PAPER

CHEMICAL, NUTRITIONAL, PHYSICAL AND ANTIOXIDANT PROPERTIES OF *PECORINO D'ABRUZZO CHEESE*

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ABSTRACT

Proximate composition, minerals, cholesterol, fat soluble vitamins, fatty acids (FA), colour and antioxidant properties of *Pecorino d'Abruzzo* cheese, manufactured at three different months, were investigated. Protein, calcium ($P \leq 0.05$) and polyunsaturated FA content was higher in winter samples than in summer, whereas cholesterol and total fat were lower. Summer cheese samples showed a lower content in saturated FA (55.4%, $P \leq 0.05$) and higher content of monounsaturated FA (38.3%, $P \leq 0.05$) than winter samples. Colour and FT-IR spectra varied ($P \leq 0.05$) seasonally. The dairy products supplied a good level of coverage (%) of some nutrients that are daily required according to Italian recommendations.

Keywords: fatty acids, antiradical activity, cholesterol, minerals, colour parameters, fat soluble vitamins

1. INTRODUCTION

Traditional food products, closely linked to a system of historic and cultural traditions and connected to a specific geographical area, have an important role in maintaining and supporting agro-biodiversity as well as sustainability. Traditional food products are a good source of nutrients, and the levels thereof can be influenced by a range of parameters related to the environment, as well as to food preparation and processing.

Generally speaking, *Pecorino* is a term indicating cheeses made of raw or thermised ewes' milk, among which there are cheeses with a defined geographical origin or labelled as Protected Designations of Origin (PDO) reporting also the specification of the Region, e.g., *romano, sardo, siciliano*.

In this study, the name of "*Pecorino d'Abruzzo*" cheese indicates an Italian traditional food product, recognised by the Italian Ministry of Agriculture, Food and Forest (MiPAAF). This hard or semi-hard cheese, made from ewes' milk, is a local produce linked to the sheep breeding tradition of the homonymous Italian region (*Abruzzo*) and it is processed according to a traditional cheese-making technique in farms located in mountain areas.

The quality of a product is expression of its own nutrient composition. The information on the physicochemical and nutritional characteristics of "*Pecorino d'Abruzzo*" is scarce or currently not available. In addition, there are many literature references on Fatty Acid (FA) profile and Conjugated Linoleic Acid (CLA) contents applying to *Pecorino* cheese in general, however the information on seasonal variation of other nutrients is very limited.

According to some authors, variations in milk and cheese ewe's composition may occur due to different factors. RAYNAL-LJUTOVAC *et al.* (2008) reported that protein content for ewes' milk can be different depending on the stage of lactation, season, age and animal feeding. JAEGGI *et al.* (2005) studied the influence of seasonal changes in ovine milk, in details, on the composition and yield of hard-pressed cheese, and found significant differences in the physico-chemical characteristics of the ovine milk collected at different times of the year (February, May and August), as well as in the cheese produced with it. TODARO *et al.* (2014) also reported that the season greatly affects the quality of milk and cheese, as it is the case of the milk and cheese produced by sheeps in *Valle del Belice*, mainly fed at pasture for most of the year. ADDIS *et al*. (2015) analyzed Pecorino cheeses manufactured from March to June and they also found that the month of production affected cheese composition. PRANDINI *et al.* (2011) reported that ewes' milk and products thereof are richer in CLA than other cheeses (e.g. cows, goats). NUDDA *et al*. (2005) reported substantial differences in the fatty acids pattern in ewes' cheeses from March to June, and explained that the progressive variation of fatty acid profile was due to the temporal variation of the animal diet, in terms of grass matured. The most significant seasonal variations were found by DE LA FUENTE *et al.* (2009) in polyunsaturated FA (in particular CLA and linolenic acid) contents, with the highest values occurring in spring and summer and the lowest in winter.

Based on the above, this research, that falls within the framework of the Italian project "TERRAVITA" (MiPAAF), investigated the difference between chemical, physical and nutritional characteristics of "*Pecorino d'Abruzzo*" cheeses manufactured at different months (February, July, December). In particular, macronutrients, minerals (sodium, calcium, potassium, magnesium and phosphorous), vitamin A and E, cholesterol, antioxidant properties and colour (CIE L*a*b*) parameters were studied, in order to update available data on composition and nutritional properties of this dairy product and investigate how and/or if these results are influenced by the manufacturing season.

Finally, a qualitative analysis of the major functional groups was also performed by Fourier Transformed Infrared Spectroscopy (FTIR) on Attenuated Total Reflectance (ATR) to show the fingerprint of these cheeses.

2. MATERIALS AND METHODS

2.1. Samples

Traditional *Pecorino* cheeses were supplied by a farm located at *Anversa degli Abruzzi*, in the province of *L'Aquila* (Italy), at 40 km from the two most important National Parks of the region: the Majella Park and the National Park of *Abruzzo*. This organic farm was founded in 1977 and generation by generation followed traditional farming practices, typical of that geographical area, in the production of their products.

The *Pecorino d'Abruzzo* cheeses samples are made of raw organic ewes' milk, rennet and salt, and are sold with the label "made with organic". They contain, apart from water and salt, at least 95% percent organically produced ingredients. Besides the manufacturer's name - or transformation/distribution company - the product shows in the label the code of the national control expressly authorized by the Italian Ministry of Agriculture, Food and Forestry (MiPAAF) (EU REGULATION, No. 271, 24 March 2010).

In this research, *Pecorino d'Abruzzo* cheeses, made by ewes' milk collected in different seasons, were studied: the PEC-A and PEC-C batches belonged to cheeses manufactured in winter time (February and December, respectively), whereas the PEC-B batch was produced in summer (July). For each batch (PEC-A, PEC-B and PEC-C), three different samples were analyzed.

Regarding the manufacture of *Pecorino d'Abruzzo* cheeses, after milking the ewes' milk is warmed up to 38°C, and natural microflora and lamb rennet paste are added. The milk is left curdling for less than one hour, and then the curd is broken, pressed with hands and fitted into special baskets (*'fiscelle' in Italian*) to facilitate whey drainage. Afterwards, it is salted and stored into ripening rooms. If the maturing process lasts over one month, the rounds periodically grease with olive oil.

After purchase, the cheeses under investigation were sampled in appropriate aliquots and stored at -20°C until analysis.

2.2. Chemicals

All used reagents were of HPLC grade or at least of the highest available purity. Standards were obtained from Sigma Aldrich (St Louis, MO, USA) and Merck KGaA (Darmstadt, Germany). Ultrapure water, of the grade required for critical laboratory applications, was prepared by an ion exchange system to >18 mΩ resistivity (ElgaPurelab Ultra, Veolia, UK).

