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TESI DI DOTTORATO DI RICERCA

**A research to support  
an alpine monobreed cheese**

**Montasio PDO-PDM “Solo di Pezzata Rossa Italiana”**

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## ABSTRACT

The thesis work has allowed to create the scientific and technical context for the production of cheese Montasio PDO-PDM "*Solo di Pezzata Rossa Italiana*" and to study the variability range of the product.

The first part of the thesis consists on a bibliographical research which describes the situation of dairy farming on the Alps and the main factors affecting the quality of the labelled cheese.

The second part of the work consists in an evaluation of the potential production context of Montasio PDO-PDM monobreed, based on a farm surveys.

The third part is essentially composed of three scientific papers. A first experiment was aimed to assess the variability range of the qualitative characteristics of Montasio PDO cheese, made from milk of Italian Simmental cows (grazing on high altitude pasture or kept indoor and fed a hay-based diet) in two periods (early July and late August) and ripened two or six months. Pasture-derived milk and cheese presented higher fat and lower protein content than hay-derived ones. Rearing systems also affected cheese colour and some textural parameters. In addition, it showed lower level of total saturated fatty acids, and higher level of mono and polyunsaturated fatty acids than hay cheeses. Consumers perceived the difference of cheeses in terms of colour and holes, but they expressed a similar overall liking. More limited effects of period and ripening time were observed.

A second work was aimed to assess whether the information about production system (cow breed and rearing system) may modify the consumer liking of cheese. A traditional Montasio "*Solo di Pezzata Rossa Italiana*" and a pasture-derived Montasio monobreed were analyzed. A consumer test was carried out in two sessions. In the first, both cheeses were tasted by consumers in blind condition (Perceived liking). Then they were asked to read information

about the production system and to give their liking expectation (Expected liking). In the second session, the same consumers tasted cheeses with linked information (Actual liking). Despite the similar Perceived liking average score, it was possible to identify consumers' clusters with differential liking for the two types of Montasio PDO, that were characterized by different physico-chemical properties. Consumers express a high expectation for Montasio monobreed and even more for pasture cheese. The Actual liking of Montasio monobreed was similar and not different from the Expected liking (complete assimilation of information about breed). For pasture cheese the assimilation was complete for consumers who have expressed a positive Perceived liking for it, at least twice as much than other cheese. For the rest of consumers, both information and intrinsic properties played a significant role in Actual liking of cheese.

A third trial was aimed to characterize the volatile fraction and the sensory properties of Montasio cheese produced from milk of Italian Simmental cows grazing on alpine pastures. Seventy two cows grazing on two alpine pastures, that differ for botanical composition, receiving two different levels of supplement. Sixty-two volatile compounds, including alcohols (11), aldehydes (6), ketones (13), esters (5), hydrocarbons (3), organic acids (7), phenolic compounds (4), monoterpenes (7), sesquiterpenes (1), sulfur compounds (2), and others volatiles (3) were detected. The trained assessors described the experimental cheeses with an intense color, a small and evenly distributed eyes, an intense odour and flavour of milk - sour milk and cow and a tender and creamy texture. No effect was recorded for the supplement level. Pasture type has an effect on the volatile fraction, allowing to distinguish the cheeses, without evident effects on the organoleptic perception of assessors.

In general, this thesis is proposed as a scientific support for the valorization of the product along all the chain, from the effect of production techniques on the intrinsic quality of product to its promotion through the assessment of the positive externalities of mountain farms.







## 1. INTRODUCTION <sup>(\*)</sup>

The Italian Alps are characterized by a great variety of environments and socio-economic situations, which are quite difficult to standardize. This consideration can be applied also to the dairy sector, strongly linked to old traditions and local resources utilization. In the recent past, intensive production systems have gradually replaced the traditional ones, resulting in the increase of farm production and income. At the same time intensification processes caused environmental problems, abandonment of marginal lands and biodiversity losses (Cozzi et al., 2006; Ramanzin et al., 2009; Sandrucci et al., 2010).

Today, the competitiveness of alpine production systems cannot be based simply on the ability to reduce production costs. It is necessary to focus on products quality and activities diversification, as well as on the ability to evoke the production area and its environmental, historical and cultural values (Dovier, 2004; Tregear et al., 2007).

Alpine production constraints can be transformed into competitive advantages. In fact, consumers show a strong interest on modern issues, such as environmental sustainability, “local identity” products, food safety and animal welfare. They need to recognize, in mountain products, these characteristics that French effectively summarize within the concept of *terroir*. According to Grappin and Coulon (1996), this term defines a territorial production system characterized by particular environmental conditions, animals able to use the local resources and sustainable agricultural practices. It is, therefore, a synthesis between *savoir* (to know) and *savoir faire* (know how to do), that expresses well the interaction that exists between territory and human factor.

<sup>(\*)</sup> This chapter is based on the paper:

Bovolenta S., Dovier S., Romanzin A., 2011. **Sistemi produttivi lattiero-caseari nell'areale alpino italiano**. In: (E. Piano Ed.) *Pascoli e formaggi d'Alpe* (CRA-FLC, Lodi, Italy), 5-18.

Alpine farms are well suited to this production approach which allows to realize “positive externalities” and consequently to access to CAP payments. As a matter of fact European Parliament decisions are oriented to remunerate management farm choices related to real benefits for the society. The European policies for the development of the mountain seem to aim, in particular, on the dairy sector, the “short chains” and the product quality. Mountain products will also benefit from an optional label, which can guarantee their origin, linked to the existing system of Protected Designation of Origin (PDO) and Protected Geographical Indications (PGI) (Regulation EU 1151/12).

## **1.1. Dairy sector in Italian Alps**

### *1.1.1. Alpine dairy farms*

Dairy livestock is still a leading sector for agricultural economy in the Italian Alps, except for Trentino Alto Adige region where products diversification - especially fruit and wine - reduced its role. As a result of the intensification process, dairy farms radically changed i.e. reduction of farms number, increase of head number per farm, increase of milk yield per farm, animal genetic improvement, resources utilization a progressive abandonment of meadows and pastures, and consequently an increased use of extra farm forages and concentrates (Table 1.1).

In 2009/2010 milk campaign the dairy farms located in the Italian alpine area were 12,118 (63.6% of those in Italian mountains and to 30.1% of the farms in Italy). Marketed milk yield was 1,067 thousand tons (58.2% of Italian mountain production and 9.8% of Italian overall production). In the last decade the average milk yield per farm has doubled, from 41 to 88 tons/year (Pieri, 2010). In the

Italian Alps about 220,000 dairy cows are farmed (National Livestock Database, modified) and the average milk yield per cow is 4.9 tons/year.

**Table 1.1 - Number of dairy farms and their marketed milk yield in mountain areas of Italian alpine regions (Source: Milk Observatory on Agea data; Pieri, 2010)**

	1995/96			2009/10		
	Farms (n.)	Milk yield (.000 t)	Milk yield / farms (t)	Farms (n.)	Milk yield (.000 t)	Milk yield / farms (t)
Region:						
<i>Valle d'Aosta</i>	1.585	46	29	993	44	45
<i>Piemonte</i>	2.234	66	29	897	80	90
<i>Lombardia</i>	3.633	128	35	1.814	164	90
<i>Trentino A.A.</i>	9.122	407	45	6.646	512	77
<i>Veneto</i>	3.896	198	51	1.360	206	151
<i>Friuli V.G.</i>	1.265	40	32	408	61	149
Total:						
<i>Alps (*)</i>	21.735	885	41	12.118	1.067	88
<i>Mountains</i>	41.270	1.643	40	19.042	1.834	96
<i>Italy</i>	97.044	10.403	107	40.199	10.876	271

(\*) Ligurian Alps are excluded

The alpine dairy farms present different levels of intensification and integration with the territory. In intensive farms, cattle - mainly Holstein and Brown Swiss breeds - are bred in loose housing stables located in valley and fed with hay (also of extra farm origin) and concentrates. Cattle calving are distributed throughout the year because of industrial dairy plant requirements. The number of alpine farms that practice the traditional livestock system, characterised by high altitude pasture utilization during summer, milk processing in small farms and dairy products sold in farm shops is decreasing. A small

number of farms use the pasture at different altitudes for exploit vegetation gradient and guarantee animal nutrition transition. In these farms, during the winter the milk is delivered to dairies, while in summer is transformed in high altitude farms ("*malga*" or "*alpeggio*") and is sold directly.

### *1.1.2. Dairy plants and products in Italian Alps*

In the last century dairy plants in alpine area were usually small sized and worked only during winter period. In recent times the number of dairy plants decreased and their average size increased, resulting in safety and hygiene improvement as well as in standardization of milk yield and quality. Even the milk produced in the high altitude farms it is not processed *in loco*, but is delivered to dairies, that do not always transform it separately from that produced in the stalls of valley.

The process of standardization of mountain products led to the closure of marginal farms, which must support higher production costs, and to the intensification of farming in the most favorable valleys. In certain alpine areas (e.g. Trento Province), however, traditional farms have been associated in cooperative dairies producing PDO and other typical cheeses. Generally, these cooperatives paid a milk price higher than that paid by the cooperative of lowland, and in this way contributed to reducing the economic handicap of the mountain farms. The network of cooperative dairies seems to play an important role also for the intensive mountain farms, since a remarkable proportion of them have been able to enter these high value milk transformation chains. The destination for these productions from intensive systems is still controversial, because this milk is produced mostly by selected breeds, fed diets rich in concentrates imported from other areas, and has a lower suitability for processing than the milk produced by local breeds (Sturaro et al., 2013).

Most of alpine milk is processed into dairy products. A large part of these products are included in the Italian “Traditional Food Product” (*Prodotti Agroalimentari Tradizionali* - PAT; D.M. Mipaf 08.09.99 n. 350; D.M. Mipaf 25.06.13 n. 147) list (173 totally alpine of 457), while some others obtained PDO label (Reg. EU 1151/12). Italian PDO cheeses are 37, of which 10 (will soon be 11) are produced only in the alpine area and 11 partially (Table 1.2).

**Table 1.2 - PDO Italian cheese totally (T) or partially (P) alpine and relative production referable to mountain area (t/year)**

PDO cheese	Production area	Production in mountain area (*)
<i>Asiago</i>	P	418
<i>Bitto</i>	T	290
<i>Bra</i>	P	161
<i>Castelmagno</i>	T	227
<i>Fontina</i>	T	4.473
<i>Formai de Mut dell’Alta Val Brembana</i>	T	71
<i>Gorgonzola</i>	P	-
<i>Trentingrana (Grana Padano)</i>	T (P)	3.515
<i>Montasio</i>	P	450
<i>Monte Veronese</i>	P	-
<i>Murazzano</i>	T	15
<i>Piave</i>	T	2.356
<i>Provolone Valpadana</i>	P	-
<i>Quartirolo Lombardo</i>	P	-
<i>Raschera</i>	P	88
<i>Spessa delle Giudicarie</i>	T	150
<i>Stelvio</i>	T	1.112
<i>Taleggio</i>	P	-
<i>Toma Piemontese</i>	P	-
<i>Valle d’Aosta Fromadzo</i>	T	4
<i>Valtellina Casera</i>	T	1.360
<i>Puzzone di Moena (request)</i>	T	396

(\*) For cheeses partially alpine has been reported the data relating to the products with indication "Mountain Product" (*Asiago* and *Montasio*) or "di Alpeggio" (*Bra* and *Raschera*).  
Source: Consortiums for the Protection of PDOs, 2010.

Most of alpine PDO cheeses are sold exclusively in local markets and are often part of the tourist offer. Some have an extra-regional spread, such as Fontina and Trentingrana (geographical indication which identifies Grana Padano cheese produced in Trento province) among the totally alpine and Asiago and Taleggio among the partially alpine. In general, due to the costs related to the operation of supervisory bodies, the PDO represents a useful tool mainly for food chains well organized and able to produce adequate amounts of product.

A specific initiative to enhance mountain products is the establishment of the Register of Mountain Products (MP; L. 289/02, art. 85; D.M. Mipaf 30.12.03), which aims to “protect the originality of the historical and cultural heritage of mountain areas, through the enhancement of their protected products”. The MP label is granted to PDO and PGI products whose area of production, processing and ripening takes place in a mountain area. When the area of production of the PDO or PGI is completely contained within a mountain area, the label “Prodotto della Montagna” may be added to the product once it is registered in the Register of Mountain Products. If only part of the production is made in a mountain area, the label may only be used for the products obtained from that area. However, it is widely believed (Santini et al., 2013) that this national label does not give sufficient guarantees of transparency to consumers.

One of the first PDO cheeses that used this label is the PDO Asiago, followed by the PDO Montasio. In fact, this opportunity, such as that offered to Trentingrana, seems interesting to differentiate mountain cheeses within the PDO partially alpine.

Recently, with a view to make the mountain products on the market more clearly identifiable for the consumer, EU institutions legislated on a common definition of an optional quality term, “Mountain Products”, in the labelling of agricultural products. According to the Regulation EU 1151/2012 of the European Parliament this label shall only be used to describe products in respect of: “a) both the raw materials and the feedstuffs for farm animals come

essentially from mountain areas; (b) in the case of processed products, the processing also takes place in mountain areas” (Article 31). European Commission will soon adopt implementing acts setting derogations to the general principles of the new Regulation in order to take into account the specificities of the different sectors involved.

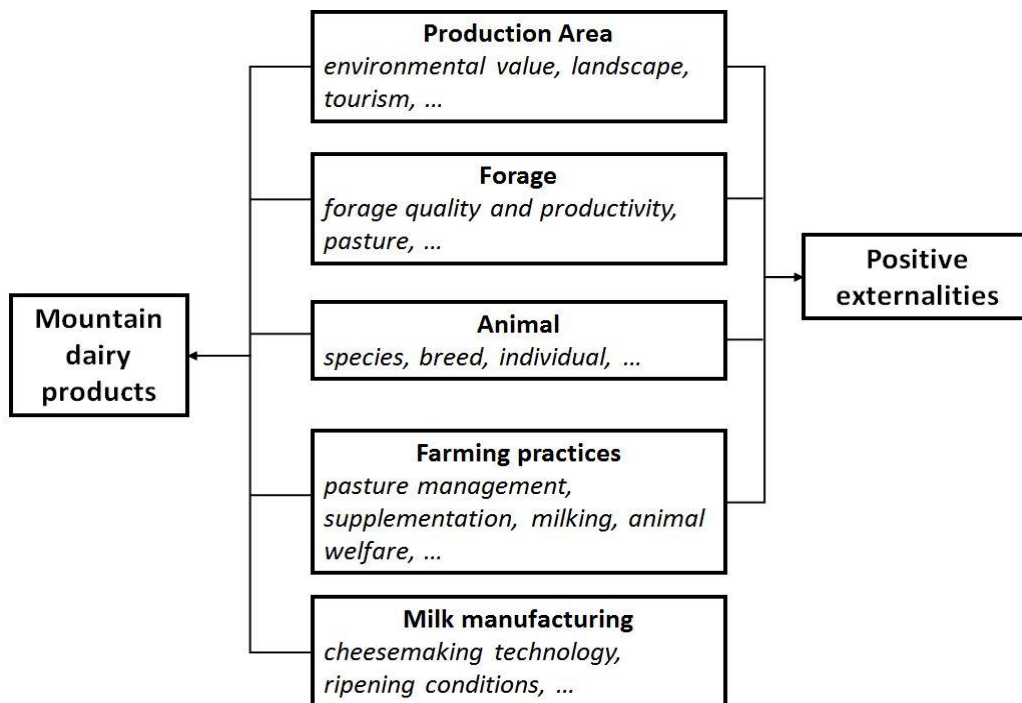
In addition to PAT and PDO cheeses, recently some labels linked to production area (e.g. “Südtirol Quality”, “Saveurs du Val d'Aoste”, ...) or to a single animal breed have spread. In addition to the historical link animal breed-product of the Valdostana breed with the PDO Fontina, in recent years, similar initiatives are increasing. Examples are the trademarks “Di Sola Bruna” (Italian Brown breed), “Formaggio Razza Rendena” (Rendena breed) and “Solo di Pezzata Rossa Italiana” (Italian Simmental breed).

