observed between farms for the summer cheeses. Probably, other factors than the phenotypic/genetic ones can have affected the FRAP results. No difference was observed as far as the SH content in the WSE of mountain cheeses produced in summer or winter season, but Farm B cheeses were almost double in SH content with respect to the ones from Farm A. These outcomes, support the hypothesis of the herd genetics (IB/PR and IB, respectively for Farm A and Farm B) that most probably played a relevant role on the proteolysis process occurred during the ripening period (Mariani et al. 2002). The TP content found in this study cannot be directly compared with similar research focussed on alpine bovine cheeses, since similar studies including this variable were not found in the literature. However, the TP content of some Italian cheeses produced in hilly and/or mountain areas of Sardinia and Sicily was recently assessed by Di Trana et al. (2022). Compared to the data reported by Di Trana et al. (2022), the TP content of the alpine cheese samples investigated in this study laid in the range recorded for the Sardinian 'Casizolu del Montiferru' cheese (298–365 mgGA/100g, no statistical difference recorded for cheeses produced in winter, spring or late summer), but were lower than the values reported for 'Caciocavallo Palermitano' cheese in winter (352 mgGA/100) or in spring (465 mgGA/100g). In this study, the TP content did not depend upon the factor 'farm' or 'season'; however, a significant farm-to-season interaction was recorded. Noteworthy, the numerical increase of the TP content in Farm B summer cheeses (+31.9% on average), in comparison with the winter ones, paralleled the observed enhancement of the antioxidant activity assayed by the FRAP test. This fact suggests a direct relationship between the TP content and the anti-oxidant activity assayed by the FRAP test in cheeses (Chávez-Servín et al. 2018).

Multivariate analysis allowed identifying a tendency of samples to grouping based on both seasonality and farm. The original variables that accounted for most of the variance were associated to lipid quality (31 FAs) and to the antioxidant capacity of cheese (ABTS-SA). Within the same farm, a clear separation between cheeses manufactured in different seasons was observed, with summer cheese associated to higher scores for many FAs previously indicated as markers of pasture and/or fresh grass intake (namely, α-linolenic acid, *c9t*11-CLA, *cis*-vaccenic acid, *t*16:1, vaccenic acid) and the ABTS-SA. This indicated a relevance of seasonality, influencing the quality of alpine cheeses analysed in this study, mainly linked to the possibility to fed cows with nutritionally valuable forages during summer. Furthermore, a clear separation was observed between cheeses produced in the two farms during summer, with cheese from Farm A positively associated to several FAs indicative of higher ruminal activity (namely, oleic acid, 17:0, 18:0, 22:0, 20:0, *iso*15, 24:0, vaccenic acid, *t*-16:1, *anteiso*15, *c9t*11-18:2, *cis*-vaccenic acid, palmitoleic acid). This outcome suggested that, within the cheese summer production analysed, an added value was advisable in cheese manufactured with milk of cows exclusively grazing alpine pasture. On the other hand, a less noticeable

separation was observed between the two farms during winter, when the management conditions were more similar in both farms.

Though the results presented in this manuscript are only representative for the farms involved in the study, an overall superior quality of summer cheeses, particularly those produced by exclusively grazing cows (Farm A, during summer), was advisable by means of the combination of all the parameters analysed in this study, some of which could be worthy of further study with the aim of tracing different type of alpine cheese productions.

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Ethical approval

This article does not contain any studies with human or animal subjects.

Disclosure statement

The authors declare no conflicts of interest exist in the submission of this manuscript.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

Astrup A, Magkos F, Bier DM, Brenna JT, de Oliveira Otto MC, Hill JO, King JC, Mente A, Ordovas JM, Volek JS, et al. 2020. Saturated fats and health: a reassessment and proposal for food-based recommendations: JACC state-of-the-art review. J Am Coll Cardiol. 76(7):844–857.

Basiricò L, Catalani E, Morera P, Cattaneo S, Stuknytė M, Bernabucci U, De Noni I, Nardone A. 2015. Release of angiotensin converting enzyme-inhibitor peptides during in vitro gastrointestinal digestion of Parmigiano Reggiano PDO cheese and their absorption through an in vitro model of intestinal epithelium. J Dairy Sci. 98(11): 7595–7601.

Benzie IFF, Strain JJ. 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Biochem. 239(1):70–76.

Bergamaschi M, Cipolat-Gotet C, Stocco G, Valorz C, Bazzoli I, Sturaro E, Ramanzin M, Bittante G. 2016. Cheesemaking in highland pastures: milk technological properties, cream, cheese and ricotta yields, milk nutrients recovery, and products composition. J Dairy Sci. 99(12):9631–9646.

Branciari R, Ranucci D, Trabalza-Marinucci M, Codini M, Orru M, Ortenzi R, Forte C, Ceccarini MR, Valiani A. 2015. Evaluation of the antioxidant properties and oxidative stability of Pecorino cheese made from the raw milk of ewes fed Rosmarinus officinalis L. leaves. Int J Food Sci Technol. 50(2):558–565.

