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Chemical characterization of 'Pecorino Di Farindola' cheese during ripening

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PAPER

Abstract

This study evaluated the nutritional and sensorial characteristics of Pecorino di Farindola cheese at different commercial ripening stages. Moreover, in order to assess effectively the peculiar features of this product, the evolution of proteolysis and lipolysis, together with that of free amino acids (FAAs), was studied throughout ripening. A marked proteolysis of Pecorino di Farindola was found. At the end of ripening, FAAs with the highest content were glutamic acid, valine, leucine and lysine. Long-ripened cheeses had a light spicy feature that distinguishes them from other Italian Pecorino cheeses.

Keywords: free amino acids, lipolysis, Pecorino di Farindola, pig rennet

Introduction

The term 'Pecorino' refers to a cheese obtained from ewe milk, in most cases of protected origin. In Italy, however, several cheeses, even not of protected origin, have typical features and are prepared in limited geographical areas (Coda *et al.,* 2006). Pecorino cheeses are often produced traditionally in central and south Italy and are characterised by different ripening stages (Di Cagno and Gobbetti, 2011; Schirone *et al.,* 2011), and the use of different milk, rennet and technology of production (Gobbetti, 2016). 'Pecorino di Farindola' is a traditional food product (Prodotto Agroalimentare Tradizionale – PAT, published in the Official Gazette of the Italian Republic on 20th February 2020, General series n. 42 , Ordinary supplement n. 9) and has a limited production in the eastern part of the Gran Sasso area, Abruzzo, Italy. This is an original Pecorino cheese, since it is made from pig rennet that gives the cheese particular flavours and taste. The milk comes from sheep of Pagliarola Appenninica breed, which are bred in the wild, with a limited milk production. The cheese can be found at a short ripening time (3 months), demonstrating a soft texture and a yellow crust, or at a long ripening time (over 12 months), having a harder texture, a more intense and spicy flavour and a darker crust (Schirone *et al.,* 2011). The cheese-making process is reported by Schirone *et al.* (2011). It starts from raw milk without the addition of natural cultures or selected starters, added with porcine rennet. The microbiota, derived mostly from mesophilic lactobacilli coming from the raw milk and the cheese-making environment, plays an important role during ripening, contributing to the development of typical aromas of this cheese (Aquilanti *et al.,* 2007; De Angelis *et al.,* 2001; Tofalo *et al.*, 2015). The chemical and microbiological features of 10 Pecorino di Farindola cheeses coming from different dairies of the Consortium, at 90 days of ripening, are reported by Schirone *et al.* (2011). Information about the chemical and microbiological characteristics of Pecorino di Farindola, together with that of proteolytic and lipolytic phenomena during its ripening process is still limited (Di Giacomo *et al.,* 2013; Suzzi *et al.,* 2014; Tofalo *et al.,* 2015). The aim of this work is to have a deeper insight into the chemical and nutritional characterization of the Pecorino di Farindola cheese and to evaluate its proteolysis and lipolysis at different ripening stages.

Materials and Methods

Sample collection and preparation

Three batches of Pecorino di Farindola cheeses were analysed. The three batches were different according to ripening times: three samples were ripened for 3 months (3R), three for 5 months (5R) and three for 12 months (12R). Samples came from a dairy in the area of Pecorino of Farindola, which includes nine towns located within the provinces of Pescara and Teramo (reported in the 'Production Disciplinary of Pecorino di Farindola'- https://www.pecorinodifarindola.it/disciplinare/). All cheeses were produced following the cheese-making phases stated in the production disciplinary. The 3- and 5-month cheeses were produced during spring and summer, respectively, while the 12-month ripened samples in the autumn season. Cheeses were grounded and carefully mixed. Three bulk samples were prepared by combining the samples of each ripening month and stored at –20°C until analysis.