## **1.2. Factors affecting the quality of dairy products in mountain areas**

Dairy products standardization is due to farming practices intensification and to industrialization of dairy manufacturing. The adoption of precise constraints in product specification can maintain (or improve) product-territory relationship, highlighting organoleptic originality and positive externalities of dairy products. Factors affecting quality of alpine dairy products can be classified in five main categories: (i) production area (e.g. environmental value, tourist importance, etc.); (ii) forage component of animal diet (e.g. forage quality and productivity, pasture, etc.); (iii) animal (e.g. species, breed, etc.); (iv) farming practices (e.g. pasture management, concentrate supplementation, animal welfare, etc.); (v) milk manufacturing (e.g. technology, storage conditions, etc.) (Figure 1.1). The four first strongly affect also positive externalities (e.g. carbon sequestration, soil protection, water quality, fire and avalanche protection,

landscapes, biodiversity, outdoor recreation, rural communities and cultural heritage, etc.) of alpine farms.

In recent years several researches were carried out to provide scientific data on subjects listed above, resulting in a great number of reviews and meetings (AAVV, 2000; Agabriel et al., 2004; Biala et al., 2005; Bovolenta et al., 2006; Gasperi and Versini, 2005; Grappin and Coulon, 1996; Peeters and Frame, 2002; Piano, 2010).



**Figure 1.1. Factors affecting the quality of dairy products and the positive externalities of alpine farms**

### 1.2.1 Forage

The forage is the main link between a territory and a dairy product. The quantity and quality of forage, used in animal feeding, significantly affect the nutritional and organoleptic properties of cheeses.



In mountain regions, usually, are produced forages with nutritional qualities lower than those produced in lowlands. A reduced presence of *Fabaceae*, due to lower soil fertility and a shorter growing season typical of the alpine environment, leads to a reduction of the protein content in the grass (Minson, 1990). This affect, other things being equal, milk coagulation time and curd firmness (Leiber et al., 2005). On the other hand, high aromatic value of the mountain forages, due to the floristic richness of meadows and pastures, gives specific sensory characteristics to the local dairy products (Martin et al., 2005).

Several studies highlight the positive effects of direct use of the herbage on the quality of animal products. Significant differences are found in the sensory properties - color, smell, taste, flavor and texture - of cheeses made from the milk of cows fed with green forages rather than dried or silage (Kalac and Samkova, 2010; Kalac, 2011). Generally, cheeses made with pasture milk were more yellow, with a less hard texture and a stronger taste than those made with winter milk (Bovolenta et al., 2005; Martin et al., 2005). However, Buchin et al. (1998) showed a weaker effect of pasture diet on cheese flavour when the milk is previously pasteurized.

The differences in color linked to forage preservation are mainly due to variable amounts of pigments. The most studied in cow milk is  $\beta$ -carotene that depends directly on  $\beta$ -carotene content of forages. In fact, since  $\beta$ -carotene is highly UV-sensitive, it is degraded during grass drying process proportionally to the degree of light exposure. The type of diet, therefore, has a marked effect on carotene content in milk, thus on the color of cheeses (Coulon et al., 2004; Noziere et al., 2006). So, in preserved diets, dairy products made with milk from grass silage are more red-yellow than those made with hay milk, particularly when the hay was left on the ground for a long time (Piasentier and Martin, 2005).

The main effect of forage on the texture of cheese was ascribed on the milk fat composition, which is closely linked to the nature of forage. In particular, milk

fat from pasture diets, compared with hay diets, has a higher proportion of mono and polyunsaturated fatty acids (MUFA and PUFA) and a lower proportion of saturated fatty acids (SFA). The lower melting point of MUFA and PUFA may produce a more fluid fat and consequently softer cheeses (Chilliard et al., 2001).

In addition, are well known the effects of vegetation type and phenological stage of meadows and pastures on texture and flavor of cheese (Buchin et al., 1999; Cabiddu et al., 2009; Collomb et al., 2002; Farruggia et al., 2008). De Noni and Battelli (2008), in a trial conducted during summer transhumance on three pastures at different altitudes, emphasize the importance of the botanical composition of pastures in promoting CLA biosynthesis and in enriching the terpene profiles of milk. The effects of botanical composition on the hydrocarbon and fatty acid composition of cheese was investigated by Povolo et al. (2012; 2013) on two similar pastures in Italian Alpine region. The different concentrations were explained, by the authors, with a different rumen environment created by botanical composition of two pastures.

From a nutritional point of view, dairy products derived from grazing cows are particularly rich in antioxidants compounds such as vitamin E, polyphenols and carotenoids. Weiss (1998) reported that fresh forages contain substantial amounts of  $\alpha$ -tocopherol (80 to 200 IU/kg), but vitamin E concentrations in hay and silage are 20 to 80% lower. Permanent pastures, especially those with high contribution of dicotyledonous species, are particularly rich in polyphenols with different content in relation to the herbage phenological stage (Fraisie et al., 2007; Reynaud et al., 2010). In the bovines a high concentration of carotenoids, mainly  $\beta$ -carotene, is accumulated from the grass and transferred to milk. These compounds are useful for human health (antioxidant, UV skin and macula protection) and are involved in sensory characteristics of dairy product (yellow and red color). Carotenoids are found in higher concentrations in milk produced on pasture. In hay, instead, strongly decreases their concentration mainly due to

solar radiations and to the presence of oxygen (Calderon et al., 2006; Noziere et al., 2006).

Even the fatty acid profile seems to be positively affected by diets based on fresh forages. Dairy products from fresh forage are characterized by a higher content of PUFA (Chilliard et al., 2007; Dewhurst et al., 2006; Revello Chion et al., 2010), which are able to decrease the risks of cardiovascular diseases, and conjugated linoleic acid (CLA) (Chilliard et al., 2001; Kelly et al., 1998), which are assigned an antitumor, immunomodulatory and antidiabetic activities. This is partly due to the inhibitory effect of alpine pasture on the last step of ruminal fatty acid biohydrogenation (Khiaosa-ard et al., 2011).

Over and above the effects on the quality of products, it is clear that the use and the conservation of meadows and pastures in mountain area is important for the protection of biodiversity and landscape as well as for the contribute to the tourism (Gusmeroli et al., 2006; Schirpke et al., 2013). Mountain dairy farms, in addition to providing a public service that is repaid in the agri-environmental measures of the Rural Development Programmes (RDP), creates conditions to sell their products in local markets, thus increasing the added value.

### *1.2.2. Animal*

It is well known that the chemical and technological properties of milk vary according to species, breed and the individual, with particular regard to genetic variants of casein and lacto-globulin, together with the effect of the number of lactations, the physiological stage and diseases of dairy cows (Coulon et al., 2004; Kelsey et al., 2003; Malossini et al., 1996).

The physiological stage, except for the first and the last stage of lactation, seems to have only limited effects on dairy products (Calderon et al., 2007; Penasa et al., 2014). Conversely mastitis, in addition to causing a reduction of the

index of casein, also have some effect on the quality of the cheese (Auldism et al., 1996; Chen et al., 2010).

The effects on the quality of the cheese are clear with regard to the species, while they are less pronounced within species. In cheeses made with whole milk, the texture varies in relation to fat content, due to the different fat/protein ratio of milk processed (Foegeding and Drake, 2007; Pereira et al., 2009). Skimming, even partial, of the milk greatly reduces the differences between cheeses of different breeds (Coulon et al., 2004).

The index of casein and genetic variant B of K-casein are positively correlated with cheese yield (Delacroix-Buchet et al., 1993; De Marchi et al., 2007; Macheboeuf et al., 1993), with limited effects on sensory properties. Some authors report effects, on the texture and taste, of genetic variants of  $\beta$ -casein in dairy cows (Martin, 1998). Auldism et al. (2002) underline that milk from the dual-purpose cows is the most suitable for the production of high quality cheese.

The performances of grazing cows depend on their ability to select and ingest an adequate diet to meet their nutrient requirements. The local breeds, which are more adapted to environmental conditions, have developed a more efficient utilization of intake and a lower locomotion cost. Aharoni et al. (2009) have shown that the local cows (Baladi breed) graze more hours per day and walk longer distances with respect to the selected breed (Beefmaster x Simford).

Also, diet selection may be significantly affected by breed. Dumont et al. (2007a) recorded a limited effect of breed on diet selection, among which a faster biting rate of the Salers compared with commercial breed (Charolais), probably due to a relatively lower selectivity of traditional breed. Braghieri et al. (2011) observed a higher percentage of toxic plants selected by Podolian cows. Native animals that have coevolved with the local vegetation have developed the ability to detoxify toxins of plants.

In general, the breed is not a decisive factor for sensory properties of cheeses other things being equal, although it is difficult to assess separately its

effect. For this reason cows breed, usually, is not a constraint imposed in the product specifications. However, it is necessary to consider the ability of adaptation to the mountain area of the different breeds (Bertoni et al., 2001; Mattiello et al., 2011). The local breeds have been developed with the territory and with human activities, therefore they are able to use the forage resources of the mountain (Battaglini et al., 2006; Weißensteiner, 2010). The preservation of local breeds is recognized as one of the main positive externalities of the agro-environmental measures in the RDP.

Another useful tool for the preservation of local breeds is the monobreed products. Their diffusion is having a significant positive impact on the rural economies and on the conservation of biodiversity (Gandini and Villa, 2003).

### *1.2.3. Farming practices*

Farming practices, especially the feed rationing, the management of grazing animals and supplementation can play a key role in the enhancement of dairy products.

The pastures, especially those located at high altitude, may not always be able to meet the nutritional requirements of lactating cows, increased by the movement involved and the low temperatures. Under these conditions, there is often a decrease in milk production normally accompanied by a failure to recover the body condition (Malossini et al., 1995). Therefore, the supplementation of grazing cows can be a key tool to support milk production at pasture, cheesemaking properties and cheese quality (Berry et al., 2001; Combs et al., 2007). On the other hand, animals respond to high levels of supplements with a reduction of herbage intake through the substitution rate mechanism (the decrease of pasture intake per kilogram of supplement) (Bovolenta et al., 2009; Dillon, 2006; Perez-Prieto et al., 2011a).

Dry matter intake, the main factor of milk production in grazing systems, is primarily controlled by pasture allowance, which is in turn linked to sward height and stocking rate (the number of animals allocated to an area of land) (Maher et al., 2003). Several studies have shown the effect of pasture allowance on grazing behaviour of cows, pasture intake, milk yield and composition (Delaby et al., 2003; Dumont et al., 2007b; Macdonald et al., 2008; Perez-Prieto et al., 2011b; Pulido et al., 2010).

In mountain farms, more and more often, births are spread throughout the year, thus complicating the management of animals in traditional transhumant systems (Garcia and Holmes, 1999; Roche et al., 2011). The feeding of the cows in early lactation is difficult, or even impossible, to pasture, due to the high energy requirements. In this situation increases the needs for maintenance of the cows and the ingestion of grass proves to be the limiting factor of production in particular for the selected breeds.

A lack of knowledge of grazing may severely reduce the beneficial effects of the utilization of Alpine pasture as well as those induced by direct consumption of grass (Bovolenta et al., 2002a, 2002b, 2009; Ventura, 2005). In this direction, the findings of some experiments carried out in the Italian Alps provide useful guidelines for the rational management of forage resources. The issue has been addressed in an interdisciplinary way, through the characterization of the pastures, the study of the techniques of management of grazing animals and their effects on the quality of milk and cheese (Bovolenta et al., 2006; Cavallero et al., 2007; Gasperi and Versini, 2005; Pasut et al., 2006; Piano, 2010; Ziliotto et al., 2004). This approach allows to meet the nutritional requirements of the animals, to improve the meadows and pastures, to get milk and cheeses with organoleptic characteristics related to forage and to ensure animal welfare (Bovolenta, 2008; Dovieer et al., 2008).

In addition to farming practices related to nutrition and reproduction, also the hygiene of the milking barn or in the pasture, plays an important role on the

quantitative and qualitative characteristics of milk (Bertoni et al., 2001; Ventura et al., 2004).

Finally, some studies show that keeping animals in mountain tie-stalls, as well as the practice of seasonal transhumance to the high alpine pastures, do not always give good results in terms of animal welfare (Comin et al., 2011; Corazzin et al., 2009; 2010). Mattiello et al. (2009) have highlighted some welfare problems in tie-stalls related to structural causes. However, the presence of good farmers can help to compensate, at least partially, the structural lacks.

Farming practices are the best tool to achieve a balance between production targets and positive externalities, such as the environmental protection, the hydro-geological structure, the reduction of pollutant emissions, the conservation of animal and plant biodiversity and the provision of cultural services.

#### *1.2.4. Cheesemaking and ripening*

The cheesemaking techniques are the factor that most of the others can affect the organoleptic characteristics of dairy products. The transfer of the quality properties from milk - linked to forage, animal and farming practices - to cheese, is strictly dependent on the conditions of refrigeration, processing and ripening.

Among the alpine dairy products, there are some that provide techniques more respectful of the milk characteristics than the other. For example, the organoleptic characteristics of Fontina cheese are very related to those of milk (Giannino et al., 2009; Renna et al., 2009). Instead, Gorgonzola cheese is almost completely independent from the raw material and its typicality is linked exclusively to the production techniques.

In the raw milk cheeses that use natural starter cultures, the large microbiological biodiversity existent in bulk milk (derived from forage, farm,

milking and dairy) is transferred to the ripened cheese (Lodi et al., 2000). It has been demonstrated that raw milk cheeses have more intense and typical flavour than pasteurized milk cheeses (Albenzio et al., 2001; Demarigny et al., 1997), and, besides modification connected to rennet coagulation properties and indigenous milk enzymes, the main differences concern the large number of bacteria and related enzymes associated with raw milk (Poznanski et al., 2004).

The ripening process can affect much the quality of product because it is able to amplify its strengths and weaknesses. In particular, temperature, humidity and the length of ripening may affect the texture, flavor and aroma of the cheese (Addeo et al., 1998; Agabriel et al., 2004).

In the product specifications, technique of cheesemaking are usually well regulated because their effects are well known, unlike what happens for the factors involved in the production of milk.

### **1.3. Can PDO cheeses be considered as “local identity” products?**

PDO label covers agricultural products which are produced, processed and prepared in a given geographical area complying with a specific product specification (Reg. EU 1151/2012). This results in a detailed description of raw material characteristics, production process and chemical-organoleptic quality of product. Then the analysis of product specifications for PDO cheeses allows to verify the use of production features making a product as a “local identity” one, i.e. not reproducible outside the production area and linked to local traditions. In Table 1.3 are shown the main constraints established by product specification for some alpine PDO cheeses.

Product specification for Fontina PDO cheese is the only requiring tight constraints for all factors analyzed, from production area to cheese storage conditions. In particular, it is the only one obliging the use of a specific breed



(Valdostana). As a consequence the breed, despite the limited production average (3.7 tons per lactation), was not interested by the strong animal number reduction affecting all alpine breeds.

Instead, other PDO specifications enhance only some of the factors taken into consideration. Bitto PDO cheese is entirely produced and manufactured on high altitude pastures of the production area with cows' milk from a single milking and a small amount of goat milk. However, the use of specialized cattle breeds (mainly Brown Swiss) resulted in concentrate supplementation on pasture, once not used. These issues led to the creation of Bitto cheese “Valli del Bitto” (Slowfood Presidium), which requires a limited area of production, hand milking, use of local goat's milk (Orobica breed) and the prohibition of the use of supplements and selected starters. Castelmagno PDO cheese is produced both in valley and on high altitude pasture. The origin is specified on different cheese packaging. In both products, the use of Holstein breed is forbidden.

