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Master Degree in Valorization and Sustainable Development of Mountain Areas

Valorization of Milk Chain as Tool to Support Competitiveness of Mountain Farms

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1 Introduction

1.1 Importance of Mountain Pastures

1.1.1 Transhumant Pastoralism in EU

Transhumant pastoralism is a form of livestock mobility characterized by seasonal migrations between different grazing areas, it has historically shaped the landscapes and rural economies of Europe. Transhumance is distinct from nomadism due to its cyclical movements between fixed points at different altitudes (Liechti and Biper, 2016). This practice remains vital in many European regions, particularly in mountainous areas, where it supports biodiversity conservation, carbon sequestration, and the production of high-quality dairy products (García-Martínez et al., 2009).

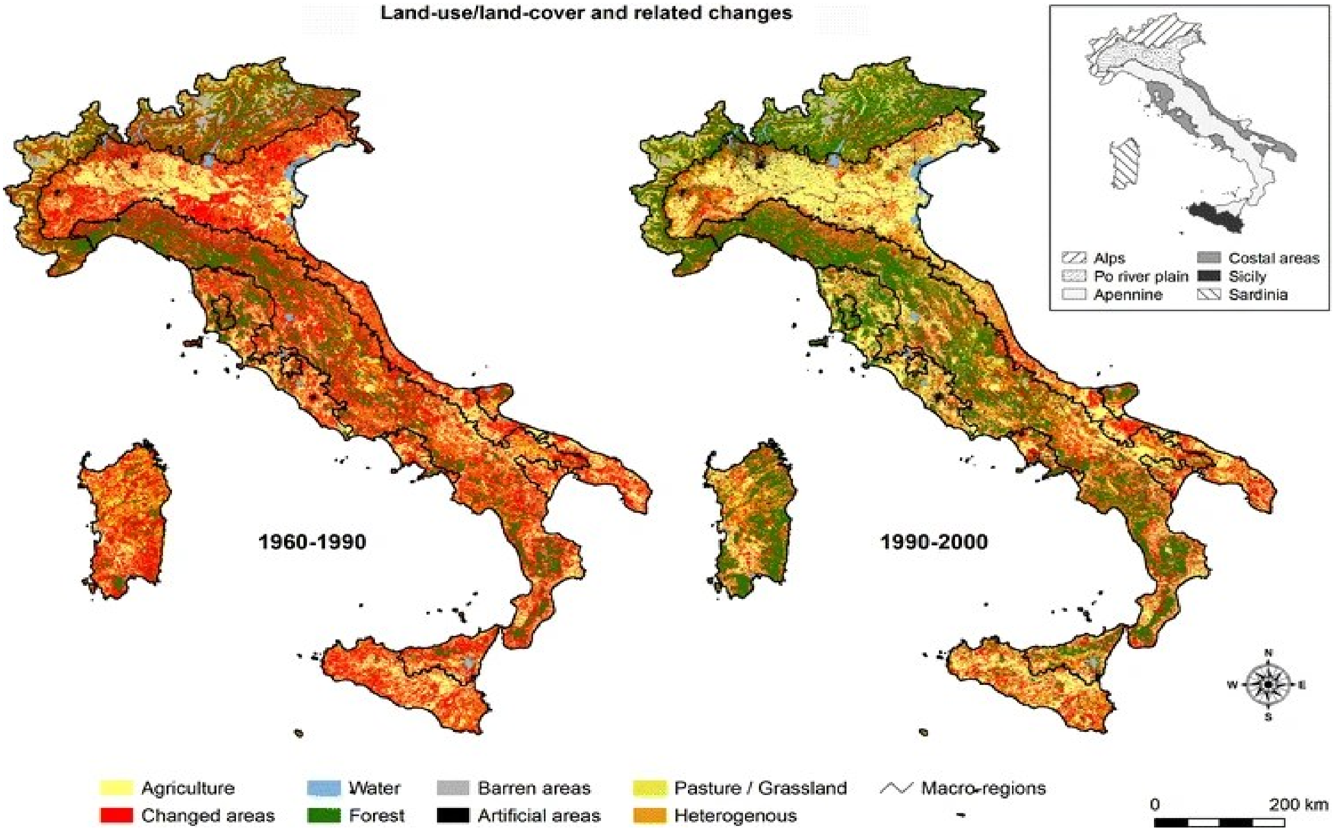
Across the EU, it is estimated that more than 30 million hectares are under grazing management, with notable transhumant systems found in the Iberian Peninsula, the Carpathian Mountains, and the Alps (Pastoralist Knowledge Hub – FAO). These systems sustain semi-natural grasslands, which are critical for ecological functions such as soil stabilization, water cycle regulation, and fire prevention (Liechti and Biber, 2016; MacDonald et al., 2000). Moreover, traditional transhumance networks, such as Spain's Vías Pecuarias and Italy's Tratturi, continue to provide routes for seasonal migrations, ensuring livestock access to diverse forage resources throughout the year (Pastoralist Knowledge Hub - FAO).

In Italy, transhumant pastoralism has been a foundational element of agro-pastoral systems, particularly in the Alps and the Apennines. The country's drove roads (tratturi) have historically facilitated livestock mobility, enabling pastoralists to optimize pasture use across different elevations (Bernués et al., 2011). The Apennine regions, as well as areas in Sardinia and Sicily, continue to practice transhumance, preserving traditional breeds that have adapted to extensive grazing conditions (Orlandi et al., 2016).

1.1.2 Drivers of Change in Mountain Areas

In the last 50 years, European rural areas have experienced profound changes in agricultural systems and land use. These transformations have been particularly impactful in mountain regions, where livestock farming has historically been crucial in sustaining rural economies (García-Martínez et al., 2009). The abandonment of pastures in the Alps over the last two decades can be attributed to a combination of socio-economic, environmental, and climatic factors.

Figure 1.1



Falcucci et al., 2007

One of the primary drivers of pasture abandonment is the socio-economic shift in rural areas, characterized by depopulation and the marginalization of traditional agricultural practices. As younger generations migrate to urban centers in search of better economic opportunities, the traditional agro-pastoral lifestyle has diminished significantly. This trend has led to a reduction in livestock numbers and the cessation of seasonal grazing practices, which were once vital for maintaining the alpine landscape (Baur and Nax, 2018; Targetti et al., 2010; Dibari et al., 2020). The modernization of farming practices has also contributed to this decline, as many farmers have transitioned to more lucrative agricultural activities, further neglecting the maintenance of pastures (Kušar and Komac, 2019).

Environmental changes, particularly the natural reforestation process, have been accelerated by the abandonment of pastures. When grazing is reduced or eliminated, vegetation succession occurs, leading to the encroachment of shrubs and trees into previously open grassland areas (Tocco et al., 2013; Brambilla and Pedrini, 2016).

In the Alps, shrub expansion has been particularly pronounced, with species such as *Alnus viridis* rapidly colonizing abandoned pastures, reducing forage availability, and altering nitrogen cycles (Bühlmann et al., 2016).

This shift has not only decreased grazing areas but also impacted biodiversity, as many plant species dependent on grazing pressure have been replaced by dominant woody species (Caviezel et al., 2017).

Climate change is another critical factor influencing pasture abandonment. Rising temperatures and changing precipitation patterns have affected the viability of traditional grazing practices in the Alps. Studies indicate that climate warming may facilitate the upward movement of tree lines, which can encroach upon grasslands and reduce the area available for grazing (Tudoroiu et al., 2016; Dullinger et al., 2003). Additionally, the increased frequency of extreme weather events can make pastoral activities more challenging, leading to further abandonment (Chamberlain et al., 2013).

1.1.3 Alpine Pasture: A Source of Ecosystem Services

Human management of alpine pastures holds a fundamental role in the cultural, environmental, and economic fabric of mountainous regions. These landscapes provide a multitude of ecosystem services that are crucial for both human livelihoods and environmental health in the alpine mountain range.

Multifunctionality is the key factor in land planning, through this it is possible to maximize the value of these pastures, since in this environment all the ecosystem services are interconnected and can't be provided successfully but as a whole (Maldonado et al., 2019). Also this bundle of ecosystem services contributes to maintaining a fragile socio-economic balance on which alpine areas are based. However, achieving a balance among all the ecosystem functions is complex; trade-offs are inevitable, and it is not feasible to maximize all ecosystem services at the same time. The primary challenge lies in managing these pastures in a way that sustains their multifunctionality and heterogeneity, while acknowledging the constraints imposed by natural conditions and the limited capacity of livestock farming as the primary management tool (Pauler et al., 2024).

Talking about pastures managed through livestock grazing there are three main ecosystem services we should highlight: biodiversity conservation, carbon stock, provisioning of high quality products (Leiber et al., 2017).

Biodiversity Conservation

Biodiversity is considered the supporting ecosystem service, this is one of the pillars on which the pasture habitat can be maintained in all its characteristics (nutrient cycle, water cycle, species richness), and so sustain the provision of all the other ecosystem services.

The state of health of this type of habitat can be detected through an important indicator, plant species richness, and this is influenced by two main factors: landscape and local characteristics. The role of the landscape factors is debated by the scientific community, with the exception of the influence of fertilizers that is considered to be strong on the variability of plant species in grasslands. The local characteristics of the pasture (such as type of management, abiotic traits and soil features) are recognized as an important input for habitat's health indicators variability like plant species richness (Marini et al., 2008). Among local factors, it has been demonstrated how good livestock management can have a positive impact on plant species variability and in counteracting invasive species. In fact, grazing animals are both enhancing nutrient availability and regulating light access (Gavrichkova et al., 2022). In this way, traditional livestock management can enhance pasture's plant species richness, promoting its heterogeneity and supporting a wide range of animal species including many that are endemic or threatened; (Fuhlendorf and Engle, 2001). This biodiversity, in turn, enhances the resilience of the ecosystem, allowing it to better withstand and adapt to environmental changes such as climate variability or extreme weather events.

From an environmental perspective, the preservation of biodiversity in these grazed pastures is intrinsically linked to the overall health of the ecosystem. Grazing by cows and goats helps maintain open landscapes by controlling the growth of dominant plant species, which can otherwise outcompete others and reduce plant diversity. This activity is crucial for maintaining soil stability and reducing erosion, as diverse plant root systems play a key role in holding soil in place. Moreover, grazing contributes to the hydrological balance by promoting water infiltration and reducing surface runoff. The mosaic of different vegetation types, including grasses, herbs, and low shrubs, creates a complex habitat that supports a wide range of pollinators, insects, birds, and other wildlife, thereby maintaining ecological networks that are essential for the functioning of these ecosystems (Chamberlain et al., 2013; Tocco et al., 2013).

It has been demonstrated how a wise grazing management plan is fundamental to obtain a positive impact on the biodiversity state of conservation of the pasture, and particularly the rotational grazing system (in contrast with the continuous grazing system) was found to be equilibrating the proportion of eutrophic, mesotrophic and oligotrophic plant species (Pittarello et al., 2019).

Carbon Stock

Grasslands and alpine pastures are playing a crucial role in the global carbon cycle. Soil is the second-largest carbon pool on Earth, containing about 1,550 Pg of organic carbon (double the amount found in the atmosphere) (Batjes N. H., 1996). The management of grasslands, especially in mountain regions, becomes critical for maintaining and enhancing carbon sequestration, which can contribute to mitigating climate change. However, the role of these grasslands in the carbon cycle changes in different environmental conditions, such as weather variability or land management practices, so that they can shift from being carbon sinks to carbon sources. In this context, the weather affects the season length and consequently the number of harvests or grazing passages (Zeeman et al., 2010). This brings that the CO_2 net exchange is influenced by the weather as well as by management factors, like the number of harvests during the season and quantity of manure input. Also warmer or wetter conditions can extend the grazing season, increasing grass biomass production and affecting the potential for carbon uptake. Over time, appropriate management can enhance the soil organic carbon content and contribute to a stable carbon sink, even under changing environmental conditions (Zeeman et al., 2010).

Furthermore, soil management strategies, such as shrub clearing in abandoned pastures, have been shown to increase carbon storage and total nitrogen storage. Cortijos-López et al., 2024 demonstrates that converting abandoned shrublands into managed pastures improves soil quality and enhances carbon and nitrogen sequestration, particularly in alkaline soil environments. In the context of climate change, the effective management of mountain pastures can offer a crucial mitigation strategy. By preventing shrub encroachment, maintaining grazing activity, and ensuring appropriate timing of agricultural inputs, farmers can help stabilize and increase carbon stocks in soils. This approach not only enhances the ecological function of pastures but also supports their role as vital carbon sinks in the broader landscape, contributing to global efforts to combat climate change (Cortijos-López et al., 2024).

Provision of High Quality Dairy Products

First aspect of alpine pastures to be highlighted, in terms of provision of food, is that these lands are located in areas considered as non-arable, mainly because of their steepness, but also due to low accessibility, scarce soil quality and adverse climate (Leiber et al., 2017). Ruminant livestock grazing is the most efficient way to exploit these lands; thanks to their digestive physiology they are able to transform vegetal fibers into high quality proteins (Clauss et al., 2010). Quality of dairy products coming from livestock grazing in the pastures is directly related to the pastoral value (forage quality and productivity) of the pasture itself (Aprea et al., 2016; Coppa et al., 2011), consequently this is the main indicator to quantify the provisioning ecosystem service within an alpine pasture. The quality of pasture forage directly influences livestock health and dairy production. Proper grazing management ensures a balance between forage supply and livestock demand, optimizing pasture productivity over time (Perotti et al., 2018). RGS has been demonstrated to enhance forage yield and quality by preventing selective overgrazing, thereby maintaining a nutritionally rich and diverse plant community (Probo et al., 2016).

Conversely, CGS leads to an imbalanced botanical composition, where less palatable plant species proliferate due to selective overgrazing, reducing overall forage quality (Bühlmann et al., 2016). This, in turn, affects dairy production by lowering the nutritional intake of livestock and increasing reliance on supplementary feed.

1.1.4 Role of Grazing Livestock in Pasture

These ecosystem services (in particular the biodiversity level) within the context of livestock managed pastures appeared to be more influenced by abiotic factors (like yearly precipitation and temperatures, morphological characteristics of the site, elevation) than by the grazing livestock input (Pittarello et al., 2020). Nevertheless, in this environment, livestock management is the only lever to interact with the ecosystem, still it is fundamental to set a wise grazing management plan to obtain a positive impact. Studies have demonstrated that improper grazing—either through overgrazing or insufficient grazing—leads to pasture degradation, reducing forage quality and overall biodiversity (Bühlmann et al., 2016; Pittarello et al., 2019).

Among the different grazing strategies, Rotational Grazing Systems (RGS) have been identified as the most effective type of GMP. Unlike continuous grazing, which allows unrestricted livestock access to pasture, RGS involves dividing the grazing area into paddocks, where livestock rotate periodically. This method prevents overexploitation of forage, allows for plant regrowth, and promotes a balance between eutrophic, mesotrophic, and oligotrophic plant species, ultimately optimizing pasture conditions (Probo et al., 2016; Perotti et al., 2018). Research has shown that rotational grazing enhances soil nutrient content, increases the palatability of forage, and improves ecosystem resilience to climatic variations (Gavrichkova et al., 2022).

More specifically, a wise grazing management plan has the opportunity to counteract shrub encroachment and favor a more balanced spreading of nutrients in the soil of the pasture in the long term. Since A. viridis leaves contain moderately high tannins levels (Stević et al., 2010), they can have low digestibility and palatability, preventing their use as a forage resource for production-oriented livestock (Kumar and Vaithiyanathan, 1990; Besharati and Taghizadeh, 2011). However, robust livestock breeds have the ability to digest lignified and tannin-rich vegetation through their tannin-tolerant rumen bacteria (Manousidis et al., 2016). Previous studies have shown that robust cattle breeds, such as Highland cattle, are able to feed on shrub species foliage with low forage quality (Pauler et al., 2020;

Svensk et al., 2021). The exploitation of woody species-encroached areas by Highland cattle can help controlling shrub and tree cover over time, by combining the effect of direct foliage consumption, trampling, and the mechanical breaking of branches, thanks to their long horns, and could potentially lead to the slow opening of the canopy, allowing the recolonization of typical pasture species in the long-term (Probo et al. 2016; Pauler et al., 2020; Svensk et al. 2022). In addition to their use to limit A. viridis encroachment, robust livestock could be used to balance the level of nitrogen in shrub-encroached areas and adjacent pastures. Indeed, nutrients can be transported through animal excretions, by taking in nutrients while foraging, and returning them through urine and dung excretions (Haynes and Williams, 1993; Schnyder et al., 2010). Up to 95% of the nitrogen ingested by grazing animals can be excreted, mostly in urine (Svensk et al., 2023). Cattle activity (e.g., grazing, resting) is usually affected by topographic, vegetation and management factors (Probo et al., 2014; Homburger et al., 2015). Thus, the spatial distribution of dung pats and urine is not uniform and excretions are mainly deposited in resting areas, which are usually flat areas with low shrub cover (White et al., 2001; Kohler et al., 2006; Koch et al., 2018). As a consequence, nutrients can be spatially redistributed from grazing areas, where they are taken in, to resting areas, where they are deposited (Kohler et al., 2006). Therefore, in A. viridis encroached pastures subjected to targeted grazing with Highland cattle, we expect an active N translocation from shrub-encroached to open and flat areas. A. viridis-encroached areas can have a high N level in the vegetation, due to the high N content of A. viridis leaves (Bühlmann et al., 2016) and that of the understory herbaceous vegetation. Indeed, the understory of herbaceous vegetation is characterized by significantly higher N content than that of both nutrient-rich and nutrient-poor pastures in the surroundings (Zehnder et al., 2017). Moreover, condensed tannin-rich species, such as A. viridis, may induce proportionally higher nitrogen excreted through dung than through urine (Svensk et al., 2023), thus limiting ammonia volatilization from urea, and making nitrogen more available for plant utilization on the long-term (Berry et al., 2001)

Another important and much discussed impact of grazing livestock is the carbon footprint, factor which influences much the overall environmental sustainability of the livestock farm. Extensive grazing systems have been associated with lower greenhouse gas (GHG) emissions compared to intensive livestock farming, particularly when implemented with sustainable grazing strategies (García Criado et al., 2020). However, inefficient grazing management can exacerbate carbon emissions by increasing the reliance on supplemental feed and reducing soil carbon sequestration potential (Farruggia et al., 2014).

Feed self-sufficiency plays a crucial role in determining the carbon footprint of dairy farms. Farms that rely heavily on external feed sources, particularly soybean-based concentrates, contribute to significant land use changes and associated emissions (Guerci et al., 2014). The inclusion of emissions from land use change in carbon accounting highlights the disproportionate impact of feed importation, making on-farm forage production a key strategy for reducing overall GHG emissions (Wassen et al., 2005).

1.2 Milk and Derived Products

Quality of dairy products is a complex topic, the characteristics of raw material affect all derived products' quality, and milk can change much under several influencing factors. Here follows a characterization of goat and bovine milk physicochemical and nutritional composition.

1.2.1 Bovine Milk

Bovine milk is a complex biological fluid composed of water, lipids, proteins, carbohydrates, vitamins, and minerals. Its composition varies significantly depending on factors such as breed, stage of lactation, diet, and overall animal health (Fox, 2011). Understanding these compositional variations is crucial for optimizing dairy production and ensuring high-quality milk for human consumption.

Table 1.1: Milk composition and main health effects of the components.

Milk component	**Concentration in 1 l whole milk**[a]	**Health effects**
Fat	33 g/l	Energy rich
Saturated fatty acids	19 g/l	Increase HDL, small dense LDL, and total cholesterol. Inhibition of bacteria, virus
Oleic acid	8 g/l	Prevent CHD, gives stable membranes
Lauric acid	0.8 g/l	Antiviral and antibacterial
Myrisitc acid	3.0 g/l	Increase LDL and HDL
Palmitic acid	8 g/l	Increase LDL and HDL
Linoleic acid	1.2 g/l	Omega-6 fatty acid
Alpha linolenic acid	0.75 g/l	Omega-3 fatty acid
Protein	32 g/l	Essential amino acids, bioactive proteins, peptides. Enhanced bioavailability
Lactose	53 g/l	Lactosylation products
Calcium	1.1 g/l	Bones, teeth, blood pressure, weight control
Magnesium	100 mg/l	For elderly, asthma treatment
Zinc	4 mg/l	Immune function. Gene expression
Selenium	37 ug/l	Cancer, allergy, CHD
Vitamin E	0.6 mg/l	Antoixidant

Vitamin A	280 ug/l	Vision, cell differentiation
Folate	50 ug/l	DNA synthesis, cell division, amino acid metabolism
Riboflavin	1.83 mg/l	Prevent ariboflavinosis
Vitamin B_{12}	4.4 ug/l	Key role in folate metabolism

Haug et al., 2007

Bovine milk lipids constitute approximately 3–6% of the total milk composition. The lipid fraction of milk primarily consists of triacylglycerols, which account for about 95–98% of total milk lipids, followed by smaller amounts of phospholipids (~1%), diglycerides (~2%), cholesterol (<0.5%), and free fatty acids (<0.5%) (Fox et al., 2015). The fatty acid profile of bovine milk is largely influenced by diet, and microbial biohydrogenation in the rumen plays a pivotal role in determining the proportions of saturated and unsaturated fatty acids (Vlaeminck et al., 2006). Short- and medium-chain fatty acids (C4:0–C10:0), unique to ruminant milk, are synthesized de novo in the mammary gland from precursors such as acetate and β-hydroxybutyrate, while long-chain fatty acids (≥C16:0) are derived from dietary lipids and body fat reserves (Palmquist et al., 1993). Ruminant milk generally contains low levels of polyunsaturated fatty acids due to extensive biohydrogenation of dietary unsaturated fatty acids by rumen microbes (Jahreis et al., 1997). However, an exception to this process is conjugated linoleic acid, which is produced as an intermediate in the ruminal biohydrogenation of linoleic acid and subsequently synthesized in the mammary gland (Griinari et al., 2000). The physicochemical properties of milk fat are also influenced by the organization of lipids into milk fat globules, which range from 0.1 to 20 μm in diameter, with an average size of 3–4 μm (Singh, 2006). These globules are enveloped by the milk fat globule membrane (MFGM), a structure composed of phospholipids, glycoproteins, and enzymes that stabilize the emulsion and facilitate fat digestion (Lopez, 2011). The presence of cryoglobulins in bovine milk accelerates creaming, promoting rapid fat separation compared to the milk of other dairy species (Fox, 2011). Additionally, bovine milk is distinguished by its carotenoid content, which imparts a characteristic yellow hue to dairy products—a feature less pronounced in the milk of other ruminants such as sheep, goats, and buffalo due to species-specific differences in carotenoid metabolism (Nozière et al., 2006).