Chemical–physical analysis

For each ripening stage, each sample was analysed in triplicate. Cheese samples were analysed, following the international methods of Association of Official Analytical Chemists (AOAC, 2000), for fat (method: 933.05), protein (method: 920.123), moisture (method: 948.12) and ash (method: 935.42). Proteolysis was assessed by determining the content of water-soluble nitrogen (SN) and non-protein nitrogen (NPN), as done in Niro *et al.* (2014). The amount of SN and NPN, expressed as a percentage of total nitrogen (TN) (SN/TN% and NPN/TN%) indicates the extent of proteolysis. The nitrogen content was determined by the Kjeldahl method (AOAC, 2000; method: 920.123). Free amino acids (FAAs) were analysed by a Biochrom 30 series Amino Acid Analyzer (Biochrom Ltd., Cambridge Science Park, UK), with a Li-cation-exchange column (20 × 0.46 cm). A mixture of basic, acid and neutral amino acid (AA) of a known concentration (Sigma Chemical Co., St. Louis, MO) was used as standards. The FAA extraction procedure is reported in Niro *et al.* (2017a). Lipolysis was expressed as Acid Degree Value (ADV) (Deeth and Fitz-Gerald, 1976).

Sensorial analysis

Each sample was evaluated for three times. Samples were evaluated by a panel comprising 10 trained components. Sensory evaluation was conducted according to the Etana method described by Bozzetti *et al.* (2004) modified by Chiavari *et al.* (2006). The evaluated attributes were flavour and aroma: odour intensity, aroma intensity, hardness, solubility, sweet, salty, bitter, spicy and acidic. The definition of the descriptive attributes is reported in Niro *et al.* (2014). Samples were served at room temperature. The intensity of each attribute was rated on an increasing scale from 1 to 10 (from absence to maximum).

Statistical analysis

An ANOVA was applied to the data. Least significant differences were obtained using the least significant difference test ($P < 0.05$).

Results and Discussion

The chemical composition of different Pecorino cheeses (g/100 g fresh weight), at different ripening stages, is reported in Table 1. The moisture value ranged from 29.6% in 3R samples to 28.5% in 5R and 23.2% in 12R samples. Significant differences ($P < 0.05$) were found in proteins and fats, depending on different raw milk, which, in 3R and 5R samples, came from grazing sheep, while in 12R samples from sheep fed with forage. Different ash values could depend on the variability of salting process. The cheese composition is in accordance with the values reported in the 'Production Disciplinary' and the ranging values are from literature for Pecorino di Farindola (Bellocci *et al.,* 2018). Schirone *et al.* (2011) and Tofalo *et al.* (2015) have reported lower fat values in several Pecorino di Farindola cheeses. Different Pecorino cheeses were found to have similar or higher fat contents (Coda *et al.*, 2006; Di Cagno *et al.*, 2007).

The proteolysis and lipolysis indices are shown in Table 2. The value of SN/TN% increased significantly during ripening, ranging from 32.5% at 3 ripening months (3R)

Table 1. Proximal composition of Pecorino di Farindola cheeses at different ripening times (g/100-g fresh weight) (mean ± S.D).

Ripening time	Moisture	Proteins	Fats	Ash
3 months	29.6 ± 0.04^{a}	25.7 ± 0.29^{a}	39.0 ± 0.09^{a}	4.1 ± 0.03^{a}
5 months	28.5 ± 0.03^{b}	27.7 ± 0.16^{b}	36.3 ± 0.12^{b}	5.4 ± 0.02^{b}
12 months	23.2 ± 0.08^{c}	31.6 ± 0.27^{c}	40.1 ± 0.27^{c}	5.0 ± 0.00^{c}

Different letters within the same column indicate a significant difference ($P < 0.05$).

Table 2. Proteolysis and lipolysis indices of Pecorino di Farindola cheeses at different ripening times (mean ± SD).

