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# Changes in the nutritive quality, fatty acid and terpenoid profile of milk and "Plaisentif" cheese from nine Italian Alpine dairy farms during two grazing seasons

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## Abstract

Traditional food products have been gaining importance over the last few years. The interest in such products is linked to the consumers' awareness of the importance of local cultures and low-processed food and of the environmental impact of food production systems. These products have been identified by the European Community as a way of preserving tradition and different cultures and as a way of enhancing local economies. Indeed, specific regulations have been set up for these reasons. Plaisentif is a traditional cheese that is produced in two valleys, named the Chisone and Susa valleys (Northwest Italy), of the Piedmont region. A survey was conducted over two different grazing seasons on nine Alpine dairy farms, where Plaisentif is produced. The aim was to characterize the chemical composition, fatty acid profile, and terpene content of milk and Plaisentif cheese, known as the "cheese of the violets", which is produced from the raw milk of cows fed on pastures when violets are in bloom. The fatty acid profile of the cheese was found to be similar to that of other cheeses obtained from pasture breeding, and conjugated linoleic acid and fatty acids, derived from pasture herbs, were found to be present at higher levels than in other Italian cheeses. Terpenes, which are generally derived from herbal feeds, have also been found to be present, and they can be a reliable marker of pasture grazing. This study is the first attempt to characterize the Plaisentif cheese profile. This is a crucial step to valorize the product in order to enhance its production and thus to give more opportunities to the valleys in Piemonte that produce it, as well as to encourage continuity of local traditions.

**Key words:** PDO, protein, lipid, fatty acid, terpene, cheese, milk.

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## Introduction

Several studies have highlighted the remarkable biodiversity of pastures and the high content of beneficial fatty acids (FAs), including terpenes, conjugated linoleic acid (CLA), and n-3 series polyunsaturated FAs (PUFAs), in dairy products derived from grass-fed ruminants (Dewhurst *et al*., 2006; Paszczyk *et al*., 2016). The relationship between highland botanical species and the chemical composition of dairy products has been examined in various studies, as reviewed by Kilcawley *et al.* (2018).

In Europe, such studies have been conducted in Switzerland (Collomb *et al*., 2002) and in several sites in the Italian Alps (Zeppa *et al*., 2003; Revello-Chion *et al.*, 2010). A wide range of cheeses, closely linked to their place of origin, are produced in Italian mountain regions according to ancient dairy traditions. These cheeses are often considered niche products, and they share certain key features, such as production by small-scale artisans within a confined area, high variability in production and a limited number of final products.

Plaisentif, a hand-crafted, semi-hard cheese originating from two Piedmont valleys in the northwestern Italian Alps, is one of these traditional cheeses. Its distinctiveness lies in the fact that it is produced exclusively from the milk of cows that graze above 1800 meters during the violet flowering period, that is, June-July (Pattono *et al*., 2022). A study was undertaken to investigate the chemical composition, FA profile, and terpene content of milk and Plaisentif cheese in an attempt to support environmental conservation and foster the economic revitalization of local communities while safeguarding the heritage of this traditional Alpine cheese. Sampling was carried out on nine Alpine farms situated at various altitudes in the Chisone and Susa valleys (Piedmont Region, northwest Italy) during two successive grazing seasons. The findings could be useful to obtain a more comprehensive characterization of this artisanal product.

## Materials and Methods

### Experimental design

The study was conducted during the grazing periods of two consecutive (T1 and T2) years on 9 dairy farms in the Alps in northwest Italy, at altitudes ranging from 1600 to 2100 m a.s.l.

### Milk and cheese sampling

Milk samples were collected on the same day as the pasture surveys were conducted to ensure a close correlation between the consumed botanical species and the characteristics of the dairy products. The samples were immediately frozen, freeze-dried, and stored at -20°C until FA extraction. After 60 days of ripening, cheese samples were cut into wedges and stored at -20°C.