About Grana Padano, Asiago and Montasio PDO cheeses, produced mainly in lowland and in a small Alpine area, the strategies to improve mountain products were different. The first one obtained a specific geographic indication “Trentino” for cheese produced in the Trento province (entirely alpine) with milk coming from the same province and from some municipalities of Bolzano province. Both Asiago and Montasio terms refer to two famous alpine uplands in Veneto and Friuli Venezia Giulia regions, respectively. However cheeses are produced largely in lowlands. Therefore the MP label utilization allowed to recognize mountain from lowland productions.

**Table 1.3 - PDO cheeses in Alps and main constraints established by product specification**

<b>Cheese - Label</b>	<b>Production area</b>	<b>Forage</b>	<b>Animal</b>	<b>Farming Practice</b>	<b>Milk manufacturing</b>
Fontina PDO – MP	Valle d’Aosta	only regional hay and grass, no silage	only Valdostana breed	concentrate: limitation for each feed	whole milk of 1 milking, > 80d ripening at 90% humidity
Bitto PDO – MP	high altitude pastures in SO province	only pasture and hay (if necessary)	local cattle and goat breeds	concentrate: max. 3kg/d	whole milk of 1 milking, goat milk <10%, >70d ripening
“Valli del Bitto” Slowfood Presidium	Valli del Bitto, Val Brembana e Val Varrone high altitude pastures	<i>id.</i>	local cattle breeds and Orobica goat breed	no concentrate supplementation, hand milking	<i>in loco</i> , no selected starters, 10-20% goat milk
Castelmagno PDO - MP	3 municipalities in CN province	forage >30% from PDO area, no silage	several cattle breeds, goat and sheep	no particular constraints	raw milk of max. 4 milking, goat milk <30%, >60d ripening
Castelmagno di Alpeggio PDO - MP	High altitude pastures of the 3 municipalities	<i>id.</i>	<i>id.</i>	<i>id.</i>	<i>in loco</i> , above 1000 m asl
Grana Padano PDO	34 provinces of North Italy	forage min. 75% from PDO area	cattle breeds	concentrate: max. 50%	raw milk, 1-2 milking, >9 months ripening
Grana Padano PDO “Trentino” (Trentingrana)	TN province and some municipalities in BZ province	no silage	<i>id.</i>	<i>id.</i>	no lysozyme
Asiago PDO	VI, TN, PD (partially) and TV (partially) provinces	no particular constraints	cattle breeds	no particular constraints	1-2 milking, >20d ripening for “Pressato” and > 60d for “d’Allevato”
Asiago PDO - MP	mountain area of PDO provinces	no silage	<i>id.</i>	<i>id.</i>	no lysozyme, >30d, > 90d ripening respectively
Montasio PDO	Friuli V.G. region and NW Veneto region	no particular constraints	cattle breeds	no particular constraints	max. 4 milking, >60d ripening
Montasio PDO-MP	mountain area of PDO area	<i>id.</i>	<i>id.</i>	<i>id.</i>	<i>id.</i>

At present, consumers show a strong interest on innovative subjects i.e. link between products and territory, environmental issues and animal welfare (Gandini and Villa, 2003; Martelli, 2009). The challenge is increasing consumers' capacity to recognize in alpine products a set of *terroir* characteristics. The term *terroir* was defined for wine sector by French researches and then extended to other product to identify a production system based on peculiar environmental conditions, animal ability to exploit local resources and sustainable agricultural practices (Grappin and Coulon, 1996). Although not exhaustive, this analysis revealed that the potentialities linked to *terroir* for Italian alpine dairy products are not exploited enough. The production area and processing techniques are always well defined, as results of product specifications constraints, while fodder, animal characteristics and farming practices are not enhanced enough.

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## 2. AIM OF THE WORK

The thesis is part of a regional project that has the objective to study and promote a new dairy product, a Montasio protected designation of origin (PDO) produced in mountain area (PDM) with milk of Italian Simmental cows (“Solo di Pezzata Rossa Italiana”).

The study was developed as follow:

- I. evaluation of the potential production context;
- II. study of the product range of variability with particular regard to qualitative characteristics;
- III. assess the effect of information on the product origin on the consumers liking;
- IV. evaluation of the effect of pasture type and supplement level on the quality of milk and cheese.

In general, this thesis is proposed as a scientific support for the valorization of the product along all the chain, from the effect of production techniques on the intrinsic quality of product to its promotion through the assessment of the positive externalities of mountain farms.



### 3. MONTASIO PDO-PDM “SOLO DI PEZZATA ROSSA ITALIANA” (\*)

In order to strengthen the link between PDO Montasio, mountain area of *Friuli Venezia Giulia* and local breed Italian Simmental, has been developed the new product Montasio PDO-PDM “*Solo di Pezzata Rossa Italiana*”, taking advantage of product specifications that already exist.

#### 3.1 Production context

##### 3.1.1 Territory - The dairy farming in mountains area of Friuli Venezia Giulia

The livestock sector in mountain area of Friuli Venezia Giulia Region (FVG) follows general trends of the Alpine region (Table 3.1; ISTAT, 2013). This region has seen a gradual decrease of the area at pastures and meadows, until the disappearance of 60% of the hectares in 20 years, from just over 30000 ha in 1990 to about 12000 in 2010. This decrease is probably linked to the drastic reduction of the total number of farms, from nearly 1600 in 1990 to 406 in 2010 (-74,6%), and more particularly to the reduction of the farms that use, during the summer, the pastures, favoring the gradual reforestation of meadows and pastures.

To date, dairy farms in mountain area of FVG take precedence over other types of cattle farming. In 2010, out of 406 farms with a number of animals bred of 6323, 323 were dairy farms with 3463 dairy cows bred. Therefore, also the number of dairy farms is progressively reduced from 1446 in 1990 to only 323 in 2010 (-78%). However, the number of cows per dairy farm is increased from 4.2

(\*) A part of this chapter has been submitted and accepted by JM FAO CIHEAM 2014  
*Romanzin A., Corazzin M., Fontana C., Piasentier E., Bovolenta S. Montasio PDO cheese  
'Mountain Product' and 'Only Italian Simmental breed'*

to 10.7 (+156.7%). Thus confirming the overall trend of the increase of companies' size, mainly due to the closure of smaller farms.

A study carried out on a sample of 327 dairy farms (ERSA FVG, 2008), out of about 400 present in mountain area of *Friuli Venezia Giulia*, has shown that: over 90% are tie-stalls; 43% of farms, corresponding to 31% of dairy cows, use alpine pastures during the summer months. Over 60% of farmers are over 55 years old. On average, farms have a UAA of 15.8 ha and bred 17.2 LU, of which 13.2 represented by dairy cows. The average milk quota delivered to dairy and/or directly sold, amounts to 62 t/year. To a reduction in number of farms is inevitably followed the closure of many small dairies. In the mountain area only 8 dairies have survived with little more than 200 members who deliver about 8000 t of milk per year.

**Table 3.1. Livestock sector in mountain area of Friuli Venezia Giulia Region (ISTAT, 2013)**

Census year	1982	1990	2000	2010	1990-2010 (%)
<b>Pastures and meadows (ha)</b>	38908	31476	24224	12178	-61.3
<b>Bovines (n.):</b>					
Farms	2913	1598	641	406	-74.6
Heads	12618	10084	7101	6323	-37.3
Heads/Farm	4.3	6.3	11.1	15.6	+146.8
Dairy farms	2711	1466	541	323	-78.0
Dairy cows	7507	6122	4097	3463	-43.4
Cows/Dairy farm	2.8	4.2	7.6	10.7	+156.7

### 3.1.2 Product - The Montasio PDO cheese

In FVG Region there are a lot of typical dairy products. Some of them use national or local labels to certify their typicality. For example, 15 regional cheeses fall into the list of PAT ("*Prodotto Agroalimentare Tradizionale*") and 2

have been recognized of Slow Food Presidia ("*Formadi Frant*" and "*Formaggio di Latteria Turnaria*").

Traditional dairy products can be certified at European level through the PDO and PGI labels. As regards in particular the *Friuli Venezia Giulia*, the only dairy product with a European label is Montasio PDO cheese, however for its production also involves part of the Veneto Region (13 out of 43 producers, *Consorzio Tutela del Formaggio Montasio*). Montasio belongs to the group of alpine cheeses and takes its name to a mountain (Jôf Montasio 2754 asl) and to the well-known plateau (on which is located Malga Montasio 1500-1700 asl), but for the most part is produced in lowland.

The Register of the Mountain Products ("*Prodotto della Montagna*") was founded by a national law (L. 289/02, Art. 85, Mipaf 30/12/03) to further characterize the products with PDO and PGI labels, which may include in their product specification this option. Montasio PDO has requested and obtained the amendment of the specification which among other things says: "Was admitted the opportunity to uniquely identify the cheese Montasio PDO "*Prodotto della Montagna*", through the affixing of a label on the rind which will be labeled "PDM", if the entire production cycle, from the production of milk to the ripening for at least 60 days, takes place in mountain area as defined by national legislation, and included in the area of PDO production". Therefore, the product specification does not require further constraints for the production of Montasio PDO-PDM, with the exception of the territorial limitation with regard to the national legislation.

Examples of dairies that use this new label are the Farmers Cooperative of Valcanale located in Ugovizza and Artelatte in Ovaro. However, most of Montasio PDO-PDM is produced in Veneto Region, in particular by Lattebusche in Belluno Province (*Consorzio Tutela del Formaggio Montasio*). Association of Breeders of Friuli Venezia Giulia (AAFVG) conducted an interesting initiative

which has started the production in Malga Montasio of Montasio PDO-PDM cheese (origin verifiable by the code 000 affixed to the rind).

### 3.1.3 Animal – Italian Simmental breed and monobreed products

The cattle breeds reared in mountain area of Friuli Venezia Giulia are mainly Simmental (72%), followed by Brown (14%) and Holstein (9%). In many farms, especially small ones, can be found cows of different breeds and in mountain area are widespread hybridization with the beef breeds (e.g. Belgian Blue) in order to obtain a greater income from the sale of calves (Venerus, 2008).

Table 3.2 shows the numerical trend of the different breeds of cattle found in Carnia, taken as area representative of FVG mountain, in the years 2004-2009. The number of Italian Simmental cows (IS) is progressively increased to the detriment of Brown and Holstein and reached, in 2009, a number of 255 heads that are about 38% of the animals reared in Carnia (Piasentier et al., 2010).

**Table 3.2. Trends in the number of cows of different breeds in Carnia (Piasentier et al., 2010)**

Trend						
	Brown	Holstein	Hybrids	Simmental	Others	Total
<b>2004</b>	358	55	11	171	6	601
<b>2005</b>	269	53	15	215	5	557
<b>2006</b>	297	45	31	238	5	616
<b>2007</b>	274	83	38	232	6	633
<b>2008</b>	253	120	46	246	5	670
<b>2009</b>	248	116	41	255	5	665
<b>2009-2004</b>	-110	61	30	84	-1	64



Simmental is a dual-purpose cattle breed well adapted to different environments, it stands out for its resistance to disease, especially to mastitis, and high fertility (100 days average post-partum to conception interval; AIA, 2010). This breed is able to achieve medium-high levels of production even in marginal environments and in mountains (Piasentier et al., 2010). It is precisely in mountain that Simmental is gradually imposing, through the ability to grazing and the hardiness that comes from its alpine origins.

In Italy, the Simmental breeding began in Friuli through a cross of replacement on the local cattle population. This has meant that for years the breed has been called “Friulian Simmental”, taking the adjective “Italian” only in 1986 (D.P.R. n° 1134/86), following the spread throughout the country.

This breed offers to the farmers the opportunity to diversify mountain productions and to the consumers the opportunity to recognize these products in an extremely undifferentiated market. The ANAPRI (National Association of Cattle Breeders of Italian Simmental breed) since 2008, according to a product specification, gives the label “*Solo di Pezzata Rossa Italiana*” for the dairy products or meat from animals recorded to Herd Book. Regarding dairy products, the specification provides that they must be obtained exclusively from raw milk of IS cows with the only addition of rennet and salt.

Recently, on the basis of an agreement, the Consortium of Montasio cheese has recognized this monobreed label and allows to pair it with own. The production of cheese monobreed will therefore be subjected to more restrictive constraints of the two specifications.

Therefore, in FVG exists the possibility to combine the product Montasio PDO to the mountain territory and to the most representative breed in a single product: Montasio PDO-PDM “Solo di Pezzata Rossa Italiana”.

### 3.2 A project to support

The interest at regional level for this new product, and the potential positive impact that its adequate valorization can lead to dairy farming in Friulian mountains, have materialized in a project initially funded by the “*Comunità Montana del Gemonese, Val Canale e Canal del Ferro*” (2011-2012) and later by the “*Centro di Ricerca e Innovazione Tecnologica in Agricoltura*” (CRITA; 2012-2015).

The aim of the project is to create the scientific and technical context for the production of cheese Montasio PDO-PDM “*Solo di Pezzata Rossa Italiana*”. The project involves actions designed to verify, first, the availability of the operators at local level and, secondly, to assess the possible options for the livestock component, the techniques of cheesemaking and ripening and the promotion of the product.

For experimental purposes, among the potential producers presents on the territory, have been identified two dairies in order to ensure production from winter (cows kept indoor and milk processed in the Dairy of Ugovizza) and from summer (cows grazing on alpine pasture and milk processed in Malga Montasio).

#### *3.2.1 Farmers Cooperative of Valcanale – Dairy of Ugovizza and farms of Valcanale - Canal del Ferro*

From a preliminary valuation of the potential productive context it was noted that few dairies produce Montasio PDO in the regional mountain area. Among these, our choice fell on the Dairy of Ugovizza, as the farms which confer the milk bred for 90% Italian Simmental cows and the Dairy had already shown its intention to convert part of the production in monobreed products.

The production of Montasio PDO-PDM “*Solo di Pezzata Rossa Italiana*” was therefore started separating the milk of Simmental cows and cheesemaking raw

milk according to the product specifications of Montasio and ANAPRI. Based on an agreement between Dairy, ANAPRI and Consortium of Montasio has been designed a *pelure* to be affixed to shapes of Montasio monobreed, that will display the various information useful to the consumers.

The Dairy of Ugovizza is the last dairy cooperative of Valcanale - Canal del Ferro, with a potential source from 8 municipalities (Chiusaforte, Dogna, Malborghetto Valbruna, Moggio Udinese, Pontebba, Resia, Resiutta and Tarvisio). The cooperative has 23 members, which represent almost the totality of the livestock farms of the valleys.

Traditionally the dairy farming in these valleys has been the main source of livelihood for the population and has also been essential for development of local traditions and for formation of the typical alpine landscape. The dairy farming has always been widespread in these areas and is still characterized by the vertical transhumance of cattle. During the winter season the animals are kept indoor in the stables and the milk is conferred to dairy, while in summer cows are often brought to the mountain pasture, and the milk is processed in *malga*.

A first assessment of the farms, that confer the milk to the Dairy of Ugovizza, was carried out using the data collected from AAFVG and some direct surveys. For the collection of data has been prepared a questionnaire for farmers with questions regarding structural characteristics, arable land, livestock, management, productions and other useful information for the evaluation of the technical-economic context. The collected data will be useful for technical assessments that, in a view of global quality of the product, could be used to increase its value. Examples in this regard can be fodder self-sufficient, environmental sustainability, animal welfare, etc...

The surveys were held during 2013 and the data were subsequently processed. The farms were divided according to their size, which is considered as the number of lactating cows in the herd. The farms were separated into three

groups: 0 to 10, 11 to 20 and over 20 cows. In addition to the questionnaire for the assessment of economic and environmental sustainability, have also been used two methods for assessment of animal welfare: Welfare Quality (WQ; Welfare Quality, 2009) and Animal Needs Index (ANI-35L; Bartussek, 2000).