Milk fat composition plays a critical role in determining the sensory and technological properties of dairy products. Increased levels of FFAs, particularly volatile short-chain fatty acids, contribute to the development of distinctive flavours in aged cheeses, while excessive FFA concentrations can lead to rancidity and off-flavours in milk and dairy products (Collomb et al., 2002; Haug et al., 2007).

Bovine milk contains approximately 32 g of protein per liter, making it a valuable source of essential amino acids with high biological value (Haug et al., 2007). Nitrogen present in milk is distributed among caseins, whey proteins, and non-protein nitrogen compounds, each serving distinct physiological functions (Holt et al., 2013).

Caseins, which constitute approximately 80% of total milk proteins, play a critical role in calcium and phosphate transport and are responsible for the formation of a clot in the stomach, optimizing digestion efficiency in neonates (Fox, 2011). Caseins are phosphoproteins that exist as colloidal micelles, stabilized by calcium phosphate, and remain insoluble at pH 4.6, a property that facilitates their curdling during cheese production (Fox et al., 2015). The casein/whey protein ratio is a characteristic feature of different species, with ruminant milk (e.g., bovine, ovine, caprine, and buffalo) typically exhibiting a casein content of about 80%, whereas in human and equine milk, this proportion is closer to 50% (Fox et al., 2015).

Whey proteins, which account for the remaining 20% of milk proteins, are globular and more water-soluble than caseins. The primary whey protein fractions include β-lactoglobulin, α-lactalbumin, bovine serum albumin, and immunoglobulins, each contributing to immune defence and metabolic regulation (Swaisgood, 1995). Whey proteins are known for their rapid digestion rate, leading to a fast postprandial increase in plasma amino acid concentrations, making them particularly beneficial for muscle protein synthesis and metabolic health (Boirie et al., 1997).

Lactose is the primary carbohydrate in bovine milk, representing approximately 4.8% of its total composition (Fox, 2011). This disaccharide, unique to milk, consists of glucose and galactose linked by a β-1,4-glycosidic bond and serves as a major energy source for neonatal mammals (Fox et al., 2015). The concentration of lactose remains relatively stable across dairy species, with cow, buffalo, goat, and sheep milk containing between 4.5% and 5.0% lactose, largely due to its role in regulating the osmotic balance of milk secretion (Atkinson et al., 1995). Unlike other milk components, lactose content is minimally influenced by breed, diet, or individual variability, as its synthesis is closely linked to the osmotic pressure of blood plasma, which remains physiologically constant (Gänzle, 2014). However, lactose concentration can decrease during late lactation and in cases of mastitis, as udder infections disrupt normal milk synthesis and secretion processes (Hernández-Castellano et al., 2014).

As a reducing disaccharide, lactose contributes to the slightly sweet taste of milk and plays a fundamental role in dairy processing, particularly in fermentation and Maillard reactions during heat treatments (Fox et al., 2015). The enzymatic hydrolysis of lactose by β-galactosidase (lactase) yields its constituent monosaccharides, which are more readily absorbed by the intestine; insufficient lactase activity in humans results in lactose malabsorption, a common condition worldwide (Swallow, 2003).

In addition to lactose, bovine milk contains trace levels of oligosaccharides (OSs), complex carbohydrates composed of 3 to 10 monosaccharide units, including fucose and N-acetylneuraminic acid (Gänzle, 2014). These oligosaccharides, which were predominant in early mammalian evolution, are thought to play a protective rather than a nutritive role, acting as prebiotics and influencing gut microbiota development (Urashima et al., 2012).

1.2.2 Goat Milk

Several factors contribute to the growing demand for goat milk, including its widespread domestic consumption, the interest of consumers in gourmet dairy products, its suitability for individuals with cow milk allergies or lactose intolerance, and its potential health benefits due to higher oligosaccharide and nucleotide content compared to bovine milk (Alférez et al., 2001; Haenlein, 2004).

The composition of goat milk is influenced by numerous factors, including breed, nutrition, environmental conditions, lactation stage, parity, and seasonality (Clark and Mora García, 2017). While the overall content of main components of goat milk—total solids, fat, crude protein, lactose, and ash—is comparable to that of cow milk, key differences exist in the structure and concentration of specific components, affecting its digestibility, flavour, and processing behaviour (Amigo and Fontecha, 2011).

Table 1.2: Average nutritional values of Goat milk.

	Average (%, w/w)	Range (%, w/w)
Total solids	12.90	9.95–21.5
Fat	4.10	2.46–7.76
Crude protein[a]	3.50	2.49–5.06
Casein	2.90	2.33–4.63
Lactose	4.50	3.62–6.30
Ash	0.80	0.69–0.89

Amigo and Fontecha, 2011

Fats are among the most critical components of goat milk, influencing its nutritional value, sensory properties, and technological applications in dairy products (Amigo and Fontecha, 2011). The fatty acid profile of goat milk exhibits substantial differences compared to bovine milk, primarily due to its elevated concentrations of short-chain fatty acids and medium-chain fatty acids. Short chain fatty acids account for 15–18% of the total fatty acids content in goat milk, compared to only 5–9% in bovine milk. This difference arises from variations in the polymerization of acetate by rumen bacteria in goats, significantly contributing to the distinctive aroma and flavour of goat dairy products, particularly cheeses (Haenlein, 2004; Chilliard et al., 2003).

A notable characteristic of goat milk fat is its content of branched-chain free fatty acids with fewer than 11 carbon atoms, which are virtually absent in bovine milk. These branched chain fatty acids are responsible for the characteristic "goaty" flavour of goat-derived dairy products, further distinguishing them from their bovine counterparts (Skapetas and Bampidis, 2016). Additionally, goat milk fat is rich in conjugated linoleic acid (Park et al., 2007).

From a metabolic perspective, the medium-chain triacyl glycerides in goat milk, primarily composed of short chain fatty acids, hold particular significance due to their rapid digestion and absorption, making them highly beneficial for individuals with metabolic disorders, malabsorption syndromes, or impaired lipid metabolism (Alférez et al., 2001).

In terms of structural organization, the lipids in goat milk exist in the form of fat globules, which remain dispersed due to the absence of agglutinin—a glycoprotein responsible for fat globule aggregation in bovine milk (Park et al., 2007). Goat milk fat globules are not only more numerous but also smaller in size, with an average diameter of 2.76 μm, compared to 3.51 μm in bovine milk. This reduction in fat globule size is advantageous for digestion, facilitating lipid metabolism and enhancing the bioavailability of fat-soluble nutrients (Hernández-Castellano et al., 2014).

The protein composition of goat milk is broadly similar to that of other ruminants, with caseins and whey proteins being the predominant components. The primary caseins include κ-casein (κ-CN), β-casein (β-CN), αs1-casein (αs1-CN), αs2-casein (αs2-CN), and γ-casein (γ-CN), while the main whey proteins comprise β-lactoglobulin (β-Lg), α-lactalbumin (α-La), serum albumin, and immunoglobulins (Amigo and Fontecha, 2011). A strong homology (80–90%) exists between goat and cow milk proteins, but the relative distribution of individual caseins in goat milk is highly variable due to extensive genetic polymorphism at the casein loci (Clark and Mora García, 2017).

A defining characteristic of goat milk proteins is the complex genetic variability of αs1-casein, which significantly influences its physicochemical properties and processing behaviour. Over 18 alleles have been identified for αs1-CN, classified into ten distinct protein variants (αs1-CN A, B1, B2, B3, C, D, E, F, G, and O), each associated with different expression levels of αs1-casein. The relative abundance of αs1-casein variants varies between goat breeds, impacting milk coagulation properties, cheese yield, and textural characteristics of goat dairy products (Ambrosoli et al., 1988). Compared to bovine milk, goat milk is generally lower in αs1-CN but richer in β-CN, which affects its digestibility and allergenic potential (Haenlein, 2004). β-casein in goat milk occurs in two phosphorylation levels, β1-CN and β2-CN, with six and five phosphoseryl residues, respectively. Historically considered monomorphic, β-CN in goats has been found to exhibit genetic variation, with a null allele identified in certain breeds such as Garganica, Creole de Guadeloupe, and Granadina (Park et al., 2007). Additionally, at least seven genetic variants of κ-casein have been identified (Pulina et al., 2018). These genetic polymorphisms contribute to significant variability in goat milk's technological properties, particularly its suitability for cheesemaking.

Beyond caseins, goat milk contains whey proteins that are structurally distinct from those in bovine milk. Two genetic variants of β-Lg have been identified in goats, with a lower net charge compared to their bovine counterparts. α-La in goat milk also exhibits differences in charged amino acids, with two variants reported (Clark and Mora García, 2017).

An interesting distinction of goat milk is its elevated non-protein nitrogen content compared to bovine milk. Non-protein nitrogen includes various nitrogenous compounds such as nucleosides and nucleotides, which play a role in neonatal nutrition and immune function (Park et al., 2007).

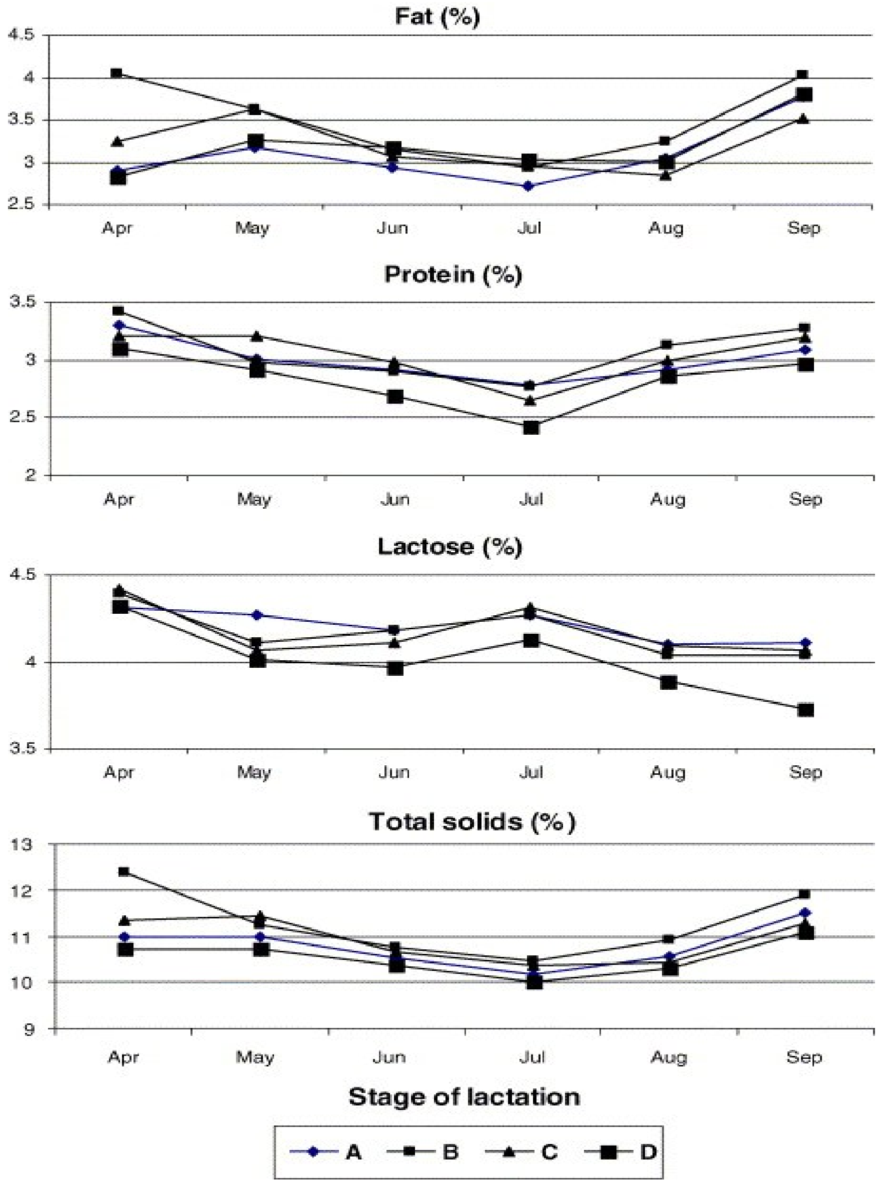
Lactose is the predominant carbohydrate in goat milk, although its concentration is 0.2–0.5% lower than that found in cow milk (Amigo and Fontecha, 2011; Park et al., 2007). In addition to lactose, goat milk contains various oligosaccharides, glycopeptides, glycoproteins, and nucleotide sugars, which play a significant role in neonatal nutrition and gut health (Albrecht et al., 2014).

Goat milk stands out for its high oligosaccharide content, which surpasses that of cow milk. These oligosaccharides act as prebiotics, stimulating the growth of bifidobacteria in the infant gut and contributing to the development of the intestinal microbiota (Urashima et al., 2001). Additionally, they serve as protective agents against pathogens by preventing bacterial adhesion to intestinal mucosa (Gopal & Gill, 2000).

1.2.3 Mountain Milk and Cheese

Mountain farms are characterized by specific environment and livestock management practices, namely the breed of the animals, and feeding type. Most represented management type in mountain areas is the extensive-transhumant one, characterized by grazing animals and feeding integration mostly based on hay. The nutritional composition of milk is significantly affected by the feeding regime of dairy animals. For instance, diets rich in energy and protein are crucial for optimal milk production. Insufficient nutrient supply can lead to weight loss in lactating cows, as they mobilize body reserves to meet the demands for milk protein and fat synthesis.

Figure 1.2: Changing of main goat milk components along the lactation period of 4 groups of Alpine goats on diet basis (A: confined with hay and high concentrate; B: grazing with high concentrate; C: grazing with low concentrate; D: grazing with no concentrate (Soyral et al., 2004).



It has been demonstrated how the higher overall movement of the animals during the day to obtain the same amount of food can affect milk's yield in the context of mountain area, since more energies are used for the movement rather than for the metabolism and so leading to lower yield (Saha et al., 2019; Ungureanu-Iuga et al., 2024). Environmental conditions are also playing an important role, particularly studies (Zhu et al., 2020; Marumo et al., 2022) confirm how high temperatures and humidity can cause stress in the animals and so lower bloodstream in the udder, with consequent lower milk yield. The lactation period is an easily predictable factor for both mountain and lowland farms, generally the yield in correlation with this input is characterized by the typical lactation curve, reaching the maximum yield after the first month of the lactation period (Fox, 2011).

Yield

Milk yield in livestock managed under seasonal transhumance systems is influenced by multiple factors, including stocking density, nutritional availability, lactation stage, and seasonal variations in forage quality. Generally, high-yielding dairy cows prioritize milk production over body condition, leading to energy repartitioning when forage availability is suboptimal (Bovolenta et al., 2008). However, in well-managed alpine pastures, animals can maintain milk production despite variations in stocking density and supplemental feeding strategies (Kasapidou et al., 2023).

Studies have shown that milk yield exhibits significant fluctuations throughout the lactation period. During early lactation (March–May), higher levels of fat, protein, and non-fat solids are observed, while a decline occurs in mid-summer (June–July), followed by a subsequent increase towards the end of lactation (Soryal et al., 2004; Strzałkowska et al., 2009). These variations are primarily attributed to seasonal changes in pasture composition and energy availability, as lactating cows and goats switch from winter feed, consisting mainly of hay and concentrates, to a diet dominated by fresh alpine forage (Soryal et al., 2004).

An inverse relationship between milk yield and fat and protein concentrations has been reported, commonly referred to as the "dilution effect" (Lock & Garnsworthy, 2003). During peak lactation, milk production increases, leading to a transient reduction in fat and protein content, while later in lactation, as yield declines, the concentration of these components rises (Sandrucci et al., 2018). This dynamic is particularly relevant in mountain dairy systems, where variations in milk composition directly impact cheese yield and quality attributes such as texture and smoothness (Soyral et al., 2004).

Furthermore, hygienic quality indicators such as total viable counts (TVCs) show lower levels towards the end of lactation due to improved environmental conditions and decreased microbial contamination from handling equipment and udder surfaces (Lianou et al., 2021). Proper pasture management, rotational grazing, and supplementation with energy-dense feed during peak lactation can help mitigate the decline in milk yield and ensure optimal milk composition for dairy processing.

Proteins

The protein composition of cow and goat milk from pasture-based systems is influenced by various environmental and physiological factors, including altitude, breed, lactation stage, and diet composition (Fox et al., 2015; Barlowska et al., 2020). In goats, studies have shown that milk protein concentration tends to decrease with increasing altitude due to lower nitrogen availability in alpine pastures, affecting protein synthesis in the mammary gland, and also under the influence of levels of concentrate supplementation (Peña-Avelino et al., 2023). However, other research indicates that in certain breeds, such as Saanen goats, milk protein content is higher in mountainous regions, likely due to differences in forage composition and production systems (Barlowska et al., 2020). Similarly, cow milk from pasture-fed animals tends to have a slightly lower protein concentration than that from intensively fed cows, yet it exhibits a more favourable protein profile for cheesemaking, with higher casein content and improved coagulation properties (Martin et al., 2002). Additionally, the natural grazing diet of pasture-fed animals is associated with increased levels of bioactive peptides and essential amino acids, which can enhance the nutritional value and sensory attributes of cheeses (Korhonen and Pihlanto, 2006). Overall, the variability in milk protein composition between cow and goat milk from pasture-based systems underscores the importance of breed selection, altitude, and forage quality in optimizing milk for artisanal cheesemaking.

Fat Content

Milk and cheese derived from grazing systems in mountain pastures exhibit a superior fatty acid profile compared to those produced in lowland systems or from animals fed a hay-based diet. Various studies have demonstrated that milk from pasture-fed ruminants contains a more favourable composition of fatty acids, particularly in terms of human health benefits, due to its higher levels of polyunsaturated fatty acids (PUFAs) and conjugated linoleic acids (CLAs) (Coppa et al., 2012; Chilliard et al., 2007). The unique botanical diversity of alpine pastures plays a crucial role in this composition, as fresh herbage is richer in essential fatty acids compared to conserved forages such as hay (Wyss, 2012; Agradi et al., 2020). In both milk and cheese from mountain pastures, saturated fatty acids (SFAs) constitute the predominant lipid fraction, followed by monounsaturated fatty acids (MUFAs) and omega-6 (n-6) PUFAs, while omega-3 (n-3) PUFAs and CLA stereoisomers are present in lower proportions but still contribute significantly to the nutritional value (Agradi et al., 2020). The most abundant fatty acids detected in these dairy products include palmitic acid (C16:0), oleic acid (C18:1 n-9c), myristic acid (C14:0), and stearic acid (C18:0). Importantly, the n-6/n-3 ratio in mountain pasture milk and cheese remains below 2.5, a threshold considered beneficial for cardiovascular health (Nozière et al., 2006).

The fatty acid profile of milk and cheese is a strong indicator of the farming system employed, with specific compounds serving as biomarkers of grazing-based production. Linoleic acid (C18:2 n-6c), α-linolenic acid (C18:3 n-3), and pentadecanoic acid (C15:0) have been identified as the key discriminating factors between pasture-based and non-pasture-based dairy products (Coppa et al., 2012). These differences arise primarily from dietary intake, as fresh forages are naturally rich in C18:3 n-3 and C18:2 n-6c, while the process of hay drying and storage leads to significant losses of these essential fatty acids due to oxidative degradation and leaf loss (Wyss, 2012).

The higher intake of fresh pasture in mountain systems enhances the deposition of PUFAs in milk and their subsequent conversion into bioactive lipid metabolites such as CLA isomers (n-9c, n-11t), vaccenic acid (C18:1 n-7), and eicosapentaenoic acid (C20:5 n-3). These metabolites have been widely recognized for their potential anti-inflammatory and cardioprotective properties (Haug et al., 2007). Conversely, cows fed a hay-based diet exhibit a reduced ruminal production of these beneficial compounds due to a lower presence of their precursors in the diet (Nozière et al., 2006).

Another key factor influencing lipid composition in pasture-based milk and cheese is the proportion of C15:0, a saturated fatty acid derived either from specific plant species found in alpine pastures or from microbial fermentation in the rumen.

Studies suggest that a higher forage-to-concentrate ratio in pasture-fed cows stimulates greater bacterial synthesis of C15:0, thereby increasing its concentration in milk (Agradi et al., 2020). This fatty acid has gained interest for its potential role in metabolic health, as it has been associated with improved lipid metabolism and anti-diabetic properties in human nutrition (Haug et al., 2007; Coppa et al., 2012).
Overall, the lipid composition of dairy products from mountain pastures is strongly influenced by grazing patterns, forage diversity, and the altitude-related characteristics of the farming environment. The enrichment of these products in essential fatty acids, CLAs, and bioactive lipids provides both technological advantages for cheese production and enhanced nutritional value for human consumption.

