Nutritional characteristics and volatile components of sheep milk products during two grazing seasons

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Highlights

- The pasture season influenced the composition of sheep's milk and dairy products
- Spring dairy products were richer in calcium
- Spring milk and cheese had more omega-3 fatty acids and more vaccenic acid
- Spring cheese had more volatile components linked to alcoholic and floral notes

Abstract

Feeding is an important factor that affects the quality of animal products and also has a major impact on regional and local dairy products in terms of origin and quality. This study investigated the effects of pasture on nutritional characteristics and volatile components of sheep's milk, yoghurt and cheese produced in central Italy. During two grazing seasons (winter and spring) aliquots of fodder, yoghurt, fresh cheese and the corresponding ripened cheese were taken and analysed in relation to nutritional composition and volatile compounds. The grazing season influenced the chemical composition of the sheep's milk and dairy products, giving the cheese from the spring pasture a significantly higher dry matter content (61.26% vs 52.85%). Spring dairy products were also significantly higher in calcium (milk 0.40% vs 0.19%; yoghurt 0.23% vs 0.18%; cheese 0.64% vs 0.41%), while milk and cheese had significantly higher omega-3 fatty acids (milk 1.05 vs 1.61 g/100g of total fatty acids; cheese 1.09 vs 1.76 g/100g of total fatty acids) especially cis-9, cis-12, cis-15 C18:3 (milk 1.31 vs 0.66 g/100g of total fatty acids; cheese 1.43 vs 0.70 g/100g of total fatty acids). Spring cheese also contained more than twice the amount of trans-11 C18:1 (2.92 vs 1.20 g/100g of total fatty acids) compared to the winter cheese. The higher linolenic acid and omega-3 content in milk were presumably related to the higher content of linolenic acid in the pasture.

Of the 16 terpenes detected in pasture, α-pinene, β-myrcene, α-terpinene, limonene, α -cubebene were also observed in yoghurt, while alpha terpinene and limonene were detected in cheese. Neither the terpene profile of the yoghurt nor that of the cheese showed significant variations according to the season or ripening. In addition in the cheese, the spring pasture led to an increase in the number of components responsible for alcoholic and floral notes (esters, alcohols) and a reduction of components linked to a cheesy sharp aroma (acids).

Key words: pasture, sheep milk, fatty acids, volatile compounds

1.Introduction

Feeding affects the quality of animal products (Martini et al., 2010; Morand-Fehr et al., 2007) and is also key in terms of the feature characterizing typical dairy products that are in some way associated with a regional or local origin and quality.

Dairy foods from grazing animals are perceived by most consumers and producers as high-quality foods with ethical links such as animal welfare and environment safeguard.

In the Mediterranean area, the dairy sheep sector is mainly based on local breeds reared under extensive or semi-extensive management and feeding is traditionally based on the use of artificial, natural or semi-natural pastures.

In southern Europe, there has been a decrease in pastures and a diffusion of intensive farming systems which has also been due to the spread of foreign high-yielding sheep breeds (Salari et al., 2018). There has also been a decrease in the "typicality" and quality of some cheeses (Morand-Fehr et al., 2007).

Pasture feeding and green forage are known to enhance the content of the health beneficial components in milk fat (Mele, 2009; Nudda et al., 2005). The pasture also contains volatile components (flavour, terpenes) that contribute to the flavour of the cheese.

The development of flavour in dairy products is related to several components, some of which are the volatile compounds derived from the primary products of lipolysis, glycolysis and proteolysis by exogenous and endogenous enzymes. The volatile components in dairy products are also affected by milk quality, processing operations (coagulation of the milk, cheese ripening), and microbial activity (Fox, Guinee, Cogan, & McSweeney, 2017). In addition, environmental factors, soil characteristics, botanical composition (terpens synthesized by plants and other chemical compounds that derive from the lipolysis and proteolysis of vegetables) and geographical location, as well as the phenological stage of each grass species contribute to the high diversity in volatile components in the pasture (Mariaca et al. 1997).

The nutritional quality of milk and cheese are also affected by seasonal fluctuations of the pasture, the level and quality of herbage intake by grazing animals, the quality of the grass species and the vegetative phase (Chilliard et al., 2007, Molle et al., 2003).

The aim of this study is to investigate the effects of the pasture season on the nutritional characteristics and volatile components of sheep's milk, yoghurt and cheese produced in central Italy.

2. Materials and methods

2.1 Samplings

The farm involved in the study rears Massese breed ewes and is located in central Italy (GPS N 43.63.80 – E 10.37.91), 1 m above sea level.

Pasture samples were taken in winter (January -February) and spring (March April) 2015. The average daily temperature and air humidity were 8 °C and 79% in winter, and 15°C and 77.8% in spring.

Grass was cut from 0.5 m2 plots in six random areas and the botanical composition was determined by hand separation, and expressed as g of single species on 100 gr of total sample.

Three bulk milk samples were also taken from sheep grazing in the two seasons considered. In addition, during the two periods, yoghurt, fresh cheese (24 h after cheese making) and 30-day ripened cheese were taken from the same milk. For each cheese, three loaves were sampled at 24h and 30 days and two sub samplings were carried out on each loaf. Regarding the yoghurt, three aliquots were taken for each batch and were analysed in duplicate.

2.2 Manufacturing processes

Raw milk was heated to 35 °C, into which a liquid bovine rennet and Streptococcus thermophilus starters (Biotec Fermenti-Lodi, Italy) were injected. The curd was then broken, purged and salted by hand. Cheeses were ripened at the dairy for 30 days at 10 ° C and 85% relative humidity.

Regarding the yoghurt, whole pasteurized milk was heated at a temperature of 40 ° C, lactic ferments were then inoculated by adding a yoghurt starter (0.5 kg of inoculum for every 8 L of milk) containing *Streptococcus thermophilus* and *Lactobacillus delbrueckii subsp. Bulgaricus* (Biotec Fermenti-Lodi, Italy). The incubation of the yoghurt lasted 10-15 h at 40 ° C.

2.3 Chemical and fatty acid composition of sheep milk yoghurt and cheese

All the yoghurt, fresh and ripened cheese samples were analysed in relation to dry matter and proteins following the AOAC method (2004). The Ca, P and Mg content were determined by atomic absorption spectroscopy and ultraviolet–visible spectroscopy according to Horwitz (2000) and Murthy and Rhea (1967). The fat was gravimetrically determined after extraction following Rose-Gottlieb's method. After extraction, methyl esters of fatty acids were prepared using methanolic sodium methoxide according to Christie (1982) for gas chromatography. A Perkin Elmer Clarus 480 (Perkin Elmer, Norwolk, CT, USA) equipped with a flame ionization detector and a capillary column were used (60 m× 0.25 mm; film thickness 0.25 mm (ThermoScientific TR-FAME 60 m x 0.25 mm ID; film thickness 0.25 µm, Fisher Scientific UK).