2.3. Analytical Methods

2.3.1 Proximate composition

Water, protein, fat and ash contents were determined according to the Italian Official methods of Cheeses analysis (1986), and total carbohydrates were determined by difference to 100 of macronutrient contents. Total energy was calculated using the conversion factors according to Regulation (EU) No. 1169/2011.

The designation according to firmness characteristics was also calculated by the Moisture on Fat Free Basis (MFFB %), i.e. percent ratio between weight of moisture in the cheese and difference between total weight of cheese and weight of fat in cheese, in accordance to the Codex Standard for cheese (CODEX STAN 283-1978, Amendments 2006, 2008, 2010, 2013).

2.3.2 Mineral

Calcium, magnesium and phosphorous were determined according to the AOAC method (AOAC, 2002), which was also applied for potassium and sodium. The samples were analyzed after ashing: 1.0 g of sample was weighed into platinum crucibles and ashed in the furnace at 525C° for 16 hours. Calcium, sodium, potassium and magnesium were determined using an Atomic Absorption Spectrometer (Perkin Elmer Model: A. Analyst 300, Norwalk, CT), while phosphorus was measured at 400 nm by a spectrophotometer (Shimadzu Model: 1800 Tokyo, Japan).

2.3.3 Fatty Acid

The extraction and determination of fatty acids were performed according to a modified version of the method by PRANDINI *et al.* (2007). 2 g of sample were dissolved in ethanol/water (2:1, v/v) and extracted twice with diethyl ether/petroleum ether mixture (1:1 v/v). Fatty acid methyl esters (FAME) were prepared according to the methods of PRANDINI *et al.* (2007). The samples were analysed by GC (Clarus 500-Perkin Elmer) equipped with flame ionization detector (FID), using a fused silica capillary column SP 2380 (Supelco), 60 m x 0.32 mm x 0.2 μm film thickness and helium carrier gas. The oven temperature was programmed as follows: initial temperature 60°C for 5 min, 5°C/min to 180°C and then 3°C/min to 240°C. The injection and detector temperatures were held at 240°C and 260 °C, respectively.

2.3.4 Unsaponifiable fraction

α-tocopherol, β-carotene, *trans* retinol and cholesterol were determined according to PANFILI *et al.* (1994). Briefly, samples were subjected to alkaline digestion and extracted twice with hexane/ethyl acetate (9:1, v/v). The organic phase was collected and evaporated to dryness. The residue was dissolved in 2 ml mobile phase (2-propanol 1% in n-hexane), injected and analysed by an HPLC analytical system Alliance (Waters Model: 2695, Milford, MA). In the chromatographic procedure, a Phenomenex, Kromasil 5μm Si 250x4.6 mm, a fluorescence detector (Waters Model: 2475, Milford, MA, USA) and UV/VIS detector (Waters Model: 2487. Milford, MA, USA) connected in series were utilized to determine cholesterol (208 nm), β-carotene (450 nm), α-tocopherol (excitation 280 nm, emission 325 nm) and retinol (excitation 325 nm, emission 475 nm).

2.3.5 Degree of Antioxidant Protection (D.A.P.)

The *DAP index* was calculated as a molar ratio between Antioxidant Compounds (AC) and Oxidation Target (OT), as follows (PIZZOFERRATO *et al.*, 2007):

$$D.A.P. = \frac{\sum_{i=1}^{n} A.C._{i}(n^{\circ} moles)}{O.T.(n^{\circ} moles)}$$

Generally, α-tocopherol and β-carotene, when present, are used as AC, while cholesterol content was used as OT molecule.

2.3.6 Radical scavenging activity

The radical scavenging activity was evaluated on fat-soluble vitamins-rich extracts dissolved in 2-propanol 1% in n-hexane by the DPPH assay according to the methods of BRAND-WILLIAMS *et al.* (1995), with appropriate modifications. The radical scavenging activities of these extracts were evaluated towards the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) as follows: 1.5 ml of the extract was reacted with 1.5 ml of DPPH radical (60μM), the decrease in absorbance at 515 nm for 400min was monitored and ethyl acetate was used as blank. The control was prepared using 1.5 ml of 2-propanol 1% in n-hexane. The percentage of inhibition of DPPH was calculated using the formula: % Radical scavenging activity (Inhibition of DPPH activity) = $[(Abs_{control}-Abs_{sample}) / Abs_{control}]$ *100; where $Abs_{control}$ is the absorbance of the reaction control and Abs_{sample} is the absorbance in the presence of the sample measured at 400 min, and then the radical scavenging activity was expressed as μmol α-tocopherol/100g.

2.3.7 Colour measurements

The CIE L, a* and b* measurements were made on grated and sliced samples using an handheld tristimulus colorimeter (Konica Minolta CR-400, Minolta Limited, Milton Keynes, UK) controlled with the software CM-S100w (SpectraMagic NX) with D65 illuminant and 10° viewing angle, calibrated with a white tile. The following parameters were determined: L* value, that is an indicator of luminosity (the degree of lightness) and varies from black to white; the a* value, indicator of green (negative values) and red (positive values); the b* value, indicator of blue (negative values) and yellow (positive values); Hue angle (h) and Chroma (C*) colour intensity, which is the distance of a colour from the origin (a*=b*=0) in the a*, b* space.

2.3.8 Fourier Transform Infrared (FTIR) analysis

The FTIR spectra were acquired according to LERMA-GARCÍA *et al.* (2010). Different slices of each sample were examined by FT-IR (Nicolet iS10 Thermo Fisher Scientific, USA) with an ATR (Attenuated Total Reflectance) accessory (with diamond crystal). Each sample was placed in ATR surface and pressed with the clamp to obtain a thin layer. All infrared spectra were recorded (32 scans/sample) in the range between 4000 and 700 cm^{-1} at a resolution of 4 cm^{-1}, with automatic atmospheric suppression. Data were elaborated with Omnic 8.2.0.387 (Thermo Fisher Scientific Inc., USA) software.

2.3.9 Statistical analysis

Analysis of variance, one-way ANOVA test with the Tukey's Post hoc comparison, was used for multiple comparison of mean values of nutrients and parameters regarding cheeses produced at different months (February, July, December). P-values ≤0.05 were considered significant. All statistical analyses were performed using the KaleidaGraph 3.6 software (Synergy Software, Reading, PA. USA).