Table 3.3 shows the different categories of animals bred in farms that give the milk to the Dairy of Ugovizza. In the farms surveyed are raised a total of 426 heads divided into dairy cows, heifers, calves and bulls. The average percentage of Simmental animals bred is closer to 90% of the total, as out of a total of 426 animals bred, well 381 belong to this breed.

**Table 3.3. Animals bred in farms that give milk to the Dairy of Ugovizza (n = 20)**

<b>Heads</b>	<b>Total (n)</b>	<b>Simmental (n)</b>	<b>Simmental (%)</b>
Cows	276	246	89.1
Heifers	73	70	95.9
Calves 0-6 months	54	46	85.2
Calves 6-12 months	20	18	90.0
Bulls	7	5	71.4
<b>Total heads</b>	<b>426</b>	<b>381</b>	<b>89.4</b>

Are reported in Table 3.4 the hectares of UAA (Utilized Agricultural Area) of the same companies. The data are organized according to farm size, type of management (property, rent or others - which usually means free use -) and the type of use (defined as pastures / meadows and wood which are the two main categories and others).

The total UAA of farms in the Valcanale - Canal del Ferro that give the milk to Ugovizza is higher than 600 ha, while the average area of farms is equal to 35.6 ha. The larger farms, i.e. those with a higher number of animals in

production, also have a greater UAA (47.2 against 29.0 ha of the smaller farms) however, most of this surface is rented or on free loan (45.31 ha), while smaller farms, although with a lower UAA, have a greater ownership land (6.7 vs 1.8 ha of the larger farms). With regard to the use, most of the UAA is cultivated with pastures and meadows (402.4 ha), while secondly is occupied by woods (223.1 ha).

**Table 3.4. UAA (ha) of farms that give milk to the Dairy of Ugovizza (n=18)**

	Total farms	Average of farms	SD	n° lactating cows		
				0-10	10-20	> 20
<b>UAA (ha)</b>	641.22	35.62	4.79	29.04	38.21	47.15
<b>Management:</b>						
property	91.30	5.07	1.41	6.65	4.66	1.84
rent	152.53	8.47	2.87	2.42	12.96	14.14
others	397.39	22.08	4.46	19.97	20.59	31.17
<b>Use:</b>						
pastures and meadows	402.38	22.35	2.76	18.20	23.38	31.04
wood	223.06	12.39	2.12	9.89	14.51	14.11
others	15.78	0.88	0.29	0.94	0.32	2.00

About 75% of the farms that give the milk to the Dairy of Ugovizza carrying out the vertical transhumance by leading cattle in alpine pastures during the summer (Table 3.5). Some of these lead lactating cows in pastures owned or rented and process the milk directly, while others take indoor lactating cows and giving milk to the Dairy, the latter usually bring in high altitude pastures only heifers and dry cows.

The period of mountain pasture (alp) is about 100 days, which corresponds to the average of 90 days in alpine *malghe* managed by third parties and the longer periods of pasture in *malghe* managed directly. In addition, more than half of farms graze their heads in the pastures of valley in the periods before and after the alp for a total of about 60 days.

**Table 3.5. Use of pastures (Alp and Valley) from farms that give milk to the Dairy of Ugovizza (n=18)**

	Alp			Valley		
	farms (%)	heads (%)	days/year	farms (%)	heads (%)	days/year
Lactating cows	75.0	55.9	100	57.9	71.1	51
Dry cows and heifers	73.7	66.7	108	63.2	70.7	54

**Table 3.6. Forage use by farms that give milk to the Dairy of Ugovizza (n=19) <sup>(1)</sup>**

Forage	Total	n° lactating cows		
		0-10	10-20	> 20
<b>Type:</b>				
Hay <sup>(2)</sup>	69.5	64.7	73.0	68.4
Grass silage	30.5	35.3	27.0	31.6
<b>Origin:</b>				
Produced	80.9	100.0	87.1	66.0
Bought	19.1	0.0	12.9	34.0

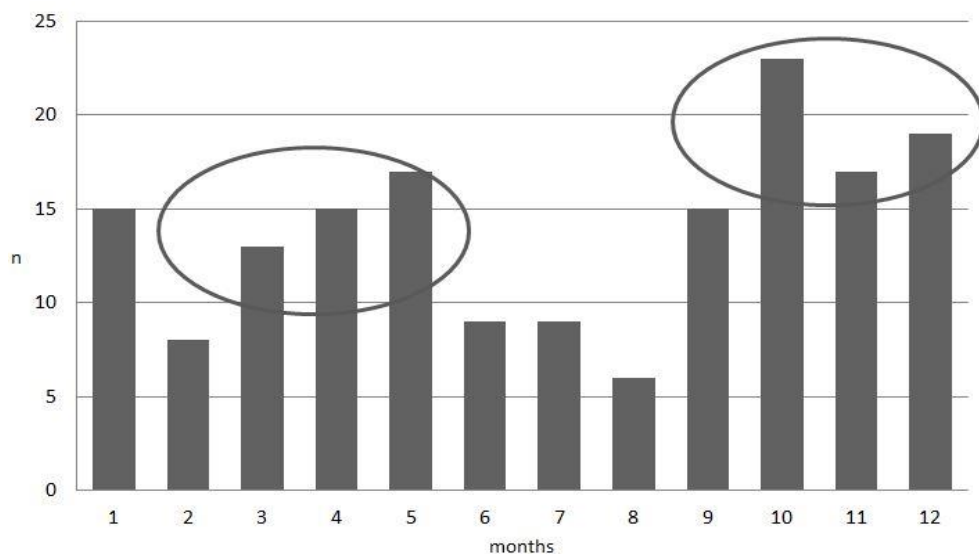
<sup>(1)</sup> Includes only the cut forage, not the grazed

<sup>(2)</sup> Includes polyphyte and alfalfa hay

In the farms considered the forage component of the diet (Table 3.6) is based for two-thirds on the hay, usually coming from the first cut, while for one-third on grass silage, usually derived from the second or in some cases even the third cut.

The forage self-sufficiency of small farms is equal to 100%, which means that they do not buy forage from outside. The larger farms have lower self-sufficiency of forage, until reaching the purchase of approximately one-third of the forage used. Purchased forages, often represented by alfalfa hay, usually comes from other regions.

The births are concentrated in two periods of the year (Figure 3.1), spring and autumn-winter, reflecting different management requirements. Usually farms that concentrate births in spring are those that have an alpine pasture owned and prefer to process larger quantities of milk in dairy products that sell directly into *malga*. Other farms, concentrating the births in the late autumn, avoid to lead cows to pasture at the peak of lactation.



**Figure 3.1. Distribution of births in 2012 in farms that give milk to the Dairy of Ugovizza (n=19)**

From the assessment of animal welfare by the method of Welfare Quality have been obtained overall scores of “enhanced” for 6 farms on 19 and “acceptable” for the other 13. No farm had negative evaluations. Instead, as regards the average scores of the welfare principles (Table 3.7), it can be noted that there are scores rather high for feeding and health of the animals (63.3 and 65.7 respectively), while the lowest score was found in animal housing (31.3). This is due to the fact that all farms considered had tie-stall, a housing system that is particularly penalized by this method.

The second method of evaluation (ANI-35L) takes into account five main aspects of farming: locomotion, social interaction, flooring, light and air, stockmanship. The overall value obtained is equal to 22.2 points, which corresponds to farms which can be defined as “average suitable”.

**Table 3.7. Assessment of animal welfare in farms that give milk to the Dairy of Ugovizza (n=19)**

	Average	Error	n° lactating cows		
			0-10	10-20	> 20
<b><i>Method Welfare Quality</i></b>					
Feeding	63.3	6.20	61.3	66.0	62.6
Housing	31.3	1.55	33.4	29.5	30.4
Health	65.7	4.29	67.7	57.5	75.9
Behaviour	39.0	2.12	39.1	39.3	37.9
<b><i>Method ANI-35L</i></b>					
Locomotion	2.6	0.11	2.6	2.4	3.0
Social interaction	2.9	0.09	3.0	2.7	2.9
Flooring	4.8	0.13	4.8	4.9	4.6
Light and air	4.8	0.27	4.9	4.5	5.1
Stockmanship	7.2	0.14	7.3	7.0	7.4
<b>ANI-35L<sup>(1)</sup></b>	<b>22.2</b>	<b>0.48</b>	<b>22.5</b>	<b>21.5</b>	<b>23.0</b>

<sup>(1)</sup>Total of the partial scores



The two evaluation methods are quite different, because ANI-35L takes into account the parameters of technical-structural type, while Welfare Quality gives more attention to the evaluation of the animals. However, both methods have found no significant differences between farms of different sizes.

**Table 3.8. Milk delivered and processed in Dairy of Ugovizza in 2012**

Month	Farms	Milk delivered	Milk Simmental delivered	Fat	Protein	Price	Milk for Montasio PDO-PDM	Montasio PDO-PDM shapes
	n°	L/d	L/d	%	%	€/L	%	n°
Jan	21	2183	1967	3.93	3.25	0.44	21.4	204
Feb	21	2236	2014	3.95	3.27	0.46	34.7	311
Mar	21	2390	2133	3.84	3.21	0.45	36.2	366
Apr	21	2601	2289	3.95	3.16	0.45	31.9	350
May	23	2709	2377	3.92	3.13	0.42	34.1	401
Jun	19	2206	1951	3.84	3.10	0.42	34.8	319
Jul	11	1418	1268	3.74	3.02	0.49	18.7	113
Aug	9	1476	1327	3.77	3.01	0.48	27.1	174
Sep	13	1578	1439	3.96	3.18	0.49	20.0	138
Oct	17	1826	1659	3.95	3.24	0.43	13.3	108
Nov	19	2087	1893	4.01	3.24	0.44	9.2	77
Dec	19	2321	2090	3.99	3.29	0.44	22.9	221
Year	n°	L (.000)	L (.000)	%	%	€/L	%	n°
<b>2012</b>	<b>23</b>	<b>763</b>	<b>683</b>	<b>3.91</b>	<b>3.18</b>	<b>0.45</b>	<b>26.2</b>	<b>2782</b>

Table 3.8 shows the data for the milk delivered and processed in the Dairy of Ugovizza in 2012. In the months of mountain pasture (from July to September) only few farms delivering milk to the Dairy of Ugovizza, with a minimum of 9 in August. The other farms carrying out the vertical transhumance and processing the milk directly in *malga*. Consequently, also the amount of milk delivered is

lower in the summer, from an average of about 2500 liters per day during the winter months it gets a little more than 1400 liters per day in the summer. The milk from Simmental cows is a little less than 90% of the total, and it also reflects the values shown in Table 3.3, where it is reported that 89.1% of dairy cows are IS breed.

The delivered milk had 3.91 % of fat and 3.18 % of protein content on average. The milk is paid 0.45 € per liter on average, although the values vary according to its quality defined by fat and protein content, somatic cell count and clostridia. The price of milk is highest in summer, in order to encourage farmers to keep lactating cows in farm or in any case give the milk to the Dairy rather than transform it directly, to ensure a production more continuous throughout the year. About a quarter of the milk delivered (26.2%), equal to about 200000 liters, is processed in Montasio PDO-PDM, for a total of 2782 shapes in 2012.

### 3.2.2 Malga Montasio

With regard to the experiments in mountain pasture, in order to evaluate also an alp cheese, was chosen Malga Montasio since it is the only alp already producing Montasio PDO-PDM cheese. In addition in it are bred mainly Italian Simmental cows.

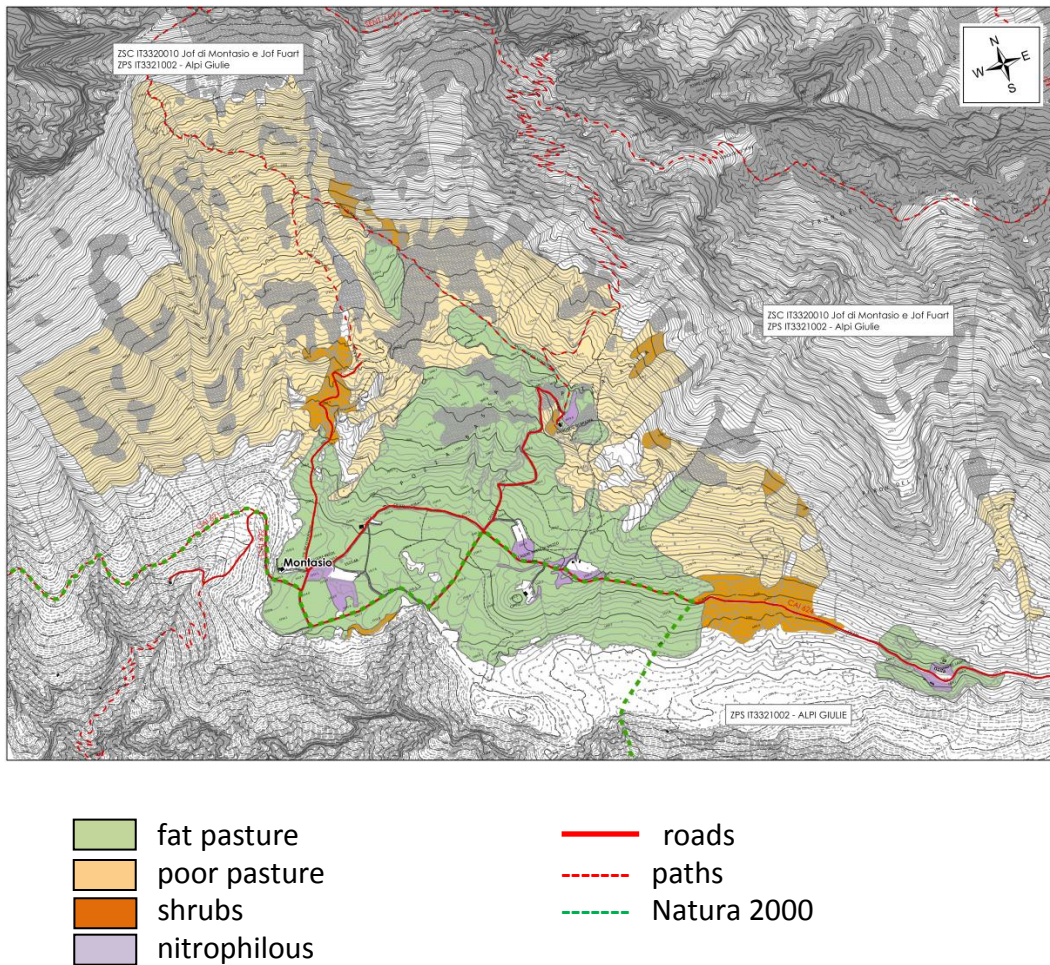
Malga Montasio is situated on the homonymous plateau in the municipality of Chiusaforte (Udine Province). It includes many pastures (nearly 500 hectares), facilities for animals housing, milk processing and cheese ripening. The area is owned by the “*Associazione Tenutari Stazioni Taurine ed Operatori di Fecondazione Artificiale*” but is managed by AAFVG. Breeders Association of FVG, who has long collaborated with the Dairy of Ugovizza and the University of Udine, has ensured the necessary logistical and analytical support for the experimental tests.

On Malga Montasio are reared every summer (from June to September) dairy cows and young cattle, mainly of Simmental breed. Are raised, to a lesser number, other breeds such as Holstein, Brown and hybrids. In 2013, 339 cattle have been bred, including 140 dairy cows from 30 different municipalities in the Friuli and belonging to more than 50 members of AAFVG.