Vitamins and Other Components

Compared to milk from animals raised in lowland systems, pasture-based dairy products contain higher levels of fat-soluble vitamins, including vitamin A, vitamin E (α-tocopherol), and beta-carotene, as well as essential minerals such as calcium, magnesium, and selenium (Martini et al., 2021; Nozière et al., 2006).
Among fat-soluble vitamins, α-tocopherol, the biologically active form of vitamin E, plays a crucial role in maintaining oxidative stability in milk and dairy products (Cervinkova et al., 2016). Studies have shown that pasture-fed animals have higher α-tocopherol concentrations in their milk compared to those receiving conserved forages, as fresh pasture is a primary source of this antioxidant (Nozière et al., 2006).
Phytosterols, bioactive compounds found in plants and absorbed into ruminant milk through diet, contribute to the nutritional value of dairy products from pasture-fed animals. While the presence of phytosterols in cow milk remains relatively low and is unaffected by diet, goat and sheep milk from mountain pastures contains a higher concentration of these compounds, which are known for their cholesterol-lowering effects (Bresson et al., 2008). Furthermore, mountain pasture-derived dairy products contain higher levels of lanosterol and desmosterol, sterols involved in cholesterol biosynthesis, with potential protective effects against cardiovascular diseases (Zhao et al., 2015).

The impact of cheese production on vitamin and mineral retention varies depending on processing methods. While α-tocopherol and beta-carotene are partially lost during cheese production due to fat partitioning into whey, the retention of calcium and phosphorus remains high due to their role in the formation of the caseins structure, particularly in aged cheeses such as Pecorino and other mountain pasture cheeses (Fox et al., 2015).

1.3. Aroma of milk and derived products of mountain productions

The volatilome of milk and cheese is influenced by several productive inputs, namely the breed of the animal, diet and cheesemaking practices. The breed of the animals plays a significant role in determining the quality of milk and, consequently, the cheese produced. Different breeds exhibit variations in milk composition, particularly in fat and protein content, which are critical for flavor development during cheese ripening. For instance, studies have shown that the milk from Brown Swiss cows, which are commonly found in alpine regions, tends to have a higher fat content, leading to a richer flavor profile in the resulting cheese, also taking into account that biochemical processes involving fatty acids play a major role in the creation of relevant volatile organic compounds (Bergamaschi et al., 2015; Ianni et al., 2020). Additionally, the diet of these animals, both directly and indirectly, impacts the transfer of VOCs from grass to milk and subsequently to cheese (Aprea et al., 2016). First of all, the switch from hay fed to grazing management affects directly the flavor profile of the milk and consequently the cheese produced. In fact, it has been largely demonstrated (Mariaca et al., 1997) how terpenes, especially sesquiterpenes, present in vegetal organisms, are mostly unmodified by the ruminal bacterial flora. Studies indicate that cheeses made from milk of grass-fed cows exhibit distinct flavor notes, often described as herbal or floral, which are absent in hay-fed counterparts (Abilleira et al., 2011; Coppa et al., 2011). Seasonal variations of environmental factors such as altitude and weather conditions can affect the nutritional quality of the forage, thereby impacting the flavor profile of the cheese (Aprea et al., 2016).

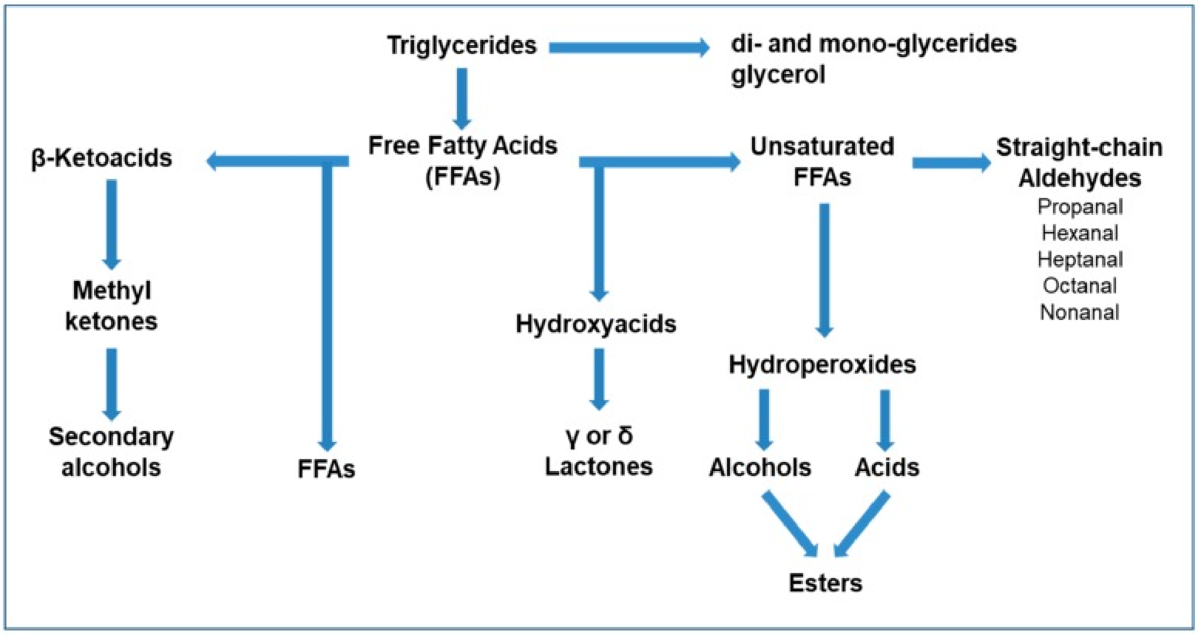
The cheesemaking process itself is crucial in shaping the volatilome of the final product. Key practices include the timing and temperature of fermentation, the addition of ferment and rennet, and the salting method. For example, higher ripening temperatures have been shown to enhance the expression of genes related to proteolysis and lipid metabolism, resulting in increased production of free amino acids and fatty acids, which are precursors to various VOCs (Filippis et al., 2016). The choice of starter cultures and the conditions under which they are allowed to ferment also play a pivotal role in flavor development. Specific strains of lactic acid bacteria can produce distinct profiles of VOCs, influencing the sensory characteristics of the cheese (Şahingil et al., 2014).

1.3.1 Biochemical Processes of Volatile Compounds in the Cheese

The formation of volatile compounds in cheese results from a complex series of biochemical transformations, primarily driven by enzymatic and microbial processes during milk fermentation and cheese aging. These processes convert various milk components—such as lipids, proteins, and carbohydrates—into a wide array of volatile organic compounds that contribute to cheese's characteristic aroma and flavor.

Lipolysis, the enzymatic breakdown of lipids, plays a crucial role in producing volatile compounds in cheese. Enzymes like lipases, originating from both the milk and starter bacteria, hydrolyze milk fat (primarily triglycerides) into free fatty acids, which are then further transformed into volatile compounds through oxidation, reduction, and esterification reactions. These fatty acids give rise to a range of volatiles, including methyl ketones, lactones, and esters, which impart characteristic buttery and fruity notes to cheese (Ianni et al., 2020). This process is particularly pronounced in cheeses derived from milk of grazing animals, as the fatty acid profile varies with forage types, resulting in a distinctive range of aroma compounds, especially when rich in omega-3 and conjugated linoleic acid (CLA) (Kilcawley et al., 2018).

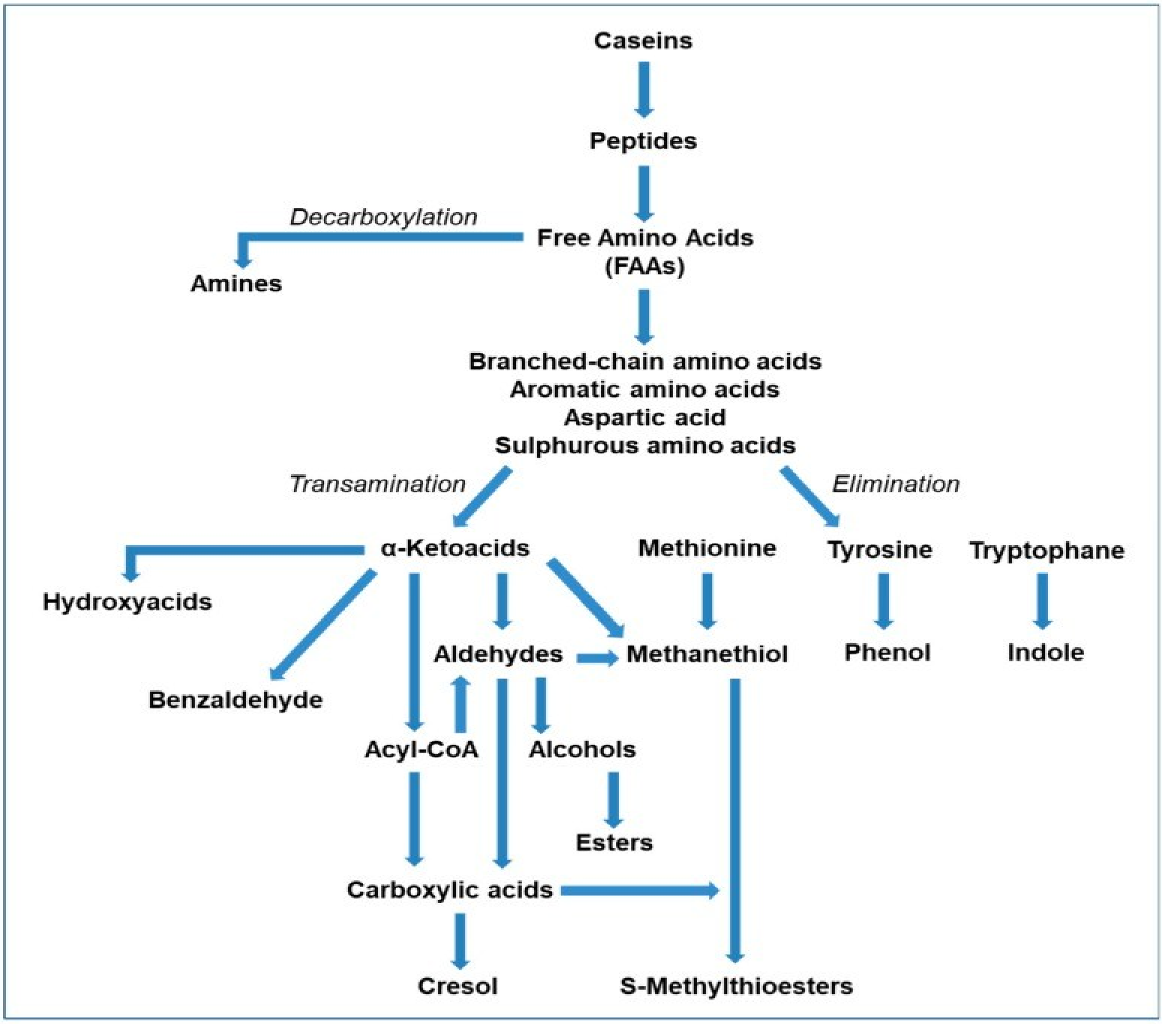
Figure 1.3: Volatile compounds genesis from lipidic fraction.



Ianni et al., 2020

Proteolysis, the breakdown of milk proteins (mainly caseins), is another essential process in flavor development. Proteolytic enzymes from milk, bacteria, and rennet contribute to this breakdown, producing peptides and free amino acids, which are further degraded into volatile compounds. The amino acids are catabolized into various volatile compounds through deamination, decarboxylation, and transamination pathways, resulting in a variety of sulfur compounds, aldehydes, alcohols, and carboxylic acids that provide both savory and pungent flavors. In particular, amino acids like methionine and leucine are precursors to sulfur compounds and branched-chain aldehydes, which contribute to the complex aroma profiles typical of ripened cheeses (Ungureanu-Iuga et al., 2024; Ianni et al., 2020).

Figure 1.4: Volatile compounds genesis from protein fraction.



Ianni et al., 2020

Glycolysis and subsequent carbohydrate fermentation are initiated by lactic acid bacteria, transforming lactose in milk into lactic acid and various aroma compounds, such as diacetyl, acetoin, and acetaldehyde, which correspond to buttery and creamy notes. These compounds contribute significantly to the early flavor development in fresh cheeses and set the foundation for subsequent transformations during ripening. Additionally, these microbial activities enhance the texture and pH balance of the cheese, which influence the solubility and release of volatile compounds over time (Valdivielso et al., 2016).

The microbial populations in cheese—such as lactic acid bacteria, propionibacteria, and yeasts—play a crucial role in producing and modifying volatile compounds. These microbes introduce additional layers of complexity to cheese flavor through various metabolic activities that generate alcohols, aldehydes, ketones, and other aroma-active compounds. The specific microbial consortia vary between cheese types and production conditions, creating unique sensory profiles that are often specific to particular regions and production methods (Ianni et al., 2020).

1.3.2 Terpenes: Origin, Classification, and Biochemical Role in Plants

Terpenes are a diverse group of lipophilic aliphatic compounds derived from the secondary metabolism of plants, playing essential roles in plant physiology, ecological interactions, and biochemical defence mechanisms. These compounds, synthesized via the mevalonate pathway in the cytoplasm and the methylerythritol phosphate pathway in plastids, are constructed from isoprene (C5H8) units and classified based on their structural complexity. The primary categories include monoterpenes (C10), sesquiterpenes (C15), diterpenes (C20), triterpenes (C30), and polyterpenes (C40 and above) (Ajikumar et al., 2008; Ungureanu-Iuga et al., 2024).

The ecological role of terpenes varies significantly among plant species. Many function as allelochemicals, influencing plant-plant interactions and acting as attractants for pollinators (Mariaca et al., 1997). Others serve as defence compounds against herbivores and pathogens due to their antimicrobial, antifungal, and antiviral properties (Ajikumar et al., 2008). The synthesis of terpenes is closely regulated by environmental conditions such as light intensity, temperature, altitude, and soil composition, which influence the plant's metabolic pathways and secondary metabolite production (Mariaca et al., 1997; Valdivielso et al., 2016).

Certain plant families exhibit a higher abundance of terpenes, particularly dicotyledonous families such as Lamiaceae, Asteraceae, and Apiaceae, which accumulate significant levels of monoterpenes and sesquiterpenes. In contrast, monocotyledons such as Poaceae contain lower terpene concentrations (Bugaud et al., 2001). Highland pastures, characterized by a predominance of dicotyledonous plant species, have been shown to produce forage with a significantly higher terpene content compared to lowland pastures dominated by grasses. Studies on mountain cheeses, such as Beaufort, Gruyère, and Etivaz, have identified a range of terpenes, including β-myrcene, linalool, limonene, α-phellandrene, α-terpinene, δ-3-carene, p-cymene, β-pinene, α-copaene, and α-humulene, which are associated with the botanical diversity of high-altitude pastures (Mariaca et al., 1997; Bugaud et al., 2001).

1.3.3 Terpene Stability and Degradation Processes

The stability of terpenes is influenced by both environmental and processing factors. Monoterpenes, due to their low molecular weight and high volatility, are particularly susceptible to degradation via oxidation and hydrolysis, especially during the drying and storage of forages. In contrast, sesquiterpenes, which have a higher molecular weight, are more stable and tend to persist in dried forages and dairy products (Mariaca et al., 1997).

Environmental conditions such as altitude, temperature fluctuations, and soil composition affect terpene biosynthesis. Alpine pastures, with their extreme temperature variations and high UV exposure, have been shown to induce increased terpene production in plants as a response to oxidative stress (Bugaud et al., 2001). During the wilting and haymaking process, oxidative degradation leads to significant losses of monoterpenes, whereas sesquiterpenes are more resistant to degradation and can still be detected in forages and milk (Mariaca et al., 1997). Furthermore, the phenological stage of plants at the time of grazing significantly impacts terpene composition, with mature plants exhibiting different terpene profiles compared to younger plants (Mariaca et al., 1997).

1.3.4 Absorption via the Intestinal and Pulmonary Pathways

These compounds are not synthesized by the cows themselves but are derived from the terpenoid-rich plants in their diet (Ungureanu-Iuga et al., 2024). There have been hypothesized two biological paths in ruminants for the terpenes to be absorbed by the organism and transferred to the udder and then to the milk: through ingestion and inhalation (Faulkner et al., 2018; Faccia, 2022). Terpenes can be brought to the blood flow through the lungs absorption (after inhalation in high terpenes concentration air conditions) or either through intestine absorption.

Once ingested by ruminants, terpenes undergo various biochemical processes before being excreted in milk. Their absorption depends on factors such as lipophilicity, molecular weight, and interactions with ruminal microbiota. Due to their hydrophobic nature, terpenes preferentially bind to dietary lipids, particularly triglycerides and phospholipids, which facilitate their solubilization and transport within the digestive system (Faulkner et al., 2018). Within the rumen, terpenes are subjected to mechanical and enzymatic degradation, and their stability is influenced by microbial metabolism. Monoterpenes, being smaller and more volatile, tend to undergo greater microbial biotransformation than sesquiterpenes, which exhibit higher molecular stability and are more likely to persist through digestion (Ajikumar et al., 2008).

Beyond their role as aroma compounds, terpenes may also influence the fatty acid composition of milk. Some studies indicate that terpenes can inhibit key enzymatic reactions in ruminal lipid metabolism, specifically by slowing down the activity of fatty acid amide hydrolase and fatty acid amide hydroxylase (Ungureanu-Iuga et al., 2024). This effect may reduce the biohydrogenation of polyunsaturated fatty acids (PUFAs) in the rumen, leading to a higher proportion of unsaturated fatty acids in milk and dairy products (Ungureanu-Iuga et al., 2024).

1.3.5 Terpenes and Flavour

<u>Table 1.3</u>: List of terpenes usually identified in dairy products and corresponding odour. Adapted from Mariaca et al., 1997

Compound	Odour Descriptors
β-Myrcene	Fragrant, fresh, hop note

Compound	Odour Descriptors
cis-β-Ocimene	Fragrant, sweet, fresh, herbaceous
trans-β-Ocimene	Pleasant, warm, herbaceous
4-trans-6-trans-Alloocimene	Spicy, herbaceous, diffusive
Linalool	Flowery, citrus-like, sweet, woody
α-Cyclocitral	Lemon-like
β-Cyclocitral	Minty, fruity, green
Limonene	Fruity, citrus-like, fresh, sweet
1,8-Cineole	Balsamic, sweet, fresh, eucalyptus
α-Phellandrene	Citrus-like, fresh, herbaceous, sweet
α-Terpineol	Flowery-fruity, earthy, terpenic, lilac-like
α-Terpinolene	Flowery, fragrant, sweet
α-Terpinene	Citrus-like, woody, terpeny
γ-Terpinene	Citrus-like, herbaceous, terpeny
δ-3-Carene	Sweet, fruity, pungent
δ-4-Carene	Kerosene-like, citrus, bergamot-like
p-Cymene	Weak, spicy herbaceous, citrus-like, fresh
Sabinene	Fragrant, woody, resinous
trans-Sabinene Hydrate	Fresh, minty
α-Thujene	Woody, warm, resinous
Bornyl Acetate	Eucalyptus, minty, fresh, woody
Endo-Borneol	Camphor, dry, woody
Camphene	Camphoraceous, mothball note
Tricyclene	Woody, resinous, conifer-like
α-Pinene	Fragrant, fresh, conifer-like
β-Pinene	Fragrant, fresh, conifer-like

Compound	Odour Descriptors
Trans-Pinocarveol	Warm, woody, balsamic, slightly fennel-like
Myrtenol	Camphor, malty, medicinal
α-Fenchene	Fresh, camphor-like, fruity
α-Cubebene	Fruity, sweet, citrus-like
α-Copaene	Fresh, earthy, mango-like
α-Humulene	Hop-like, musty, woody
trans-β-Caryophyllene	Sickly sweet, wallflowers, woody-spicy

In Table 1.3 are listed many examples of terpenes found in mountain dairy products, associated with the typical odours they apport to the product. Terpenes related odours are described as characteristic of mountain dairy products, but also have high odour detection threshold, which makes them relevant in determining sensory properties of dairy only when they accumulate in very high concentrations (Kilcawley et al., 2018).

1.3.6 Mountain Product Label and Terpenes as Biomarkers

As already mentioned, seasonal transhumance is a decaying practice, and generally traditional livestock farms number is decreasing all over Europe and particularly in Alpine regions (Falcucci et al., 2007). Lowland traditional livestock farms with suitable conditions are intensifying the production, preferring confined rearing and silage and concentrate feedings rather than hay and highland pastures, in order to maximize the profits (Faccioni et al., 2019). Seasonal transhumant farms seem to be no longer economically competitive on the market, since they cannot afford to sell products at prices as low as the lowland dairies can sell (Faccioni et al., 2019; Ramanzin et al., 2014). In this context origin and quality certifications are a strong tool that EU institutions can use to support mountain farmers category. On one hand they can give mountain products high recognizability (and so consumer's willingness to pay higher prices) (Bryła, 2017), on the other hand can function as a discrimination to better direct European funds only to transhumant livestock farms.

Consumer perception of food products is based on both intrinsic and extrinsic characteristics, so both on physical properties of the product and on information provided about the product (Fernqvist et al., 2014). For example, EU quality labels such as PDO (Protected Denomination of Origin) and PGI (Protected Geographical Indication) purchase is proved to be strictly correlated to food origin interest, so many consumers put those products in the premium price category (Bryła, 2017). Anyway, PGI sign emphasizes the historical denomination, rather than the relationship with the territory of origin, since only one stage of the production chain is requested to be carried out in the denomination region. PDO products, among all EU signs, are the most strictly related to the place of production, since every part of production and processing must take place in the denomination region (Regulations on geographical indications and quality schemes – European Commission). These two EU signs are still too wide to be able to address economic issues related to a specific category like the one of highland pastures-related farms; hence, in order to address pastoral lands abandonment, the European Union focalized on promoting and developing mountain food production, eventually creating a new Mountain Product Label (Tebby et al., 2010; Bentivoglio et al., 2019). Optional quality term "Mountain Product was adopted in 2012 by the EU with the Regulation n. 1151/2012, and specific condition utilization of the EU label are defined by the national implementations of the EU Regulation n. 665/2014. (Regulations on geographical indications and quality schemes – European Commission).
Several studies reported how consumers are more and more interested in external factors related to sustainability, production method, ethics, origin, ecology (Fernqvist and Ekelund, 2014). Moreover, consumers recognize mountain production as related to higher sustainability and as embedding a trade-off between productive, social and environmental needs (Zuliani et al., 2018). Still high quality is a fundamental trait of mountain product for consumers, considering it as the most important ecosystem service provided by mountain pastures (Pachoud et al., 2020). Endrizzi et al., 2021, in a taste test of lowland and pasture derived Puzzone di Moena cheese, reported that external information given about the product had stronger effect than physical properties of the cheese. Particularly they found that cheese presented as "mountain pasture product" received statistically higher

scores. This confirms how strong such a tool can be in terms of recognizability and of competitiveness.
Still, either in EU Regulations or in National Implementations, there isn't discrimination between intensive livestock farming and mountain pastures traditional farming, hence even EU Mountain Product labelling system is still too wide. In this context, where a strong scientific support to the certification and monitoring system is needed, terpenoid compounds have the possibility to play a central role as biomarkers for the traceability of dairy products. As mentioned previously, many studies confirmed higher concentrations of terpenes in milk and cheese deriving from grazing animals (Ungureanu-Iuga et al., 2024; Valdivielso et al., 2016; Bovolenta et al., 2014; Kilcawley et al., 2018), and even found correlation of certain terpene compounds with specific botanical compositions, hypothesizing the possibility to trace dairy products up to the morphological and phenological properties of the pasture (Zeppa et al., 2005), so possibly discriminating highland pasture derived milk from lowland derived milk. In this way funds and policies could aim at supporting mountain farms where ecosystem services are maximized.