The helium carrier gas flow rate was 1mL·min–1. The oven temperature program was as follows: level 1, 50°C held for 5 min, level 2, 50 to 140°C at 3°C·min–1 then held for 2 min, level 3, 140 to 240°C at 1°C·min–1 then held for 10 min. The injector and detector temperatures were set at 270 and 300°C, respectively. The peak areas of individual fatty acids (FA) were identified using an FA standard injection (Food Industry FAME Mix – Restek Corporation • 110 Benner Circle • Bellefonte, PA16823) and quantified as a percentage of the total FA. In addition, nonadecanoic acid methyl ester (C19:0 Restek Corporation • 110 Benner Circle• Bellefonte, PA 16823) was also used as an internal standard.

Milk fatty acid classes of saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), short chain (SCFA), medium chain (MCFA) and long chain (LCFA) fatty acids were calculated as follows: SFA=Σ C4:0, C6:0, C8:0, C10:0, C11:0, C12:0,C13:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0,C21:0, C22:0, C23:0, C24:0; MUFA=Σ C14:1,C15:1, C16:1, C17:1, trans-9 C18:1, trans-11 C18:1, cis-9 C18:1, C20:1, C22:1, C24:1; PUFA=Σ trans-9,trans-12 C18:2, cis-9,cis-12 C18:2, cis-

6,cis-9,cis-12 C18:3, cis-9, cis-12,cis-15 C18:3 n3, C20:2, cis-11,cis-14,cis-17 C20:3, C20:4, cis-8, cis-11, cis-14-C20:3, C20:5, C22:2, C22:5, C22:6. SCFA=Σ of the fatty acids from 4 to 10 C; MCFA=Σ of the fatty acids from 11 to 17 C; LCFA=Σ of the fatty acids from 18 to 24 C.

2.4 Volatile Fraction Composition

Each aliquot of fodder, yoghurt, fresh cheese and the corresponding ripened cheese were analysed in relation to the aromatic profile by means of solid phase micro extraction with gas chromatography-mass spectrometry (SPME-GC / MS).

A divinylbenzene/carboxen/polydimethylsiloxane, 50/30 µm, 2-cm-long fibre was used to collect volatile fractions by SPME. The fiber was conditioned at 270°C for 30 min in a GC split/splitless injector before analysis. Fifteen grams of yoghurt, 4 g of cheese, or 4 g of pasture, roughly cut into small pieces shortly before the analysis, were weighed in a 20 mL crimp-top vial, and sealed with an aluminium cap provided with a pierceable septum (23 × 75 mm, Varian, Palo Alto, CA). Dairy samples were allowed to equilibrate to 50°C in a thermostatic bath for 5 min, and the fiber was exposed to the headspace for 30 min. Volatile compounds were extracted from the pasture, maintaining the sample at room temperature, and exposing the fiber to the head-space for 15 min.

The gas chromatographic analysis of volatile compounds adsorbed on the SPME fiber was carried out with a CP-WAX 52CB capillary column (Varian; 60 m long, 0.32 mm i.d., 0.5 µm film thickness).

A Hewlett Packard (Palo Alto, CA) 5890 series II gas chromatograph coupled with a Hewlett Packard 5989A mass spectrometer was used. To achieve sharper peaks, the split/splitless injector was equipped with a 0.75 mm i.d. inlet liner (Supelco, Bellefonte, PA).

During the injection phase, a 3-min splitless mode was applied and the injector temperature was held at 250°C. Oven temperature was held at 40°C for 8 min, programmed to 220°C at a rate of 4°C/min, and held at 220°C for 20 min. Helium was used as a carrier gas at a flow rate of 1.0

mL/min. The MS temperatures adopted were as follows: interface 220°C, source 200°C, quadrupole 100°C. Acquisition was performed in electron impact (EI) mode (70 eV) by 1.6 scans per second and the mass range used was m/z 35 to 270.

The identification of volatile compounds was performed with the following criteria: comparison with the mass spectra of the Wiley library (McLafferty and Stauffer, 1989), injection of authentic standards analyzed under the same GC-MS conditions, calculation of retention indices (RI) followed by comparison with those obtained from both authentic standards and the literature (Jennings and Shibamoto, 1980; Acree and Arn, 2004).

2.5 Statistic analysis

Data processing was performed using JMP V.11.0 software, according to the following linear models:

• For cheese:

$yijk = \mu + Ri + Sj + Ri \times Sj + \varepsilon ijk$

where:

$yijk$= dependent variable

μ: mean

Ri: fixed effect of the ith ripening factor (fresh, ripened).

Sj: fixed effect of the jth season (winter, spring).

εijk: random error.

• For forage, milk and yoghurt:

$yij = \mu + Sj + \varepsilon ij$

where

yij= dependent variable

µ: mean

Sj: fixed effect of jth season (winter, spring).

εij: random error.

3. Results

3.1 Chemical and fatty acid composition of sheep milk, yoghurt and cheese

The effects of the season on the nutritional composition of milk and yoghurt are summarized in Table 1. Spring milk was poorer (P <0.01) in protein (Table 1), while both spring milk and yoghurt were richer (P <0.001) in calcium.

As for the fatty acid profile, the spring milk (Table 1) also contained higher quantities of C8:0 (P <0.05), C10:0 (P <0.05), and almost double the content of cis-9,cis-12,cis-15 C18:3 (□-linolenic acid) (P <0.001) as well as a higher (P <0.001) overall content of omega-3 PUFA, compared to the winter milk. Both the spring milk and yoghurt showed higher C18:0 (P <0.01) and trans-11 C18:1 (P <0.001).

On the other hand, both the winter milk and yoghurt showed a significantly higher content of several saturated fatty acids (C4:0, C11:0, C15:0, and C24:0), mono-unsaturated acids (C14:1, C15:1, C16:1, C17:1 and cis-9 C18:1), and of trans -9,trans-12, C18:2, cis-9,cis-12, C18:2, cis-6, cis-9, cis-12 C18:3, C22:6 (P <0.01) and of PUFA (P <0.05; P <0.001 respectively) omega-6 (P <0.01; P <0.001).

In addition, in the milk, MCFA (P <0.01) such as C13:0 (P <0.01), C16:0 (P <0.05), C17:0 (P <0.001), and also cis-9 C18:1 (P <0.05), cis-9, trans-11 C18:2 (rumenic acid) (P <0.01), C20:0 (P <0.01), C22:2 (P <0.01), C22:5 (P <0.01) were more present in the winter.

In terms of the nutritional quality of the cheese (Table 2), compared to the winter products the products derived from the spring pasture showed significantly higher quantities of dry matter (P <0.001), protein (P <0.001), calcium (P <0.05) and phosphorus (P <0.001) .

In terms of the fatty acid composition, the spring cheese contained less (P<0.05) total SFA (except for C6:0, C8:0, C10:0 and C18:0), more (P<0.05) long chain MUFA (such as trans-11 C18:1 and C20:1). In particular, the spring cheese contained more than twice the amount of trans-11 C18:1 (P<0.01) compared to the winter cheeses. Similarly, LCFAs were more present in spring cheese (P <0.01), and of these there were higher trans-9,trans-11 C18:2 (P<0.05), C20:3, cis-9,cis-12,cis-15 C18:3 and also more total omega-3 PUFAs (P<0.01) and a lower omega-6/omega-3 ratio (P<0.01).