### Chemical analysis

An aliquot from each pooled milk sample was used, in duplicate, to determine the dry matter content, according to the AOAC protocols (1990). Another aliquot of 200 g was refrigerated, freeze-dried, and stored for qualitative analyses. Freeze-dried samples were also analyzed for nitrogen (AOAC #984.13) and ash (AOAC #923.03). The lipid content was determined using the Hara and Radin method (1978). All the analyses were performed in duplicate.

### Fatty acid analysis

The FAs in the freeze-dried milk and cheese samples (2 g) were analyzed according to Peiretti *et al.* (2010). FA methyl esters in hexane were injected into a gas chromatograph (Dani Instruments S.P.A. GC 1000 DPC; Cologno Monzese, Italy), equipped with a flame ionization detector, a PTV injection port, and a Supelcowax-10 fused silica column (60 m × 0.32 mm, 0.25 µm). Peaks were identified using methyl ester standards (Supelco and Restek Corp.), and the data were expressed as g/100 g of total FA. All the analyses were conducted in duplicate.

### Terpene analysis

A Terpene analysis was performed on milk and cheese samples, without solvent extraction, using dynamic headspace extraction (Dani Instruments S.p.A., Cologno Monzese, Italy), followed by gas chromatography-mass spectrometry (Agilent Tecnologies Santa Clara, CA, USA), as described by De Noni and Battelli (2008). The data were expressed in arbitrary units, as $\log_{10}$ of the peak area of the corresponding selected ion. All the analyses were conducted in duplicate.

### Statistical analysis

Variability in the chemical composition, FA profiles, and terpene contents of the milk and cheese was assessed using an analysis of variance to test the effect of farm location. SPSS software (version 11.5.1; SPSS Inc., Chicago, IL, USA) was used for this purpose. Post-hoc comparisons were performed using Tukey's test. Statistical significance was set at $p<0.05$.

## Results and Discussion

### Chemical composition of the milk and cheese

The chemical composition of the milk and Plaisentif cheese, in terms of dry matter, lipid, crude protein, and ash content, is reported in Tables 1 and 2, respectively, for the two grazing seasons. The lipid, crude protein, and ash content of the milk showed significant differences between farms during the first grazing season, while only the dry matter content differed significantly in the second season. Conversely, all the parameters pertaining to the cheese (except the dry matter in the second season) showed significant differences between farms. These variations may reflect the influence of the pasture composition and cheese production conditions on the nutritional traits (Gai *et al*., 2014).

### Fatty acid profile of the milk and cheese

The results pertaining to the FA profile of the milk and cheese are shown in *Supplementary Tables 1 and 2*. The first noteworthy observation is the marked variability between the producers, a trend that is consistent with the findings of Agradi *et al*. (2020) for other traditional cheeses, and with the microbiological data reported by Pattono *et al.* (2022). This variability could stem from differences in the pasture composition, microbial variety, and, albeit to a lesser extent, cheesemaking practices (Dalmasso *et al*., 2016; Bionaz *et al*., 2020).

Interestingly, the proportion of statistically significant FAs remained relatively consistent for the 2 years in both the milk and cheese, a pattern that has also been observed in other studies (Formaggioni *et al*., 2020). Minor differences observed between the milk and cheese could be due to partial triglyceride hydrolysis and lipase activity during ripening and aging (Eisenstecken *et al*., 2021).

Overall, the most abundant FAs in both the milk and cheese samples for both years were saturated FAs (SFAs), followed by monounsaturated FAs (MUFAs) and n-6 PUFA, with n-3 PUFA being the least represented. Similar distributions have been reported in other works (Corazzin *et al.*, 2019; Agradi *et al*., 2020; Formaggioni *et al*., 2020), although with a lower SFA and higher PUFA content.

The most prevalent SFAs were C16:0, C18:0, C14:0, C4:0, C12:0, C10:0, and C6:0, although their relative proportions were found to vary in other studies. For example, Agradi *et al*. (2020) reported higher C16:0 and C14:0 levels. Instead, although the C18:0 content was similar in the milk, it was slightly lower in the Plaisentif cheese.