2. Aim of the Study

The objectives of this study were several. First of all to evaluate how the transhumance to highland pastures and the consequent change in activity and feeding daily routine affected the quality of goat and bovine milk, in terms of yield and composition. Second, to analyze how the volatile profiles of goat and bovine milk and curd and goat cheese were influenced by the changes in environment, management and diet due to the seasonal transhumance to highland pastures.

Finally, we focused on terpene fraction of the volatilome, looking for the possibility to use these as biomarkers to trace a mountain product deriving from grazing animals, like the goat Formaggella cheese, which still isn't widely recognized in the market, as it is not labeled with any kind of certification. We think that setting a scientifically solid system of certification of mountain products is an important step to make, in order to give a practical support to the mountain livestock farming system.

3. Materials and Methods

3.1. Study Area Description

3.1.1 Location

The present study was conducted at Azienda Agricola Prestello, a dairy farm located in Prestine, a small village in the municipality of Bienno in Valle Camonica (Brescia province, Northern Italy). The region is characterized by a strong tradition of transhumant pastoralism, with seasonal movement of livestock between lowland winter stabling areas (about 600 m a.s.l.) and high-altitude summer pastures. During the winter months, the farm operates in the vicinity of Prestine, where it manages:

The transhumance season in 2023 began in mid-June and lasted until late August; depending on weather conditions this period might vary from one year to another. During this period, livestock and cheese production were relocated to Malga Prato, situated in Valle di Campolaro at approximately 1650 meters a.s.l., this was the site where the cheesemaking tools were set in the summertime. Subsequently animals were moved to Malga Varicala in the Valle di Crocedomini at approximately 1800 meters a.s.l., where animals were held only to be milked and to rest overnight, having access to drinkable water. The higher-altitude pastures extend from Malga Prato up to Passo Crocedomini, this area is fully integrated within the territory of Parco Regionale dell’Adamello.

3.1.2 Characteristics of the Livestock Farm

Azienda Agricola Prestello is a family dairy farm, where four family members work full-time. The farm is specialized in raw milk cheese production, processing both cow and goat milk following traditional artisanal methods. The company manages 50 Italian Brown cows of which 35 milking and 80 Bionda dell'Adamello breed goats of which 60 milking. The activity is mainly based on dairy production, in particular the produced cheese are Silter DOP, Fatulì (a goat's cheese smoked with juniper wood) and Nostrano cheese, which are the equivalent of the Silter but outside the official denomination. This study focused on bovine milk and curd destined to Silter and Nostrano production, and on goat milk, curd and Formaggella cheese.

The entire cheese production process relies on raw milk and traditional copper-lined stoves. Cutting tools and stirring steel devices are used, while a mechanized stirrer is used to maintain milk movement during heating.
500-600 liters of partially skimmed cow milk were processed to produce two wheels of Silter/Nostrano cheese during pasture period, while along lowland period around 800 liters were processed to realize three wheels. Silter and Nostrano cheese were produced using a partially skimmed cow's milk, collected from two daily milkings. The evening milk was stored in cooling tanks at a temperature not exceeding 8-10°C, allowing the cream to rise naturally overnight. The following morning, the skimmed evening milk was mixed with the freshly milked morning milk, forming the base for cheese production. The milk was gently heated to approximately 40°C, then whey starter and rennet were added, initiating coagulation, which lasted around 50 minutes. Once the curd reached the desired consistency, it was cut in two stages: first with a "spada" (sword-like knife) and then with a "spino" (curd breaker) until the granules reached a corn kernel size.
The curd was cooked at 50°C, rested for about 20 minutes, and gathered into cloths before being pressed into 2 (or 3) specific moulds to eliminate excess whey. The newly formed wheels are immersed in a brine bath for 5 days, before beginning the aging process, which lasts a minimum of 8-10 months in a cold storage room at 12-14° C.
For goat's cheeses around 100 liters of whole goat's milk were processed to make between 20 and 25 goat's cheeses (then eventually smoked later to make Fatulì). The freshly collected milk was heated to about 40°C, and graft and rennet were added to facilitate coagulation, lasting about 50 minutes. Once the curd was formed, it was cut with a "spino", ensuring uniform curd particles. The curds were then manually transferred into small moulds. The cheeses underwent salting and aging under controlled conditions. After being immersed in brine (5-8 h), they were left to mature for up to 30 days in a cold storage room at 12-14° C.
Fatulì goat cheeses were smoked using juniper wood after a specific period of aging (25-40 d).

3.2 Animal Management and Feeding Regimes

3.2.1 Feeding

During the lowland period cows were fed with the use of TMR, mixing a proportion of first cut hay, second cut hay and alfalfa hay, with concentrate feedings. Goats, instead, were kept in the barn, during the lowland period, and the rations were made up hay (ad libitum) and concentrate (about 1 kg/d), without possibility to access to a paddock area.

In the pasture period cows (June to September) were held in a fenced open pasture during the night, with constant access to drinkable water. Then cows were left to graze in the daytime, with a kind of rotational grazing, changing pasture patch in average every 3 days, but still having access to the previously grazed patch. During 2 milking period cows were fed with 1.5-2 kg of concentrate per milking.

3.2.2 Cattle Management

In the winter period animals were held in Prestine (BS), where the main cheese production facility is located. Cows were held in a medium sized barn, with a central corridor for feeding displacement, parallel individual stations to access the feedings, and a wide resting area.

A connected milking parlor was used for milking (twice a day) and milk collection, from where milk was directly carried in the dairy tanks for cream separation.

During Summer, animals were brought to highland pasture, to Malga Prato and Malga Varicla. No recovery for the animals were allowed.

Cows were milked twice a day, in the early morning and in the late evening, using the milking cart during the summer period.

3.3. Sample Collection

Three samples of milk and three sample of curd of both cow and goat were gathered with falcon vials for volatiles analysis. Cow milk was gathered from the partially skimmed bulk milk at the moment it was poured in the stove, at a temperature ranging between 12 and 18°C. For goat milk the protocol was the same, but it came from the morning milking process, so it was whole milk. Milk samples were always taken from the bulk milk before ferments or rennet were taken. Subsequently milk was refrigerated at a temperature of 4°C and finally put in a freezer at a temperature of -20°C and stocked until extraction and analysis of the volatiles.

Both goat and cow curd samples were collected in the moment of the laying in the moulds, putting a mix of the wheels in each falcon vial, for cow curd were picked pieces of curd from several random zones of the wheel. The samples, like what done for the milk, were then refrigerated for 5 to 6 hours and the stored in the freezer at -20°C until extraction and analysis of the volatiles.

Goat cheese samples of the corresponding wheels were taken after 30 days and then stored in the freezer at -20°C until extraction and analysis of the volatiles.

For quality analysis of milk, each time three samples of goat bulk milk and both cow partially bulk skimmed milk and milking whole milk were collected in plastic vials with preserver (sodium azide) and refrigerated at 4°C for 1-2 days and finally A.R.A.L. laboratory (Crema, CR).

Determination of volatilome profiles was conducted by using Solid Phase Microextraction combined to Gas Chromatography Mass Spectrometry detection (SPME- GC/MS) according to Panseri et al, 2009 and Faustini et al., 2019. VOCs results were compared using parametric ANOVA with Tukey’s post hoc test.The significance threshold was set at $P < 0.05$.

For analysis regular samplings of both goat and cow milk were effectuated every two weeks along the period from March to August 2023; then on the months of October, November and December samples were collected only on bovine milk.

For volatiles analysis on milk and curd of both cow and goat samples were collected every three weeks from late March to late June, and then every two weeks until the end of July.

Goat cheese samples for volatiles analysis were taken in 5 dates, 2 in the lowland period (March and April) and 3 in the Summer period (June and July).

4 Results and Discussion

4.1 Milk Composition

4.1.1 Goat Milk Composition

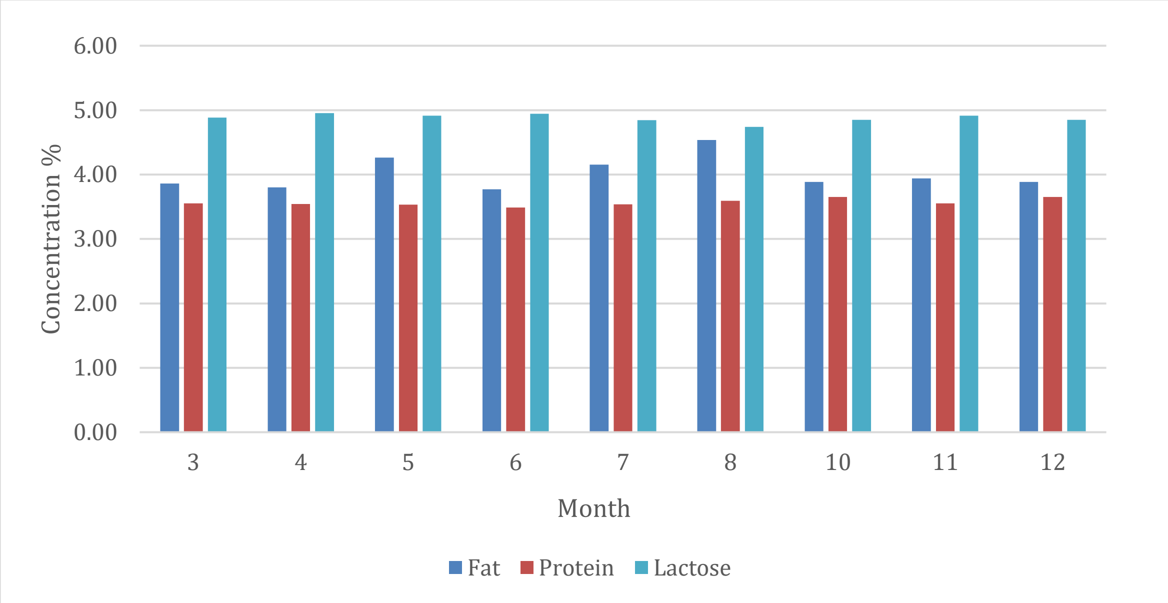
The overall means, standard deviations and ranges of the major goat and cow milk components are shown in Table 4.2., while goat and cow milk components per month are shown in Table 4.1.

Table 4.1: main goat and bovine milk components mean values on each month of the considered period divided into whole and skimmed milk.

MONTH	MILK						
	FAT (%)	PROTEIN (%)	CASEIN (%)	CASEIN (% PROTEIN)	LACTOSE (%)	UREA (mg/dL)	Somatic Cell Count (log10/mL)
GOAT							
3	2.63	3.33	2.53	76.0%	4.34	24.10	6.20
4	2.81	3.40	2.55	75.0%	4.34	40.60	6.16
5	2.53	3.36	2.56	76.0%	4.40	28.65	6.10
6	3.55	3.31	2.49	75.2%	4.22	30.80	6.21
7	3.67	3.34	2.53	75.6%	4.16	31.08	6.16
8	3.30	3.55	2.63	74.2%	4.10	34.87	6.36
COW							
3	3.86	3.55	2.79	78.4%	4.89	20.30	4.96
4	3.80	3.55	2.80	78.9%	4.95	21.53	4.71
5	4.26	3.53	2.80	79.1%	4.91	23.63	5.19
6	3.77	3.49	2.77	79.4%	4.94	23.97	4.86
7	4.15	3.54	2.79	78.9%	4.84	22.42	5.23
8	4.54	3.59	2.82	78.4%	4.74	20.87	5.61
10	3.89	3.65	2.87	78.5%	4.85	19.90	4.99
11	3.94	3.55	2.81	79.1%	4.91	22.72	4.92
12	3.89	3.65	2.87	78.5%	4.85	19.90	4.99

Goat milk components mean values fell within the general quantities reported by other studies on goats reared under experimental conditions in the same period of the year (Soryal et al., 2004) and resulted lower than the one reported by other studies conducted in mountainous areas (Sandrucci et al., 2018; Saha et al., 2019; Peña-Avelino et al., 2023; Kasapidou et al., 2023). Usually, Bionda dell'Adamello goat breed used in this study, as minor local breed has lower milk yield and consequently higher values of fat and protein were expected.

Figure 4.1: Mean values of fat, protein and lactose content concentration in bovine milk from March to December.



It is evident how the mean value of fat was lower than the mean value of protein (3.20% and 3.38% respectively). This was in accordance with several studies conducted in similar conditions, who reported notable to severe fat/protein ratio reversion, as known this is related to worsened cheese-making properties such as lower cheese yield and worse sensorial properties (Sandrucci et al., 2018; Soryal et al., 2004). This would be confirmed also by Sandrucci et al., 2018, who reported a significant correlation between fat/protein ratio reversion and milk with a Somatic Cell Count (SCC) above 5.8 log10/ml.

As said previously, the lactation stage affects main components of goat milk, particularly Sandrucci et al., 2018 and Kasapidou et al., 2023 reported that fat and protein values, after an increase in the first weeks of lactation, reached the lowest levels in the hottest season (July and August), and then were at the highest in the late lactation stage (September). Nevertheless, in this study only fat seemed to be considerably affected by the lactation period (range from 2.53% to 3.67%), while protein and lactose content showed only slight changes along the considered period. Highest values of fat in this study were recorded in the months of June and July, this is in contrast with Sandrucci et al., 2018 and Kasapidou et al., 2023, who found July and August as the months with lowest fat values.

Protein content was essentially unchanged throughout the period considered (mean value 3.38%) with the exception of August in which proteins were at 3.55%.

Table 4.2: Total mean values of main milk components. For bovine milk are presented the data of bulk milk, whole milk and skimmed milk.

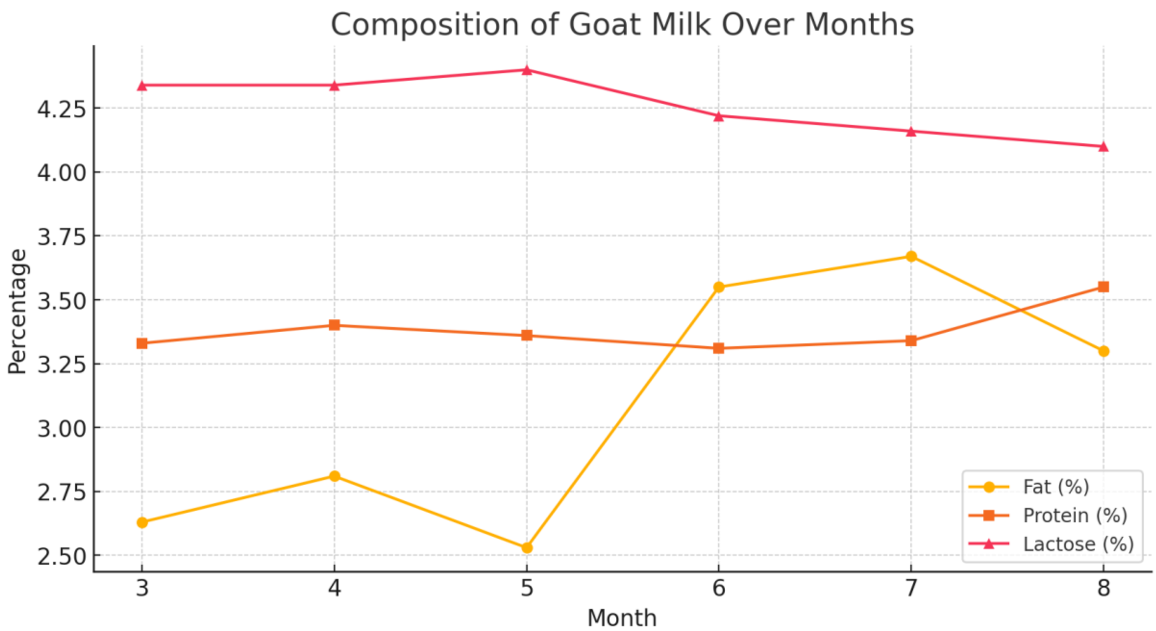
		FAT (%)	PROTEIN (%)	LACTOSE (%)	CASEIN (%)	CASEIN (% PROTEIN)	UREA (mg/dL)	Somatic Cell Count (log10/mL)	SKIM TOTAL SOLIDS (%)	TOTAL SOLIDS (%)
GOAT	**mean**	**3.20**	**3.38**	**4.24**	**2.54**	**75.3%**	**31.7**	**6.19**		
	SD	0.47	0.10	0.12	0.05	0.9%	5.0	0.08		
COW	**mean**	**3.12**	**3.63**	**4.91**	**2.87**	**79.1%**	**22.9**	**4.86**	9.24	12.35
	SD	0.89	0.14	0.13	0.12	0.7%	3.9	0.34	0.22	0.84
MILK	**mean**	4.06	3.56	4.88	2.81	78.9%	22.7	5.05	9.14	13.20
	SD	0.39	0.08	0.11	0.06	0.6%	3.3	0.34	0.12	0.39
SKIM MILK	**mean**	2.54	3.68	4.93	2.91	79.1%	23.1	4.75	9.30	11.84
	SD	0.56	0.15	0.13	0.13	0.7%	4.3	0.29	0.24	0.60

Notably this value is influenced by SCC, particularly high SCC make immunoproteins increase. Higher protein content enhances LAB activity, and so increasing milk’s acidifying properties.

According to Ambrosoli et al., 1988 casein content in goat bulk milk was found to be low and milk to have low coagulation capacity, consequently affecting cheese yield. This was evident compering our data on casein content in goat milk (75.3% of total protein content) comparing it with casein content in cow milk (79.1% of total protein content). The lower casein content in goat milk might have been a consequence of the genetic polymorphism related to the different caseins expression, namely αs1, αs2 and k-casein, which leads to different quantities of these caseins in different goat breeds (Amigo and Fontecha, 2011). This was confirmed by Ambrosoli et al., 1988, who compared milk composition and lactodynamographic parameters of two milks characterized by different levels of expression of αs1-casein, both in Alpine and Saanen goat breed; in the high type milk sample were registered higher concentration of total solids, casein, total protein and P content, and also higher scores for curd firmness, in both Saanen and Alpine breed. Curd firmness is associated with good whey sineresis and consequently good predisposition to cheese ripening. Moreover both Ambrosoli et al., 1988 and Barłowska et al., 2020

found that higher content of casein led to longer coagulation time (probably because of lower Ca ions availability) and firmer curd.
SCC resulted being quite stable during the considered period, with a mean value of 6.19 log10/ml. This was in contrast with Sandrucci et al., 2018, who found great variability in the SCC value. This might be related to the fact that in their study also a little percentage (about 8%) of the farms operated hand milking, which can be characterized by lower hygienic procedures. Sandrucci et al., 2018 also reported that goats belonging to small herds (less than 45 milking goats) and goats kidding in the first six months of the year were the least subject to have high SCC levels, compared with medium and large herds and with goats kidding in July or August.

Figure 4.2



Considering the transition from stable rearing to mountain pasture grazing, data were in contrast with what we expected. In this study there were not notable differences in milk protein content between the stable rearing period (from March to middle June) and the grazing period (from July to early September). This data were in line with Soryal et al., 2004, who reported no substantial variation in protein content in milk from 3 different experimental groups of goats (3.00% stable with hay and high concentrate, 3.04% grazing with high concentrates and 3.04% grazing with low concentrates). In the same study a slight decrease in protein content was noticed (2.84%) in milk from the grazing without concentrates group, while Peña-

Avelino et al., 2023 found a consistent decrease in protein content between the milk from two different experimental groups, one grazed in lowland pasture without supplement (3.84%) and one grazed in mountain without supplement (3.58%). This might lead to the importance of diet integrations to support the nutritional transition from stable rearing to pasture grazing.
Fat content, instead, was found to be increased in the grazing period comparing it with the stable rearing period (3.5% and 2.65% respectively). This was consistent with what reported by Peña-Avelino et al., 2023, who found fat content increasing from 4.38% in lowland pasture milk, to 4.6% in mountain pasture milk. This evidence can be easily related to the higher activity level of animals in mountain pasture, which are characterized by high fiber and lower energy availability, so leading to higher levels of lipid mobilization (Chilliard et al., 2003). Also, it has been demonstrated how milk fat content is positively correlated to the quantity of dietary lipid intake, so this might also suggest that fat content increasing is related to lipid content of grass. Still must be taken into account that fat is supposed to be higher in the late stage of lactation, and this was confirmed by several studies (Sandrucci et al., 2018; Kasapidou et al., 2023; Peña-Avelino et al., 2023), who generally reported high values at the beginning of the lactation, followed by a decrease in the months of July and August, to reach the highest values in September.
Lactose content was recorded to be decreased in the pasture grazing period compared to the stable rearing period (4.36% mean value vs 4.16% respectively); this was in accordance to what Soryal et al., 2004 found, in fact in their study the group which followed a no-concentrates diet produced the milk with the lowest lactose content value (4.01% in grazing without concentrates group vs 4.18% in grazing with high concentrates group). Generally, lactose was found to be the highest at the end of lactation, with minimum values in the months of June and July (Sandrucci et al., 2018; Soryal et al., 2004), but Kasapidou et al., 2023 reported decreasing values of lactose along the lactation.