3. RESULTS AND DISCUSSIONS

3.1. Proximate composition

In Table 1 proximate composition, percentage ratio of fat/protein and Moisture/Fat Free Basis (MFFB), energy values, pH, mineral contents, calcium (Ca), phosphorous (P), sodium

(Na), potassium (K), magnesium (Mg) and the molar ratio of calcium/phosphorous of traditional *Pecorino d'Abruzzo* cheeses are reported. In all samples, the pH values were moderately acid, and a slightly but not significant increase from February to December was observed according to cheese manufacturing season. These pH values were higher when compared with those reported by ADDIS *et al.* (2015) for PDO *Pecorino Romano* cheeses and by JAEGGI *et al.* (2005) for ovine hard-pressed cheese. TODARO *et al.* (2014) found that there were slight changes in milk pH during the year, although it was lower in summer and spring. As shown in Table 1, the samples in February (PEC-A) exhibited a higher value of water 47.5 g/100g and a lower value of protein, fat and ash content than those produced in July (PEC-B) and December (PEC-C).

Table 1: Proximate composition (g/100g), percentage ratio of fat/protein and Moisture/Fat Free Basis (MFFB)°, energy values (kcal/100g and kJ/100g)°°, pH, mineral contents (mg/100g) and molar ratio of calcium/phosphorous on a fresh matter (F.M.) and dry matter (D.M.) basis of traditional *Pecorino d'Abruzzo* cheeses.

Samples	**PEC-A**	**PEC-B**	**PEC-C**	**PEC-A**	**PEC-B**	**PEC-C**
Month of production	**February**	**July**	**December**	**February**	**July**	**December**
		F.M. (%)			**D.M. (%)**	
water (g/100)	47.5±0.0^a	39.1±0.0^b	36.9±0.0^c			
° MFFB (%)	64.2	57.4	54.9			
*** protein (g/100g)**	21.6±0.1^c	23.2±0.1^b	25.1±0.1^a	41.2±0.1^a	38.0±0.1^c	39.8±0.1^b
fat (g/100g)	26.1±0.1^c	32.0±0.2^b	32.8±0.1^a	49.6±0.2^b	52.5±0.3^a	51.9±0.2^a
ash (g/100g)	3.5±0.0^c	4.1±0.0^b	4.8±0.0^a	6.7±0.1^b	6.7±0.0^b	7.6±0.1^a
**** carbohydrates**	1.3	1.7	0.4			
fat/protein (%)	1.2	1.4	1.3			
°° energy kcal (kJ)	327 (1355)	387 (1606)	397 (1645)			
pH	5.4±0.0	5.6±0.1	5.8±0.0			
Ca (mg/100g)	669.2±5.9^b	661.0±4.3^b	720.0±5.3^a	1273.8±11.2^a	1085.0±7.1^c	1141.9±8.5^b
P (mg/100g)	458.6±1.9^c	484.9±6.4^b	504.0±0.7^a	872.9±3.7^a	795.9±10.5^b	799.3±1.1^b
Na (mg/100g)	580.5±5.1^c	840.5±0.7^b	1074.5±2.0^a	1104.9±9.8^c	1379.5±1.2^b	1704.0±3.1^a
K (mg/100g)	84.0±0.2^a	80.3±0.1^b	76.7±1.1^c	159.9±0.4^a	131.8±0.1^b	121.6±1.7^c
Mg (mg/100g)	41.0±0.4	39.5±0.9	39.2±0.6	78.0±0.7^a	64.8±1.6^b	62.2±0.9^b
molar ratio Ca/P	1.1±0.0	1.1±0.0	1.1±0.0			

Data are means ± Standard Deviation (SD) of triplicate analyses; values in the same row with different superscript letters are significantly different (P≤0.05).
°Moisture/Fat Free Basis (%): in accordance to the Codex Standard for cheese (CODEX STAN 283-1978);
*proteins: total nitrogen 6.38;
**carbohydrates calculated by difference to 100 of macronutrient contents;
°°energy calculated using the conversion factors according to Regulation (UE) No. 1169/2011.

However MFFB ranged between 54% and 69% for all samples, and in terms of firmness it designated samples as firm or semi-hard cheeses. On the other hand, when taking into consideration the ripening times, cheeses can be designated as unripened/fresh (CODEX STAN 283-1978).

Generally, macronutrient content of commercial *Pecorino* cheeses, as reported by RAYNAL-LJUTOVAC *et al*. (2008), is very wide. Fat and protein contents are reported in literature to range 29.0 to 36/37 g/100g and 25.0 to 28.0 g/ 100g, respectively, in different ewe milk cheeses (*Pecorino cheeses*). In a study of MANZI *et al.* (2007) on different PDO Italian cheeses (*Pecorino romano, Pecorino toscano, Pecorino sardo*), the following ranges were found: 23.8 - 40.8 g/100g for moisture content, 21.8 - 30.3 g/100g for protein content 30.8 - 40.2 g/100g for fat content and 3.4 - 10.1 g/100g for ash. So, the macronutrients of *Pecorino d'Abruzzo* cheeses within this study had lower values than those obtained by MANZI *et al.* (2007) for the analysed PDO Italian *Pecorino* cheeses.

RAYNAL-LJUTOVAC *et al.* (2008) reported that the protein content for ewes' milk can range between 4.75 g/100g and 7.20 g/100g and that the main non-individual factors of protein content variation were the stage of lactation, season, age and feeding.

JAEGGI *et al.* (2005) found that the ewe's milk produced in February had a higher fat and a lower protein content (37.19% and 24.84%, respectively) with a lower casein:fat ratio than milk used in May (35.46%; 26.59%) and August (35.70%; 26.13%); and the same applied to cheeses. Moreover, TODARO *et al.* (2014) reported that the monthly levels of fat in milk showed a decreasing trend from January to April, and then an increase until reaching a maximum value in July/August. This result could explain the lowest ($P \leq 0.05$) fat content among *Pecorino d'Abruzzo* samples in February. However, as reported by TODARO *et al.* (2014), the higher fat content in summer cheese depends on the higher percentage of fat observed in summer ewes' milk, and this fact is the consequence of milk concentration in late lactation and a high level of dry and fibrous forage in ewes' diet.

ADDIS *et al.* (2015) analyzed several samples of *Pecorino* cheeses manufactured at different times of the year (from March to June) and found that the cheeses produced in late winter and spring were characterized by a less fat and salt content and a higher protein level with respect to those produced in early summer.

The outcomes by ADDIS *et al.* (2015) and TODARO *et al.* (2014) are in accordance with this study, and probably they could explain the differences observed and also expressed on a dry matter (D.M.) basis, for protein, fat and some mineral (Ca, P, K, Mg) concentrations (Table 1). In particular, as regards mineral content, the samples produced during winter had a major protein and Ca ($P \leq 0.05$) content than those manufactured in summer.