Bütikofer U, Meyer J, Sieber R, Walther B, Wechsler D. 2008. Occurrence of the angiotensin-converting enzyme-inhibiting tripeptides Val-Pro-Pro and Ile-Pro-Pro in different cheese varieties of swiss origin. J Dairy Sci. 91(1):29–38.

Chávez-Servín JL, Andrade-Montemayor HM, Velázquez Vázquez C, Aguilera Barreyro A, García-Gasca T, Ferríz Martínez RA, Olvera Ramírez AM, de la Torre-Carbot K. 2018. Effects of feeding system, heat treatment and season on phenolic compounds and antioxidant capacity in goat milk, whey and cheese. Small Rumin. Res. 160: 54–58.

Chilliard Y, Glasser F, Ferlay A, Bernard L, Rouel J, Doreau M. 2007. Diet, rumen biohydrogenation and nutritional quality of cow and goat milk fat. Eur J Lipid Sci Technol. 109(8):828–855.

Danaei G, Lu Y, Singh GM, Carnahan E, Stevens GA, Cowan M, Farzadfar F. 2014. Cardiovascular disease, chronic kidney disease, and diabetes mortality burden of cardiometabolic risk factors from 1980 to 2010: a comparative risk assessment. Lancet Diabetes Endocrinol. 2:634–647.

Davis H, Chatzidimitriou E, Leifert C, Butler G. 2020. Evidence that forage-fed cows can enhance milk quality. Sustain. 12(9):3688.

De La Torre S, Vincente F, Royo L. 2018. Dairy cows eating grass produce milk with a higher proportion of fat-soluble antioxidants. In: Sustainable Meat and Milk Production from Grasslands. Proceedings of the 27th General Meeting of the European Grassland Federation. Cork.

Dewhurst RJ, Shingfield KJ, Lee MRF, Scollan ND. 2006. Increasing the concentrations of beneficial polyunsaturated fatty acids in milk produced by dairy cows in high-forage systems. Anim Feed Sci Technol. 131(3-4):168–206.

Di Trana A, Di Rosa AR, Addis M, Fiori M, Di Grigoli A, Morittu VM, Spina AA, Claps S, Chiofalo V, Licitra G, et al. 2022. The quality of five natural, historical Italian cheeses produced in different months: gross composition, fat-soluble vitamins, fatty acids, total phenols, antioxidant capacity, and health index. Animals. 12(2):199.

Dimick PS, Kilara A. 1983. Photooxidative changes in milk and amino acids. Kieler Milchwirtsch Forschungsberichte. 35:289–299.

Elgersma A. 2015. Grazing increases the unsaturated fatty acid concentration of milk from grass-fed cows: a review of the contributing factors, challenges and future perspectives. Eur J Lipid Sci Technol. 117(9):1345–1369.

Ellman GL. 1959. Tissue Sulfhydryl Groups. Arch Biochem Biophys. 82(1):70–77.

Esposito G, Masucci F, Napolitano F, Braghieri A, Romano R, Manzo N, Di Francia A. 2014. Fatty acid and sensory profiles of Caciocavallo cheese as affected by management system. J Dairy Sci. 97(4):1918–1928.

Formaggioni P, Malacarne M, Franceschi P, Zucchelli V, Faccia M, Battelli G, Brasca M, Summer A. 2020. Characterisation of Formaggella della Valle di Scalve cheese produced from cows reared in valley floor stall or in mountain pasture: fatty acids profile and sensory properties. Foods. 9(4):383.

Franciosi E, Schiavon S, Poznanski E, Cavazza A. 2004. Biodiversità batterica in latti destinati alla produzione di formaggi nostrani trentini. In: Il Sistema Delle Malghe Alpine: Aspetti Agro-Zootecnici, Paesaggistici e Turistici. San Michele all'Adige (TN): Società per lo studio e la valorizzazione dei sistemi zootecnici alpini; p. 148–159.

Gómez-Ruiz JA, Ramos M, Recio I. 2002. Angiotensin-converting enzymeinhibitory peptides in Manchego cheeses manufactured with different starter cultures. Int Dairy J. 12(8): 697–706.

Gómez-Cortés P, Juárez M, de la Fuente MA. 2018. Milk fatty acids and potential health benefits: an updated vision. Trends Food Sci Technol. 81:1–9.

Hanus O, Samkova E, Křížova L, Hasoňova L, Kala R. 2018. Role of fatty acids in milk fat and the influence of selected factors on their variability—a review. Molecules. 23(7): 1636–1632.

Hilario MC, Puga CD, Ocaña AN, Romo FPG. 2010. Antioxidant activity, bioactive polyphenols in Mexican goats' milk cheeses on summer grazing. J Dairy Res. 77(1): 20–26.

Iwaniak A, Mogut D. 2020. Metabolic syndrome-preventive peptides derived from milk proteins and their presence in cheeses: a review. Appl Sci. 10(8):2772.