4.1.2 Cow Milk Composition

A summary of the functional controls of individual cows is shown in Table 4.3.

Mean values of whole cow milk in the whole considered period (from March to December 2023) were comparable with similar studies conducted in mountainous areas (Bovolenta et al., 2008; Tata et al., 2022).
Protein content was quite stable all along the studied period (minimum value was 3.49% and maximum value was 3.65%), this fact was in contrast with Saha et al., 2019 who reported a constant increase of protein content throughout the lactation period in the experimental group hold in permanent farm, and a slight decrease followed by a constant increase, reaching the maximum value in October in both experimental groups. Also Farruggia et al., 2014 found a significant increase of protein content in relation with both the period of the year and the type of pasture management, reporting a considerable increase in protein content of milk from rotational grazing cows, particularly in the last period (early August to late September). In this last case this variability might be due to the absence of supplemental nutrients other than sward.

Table 4.3: Test-day milk yield of individual cow milk reported as mean values of each component per month.

MONTH	Mean MILK	Mean YIELD	Mean FAT	Mean PROTEIN	Mean SCS	Mean EVM MILK
2023	**27.3**	**157**	**4.09**	**3.71**	**4.73**	**7595**
1	26.3	26	4.15	3.80	4.75	7133
3	27.4	27	3.87	3.46	4.58	7333
4	30.6	27	4.42	3.89	4.57	7765
5	31.9	26	3.99	3.55	4.72	8214
6	27.4	17	4.03	3.75	4.71	7944
10	22.3	16	3.90	3.62	4.99	7550
12	21.1	18	4.18	3.98	4.93	7235
2024	**28.7**	**91**	**3.98**	**3.68**	**4.74**	**7535**
1	26.3	13	4.06	3.66	4.94	7355
2	28.4	26	4.00	3.75	4.77	7310
3	31.6	25	3.76	3.48	4.60	7841
4	27.4	27	4.12	3.83	4.73	7546
Total	**27.8**	**248**	**4.05**	**3.70**	**4.73**	**7576**
Average 1 Mar23-Jun23	28.7	24.60	4.09	3.69	4.67	7678
Average 2 Oct23-Apr24	26.2	20.83	4.00	3.72	4.83	7473

The fat content of ruminant milk is influenced by a combination of intrinsic factors—such as species, breed, genotype, pregnancy, and stage of lactation—as well as extrinsic factors, particularly environmental and nutritional conditions (Leiber et al., 2014; Ianni et al., 2020). Within a given species, the effects of breed and genotype on milk fat are significant but relatively limited, as they require long-term genetic selection to induce notable changes (García-Martínez et al., 2009).

The stage of lactation also plays a crucial role in modulating milk fat content and fatty acids composition, particularly in early lactation when cows mobilize body fat reserves to compensate for the energy deficit associated with peak milk production (Kilcawley et al., 2018). However, this effect is temporary, typically lasting only a few weeks each year.

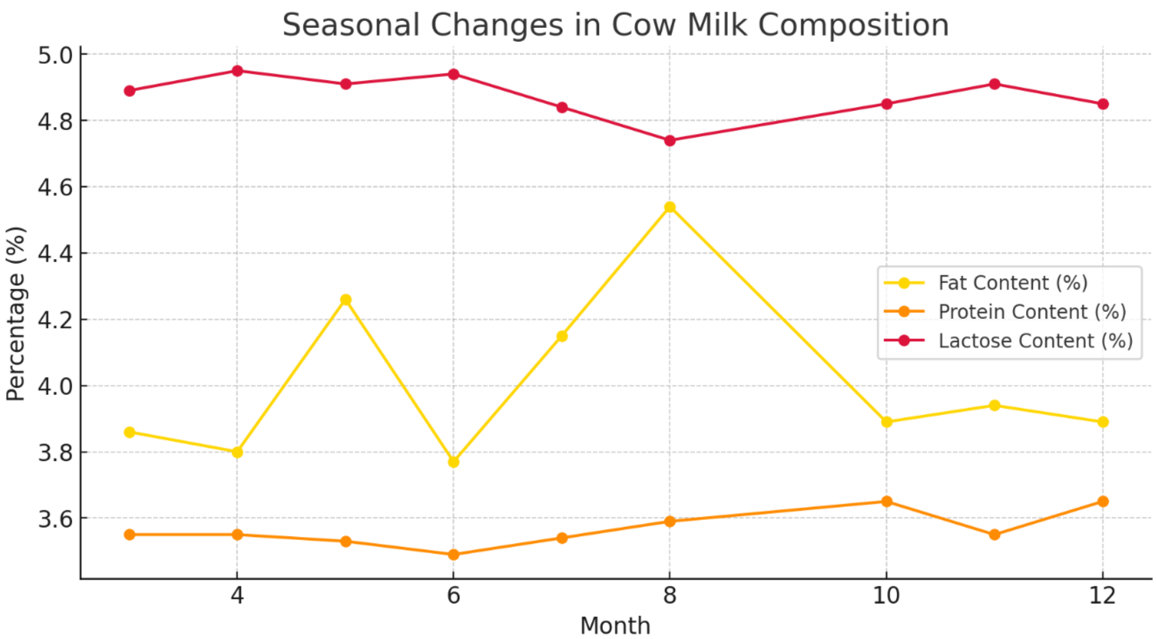
In contrast, seasonal variations in milk are much more pronounced and are primarily driven by dietary changes (Farruggia et al., 2014; Coppa et al., 2011). Due to its rapid and cost-effective impact, nutrition remains one of the most efficient strategies for farmers to modulate milk fat content. The most significant changes can be achieved by altering forage sources, particularly through the inclusion of fresh pasture, which is richer in alpha-linolenic acid (Chilliard et al., 2003).

Fat content in this study was highest in May (4.26%), July (4.15%) and August (4.54%). Curiously these months were also the ones with the highest scores for somatic cells, so there might be a positive correlation between fat content and SCC, fact that was reported by Niero et al., 2018 as well. Anyway this fat content trend is confirmed by Saha et al., 2019, who reported July and August to be the months with the highest fat content mean values. Farruggia et al., 2014 reported the same trend for fat and protein content, finding a higher correlation with period of the year then for type of system (minimum 39.0 and 37.9, maximum 42.6 and 39.4 g/Kg respectively for low and high LU/ha), this would confirm the trend to be more related to the lactation stage than to environmental or management factors. Nevertheless this was in contrast with Bovolenta et al., 2008, who found a significant correlation only between fat content and supplement level. Another fact must be underlined: in our study the months of July and August, which reported higher levels of fat content, correspond to the time in which cows have been grazing in the steepest slopes, presumably causing higher fat mobilization (Niero et al., 2018), hence higher fat content values in the milk might also be explained by management factors.

SCC mean values were quite stable along the considered period, with the exceptions of values registered in May, July and August (as mentioned before), with a total mean score of 5.05 log10/ml. Saha et al., 2019 reported a noticeably lower total SCC mean value (3.5 log10/ml) than the one registered in our study, and the mean values per month were increasing esponentially for the stable experimental group from July (slightly over 1.5 log10/ml) to October (over 3 log10/ml), and stable around 4 log10/ml for the grazing experimental group. Bovolenta et al., 2008 reported a significant correlation between SCC and stocking density, particularly Brown cows grazing with a 0.7 LU/ha had significantly higher SCC values than Brown cows grazing with a 1.4 LU/ha; in our study there was not strict stocking density control (fences were moved each week to give the cows access to additional portion of the pasture), so findings from Bovolenta et al., 2008 might suggest that morphology of the terrain could have brought to a change in the activity of the cows and so to an increase in the stocking density (and so the SCC values) in the months of July and August.

Lactose is notably the least component affected by environmental, management or lactation factors, and this was confirmed by several studies similar to ours (Bovolenta et al., 2008; Niero et al., 2018; Tata et al., 2022), who reported similar total mean values to the ones registered in our study.

Figure 4.3



Transhumance to summer alpine pastures significantly influences the physiological, social, feeding, and nutritional status of cows, leading to variations in milk yield and composition. The transition from indoor stabling to outdoor grazing involves a shift from a controlled diet with constant rations to a pasture-based diet, which is often characterized by lower nutritional value, high fiber content, and seasonal fluctuations in forage availability (García-Martínez et al., 2009; Farruggia et al., 2014). Additionally, cows increase their physical activity due to long-distance walking on steep slopes, which leads to greater energy expenditure and may limit grass intake, thereby affecting their overall nutritional status (Leiber et al., 2014; Pauler et al., 2024). Several studies have associated alpine transhumance with a reduction in voluntary feed intake, which can result in lower energy availability for metabolic processes (Farruggia et al., 2014; Coppa et al., 2011; Ianni et al., 2020). Most notable data is the fat content, since this component was higher by 5.3% in the grazing period compared to the stable rearing period. This finding is in accordance with several studies who reported a significant increase in fat content in comparing milk from grazing cows to milk from stable reared cows (Niero et al., 2018; Tata et al., 2022; Saha et al., 2019). In relation to the pastoral value, we can assume that herbs were richer in PUFA, that is the metabolic basis for fatty acids biosynthesis in the gastro-intestinal tract of cows, and consequently milk resulted in having higher fat content value.

The negative impact of alpine pasturing on milk protein content has been widely documented, with studies suggesting that factors such as hypoxia at high altitudes and nutrient deficiencies may contribute to this decline, despite increasing supplementation with concentrates (Farruggia et al., 2014; Ianni et al., 2020; Coppa et al., 2011). Similarly, a reduction in lactose content following transhumance to summer alpine pastures has been reported by multiple authors (Bergamaschi et al., 2015; Valdivielso et al., 2016), although some studies indicate no significant variation in lactose concentration. Protein content and lactose content weren't particularly affected by the switch to grazing management, they were decreased by 1.1% and 1% respectively. Niero et al., 2018 and Tata et al., 2022 reported comparable results, with slight increase of protein content (+1.8% and +2%) and no changes in lactose content. Saha et al., 2019 had even greater losses in protein content during the grazing period, confirming that transhumance might affect milk components even with supplemental feedings. Anyway, in our study also urea content (which is an useful indicator of the correct level of supplementation of the diet with proteic feedings) remained substantially constant, suggesting that supplemental feedings were properly managed.

Finally SCC resulted quite increased in the grazing period (+4.8%), which was confirmed by Saha et al., 2019, who reported similar data comparing grazing cows' milk to permanent farm cows' milk and finding then similar SCC values in October, when grazing cows were back in stable rearing conditions.

4.2 Volatile Compounds

All volatile compounds detected in goat and cow milk and curd, and in goat cheese, are shown in tables 4.1, 4.2, 4.3, 4.4 and 4.5, all of them are reported as a proportion of the standard compound that was used as column of injection, with a chosen value of 400.

4.2.1 Goat Milk

A total of 32 compounds was detected in goat milk, these are divided into 7 categories: aldehydes, ketones, esters, alcohols, terpenes, free fatty acids and miscellanea.

Among non-terpenes compounds, most abundant category was the one of ketones. This is in accordance with other studies, even if such great concentration difference with other categories wasn't reported (Faulkner et al., 2018; Coppa et al., 2011; Faustini et al., 2019). Non-terpene compounds were quite affected by the rearing management type, with particular concentration changes in 12 volatile compounds, namely: 3-methyl-Butanal, Hexanal, 2-Propanone, 2,3-Butanedione, 3-Hydroxy-2-Butanone, Acetic acid-Ethyl Ester, Butanoic acid methyl ester, 3-Methyl-1-Butanol, Acetic acid and Dimethyl ester.

All the categories, except for terpenes, were negatively affected by the switch to mountain pasture, this might be related to high volatility of certain compounds and so loss of these due to longer times occurring from milking to cheesemaking during the pasture period. Nevertheless this loss in terms of concentration is not necessarily a loss in terms of sensorial characteristics and acceptability, since transformations processes occurring in lipids and proteins during cheesemaking and ripening lead to the formation of new volatile compounds (along with the increasing in concentration of them due to water losses), also considering different odour detection thresholds of the aromas. High variability of non-terpene volatile compounds in cow milk —along with diet, livestock management type and seasonality— was reported by many studies (Bugaud et al., 2001; 2011; Faulkner et al., 2018; Faustini et al., 2019), nevertheless Coppa et al., 2011 and Kilcawley et al., 2018 found changes in a smaller part of the total detected non-terpene volatiles.

Aldehydes

Aldehydes, having a low odour detection threshold, are recognized to have a great impact on the overall sensorial characteristics of milk and even more of cheese (Kilcawley et al., 2018).

Most affected aldehydes were 3-methyl-Butanal and Hexanal, the least wasn't detected at all in lowland goat milk. Short chain aldehydes like Hexanal are recognized to be the result of lipid oxidation, particularly unsaturated fatty acids like linoleic acid. This would confirm the presence of Hexanal in pasture milk and absence in the lowland one, since fresh pasture in notably richer in PUFA and MUFA (Kilcawley et al., 2018). Heptanal is a short chain aldehyde as well, it was detected with very low concentration in lowland goat milk, its concentration was higher in pasture milk but still the increase was not significant. It must be noted that Faulkner et al., 2018 found higher concentrations of Hexanal in milk from cows fed concentrates than in pasture milk, this corresponding with higher levels of oleic acid and linoleic acid in concentrate feeding. Considering that alcohols derive from the corresponding aldehydes through oxidation process, it would be expected to find progressively higher concentrations of these alcohols and decreasing concentrations of short chain aldehydes with the ongoing of the ripening of cheese. 3-methyl-Butanal was detected in much greater concentration in lowland milk than in pasture milk. Faustini et al., 2019 found that seasonality impact was more relevant than differences in feeding types, differences which were mainly constituted by the quantity of hay (minimum 50% for Parmigiano Reggiano milk and no minimum level for Grana Padano milk). 3-methyl-Butanal is a branched chain aldehyde, which formation is related to the metabolism of amino acids, particularly Leucine, this might lead to a possible higher concentration of this free amino acid in the diet during the lowland period (Kilcawley et al., 2018).

Ketones

Greatest part of ketones, like aldehydes, creates through oxidation of PUFA and MUFA, so the expectations would be to find these volatile compounds in higher concentration in pasture milk. This was not confirmed, since most of the volatiles in this category were negatively affected by pasture management. This was the case only for 2-Propanone, which was the most affected by the switch from lowland to pasture together with 2,3-Butanedione, 3-hydroxy-2-Butanone and 2-Butanone. Contrarily 2,3-Butanedione was found in higher concentration in lowland milk, decreasing significantly in pasture milk, and this was reported for all the other ketones but 2-Propanone. This is in contrast with Faustini et al., 2019, who reported a significant correlation between 2,3-Butanedione quantity and livestock management system, in particular found lower concentration of this volatile compound in milk from cows fed mainly with concentrates and with low fraction of hay. 2-Butanone and 3-Hydroxy-2-Butanone were detected in considerably higher concentration in the milk from hay and concentrate feeding, this is in accordance with several studies which reported the same (Kilcawley et al., 2018). This could be explained by the fact that 2-Butanone and 3-Hydroxy-2-Butanone are formed by carbohydrate metabolism, and notably concentrates and hay have higher carbohydrate/protein ratio than sward. This is confirmed by Faulkner et al., 2018, who found much less concentration of 3-Hydroxy-2-Butanone in milk derived from sole grass fed cows, while Faustini et al., 2019 reported not significant changes of concentration between Grana Padano milk and Parmigiano Reggiano milk.

Esters

Both esters detected, Acetic acid ethyl ester and Butanoic acid methyl ester were substantially decreased by the switch to pasture feeding. Faulkner et al., 2018 confirmed what reported on Acetic acid ethyl ester, reporting that it was undetected in milk from cows grazing without supplements, and maximum concentration of it in TMR (Total Mixed Ration) milk. Faustini et al., 2019, comparing milk from hay and concentrate feeding with milk from mostly hay feeding, didn’t find much variability in concentration of Butanoic acid methyl ester, finding it more correlated with seasonality than to management system and diet. Several studies reported instead higher concentrations of esters in the pasture milk (Coppa et al., 2011; Kilcawley et al., 2018). The biochemical paths bringing to esters formation are mainly two: esterification and alcoholysis. The first interests alcohols and carboxylic acids, the second involves alcohols and acylglycerols or Acyl-CoAs from, deriving essentially from the metabolism of lipids. Origin of esters was studied and it was found that this category of volatile compounds can be transferred directly by the plants, or also formed during insiling through biosynthesis through fermentation, so this could explain both results (Kilcawley et al., 2018).

Alcohols

All of three alcohols detected in goat milk were primary alcohols, which generally derive from the corresponding aldehydes, which are easily reduced to acid and subsequently to alcohols (Kilcawley et al., 2018). 3-Methyl-1-Butanol was particularly negatively affected by the pasture feeding and management; must be noted that this was probably deriving from 3-methyl-Butanal, which went through a decrease in concentration comparable to the corresponding alcohol, we could expect an increase in 3-methyl-1-Butanol along with the cheesemaking process and following ripening process. Still aldehydes have a lower odour detection threshold and consequently a higher odour activity, so they might remain of greater importance even if present at lower concentrations.

Ethanol was also considerably decreased in pasture milk in comparison with lowland milk, this is in accordance with some studies which reported higher presence of ethanol in concentrate fed livestock (Kilcawley et al., 2018). This might be due to high concentration of ethanol in silages as a consequence of microbial fermentation, suggesting a direct transfer from feed to milk. Nevertheless this is not confirmed by other studies which found no correlation between ethanol concentration and feeding (Faustini et al., 2019; Faulkner et al., 2018).

2-Methyl-1-Propanol wasn't much affected by the management change, and this was confirmed by Faustini et al., 2019, who reported a constant concentration.

Free Fatty Acids

Carboxylic acids are known to be very important in the formation of dairy products flavor profile, and particularly cheese sensorial properties. These compounds can originate by several biochemical paths, namely lipid oxidation, carbohydrate or amino acid metabolism or even through direct transfer from feed to milk. Often they are the most abundant category among the volatile compounds. This was not the case since acids were characterized by quite low concentration in goat milk, with the exception of Acetic acid. This was long way the most interesting result, in fact it was almost 25 times lower in pasture goat milk than in lowland goat milk. Faulkner et al., 2019 found similar data on Acetic acid in TMR fed cow milk comparing it with grass and grass/clover fed cow milk. This data might be explained by a great intake of this acid directly through the supplement feedings, due to microbial fermentation of insilates, or even by rumen carbohydrates fermentation (being the winter diet characterized by higher content of sugars) or by lipid oxidation (Kilcawley et al., 2018).

All other volatile compounds within this category were present in lower or same concentration in pasture milk than in lowland milk. Other volatile acids significantly negatively affected by the switch from confined rearing to mountain pasture were Butanoic acid and 3-methyl-Butanoic acid. This is in contrast with Coppa et al., 2011, who found progressively higher concentrations of Butanoic acid in hay fed, rotationally grazing and continuously grazing cow milk, while is in accordance with Faulkner et al., 2018, who reported higher quantities of Butanoic acid in the TMR feeding regime milk. Butanoic acid aroma is described as goaty, with positive or negative impact depending on the type of dairy product, this is one of the characterizing flavors of goat milk and cheese. Anyway it’s a substrate from which several other volatile compounds can form through oxidation.

Butanoic acid is neo-synthesized in the rumen, since goat milk fatty acid profile changes much under lipids supplements to the diet, probably the change in Butanoic acid concentration between lowland and pasture milk is due to lipid profile of feeding (Chilliard et al., 2003).

3-Methyl-Butanoic acid was detected with higher concentration in lowland milk, this was in accordance with Faulkner et al., 2018, who reported far higher concentration of this volatile in the TMR regime milk. This acid is originated from amino acid metabolism or from reduction of 2-methyl Butanal, even if other studies proposed the possibility of a direct transfer from the diet of both acetic, butanoic and 3-methyl butanoic acids (Kilcawley et al., 2018). So concentration of this carboxylic acid, as well as of other branched chain fatty acids, might be related to easy digestible protein availability in the feeding.

Table 4.4: Volatile compounds detected in bulk goat milk according to their chemical classes.

		Lowland		Pasture	
R.T.	**Compounds***	**average (n=7)**	**s.e.**	**average (n=9)**	**s.e.**
	Aldehydes				
3.31	3-methyl-Butanal	113.58	14.65	18.44	2.17
8.6	Hexanal	n.d.	-	6.92	1.14
13.62	Heptanal	0.02	-	0.63	0.07
23.57	Benzaldehyde	1.34	0.25	0.24	0.04
tot		**114.94****		**26.24**	
	Ketones				
2.22	2-Propanone	219.25	17.16	602.59	20.03
3.03	2-Butanone	8.5	2.83	2.49	0.19
4.49	2-Pentanone	2.93	0.16	0.63	0.07
4.67	2.3-Butanedione	25.59	5.24	4.23	0.63
7.85	2,3-Pentanedione	n.d.	-	0.7	0.13
13.5	2-Heptanone	1.43	0.22	0.37	0.01
17.36	3-Hydroxy-2-Butanone	670.51	160.78	44.67	7.23
tot		**928.22****		**655.68**	
	Esters				
2.34	Acetic acid. methyl ester	n.d.	-	n.d.	-
2.92	Acetic acid. ethyl ester	17.47	3.3	2.14	0.28
4.84	Butanoic Acid. methyl ester	43.58	10.68	0.52	0.06
tot		**61.05****		**2.66**	
	Alcohols				
3.65	Ethanol	59.26	6.97	38.95	1.33
10.17	2-Methyl-1-propanol	4.79	0.32	7.07	0.31
15.09	3-methyl-1-butanol	83.44	11.82	27.17	3.96
tot		**147.5****		**73.18**	

	Terpenes				
5.79	α-Pinene	n.d.	-	126.6	8.95
7.41	Camphene	n.d.	-	6.73	0.57
9.19	β-Pinene	n.d.	-	5.48	0.28
11.3	Limonene	n.d.	-	0.73	0.05
25.31	trans-Caryophyllene	n.d.	-	0.13	0.01
25.47	Patchulen	n.d.	-	n.d.	-
tot				**139.67****	
	Free Fatty Acids				
21.82	Acetic acid	235.51	25.4	9.6	0.77
23.96	Propanoic acid	0.92	0.21	0.18	0.01
24.65	2-methyl-propanoic acid	3.14	0.68	0.23	0.03
25.88	Butanoic acid	16.12	2.23	3.67	0.42
26.74	3-methyl-butanoic acid	8.77	1.32	0.79	0.09
28.04	Pentanoic acid	0.04	0.01	0.05	-
30.05	Hexanoic acid	1.97	0.33	0.68	0.06
32.87	Octanoic acid	0.25	0.03	0.13	0.01
tot		**266.72****		**15.33**	
	Miscellanea				
1.88	Dimethyl sulfide	146.67	52.53	45.28	7.34
28.29	Acetamide	0.11	0.01	0.09	0.01
30.83	Dimethyl sulfone	8.34	0.81	7.79	0.5
tot		**155.12****		**53.16**	

*=data are expressed as ng ml-1 of internal standard equivalents; means with (**) are significantly different ($p < 0.05$).