3.2. Minerals

The content of Ca, Na and K was significantly ($P \leq 0.05$) different among all samples (Table 1), whereas a significant difference ($P \leq 0.05$) for P and Mg contents was found only among samples of cheese produced in February and in those produced in July and December. On dry matter basis, the samples produced in February exhibited the highest value for minerals, such as Ca, P, K and Mg (1273.8: 872.9: 159.9: 78.0 mg/100g, respectively) and the lowest value for Na (1104.9 mg/100g). RAYNAL-LJUTOVAC *et al.* (2008) also reported for sheep milk cheeses that Mg content is less variable, by showing a mean value of 80 mg/100g D.M. that is higher than what was obtained in this study for *Pecorino d'Abruzzo* samples (on average 68.3mg/100 D.M.).

However, depending on the pH obtained during the cheese-making process, Ca and P content ranged between 661.0 and 720.0 mg/100g and 458.6 to 504.0 mg/100g, respectively. Regarding the mineral components, few data are available in literature for

sheep milk cheeses. As for the other investigated nutrients, the data obtained for the mineral composition of *Pecorino d'Abruzzo* samples were lower than those reported by literature. For instance, in PDO Italian cheeses, such as *Pecorino romano, Pecorino sardo* and *Pecorino toscano,* the average Ca content was, respectively, 938.5, 940.4 and 860.0 mg/100g, and P content was 634.5, 714.8 and 658.9 mg/100g (MANZI *et al.*, 2007).
In all samples the percentage ratio of fat/protein and the molar ratio Ca/P were always higher than 1.0 (Table 1). These results may be easily explained by the high content in fat, protein and minerals of the ewes' cheeses under investigation that thus resulted a food with high nutritional value.
The experimental results of this study matched ADDIS *et al.* (2015) that reported that ewes' milk produced in June and July is characterized by a higher fat content, generally not offset by an equal increase in protein content. The fat to protein ratio thus ranges from 1.03 to 1.13 in March/April, whereas in June/July it exceeds 1.20 (ranging from 1.22 to 1.35).

3.3. Unsaponifiable fraction

In Table 2, cholesterol, α-tocopherol, *trans*-retinol and β-carotene contents, and the Degree of Antioxidant Protection (DAP exp-3) in samples of traditional *Pecorino d'Abruzzo* cheese are shown.

Table 2: Cholesterol (mg/100g), α-tocopherol, *trans*-retinol, β-carotene contents (μg/100g) and Degree of Antioxidant Protection (DAP exp-3) on a fresh matter (F.M.) and dry matter (D.M.) basis of traditional *Pecorino d'Abruzzo* cheeses.

Samples	PEC-A	PEC-B	PEC-C	PEC-A	PEC-B	PEC-C
Month of production	February	July	December	February	July	December
		F.M. (%)			D.M. (%)	
cholesterol	72.9±0.4^{c}	96.1±1.1^{a}	81.1±0.0^{b}	138.7±0.8^{b}	157.7±1.9^{a}	128.6±0.0^{c}
α-tocopherol	769.2±4.8^{c}	898.8±2.0^{b}	945.9±5.5^{a}	1464.0±9.2^{b}	1475.3±3.3^{ab}	1500,1±8.8^{a}
trans -retinol	308.3±0.7	299.2±18.5	309.8±3.4	586.9±1.4^{a}	491.1±30.4^{b}	491.3±5.4^{b}
β-carotene	nd*	nd*	nd*			
DAP (exp-3)	9.5±0.1	8.4±0.1	10.5±0.1			

Data are means ± Standard Deviation (SD) of triplicate analyses; values in the same row with different superscript letters are significantly different (P≤0.05);
*nd= not detectable; β-carotene detection limit 0.16ng/20 μl injected.

As regards cholesterol content, cheese samples exhibited on average a value of 83.4 mg/100g, and PEC-B cheeses (summer produces) had on average the highest cholesterol content (96.1 mg/100g). However, the amount of cholesterol is quite variable between winter and summer samples, and the values ranged in accordance with the data obtained by MANZI *et al*. (2007) on different PDO Italian Pecorino cheeses.
The winter samples had significantly a lower cholesterol value than those manufactured with milk produced in summer (P≤0.001), even when expressed on dry matter basis (Table 2). The cholesterol content seems therefore to be influenced by the manufacture month.

Regarding fat-soluble vitamins, there was a great variability in this study: α-tocopherol ranged between 945.9 and 769.2 μg/100g and *trans* retinol between 299.2 and 309.8 μg/100g. As expected, the β-carotene was not detectable in all studied cheese samples (NOZIERE *et al*., 2006).

Upon comparison of cheeses, PEC-C samples (December) showed the highest values for α-tocopherol, whereas no significant differences (P≤0.05) were observed for *trans* retinol. On the contrary, significant (≤0.05) differences for both α-tocopherol and *trans* retinol were found (Table 2) by comparing cheeses produced in February with the other samples on dry matter basis. This result could be explained by literature data. According to MANZI *et al.* (2007), in some *PDO* Italian cheeses, the average α-tocopherol content was 850 μg/100g and the range of variability for *trans* retinol was very wide: 322.1 to 545.9 μg/100g in *Pecorino romano*, 256.2 to 484.6 μg/100g in *Pecorino sardo* and 337.4 to 602.4 μg/100g in *Pecorino toscano*.

CALDERÒN *et al.* (2007) reported that the concentration of Vitamin E in cow milk was linearly related to the experimental amount of feed in the diet, and milk characteristics differed according to season. The vitamin E content in milk was higher during the grazing period (May-September) than during the winter feeding period (February-March), and this fact was linked to the proportion of grazed grass or grass silage in the forage, unlike vitamin A (AGABRIEL *et al*., 2007). Indeed, REVILLA *et al*. (2014) investigated the effects of milk origin (i.e., sheep, goat and cow) and season on the vitamin composition of different types of cheese, and found that seasonality had a significant effect on the concentration of vitamin A (i.e. higher during summer and autumn), but not on vitamin E. They also reported that the seasonal changes in animal feeding practices were the main reason for the differences observed.

So, in this study, the main chemical characteristics of *Pecorino d'Abruzzo* cheeses also seem to be affected by the month of manufacture; there is, in fact, a significant (P≤0.05) effect of seasonal change on cheese composition.

The Degree of Antioxidant Protection (DAP), that is a parameter to estimate the potential oxidative stability of fat in foods, has been also evaluated (Table 2). This parameter is related to farm systems and/or cheese manufacture. As reported by PIZZOFERRATO *et al*. (2007), it can discriminate products from grazing and zero-grazing feeding systems, and when this value is ≥7.0 the pasture is predominant (grazed herbage exceeds 15% in animals' total diet). In this study, the DAP reached the highest values in winter samples (PEC-A and PEC-C), although it can be stated that in all samples the values were always ≥7.0.