MCFA (P<0.05) such as C11:0, C14:0 (P<0.01), C15:0 (P<0.05), C15:1(P<0.001), C16:0, C17:0 (P<0.05), C17:1(P<0.001) were higher in the winter. In addition cis-9 C18:1 (P<0.05), cis-9,cis-12 C18:2 (linoleic acid) (P<0.01), its isomer cis-9,trans-11 C18:2 (P <0.01), cis-6,cis-9,cis-12 C18:3 (P<0.05), C22:0, C24:0, and C22:6 (P<0.05) were also more present in the winter.

As expected, the ripening significantly affected the dry matter (P<0.05), proteins (P<0.001), and fat (P<0.05) content.

The ripening led to an increase (P<0.05) in the SCFA and in particular C6:0 (P<0.01) and C8:0 (P<0.05) and decreases (P<0.001) in MUFAs including C15:1 (P<0.01), C17:1(P<0.001), cis-9 C18:1 (P<0.01) and C24:1 (P<0.01). Decreases in C11:0 (P<0.05), cis-9,trans-11 C18:2 (P<0.05), cis-6,cis-9,cis-12 C18:3 (P<0.05), C20:3 (P<0.001), and C20:4 (P<0.05) were also found.

Finally, the interaction between the season and ripening was significant for SFA (P<0.01) and MUFA (P<0.05) and for C11:0 (P<0.05), C20:1 (P<0.05) fatty acids.

3.2 Volatile compounds in the pasture, sheep milk, yoghurt and cheese

A volatile composition rich in alcohols and aldehydes was found in the pasture samples (Table 3), while the main compounds in yoghurt (Table 4) and cheese (Table 5) were ketones and acids, respectively. Regarding the aldehydes, cis 2-hexenal was found the most in the fodder, and acetaldehydes were the main ones found in the yoghurt. In cheeses the only aldehyde detectable was nonanal. In our study the total content and the profile of the aldehydes showed no significant differences in the season in the substrates analysed (Tables 3, 4, 5). The only exception was the n-hexanal present in significantly (P<0.01) higher quantities in the spring pasture (Table 4).

Significant differences in the ketone contents in the seasons were observed in all the substrates analyzed.

In particular, 2-butanone (P<0.05), 2-propanone (P<0.05) and 1-octen-3-one (P<0.05) were significantly greater in the spring pasture (Table 3).

The ketones were generally highly represented in the yoghurt, and the quantities of 3-hydroxy, 2-butanone were high in both seasons. In the yoghurt (Table 4) a greater (P<0.01) content of 2, 3-pentanedione was detected in the spring. In terms of the cheese (Table 5), the spring season produced higher quantities of 2-butanone (P<0.001). Ripening also influenced the variations in several individual ketones with higher quantities of 2-butanone (P<0.001) and 2-pentanone (P<0.05) in the ripened cheese, while 3 hydroxy, 2-butanone was higher (P<0.05) in the fresh cheese.

The interactions between ripening and season were significant for 2-butanone (P<0.001), 2-nonanone (P<0.05).

In the cheese, the total content of esters (Table 5) was linked to the season with significantly (P<0.05) higher values in the spring than in winter. In particular, ethyl acetate was predominant (P<0.001) in fresh cheese and in the spring. The interaction between ripening and season was also significant (P<0.001) for this compound.

Regarding alcohols, the winter pasture (Table 3) contained significantly higher quantities of trans-2-penten-1-ol ($P<0.01$), 1-hexanol ($P<0.05$), cis-3-hexen-1-ol ($P<0.05$), and 2-heptanol ($P<0.01$), while cis-2-penten-1-ol ($P<0.01$) and 1-octen-3-ol ($P<0.01$) were more present in the spring period.

Seasonal differences in the alcohol content were not detectable in the yoghurt (Table 4).

In the cheese (Table 5), the main alcohol was ethanol and the content of total alcohol was higher ($P<0.01$) in the spring when higher quantities of 2-butanol ($P<0.001$), 3-methyl-1-butanol ($P<0.01$), 3-methyl-1-pentanol ($P<0.01$) and phenethyl alcohol ($P<0.05$) were detected. Ethanol ($P<0.001$), 3-methyl-1-butanol ($P<0.001$), 3-methyl-1-pentanol ($P<0.01$) decreased significantly with ripening. On the other hand, 2-butanol ($P<0.001$), 2-pentanol ($P<0.01$), and 2-heptanol ($P<0.01$) significantly increased in the ripened compared to the fresh cheese.

The most represented volatile acid in yoghurt (Table 4) was acetic acid with variations in seasons, while hexanoic and octanoic were more present in the cheeses (Table 6). In the cheese a greater ($P<0.05$) content of 3-methylbutanoic acid was detected in the winter. Ripening positively ($P<0.01$) affected the acid content and in particular butyric acid ($P<0.001$), 3-2-methyl-butanoic ($P<0.05$), propanoic acid ($P<0.05$), 2-methyl-propanoic acid ($P<0.01$) and hexanoic acids ($P<0.001$) which were higher in ripened compared to the fresh cheese.

The terpene analysis in the fodder highlighted 16 different compounds (Table 3) with significantly higher quantities of 2-carene ($P<0.01$) and limonene ($P<0.01$) in the spring, while γ-terpinene ($P<0.001$) and caryophyllene ($P<0.05$) were more present in the winter pasture.

Five of the terpene compounds in the pasture (α-pinene, β-myrcene, α-terpinene, limonene, α -cubebene) were also observed in the yoghurt (Table 4), while in the cheese (Table 5) alpha terpinene and limonene were detected.

Neither the terpene profile of the yoghurt nor that of the cheese showed significant variations in the season or with the ripening.

Regarding hydrocarbons, p-xylene was greater ($P<0.05$) in the winter pasture, while no significant differences in hydrocarbons were observed in the dairy products.

4. Discussion

4.1 Chemical and fatty acid composition of sheep milk, yoghurt and cheese

The higher content of t-11 C18:1 in all the spring dairy products analysed is interesting from the point of view of human nutrition. In fact, this fatty acid is converted into rumenic acid in humans, and the latter has been shown to be an anticarcinogenic and antiatherogenic compound and to influence body composition and fat metabolism in animals (Turpeinen et al., 2002). The content of t-11 C18:1 in the cheese was not affected by ripening. We also found about twice the content of linolenic acid in the milk and cheese in the spring. The higher linolenic acid was presumably related to the higher content of this fatty acid in the spring fodder which was richer in Leguminosae (Table 6).