Ripening time	Proteolysis indices		Lipolysis index
	SN/TN%	NPN/TN%	ADV (meq KOH/100-g fat)
3 months	32.5 ± 0.75[a]	12.1 ± 0.61[a]	2.9 ± 0.34[a]
5 months	33.7 ± 1.91[a]	13.8 ± 1.68[b]	4.0 ± 0.23[b]
12 months	37.6 ± 0.12[b]	19.6 ± 0.24[c]	7.0 ± 0.05[c]

Different letters within the same column indicate a significant difference ($P < 0.05$).

to 37.6% at 12 ripening months (12R). This value is an indicator of hydrolysis of casein caused by the action of rennet and milk proteases present at the beginning of the ripening process. The SN is very variable for composition, including high-, medium-, low-molecular weight peptides and AAs. Moreover, a significant part of SN is produced during curd acidification and, consequently, it is partly lost into the water or brine (Alichanidis and Polychroniadou, 2008). The breaking off of casein and high- and medium-molecular mass peptides by microorganisms, endogenous enzymes and rennet into low-molecular mass peptides and AAs, which are soluble in 12% trichloroacetic acid (TCA), is expressed by the NPN/TN% (Corradini, 1995). This value increased significantly from 12.1% in 3R samples to 19.6 % in 12R samples. These results demonstrated a marked proteolysis of Pecorino di Farindola cheese, similar of that of common Italian Pecorino cheeses (Di Cagno and Gobetti, 2011). The principal proteolytic agents in the curd are coagulant, microbial proteinases and peptidases and indigenous milk proteinase (plasmin) (Di Cagno and Gobetti, 2011). The proteinases and peptidases of rennet are among some of the proteolytic enzymes acting during the cheese-making process and the ripening phase (Fox and Stepaniak, 1993). Tofalo *et al.* (2015) found faster casein breaking off in Pecorino di Farindola cheeses, attributed to higher proteolytic activity of the enzymes of pig rennet. On the contrary, a lower proteolytic activity of pig rennet than that of calf rennet was found by Di Giacomo *et al.* (2013) in Pecorino di Farindola cheese samples.

Table 3. Free amino acid (FAA) content of Pecorino di Farindola cheeses at different ripening times (mg/100-g fresh weight).

FAA	3 months*	5 months	12 months
Aspartic acid	10.74[a]	32.20[b]	45.26[c]
Threonine	3.24[a]	10.06[b]	17.49[c]
Serine	0.00[a]	5.68[b]	11.01[c]
Asparagine	10.15[a]	13.27[b]	54.20[c]
Glutamic acid	15.36[a]	99.14[b]	146.21[c]
Glutamine	4.56[a]	4.01[a]	9.64[b]
Glycine	4.34[a]	6.45[a]	20.89[b]
Alanine	11.35[a]	14.13[b]	43.82[c]
Valine	30.71[a]	42.08[b]	88.03[c]
Methionine	7.95[a]	14.62[b]	33.68[c]
Isoleucine	12.03[a]	25.50[b]	60.10
Leucine	42.97[a]	57.66[b]	115.54[c]
Tyrosine	0.00[a]	2.27[b]	12.41[c]
Phenylalanine	26.75[a]	37.07[b]	63.56[c]
γ-Aminobutyric acid	21.55[a]	30.02[b]	56.98[c]
Ornithine	14.33[a]	15.08[a]	10.64[a]
Lysine	20.34[a]	38.92[b]	115.87[c]
Histidine	0.00[a]	9.13[b]	31.15[c]
Total	236.37[a]	457.28[b]	936.48[c]