Dairy cows are grazing day and night, driven by herdsmen during the day and confined to fenced pastures, adjacent to the stables, at night. The stables are used only for the two milkings and for the veterinary assistance. In the last years have been used the pastures Pecol, Parte di Mezzo and Larice, the latter only with heifers.

The production of milk during summer ranging from 16-18 quintals per day in June to 8-10 in September. Milk is processed into the dairy of *malga* to produce Montasio cheese, *caciotta* cheese, *ricotta*, yogurt and butter.

A primary aim of the trial in Malga Montasio was to create a physiognomic map of the pasture vegetation. Collaboration with a botanist has allowed to obtain geo-referenced map, combining information derived from aerial images and the recognition of the vegetation type on the ground.



**Figure 3.2.** Physiognomic map of pasture vegetation in Montasio plateau (Pasut, 2013)

On the basis of preliminary studies conducted by Pasut (2013), pasture vegetation can be divided into three areas (Figure 3.2) sufficiently homogeneous:

- fat pasture, is the lower part of the pasture, flat or only slightly inclined, and is characterized by several species with a good forage value as *Phleum alpinum*, *Festuca pratensis* and *Poa alpina* and other species typical of a medium fertile pasture, as several species of clover (*Trifolium badium*, *T. repens*, *T. pratense*), *Lotus corniculatus*, *Alchemilla vulgaris*, *Crepis aurea* and *Leontodon hispidus*;

- poor pastures, present on the steep slopes in the upper part of the pasture, is characterized by a greater rusticity and species such as *Sesleria coerulea*, *Koeleria pyramidata* and *Nardus stricta*;

- nitrophilous phytocoenoses: *Deschampsia caespitosa* and *Veratrum lobelianum*, which is present around the stables and is a cenosis derived from *Poion alpinae*, in the presence of a strong eutrophication of the soil. *Rumex alpinus*, which is found mainly near the stables of *Parte di Mezzo* and *Larice*, and form a closed population.

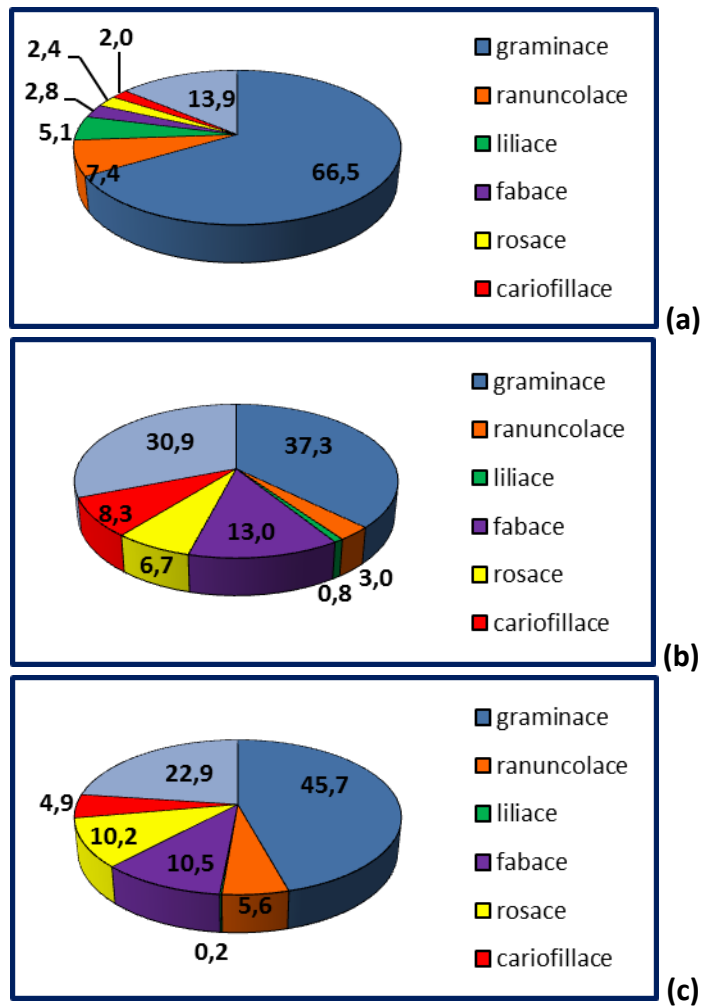
For the experiments were selected two representative pastures of the Malga. The first is a fat pasture at 1500 m asl, the second is a poor pasture situated at an average altitude of 1700 meters. The amount of grass available to cows was estimated by cutting, in each of the identified pastures, 14 strips of 10 x 0.10 m at 4 cm in height, using electric shears. On the samples was carried out a botanical assessment in weight.

The grass effectively consumed by grazing cows was estimated by the "hand plucking" method (Pulido and Leaver, 2001) which consists in simulating the bite of cows and thereby to collect the herbage adjacent to an individual grazing cow and at the same height. For 3 consecutive days and for each pasture ("Fat" and "Poor") were taken samples of the grass consumed by 6 cows for each

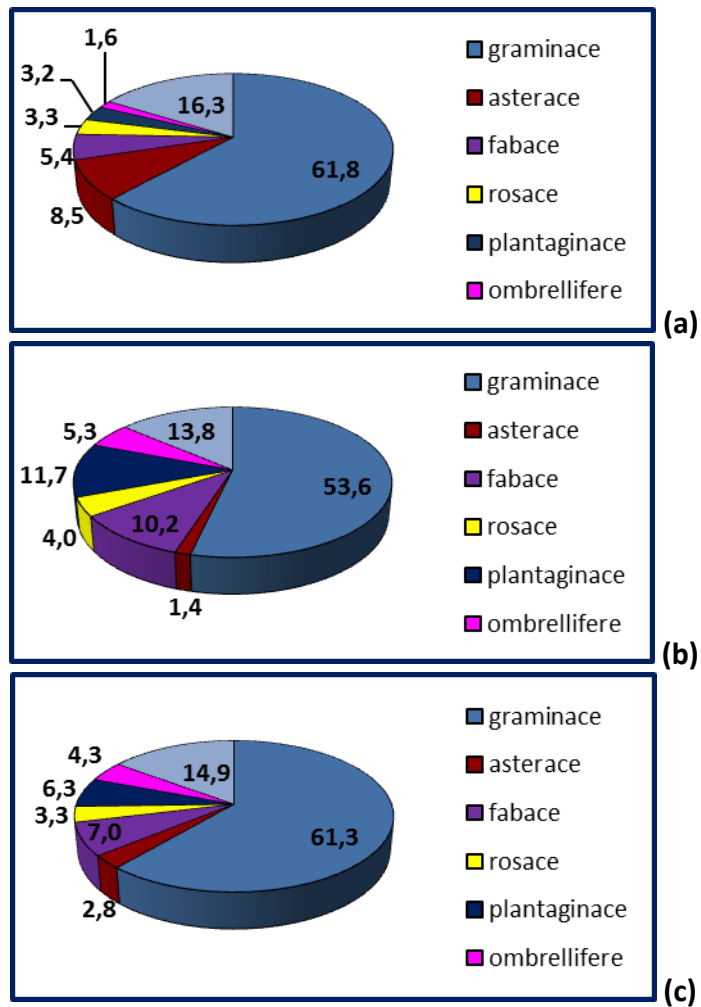
experimental group (supplement level “High”: 3 kg/day; “Low”: 1.5 kg/day). Even on these samples was evaluated the botanical composition by weight.

Following figures show the botanical compositions of the grass available **(a)** and consumed by cows with integration “High” **(b)** and “Low” **(c)**, respectively for grazing “Fat” (Figure 3.3) and “Poor” (Figure 3.4).

As can be seen from figures, both pastures are characterized by a predominance of *gramineae* (66.5 and 61.8%) but considerable differences between the other families was recorded. On the first pasture families more appetite were *fabaceae*, *rosaceae* and *caryophyllaceae* while the families less appetite were *liliaceae*, *asteraceae*, *polygonaceae* and *ranunculaceae*. On the second pasture cows have been selected mainly *umbelliferae*, *plantaginaceae* and *fabaceae* and less *asteraceae*, *hypericaceae* and *ranunculaceae*. The cows have ingested large amounts of *gramineae* (on average 93% of available) in poor pasture as opposed to fat pasture where this percentage was reduced to 62% of grass available. The two experimental groups of cows have selected differently between the various botanical families. In fact, cows with “High” supplement level have generally selected less *gramineae* and botanical families unappealing and most *fabaceae*, *caryophyllaceae* and *plantaginaceae* compared to the group “Low”.



**Figure 3.3.** Fat pasture: botanical composition of grass available (a), of grass consumed by cows with High supplement level (b) and by cows with Low supplement level (c).



**Figure 3.4.** Poor pasture: botanical composition of grass available **(a)**, of grass consumed by cows with High supplement level **(b)** and by cows with Low supplement level **(c)**.



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## **4. FACTORS AFFECTING CHEMICAL, REOLOGICAL, AROMATIC AND SENSORY PROPERTIES OF MONTASIO PDO-PDM MONOBREED**

### **4.1 Effect of rearing system (mountain pasture vs indoor) of Simmental cows on milk composition and Montasio cheese characteristics**

A first experimental work was conducted in 2011-2012 with an agreement between the Department of Agriculture and Environmental Science and the “*Comunità Montana del Gemonese, Val Canale e Canal del Ferro*” (ProMo project) and a collaboration of AAFVG.

The trial was aimed to assess the range of variability of the qualitative characteristics of Montasio PDO cheese, made from milk of Italian Simmental cows (grazing to pasture or kept indoor).

The results have been partially presented at: Romanzin A., Corazzin M., Saccà E., Sepulcri A., Bovolenta S., 2012. Quality of Montasio cheese from Italian Simmental cows grazing on mountain pasture or reared indoor. In: P. Golinski, M. Warda and P. Stypinski (Eds) *Grassland: a European resources?*. Proceedings of the 24<sup>th</sup> General Meeting of the European Grassland Federation, Lublin, Poland, 3-7 June (Polish Grassland Society, Poland), *Grassland Science in Europe*, 17, 414-416; and published as: Romanzin A., Corazzin M., Piasentier E., Bovolenta S., 2013. Effect of rearing system (mountain pasture vs. indoor) of Simmental cows on milk composition and Montasio cheese characteristics. *Journal of Dairy Research* (Cambridge University Press, UK), 80, 390-399.

# **Effect of rearing system (mountain pasture vs indoor) of Simmental cows on milk composition and Montasio cheese characteristics <sup>(\*)</sup>**

## **Abstract**

Dairy cattle in the Alps are traditionally maintained on high altitude pastures during summer. In recent decades, however, many farmers prefer to maintain the cows always indoor with a hay-based diet. Many authors have shown that the forage type is able to modify the characteristics of milk and cheese. Recently the product specification of PDO Montasio allowed differentiation between mountain cheeses and other products. Aim of this trial is to study the effect of rearing system on the characteristics of milk and cheese produced in this context. One hundred and twenty Simmental dairy cows were considered, 60 grazed on high altitude pasture, and 60 kept indoor and fed a hay-based diet. Cheese production was repeated in two periods (early July and late August) and ripened two and six months. Pasture-derived milk and cheese presented higher fat and lower protein content than hay-derived ones. Rearing systems also affected cheese colour. Textural parameters, hardness, gumminess and chewiness were found to be higher in pasture-derived cheese. In addition, it showed lower level of total saturated fatty acids, and higher level of mono and polyunsaturated fatty acids than hay-derived cheeses. Consumers perceived the difference of cheeses in terms of colour and holes, but they express a similar overall liking. More limited effects of period and ripening time were observed.

<sup>(\*)</sup> This chapter has been published as:

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*Keywords:* Mountain pasture, indoor housing, Simmental cows, milk, Montasio cheese.

## **Introduction**

In the alpine mountains, the traditional breeding system of dairy cattle involves the direct use of high altitude pastures during summer. In addition to the traditional system the percentage of farms keeping animals in the stable all year with a hay-based diet is increasing.

Many studies have shown that, other factors being equal, the forage component of the diet is able to modify milk and raw milk cheeses characteristics. From a nutritional point of view, pasture-derived dairy products seem particularly interesting when assessed in relation to anti-oxidant substances content such as vitamin E, polyphenols and carotenoids (Lucas et al. 2006; Noziere et al. 2006). Furthermore, the fatty acid (FA) profile is favorable to human health being characterised by a higher content of polyunsaturated fatty acids (PUFA), able to decrease the risk of cardio-vascular origin, and acid conjugated linoleic acid (CLA), which seem to be involved in anti-tumour, immuno-modulatory and anti-diabetic activity (Dewhurst et al. 2006). In effect, the hay- making process, i.e. mechanical damage to plant tissues combined with air access, causes extensive oxidation of PUFA (Kalac & Samkova, 2010). Even the sensory properties – colour, smell, aroma, flavour and texture – of cheeses made with pasture-derived milk rather than dry forages may be different (Coulon et al. 2004). These characteristics are also modified during ripening because of the different enzymatic processes, including proteolysis and lipolysis, which play a key role in texture and aroma development, typical of the product (McSweeney & Sousa, 2000).

In order to increase the market value of mountain dairy products, in particular quality-labelled products, it is important to test the ability of consumers to

recognise and appreciate these differences. Montasio cheese is one of the most important Protected Designation of Origin (PDO) products in North-East Italy. However, although it takes its name from a mountain plateau, it is produced and manufactured largely in lowlands. Several papers about Montasio cheese have been published highlighting its chemical, nutritional and organoleptic characteristics (Innocente et al. 2002, 2013; Marino et al. 2003), while there are no studies on the effect of the forage component of the diet. Recently, in order to valorise the mountain cheese and to link it to the breed more present in this area, the production of Montasio PDO was combined with two additional labels: 'Mountain Product' and 'Only Simmental breed'.

The aim of the study was to compare the quality properties of this cheese produced from milk of Italian Simmental cows grazing on mountain pasture or fed indoor with a hay-based diet.

## **Materials and methods**

### *Animals and rearing systems*

The trial was carried out using 120 lactating Italian Simmental cows, registered on the herd-book (milk production:  $19.2 \pm 0.72$  kg/d, number of lactations:  $2.7 \pm 0.17$ ; stage of lactation:  $183 \pm 13.1$  d in milk).

Sixty dairy cows (Group MP) were maintained on mountain pasture (Malga Montasio, Udine, Italy; lat.  $46^{\circ}24'45''\text{N}$ , long.  $13^{\circ}25'53''\text{E}$ ; altitude 1500–1800 m) characterised by a Poion alpinae alliance (main species: *Phleum alpinum*, *Festuca pratensis*, *Poa alpina*, *Trifolium repens*, *Trifolium pratense*, *Leontodon hispidus*). Cows were allowed to pasture day and night and milked twice a day. At milking, cows were supplemented with 2 kg head/d of concentrate (based on maize, barley, beet pulp, soybean and wheat).

The other 60 cows (Group I) were reared indoors at lowland altitude and fed with alfalfa hay, permanent meadow hay characterised by a *Arrhenatheretum elatioris* association (main species: *Arrhenatherum elatius*, *Dactylis glomerata*, *Phleum pratense*, *Poa pratensis*, *Festuca rubra*, *Festuca pratensis*, *Trifolium repens*, *Trifolium pratense*) and concentrate (based on maize, barley, soybean and bran). The average forage to concentrate ratio was approximately 60:40.

### *Cheese making and ripening*

In two periods, early July (J) and late August (A), milk from the evening milking (cooled to 10 °C) pooled with milk from the morning was processed for three consecutive days in accordance with the product specification of PDO Montasio cheese.

In the vat, milk was heated to 32 °C and then 7.5 g natural starter/kg of milk was added (natural starter: microflora naturally present in raw milk and cultured in appropriate conditions for a day). After 25 min of incubation the rennet (Clerici Sacco, 96% chymosin) was added. Firming time (between the addition of rennet and the beginning of cut of the curd) was 20 min. The curd was cut to obtain pellets of size comparable to grain of rice, then it was cooked at 45 °C for 30 min. The curd was blended out fire for 20 min and left to stand. After draining the whey, it was placed in moulds and pressed. The curd at the time of extraction had an average pH 6.30 (43 °C), while at 8 h to extraction from the vat, it had pH 5.50 at 25 °C.