In support of this hypothesis, Meľuchová et al., (2008) highlighted that linolenic acid in milk is linked to its content in pasture. Prado et al., (2019) also found a higher duodenal C18:3 flow related to Leguminosae intake in ruminants. The higher trans-11 C18:1 in spring dairy products may be due to the higher intestinal absorption of t-11 C18:1 which has also been reported as being positively dependent on C18:3 intakes (Prado et al., 2019).

The higher oleic and α-linoleic acid in winter in all the products analysed may be due to the greater presence of this fatty acid in the winter pasture, since the origin of these fatty acids is almost exclusively dietetic (Meľuchová et al., 2008).

The higher quantities of cis-9,trans 11 C18:2 in all dairy products in winter are presumably related to the seasonal variations in linoleic acid in pasture lipids as also reported by Meľuchová et al. (2008). In fact conjugated linoleic acids (CLA) originate from the precursor cis-9,cis-12 C18:2, by the action of the enzymeΔ9 desaturase (stearoyl-coA desaturase). The higher content of cis-9,trans 11 C18:2 in winter differs from findings observed by Cabiddu et al. (2005) in grazing sheep.

The higher quantities of dry matter, proteins, and calcium found in the cheese derived from spring pasture could be related to the higher calcium content detected in the spring milk (Table 1). In fact, a higher level of total calcium in milk increases cross-linking between casein micelles (Choi et al., 2008) which retain less water, as confirmed by the greater drop in the average weight detected in the spring (winter 12% vs 14% in spring) (data not shown).

The nutritional profile of spring cheese is interesting due to the higher content of some fatty acids that are considered beneficial for human health (cis-9,cis-12,cis-15 C18:3 and total omega-3) and the low content of SFA. In fact, a reduction in saturated fats and a lower omega-6/omega-3 ratio in the human diet is advisable in order to prevent obesity (Simopoulos, 2016) and for a healthy diet.

Regarding the ripened cheese, the chemical composition was in line with the values of pecorino cheese ripened for 30 days, while the changes found in SCFA and MUFA, in cis-9 C18:1 and cis-9,trans-11 C18:2 are in agreement with Buccioni et al., (2012) in pecorino cheese, who ascribed these trends to biohydrogenation or of double bond isomerisation.

4.2 Volatile compounds in the pasture sheep milk, yoghurt and cheese

In line with the literature, acetaldehyde was one the main volatile component aldehydes found in the yoghurt. Acetaldehyde participates in the formation of the flavour and is an indispensable aroma (green apple or nutty flavour) compound in yoghurt (Cheng, 2010). Regarding C6 compounds such as n-hexanal, cis-3-hexenal, trans-2-hexenal, found in dairy products, they and the corresponding alcohol and ester derivatives are collectively named "green leaf volatiles" because they have a characteristic green leaf odour (Kunishima et al., 2016).

2-nonenal, which was detected in the yoghurt and cheese, has been reported to be characteristic of cheese and milk derived from pasture (Carpino et al., 2004). However it is extremely difficult to ascertain the source of many aldehydes (and ketones). In fact, some aldehydes likely originate directly from the forage, some are formed during transformation processes (e.g. acetaldehyde of yoghurt) (Kalaˇc 2011) derived from the oxidation of linoleic and linolenic acid present in plants or in milk fat (Kilcaweley et al., 2018).

With regard to cheese, although aldehydes are "odor active" compounds that have a sensory influence, they are transitory compounds which are easily metabolized to alcohol.

Ketones derive from lipid oxidation generated by linoleic and arachidonic acid and contribute to mushroom odor (Li and Wang, 2016). Evidence exists for the direct transfer of some ketones from the diet into milk, but there appear to be no obvious trends. 3-hydroxy-2-butanone, which was the main keton found in yoghurt and fresh spring cheeses in our study, has been reported as mainly deriving from the metabolism of carbohydrate present in grass (Kilcawley et al., 2018).

Higher total ester and ethyl acetate contents were found in the fresh and spring cheeses. Esters contribute to the aroma of dairy products by giving them floral and fruity aromatic notes, also lightening the pungent and bitter notes of fatty acids and amines, respectively (Pinho et al., 2003). The ester content in dairy products is dependent on the content of primary alcohols and carboxylic acids (Kilcawley et al., 2018).

The branched chain alcohols we detected in the yoghurt and cheese are formed by the reduction of branched chain aldehydes, which likely play an important role in the aroma, giving alcoholic and/or floral notes to dairy products (Pinho et al., 2003; Fernandez-Garcia et al. 2004). The ethanol found in fresh cheese probably derives from the reduction of acetaldehyde (Fernandez-Garcia et al. 2004).

The decrease in alcohol found in the cheeses is due to the ripening, when alcohol binds fatty acids to form esters, as also reported by Conte et al. (2015). Seasonal differences in the content of some alcohols have been reported in cheese especially for primary and secondary alcohols (Fernandez-Garcia et al. 2004).

Acids are some of the main volatile fractions of pecorino cheese and are an important component of the flavour. Linear acids are generally produced from the lipolysis of milk fat and are the main contributors to the flavour of aged Italian cheese (Ziino et al., 2005).

In our study most of the volatile acids found in the ripened cheese were fermentative. In addition the increase in acids found with ripening is in agreement with Conte et al. (2015) who reported a lipolytic activity during the maturation period.

The butanoic acid found in our samples of ripened cheese, is a compound with a cheesy sharp aroma and plays an important role in the flavour of many cheese types (Curioni and Bosset, 2002). Hexanoic acid is also a characteristic flavour component of Grana Padano, Roncal cheese, and goat cheeses and is perceived to have a very mild or strong sharp, goat-like smell (Randazzo et al., 2008; Conte et al., 2015).

Organosulfurs are compounds with a high odour and intense aroma (Falchero et al., 2010) linked to the high protein/non-fibrous carbohydrate ratio typical of grass diets which enhance protein deamination by rumen microbes (Coppa et al., 2011). The dimethyl sulfide found in winter cheeses probably arises from the amino acid methionine found in the grass.

The composition of the terpenes in the products seems to be linked to the type of pasture (Table 6), consisting above all of the genus Lolium in both seasons (49.14 g and 44.14 in 100 g of fresh grass in winter and spring respectively). In fact, similarly to findings reported by Fedele et al. (2004) in the milk of goats fed on lolium pasture, the dominant terpenes were limonene and α-Pinene.

There were fewer terpene compounds detected in the cheese, linked to a balsamic and fruity aroma than those found in the pasture. Cheese- making may thus have induced the most important changes in the terpene profiles, as reported by Cornu et al., (2005).

Variations in the terpene content in both cow's and sheep's milk and cheeses (Revello Chion et al., 2010; Valdivielso et al., 2016) have been reported in relation to the type of pasture (mountainous, hilly) and to the animals' feed. However, it is still unclear as to whether these compounds would be useful as biomarkers for the traceability of ruminant diets under real conditions.

The origin of hydrocarbons in milk and cheese is still not well-understood. Buchin et al. (1998) found that the hydrocarbon content was higher in cheese produced from pasture-fed cows than milk cows fed a hay-based diet.