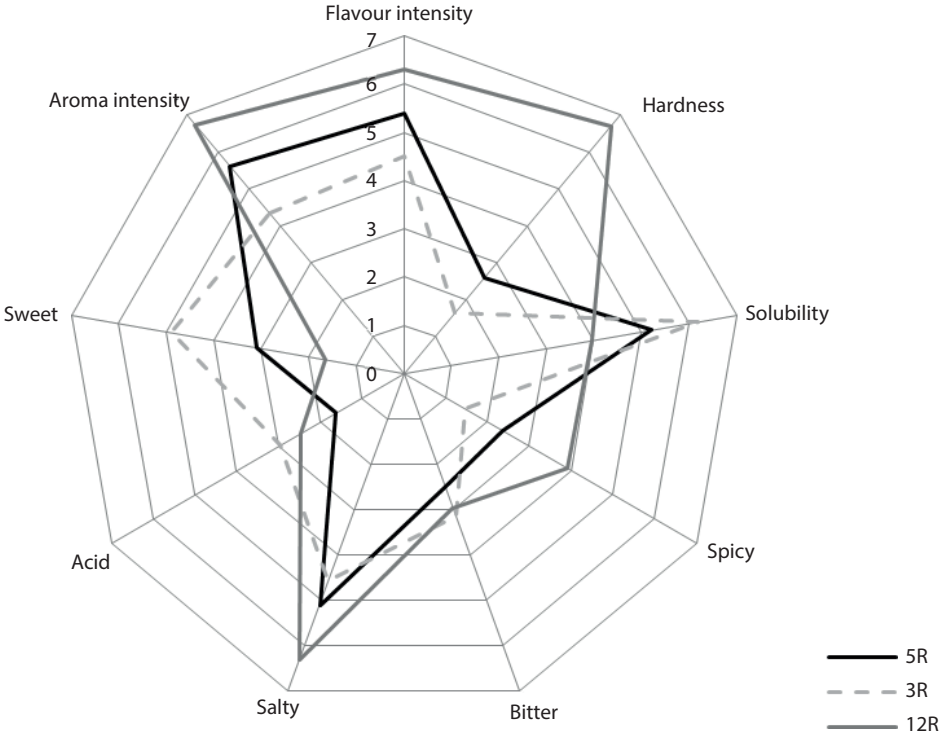
*Different letters within the same raw indicate a significant difference ($P < 0.05$).

A measure of lipolysis is represented by the determination of ADV. This value is a measure of the content of free fatty acids (FFA) dissolved in a certain amount of fat by lipases that can be correlated to the sensorial quality of finished products (Deeth and Fitz-Gerald, 1976; Mcsweeney and Sousa, 2000). The 3-, 5- and 12-month ripened cheeses demonstrated an ADV value of 2.9, 4.0 and 7.0 meq KOH/100-g fat, respectively (Table 2). Similar values were reported for ewe's cheeses (Georgala *et al.,* 2005), while higher indices were found in mixed cow/ewe Caciocavallo cheeses by Niro *et al.* (2014).

The evolution of FAAs of cheeses during ripening is shown in Table 3. The concentration of total free amino acids (TFAA) increased significantly ($P < 0.05$) to 936.48 mg/100 g at 12 months of ripening. At different ripening stages, the average TFAA content is in accordance with literature data for other Italian Pecorino cheeses (Coda *et al.*, 2006). No proline and arginine were found in all analysed samples. The latter evidence could be due to its consumption by bacteria; in fact, many species of LAB are able to convert arginine to citrulline and ornithine (Diana *et al.,* 2014; Niro *et al.,* 2017a). During ripening, the content of single FAAs increased, with the exception of ornithine. As also reported by Di Giacomo *et al.* (2013), at 12-month ripening, the highest FAAs found were glutamic acid (Glu), valine (Val), leucine (Leu) and lysine (Lys) in Pecorino di Farindola cheese at 6-month

ripening and in long-ripened Pecorino cheese (Coda *et al.,* 2006; Mangia *et al.,* 2008). Glutamic acid is one of the most abundant amino acids in milk protein and, as almost free, in mature cheeses (Redruello *et al.,* 2020) and is strictly correlated with the 'umami' taste (McSweeney and Sousa, 2000).