Kang ZQ, Yang Y, Xiao B. 2020. Dietary saturated fat intake and risk of stroke: systematic review and dose–response meta-analysis of prospective cohort studies. Nutr Metab Cardiovasc Dis. 30(2):179–189.

Khan IT, Nadeem M, Imran M, Ullah R, Ajmal M, Jaspal MH. 2019. Antioxidant properties of Milk and dairy products: a comprehensive review of the current knowledge. Lipids Health Dis. 18(1):13.

Leiber F, Kreuzer M, Nigg D, Wettstein HR, Scheeder MRL. 2005. A study on the causes for the elevated n-3 fatty acids in cows' milk of alpine origin. Lipids. 40(2):191–202.

Lin MY, Yen CL. 1999. Antioxidative ability of lactic acid bacteria. J Agric Food Chem. 47(4):1460–1466.

Lopez A, Bellagamba F, Savoini G, Moretti VM, Cattaneo D. 2022. Characterization of fat quality in cow milk from alpine farms as influenced by seasonal variations of diets. Animals. 12(4):515.

Marcone S, Belton O, Fitzgerald DJ. 2017. Milk-derived bioactive peptides and their health promoting effects: a potential role in atherosclerosis. Br J Clin Pharmacol. 83(1): 152–162.

Mariani P, Summer A, Formaggioni P, Malacarne M. 2002. La Qualità Casearia Del Latte Di Differenti Razze Bovine. La Razza Bruna. 1:7–13.

Moon J-K, Shibamoto T. 2009. Antioxidant assays for plant and food components. J Agric Food Chem. 57(5): 1655–1666.

Morandi S, Silvetti T, Tamburini A, Brasca M. 2016. Changes in oxidation-reduction potential during milk fermentation by wild lactic acid bacteria. J Dairy Res. 83(3):387–394.

Mozaffarian D. 2019. Dairy foods, obesity, and metabolic health: the role of the food matrix compared with single nutrients. Adv Nutr. 10(5):917S–923S.

Nestel PJ, Mori TA. 2022. Dairy foods: is its cardiovascular risk profile changing? Curr Atheroscler Rep. 24(1): 33–40.

Niero G, De Marchi M, Masi A, Penasa M, Cassandro M. 2015. Short communication: Characterization of soluble thiols in bovine milk. J Dairy Sci. 98(9):6014–6017.

Perna A, Intaglietta I, Simonetti A, Gambacorta E. 2015. Short communication: Effect of genetic type on antioxidant activity of Caciocavallo cheese during ripening. J Dairy Sci. 98(6):3690–3694.

Perna A, Intaglietta I, Gambacorta E, Simonetti A. 2016. The influence of casein haplotype on quality, coagulation, and yield traits of milk from Italian Holstein cows. J Dairy Sci. 99(5):3288–3294.

Rashidinejad A, Birch EJ, Sun-Waterhouse D, Everett DW. 2013. Effects of catechin on the phenolic content and antioxidant properties of low-fat cheese. Int J Food Sci Technol. 48(12):2448–2455.

Rafiq S, Gulzar N, Sameen A, Huma N, Hayat I, Ijaz R. 2021. Functional role of bioactive peptides with special reference to cheeses. Int J Dairy Technol. 74(1): 1–16.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 26(9–10):1231–1237.

Santiago-López L, Aguilar-Toalá JE, Hernández-Mendoza A, Vallejo-Cordoba B, Liceaga AM, González-Córdova AF. 2018. Invited review: Bioactive compounds produced during cheese ripening and health effects associated with aged cheese consumption. J Dairy Sci. 101(5): 3742–3757.

Shaiban M, AL-Mamary M, AL-Habori M. 2006. Total antioxidant activity and total phenolic contents in Yemeni smoked cheese. Sci York. 12:87–92.

Sieber R, Bütikofer U, Egger C, Portmann R, Walther B, Wechsler D. 2010. ACE-inhibitory activity and ACE-inhibiting peptides in different cheese varieties. Dairy Sci Technol. 90(1):47–73.

Sturaro E, Marchiori E, Cocca G, Penasa M, Ramanzin M, Bittante G. 2013. Dairy systems in mountainous areas:

farm animal biodiversity, milk production and destination, and land use. Livest Sci. 158(1–3):157–168.

Vargas-Bello-Pérez E, Márquez-Hernández RI, Hernández-Castellano LE. 2019. Bioactive peptides from milk: animal determinants and their implications in human health. J Dairy Res. 86(2):136–144.

Vlaeminck B, Fievez V, Cabrita ARJ, Fonseca AJM, Dewhurst RJ. 2006. Factors affecting odd- and branched-chain fatty acids in milk: a review. Anim Feed Sci Technol. 131(3-4):389–417.

Wang YC, Yu RC, Chou CC. 2006. Antioxidative activities of soymilk fermented with lactic acid bacteria and bifidobacteria. Food Microbiol. 23(2):128–135.