Differences in FA profiles can be attributed to pasture variability, geographic factors, and cow breed (Agradi *et al*., 2020; Bionaz *et al*., 2020; Eisenstecken *et al*., 2021). The presence of C18:1n-9, together with C15:0, appears to be a distinguishing feature of Italian Alpine cheeses (Eisenstecken *et al*., 2021).

A comparison of the milk and cheese from the same producers over two grazing seasons revealed minimal changes in the FA profiles (*e.g.*, for C6:0 and C12:0), thus suggesting that the cheesemaking process used for Plaisentif has a limited impact on the FA profile — an observation that confirms those of Eisenstecken *et al*. (2021).

Palmitic acid (C16:0) and oleic acid (C18:1n-9) were the most abundant FAs in both the milk and cheese samples. The proportion of C16:0 in the milk was 25.97±1.56% in the first season and 24.47±1.01% in the second, while it was 24.98±1.22% in the cheese in the T1 and 24.24±1.32% in the T2. High levels of this FA have been associated with pasture-based feeding systems (Mitani *et al*., 2015). Stearic acid (C18:0) and myristic acid (C14:0) were the third and fourth most prevalent FAs. Palmitic acid (C16:0) is known to be a characteristic of milk derived from animals that graze on high-altitude pastures (Peiretti *et al*., 2020). C18:1n-9 was the most prominent MUFA, with the milk containing 21.99±1.94% (T1) and 21.79±1.74% (T2), and the cheese containing 22.59±1.41% (T1) and 23.08±2.03% (T2).

Linoleic acid (C18:2n-6) was the most abundant PUFA for both sampling years: 2.30±0.26% and 2.39±0.35%, respectively, in the milk and 2.36±0.27% and 2.00±0.9% in the cheese. These values are slightly higher than those reported by Agradi *et al*. (2020).

Principal component analysis showed that C18:1n-9 and C18:2n-6 were significantly associated with specific grazing farms. CLA1 was more abundant than CLA2, although the significance of this ratio remains unclear. Plaisentif cheese has consis-



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tently shown higher CLA concentrations than other traditional cheeses (Formaggioni *et al*. 2020). Although these compounds were present in small amounts, they are known for their health-promoting properties (Eisenstecken *et al*., 2021).

## Terpene profile of the milk and cheese

α-pinene, camphene, β-pinene, δ-3-carene, allo-ocimene, p-cymene, and limonene were the main terpenes found in both the milk and cheese samples (Tables 3 and 4). All of these terpenes have been identified in Alpine plants (Mariaca *et al*., 1997) and are transferred directly from the pasture to milk and cheese (Cifuni *et al*., 2022). The differences in the terpene content were statistically significant, except for δ-3-carene (milk and cheese), limonene (milk), and camphene (cheese) for the second year samples.

α-pinene was the most abundant, with mean values of 6.26±0.26 (T1) and 5.94±0.58 (T2) in the milk, and 6.35±0.33 (T1) and 5.35±0.48 (T2) in the cheese. These levels are comparable with those found in Montasio PDO cheese (Aprea *et al*., 2016). The high concentration of α-pinene during summer reflects its prevalence in Alpine pastures during that season (Peiretti *et al*. 2020). The variability in the terpene content observed between the producers likely reflects differences in pasture composition, cheesemaking practices, plant phenological stages, management systems, and geographic location (Valdivielso *et al*., 2017; Cifuni *et al*., 2022). These differences may also serve as quality markers and as an aid in product traceability within the PDO system. The terpenes also varied according to the grazing period. The early summer cheeses contained higher levels of D-limonene, δ-3-