Leu, phenylalanine (Phe) and Lys e Val can be found in other long-ripened cheeses such as Caciocavallo cheeses (Corsetti *et al.,* 2001; Niro *et al.,* 2017b; Succi *et al.,* 2016), Idiazabal (Barcina *et al.,* 1995), Picante (Freitas *et al.,* 1998), Serra da Estrela (Tavaria *et al.,* 2003), Teleme (Pappa and Sotirakoglou, 2008), goat cheeses (Poveda *et al.,* 2016) and Italian hard cheeses (Niro *et al.,* 2017b). Leu, with the other branched chain AA, isoleucine (Ile) and Val, the aromatic AA, Phe and tyrosine (Tyr), and metionine (Met) are the main precursors of key aroma compounds (Yvon and Rijnen, 2001). Some authors have attributed the bitter flavour in cheese to the concentration of arginine (Arg) (Barcina *et al.,* 1995; Pappa and Sotirakoglou, 2008). In all samples, ornithine (Orn) and γ-aminobutyric acid (GABA) were found; they do not originate from casein but are the products of microbial metabolism and are established functional AAs (Diana *et al.,* 2014; Tofalo *et al.*, 2019). As for GABA, its anti-hypertensive and anti-diabetic properties and its



	3R	5R	12R
Flavour intensity*	a	b	c
Hardness	a	b	c
Solubility	a	a	b
Spicy	a	b	c
Bitter	a	a	a
Salty	a	a	b
Acid	a	a	a
Sweet	a	b	c
Aroma intensity	a	b	c

*Different letters within the same row indicate a significant difference (P < 0.05)

Figure 1. Sensorial profile of Pecorino di Farindola cheeses at different ripening times. 3R: three-month ripening; 5R: five-month ripening; 12R: 12-month ripening.

ability to reduce stress and anxiety are widely recognised (Redruello *et al.,* 2020; Tofalo *et al.,* 2019). GABA is synthesised by glutamate decarboxylase through the decarboxylation of L-glutamate; milk origin, milk treatment, proteolytic activity, fat content, texture, ripening time and climate are reported to be the key factors governing its accumulation (Redruello *et al.,* 2020). Tofalo *et al.* (2019), in Pecorino di Farindola cheeses made from pig rennet, reported similar amounts of GABA than those found in this study, higher than the values of different commercial cheeses.

Regarding sensorial analysis, all samples were characterised by low acid and bitter attributes and a high salty score (Figure 1). As reported by Suzzi *et al.* (2014), cheeses made from pig rennet demonstrated the lowest elasticity, bitter taste and fruity and hay flavour intensities, compared with the cheeses made from calf and kid rennet. These data were also confirmed by Di Giacomo *et al.* (2013). Low-ripened cheeses had a higher solubility, and tasted sweeter, less hard and less spicy than the corresponding long-ripened cheeses ($P < 0.05$). Flavour and aroma intensity increased during ripening, reaching the highest score in Pecorino at 12-month ripening ($P < 0.05$). The 12-month ripened cheeses had a light spicy feature that distinguishes them from other Italian Pecorino cheeses, probably because of the use of pig rennet instead of the commonly used lamb rennet. As reported by different authors (Di Giacomo *et al.,* 2013; Kindstedt *et al.,* 2004), cheeses with a more evident lipolysis differ for strong flavours. The effects of FAAs on taste and flavour are reported by McSweeney and Sousa (2000) and Yvon and Rijnen (2001). In raw milk cheeses, similar to Pecorino di Farindola, the native microbiota may have played an important role and contributed to the distinct sensorial characteristics (McSweeney and Sousa, 2000; Niro *et al.,* 2014).

Conclusions

Different Pecorino di Farindola cheeses were characterised by certain variability because of different composition of the used raw milk, the low standardization of the cheese making process and different salting and ripening conditions. The use of raw ewe's milk and pig rennet contributed to the peculiar features of lipolysis and proteolysis and the sensorial attributes of the Pecorino of Farindola cheese. Sensorial analysis confirmed Pecorino of Farindola as sweet, lightly spicy and never bitter cheese, even when the ripening is extended a distinctive feature that is appreciated by the consumer.

Data emerging from this work could add new knowledge to the investigations of this cheese, giving an effective characterisation of the final nutritional and sensorial quality of the product. An additional investigation could be conducted to have a deeper insight into the fatty acid evolution and the influence of lipolysis and proteolysis on the profiles of volatiles during ripening.

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