5. Conclusions

The pasture season influenced the chemical composition of sheep's milk and dairy products. In fact, a higher dry matter, protein, calcium and phosphorus content and higher beneficial fatty acids, such as omega-3 and vaccenic acid, characterized the cheese derived from the spring pasture. Spring cheese also has more components responsible for alcoholic and floral notes (esters, alcohols) and less components linked to a cheesy sharp aroma (acids). The terpene compounds detected in the cheese and yoghurt were linked to balsamic and fruity aromas. The effect of the seasonal differences on the consumer' preference should be further studied.

Conflicts of interest

Authors declare that there are no conflicts of interest.

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Table 1. Nutritional composition and fatty acid profile of milk and yoghurt (g / 100 g of total fatty acids).

		Milk				Yoghurt			
		Winter	Spring	SE[1]	P value	Winter	Spring	SE[1]	P value
ss	%	21.46	17.03	1.06	0.0736	19.67	18.18	0.11	0.0015
protein		6.17	5.48	0.07	0.0030	5.72	5.68	0.60	0.0842
Fat		6.32	5.56	0.39	0.0571	7.95	6.56	0.89	0.7122
Ca		0.19	0.40	0.04	<0.0001	0.18	0.23	0.01	0.0049
Mg		0.02	0.03	0.00	0.1161	0.02	0.02	0.00	0.1161
P		0.20	0.38	0.00	<0.0001	0.20	0.17	0.00	0.0037
C4:0	g / 100 g	2.24	1.54	0.10	0.0088	2.12	1.48	0.06	0.0008
C6:0	fatty acids	1.67	1.72	0.07	0.6371	1.47	1.59	0.01	0.5133
C8:0		1.77	2.17	0.07	0.0137	1.64	2.00	0.06	0.0782
C10:0		6.14	6.99	0.15	0.0187	6.21	6.68	0.20	0.1949
C11:0		0.35	0.05	0.02	0.0010	0.44	0.00	0.07	0.0340
C12:0		3.86	4.19	0.10	0.0992	3.91	4.08	0.10	0.4608
C13:0		0.12	0.08	0.01	0.0083	0.12	0.08	0.01	0.1726
C14:0		10.71	10.72	0.25	0.9702	10.76	11.19	0.11	0.1287
C14:1		0.58	0.11	0.02	<0.0001	0.51	0.12	0.01	0.0002
C15:0		1.70	1.02	0.02	<0.0001	1.34	1.12	0.03	0.0175
C15:1		0.41	0.01	0.02	<0.0001	0.39	0.01	0.01	0.0002
C16:0		27.15	23.92	0.53	0.0177	25.17	25.44	0.09	0.2147
C16:1		0.95	0.37	0.04	0.0011	0.79	0.27	0.02	<0.0001
C17:0		0.97	0.61	0.02	0.0003	0.70	0.65	0.01	0.0648
C17:1		0.46	0.20	0.01	<0.0001	0.39	0.21	0.02	0.0102
C18:0		10.90	14.38	0.39	0.0036	12.98	14.98	0.07	0.0001
C18:1trans-9		0.25	0.27	0.02	0.6451	0.18	0.28	0.04	0.1408
C18:1 trans-11		0.86	3.60	0.06	<0.0001	2.14	3.75	0.07	0.0004
C18:1 cis-9		21.87	18.81	0.53	0.0169	21.34	20.65	0.29	0.4124
C18:1 cis-11		0.38	0.30	0.02	0.0728	0.36	0.31	0.04	0.6914
C18:2 trans-9,trans-12		0.68	0.11	0.05	0.0022	1.23	0.10	0.05	0.0001
C18:2 cis-9,cis-12		1.88	1.40	0.04	0.0017	1.84	1.45	0.02	0.0003
C18:3 cis-6,cis-9,cis-12		0.12	0.01	0.00	0.0022	0.05	0.01	0.01	0.0083
C20:0		0.14	0.28	0.04	0.1063	0.17	0.28	0.02	0.0357
C20:1		0.02	0.02	0.00	0.9721	0.07	0.02	0.01	0.0358
C18:3 cis-9,cis-12,cis-15		0.66	1.31	0.03	0.0002	1.24	1.49	0.05	0.0680
C18:2cis-9,trans11		1.16	0.92	0.02	0.0043	0.95	0.99	0.01	0.1286
C21:0		0.40	0.04	0.13	0.1521	0.23	0.02	0.02	0.0087
C20:2		0.04	0.01	0.01	0.0936	0.03	0.00	0.01	0.0951
C20:3 cis-8,cis-11, cis-14-		0.05	0.01	0.01	0.1104	0.03	0.02	0.01	0.3107
C20:4		0.11	0.12	0.00	0.4365	0.09	0.11	0.01	0.2019
C20:3 cis-11,cis-14,cis-17		0.03	0.04	0.01	0.6740	0.03	0.00	0.01	0.0953
C22:0		0.27	0.15	0.02	0.0054	0.22	0.15	0.01	0.0645
C22:1		0.04	0.00	0.00	0.0104	0.04	0.00	0.01	0.0067
C20:5		0.06	0.07	0.01	0.4761	0.07	0.08	0.01	0.7560
C23:0		0.08	0.06	0.00	0.0574	0.11	0.06	0.01	0.1548
C22:2		0.21	0.10	0.01	0.0017	0.13	0.12	0.02	0.7801
C24:0		0.13	0.01	0.00	0.0019	0.11	0.00	0.01	0.0268
C24:1		0.04	0.08	0.01	0.0520	0.05	0.06	0.01	0.9671
C22:5		0.19	0.13	0.01	0.0063	0.14	0.14	0.01	0.7123
C22:6		0.11	0.05	0.01	0.0083	0.11	0.05	0.01	0.0348
SCFA[2]		11.83	12.47	0.38	0.3059	11.45	11.75	0.24	0.6250
MCFA[2]		47.25	41.26	0.88	0.0094	44.52	43.16	0.25	0.1086
LCFA[2]		40.69	42.32	1.03	0.3351	43.97	45.08	0.38	0.2594
SFA[2]		68.48	67.96	1.20	0.7307	67.61	69.81	0.31	0.0596
MUFA[2]		25.97	23.83	0.53	0.0690	26.37	25.64	0.31	0.4968
PUFA[2]		5.32	4.31	0.16	0.0201	5.96	4.54	0.24	0.0002
PUFA omega-3		1.05	1.61	0.03	0.0006	1.60	1.75	0.18	0.1976
PUFA omega-6		2.84	1.66	0.11	0.0016	3.20	1.67	0.09	0.0002
Omega-6/omega-3		2.07	1.03	0.04	<0.0001	2.04	0.96	0.08	0.0029

[1] Standard Error;

[2] SCFA= Short Chain Fatty Acids (C<12), MCFA= Medium Chain Fatty Acids (12 ≤ C < 18), LCFA= Long Chain Fatty Acids (C≥18), SFA= Saturated Fatty Acids, MUFA= Monounsaturated Fatty Acids, PUFA= Polyunsaturated Fatty Acids.