Fresh cheeses were then placed in brine for 24 h (18% salt concentration). Subsequently the salt was put once on one side of the cheeses that then were stacked. Cheese shape was cylindrical, about 6 kg weight and 70 mm height. Two cheeses for each cheese making were ripened for 60 (RT60) and 180 (RT180) days in a ripening cellar with controlled temperature (12 °C) and humidity (85%) until analysed.

### *Chemical and rheological analysis*

On samples of milk were made the following determinations: fat, protein and lactose (IDF, 2000), urea (AOAC, 2000) and somatic cell count (SCC; Foss-o-Matic, Foss Electric). SCC data was analysed as somatic cell score (SCS)= $\log_2$  (SCC/100000)+3 (Schutz, 1994).

The experimental cheeses were analysed for: dry matter (DM) using a gravimetric method, fat according to the Schmidt-Bondzynski-Ratzlaff method, total nitrogen (TN) and soluble nitrogen at pH 4-6 (SN) by the Kjeldhal method (AOAC, 2000). The ripening index (RI) was calculated as ratio (x100) between SN and TN. Protein content was obtained multiplying TN $\times$ 6.38.

A spectrophotometer (Minolta CM2600d) was used for measuring colorimetric parameters: L\*, a\* and b\*.

The rheological properties of cheeses were evaluated with a Texture Analyser (TA Plus, Lloyd Instruments, UK) using the procedure described by Bourne (1978) and modified by Gunasekaran & Ak (2003). Texture Profile Analysis (TPA) was applied to the cylinders of cheese (20 mm in diameter and 20 mm height) compressed axially in two consecutive cycles, with a deformation of 50% of the original height and applying a force of 100 mm/min. The rheological parameters analysed were: hardness, cohesiveness, adhesiveness, springiness, gumminess and chewiness.

For fatty acids (FA) analysis milk was centrifuged at 17800 g for 30 min at 8 °C, milk cream was stored at  $\pm$ 20 °C until analysed. Lipid extraction of milk cream and cheese fat was performed according to Hara & Radin (1978). FA were transesterified with sodium methoxide according to method of Christie (1982) and modify by Chouinard et al. (1999).

FA methyl ester in hexane were then injected into a GC system (model HRGC 5300 Carlo Erba, IT) with 1:50 split mode. Separation was performed with a SP-2380 fused silica capillary column (60 m $\times$ 0.25 mm $\times$ 0.25  $\mu$  M , Supelco,



Bellefonte, US). Helium was used as carrier gas. Oven temperature was programmed from 50 to 230 °C and held for 70 min; injector and detector were set at 250 °C. Each peak was identified by pure methyl ester standards (Supelco 37 Components FAME Mix; Fluka CLA 10E, 12Z and 9Z, 11E; Sigma Methyl Trans-Vaccenate) and quantified with Fluka Nonadecanoic acid as internal standard. The milk and cheese FA composition was expressed as g/100 g of identified FA.

### *Consumer test*

The consumer test was performed in four subsequent sessions, according to UNI ISO 8589:1990, using 280 consumers of cheese (151 men and 129 women; mean age of 40 years; 85% eat cheese more than once a week).

The consumers not previously informed about cheeses origin were asked to record their overall liking (Labelled Affective Magnitude scale; LAM from -100 to +100; Cardello & Schutz, 2004), and intensity scores (Just About Right scale; JAR from 1 to 5, with 3 point for the ideal of typicality for each descriptor; Chambers & Baker Wolf, 1996) for colour, holes, smell, taste and structure. JAR data were expressed as frequency.

### *Statistical analysis*

The statistical analysis was performed using the free software R version 2.14.1. Normality of data distribution was tested by the Shapiro-Wilk test. Milk data were subjected to two-way ANOVA with rearing systems (RS; MP and I) and period (P; J and A) as fixed factors. Cheese chemical composition, texture and overall liking were analysed using the general linear model (GLM) repeated measures procedure considering the ripening times (RT; RT60 and RT180) as within- subject factors and RS and P as the between-subject factors. Also the triple interaction was considered, but not reported in Tables because it never

reached the level of significance. When an ordinal interaction from the perspective of a factor was significant, the main effect of the same factor was discussed (Keppel, 1973). Student's t-tests were performed for overall liking data. The frequency distributions for JAR scales were compared using Stuart-Maxwell and McNemar tests as proposed by Stone & Sidel (2004). Data were also processed by Principal Component Analysis (PCA) carried out using The Unscrambler X version 10.2 (Camo Software AS, Oslo, Norway). Data were weighted with  $1/SD$  and the full cross-validation method was used.

## **Results and discussion**

### *Milk composition*

MP milk had a higher fat and lower protein content than I milk (Table 4.1). As well known the high levels of energy in the diet of animals kept indoor reduce fat content in milk, reducing the synthesis of acetic acid in the rumen while maintaining a high protein content (Bargo et al. 2003; Delaby et al. 2003). In addition, the lower protein level in MP milk could be due to low energy supply and hypoxia, which are characteristic on high mountain pastures (Leiber et al. 2006).

Urea content was slightly higher in I. The results obtained in both rearing systems fall in the range of values reported by Jonker et al. (1998) and are indicators of the correct balance of the ration. SCS of MP milk, result comparable with that of I milk, with an average value of 230700 units/ml. This value is lower than those reported by Bovolenta et al. (2008, 2009) but similar to that recorded by Comin et al. (2011) in similar conditions in alpine pastures. Surprisingly MP milk showed lower total bacteria count, both values are well below the limit of 100000 cfu/ml.

**Table 4.1.** Characteristics of milk, n = 24

	Rearing System <sup>†</sup>		Period <sup>‡</sup>		SEM	Significance <sup>§</sup>		
	MP	I	J	A		RS	P	RS×P
Fat (%)	3.97	3.85	3.89	3.94	0.026	*	ns	ns
Protein (%)	3.30	3.47	3.36	3.41	0.004	**	**	**
Lactose (%)	4.63	4.80	4.74	4.69	0.004	**	**	ns
Urea (mg/100ml)	22.12	24.25	20.26	26.11	0.444	*	**	ns
SCS <sup>¶</sup> (units)	4.20	4.37	4.37	4.20	0.067	*	*	**
Total bacteria (10 <sup>3</sup> cfu/ml)	10.2	34.2	25.0	19.3	3.82	*	ns	ns

<sup>†</sup> MP: mountain pasture, I: indoor;

<sup>‡</sup> J: July, A: August;

<sup>§</sup> RS: Rearing System, P: Period, \*:  $P < 0.05$ , \*\*:  $P < 0.01$ , ns: not significant;

<sup>¶</sup> SCS: Somatic Cell Score

As expected, from J to A, there was an increase of milk fat, protein and urea contents. These changes in milk composition are due to the effect of lactation stage as explained also by Coulon et al. (1998).

#### *Milk fatty acid profile*

In general, MP cheese had lower levels of short and medium chain saturated fatty acids (SFA), with the exception of C4:0 (Table 4.2). MP milk was richer in C18:3 n-3 and its biohydrogenation products (trans11-C18:1 and C18:0) than I milk. Also CLA isomers, which derived both from desaturation of trans11-C18:1 in mammary gland and from biohydrogenation of PUFA in rumen (Rutkowska et al. 2012), was higher in MP than I milk. MP milk showed higher values of PUFA and lower values of SFA than I milk, in agreement with the results of several studies (Dewhurst et al. 2006; Coppa et al. 2011). Despite over the 80% of dietary PUFA could be hydrogenated in rumen (Scollan et al. 2001), these results could be due to the high content of PUFA n-3 of the fresh forage (50–75% of total FA; Elgersma et al. 2006), and by the high losses of these FA during hay making (Kalac & Samkova, 2010). However Collomb et al. (2008) and Khiaosa-ard et al. (2011) explained the high level of C18:3 n-3 of milk of grazing cows as a

possible consequence of the interaction among many factors such as: pasture feeding, alpine hypoxia condition of animals, and a reduced ruminal biohydrogenation due to possible presence of polyphenols or terpenoids in fresh forage. Moreover, these differences may have been increased by the different levels of concentrate offered to the two groups of animals (Bovolenta et al. 2009; Khiaosa-ard et al. 2010).

Odd and branched chain fatty acids (OBCFA) were higher in MP than I milk, in agreement with the findings of Looor et al. (2005). OBCFA derived mainly from rumen bacterial, and they could be related to change in the substrate for microbial populations of rumen. Short chain fatty acids (SCFA; C4:0–C10:0) were similar in both RS, while Medium chain fatty acids (MCFA; C11:0–C16:0) were lower in MP than I milk. Despite part of C16:0 and, to lesser extend to C14:0, can derived from circulating lipids, short and medium fatty acid can be used to evaluated the mammary de novo FA synthesis (Glasser et al. 2005). In our trial, higher level of dietary C18:3 n-3 and subsequent higher mammary uptake of this FA could have induced an inhibition effect on FA synthesis in agreement with the findings of Yang et al. (2012).

The differences between cheese making periods were less evident than those between rearing systems. J milk showed higher levels of C14:0, C16:0 and lower level of CLA isomers with respect to A milk. However a disordinal interaction between experimental factors was found, which was largely a response to greater difference between periods of C14:0 (J: 13.65 vs. A: 11.88) and C16:0 (J: 33.02 vs. A: 31.26) in I milk than in MP milk, and to greater period difference of CLA isomers in MP milk than in I milk (J: 1.35 vs. A: 1.76). These results are in agreement with Coppa et al. (2010) who found a limited effect of season on the level of these FA of milk from cows grazing on mountain pasture, with the exception of CLA that increased from July to September. In general, J milk presented slightly higher SFA and lower PUFA content.

**Table 4.2.** Milk fatty acids profiles (g/100g of total fatty acids), n = 24

	Rearing System <sup>†</sup>		Period <sup>‡</sup>		SEM	Significance <sup>§</sup>		
	MP	I	J	A		RS	P	RS×P
C4:0	2.99	2.47	2.73	2.74	0.032	**	ns	**
C6:0	1.69	1.62	1.69	1.62	0.029	ns	ns	**
C8:0	1.03	1.11	1.10	1.04	0.020	ns	ns	ns
C10:0	2.52	3.03	2.86	2.68	0.055	**	ns	ns
<i>cis</i> 9-C10:1	0.18	0.24	0.22	0.20	0.013	ns	ns	ns
C11:0	0.11	0.08	0.10	0.09	0.011	ns	ns	ns
C12:0	3.06	3.81	3.54	3.33	0.056	**	ns	ns
<i>iso</i> -C13:0	0.03	0.05	0.04	0.05	0.005	ns	ns	*
<i>cis</i> 9-C12:1	0.02	0.05	0.06	0.02	0.005	*	**	ns
<i>iso</i> -C12:1	0.07	0.08	0.07	0.08	0.003	ns	ns	ns
C13:0	0.08	0.10	0.09	0.08	0.003	*	ns	ns
C14:0	11.00	12.77	12.27	11.49	0.099	**	**	**
<i>iso</i> -C15:0	0.24	0.23	0.22	0.24	0.017	ns	ns	*
<i>cis</i> 9-C14:1	1.51	1.35	1.45	1.41	0.013	**	ns	**
<i>anteiso</i> -C15:0	0.00	0.03	0.01	0.02	0.005	*	ns	ns
C15:0	1.34	1.22	1.29	1.27	0.010	**	ns	**
C15:1	0.30	0.28	0.29	0.28	0.005	ns	ns	ns
C16:0	27.51	32.14	30.20	29.44	0.148	**	*	**
<i>trans</i> 9-C16:1	0.65	0.42	0.54	0.52	0.007	**	ns	ns
<i>cis</i> 9-C16:1	1.20	1.32	1.26	1.26	0.014	**	ns	ns
<i>iso</i> -C17:0	0.50	0.44	0.47	0.47	0.008	**	ns	ns
<i>anteiso</i> -C17:0	0.04	0.07	0.06	0.05	0.009	ns	ns	**
C17:0	0.73	0.58	0.68	0.63	0.021	**	ns	ns
<i>cis</i> 9-C17:1	0.28	0.23	0.26	0.25	0.006	**	ns	*
C18:0	11.21	10.42	10.73	10.89	0.110	**	ns	ns
<i>trans</i> 9-C18:1	1.24	0.31	1.19	0.36	0.034	**	**	**
<i>trans</i> 11-C18:1	3.62	1.59	1.88	3.34	0.064	**	**	**
<i>cis</i> 9-C18:1	22.36	21.66	21.57	22.45	0.158	ns	*	**
<i>cis</i> 11-C18:1	0.72	0.62	0.64	0.70	0.011	**	*	ns
C18:2 <i>n</i> -6	0.61	0.38	0.48	0.51	0.065	ns	ns	ns
C18:3 <i>n</i> -6	0.13	0.12	0.09	0.16	0.012	ns	*	*
C18:3 <i>n</i> -3	1.25	0.42	0.79	0.87	0.021	**	ns	*
ΣCLA <sup>¶</sup> isomers	1.55	0.51	0.93	1.14	0.029	**	**	**
C20:3 <i>n</i> -3	0.07	0.09	0.06	0.10	0.002	**	**	ns
C22:0	0.09	0.06	0.07	0.08	0.003	**	*	ns
C20:4 <i>n</i> -6	0.07	0.13	0.08	0.13	0.009	**	*	ns
SFA <sup>¶</sup>	64.16	70.20	68.14	66.22	0.218	**	**	ns
MUFA <sup>¶</sup>	32.16	28.15	29.43	30.88	0.171	**	**	*
PUFA <sup>¶</sup>	3.69	1.65	2.43	2.90	0.077	**	*	ns
OBCFA <sup>¶</sup>	3.15	2.83	3.02	2.97	0.010	**	*	**
SCFA <sup>¶</sup>	8.42	8.47	8.60	8.29	0.123	ns	ns	**
MCFA <sup>¶</sup>	47.11	53.91	51.44	49.59	0.247	**	**	**
LCFA <sup>¶</sup>	44.47	37.62	39.97	42.13	0.332	**	*	*
<i>n</i> -3	1.32	0.50	0.85	0.97	0.022	**	*	*
<i>n</i> -6	0.82	0.64	0.65	0.80	0.073	ns	ns	ns
<i>n</i> -6/ <i>n</i> -3	0.63	1.26	0.78	1.11	0.061	**	*	**

<sup>†</sup> MP: mountain pasture, I: indoor;

<sup>‡</sup> J: July, A: August;

<sup>§</sup> RS: Rearing System, P: Period, \*:  $P < 0.05$ , \*\*:  $P < 0.01$ , ns: not significant;

<sup>¶</sup> CLA: conjugated linoleic acids, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, OBCFA: odd and branched chain fatty acids, SCFA: short chain fatty acids, MCFA: medium chain fatty acids, LCFA: long chain fatty acids

### Cheese composition and texture analysis

As expected in relation to the composition of milk, MP cheeses showed higher fat and lower protein content than I cheeses (Table 4.3). The values of ripening index did not vary between rearing systems and were similar to those reported for Montasio PDO cheese (Innocente et al. 2002). The increase of 4.1 points in the ripening index from RT60 to RT180 is in agreement with data reported by Coppa et al. (2011) on Cantal cheese.