**Table 1.** Chemical composition (g/kg fresh matter) of the milk during the two grazing seasons.

| | GS | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | SEM | p |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dry matter | T1 | 126.8 | 129.1 | 131.1 | 126.5 | 120.7 | 127.6 | 121.1 | 133.3 | 118.4 | 1.51 | 0.252 |
| | T2 | 139.8$^{abc}$ | 132.8$^{ab}$ | 131.2$^{ab}$ | 124.0$^{a}$ | 135.4$^{abc}$ | 134.8$^{abc}$ | 143.8$^{bc}$ | 130.2$^{ab}$ | 152.3$^{c}$ | 2.08 | 0.008 |
| Lipid | T1 | 22.0$^{ab}$ | 25.1$^{abcd}$ | 31.3$^{bcd}$ | 23.0$^{abc}$ | 32.3$^{cd}$ | 34.4$^{d}$ | 18.7$^{a}$ | 26.9$^{abcd}$ | 22.2$^{abc}$ | 1.31 | 0.002 |
| | T2 | 37.1 | 26.6 | 28.5 | 26.9 | 36.8 | 26.7 | 40.7 | 25.4 | 40.2 | 2.14 | 0.494 |
| Crude protein | T1 | 32.2$^{abc}$ | 32.9$^{bc}$ | 34.3$^{c}$ | 26.4$^{a}$ | 27.1$^{ab}$ | 29.5$^{abc}$ | 33.0$^{b}$ | 34.0$^{b}$ | 28.7$^{abc}$ | 0.73 | 0.002 |
| | T2 | 31.6 | 29.6 | 33.6 | 31.4 | 32.1 | 34.5 | 32.3 | 31.0 | 31.2 | 0.45 | 0.343 |
| Ash | T1 | 6.66$^{ab}$ | 7.00$^{bc}$ | 6.94$^{b}$ | 6.99$^{bc}$ | 7.12bc | 6.31$^{a}$ | 6.97$^{bc}$ | 7.43$^{c}$ | 6.67$^{ab}$ | 0.08 | <0.001 |
| | T2 | 7.13 | 6.90 | 7.16 | 6.93 | 6.67 | 7.22 | 7.42 | 6.97 | 7.19 | 0.08 | 0.603 |

GS, grazing season; $^{a-d}$means in the same row with different superscripts differ (p<0.05).

**Table 2.** Chemical composition (g/kg fresh matter) of the Plaisentif cheese during the two grazing seasons.

| | GS | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | SEM | p |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Dry matter | T1 | 577.7$^{ab}$ | 597.5$^{bc}$ | 646.4$^{d}$ | 561.0$^{a}$ | 610.5$^{c}$ | 568.5$^{ab}$ | 611.3$^{c}$ | 566.0$^{a}$ | 568.5$^{ab}$ | 6.69 | <0.001 |
| | T2 | 642.9 | 666.6 | 713.8 | 653.0 | 663.0 | 686.9 | 653.7 | 662.0 | 610.0 | 8.46 | 0.230 |
| Lipid | T1 | 195.0$^{ab}$ | 201.3$^{abc}$ | 288.9e | 182.6$^{a}$ | 221.7$^{c}$ | 224.0c | 259.3$^{d}$ | 201.6$^{abc}$ | 210.0$^{bc}$ | 7.81 | <0.001 |
| | T2 | 300.0$^{ab}$ | 282.8$^{a}$ | 353.7$^{a}$b | 349.8$^{ab}$ | 362.5$^{ab}$ | 377.2$^{bc}$ | 462.7$^{c}$ | 301.0a$^{b}$ | 365.7$^{ab}$ | 12.9 | <0.001 |
| Crude protein | T1 | 230.2$^{ab}$ | 235.9$^{ab}$ | 247.2$^{ab}$ | 200.8$^{a}$ | 210.6$^{ab}$ | 215.6a$^{b}$ | 215.0$^{a}$b | 266.6$^{b}$ | 207.2$^{ab}$ | 5.63 | 0.041 |
| | T2 | 151.4$^{ab}$ | 191.9d | 156.5$^{abc}$ | 156.3$^{a}$b$^{c}$ | 162.9$^{abcd}$ | 134.3$^{a}$ | 155.7$^{abc}$ | 181.9$^{c}$d | 176.0$^{bcd}$ | 4.21 | 0.001 |
| Ash | T1 | 26.84$^{c}$ | 24.38$^{ab}$ | 24.48$^{ab}$ | 23.44$^{a}$ | 24.77$^{b}$ | 27.69$^{c}$ | 23.39$^{a}$ | 23.46$^{a}$ | 24.26$^{ab}$ | 0.35 | <0.001 |
| | T2 | 66.1$^{d}$ | 54.8$^{bc}$ | 59.8$^{cd}$ | 57.8$^{bcd}$ | 49.4$^{ab}$ | 49.5$^{ab}$ | 43.6$^{a}$ | 65.1$^{d}$ | 63.6$^{cd}$ | 1.85 | <0.001 |

GS, grazing season; $^{a-d}$means in the same row with different superscripts differ (p<0.05).