Miscellanea

Of the three volatiles detected in this category (Dimethyl sulfide, Acetamide and Dimethyl sulfone) only Dimethyl sulfide was affected by the switch from lowland to mountain pasture. Contrarily, Faustini et al., 2019 found the presence of this sulfurous compound related to seasonality rather than to diet, while Faulkner et al., 2018 didn't report any change in dimethyl sulfide along with diet regime changes. This volatile compound is originated from methionine and cysteine metabolism in the rumen, so its presence in goat milk might be due to high protein/non fibrous carbohydrates ratio.

Terpenes

This is the only category where we found total absence of compounds in lowland milk and instead a considerable concentration in pasture milk. Particularly the most represented compound was α-Pinene, followed by Camphene and β-Pinene and then, with not considerable concentration, Limonene and Trans-Caryophyllene.

Influence of dietary regime on terpenes concentration in milk is confirmed for most of the terpene compounds by many studies (Ungureanu-Iuga et al., 2024; Coppa et al., 2011; Abilleira et al., 2010; Faulkner et al., 2018; Kilcawley et al., 2018).

Monoterpenes were the most represented and most abundant in the category of terpenes compounds with 99.9%, and α-Pinene accounted for 90.6% of total terpenes. Even if this unbalance wasn't as high as in our study, higher abundance of monoterpenes is confirmed by other studies. Terpenes are characterized by higher odour detection threshold, so in this case this category is probably not contributing much to the overall sensorial properties of milk, since concentration of the compounds is quite low comparing it to other categories with lower odour detection threshold (i.e. aldehydes).

Abilleira et al., 2010 detected monoterpenes in both hay, TMR and grass fed sheep milk while they didn't detect sesquiterpenes but in grass fed sheep milk. Kilcawley et al., 2018 instead reported higher sesquiterpene/monoterpene ratio in highland milk (Mariaca et al., 1997). Anyway this data couldn't be analysed in our study since no terpenes were detected in lowland milk. This is probably due to hay quality, since concentration of terpenes in hay is strictly related to plant species (monocotyledons terpene content is lower than dicotyledons') and stocking conditions. Also such unbalanced sesquiterpenes/monoterpenes reported in goat milk might be related to species composition and phenological stage of single patches in which animals grazed in the period of sampling (considering that terpenes disappear in milk within few days if not present in the diet, and in this study milk sampling was effectuated every 2 weeks) (Kilcawley et al., 2018; Mariaca et al., 1997).

About terpene fraction composition, Kilcawley et al., 2018 and Coppa et al., 2011 found β-Pinene and Limonene has the most common and most abundant in both fresh grass, hay and silage fed cows milk; this is contrast with our finding, having detected Limonene and β-Pinene in far lower concentration than α-Pinene.

4.2.2 Goat Curd

Volatiles detected in goat curd are listed in Table 4.2.

A total of 33 volatile compounds was detected, while in goat milk 32 were found. Also some new compounds were found in curd that were not present in the milk, namely dimethyl sulfoxide, patchoulen, heptanal, 1-butanol-3-methyl acetate; while others present in milk weren't detected in curd: propanal, 2,3-pentanedione, 2-butanone.

General concentration of volatiles was higher in the curd than in the milk, with the exception of miscellanea (which registered an overall decrease) and ketones during the pasture period (which decreased in comparison to pasture milk). This was the same trend followed by volatile profile of cow milk through the process of cheesemaking until the forming of the cheese masses, with the exception of ketones that were significantly incremented from cow milk to cow curd, and aldehydes that were halved in cow curd. The main differences between cow and goat cheesemaking processes are that goat bulk milk is whole milk, while cow's is skimmed, and that goat curd wasn't brought over 40°C, while cow curd reached about 50°C.

Aldehydes

Most abundant compound was by far 3-methyl-Butanal, and the same was registered for cow curd, and confirmed also by several studies (Sulejmani and Hayaloglu, 2016; Sahingil et al., 2014).

Both in pasture and lowland goat curd 3-methyl-Butanal had an increment in concentration of about 10 times comparing it with goat milk. This might be explained by the fact that this compound can originate through reduction of branched chain fatty acids, which notably are more abundant in goat milk than in other species' milk (Amigo and Fontecha, 2011; Kilcawley et al., 2018). Also the addition of rennet might have enhanced the proteolysis, increasing amino acids substrates from which 3-methyl-Butanal can originate (Kilcawley et al., 2018).

Benzaldehyde wasn't affected at all by the cheesemaking process. Hexanal wasn't affected in by the cheesemaking process during the lowland period, while it increased considerably in the pasture period. It is interesting to notice that in cow curd hexanal was not detected, even if it was present in considerable concentration in pasture cow milk; this might lead to think that temperature of curd heating had a role in bringing to this result, like also suggested by Sulejmani and Hayaloglu, 2016. Propanal was present only in goat curd, and it was doubled in concentration in the pasture period. This could be probably explained by the higher values of fat content in the pasture period, since Propanal derives from lipid oxidation (Kilcawley et al., 2018).

Ketones

Ketones followed a similar trend to the one we saw in cow milk between lowland and pasture period, with the exception of 2-Pentanone and particularly 3-Hydroxy-2-Butanone, which in cow curd constituted by far the most abundant volatile compound for both lowland and pasture period. In particular the highest concentration of 3-Hydroxy-2-Butanone in cow curd was recorded in the pasture period, while in goat curd concentration of this compound dropped drastically. This volatile is mainly derived from carbohydrate or amino acid metabolism, hence we might assume there were substantial differences between goat and cow diet during the pasture period, probably goats couldn't reach a satisfying intake of protein during this period (Kilcawley et al., 2018). Contrarily Valdivielso et al., 2016 found that 3-Hydroxy-2-Butanone reached maximum concentration in sheep cheese produced during the period of early mountain grazing (June). Also Sulejmani and Hayaloglu, 2016 didn't find any correlation between 3-Hydroxy-2-Butanone content and curd heating temperature, leading us to exclude the influence of this factor on the differences between cow and goat curd. However 3-Hydroxy-2-butanone followed the same trend along with switch from lowland to pasture in both goat milk and curd. It remained at the same concentration in lowland milk and curd, and was increased in both milk and curd but in lower proportion in the curd. Anyway this increment in pasture period goat curd was confirmed by Bovolenta et al., 2014, who found higher the highest concentration of 2-Propanone in Montasio ripened cheese produced with energy-poor pasture. 2-Propanone was expected to increase during the cheesemaking process due to water losses through whey, the fact that it remained stable during the lowland period and even decreased in pasture period might be attributed to a reduction of this compound to form 2-methyl-1-Propanol, which increased considerably during the cheesemaking process (Ianni et al., 2020).

2-Pentanone and 2-Heptanone apparently weren't affected by cheesemaking process, having a slight increase from goat milk to curd both in lowland and pasture period.

2,3-Butanedione was found in much higher concentration in lowland goat curd than in pasture goat curd. We registered the same in goat milk, but with a lower increment from lowland to pasture. Bovolenta et al., 2014 confirmed this trend, reporting higher concentration of 2,3-Butanedione in ripened Montasio cheese from high supplement levels to diet; nevertheless they also found high concentration of 2,3-Butanedione in the same type of cheese from energy-poor pasture. Aprea et al., 2016 reported the same result, but they didn't find any correlation with the supplement level.

Esters

Acetic acid ethyl ester was the most abundant ester in both pasture and lowland goat curd. This compound had a greater increment along with the cheesemaking process during the lowland period, while during pasture period there was only a slight increment. Kilcawley et al., 2018 confirmed this result, reporting a strong correlation between acetic acid ethyl ester and hay diet.

Butanoic acid methyl ester wasn't affected at all by cheesemaking process in the pasture period, since was detected in very low concentration in both goat milk and curd. Instead in the lowland period it was found at considerable concentration in goat milk and wasn't detected at all in goat curd.

1-Butanol-3-methyl acetate was detected only in lowland goat curd, this would be confirmed by the results of Sulejmani and Hayaloglu, 2016, who reported strong positive correlation between 1-Butanol-3-methyl acetate and curd heating temperature. We assume that the process of esterification occurring between acetic acid and 3-methyl-1-Butanol was favoured by higher temperatures reached during bulk milk heating at the beginning of cheesemaking process.

Alcohols

Alcohols were particularly affected by cheesemaking process, particularly in the lowland period, reaching by far higher concentrations in this period.

Ethanol had only a slight increase of concentration in both lowland and pasture period.
3-Methyl-1-Butanol was much increased in goat curd in comparison with goat milk. This might be explained by a parallel increase in 3-methyl-butanal, which possibly brought to the formation of the corresponding alcohol through reduction to 3-methyl-butanoic acid, which in turn reduced to 3-methyl-1-butanol (Kilcawley et al., 2018).
2-Methyl-1-Propanol was also much affected by cheesemaking process, particularly in lowland period increased by about 90 times from milk to curd, while in the lowland period increased by about 18 times. In this case enzymatic proteolysis might have had an important role, increasing the free amino acids content in the milk, which in turn might have been transformed into corresponding aldehyde which then reduced until the formation of 2-methyl-1-propanol.

Free Fatty Acids

Overall free fatty acids content in goat milk increased along with the cheesemaking process; most abundant acids, as for goat milk, were acetic acid and butanoic acid. Our results were in contrast with what Sulejmani and Hayaloglu, 2016 and Bovolenta et al., 2014, who found free fatty acids to be the most abundant category in 1 day ripened cheese and in long ripened Montasio cheese respectively. However Suljmani and Hayaloglu, 2016 confirmed acetic acid and butanoic acid to be the most abundant free fatty acids present in 1 day ripened cheese.
Acetic acid was the most affected by the cheesemaking process, and it increased particularly in the pasture period. This might be due to modifications in the serum fermentation in the pasture period, caused by different microorganisms present in the environment, leading to heterolactic fermentation during the resting time of the bulk after ferment addition and so higher concentrations of acetic acid (Sahingil et al., 2014). Must also be noted a stable concentration of Propanoic acid, which would lead us to exclude possible propionic fermentation as cause of the acetic acid increment in pasture goat curd (Ianni et al., 2014).
2-Methyl-propanoic acid appeared to be slightly decreased, particularly in lowland period, by the cheesemaking process. This might have been caused by its reduction to 2-methyl-1-propanol (Kilcawley et al., 2018).

We can observe a similar increment in butanoic acid in both goat and cow curd comparing them with corresponding milk. Probably this concentration increment was due to whey losses. Also, since butanoic acid can be originated by lipolysis, goat milk in the pasture period might have been more affected by enzymatic lipolysis after rennet addition in consequence of a higher presence of short chain fatty acids deriving from PUFA metabolism.
3-Methyl-butanoic acid was slightly increased, particularly in the pasture period. Kilcawley et al., 2018 hypothesized a direct transfer of 3-methyl-butanoic acid from diet to milk, even if this assumption is not very diffused in the studies.
Pentanoic and octanoic acids were substantially unchanged between goat milk and curd, remaining present in very low concentrations in both lowland and pasture periods.
Hexanoic acid was found in higher concentration in goat curd than in milk, yet concentration was lower in the pasture period. This would be in accordance with Kilcawley et al., 2018, who didn't detect hexanoic acid in cheese produced in the summer period, and also reported a possible correlation between silage diet and hexanoic acid presence. They also reported hexanoic acid as one of the most abundant acids in cheese together with acetic acid and butanoic acid.

Miscellanea

This category showed an overall decrease in concentration, but focusing on the single volatile compounds we can observe that acetamide and dimethyl sulfone weren't affected by cheesemaking process. We can observe instead the neo-formation of dimethyl sulfoxide, while dimethyl sulfide, contrarily of what happened in cow curd, decreased drastically along with cheesemaking process. Bergamaschi and Bittante, 2018 reported large amount of dimethyl sulfone (which originates from dimethyl sulfide oxidation) in ricotta produced from whey after cheesemaking, this would confirm a dilution of this compound from the milk through whey losses.

Terpenes

Overall terpenes concentration increased from goat milk to curd, and terpenes detected were the same found in milk plus patchoulen which wasn't present in milk. In particular this overall increase can be attributed to specific increment of camphene and limonene, while α-Pinene concentration remained stable and β-Pinene decreased. Camphene higher concentration after cheesemaking process was observed in cow curd as well, where contrarily β-Pinene increased.

Ungureanu-Iuga et al., 2024 reported higher concentrations of terpenes in cheese than in milk, confirming our results, even if results from goat cheese are of higher interest in the certification perspective. They also suggested limonene might be a product of biodegradation of sesquiterpenes by bacteria, which would explain higher limonene concentration in curd after serum fermentation addition. Bergamaschi and Bittante, 2018 also noted high correlation between skimming and limonene presence, result that might explain the absence of limonene (notably the most common terpene in all types of feeding according to Kilcawley et al., 2018) in cow milk.

Kilcawley et al., 2018 underlined the potential of biosynthesis and degradation of terpenes by both rumen and milk microflora, particularly reported degradation of β-Pinene during incubation with ruminal liquid, with neo-formation of camphene among the others. Hence we can't exclude the possibility of partial bioconversion of β-Pinene in Camphene.

Table 4.5: Volatile compounds detected in goat curd according to their chemical classes.

R.T.	Compound*	Lowland average (n=7)	s.e.	Pasture average (n=9)	s.e.
	Aldehydes				
2.07	Propanal	3.71	1.04	7.32	1.02
3.31	3-methyl-Butanal	981.59	122.16	288.6	37.19
8.6	Hexanal	0.38	0.08	1.39	0.26
23.57	Benzaldehyde	1.77	0.31	0.49	0.04
tot		**987.44****		**297.8**	
	Ketones				
2.22	2-Propanone	215.38	14.77	460.19	19.65
4.49	2-Pentanone	4.91	0.89	0.85	0.18
4.67	2,3-Butanedione	360.31	61.27	7.07	1.24
13.5	2-Heptanone	16.24	3.52	11.18	1.22
17.36	3-Hydroxy-2-Butanone	381.28	71.99	11.41	4.62
tot		**978.13**		**490.7**	

	Esters				
2.92	Acetic acid. ethyl ester	129.75	12.7	7.25	1.04
4.84	Butanoic Acid. methyl Ester	n.d.	-	0.05	0.01
10.81	1-Butanol- 3-Methyl- Acetate	2.51	0.29	n.d.	
tot		**132.26**		**7.3**	
	Alcohols				
3.65	Ethanol	90.39	5.38	80.17	9.94
10.17	2-Methyl-1 propanol	431.73	55.42	127.69	20.09
15.09	3-methyl-1-butanol	1928.77	412.41	167.64	23.09
tot		**2450.89****		**375.49**	
	Terpenes				
5.79	α-Pinene	n.d.	-	126.55	11.07
7.41	Camphene	n.d.	-	19.11	4.14
9.19	β-Pinene	n.d.	-	2.76	0.48
11.3	Limonene	n.d.	-	5.07	0.37
25.31	trans-Caryophyllene	n.d.	-	0.16	0.02
25.47	Patchulen	n.d.	-	0.17	0.02
tot		-		**153.81****	
	Free Fatty Acids				
21.82	Acetic acid	250.13	19.33	227.72	33.39
23.96	Propanoic acid	0.62	0.06	0.29	0.03
24.65	2-methyl-propanoic acid	5.1	0.54	0.63	0.08
25.88	Butanoic acid	52.03	4.19	25.9	3.31
26.74	3-methyl-butanoic acid	12.64	1.09	5.18	0.66
28.04	Pentanoic acid	0.13	0.01	0.09	0.01
30.05	Hexanoic acid	7.98	0.65	3.04	0.42
32.87	Octanoic acid	0.61	0.04	0.28	0.02
tot		**329.25**		**263.13**	
	Miscellanea				
1.88	Dimethyl sulfide	23.96	1.53	14.42	6.78
24.22	Dimethyl Sulfoxide	1.93	0.19	1.05	0.12
28.29	Acetamide	0.3	0.02	0.13	0.01
30.83	Dimethyl sulfone	7.87	0.45	8.25	0.54
tot		**34.06**		**23.86**	

*=data are expressed as ng ml-1 of internal standard equivalents; means with (**) are significantly different ($p < 0.05$).

4.2.3 Goat Cheese

A total of 72 volatile compounds was detected in goat cheese, which is more than twice the number of volatiles that were found both in goat milk and curd. This was in line with several studies, which reported a total number of volatiles detected between 56 and 84 (Panseri et al., 2008; Aprea et al., 2016; Bovolenta et al., 2014; Valdivielso et al., 2016 Sulejmani and Hayaloglu, 2016).

Overall concentration of volatiles, as expected, increased in consequence of farther losses of water content. The high number of volatiles identified represents the complexity of biochemical paths involved in the cheese ripening (Ianni et al., 2020).

Aldehydes

This group of volatile compounds was particularly affected by the period of ripening, all aldehydes found in goat curd and milk substantially disappeared in the cheese, with the exception of 3-methyl-Butanal, which was detected even in milk and curd, and Benzaldehyde. This was in accordance with Sulejmani and Hayaloglu, 2016, who reported decreasing concentration of both 3-methyl-Butanal and Hexanal in Kashkaval 90 days ripened cheese, regardless of curd heating temperature. Particularly they found significant concentration of Hexanal after 1 day of ripening and null after 60 days, which confirms also the identification of Hexanal in goat curd and no detection in 30 days ripened cheese. Generally aldehydes are reported to decrease during the first months of the ripening process, and to increase in the second stage of the process (Valdivielso et al., 2016; Sulejmani end Hayaloglu, 2016; Sahingil et al., 2014). Anyway aldehydes have notably low odour detection threshold, so they result to be important compounds in characterizing mountain cheese aroma profile, particularly the long ripened ones (Kilcawley et al., 2018; Panseri et al., 2008). This trend is clearly related to proteolysis and subsequent free fatty acids degradation occurring during the ripening process, reactions which were reported to be enhanced with higher temperatures of ripening locals (Ianni et al., 2014; Sulejmani and Hayaloglu, 2016; Sahingil et al., 2014).

3-methyl-Butanal maintained almost the same concentration from pasture goat curd to cheese, decreasing only slightly, while in lowland period the decrease was much higher. Benzaldehyde was found in pasture derived cheese and not in the lowland one. These results might be explained by higher digestible proteins level in summer pasture, which enhanced proteolysis and consequently stopped aldehydes drop in ripening cheese.

Ketones

Overall concentration of ketones was higher in pasture cheese than in lowland cheese, confirming what reported by Ungureanu-Iuga et al., 2024. In particular we found that, after reaching the lowest concentration in pasture curd, this category was at highest level in the pasture cheese. However this is in line with Aprea et al., 2016, who reported highest level of ketones in milk deriving from cows grazing poor pasture, and also was confirmed by Bergamaschi and Bittante, 2018 who found higher levels of ketones' concentration in ripened cheese compered to fresh cheese. The number of ketones identified was almost doubled from goat curd to cheese during pasture period, while 2 compounds weren't detected in the lowland period, namely 2,3-Butanedione, 5-hydroxy-2,7-dimethyl-4-Octanone.

Overall ketones that were positively affected by the switch to mountain pasture were four: 2-Butanone, 2,3-Butanedione, 2-hydroxy-3-Pentanone and 5-hydroxy-2,7-dimethyl-4-Octanone. Instead 2-Propanone, 2-Pentanone, 3-hydroxy-2-Butanone were more abundant in lowland cheese. 2-Butanone, 2,3-Butanedione and 3-hydroxy-2-Butanone are reported to be originated from metabolism of citrate residues from lactose fermentation by LAB (Ianni et al., 2020), so they might have been affected by a different rate in lactic fermentation between pasture and lowland cheesemaking processes. Other ketones instead derive from lipid oxidation, so significant differences in fat content along the lactation could have impacted their formation in the cheese.

2-Hexanone, 2-Heptanone, Butyrolactone and 2-Nonanone were substantially unchanged from lowland to pasture cheese.

Esters

In this category we found great difference between lowland and pasture cheese composition. 6 out of 10 compounds detected in cheese weren't identified in lowland period. Instead, abundance in lowland cheese was more than 10 times higher than abundance in pasture cheese. This was in accordance with Aprea et al., 2016, who found greater abundance of esters in rich pasture derived cheese. Bovolenta et al., 2014 instead didn't find significant differences in cheeses produced in rich or poor pasture with high or low supplementation levels. It's important to notice that standard errors in lowland cheese are considerably high, probably this is a consequence of the small statistic sample (n=4), so also high values of concentration found in lowland cheese might be subject to uncertainty. There were three compounds that were particularly higher in lowland cheese: acetic acid methyl ester, ethyl acetate and 1-butanol-3-methyl acetate. For example, ethyl acetate abundance was more than 30 times higher in lowland than in pasture cheese, with a standard error being equal to almost half of the mean value of abundance in lowland cheese. Both Aprea et al., 2016 and Bovolenta et al., 2014 found different results than ours, registering higher ethyl acetate abundance values in poor pasture. Acetic acid methyl ester and 1-butanol-3-methil acetate standard errors even if not as high as ethyl acetate standard error, were still considerably high.