Despite several studies on quantification of single antioxidants in cheeses are published in literature, there are only few investigations on antioxidant properties of cheeses (GUPTA *et al*., 2009). In this research, the radical scavenging activity of fat soluble vitamin-rich extracts, derived from *Pecorino d'Abruzzo* samples, was studied. The DPPH assay is a simple, quick and cheap method to evaluate the free radical scavenging activity of compounds and/or samples. This evaluation is related to the structure of the active substances present in lipophilic extracts and their possible interactions. This measurement might hence be considered an index of the antioxidant properties of cheeses. LUCAS *et al.* (2006) studied the antioxidant properties in French cheeses, such as *Abondance, Tomme de Savoie, Cantalet, Salers* and *Rocamadour* and concluded that the variation of the Total Antioxidant Activity in cheeses was probably due to variations in antioxidants, such as α-tocopherol, retinol, β-carotene. DPPH values of fat soluble vitamin-rich extracts, obtained from *Pecorino d'Abruzzo* cheese, were higher in winter than in summer samples (Fig. 1) and that thus reflected the behaviour of DAP.

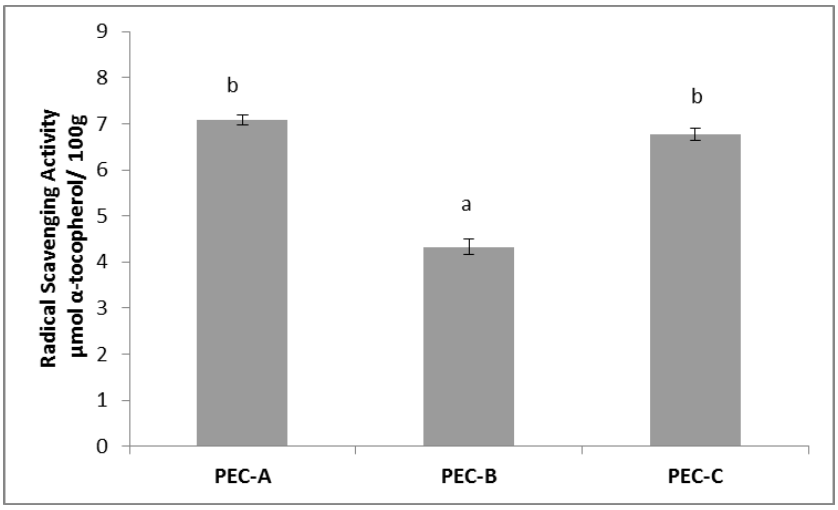

Figure 1: Radical Scavenging Activity (μmol α-tocopherol/100g) of fat-soluble vitamins-rich extracts derived from traditional *Pecorino d'Abruzzo* cheeses. Means followed by different letters are significantly different ($p \leq 0.05$).

3.4. Fatty acid composition

Fatty acid (FA) composition of traditional *Pecorino d'Abruzzo* cheeses is reported in Table 3. The samples produced during winter (December) had the highest percentage of Short and Medium Chain Fatty Acids (SCFAs, 5.25% and MCFAs 21.59%, respectively, $P \leq 0.05$), and consequently of Saturated Fatty Acids (SFAs 66.25%). On the contrary, the samples produced during summer (July) had the highest percentage of Long Chain Fatty Acids (LCFAs 81.99%, $P \leq 0.05$) and were the richest in MUFA (38.30%), primarily *all cis* and *trans* C18:1 (PEC-B samples 36.58 g/100g of total FA; $P \leq 0.05$). The cheeses produced during winter showed on average a large amount of PUFA (8.33% and 7.90% in PEC-C and PEC-A, respectively). TODARO *et al*. (2014) explained any increase in MUFA, especially oleic acid, with a major content in ewes' milk produced during summer and, hence in cheeses, as a result of the mobilization of long-chain FA from the body fat deposits of the sheep to balance the energy deficits that they undergo in summer, when the feeding regime may not be sufficient to satisfy their energy needs. DE LA FUENTE *et al.* (2009) reported that in ewes' milk there were significant seasonal variations in PUFA content with higher values in spring and summer than in winter. It is possible that factors, such as season and feeding system, could explain also in this study the differences observed. The period of cheese manufacture seems to affect the type and proportion of FA present in milk and in cheeses as well. During summer, the proportion of SCFA decreased, while LCFA increased (Table 3). In particular, SFA such as lauric acid (C12:0), myristic acid (C14:0) and palmitic acid (C16:0), that are defined atherogenic, resulted more concentrated ($P \leq 0.05$) in the *Pecorino d'Abruzzo* cheeses produced in winter. These results matched the data reported by several authors (NUDDA *et al*., 2005; TODARO *et al*., 2014; DE LA FUENTE *et al*., 2009). On the contrary, the content of stearic acid (C18:0) was higher in *Pecorino d'Abruzzo* produced in summer (15.55 g/100g of FA in PEC-B sample; $P \leq 0.05$) than in winter cheese samples (PEC-C). This result is important because it can be used for the biosynthesis of oleic acid in human metabolism, and differed from the other SFAs for the beneficial effect on blood cholesterol level: it lowers LDL-Cholesterol without affecting blood concentrations of HDL-Cholesterol (HUNTER *et al*., 2010).

Table 3: Fatty acid composition given as mean ± S.D. (g/100g of total Fatty Acids) of traditional *Pecorino d'Abruzzo* cheeses.