**Table 3.** Terpene profile of the milk fat during first and second grazing seasons.

| | GS | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | SEM | p |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| α-pinene* | T1 | 6.26$^{b}$ | 6.43$^{c}$ | 6.07$^{a}$ | 5.95$^{a}$ | 6.05$^{a}$ | 6.25$^{b}$ | 6.72$^{d}$ | 6.07$^{a}$ | 6.52$^{c}$ | 0.059 | <0.001 |
| | T2 | 6.35$^{d}$ | 5.65$^{c}$ | 5.08$^{a}$ | 7.00$^{e}$ | 5.82$^{c}$ | 6.17$^{d}$ | 6.25$^{d}$ | 5.34$^{b}$ | 5.80$^{c}$ | 0.133 | <0.001 |
| Camphene | T1 | 5.36$^{b}$ | 5.48$^{b}$ | 5.08$^{a}$ | 4.90$^{a}$ | 5.07$^{a}$ | 5.31$^{b}$ | 5.45$^{b}$ | 5.10$^{a}$ | 5.10$^{a}$ | 0.048 | <0.001 |
| | T2 | 5.23$^{e}$ | 4.50$^{c}$ | 3.91$^{a}$ | 5.51$^{f}$ | 4.65c$^{d}$ | 4.89$^{d}$ | 4.88$^{d}$ | 4.23$^{b}$ | 4.65c$^{d}$ | 0.113 | <0.001 |
| β-pinene | T1 | 5.62$^{a}$ | 5.87$^{b}$ | 5.64$^{a}$ | 5.53$^{a}$ | 5.53$^{a}$ | 5.87$^{b}$ | 6.21$^{c}$ | 5.67$^{a}$ | 5.66$^{a}$ | 0.051 | <0.001 |
| | T2 | 5.39$^{ef}$ | 4.86$^{c}$ | 4.47$^{a}$ | 6.15$^{h}$ | 5.26$^{de}$ | 5.54$^{f}$g | 5.65$^{g}$ | 4.65$^{b}$ | 5.09$^{d}$ | 0.122 | <0.001 |
| δ 3-carene | T1 | 5.62c$^{d}$ | 5.49$^{ab}$ | 5.30$^{bcd}$ | 4.96$^{ab}$ | 4.71$^{a}$ | 4.97$^{ab}$ | 5.11$^{abc}$ | 5.71$^{d}$ | 4.57$^{a}$ | 0.099 | 0.006 |
| | T2 | 4.23$^{bcd}$ | 4.00$^{abc}$ | 3.87$^{ab}$ | 4.46$^{d}$ | 4.24$^{bcd}$ | 4.41$^{cd}$ | 4.49$^{d}$ | 4.10$^{abcd}$ | 3.66$^{a}$ | 0.071 | 0.016 |
| Limonene | T1 | 5.47$^{bc}$ | 5.43$^{bc}$ | 5.53$^{bc}$ | 5.52$^{bc}$ | 5.20$^{a}$ | 5.43$^{b}$c | 5.53$^{bc}$ | 5.64$^{c}$ | 5.33$^{ab}$ | 0.033 | 0.027 |
| | T2 | 5.05$^{d}$ | 5.14$^{d}$ | 4.13$^{a}$ | 5.55$^{e}$ | 4.64$^{c}$ | 5.09$^{d}$ | 5.04$^{d}$ | 4.36$^{ab}$ | 4.53$^{bc}$ | 0.104 | <0.001 |
| p-cymene | T1 | 5.48$^{d}$ | 5.30$^{bc}$ | 5.34$^{cd}$ | 5.15$^{ab}$ | 5.10$^{a}$ | 5.85$^{e}$ | 5.90$^{e}$ | 5.99$^{e}$ | 5.33$^{cd}$ | 0.077 | <0.001 |
| | T2 | 5.11$^{c}$ | 4.77$^{abc}$ | 4.49$^{ab}$ | 5.52$^{d}$ | 4.84$^{bc}$ | 5.81$^{d}$ | 5.65$^{d}$ | 4.45$^{a}$ | 4.62$^{ab}$ | 0.120 | <0.001 |
| Allo-ocimene | T1 | 4.38$^{cd}$ | 4.50$^{de}$ | 4.15$^{ab}$ | 4.03$^{a}$ | 4.08$^{a}$ | 4.27$^{bc}$ | 4.75$^{ef}$ | 4.30$^{bc}$ | 4.60$^{ef}$ | 0.057 | <0.001 |
| | T2 | 4.36$^{cd}$ | 3.74$^{ab}$ | 3.62$^{a}$ | 5.10$^{e}$ | 4.14$^{bcd}$ | 4.15$^{bcd}$ | 4.47$^{d}$ | 3.58$^{a}$ | 3.97$^{abc}$ | 0.115 | <0.001 |