**Table 4.3.** Chemical composition, ripening index and colorimetric parameters of cheeses, n = 48

	Rearing System <sup>†</sup>		Period <sup>‡</sup>		Ripening Time <sup>§</sup>		SEM	Significance <sup>¶</sup>					
	MP	I	J	A	RT 60	RT 180		RS	P	RT	RS×P	RS×RT	P×RT
Chemical composition													
DM <sup>††</sup> (%)	69.7	67.8	68.3	69.2	66.6	70.8	0.16	**	*	**	ns	ns	**
Fat (%DM)	54.2	50.9	52.3	52.7	52.6	52.5	0.21	**	ns	ns	ns	ns	ns
Protein (%DM)	38.6	42.3	40.9	40.0	40.7	40.2	0.25	**	ns	ns	ns	ns	ns
WSN <sup>††</sup> (%DM)	0.6	0.7	0.6	0.6	0.5	0.7	0.01	**	ns	**	*	*	**
Ripening index <sup>††</sup>	13.5	14.8	14.3	14.1	12.1	16.2	0.31	ns	ns	**	*	**	**
pH	5.28	5.40	5.37	5.31	5.32	5.36	0.008	**	**	**	*	ns	**
Colorimetric parameters													
L*	75.6	77.8	76.9	76.6	77.4	76.1	0.27	**	ns	*	ns	ns	**
a*	2.2	0.6	1.2	1.5	1.4	1.4	0.04	**	**	ns	ns	**	**
b*	25.1	16.1	20.1	21.1	19.7	21.5	0.16	**	*	**	ns	ns	*

<sup>†</sup> MP: mountain pasture, I: indoor;

<sup>‡</sup> J: July, A: August;

<sup>§</sup> RT60: 60d of ripening, RT180: 180d of ripening;

<sup>¶</sup> RS: Rearing System, P: Period, RT: Ripening Time, \*:  $P < 0.05$ , \*\*:  $P < 0.01$ , ns: not significant;

<sup>††</sup> DM: dry matter, WSN: water soluble nitrogen, Ripening index:  $WSN/TN (x100)$ , TN: total nitrogen

Lightness is higher in I cheeses and decreases slightly with the ripening in agreement with Coppa et al. (2011). MP cheese have higher a\* index and b\*

index compared with I cheese. The colour of cheeses depends on high content of carotenoids in grass, which varies according to the phenological stage of the plants that compose the pasture and consequently the diet of animals (Noziere et al. 2006; Cozzi et al. 2009). The effects of period and ripening are less pronounced, although there was a statistically significant increase in a\* and b\* with period and only of b\* with ripening.

Hardness, gumminess and chewiness were significantly higher in MP cheese compared with I cheese (Table 4.4). Texture of cheeses is related to a complex interaction between chemical composition and ripening parameters. The differences in water content and holes may have caused these differences, with particular regard to hardness (Innocente et al. 2002; Gunasekaran & Ak, 2003). A cheeses were harder, more adhesives, more gummy and more chewable than J cheeses. Ripening causes in cheeses, as expected, an increase in hardness and a loss of cohesiveness and springiness in agreement with data obtained by Bertolino et al. (2011) on Castelmagno PDO cheese.

**Table 4.4.** Textural profile analysis of cheeses, n = 48

	Rearing System <sup>†</sup>		Period <sup>‡</sup>		Ripening Time <sup>§</sup>		SEM	Significance <sup>¶</sup>					
	MP	I	J	A	RT 60	RT 180		RS	P	RT	RS xP	RS xRT	P xRT
Hardness (N)	76.8	64.6	64.0	77.4	62.8	78.5	1.23	**	**	**	ns	ns	ns
Cohesiveness (x100)	53.0	54.2	53.6	53.6	57.6	49.5	0.30	*	ns	**	ns	ns	ns
Adhesiveness (N x mm)	0.99	0.96	0.75	1.19	0.94	1.00	0.038	ns	**	ns	ns	ns	ns
Gumminess (N)	40.4	34.6	34.0	41.0	36.1	38.9	0.56	**	**	*	ns	*	ns
Chewiness (N x mm)	31.8	27.4	26.8	32.3	29.5	29.7	0.44	**	**	ns	ns	*	ns
Springiness	0.79	0.79	0.79	0.79	0.82	0.76	0.002	ns	ns	**	*	ns	ns

<sup>†</sup> MP: mountain pasture, I: indoor;

<sup>‡</sup> J: July, A: August;

<sup>§</sup> RT60: 60d of ripening, RT180: 180d of ripening;

<sup>¶</sup> RS: Rearing System, P: Period, RT: Ripening Time, \*:  $P < 0.05$ , \*\*:  $P < 0.01$ , ns: not significant

### *Cheese fatty acid profiles*

Cheese processing involves negligible variations on FA profile compared with the original milk (Revello Chion et al. 2010; Table 4.5). MP cheeses presented lower level of total SFA, and higher level of MUFA and PUFA than I cheeses.

The RT mainly increases level of C4:0 and C16:0, and decrease the level of C18:0 and cis9-C18:1, with an increase of total SFA and a decrease of total MUFA. Despite the negative oxidation-reduction potential of cheese, these results could be due to oxidation of cheese lipids favoured by the action of lipase and esterase present in raw milk (McSweeney & Sousa, 2000). Surprisingly cis9trans11-CLA and trans10cis12-CLA increased from RT60 to RT180. Considering cis9trans11-CLA, the interaction RS×P was ordinal, while interaction RS×RT was disordinal. In effect, the increasing level of cis9trans11-CLA from RT60 to RT180 in MP cheese (1.45 vs. 1.60) was not found in I cheese (0.47 vs. 0.46). Other studies suggest that ripening has negligible (Werner et al. 1992; Luna et al. 2007) or at least controversial effect on CLA content. In particular, Lobos Ortega et al. (2012) observed an increase of CLA limited to the first part of the ripening in cow cheese while Lin et al. (1999) reported that, with the progress of ripening (from 3 to 6 months), there is a reduction of CLA due to enzymatic hydrogenation to MUFA and SFA.



**Table 4.5.** Cheese fatty acid content (g/100g of total fatty acids) and total weight (mg/100g of cheese), n = 48

	Rearing System <sup>†</sup>		Period <sup>‡</sup>		Ripening Time <sup>§</sup>		SEM	Significance <sup>¶</sup>					
	MP	I	J	A	RT 60	RT 180		RS	P	RT	RS ×P	RS ×RT	P ×RT
Total weight	630.5	524.1	550.8	603.7	561.5	593.1	12.28	**	ns	ns	ns	ns	ns
C4:0	2.73	2.57	3.05	2.26	2.33	2.98	0.048	ns	**	**	ns	ns	**
C6:0	1.75	1.78	1.86	1.66	1.72	1.80	0.026	ns	**	ns	ns	ns	ns
C8:0	1.11	1.21	1.19	1.13	1.16	1.16	0.012	**	*	ns	ns	ns	ns
C10:0	2.63	3.16	2.93	2.86	2.91	2.88	0.013	**	*	ns	ns	**	ns
C11:0	0.23	0.26	0.25	0.25	0.24	0.25	0.004	**	ns	ns	ns	ns	ns
C12:0	3.10	3.84	3.49	3.45	3.44	3.50	0.026	**	ns	ns	ns	**	ns
C13:0	0.08	0.10	0.09	0.09	0.09	0.09	0.002	**	ns	ns	ns	*	ns
C14:0	11.14	12.83	12.05	11.92	11.78	12.19	0.046	**	ns	**	ns	**	**
<i>iso</i> -C15:0	0.27	0.20	0.29	0.18	0.25	0.22	0.012	*	**	ns	ns	ns	ns
C14:1	1.36	1.38	1.42	1.32	1.37	1.37	0.037	ns	ns	ns	ns	ns	ns
C15:0	1.37	1.21	1.28	1.30	1.24	1.33	0.005	**	ns	**	ns	**	ns
C15:1	0.30	0.29	0.30	0.29	0.30	0.29	0.003	ns	ns	ns	ns	ns	ns
C16:0	27.78	33.10	30.54	30.34	30.08	30.80	0.056	**	ns	**	ns	ns	ns
C16:1	1.37	1.37	1.27	1.46	1.29	1.44	0.015	ns	**	**	ns	ns	**
<i>iso</i> -C17:0	0.52	0.43	0.46	0.49	0.47	0.47	0.002	**	**	ns	ns	ns	*
C17:0	0.66	0.57	0.60	0.63	0.62	0.61	0.008	**	*	ns	ns	ns	ns
C17:1	0.28	0.23	0.25	0.27	0.27	0.25	0.003	**	*	ns	ns	**	ns
C18:0	11.18	10.38	10.42	11.15	11.05	10.52	0.038	**	**	**	ns	**	*
<i>trans</i> 9/11-C18:1	5.34	2.20	4.41	3.13	3.80	3.73	0.090	**	**	ns	ns	ns	ns
<i>cis</i> 9-C18:1	22.48	21.13	21.00	22.61	22.65	20.97	0.078	**	**	**	ns	**	ns
C18:1 <i>n</i> -7	0.58	0.53	0.49	0.63	0.54	0.57	0.034	ns	ns	ns	ns	ns	ns
<i>trans</i> 6-C18:2	0.11	0.04	0.07	0.08	0.08	0.07	0.003	**	ns	*	**	**	**
<i>cis</i> 6-C18:2	0.58	0.09	0.33	0.35	0.31	0.36	0.015	**	ns	ns	ns	**	*
C18:3 <i>n</i> -6	0.18	0.13	0.15	0.16	0.17	0.13	0.003	**	ns	**	ns	ns	ns
C18:3 <i>n</i> -3	1.21	0.41	0.78	0.84	0.80	0.82	0.017	**	ns	ns	*	**	*
<i>cis</i> 9 <i>trans</i> 11-CLA <sup>††</sup>	1.53	0.46	0.96	1.03	0.94	1.06	0.013	**	*	**	**	**	ns
<i>trans</i> 10- <i>cis</i> 12-CLA <sup>††</sup>	0.08	0.02	0.03	0.06	0.02	0.08	0.010	*	ns	*	ns	ns	ns
C24:0	0.06	0.07	0.05	0.08	0.07	0.06	0.006	ns	*	ns	**	ns	ns
SFA <sup>††</sup>	64.61	71.72	68.55	67.78	67.45	68.88	0.120	**	*	**	ns	*	**
MUFA <sup>††</sup>	31.71	27.13	29.13	29.70	30.22	28.62	0.100	**	*	**	ns	**	*
PUFA <sup>††</sup>	3.68	1.16	2.32	2.52	2.33	2.51	0.032	**	*	**	ns	**	*
OBCFA <sup>††</sup>	3.10	2.64	2.87	2.87	2.86	2.88	0.008	**	ns	ns	ns	ns	ns
SCFA <sup>††</sup>	8.22	8.72	9.04	7.91	8.12	8.83	0.070	**	**	**	ns	*	**
MCFA <sup>††</sup>	46.99	54.58	50.98	50.59	50.07	51.50	0.113	**	ns	**	ns	**	**
LCFA <sup>††</sup>	44.79	36.70	39.99	41.50	41.81	39.68	0.137	**	**	**	ns	**	**
<i>n</i> -3	1.21	0.41	0.78	0.84	0.80	0.82	0.017	**	ns	ns	**	**	*
<i>n</i> -6	0.87	0.26	0.55	0.59	0.57	0.56	0.014	**	ns	ns	ns	**	*
<i>n</i> -6/ <i>n</i> -3	0.72	0.62	0.65	0.69	0.74	0.60	0.036	ns	ns	**	ns	**	*

<sup>†</sup> MP: mountain pasture, I: indoor;

<sup>‡</sup> J: July, A: August;

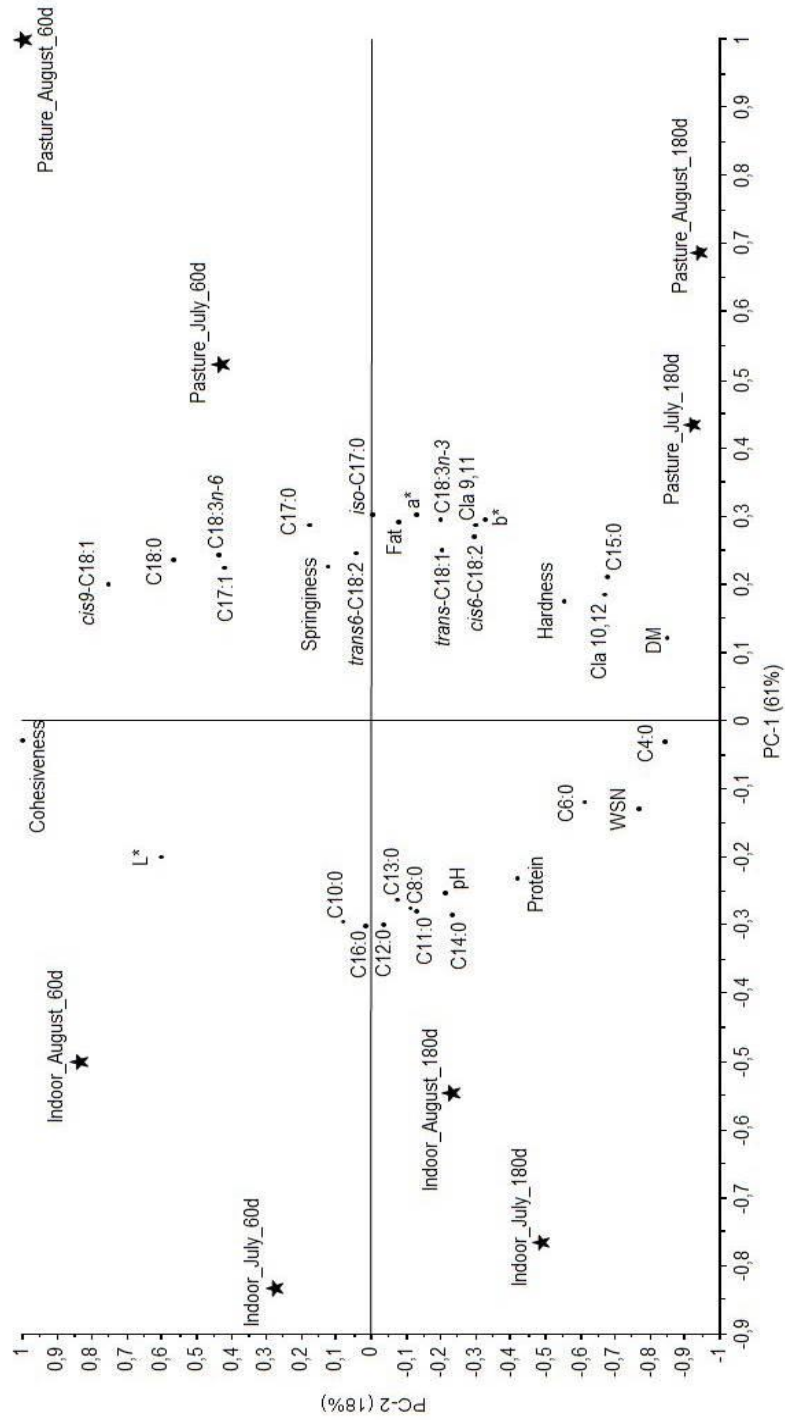
<sup>§</sup> RT60: 60d of ripening, RT180: 180d of ripening;

<sup>¶</sup> RS: Rearing System, P: Period, RT: Ripening Time, \*:  $P < 0.05$ , \*\*:  $P < 0.01$ , ns: not significant;

<sup>††</sup> CLA: conjugated linoleic acids, SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids, OBCFA: odd and branched chain fatty acids, SCFA: short chain fatty acids, MCFA: medium chain fatty acids, LCFA: long chain fatty acids

### *Principal component analysis*

Figure 4.1 provides an overall description of the chemical composition, colorimetric parameters and textural profile of the cheeses. The first Principal Component separated MP from I cheese, and it is mainly correlated with PUFA and colorimetric parameters. Instead the second Principal Component separates the cheeses according to ripening period, and it is mainly correlated with textural parameters, WSN and short chain fatty acid. The effect of period is not evident.