Samples		**PEC-A**		**PEC-B**		**PEC-C**	
Month of production		**February**		**July**		**December**	
Free Fatty Acids							
lipid numbers	*Usual names*	Means	SD	Means	SD	Means	SD
C4:0	Butyric	2.25	0.10	1.96	0.04	1.81	0.21
C5:0		0.05	0.00	0.12	0.00	0.15	0.06
C6:0	Caproic	1.37[ab]	0.09	1.23[b]	0.06	1.61[a]	0.08
C8:0	Caprylic	1.16[b]	0.09	1.09[b]	0.05	1.68[a]	0.05
C10:0	Capric	3.35[b]	0.24	3.12[b]	0.13	5.61[a]	0.13
C12:0	Lauric	2.18[b]	0.15	2.04[b]	0.08	3.34[a]	0.06
C13:0		0.06	0.01	0.04	0.00	0.06	0.00
C14:0	Myristic	8.01[b]	0.51	6.89[b]	0.17	10.44[a]	0.11
C14 :1 c9	Myristoleic	0.81[a]	0.04	0.52[b]	0.01	0.72[a]	0.03
C15:0		1.04[a]	0.05	0.75[b]	0.01	1.16[a]	0.02
C15:1		0.37[a]	0.02	0.24[b]	0.00	0.25[b]	0.01
C16:0	Palmitic	24.88[a]	1.02	21.28[b]	0.04	26.93[a]	0.25
C16 :1 c9	Palmitoleic	1.11[a]	0.05	0.69[b]	0.00	0.97[a]	0.06
C17:0	Margaric	0.83	0.03	0.68	0.00	0.85	0.12
C17:1		0.26[a]	0.01	0.18[b]	0.00	0.26[a]	0.02
C18:0	Stearic	14.23[a]	0.67	15.55[a]	0.19	11.98[b]	0.33
C18:1 (t+c)		29.28[ab]	3.25	36.58[a]	0.32	23.15[b]	1.62
C18:2, t9 t12	Linolelaidic	1.08	0.12	1.57	0.15	1.60	0.27
C18:2, c9 c12 (n-6)	Linoleic acid (LA)	3.28[a]	0.13	2.15[c]	0.02	2.69[b]	0.17
C20:0	Arachidic	0.53	0.02	0.45	0.00	0.44	0.02
C18:3 (n-6)	g-Linolenic acid	0.12[bc]	0.01	0.08[c]	0.02	0.19[a]	0.00
C:18:3 (n-3)	a-Linolenic acid (ALA)	1.41[a]	0.06	0.82[b]	0.01	1.61[a]	0.11
C20:1	Gondoic	0.08[ab]	0.00	0.10[a]	0.00	0.06[b]	0.00
C18:2, c9 t11 - CLA	Rumenic	1.38[b]	0.04	1.23[b]	0.00	1.63[a]	0.09
C20:2		0.29[a]	0.01	0.21[b]	0.00	0.29[a]	0.02
C22:0	Behenic	0.26[a]	0.01	0.17[b]	0.00	0.18[b]	0.01
C20:4 (n-6)	Arachidonic	0.12[ab]	0.00	0.08[b]	0.00	0.14[a]	0.02
C20:5 (n-3)	EPA	0.12[a]	0.00	0.08[b]	0.00	0.12[a]	0.01
C22:6 (n-3)	DHA	0.11[a]	0.00	0.10[a]	0.00	0.05[b]	0.00
ω3/ω6 ratio°		0.47		0.43		0.59	
*Σ SHORT CHAIN FATTY ACIDS (SCFA)**		*4.84*	*0.90*	*4.40*	*0.76*	*5.25*	*0.78*
*Σ MEDIUM CHAIN FATTY ACIDS (MCFA)***		*15.81*	*2.78*	*13.61*	*2.44*	*21.59*	*3.81*
*Σ LONG CHAIN FATTY ACIDS (LCFA)****		*79.35*	*8.90*	*81.99*	*9.91*	*73.16*	*8.13*
Σ SATURATED FATTY ACIDS(SFA)		*60.21*		*55.37*		*66.25*	
Σ MONOUNSATURATED FATTY ACIDS (MUFA)		*31.89*		*38.30*		*25.42*	
Σ POLYUNSATURATED FATTY ACIDS (PUFA)		*7.90*		*6.33*		*8.33*	

Fatty acids are expressed in g/100g of total fatty acid (FA). Data are means ± Standard Deviation (SD) of triplicate analyses; different letters in the same row correspond to statistical differences ($P \leq 0.05$); °ω3/ω6 ratio of total omega 3 fatty acid (ALA; EPA; DHA) and total omega 6 fatty acid (LA; γ-Linolenic acid; Arachidonic acid) *SCFA: C4:0 - to C9:0; **MCFA: C10:0 to C15:1; ***LCFA: C16:0 to C22:6.

TODARO *et al.* (2014) reported that the ewes' cheeses produced in summer were lower in saturated FA and higher in linoleic acid (LA), MUFA and ω3 PUFA than those produced in autumn and spring; these results, except for LA and ω3 PUFA content, are in accordance with the results obtained within this research study.

Among Essential Fatty Acids (EFAs), LA (C18:2 cis9, cis12 -ω6) differed significantly ($P \leq 0.05$) among samples, and reached higher values in winter cheeses (3.28:2.69 g/100g of total FA in PEC-A: PEC-C, respectively); a similar behaviour was reported for α-linolenic acid (ALA, C18:3, ω3). ALA is present in food at lower concentrations than LA, and it is an important component in cheeses for healthy nutrition (XU, 2015). Indeed, ALA and γ-linolenic acid significantly increased in cheeses produced during winter: 0.12 and 0.19 g/100g total FA for γ-linolenic acid and 1.41 and 1.61 g/100g total FA for *a*-linolenic acid in PEC-A and PEC-C, respectively.

Several studies (ASTRUP *et al.,* 2011) underline that replacing SFAs by PUFAs in the diet decreases the risk of cardiovascular diseases (CVDs) and that dietary short-term intake of a cheese naturally rich in *cis*-9, *trans*-11 CLA appears to cause favourable biochemical changes of atherosclerotic markers in comparison with commercial cheese (SOFI *et al*., 2010). As regards that, it was important to highlight in the studied cheeses the presence of long chain fatty acids (LC-PUFAs), such as Eicosapentaenoic (C20:5 -EPA) acid and Docosahexaenoic (C22:6 -DHA) acid, especially in the samples produced during late winter (February). However, the values obtained in this study for C20:5 and C22:6 were on average higher than those reported by NUDDA *et al.* (2005).

Conjugated Linoleic Acid (CLA), detected as C18:2, c9 t11 (Rumenic Acid -RA), got higher value in the samples produced in December (1.63 g/100g total FA PEC-C) than in samples produced in July. The importance of CLA in food, as shown by *in vivo* studies on animal models, is linked to positive physiological effects, in terms of reduction of the growth rate of cancer cells, improvement of the immune system, and more in general beneficial effects on health (JAHREIS *et al.,* 1999; WHIGHAM *et al.,* 2000; XU, 2015).

Recently, more information on the effects of CLA on body composition and energetic metabolism are reported by LEHNEN *et al.* (2015). The consumption of foods naturally enriched with CLA (and not supplemented) during lifetime should contribute to reducing increased adiposity and hence the risk of other diseases related to obesity. Ewe milk is the richest in CLA, among the milk of other ruminant species (except for goat); it also has the highest content on total *trans* FA, as well as *trans* Vaccenic Acid (VA), and this was season-dependent (JAHREIS *et al.,* 1999). Thus, the manufacture time, i.e. season, of *Pecorino* cheese is of paramount importance.

Apropos of that, NUDDA *et al.* (2005) reported substantial differences in the fatty acid pattern in ewes' cheeses from March to June: a decrease in the CLA e VA concentration in milk fat as lactation progressed. This was probably due to the availability of pasture feeding and a different FA composition of grass lipids. Similarly, as reported by ADDIS *et al.* (2005), the nutritional value of cheese fat, associated in particular with LA, RA and VA contents, decreased exactly with the progress of season. However, it is known that other factors, such as the period of lactation (KIM *et al*., 2009), the processing temperature (SHANTHA *et al*., 1992) or ripening time (PRANDINI *et al*., 2011; KIM *et al*., 2009) may also affect the total amount of CLA in cheeses.