GS, grazing season. *data expressed as arbitrary units of log10 of the peak area of the corresponding selected ion; $^{a-h}$means in the same row with different superscripts differ (p<0.05).

**Table 4.** Terpene profile of the Plaisentif cheese during the two grazing seasons.

| | GS | A1 | A2 | A3 | A4 | A5 | A6 | A7 | A8 | A9 | SEM | p |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| α-pinene* | T1 | 6.37$^{b}$ | 6.41$^{b}$ | 6.01$^{a}$ | 5.93$^{a}$ | 6.46$^{b}$ | 7.01$^{c}$ | 6.52$^{b}$ | 6.04$^{a}$ | 6.43$^{b}$ | 0.077 | <0.001 |
| | T2 | 5.16$^{cd}$ | 5.11$^{bc}$ | 5.27$^{d}$ | 4.97$^{b}$ | 5.58$^{e}$ | 5.74$^{f}$ | 5.72$^{ef}$ | 4.68$^{a}$ | 5.95g | 0.096 | <0.001 |
| Camphene | T1 | 5.49 | 5.53 | 5.08 | 5.07 | 5.42 | 5.63 | 5.40 | 5.25 | 5.25 | 0.057 | NS |
| | T2 | 4.12$^{b}$ | 4.38$^{c}$ | 4.09$^{b}$ | 3.74$^{a}$ | 4.38$^{c}$ | 4.48$^{c}$ | 4.50$^{c}$ | 3.83$^{a}$ | 4.48$^{c}$ | 0.068 | <0.001 |
| β-pinene | T1 | 5.91$^{c}$ | 5.70$^{b}$ | 5.64$^{b}$ | 5.47$^{a}$ | 5.96$^{c}$ | 6.25$^{d}$ | 5.93$^{c}$ | 5.94$^{c}$ | 5.72$^{b}$ | 0.054 | <0.001 |
| | T2 | 4.37$^{a}$ | 4.60$^{b}$ | 4.72$^{c}$ | 4.33$^{a}$ | 4.92$^{d}$ | 5.06$^{e}$ | 5.20$^{f}$ | 4.29$^{a}$ | 4.85$^{d}$ | 0.076 | <0.001 |
| δ 3-carene | T1 | 5.89$^{d}$ | 5.63$^{c}$ | 5.50$^{bc}$ | 5.04$^{a}$ | 5.00$^{a}$ | 5.40$^{b}$ | 5.08$^{a}$ | 5.83$^{d}$ | 5.13$^{a}$ | 0.080 | <0.001 |
| | T2 | 3.96 | 4.20 | 4.36 | 3.95 | 4.40 | 4.19 | 4.61 | 4.31 | 4.34 | 0.069 | NS |
| Limonene | T1 | 5.69$^{ab}$ | 5.98$^{ab}$ | 5.58$^{ab}$ | 5.46$^{a}$ | 5.53$^{ab}$ | 5.72$^{ab}$ | 6.05$^{b}$ | 6.80$^{c}$ | 5.64$^{ab}$ | 0.101 | 0.003 |
| | T2 | 4.08$^{b}$ | 4.36$^{d}$ | 4.38$^{d}$ | 3.97$^{a}$ | 4.40$^{d}$ | 4.55$^{e}$ | 4.84$^{f}$ | 4.19$^{c}$ | 4.42$^{d}$ | 0.059 | <0.001 |
| p-cymene | T1 | 5.79$^{b}$ | 5.49$^{a}$ | 5.38$^{a}$ | 5.32$^{a}$ | 5.56$^{ab}$ | 6.03$^{c}$ | 6.02$^{c}$ | 7.22$^{d}$ | 5.48$^{a}$ | 0.136 | <0.001 |
| | T2 | 4.35$^{b}$ | 4.52$^{c}$ | 4.54$^{c}$ | 4.19$^{a}$ | 4.56$^{c}$ | 5.24$^{d}$ | 5.55$^{e}$ | 4.28$^{b}$ | 4.60$^{c}$ | 0.104 | <0.001 |
| Allo-ocimene | T1 | 4.58$^{b}$ | 4.59$^{b}$ | 4.23$^{a}$ | 4.13$^{a}$ | 4.63$^{b}$ | 5.10$^{c}$ | 4.69$^{b}$ | 4.33$^{a}$ | 4.59$^{b}$ | 0.067 | <0.001 |
| | T2 | 3.03$^{a}$ | 3.61$^{cd}$ | 3.56$^{cd}$ | 2.98$^{a}$ | 3.42$^{bc}$ | 3.26$^{b}$ | 3.75$^{d}$ | 3.49$^{c}$ | 3.62$^{cd}$ | 0.064 | <0.001 |