Table 2. Nutritional composition and fatty acid profile of cheese (g / 100 g of total fatty acids).

		Season effect				Ripening effect				Cross
		Winter	Spring	SE[1]	P value	Fresh	Ripened	SE[1]	P value	P value
Dry matter	%	52.85	61.26	1.15	<0.0001	56.42	58.13	1.51	0.0418	0.9903
protein		19.03	21.07	0.22	0.0002	18.79	21.31	0.22	<0.0001	0.2267
fat		23.57	25.66	1.24	0.2661	22.37	26.85	1.24	0.0338	0.9772
Ca		0.41	0.64	0.05	0.0116	0.53	0.52	0.05	0.8105	0.6350
Mg		0.02	0.03	0.00	0.2215	0.03	0.03	0.003	0.6825	1.0000
P		0.55	0.66	0.01	<0.0001	0.63	0.59	0.01	0.0075	0.2810
C4:0	g/100g of fatty acids	2.44	2.77	0.10	0.0525	2.45	2.76	0.10	0.0700	0.9731
C6:0		1.84	2.18	0.07	0.0090	1.84	2.18	0.07	0.0101	0.6676
C8:0		1.90	2.30	0.08	0.0135	1.94	2.26	0.08	0.0374	0.6611
C10:0		6.28	7.08	0.24	0.0455	6.58	7.43	0.24	0.0887	0.8680
C11:0		0.26	0.17	0.02	0.0088	0.24	0.18	0.02	0.0351	0.0499
C12:0		3.86	3.96	0.07	0.3530	3.82	3.99	0.07	0.1136	0.7225
C13:0		0.11	0.10	0.01	0.5253	0.11	0.10	0.01	0.5103	0.6149
C14:0		10.46	9.96	0.10	0.0080	10.30	10.12	0.10	0.2599	0.3548
C14:1		0.58	0.50	0.02	0.0674	0.57	0.51	0.03	0.1209	0.6583
C15:0		1.60	1.22	0.09	0.0161	1.51	1.31	0.09	0.1618	0.9409
C15:1		0.37	0.31	0.01	<0.0001	0.37	0.32	0.01	0.0008	0.0945
C16:0		26.60	23.32	0.77	0.0163	25.75	24.17	0.77	0.1833	0.6504
C16:1 n7		0.80	0.60	0.06	0.0561	0.80	0.60	0.06	0.0526	0.1551
C17:0		0.92	0.69	0.05	0.0144	0.87	0.74	0.06	0.1333	0.9410
C17:1		0.42	0.28	0.02	0.0008	0.39	0.31	0.02	0.0318	0.3726
C18:0		11.65	13.62	0.52	0.0272	12.22	13.06	0.52	0.2818	0.7400
C18:1 trans-9		0.24	0.23	0.02	0.7656	0.22	0.27	0.02	0.1019	0.1782
C18:1 trans-11		1.20	2.92	0.34	0.0071	1.68	2.44	0.34	0.1502	0.7744
C18:1 cis-9		21.67	20.87	0.22	0.0314	21.84	20.70	0.22	0.0063	0.9152
C18:1 cis-11		0.42	0.33	0.04	0.1679	0.34	0.40	0.04	0.3259	0.6155
C18:2 trans-9, trans-12		0.68	1.11	0.10	0.0161	0.86	0.93	0.10	0.6412	0.1907
C18:2cis-9, cis-12		1.81	1.33	0.08	0.0030	1.62	1.52	0.08	0.4154	0.3335
C18:3 cis-6,cis-9,cis-12		0.11	0.07	0.01	0.0184	0.11	0.07	0.01	0.0474	0.6296
C20:0		0.12	0.12	0.03	0.9722	0.08	0.17	0.03	0.0976	0.6067
C20:1		0.04	0.06	0.00	0.0375	0.05	0.05	0.00	0.4432	0.0243
C18:3 cis-9,cis-12,cis-15		0.70	1.43	0.13	0.0051	0.96	1.17	0.13	0.3012	0.8086
C18:2cis-9,trans-11		1.11	0.91	0.04	0.0062	1.09	0.94	0.04	0.0242	0.8307
C21:0		0.27	0.27	0.10	0.9753	0.23	0.32	0.10	0.5538	0.5967
C20:2		0.04	0.04	0.01	0.4921	0.04	0.04	0.01	0.8371	0.2810
C20:3 cis-8,cis-11,cis-14		0.03	0.05	0.00	0.0039	0.05	0.03	0.00	0.0004	0.8513
C20:4		0.11	0.10	0.01	0.4689	0.12	0.10	0.01	0.0404	0.4931
C20:3 cis-11, cis-14, cis-17		0.06	0.05	0.01	0.3491	0.05	0.05	0.01	0.7078	0.4943
C22:0		0.28	0.20	0.03	0.0440	0.27	0.21	0.03	0.1764	0.9450
C22:1		0.02	0.01	0.00	0.3164	0.01	0.01	0.00	0.9835	0.8859
C20:5		0.05	0.06	0.01	0.7231	0.06	0.05	0.01	0.3751	0.3859
C23:0		0.07	0.07	0.00	0.2246	0.07	0.06	0.00	0.2482	0.5855
C22:2		0.19	0.11	0.03	0.0926	0.18	0.12	0.03	0.1916	0.8895
C24:0		0.16	0.12	0.01	0.0489	0.15	0.12	0.01	0.1077	0.6536
C24:1		0.04	0.04	0.01	0.3728	0.06	0.02	0.01	0.0085	0.7573
C22:5		0.16	0.14	0.01	0.0998	0.16	0.13	0.01	0.1312	0.6031
C22:6		0.11	0.08	0.01	0.0323	0.10	0.09	0.01	0.2398	0.2839
SCFA[2]		12.47	14.33	0.48	0.0246	12.60	14.20	0.48	0.0450	0.8140
MCFA[2]		46.01	41.05	1.06	0.0180	45.06	39.57	1.06	0.1445	0.7345
LCFA[2]		41.41	44.35	0.62	0.0010	42.66	43.11	0.62	0.6216	0.8056
SFA[2]		68.78	68.01	0.17	0.0154	68.21	68.59	0.17	0.0430	0.0286
MUFA[2]		25.91	26.21	0.08	0.0167	26.37	25.75	0.08	0.0002	0.0072
PUFA[2]		5.20	5.49	0.11	0.0729	5.23	5.25	0.11	0.5531	0.4429
PUFA omega-3		1.09	1.76	0.13	0.0063	1.50	1.34	0.13	0.4119	0.8239
PUFA omega-6		2.64	2.60	0.10	0.5609	2.66	2.58	0.10	0.4311	0.4865
Omega-6/omega-3		2.58	1.60	0.19	0.0074	2.26	1.91	0.19	0.2346	0.9623

[1] Standard Error;

[2] SCFA= Short Chain Fatty Acids (C<12), MCFA= Medium Chain Fatty Acids (12 ≤ C < 18), LCFA= Long Chain Fatty Acids (C≥18), SFA= Saturated Fatty Acids, MUFA= Monounsaturated Fatty Acids, PUFA= Polyunsaturated Fatty Acids.