As regards the ω3/ω6 ratio was higher in winter cheeses than in summer samples (0.59, 0.47, 0.43 for PEC-C, PEC-A, PEC-B, respectively), while a meta-analysis study on dairy products (PALUPI *et al.,* 2012) shows that the ω3/ω6 ratio is higher in summer than in winter cheeses.

The profile of fatty acid composition confirms the important role of ewes' dairy products, in particular of *Pecorino d'Abruzzo* cheese, as a natural source of PUFA and CLA in human nutrition, especially when manufactured in winter.

3.5. Nutritional information

The knowledge of the nutritional supply of some nutrients may help to drive dietary choices among consumers and understand the tight relationship between food and health. It is therefore interesting for a nutrient dense food like *Pecorino* cheese to add nutritional information (Table 4) on the level of coverage (%) for both energy and some nutrients for human adults (aged 18-59) according to Italian recommendations (SINU, 2014) for daily requirements.

Table 4: Daily coverage requirements (%) for i) calcium, phosphorous and Vitamin A, out of *Population Reference Intake* (**PRI**); ii) Vitamin E, out of *Adequate Intake* (**AI**); and iii) sodium, out of *Suggested Dietary Target* (**SDT**) for the Italian population^, and average content of minerals (Ca, P, Na), Vitamins A and E supplied by one serving (50 g) of traditional *Pecorino d'Abruzzo* cheeses (SINU, 2014).

^ Adult	*Calcium: coverage (%) out of PRI	*Phosphorous: coverage (%) out of PRI	°Sodium: coverage (%) out of SDT	*Vitamin A: coverage (%) out of PRI	°°Vitamin E: coverage (%) out of AI
Man				21.8	3.4
	34.2	34.5	20.8		
Woman				25.5	3.6
			Average content for serving (50g)		
Mineral (mg)	341.7	241.3	415.9		
Vitamin A (μg RE)				152.9	
Vitamin E (mg α-TE)					0.4

^aged 18-59
*the Italian *Population Reference Intake* (PRI) is 1000 mg/day for calcium, 700 mg/day for phosphorous; 700μg RE/day for men and 600μg RE/day for women for vitamin A (μg Retinol Equivalents);
°the *Suggested Dietary Target* (SDT) for Sodium is 2.0 g/day (corresponded to NaCl ≤ 5 g/day);
°°the *Adequate Intake* (AI) for vitamin E (mg α-Tocopherol Equivalents) is 13mg α-TE /day for men and 12mg α-TE /day for women.

From a nutritional point of view, one serving (50 g) of *Pecorino d'Abruzzo* cheese added, on average, only 185 kcal (768 kJ) to total daily calories intake (Table 1), but it is worth pointing out that 50 g of this cheese (Table 4) supply 0.4 g of sodium (1.1 g of sodium chloride), that is about 20.8% of the *Suggested Dietary Target* (SDT; ≤ 2000 mg/day), and 341.7 mg of calcium, that is, about 34.2% out of the Italian *Population Reference Intake* (PRI) for adults (1000 mg/day) daily requirements. In addition, this serving (50 g) supplies 0.44 mg of vitamin E, that cover about 3.4% and 3.6% of daily *Adequate Intake* (AI) for respectively men and women, and 152.9 μg of vitamin A, that cover in adults about 21.8% and 25.5% of *Population Reference Intake* (PRI) for men and women, respectively, in accordance with Italian recommendations (SINU, 2014).

3.6. Colour measurements

The colour is the first quality parameter evaluated by consumers and an indicator for severe heat treatments, storage, ripening times, etc.; it could also be related to bioactive compounds profile. However, few studies have been published on the evaluation of colour parameters in *Pecorino* cheeses. For example, JUAN *et al.* (2008) when investigating the effect of High-Pressure (HP) treatment at 300MPa on ewes' milk cheese in real industry conditions found that this treatment induced a colour variation, that is, a decrease in lightness and an increase in yellowness related to the structural changes of cheeses. Other authors (ROHM and JAROS, 1996; BUFFA *et al.*, 2001) studied the changes in colour parameters of cheeses during ripening time, and several changes were observed: a decrease of L and an increase of a* and b* values. The colour parameters measured in both grated (a) and sliced (b) *Pecorino d'Abruzzo* cheeses are shown in Table 5.

In this study (Table 5), all colour parameters presented significant differences (P≤0.05) among samples depending on the production period, that is, either in winter or summer, but they were not actually influenced by the shape (grated or sliced).

Table 5: Colour measuraments of a) grated or b) sliced traditional *Pecorino d'Abruzzo* cheeses.

Samples	PEC-A	PEC-B	PEC-C	PEC-A	PEC-B	PEC-C
Month of production	February	July	December	February	July	December
		a) grated			b) sliced	
L*	73.9±0.2[a]	69.0±0.5[c]	71.0±0.6[b]	85.7±1.1[a]	78.5±0.4[c]	80.5±0.6[b]
a*	-3.3±0.1[b]	-3.3±0.1[b]	-2.9±0.1[a]	-5.9±0.2[b]	-6.5±0.1[a]	-6.1±0.1[b]
b*	14.0±0.2[b]	17.0±0.7[a]	13.9±0.2[b]	18.3±0.5[b]	23.0±0.8[a]	17.9±1.1[b]
C*	14.4±0.3[b]	17.3±0.7[a]	14.2±0.2[c]	19.3±0.5[b]	23.9±0.8[a]	18.9±1.0[b]
h	103.3±0.1[a]	101.0±0.5[b]	101.6±0.2[a]	107.9±0.1[a]	105.7±0.5[b]	108.9±1.0[a]

*D65;
Data are means ± Standard Deviation (SD); values in the same row with different superscript letters are significantly different (P≤0.05).

In particular, all samples showed significant (P≤0.05) differences for L* (brightness). For cheeses manufactured in summer time (July), the brightness (L*) was lower, whereas the b* (yellowness) and C* (Croma) values reached higher values than those reported for the samples manufactured during winter (February and December). These results applied to both grated and sliced samples.

In the *Pecorino d'Abruzzo* samples produced during winter (December or February), the C* value tended to decrease, thus indicating that the samples became grey or looked less brilliant during this period, and suggesting that cheese might run a major risk of loosing colour in winter. Moreover, the hue angle (h), that is a qualitative attribute of colour related to the differences in absorbance at different wavelengths, resulted higher in winter samples than in the cheeses produced in July. Indeed, higher h values corresponded to lower b* values, and matched a minor yellowness in the cheeses where the β-carotene was not detectable. These are, nevertheless, preliminary results and need further investigation and more specifically targeted studies.