GS, grazing season; NS, not significant. *Data are expressed as arbitrary units of $\log_{10}$ of the peak area of the corresponding selected ion; $^{a-g}$means in the same row with different superscripts differ ($p<0.05$).

carene, myrcene, and ocimene, whereas the late-summer cheeses were richer in α-pinene, camphene, and β-pinene. Allo-ocimene was only detected in the late-summer cheese.

## Conclusions

Consumers' interest in traditional food products has grown significantly in recent years. In response to this growth in interest, the European Union and member states have supported local productions through certifications, such as PDO and PGI, which are also aimed at promoting environmental sustainability (Balogh *et al.*, 2016; Fernández-Ferrín *et al.*, 2018). These products enhance rural economies and employment, and they preserve the local cultural and culinary heritage. For these reasons, they should be protected.

Although PDO/PGI certifications are often based on geographic and procedural criteria, there is frequently a lack of detailed data on the chemical and nutritional quality of products. This study offers essential information for the characterization of Plaisentif cheese, and it provides a scientific basis for updating and strengthening its production guidelines ("*Disciplinare di Produzione*").

To the best of our knowledge, this is the first study that has analyzed samples from all the registred Plaisentif producers. The findings confirm that highland pastures are a key feature of this cheese. Most FAs reported in literature have been found in this cheese, although their concentrations vary from farm to farm, due to differences in the pastures and in the production variables, such as ripening and location (Bionaz *et al*., 2020; Eisenstecken *et al*., 2021). Furthermore, the observed presence of CLA reinforces the nutraceutical value of Plaisentif cheese (Agradi *et al*., 2020).

These findings offer a solid foundation for the improvement of production guidelines and the implementation of quality certification schemes. As demonstrated in studies on other traditional food products, a comprehensive compositional characterization strengthens product typicity and underpins the establishment of rigorous quality standards, thus ensuring the preservation of traditional food authenticity.

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*Online supplementary material*
*Supplementary Table 1. Fatty acid profile of the milk fat during the two grazing seasons.*
*Supplementary Table 2. Fatty acid profile of the Plaisentif cheese during the two grazing seasons.*

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