Esters can derive from esterification of alcohols and free fatty acids, we might hypothesize that a great part of free fatty acids present in goat curd esterified with alcohols (present in great quantities in cheese), increasing greatly esters concentration. In fact these were present in much lower concentration in curd than in cheese, in both lowland and pasture period. We might also assume that greater variability of alcohol compounds in pasture cheese was the cause of the higher number of esters detected in pasture cheese than in lowland cheese. Sulejmani and Hayaloglu, 2016 also identified a considerably lower number of esters in 1 day ripened cheese than in 60 days ripened cheese.

Alcohols

Alcohols, together with esters, were the most negatively affected category by the switch to mountain pasture management and feeding. At the same time the variability of compounds detected in lowland was way lower than that in pasture, with 10 compounds out of 14 detected only in the pasture derived cheese. The greatest abundance difference was noticed for 2-Butanol, 1-Propanol-2-methyl, 2-Pentanol, 1-Butanol-3-methyl and Ethanol. Ethanol remained with quite stable concentration, along with milk processing until cheese realization, in both lowland and pasture periods. This was confirmed by Valdivielso et al., 2016, who reported ethanol concentration to be more related to seasonality then to feeding changes.

They also reported significantly lower concentration of 2-Butanol in cheese produce with indoor feeding than with fresh pasture, even if difference between the two feeding and management types weren't as marked as in our study. Kilcawley et al., 2018 confirmed our results on 1-Propanol-2-methyl and 2-Pentanol, reporting high correlation between these compounds and diet changes. Our results on 1-Butanol-3-methyl were in line with what found by Bovolenta et al., 2014, who reported this compound as the most abundant alcohol in Montasio ripened cheese, and that it was significantly correlated with energy rich pasture.
Comparing alcohols composition of cheese and curd, we can notice that in lowland period it didn't change, in fact we found the same three compounds in both curd and cheese (Ethanol, 1-Butanol-3-methyl and 1-Propanol-2-methyl). The high complexity of alcohols composition in the pasture period was confirmed by Bergamaschi and Bittante, 2016, who compared volatile profile of pasture derived fresh and ripened cheeses, reporting 7 alcohols (among which several with very low concentration) in fresh cheese and 13 in ripened one. Also Sulejmani and Hayaloglu reported very similar results to ours, with only Ethanol and 1-Propanol-2-methyl detected in 1 day ripened cheese, and instead 7 more alcohol compounds after 60 days of ripening.
However Ethanol, 1-Propanol-2-methyl and 1-Butanol-3-methyl remained the most abundant compounds in this category, with only 2-Butanol and 2-Pentanol reached comparable concentrations.

Free Fatty Acids

Acids were the most abundant volatile category after esters, and the most abundant category in the pasture cheese. This was in line with what Sulejmani and Hayaloglu, 2016 reported on both 1 day and 60 days ripened pasture cheese, where carboxylic acids were the most abundant volatile compounds. Also Valdivielso et al., 2016 and Bergamaschi and Bittante, 2016 reported the same results.
Even in this category the difference between pasture and lowland cheese is evident, having found a total abundance of acid in pasture cheese that is equivalent to more than three times the one noticed in lowland cheese. All carboxylic acids were significantly increased by the pasture management, we noticed exceptional increase in Acetic acid, Butanoic acid, Hexanoic acid and Butanoic acid 3-methyl. Acetic acid

was the most abundant among all acids detected, this is sometimes related to propionic fermentation, which leads to the formation of off-flavours paired with exceptional size of eye in the wheel section (Ianni et al., 2020; Kilcawley et al., 2018). Nevertheless, we noticed a contained concentration of Propanoic acid, bringing to the exclusion of uncontrolled propionic fermentation. Aprea et al., 2016 reported Acetic acid, Hexanoic acid and Butanoic acid as the most abundant acids they identified, and also found a strong correlation between these three acids and poor pasture with low supplementation diet, confirming our results. Bovolenta et al., 2014 reported the same results for Acetic acid, while instead found only correlation with low supplementation for Butanoic acid and no correlation between Hexanoic acid and type of pasture or level of supplementation. Even Octanoic acid concentration was significantly higher in pasture cheese than in lowland cheese, and this result was confirmed by Valdivielso et al., 2016 who reported significantly higher concentrations of Octanoic acid in cheese produced from late grazing in mountain pasture than from indoor rearing.

Comparing free fatty acids composition in cheese and curd we can notice there was an overall increase in total abundance of acids both in lowland and pasture cheese, but the increment was way higher in pasture period. Propanoic acid was slightly increased from curd to cheese during the lowland period, while this increment was consistent in pasture cheese. This result, also considering the high acetic acid concentration, was probably caused by propionic fermentation, which still shouldn’t affect negatively the volatile profile of the cheese, since propanoic acid concentration remained contained.

Bergamaschi and Bittante, 2016 confirmed our results, reporting concentrations of single acids 2 to 6 times (depending on the specific acid) higher in ripened cheese than in fresh cheese. Sulejmani and Hayaloglu, 2016 found significant correlation of Butanoic acid and Acetic acid concentration with ripening process (from 1 to 90 days).

Miscellanea

Complexity of flavour compound is greatly represented by this group of volatiles, since 16 volatiles were identified, against 3 that were detected in curd. Once more, the difference between lowland and pasture cheese is evident, of all 16 compounds identified only dimethyl sulfone was present in lowland cheese. Anyway abundance of this category is quite low, with heterogeneity in the compounds that fall within it, with different odour detection thresholds (Ianni et al., 2020; Kilcawley et al., 2018).

Terpenes

Results confirmed the trend shown by both bovine and goat milk and curd results. Again, no terpenes were identified in lowland cheese. Overall abundance of terpenes was more than doubled in comparison to the abundance found in goat curd, data that were confirmed by Aprea et al., 2016, who reported increasing levels of terpene concentration along with longer ripening times.

All terpenes already detected in goat curd were found in cheese as well, with increased concentration, with the exception of Camphene which diminished.

New terpenes were detected, namely: α-Phellandrene, o-Cymene, α-Cubebene and Camphor. These results confirmed the general expectations about terpene resistance to biochemical processes like enzymatic degradation, fermentation and oxidation. Most interesting result is that terpenes were strongly correlated with pasture-based diet, showing presence only in the dairies produced in the Summer period (characterized by grazing in mountain pastures).

Table 4.3: Volatile compounds identified in goat cheese.

R.T.	Compund*	Lowland Average (n=4)	s.e.	Pasture Average (n=6)	s.e.
	Aldehydes				
3.29	Butanal. 3-methyl-	21.05	5.23	15.66	2.66
23.58	Benzaldehyde	n.d.	-	1.57	0.36
tot		**21.05**		**17.22**	
	Ketones				
2.22	2-Propanone	289.20	44.74	76.13	10.70
3.03	2-Butanone	133.15	31.67	647.43	120.90
4.48	2-Pentanone	149.67	38.31	131.18	16.69
4.65	2,3-Butanedione	n.d.	-	82.82	15.76
8.54	2-Hexanone	0.64	0.15	n.d.	-
13.47	2-Heptanone	62.63	6.31	62.98	4.74
17.36	Acetoin	691.69	19.19	586.39	75.98
19.53	2-Hydroxy-3-pentanone	1.49	0.37	4.73	0.85
20.56	2-Nonanone	1.81	0.27	0.41	0.13
22.44	5-Hydroxy-2,7-Dimthyl-4-Octanone	n.d.	-	27.16	4.47
25.64	Butyrolactone	0.03	0.01	0.37	0.08
tot		**1330.31****		**1619.72**	

	Esters				
2.33	Acetic acid methyl ester	421.99	137.88	41.82	9.22
2.91	Ethyl acetate	2425.31	1043.45	74.69	16.51
4.81	Butanoic acid methyl ester	0.35	0.15	28.50	6.45
6.67	Butanoic acid ethyl ester	n.d.	-	87.45	22.75
10.81	1-Butanol-3-methyl acetate	779.56	258.00	40.15	6.81
12.63	Butanoic acid 2-methyl-propil	n.d.	-	1.26	0.22
15.91	Hexanoic acid ethyl ester	n.d.	-	29.51	4.18
17.04	Butanoic acid 3-methyl-butyl ester	n.d.	-	4.47	0.69
19.32	Ethyl-2-hydroxypropanoate (Lactate)	n.d.	-	0.13	0.04
20.14	Butanoic acid 2-ethyl-1,2,3-propanetriyl ester	n.d.	-	14.07	3.33
20.65	Octanoic acid methyl ester	n.d.	-	0.71	0.17
tot		**3627.21****		**322.63**	
	Alcohols				
3.54	Isopropyl alcohol	n.d.	-	3.64	0.99
3.63	Ethanol	23.39	3.59	53.37	11.52
6.22	2-Butanol	n.d.	-	78.03	16.39
10.07	2-methyl-1-propanol	207.53	22.32	78.97	2.39
10.74	3-Pentanol	n.d.	-	1.39	0.26
11.32	2-Pentanol	n.d.	-	30.27	4.84
12.32	1-Butanol	n.d.	-	7.02	1.60
13.03	1-Penten-3-ol	2.19	0.45	1.86	0.18
15.12	3-Methyl-1-butanol	2506.36	545.51	642.69	40.84
16.59	1-Pentanol	n.d.	-	3.81	0.61
18.76	3-Methyl-2-buten-1-ol	n.d.	-	0.16	0.03
18.84	2-Heptanol	n.d.	-	2.89	0.59
19.77	1-Hexanol	n.d.	-	5.66	1.58
22.63	2,6-Dimethyl 4-heptanol	n.d.	-	0.33	0.12
tot		**2739.47****		**910.09**	
	Terpenes				
5.76	α-Pinene	n.d.	-	262.30	40.82
6.07	α-Phellandrene	n.d.	-	6.20	0.94
7.38	Camphene	n.d.	-	11.96	1.98
9.11	β-Pinene	n.d.	-	16.16	1.98
13.85	Limonene	n.d.	-	27.72	6.04
16.87	o-Cymene	n.d.	-	0.77	0.15
22.98	α-Cubebene	n.d.	-	0.07	0.02
23.27	Camphor	n.d.	-	0.44	0.07
25.27	Trans-Caryophyllene	n.d.	-	1.38	0.18
tot		-		**329.08****	

	Free Fatty Acids				
21.81	Acetic acid	420.97	92.17	1379.20	230.56
23.93	Propanoic acid	8.18	0.84	28.25	3.69
24.61	2-Methyl-propanoic acid	50.04	3.94	78.11	7.62
25.82	Butanoic acid	132.80	12.26	941.66	140.54
26.72	3-Methyl-butanoic acid	134.34	9.76	244.80	26.27
28.03	Pentanoic acid	0.90	0.20	6.65	1.25
30.04	Hexanoic acid	19.07	2.20	375.72	57.35
32.88	Octanoic acid	1.20	0.18	38.00	6.29
tot		**767.50**		**3092.39****	
	Miscellanea				
1.67	2-methoxy-2-methyl Propane	n.d.	-	2.51	0.33
1.73	Heptane	n.d.	-	0.70	0.19
1.88	Thiobis-methane	n.d.	-	2.93	0.57
2.14	Octane	n.d.	-	0.69	0.18
4.04	2-Ethyl-furan	n.d.	-	5.24	1.03
7.92	dimethyl-disulfide	n.d.	-	22.80	4.38
10.37	Ethylbenzene	n.d.	-	6.88	1.41
16.4	Styrene	n.d.	-	2.08	0.38
19.03	1.1-dimethylethyl Hydroperoxide	n.d.	-	9.26	1.29
20.72	Pyrazine trimethyl-	n.d.	-	0.10	0.03
24.23	1-Methyl-3-(3,4-dimethoxyphenyl)-6,7-dimethoxyisochromene	n.d.	-	11.47	2.39
25.42	2-Methyl-4-(2,6,6-trimethyl-1-cyclohexen-1)-2-butenal	n.d.	-	0.84	0.13
28.29	Acetamide	n.d.	-	0.64	0.09
30.83	Dimethyl sulfone	2.61	0.17	6.31	0.55
31.22	Phenyl-ethyl Alcohol	n.d.	-	28.09	7.30
32.34	Phenol	n.d.	-	0.05	0.01
33.14	3-Methyl phenol	n.d.	-	n.d.	-
tot		**2.61**		**100.59****	

*=data are expressed as ng ml-1 of internal standard equivalents ;means with (**) are significantly different ($p < 0.05$).

4.2.4 Cow Milk

A summary of the volatile profile of cow milk is shown in Table 4.2, organized with the same categories as for goat milk.

A total of 27 volatile compounds was detected in Cow milk. Comparing it with goat milk, first evident difference is the number of terpenes detected (1 vs 6), and also no esters were detected in cow milk. At the same time a higher number of volatiles was detected in the category of aldehydes.

Generally results of cow milk volatile profile are comparable to the ones of goat milk, since alle the categories but terpenes were negatively affected by the switch to mountain pasture. Overall differences between lowland and mountain pastures milk weren't as marked as in goat milk. In this case ketones were overall less affected comparing them to the goat milk case. Considering the fact that many volatiles are in some way related to lipid fraction degradation and oxidation, the different impact of rearing management on volatile profile of milk might be sought in the differences in lipid fraction content and composition, and in different microstructures between cow and goat milk. First of all, it must be considered that bulk cow milk from which samples were gathered was a proportion of both whole and skimmed milk, making lipid fraction slightly lower in cow milk than in goat milk (fat content mean values were 3.12% and 3.20% respectively). For example goat milk is richer in medium chain and branched chain fatty acids, which are responsible for the goaty flavour; also goat milk fat micelles are smaller than those present in cow milk (Amigo and Fontecha, 2011).

For cow milk 9 volatile compounds were particularly affected by rearing management type: 2-methyl-Butanal, 3-methyl-Butanal, 2-Propanone, 2,3-Butanedione, 3-Hydroxy-2-Butanone, Ethanol, 3-Methyl-1-Butanol, Acetic acid, Dimethyl Sulfide.

Aldehydes

Data for aldehydes category were very similar for cow and goat milk. Like in goat milk, Hexanal was almost undetected in cow milk during confined rearing period milk, while it was present in considerable concentration in the pasture period milk. This was the case for heptanal as well, but this compound didn't reach very high concentration. Main difference between goat and cow milk aldehydes composition was that 3-methyl-Butanal maintained considerable concentration in cow milk, while it almost disappeared in goat milk during pasture period. Also 2-Methyl-Butanal was detected in considerable quantities in the lowland milk, and almost none was found in pasture milk. Nevertheless Faustini et al., 2019 found significant differences in 2-methyl-Butanal concentration between milk destined to Grana Padano cheese and Parmigiano Reggiano cheese, which are both productions related to lowland confined livestock and to hay/concentrate based diets; Parmigiano Reggiano cows were fed with polyphyte hay, which wasn't present in Grana Padano cows, this might lead to the importance of hay type and quality more than hay to grass diet switch. Also Faulkner et al., 2018 found highest concentration of this volatile in milk derived from TMR feeding. Also, since 2-methyl-Butanal is originated through aminoacid metabolism (particularly isoleucine), availability of digestible proteins might have changed severely from hay to pasture diet (Kilcawley et al., 2018).

Ketones

Like in goat milk, we find three main volatile compounds to be significantly affected by changes in the management type and in diet: 2-Propanone, 2,3-Butanedione and 3-Hydroxy-2-Butanone. Results are quite similar to goat milk, 2-Propanone concentration increased in pasture milk comparing it with lowland milk, while 2,3-Butanedione and 3-Hydroxy-2-Butanone decreased. Main difference with goat milk was that 2-Butanone wasn't affected at all by the switch. Since some of these volatile compounds derive from lipid oxidation, higher abundance of antioxidant compounds in the pasture might have had a certain role in the drastic decrease of ketones, enhancing oxidative stability of the milk, and so lowering the concentration of substrates which would have brought to ketones formation (Kilcawley et al., 2018; Cervinkova et al., 2016; Nozière et al., 2006). Other ketones (like 3-Hydroxy-2-Butanone) are derived from carbohydrate metabolism, so typical lower carbohydrate/protein ratio of pasture might have contributed to lower concentration of this category of volatiles.

Still these results are in contrast with Ungureanu-Iuga et al., 2024, who found higher concentration of ketones in the highland milk, even if must be noted that the study was conducted on two groups which were both fed with grass, so they underlined differences mainly regarding the morphology of the territory and the management type.

Alcohols

All three volatiles detected in cow milk were also detected in goat milk, and also the trend in the transition from lowland to pasture was the same, even if with some differences. Ethanol was detected with considerably lower concentration in pasture milk than lowland milk, while in pasture goat milk it remained present in significant concentration (pasture goat milk had about the same concentration of ethanol of lowland cow milk). This might be due to differences in the goat feeding comparing it with cow feeding (?), since Kilcawley et al., 2018 confirmed the provenience of ethanol from silages; generally ethanol is recognized as a strong discriminator of diet in milk and dairy products, particularly it is more related to preserved forages than to fresh ones.

2-Methyl-1-Propanol concentration didn’t change at all between lowland and pasture milk contrarily of what happened in goat milk. 3-Methyl-1-Butanol followed the same trend in cow and goat milk having a substantial decrease in concentration with the transition to mountain pasture.

Free Fatty Acids

Carboxylic acids detected in cow milk were the same found in goat milk, namely Acetic acid, butanoic acid, propanoic acid, pentanoic acid, hexanoic acid, octanoic acid, 3-methyl-butanoic acid and 2-methyl-propanoic acid. The trend followed by acids between lowland and pasture milk was substantially the same for cow milk and goat milk, with the exception of butanoic acid, which didn’t change significantly in cow milk along with the change from lowland to pasture. Contrarily to what we expected, butanoic acid was present with higher concentration in goat than in cow lowland milk, since cow milk is known to be the one with highest content of butanoic acid among all domestic animal species’ milk (Fox, 2011). Notably goat milk, rather than cow milk, is significantly influenced by lipidic supplements to the diet, particularly levels of butanoic acid decrease with increased intake of lipids (Chilliard and Ferlay, 2004). This might explain the decrease of goat milk butanoic acid content, since lipid intake might have been decreasing in the pasture period. Butanoic acid is de-novo synthesized by the cow or also originated through lipolysis, even if a study also reported a possible direct transfer from diet to milk (Kilcawley et al., 2018). Both lipid biosynthesis and lipolysis are influenced by PUFA intake, particularly CLA, so this might have been the cause of the lower butanoic acid content in cow milk.

Acetic acid content in cow milk followed the same trend found in goat milk, showing a drastic decrease from lowland to pasture. As mentioned for goat milk, this result might be related to direct transfer of acetic acid between concentrates and milk, transfer which was decreasing along with lowered concentrate supplementation during pasture period.

3-Methyl-Butanoic acid content in cow milk was also affected by the switch to pasture.

2-Methyl-Propanoic acid is reported to be a precursor of 2-methyl-1-Propanol (Kikcawley et al., 2018), this would explain the apparent relationship between this compound and 2-methyl-propanoic acid, since the drop in 2-methyl-propanoic acid concentration in pasture goat milk corresponded to an increment of 2-methyl-1-propanol in pasture goat milk, while in pasture cow milk (where 2-methyl-propanoic acid content was stable from lowland to pasture) this increment was not registered. Other acids weren't affected by the switch to mountain pasture; this result was confirmed by several studies. Faustini et al., 2019 found no correlation between Pentanoic acid concentration and type of feeding, but instead reported a correlation between hexanoic acid and seasonality. Coppa et al., 2011 reported no variations in hexanoic and octanoic acids along with feeding changes from confined rearing with hay feeding to continuous and then rotational grazing. Faulkner et al., 2018 contrarily reported higher concentrations of hexanoic acid in TMR based diet than in grass and grass/clover based diet.

Table 4.6: Detected volatile compounds in cow milk.

R.T.	Compound*	Lowland average (n=7)	s.e.	Pasture average (n=9)	s.e.
	Aldehydes				
3.23	2-Methyl-butanal	20.12	6.84	0.41	0.06
3.31	3-Methyl-utanal	75.29	12.07	32.34	3.42
4.58	Pentanal	n.d.	-	0.04	0.01
8.6	Hexanal	0.1	0.02	3.49	0.25
13.62	Heptanal	n.d.	-	0.57	0.04
23.57	Benzaldehyde	0.3	0.08	0.01	-
tot		**95.81****		**36.87**	

	Ketones				
2.22	2-Propanone	218.46	20.01	400.4	19.9
3.03	2-Butanone	2.8	0.54	2.15	0.07
4.49	2-Pentanone	0.99	0.25	0.54	0.06
4.67	2,3-Butanedione	69.1	15.01	19.8	3.37
13.5	2-Heptanone	1.61	0.21	0.38	0.06
17.36	3-Hydroxy-2-butanone	354.69	58.9	91.89	12.65
tot		**647.67**		**515.15**	
	Alcohols				
3.65	Ethanol	32.8	4.15	6.42	0.55
10.17	2-Methyl-1 propanol	2.15	0.43	2.47	0.11
15.09	3-Methyl-1-butanol	42.37	6.65	5.28	0.49
tot		**77.33****		**14.17**	
	Terpenes				
9.19	β-Pinene	n.d.	-	1.31	0.1
tot		-		**1.31****	
	Free Fatty Acids				
21.82	Acetic acid	245.05	39.13	45.12	5.08
23.96	Propanoic acid	0.52	0.08	0.09	0.01
24.65	2-Methyl-propanoic acid	1.02	0.24	0.12	0.02
25.88	Butanoic acid	7.2	0.85	6.63	0.51
26.74	3-Methyl-butanoic acid	3.45	0.66	0.9	0.09
28.04	Pentanoic acid	0.05	0.01	0.04	0
30.05	Hexanoic acid	0.99	0.12	0.75	0.06
32.87	Octanoic acid	0.15	0.02	0.06	0.01
tot		**258.41****		**53.71**	
	Miscellanea				
1.88	Dimethyl sulfide	98.59	22.44	2.98	0.5
28.29	Acetamide	0.13	0.01	0.04	0
30.83	Dimethyl sulfone	12.9	1.63	4.1	0.3
tot		**111.62****		**7.11**	

*=data are expressed as ng ml-1 of internal standard equivalent; means with (**) are significantly different ($p < 0.05$).