3.7. Qualitative application of FTIR-ATR technique to *Pecorino d'Abruzzo* cheeses

Cheeses are heterogeneous food products, and hence show qualitative differences related to a different chemical composition, maturation process, seasonality and/or various ripening times. The FTIR-ATR technique has been widely applied in food science for

different purposes, e.g., determination of the geographical origin of cheeses, of sensory and textural properties, shelf life, as well as classification of cheeses according to the manufacture month and manufacturing technique (LERMA-GARCÍA *et al.*, 2010; KAROUI *et al.*, 2005; KOCA *et al.*, 2007; VLACHOS *et al.*, 2006). Moreover, it is worth highlighting that mid-infrared spectroscopy allows to monitor specific functional groups and smaller molecules (KOCA *et al.*, 2007).
The FTIR-ATR spectra (range 4000-700 cm^{-1}) of the studied traditional *Pecorino d'Abruzzo* cheese provided a qualitative information by characterization of typical absorption bands (Fig. 2). Indeed, several significant differences appeared on the typical absorption bands arising from amide (1718 to 1581 cm^{-1} [NH-I] and 1581 to 1483 cm^{-1} [NH-II]), from lipids (1765 to 1718 cm^{-1} [C=O ester] and 2984 to 2831 [CH_3 and CH_2]), from -NH (in the region 3300 to 2984 cm^{-1}) and -OH (3300 to 3700 cm^{-1}).

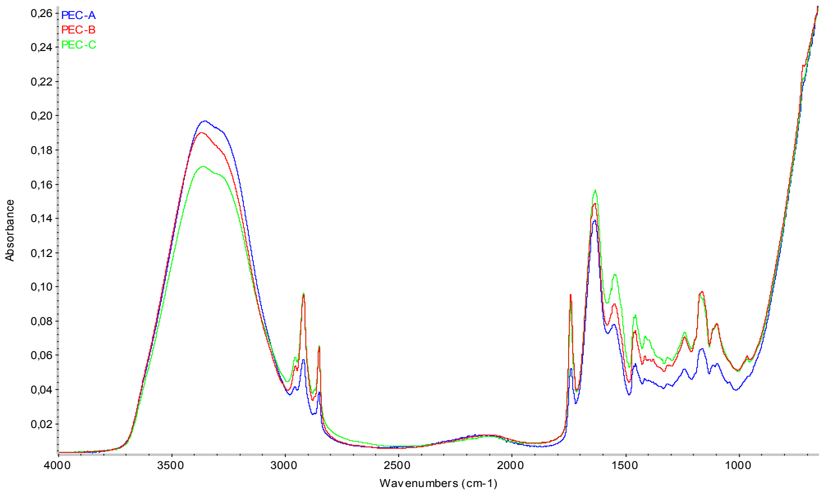

Figure 2: ATR-IR spectra average of traditional *Pecorino d'Abruzzo* cheeses.

In particular, the FT-IR qualitative study of the *Pecorino* d'Abruzzo cheeses under investigation allowed the identification of several significant (P≤0.05) changes in peak intensities in absorption bands of specific functional groups vibrations, that identify aliphatic and carbonyl stretching of acyl chain of fatty acids among samples (Table 6). However, the ratios CH_3 asym/CH_2 asym (2957 cm^{-1}/2920 cm^{-1}) and CH_3 sym/CH_2 sym (2873cm^{-1}/2851 cm^{-1}) were not significant.
Moreover, as reported in Table 6, the region of the carbonyl groups presents a maximum at 1741 cm^{-1} (VLACHOS *et al.*, 2006; ROHMAN *et al.*, 2011). The total amount of formed carbonyls or other secondary oxidation products causes a shoulder at 1728 cm^{-1} (VLACHOS *et al.*, 2006) that overlaps the stretching vibration at 1741 cm^{-1}, and thus a broadening of this peak to lower wavenumbers results. No significant differences (P≥0.05) were found among winter and summer samples when peak intensity changes are considered in this spectral area.

4. CONCLUSIONS

Results show that the main chemical characteristics of traditional *Pecorino d'Abruzzo* cheese samples, manufactured at different times of the year, are affected by the month of production (season). As expected, in this traditional dairy product there was a great compositional variability: winter samples have a major protein content (P≤0.05) and a lower cholesterol and fat content than those manufactured in summer.

Table 6: Wavelengths and peak intensities of main functional groups that identify aliphatic and carbonyl stretching of acyl chain of fatty acids of traditional *Pecorino d'Abruzzo* cheeses.

Samples		**PEC-A**	**PEC-B**	**PEC-C**
Month of production		**February**	**July**	**December**
Functional Group	Wavelength cm^{-1}		Peak Intensities	
Asym $-CH_3$	2957	$0.047±0.01^{b}$	$0.050±0.00^{b}$	$0.058±0.00^{a}$
Asym $-CH_2$	2920	$0.073±0.01^{b}$	$0.092±0.01^{ab}$	$0.098±0.02^{a}$
Sym $-CH_3$	2873	$0.028±0.00^{b}$	$0.032±0.01^{b}$	$0.037±0.01^{a}$
Sym $-CH_2$	2851	$0.046±0.01^{a}$	$0.061±0.00^{ab}$	$0.065±0.01^{b}$
C=O	1741	0.060±0.02	0.087±0.02	0.090±0.02
Oxidation shoulder peak	1728	0.040±0.01	0.046±0.00	0.047±0.01
CH_3 and CH_2 Ratio	Wavelength cm^{-1} Ratio		Peak Intensities Ratio	
Asym/Asym	2957/2920	0.663	0.548	0.597
Sym/Sym	2873/2851	0.614	0.524	0.579

Data are result of means ± Standard Deviation (SD) of at least 15 spectra; values in the same row with different superscript letters are significantly different (P≤0.05).

Cheeses produced during summer showed a lower content in SFAs and a higher content of MUFAs than those produced in winter, whereas PUFAs and CLA were higher in winter samples. However, the ratio ω3/ω6 of FA was higher in winter cheese samples than in those manufactured in summer. The different FA profiles between winter and summer samples are also confirmed by the differences observed in the peak intensities in specific absorption bands of fatty acids.

In addition, the DAP parameter, as well as the DPPH values found in fat-soluble vitamins-rich extracts derived from *Pecorino* cheeses, reached the highest values in winter samples, whereas the information about the differences of colour, e.g. lower brightness (L*) and major yellowness (b*) in summer samples, can help to improve the physical knowledge of this traditional cheese.

As regard the nutritional value, *Pecorino d'Abruzzo* cheese exhibited appreciable nutritional properties due to a good level of coverage (%) of some nutrients for human adults (aged 18-59) according to Italian recommendations for daily requirements.

This work thus contributes to improving the information about the nutritional characteristics of *Pecorino d'Abruzzo*, that is currently scarce or not available, as well as to updating the Italian Food Composition Data Base.

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