Table 3. Aromatic profile of the pasture (Areas %)

		Winter	Spring	SE[1]	P value
Aldehydes	2-Pentenal	0.00	0.17	0.06	0.1162
	n-Hexanal	0.00	1.47	0.22	0.0097
	cis-2-Hexenal	27.99	13.45	5.89	0.1561
	trans-2-Hexenal	0.99	9.67	6.44	0.3946
	Nonanal	0.00	0.45	0.14	0.039
	Benzaldheyde	0.43	0.75	0.23	0.3788
Ketones	2-Butanone (methyl ethyl ketone)	0.00	3.27	0.75	0.0364
	2-Propanone	0.00	0.65	0.16	0.0462
	2-Heptanone	0.00	0.09	0.06	0.3739
	5-Methyl-3-heptanone	0.00	1.27	0.90	0.3739
	3-Octanone	2.65	2.33	1.82	0.9088
	1-Octen-3-one	0.49	5.81	1.02	0.0214
Alcohols	trans-2-Penten-1-ol	1.38	0.00	0.16	0.0033
	cis-2-Penten-1-ol	0.00	0.60	0.06	0.0026
	1-Hexanol	6.16	3.19	0.70	0.0407
	cis-3-Hexen-1-ol	18.54	3.52	3.05	0.0257
	trans-2-Hexen-1-ol	5.25	2.78	1.23	0.2293
	2-Heptanol	2.38	0.00	0.27	0.0036
	1-Octen-3-ol	2.03	34.77	4.74	0.0082
	Benzyl alcohol	0.50	0.00	0.13	0.0534
	Phenylethyl alcohol	1.10	0.87	0.49	0.7528
	Acetic acid	0.33	0.19	0.14	0.5135
Acids	Hexanoic acid	0.42	0.40	0.08	0.8506
	2 Hexanoic acid	0.00	0.37	0.13	0.1168
Organosulfurs	Dimethyl sulfide	0.84	0.00	0.25	0.0744
Terpenes	α-Pinene	0.00	0.17	0.06	0.1184
	β-Pinene	0.00	0.03	0.02	0.3739
	β-Myrcene	1.11	2.45	1.52	0.5678
	α-Terpinene	0.00	1.97	1.35	0.3594
	2-Carene	0.00	0.30	0.04	0.0070
	Limonene	0.00	0.66	0.09	0.0064
	β-cis-Ocimene	0.00	3.09	1.30	0.1684
	ϒ-Terpinene	1.02	0.00	0.05	0.0001
	β-caryophyllene	8.25	0.28	1.47	0.0185
	β- Cyclocitral	0.00	0.65	0.17	0.0524
	β-Farnesene	0.00	1.31	0.80	0.3081
	Cedrene	0.00	0.70	0.37	0.2514
	Germacrene-D	0.00	0.42	0.17	0.1485
	Cadina-1(10),4-diene	0.40	0.00	0.15	0.1292
	ϒ-Muurolene	0.00	0.37	0.14	0.1206
	α-Amorphene	0.00	0.13	0.05	0.1359
Alkane	2-Methylbutane	4.84	0.00	0.52	0.0027
	Heptane	1.41	0.00	0.53	0.1319
Furans	2-Ethylfuran	0.64	0.38	0.11	0.2292
Hydrocarbons	3- Ethyl-1,5 octadiene	0.81	0.37	0.16	0.1251
	Ethylbenzene	0.87	0.53	0.26	0.1338
	m- Xylene, -	2.01	0.09	1.09	0.2802
	p-Xylene	1.75	0.00	0.23	0.0199
	o-Xylene,	2.50	0.00	0.54	0.0524
Aldeids		29.40	25.96	3.72	0.5489
Ketones		3.14	13.42	1.47	0.0077
Alcohols		37.26	45.74	5.81	0.3604
Acids		0.75	0.96	0.21	0.5187
Organosulfurs		0.84	0.00	0.25	0.0744
Terpens		10.78	12.54	2.71	0.6710
Alkanes		6.25	0.00	0.44	0.0006
Furans		0.64	0.38	0.11	0.2292
Hydrocarbons		11.80	1.53	4.42	0.1010

[1] Standard Error

Table 4. Aromatic profile of yoghurt (% Areas)

		Winter	Spring	SE[1]	P value
Aldehydes	Acetaldehyde	5.16	5.44	1.58	0.9090
	n-Hexanal	1.85	2.15	0.74	0.7914
	Heptanal	1.47	3.14	1.21	0.3834
	Nonanal	4.29	0.00	2.41	0.2771
Ketones	3-Hydroxy, 2-Butanone	25.66	36.74	9.23	0.4438
	2-Hydroxy, 3-Pentanone	2.13	0.00	0.76	0.1198
	2-Pentanone	10.53	15.48	1.94	0.1464
	2,3-Pentanedione	0.00	3.68	0.41	0.0032
	5-Methyl-3-Heptanone	0.00	0.39	0.27	0.3739
	2-Heptanone	1.82	0.72	0.82	0.3945
	1-Octen-3-one	17.94	0.00	12.69	0.3739
Alcohols	3-Methyl-1-butanol	0.86	5.00	3.22	0.4141
	2,3-Butanediol	1.47	0.00	1.04	0.3739
	3-Methyl-1-propanol	0.27	0.00	0.19	0.3739
	2-;Methyl- 3-pentanol	0.47	2.16	0.50	0.0776
	1-Hexanol	4.53	0.00	1.74	0.1388
	3-Hexen-1-ol	0.58	1.03	0.55	0.5958
	2-Hexen-1-ol	1.40	0.00	0.99	0.3739
	3 Octanol	1.49	0.00	1.05	0.3739
	1 Octan 3 ol	0.00	0.69	0.38	0.2662
Acids	Acetic acid	8.34	6.23	1.88	0.7458
	Butanoic acid	1.65	1.46	0.21	0.1943
	Hexanoic acid	2.64	0.00	1.34	0.2360
	Octanoic acid	0.09	0.00	0.06	0.3739
Organosulfurs	Dimethyl disulfide	0.61	0.00	0.43	0.3739
Terpenes	α-Pinene	1.69	3.33	2.12	0.6138
	β Myrcene	0.00	1.90	1.33	0.3739
	α terpinene	0.95	0.00	0.67	0.3739
	Limonene	0.91	2.46	1.40	0.4786
	α cubebene	1.08	0.00	0.77	0.3739
Hydrocarbons	2-Hydroxy, 3-methylbenzaldehyde	0.64	0.00	0.45	0.3739
	3-Ethyl 1,5-octadiene	0.12	0.00	0.79	0.3739
	Chloroform	0.00	7.99	2.41	0.2665
Aldeids		12.78	10.73	2.84	0.6363
Ketones		58.09	57.01	4.11	0.8619
Alcohols		11.07	8.88	4.34	0.7400
Acids		12.73	7.69	5.84	0.5752
Organosulfurs		0.84	0.00	0.43	0.3740
Terpenes		4.64	7.68	4.99	0.6893
Hydrocarbons		0.76	7.99	1.24	0.3729