Miscellanea

Three volatile compounds detected in this category were the same in cow and goat milk. In both cow and goat milk Acetamide was found in a very low concentration, however it didn't change between lowland and pasture milk. Dimethyl sulfide presence was even more impacted by management type change in cow milk then it was in goat milk, being more than 30 times lower in pasture milk. Dimethyl sulfone, contrarily of what was reported in goat milk, was detected in lower concentration in pasture cow milk. This result is in contrast with what Coppa et al., 2011 who didn't find any change in dimethyl sulfone concentration in milk from hay or grass based diet. Kilcawley et al., 2018 instead reviewed several studies reporting dimethyl sulfone as a great discriminator of diet. This volatile compound is originated from oxidation of dimethyl sulfide, which is metabolised starting from free amino acids in the rumen. Possibly higher concentration of dimethyl sulfide in lowland goat milk than in lowland cow milk (146.67 and 98.59 respectively) was related to higher concentration of dimethyl sulfone in lowland cow milk than in lowland goat milk (12.90 and 8.34 respectively), meaning that a greater proportion of dimethyl sulfide was oxidated to dimethyl sulfone in lowland cow milk. Also a switch in protein/available carbohydrate ratio might have changed sulphurous volatile compounds in both goat and cow milk from lowland to mountain pasture management.

Terpenes

The category of terpenoids was the one that showed most differences between goat and cow milk. β-Pinene was the only terpene detected in cow milk, and it was only present in the pasture period. Overall abundance of terpenes in cow milk was 100 times lower than terpenes abundance in goat pasture milk, and also comparing β-Pinene concentration in goat and cow milk, we can notice it is 4 times lower in cow pasture milk.

First aspect on which bring the attention is that cow bulk milk from which samples were gathered was partially skimmed, since terpenes compounds are mostly bonded to the lipidic fraction in the milk, a good amount of them might have been transferred in the cream and consequently to the butter produced in the farm.

Also this result might be attributed to the pasture management system, which was somehow rotationally grazed, but without putting fences to block the access to the patches grazed in the previous days. This might have brought cows to select grasses and monocotyledons in general, which have higher energetic value, avoiding dicotyledons which would have been richer in terpenes, while goats instead were generally held in shrub encroached and woody terrains (Mariaca et al., 1997). This would be confirmed by the absence of sesquiterpenes in cow milk, this class of terpenoids is reported to be particularly lacking in monocotyledons (Mariaca et al., 1997; Abilleira et al., 2010). Nevertheless Coppa et al., 2011 generally reported higher terpene concentration in continuous grazing system than in rotational grazing system. Also it has been confirmed how phenological stage of the pasture can impact greatly on terpene content of sward (Mariaca et al., 1997), this might have been the cause of such low concentration of terpenes in cow milk. Another explanation of this diverging results between goat and cow milk terpene presence might be related to the way chromatograms were interpreted, since this was a qualitative analysis, having very low concentrations of some compounds might have been difficult to recognize the peaks in the chromatogram and so excluding it from the compounds list.

Faulkner et al., 2018 found that terpenes were generally scarcely represented (4 trpene compounds in total) and also they didn't detect any of the terpenes detected in goat pasture milk.

4.2.5 Cow Curd

In Table 4.3 are summarized all volatile compounds detected in curd coagulated starting from partially skimmed cow milk.

This process occurred along a couple of hours and changed drastically the volatile profile of the matrix. This happened expectedly, and there were three main processes which had a role in this change: acidification consequently to the addition of serum fermentation, enzymatic degradation of macronutrients due to rennet addition, heat treatment to 50°C of the curd and loss of water and part of the volatiles along with whey syneresis.

The number of compounds detected decreased from 27 in cow milk to 22. Generally concentration of volatiles increased, probably due to water losses through whey.

Aldehydes

Of the three volatile aldehydes detected in cow milk only 3-methyl-Butanal was still present after curd formation and heating. Interesting fact was that 3-mthyl-Butanal was more than halved in pasture cow milk comparing it with lowland cow milk, while comparing curd from pasture cow milk to curd from lowland cow milk we found a concentration more than 5 time higher.

The fact that only 3-methyl-Butanal remained in this category might probably be attributed to the heating process through which the curd went. This would be confirmed by Sulejmani and Hayaloglu, 2016, who reported 3-methyl-Butanal and Hexanal as the only two aldehydes detected in 1 day ripened Kashkaval cheese, produced through curd scalding and stretching. Also we can note that in curd from goat milk, which didn't undergo such high temperature heating, aldehydes are more represented, and among these the most abundant is 3-methyl-Butanal.

Low aldehydes representativeness might be explained by a limited time of activity of the coagulant and consequently lower free amino acids content (from which aldehydes derive) (Sulejmani and Hayaloglu, 2016).

Highest decrease of aldehydes from cow milk to curd, was registered in the lowland period (95.81 for milk and 4.84 for curd), while in the pasture period aldehydes remained more stable (36.87 for milk and 26.54 for curd). This might be explained differences in the cheesemaking procedures between lowland and pasture periods, due to different environmental conditions like air temperature and temperature of the milk at the moment that cheesemaking started. Also a higher rate of conversion of aldehydes in acids might have occurred.

Ketones

After milk coagulation and curd heating to 50°C, ketones category is the most abundant of all volatile compounds category. We can also note that ketones are more abundant in the pasture curd than in the lowland curd, contrarily to what was reported for cow milk. Particularly there were 3 compounds that were significantly affected by the change from lowland to pasture: 2-Propanone, 2,3-Butanedione and 3-Hydroxy-2-Butanone.

2-Propanone slightly incremented comparing cow milk with cow curd, and it kept the same trend we observed in cow milk along with the switch from lowland to pasture. Sulejmani and Hayaloglu, 2016 didn't report any correlation between 2-Propanone concentration and temperature of curd scalding, this might lead us to think that higher concentration of this compound in cow curd than in cow milk is mainly related to water losses through whey. Bovolenta et al., 2014 confirmed that 2-Propanone together with 3-Hydroxy-2-Butanone were two most abundant ketones in a ripened cow cheese, notably this last compound gives the typical buttery aroma characteristic of ripened cheeses.

2,3-Butanedione is another compound giving buttery aroma, it was considerably decreased in the pasture curd in comparison with the lowland curd, and this trend is the same followed between lowland and pasture cow milk. Sulejmani and Hayaloglu, 2016 reported high concentration of 3-Hydroxy-2-Butanone and low presence of 2,3-Butanedione along with the ongoing ripening process, due to the action of lactic acid bacteria reducing 2,3-Butanedione, this could be expected in our study as well.

Esters

Acetic acid ethyl ester is the only compound in this category. No esters were present in the corresponding cow milk. This is in accordance with Sulejmani and Hayaloglu, 2016 who reported increasing values of concentration of acetic acid ethyl ester along with increasing temperature of curd scalding. They also reported increasing concentration of this compound with the ongoing ripening process.

Acetic acid ethyl ester presence was lower in cow pasture curd, particularly it was almost 50% lower than in lowland curd. This might be easily attributed to the diet changes between lowland and pasture, as it was also confirmed by Kilcawley et al., 2018 that esters are generally originated by plant diets.

Alcohols

Alcohol compounds detected in cow curd were the same present in cow milk, namely Ethanol, 2-methyl-1-Propanol and 3-methyl-1-Butanol. This is confirmed by Sulejmani and Hayaloglu, 2016 ì, who reported that these three compounds were the only alcohols present in 1 day ripened cheese. They also reported that these volatiles weren’t affected by the temperature of curd heating.

Comparing alcohols content between milk and curd we can note the gap between pasture and lowland period is smaller in curd, and generally alcohols concentration is higher because of water losses through whey syneresis.

Ethanol was decreased slightly in pasture cow curd comparing it with lowland curd, but still registered a consistent concentration. As mentioned before, this was a great difference between cow milk and curd, this might be due to differences in the serum

fermentation added in the pasture period, possibly leading to heterolactic fermentation and production of extra ethanol during the resting of the bulk milk.
A significant change was registered on 3-methyl-1-Butanol content between cow milk and curd, in particular it increased considerably. This might be related to the observed 3-methyl-Butanal decrease from cow milk to curd, since 3-methyl-1-Butanol derives from the corresponding aldehyde (Kilcawley et al., 2018). This increment was much higher in the pasture period (85 times higher) than in the lowland period (less than 5 times higher), having inverted the proportion between concentration in pasture and concentration in lowland from milk to curd. This could probably be attributed to enzymatic and fermenting processes occurred after rennet and serum fermentation addition.
Also 2-Methyl-1-Propanol concentration was increased comparing it with the one registered in cow milk.
Generally other studies reported that alcohols have a more important role in flavor profile of cheeses produce from hay/silages milk than cheeses from pasture milk (Kilcawley et al., 2018; Valdivielso et al., 2016)

Free Fatty Acids

Acids composition was the same in cow curd and milk, with the exception of Propanoic acid and 2-methyl-Propanoic acid, which were not detected in cow curd (however these weren't detected in considerable concentration either in cow milk). Acids presence in lowland milk wasn't significantly affected by the cheesemaking process, so we found about the same concentration of total acids in lowland milk and curd. Instead during the pasture period total concentration of acids in curd was much greater than the one registered in milk (444.08 and 53.08 respectively). Free fatty acids origin is quite heterogeneous, deriving from both carbohydrate metabolism, amino acid metabolism, plant diet, de-novo synthesis and lipolysis (Kilcawley et al., 2018) so changes in acids composition might be related to several factors. First of all Acetic acid, which was by far the most abundant organic acid registered in cow curd, can be originated through heterolactic fermentation, so differences in microbial composition of serum fermentation paired with prolonged times of resting of the bulk after ferment addition might have had a role in its high quantity in pasture cow curd comparing it with pasture milk (372.42 and 45.12

respectively). In part, also curd retention capacity might have been enhanced by the switch to pasture, since acetic acid is soluble in water fraction a higher proportion of it might have been kept inside the curd. This could be caused by higher content of fat in milk, which increased cheese yield (Soryal et al., 2004).

Butanoic acid was a little increased in curd compared to cow milk. This compound is de-novo synthesized in the rumen, hence this was probably increased in consequence of whey losses.

3-Methyl-Butanoic acid concentration gap between pasture and lowland was much greater in curd compared to the one registered in milk, this might be due to oxidation of part of the 2-methyl-butanal into 3-mthyl-Butanoic acid.

Miscellanea

The trend for this category remained substantially the same between cow milk and curd, decreasing with the same proportion from lowland to pasture.

Dimethyl sulfide concentration in curd is twice the concentration in milk, both in pasture and lowland period. We might assume this happened due to water losses with whey sineresis. Acetamide was found with very low concentration and this was the same in milk.

Dimethyl sulfone presence instead decreased from milk to curd, anyway this compound was reported to have low odour detection threshold and also was detected in ripened cheeses, so it would be expected to be still characterizing cheese flavor profile (Bovolenta et al., 2014). Also, contrarily to what we found, Aprea et al., 2016 reported higher concentration of dimethyl sulfone in cheese deriving from diets based on low energetic pasture and low supplementation.

Terpenes

Comparing terpenes detected in cow curd with those found in milk, we can notice camphene was not present in milk. More generally terpenes concentration was increased from milk to curd. Again, this was probably related to volatiles general higher concentration in consequence of whey sineresis, also considering that terpenes are generally bounded to the lipidic fraction (Faccia et al., 2022).

This was confirmed by studies which found terpenes originated exclusively from the diet, with particular presence in fresh dicotyledons (Mariaca et al., 1997; Ungureanu-

Iuga et al., 2024; Kilcawley et al., 2018). This would lead us to think that terpenes can't be newly originated in the matrix during the cheesemaking process, with some just very limited exceptions (Belviso et al., 2011).

Table 4.7: Volatile compounds identified in cow curd according to their chemical classes.

R.T.	Compounds*	Lowland average (n=7)	s.e.	Pasture average (n=9)	s.e.
	Aldehydes				
3.31	3-Methyl-butanal	4.84	1	26.54	1.25
tot		**4.84**		**26.54****	
	Ketones				
2.22	2-Propanone	365.87	33.23	491.68	4.71
3.03	2-Butanone	9.89	0.81	4.82	0.24
4.49	2-Pentanone	6.38	0.53	8.03	0.34
4.67	2,3-Butanedione	328.13	56.96	47.89	3.78
13.5	2-Heptanone	0.7	0.05	1.64	0.2
17.36	3-Hydroxy-2-butanone	1125.53	96.03	1748.04	132.42
tot		**1836.49**		**2302.09****	
	Esters				
2.34	Acetic acid methyl ester	n.d.	-	n.d.	
2.92	Acetic acid ethyl ester	19.79	3.17	9.81	0.78
tot		**19.79**		**9.81****	
	Alcohols				
3.65	Ethanol	198.64	20.55	175.93	17.2
10.17	2-Methyl-1-propanol	47.82	3.12	25.89	2.07
15.09	3-Methyl-1-butanol	204.41	19.81	449.46	19.6
tot		**450.87**		**651.28****	
	Terpenes				
7.41	Camphene	n.d.	-	0.65	0.09
9.19	β-Pinene	n.d.	-	3.17	0.07
tot		-		**3.82****	

	Free Fatty Acids				

21.82	Acetic acid	278.87	31.15	372.42	40.83
25.88	Butanoic acid	14.12	1.39	27.13	3.09
26.74	3-Methyl-butanoic acid	2.56	0.35	43.01	6.97
28.04	Pentanoic acid	0.05	0.01	0.25	0.04
30.05	Hexanoic acid	0.77	0.07	1.23	0.11
32.87	Octanoic acid	0.07	-	0.05	0.01
tot		**296.44**		**444.08****	
	Miscellanea				
1.88	Dimethyl sulfide	211.62	24.86	16.11	4.3
28.29	Acetamide	0.13	0.01	0.1	0.01
30.83	Dimethyl sulfone	5.97	0.41	3.08	0.12
tot		**217.72****		**19.29**	

*=data are expressed ad ng ml-1 of internal standard equivalents,means with (**) are significantly different ($p < 0.05$).

6 Conclusions

The present study investigated the impact of seasonal transhumance on the main components of bovine and goat milk, and on the volatile profile of bovine and goat milk, curd, with a particular focus on goat cheese and the role of terpenes as potential biomarkers for its traceability.
Results confirmed considerable differences in milk composition between the stable rearing period and the alpine grazing period. Most significant changes were observed on fat content in both goat and bovine milk. Goat milk showed the typical phenomenon of protein/fat ratio reversion, except for the period June-July-August, during which fat content mean value was higher than protein content mean value (3.5 and 3.4% respectively); bovine milk showed the highest values of fat content in the months of May, July and August.
Contrarily to what expected, protein content remained quite stable along the studied period in both bovine and goat milk, result that was attributed to a proper feeding supplementation system. Lactose content, as found in other studies, was quite constant along the period in which the study was conducted, not being affected considerably either by diet or lactation stage. Overall, we don't know on which extent milk components were affected by diet and management rather than lactation period, since the trend of main milk components was the same reported by other studies conducted on the effect of lactation period.
The volatile profiles revealed significant shifts in the concentrations of ketones, esters, and free fatty acids, with a general trend indicating lower concentrations of non-terpene VOCs in pasture-derived milk. Volatile compounds' concentration was recorded to be generally increasing along the cheesemaking process in bovine products from milk to curd and in goat products from milk to cheese, except for the categories of aldehydes and miscellanea that decreased, being probably more characterizing in long ripened cheeses.
As expected, goat cheese showed much greater number of volatile compounds (more than doubled), showing that volatile profile of cheeses is influenced by many different factors (related to environmental conditions, cheesemaking process and diet), making the correlation between it and animal's diet difficult to attribute. It must be also underlined that our statistical sample in volatiles analysis was considerably

small, particularly in cheese analysis (n=16 for milk and curd, n=10 for goat cheese). Nevertheless, the most striking difference between lowland and pasture was observed in the terpene content, which was virtually absent in products from stable-reared animals but markedly present in the ones from pasture-grazing cows and goats. In particular, goat products showed a considerable number of terpene compounds detected, number that increased in goat cheese.

Terpenes use in authentication and certification systems, such as the European "Mountain Product" label, appears promising. The ability to trace dairy products to specific feeding regimes and grazing environments through terpene profiling could offer an effective tool for valorizing traditional mountain dairy production and ensuring transparency for consumers.

Beyond their implications for product traceability, the findings of this study contribute to the broader discourse on the sustainability of mountain pastoralism. The documented differences in milk composition and volatile profiles highlight the influence of extensive grazing practices on dairy product characteristics, reinforcing the need for policies that support the viability of traditional livestock systems. Recognizing the ecological and cultural value of transhumance and integrating scientific evidence into certification schemes could enhance market opportunities for small-scale mountain dairy producers.

Strengthening the scientific basis for mountain dairy certification could play a crucial role in preserving traditional pastoral systems, promoting sustainable livestock management, and enhancing consumer trust in high-value, pasture-derived dairy products.

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7 Abstract

Mountain farming plays a crucial role in sustaining local economies, preserving biodiversity, working as a carbon sink and maintaining traditional pastoral systems and associated high quality dairy productions. In particular, grazing animals (managed through a proper grazing management plan) have a positive impact on pasture ecosystem, enhancing vegetal species biodiversity, counteracting shrub encroachment, and maximizing hay yield. Moreover it was demonstrated that mountain dairy productions are characterized by better nutritional properties, such as higher antioxidant content, favourable omega-6/omega-3 ratio, and are also characterized by overall higher consumer's acceptability comparing them to dairy deriving from intensive livestock farms.

EU origin certifications are both a marketing and political tool, having the possibility to differentiate mountain transhumant farms and intensive livestock productions, but they appear to be still too wide. This labelling system is calling for a scientific basis which could support the marketing differentiation, as well as the production system differentiation. In this context, this study tried to propose the use of terpenes compounds present in goat cheese as bio differentiator of the diet, since they were reported by several studies to be present in higher concentrations in pasture based dairy products.

The main objectives of this study were: 1) to evaluate how transhumance influences milk quality, yield, and composition in both cow and goat milk, 2) to analyse the effects of seasonal changes on volatile compounds in milk, curd, and cheese and 3) to explore the potential of terpenes as biomarkers for distinguishing mountain pasture-derived products from those obtained from stable-reared animals.

The research was conducted at Azienda Agricola Prestello, a family-run dairy farm in Valle Camonica, Northern Italy. The study focused on milk from Brown Swiss cows and Bionda dell'Adamello goats, which were reared in a transhumant system. Milk and curd samples were collected in different seasons, from both winter stable conditions and summer highland pastures. Milk composition was analysed for fat, protein, lactose, and casein content. Volatile organic compounds (VOCs) were identified using Solid Phase Microextraction-Gas Chromatography-Mass

Spectrometry (SPME-GC/MS). The analysis was conducted on samples collected every two weeks from March to August and then again in autumn for cow milk.

Both goat and bovine milk, comparing lowland and pasture period, presented substantial changes in fat content. Bovine milk fat content mean value for the pasture period was higher than in lowland period (4.15 and 3.95% respectively), this difference was even greater in goat milk fat content (3.5 and 2.65% respectively). Anyway it wasn't possible to analyse separately the impacts of diet/environment and lactation period, and the trend followed by fat content was generally in line with what reported by other studies conducted on the effect of the lactation period on milk composition. Goat milk showed greater variability of protein content along the studied period (minimum mean value 3.31% in June and maximum mean value 3.55% in August) comparing it with bovine milk (minimum 3.49 maximum 3.65%); this might be due to higher feeding ration control in cows than in goats.

Lactose content was, as expected, was substantially unchanged along the season, confirming other findings on the effects of lactation period, diet and environment on lactose content.

For what concerns volatile profiles of milk, curd and goat cheese, analysis was conducted on a quite small statistic sample (n=16 for milk and curd and n=10 for cheese), so a considerable uncertainty of the data must be taken into account. Overall volatiles abundance in both goat and bovine products, as expected, resulted increased along with the ongoing cheesemaking process due to water losses during whey syneresis and ripening. In goat milk and curd all the categories, except for terpenes, were less abundant during the pasture period. There was a switch in this pattern only with goat cheese, where abundance of some categories (ketones, free fatty acids, terpenes and miscellanea) was higher in pasture derived cheese, and number of volatiles detected in pasture cheese in each category was the same or higher than the number found in lowland cheese. This is thought to be related to a richer fat composition and higher protein/non-fiber carbohydrate ratio, enhancing variety of substrates from which volatiles originate. In bovine milk this switch was already seen between curd and milk, in fact only esters and miscellanea resulted more abundant in lowland curd than in pasture curd. In this case curd heating up to 50°C might have had a role in the modification of volatile compounds, resulting in a more even result between pasture and lowland products.

Most interesting result was about abundance and variety of terpenes identified in goat cheese, in fact 9 terpenoid compounds were detected, with a total abundance doubled comparing it to the abundance found in goat curd. Also the difference between lowland and pasture periods is evident, since no detection of any of the terpenes occurred in lowland.
Their presence suggests that they could serve as reliable markers for authenticating mountain pasture-derived dairy products. This could support quality certification schemes, benefiting traditional farming practices and enhancing the market value of these products.
This study highlights the impact of transhumance on dairy product composition, emphasizing the role of seasonal grazing in shaping milk quality. The findings support the idea that terpenes could be used to authenticate mountain-derived dairy products, providing a scientific basis for certification programs.