1 Standard Error

Table 5. Aromatic profile of cheese (% Areas)

		Season effect				Ripening effect				Cross
		Winter	Spring	ES[1]	P value	Fresh	Ripening	ES[1]	P value	P value
Aldehydes	Nonanal	7.22	0.30	5.01	0.3574	7.52	0.00	5.01	0.3198	0.3574
Ketones	2-Butanone	0.00	1.40	0.14	0.0001	0.00	1.40	0.19	0.0001	0.0001
	3-Hydroxy-2-butanone	2.57	3.58	1.39	0.6112	5.44	0.71	1.39	0.0427	0.6635
	2-Pentanone	0.23	0.32	0.16	0.6980	0.00	0.56	0.16	0.0385	0.6980
	2-Heptanone	0.87	0.91	0.38	0.9420	0.57	1.22	0.38	0.2624	0.3147
	2-Nonanone	0.47	0.35	0.24	0.7467	0.46	0.35	0.24	0.7467	0.0402
Esters	Ethyl acetate	0.00	0.78	0.09	0.0001	0.78	0.00	0.09	0.0001	0.0001
	Ehyl Butanoate	0.30	0.43	0.13	0.5052	0.44	0.29	0.13	0.4602	0.0444
	Ethyl Hexanoate	0.35	0.56	0.11	0.2165	0.45	0.46	0.11	0.9249	0.2368
	Ethyl Octanoate	0.08	0.09	0.05	0.9384	0.03	0.14	0.05	0.1330	0.4875
Alcohols	Ethanol	4.29	5.32	1.26	0.5763	9.61	0.00	1.26	0.0006	0.5763
	2-Butanol	0.00	1.82	0.25	0.0009	0.00	1.82	0.25	0.0009	0.0009
	3-Methyl, 1-butanol	1.89	4.73	0.53	0.0052	5.67	0.94	0.53	0.0002	0.0148
	3-Methyl-1-propanol	0.00	0.19	0.04	0.0052	0.19	0.00	0.04	0.0052	0.0052
	2-Pentanol	0.25	0.19	0.08	0.6180	0.00	0.44	0.08	0.0056	0.6180
	4-Amino-1-pentanol.	0.08	0.00	0.05	0.3466	0.08	0.00	0.05	0.3466	0.3466
	1-Hexanol	0.66	0.05	0.39	0.3002	0.72	0.00	0.39	0.2298	0.3002
	3-Hexen 1-ol	0.00	0.13	0.09	0.3466	0.00	0.13	0.06	0.3466	0.3466
	2-Heptanol	0.15	0.16	0.07	0.8969	0.00	0.32	0.07	0.0097	0.8969
	Phenylethyl alcohol	0.69	4.22	0.86	0.0196	3.49	1.49	0.86	0.1473	0.1240
Acids	Acetic acid	12.71	13.01	1.96	0.9171	13.33	12.40	1.96	0.7452	0.1600
	Butanoic acid	12.71	14.28	1.61	0.5094	7.39	19.60	1.61	0.0007	0.4404
	3-Methylbutanoic acid	6.36	1.11	1.23	0.0165	1.33	6.14	1.23	0.0242	0.0245
	Propanoic acid	0.23	0.29	0.12	0.6638	0.00	0.51	0.12	0.0177	0.6638
	2-Methyl-propanoic acid	1.67	0.86	0.28	0.0757	0.59	1.95	0.28	0.0092	0.0540
	Hexanoic acid	19.42	19.11	2.23	0.9242	10.42	28.10	2.23	0.0005	0.5088
	Octanoic acid	9.79	11.14	1.69	0.5784	8.92	12.02	1.69	0.2212	0.7744
	Nonanoic acid	2.48	1.55	0.68	0.3655	2.82	1.21	0.68	0.1329	0.4601
	Decanoic acid	6.85	4.74	1.50	0.3477	7.36	4.24	1.50	0.1777	0.2425
Organosulfurs	Dimethylsulfide	0.42	0.00	0.09	0.0002	0.42	0.00	0.06	0.0002	0.0002
	Dimethyldisulfide	0.20	0.00	0.10	0.1858	0.00	0.20	0.10	0.1858	0.1858
Terpenes	α terpinene	0.21	0.00	0.15	0.3466	0.21	0.00	0.15	0.3466	0.3466
	Limonene	0.00	0.23	0.16	0.3466	0.00	0.23	0.16	0.3466	0.3466
Hydrocarbons	Toluene	0.55	0.00	0.25	0.1520	0.54	0.00	0.35	0.1520	0.1520
	Ethylbenzene	1.59	0.00	0.87	0.2314	1.44	0.15	0.87	0.3227	0.3227
	o-Xilene	0.16	0.00	0.06	0.0919	0.06	0.09	0.06	0.7430	0.7430
	m-Xilene	0.18	0.00	0.06	0.0692	0.16	0.02	0.06	0.1295	0.1295
	Chloroform	2.47	7.65	2.23	0.3884	8.57	2.85	2.24	0.0665	0.0565
Aldheyds		7.22	0.30	5.01	0.3574	7.51	0.00	5.01	0.3198	0.3574
Ketones		4.15	6.58	103	0.2714	6.48	4.24	1.46	0.3085	0.3587
Esters		0.73	1.85	0.30	0.0308	1.68	0.90	0.30	0.1046	0.7118
Alcohols		8.03	16.83	1.55	0.0039	19.72	5.15	1.55	0.0002	0.2068
Acids		72.22	66.13	5.86	0.4831	52.16	86.18	5.86	0.0034	0.9002
Terpenes		0.21	0.23	0.15	0.9622	0.21	0.23	0.15	0.9622	0.1953
Organosulfurs		0.62	0.00	0.09	0.1858	0.42	0.20	0.09	0.1858	0.1858
Hydrocarbons		6.84	7.65	2.80	0.7728	12.24	3.26	2.80	0.0558	0.2146

1 Standard Error

Table 6. Pasture composition in winter and spring (g/100 g of fresh pasture)

	Winter	Spring	SE[1]	P value
Lolium	49.17	44.17	14.36	0.8105
Hordeum	0.00	1.83	1.29	0.3409
Bromus	0.00	9.00	6.36	0.3409
Trifolium repens	0.00	5.36	2.89	0.2221
Vicia	2.00	18.16	3.62	0.0102
Rumex	2.67	10.67	5.39	0.3183
Plantago	0.00	10.83	6.85	0.2894
Chicorium	12.50	0.00	5.80	0.1586
Picris	33.64	0.00	12.12	0.0776
Total Gramineae	49.16	55.00	12.25	0.7427
Total Leguminosae	2.00	23.50	4.81	0.0102
Others	48.88	21.50	12.05	0.0631

[1]:Standard Error