**Fig. 4.1.** Principal Component Analysis of chemical composition, ripening index, colorimetric parameters, textural and sensory properties of cheeses.

### *Consumer acceptance*

Consumers are usually able to discriminate between cheeses made from raw milk produced in mountain pasture and indoor (Coulon et al. 2004; Dovie et al. 2005). In this trial, assessors not previously informed about cheese origin have highlighted some peculiarities in the experimental products in relation to colour and holes (Table 4.6). In particular, MP cheeses showed a more intense colour (average: 3.9 vs. 2.7) than I, this difference decreases slightly with the ripening. These results are consistent with those obtained with the colorimetric analysis. Another distinctive characteristic between cheeses was holes, which was much less marked (average: 1.9 vs. 3.2) in P cheese than I. With regards to other parameters evaluated consumers did not find any significant difference. Despite these differences consumers have expressed a similar overall liking for MP and I assessed within periods and ripening times. The average result was  $27 \pm 1.1$  (mean  $\pm$  SE), which corresponds to a judgment of 'moderately like' (Cardello & Schutz, 2004).

The present study can provide useful data to develop future marketing strategies based on objective information. Consumers not informed about product origin have not properly appreciated pasture derived cheese. Thus, it will be necessary to support the market value of this product in order to help preserve the social and environmental role of traditional mountain farms.

**Table 4.6.** Consumer acceptance (frequency %) and overall liking (mean  $\pm$  se) of cheeses

Attribute	J, RT60 <sup>†</sup>		A, RT60 <sup>†</sup>		J, RT180 <sup>†</sup>		A, RT180 <sup>†</sup>	
	MP <sup>‡</sup>	I <sup>‡</sup>	MP <sup>‡</sup>	I <sup>‡</sup>	MP <sup>‡</sup>	I <sup>‡</sup>	MP <sup>‡</sup>	I <sup>‡</sup>
<i>Colour</i>								
1-Much too dark	10.0	0.0	24.3 <sup>A</sup>	0.0 <sup>B</sup>	18.6 <sup>A</sup>	0.0 <sup>B</sup>	17.1 <sup>A</sup>	0.0 <sup>B</sup>
2-Too dark	55.7 <sup>A</sup>	10.0 <sup>B</sup>	55.7 <sup>A</sup>	10.0 <sup>B</sup>	51.4 <sup>A</sup>	10.0 <sup>B</sup>	54.3 <sup>A</sup>	4.3 <sup>B</sup>
3-Just about right	30.0 <sup>B</sup>	62.9 <sup>A</sup>	18.6 <sup>B</sup>	52.9 <sup>A</sup>	28.6 <sup>b</sup>	58.6 <sup>a</sup>	27.1 <sup>b</sup>	50.0 <sup>a</sup>
4-Too light	1.4 <sup>B</sup>	27.1 <sup>A</sup>	1.4 <sup>B</sup>	35.7 <sup>A</sup>	1.4 <sup>B</sup>	30.0 <sup>A</sup>	1.4 <sup>B</sup>	44.3 <sup>A</sup>
5-Much too light	2.9	0.0	0.0	1.4	0.0	1.4	0.0	1.4
<i>Holes</i>								
1-Much too numerous	1.4	5.7	0.0	2.9	0.0	5.7	1.4	0.0
2-Too numerous	7.1 <sup>B</sup>	40.0 <sup>A</sup>	1.4 <sup>B</sup>	28.6 <sup>A</sup>	5.7 <sup>B</sup>	38.6 <sup>A</sup>	1.4	11.4
3-Just about right	28.6	44.3	7.1 <sup>B</sup>	51.4 <sup>A</sup>	17.1 <sup>B</sup>	47.1 <sup>A</sup>	22.9 <sup>B</sup>	52.9 <sup>A</sup>
4-Too few numerous	31.4 <sup>a</sup>	10.0 <sup>b</sup>	42.9 <sup>a</sup>	17.1 <sup>b</sup>	54.3 <sup>A</sup>	8.6 <sup>B</sup>	34.3	34.3
5-Much too few numerous	31.4 <sup>A</sup>	0.0 <sup>B</sup>	48.6 <sup>A</sup>	0.0 <sup>B</sup>	22.9 <sup>A</sup>	0.0 <sup>B</sup>	40.0 <sup>A</sup>	1.4 <sup>B</sup>
<i>Smell</i>								
1-Much too strong	1.4	1.4	1.4	0.0	1.4	0.0	4.3	0.0
2-Too strong	28.6	14.3	21.4	18.6	14.3	10.0	24.3	21.4
3-Just about right	24.3	40.0	28.6	34.3	34.3	51.4	30.0	40.0
4-Too weak	28.6	31.4	30.0	31.4	31.4	30.0	28.6	28.6
5-Much too weak	17.1	12.9	18.6	15.7	18.6	8.6	12.9	10.0
<i>Taste</i>								
1-Much too strong	7.1	1.4	8.6	4.3	4.3	5.7	7.1	4.3
2-Too strong	32.9	31.4	30.0	22.9	42.9	38.6	34.3	31.4
3-Just about right	40.0	28.6	34.3	30.0	27.1	38.6	31.4	41.4
4-Too weak	15.7	27.1	20.0	27.1	21.4	15.7	20.0	15.7
5-Much too weak	4.3	11.4	7.1	15.7	4.3	1.4	7.1	7.1
<i>Structure</i>								
1-Much too firm	0.0	0.0	1.4	0.0	1.4	0.0	0.0	0.0
2-Too firm	17.1	12.9	31.4	15.7	11.4	10.0	28.6	18.6
3-Just about right	52.9	58.6	41.4	51.4	51.4	70.0	47.1	58.6
4-Too weak	24.3	24.3	24.3	28.6	34.3	15.7	22.9	18.6
5-Much too weak	5.7	4.3	1.4	4.3	1.4	4.3	1.4	4.3
<i>Overall liking</i> <sup>¶</sup>								
	31 $\pm$	28 $\pm$	22 $\pm$	20 $\pm$	25 $\pm$	30 $\pm$	27 $\pm$	34 $\pm$
	2.7	2.9	3.1	3.3	3.5	3.4	3.3	3.0

<sup>†</sup> J: July, A: August, RT60: 60d of ripening, RT180: 180d of ripening;

<sup>‡</sup> MP: mountain pasture, I: indoor;

<sup>§ A,B</sup> Within row and within P and RT, values with different superscript letters differ at  $P < 0.01$ , <sup>a,b</sup> Within row and within P and RT, values with different superscript letters differ at  $P < 0.05$ ;

<sup>¶</sup> Expressed on a LAM scale.

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## **4.2 Montasio cheese liking as affected by information about cows breed and rearing system**

The influence of information about the product origin (cow breed and rearing system) on consumer preferences of cheese was assessed.

The results has been submitted to an international journal for publication as: Montasio cheese liking as affected by information about cows breed and rearing system (Romanzin A., Corazzin M., Favotto S., Piasentier E., Bovolenta S.).

## Montasio cheese liking as affected by information about cows breed and rearing system <sup>(\*)</sup>

### Abstract

European consumers are more and more aware by the credence attributes of foods, particularly of those of animal origin. The aim of the paper was to assess whether the information about production system may modify the consumer liking of cheese. Montasio PDO cheese (MC), usually made by milk from indoor reared cows of different breeds, was processed from pure milk of Italian Simmental (IS) cows (ISMC) or of IS cows grazing on mountain pasture (ALP-ISMC). A consumer test was carried out in two sessions on 60-day ripened cheeses. In the first, both cheeses were tasted by Montasio consumers in blind condition (Perceived liking, PL). Then the respondents were asked to read information about the breed and the rearing system and to give their Expected liking (EL). Two weeks after, in the second session, the same consumers tasted the two cheeses with the linked information (Actual liking, AL). Despite the similar PL average score (ISMC:  $21 \pm 2.3$  vs. ALP-ISMC:  $23 \pm 2.2$  points on Labeled Affective Magnitude scale,  $P > 0.05$ ), it was possible to identifying consumers' clusters with differential liking for the two types of Montasio PDO, that were characterized by different physico-chemical properties. Consumers express a high EL for ISMC ( $38 \pm 2.6$  points) and even more for ALP-ISMC ( $61 \pm 2.5$  points). The AL of ISMC ( $35 \pm 2.1$  points) was similar and statistically not different from the EL (complete assimilation of information about breed). For ALP-ISMC the assimilation was complete for consumers (29%) who have expressed a positive

<sup>(\*)</sup> This chapter has been submitted to *Journal of Dairy Research* for publication as:  
**Montasio cheese liking as affected by information about cows breed and rearing system.**  
A. Romanzin, M. Corazzin, S. Favotto, E. Piasentier and S. Bovolenta

PL for it, at least twice as much than ISMC. For the rest of consumers, both information and intrinsic properties play a significant role in the AL of the pasture-derived cheese.

*Keywords:* Montasio cheese, Simmental cows, rearing system, information, consumer preference

Sensory properties of food products are important factors in the determination of consumer preferences (Harker *et al.*, 2003; Lesschaeve & Noble, 2005; Drake, 2007). Moreover, the perception of food quality is changing rapidly and involves the production process rather than just the sensory and/or nutritional quality of the product (Becker, 2000; Grunert *et al.*, 2000; Van Rijswijk *et al.*, 2008).

In effect, according to a multidimensional concept of food quality, when consumers choose a food product they are also influenced by credence attributes, such as links to the territory of origin, identity, sustainability of the production system, ethical attributes, safety, health and more (Deliza & MacFie, 1996; Caporale & Monteleone, 2004; Siegrist, 2008; Napolitano *et al.*, 2010a; Hersleth *et al.*, 2012).

One of the methods used in the studies about the drivers of consumer behavior, involves the evaluation of the role of information which comprises several stages: a liking judgment of the product tasted without any kind of information (perceived liking), an assessment of the expectations linked exclusively to product information (expected liking), and finally an informed tasting (actual liking). If there is a difference between expected and perceived liking, consumer expectations are not met and therefore there is a “disconfirmation” (Cardello & Sawyer, 1992), which is positive if the perceived liking is higher than the expected and negative when the product is below expectations. According to the theory of assimilation (Anderson, 1973), when the actual liking of the products moves in the direction of expectations an assimilation of information occurs,

which is complete or incomplete if the actual liking reaches or does not reach expectation, respectively.

In Europe, the food information are conveyed through quality labels i.e. Protected Denomination of Origin (PDO), Protected Geographical Indication (PGI), Traditional Speciality Guaranteed (TSG) (Reg. EU 1151/2012), and Organic (Reg. EU 834/2007).

Some studies have investigated the effect of information on consumer's evaluation or on willingness to pay of labeled cheeses. For example, in Italy, Napolitano *et al.* (2010b) found similar perceived liking scores given for organic and conventional Pecorino cheeses. Garavaglia and Marozz (2012) in a study that considered Fontina PDO, a cheese produced in Valle d'Aosta (a mountain region) only with the local cattle breed (Valdostana breed), showed the positive influence of the PDO label on consumer preference, which also guaranteed a premium price to the product.

Many actions have been proposed in Italy to increase the value of mountain PDO cheeses by strengthening the link to the territory and the production chain, but the potentialities are not exploited enough. If the production area and processing techniques are always well defined, animal characteristics and farming practices are often not considered (Bovolenta *et al.*, 2011).

The aim of the paper was to assess whether the information about production process and in particular the cows breed and the rearing system, may modify the consumer liking of a PDO cheese made in small-scale traditional farms, where the use of local breeds and grasslands is widespread.

For this purpose, Montasio cheese, one of the most important PDO and traditional product in North-East Italy, has been chosen. Although it takes its name from a mountain plateau, the Montasio PDO cheese is produced largely in lowlands where different cow breeds are reared indoor. Recently, in order to increase the value of the mountain cheese and to link it to the breed more present in this area, the production of Montasio PDO was combined with two

additional labels: “Only Italian Simmental breed” and “Mountain Product”. A previous study of Romanzin *et al.* (2013) showed that consumers express a similar liking in blind condition for Montasio cheeses obtained from pure milk of Simmental cow kept indoor or grazing on mountain pasture. The present study gives an insight on the perceived liking of the Montasio cheeses and investigates its role on consumer behaviour.

## **Materials and methods**

### *Cheeses*

Cheeses under evaluation were obtained following the Montasio PDO (MC) specification, in summer period, from the milk of Italian Simmental (IS) cows maintained indoor with hay-based diet, (ISMC) or grazing on mountain pasture (ALP-ISMC).

Milk from the evening milking pooled with milk from morning milking was processed for three consecutive days (three cheese makings per type). The pooled milk collected in the vat milk was heated to 32 °C, and then was added 10 g/kg of natural starter (microflora naturally present in raw milk and cultured in appropriate conditions for a day). The rennet was added after 25 min of incubation. The curd was cut to obtain pellets of size comparable to grain of rice and it was heated at 45 °C for 30 min. The curd was then blended out fire for 20 min and left to stand then, after whey draining, it was placed in moulds and pressed. Fresh cheeses were then placed in brine for 24 h (18% salt concentration). Subsequently the salt was put once only on one side of the cheeses, then they were stacked. Cheese shape was cylindrical, about 6 kg weight and 70 mm height. Cheeses were ripened for 60 days in a ripening cellar with controlled temperature and humidity until analysed.

### *Consumer test*

Sensory analysis was performed at University of Udine, in a laboratory built according to the UNI-ISO 8589 standard. The potential respondents were asked to describe their attitudes towards cheese. One hundred twenty-four habitual consumers of Montasio PDO cheese were then selected, equally distributed by sex. The recruited subjects had a mean age of 39, from 23 to 71 years old, and 83% reported to eat cheese more than once a week.

The consumers were asked to record their overall liking on LAM scale (Labeled Affective Magnitude bi-directional scale; from -100 to +100; Cardello & Schutz, 2004). Consumers tasted cheeses presented at room temperature ( $20\text{ }^{\circ}\text{C} \pm 2$ ) as small fingers, wrapped in aluminium foil, measuring 15 x 15 mm in thickness and 50 mm long. Samples were identified using a three digit number code and the presentation followed a balanced randomization scheme in all phases.

Panel members, scheduled in groups of eight, evaluated cheeses in two sessions. In the first, both cheeses were tasted by consumers in blind condition, without any information on the products (Perceived liking). After that, panel members evaluated the expectations for cheese liking, on a LAM scale, based on information provided. ISMC was labeled as: Montasio PDO cheese “obtained from Italian Simmental cows” and “indoor rearing system”; while ALP-ISMC was labeled as: Montasio PDO cheese “obtained from Italian Simmental cows” and “mountain pasture rearing system”. The second session of the sensory test was performed after 15 days: the same consumers evaluated cheeses only after they had read the information (breed, rearing system and cheese production) linked to each sample (Actual liking).

### *Physico-chemical analysis*

Cheeses were analysed for dry matter (DM), fat content, total nitrogen (TN) and soluble nitrogen at pH 4.6 (SN) according to AOAC (2000). The ripening index (RI) was calculated as ratio (x100) between SN and TN. Protein content was obtained as  $TN \times 6.38$ .

A spectrophotometer (Minolta CM2600d) was used for measuring colorimetric parameters: lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ). The number and the percent area of the holes were evaluated on a slice of cheese cut from the middle part of the form with the exception of the sub-crust area. Digital images of the slices of cheeses were examined with an image-analyzer.

The rheological properties of cheeses were evaluated with a Texture Analyser (TA Plus, Lloyd Instruments, UK) using the procedure described by Bourne (1978) and modified by Gunasekaran & Ak (2003). Texture Profile Analysis (TPA) was applied to the cylinders of cheese (20 mm in diameter and 20 mm height) compressed axially in two consecutive cycles, with a deformation of 50% of the original height and applying a force of 100 mm/min. The rheological parameters analysed were: hardness, cohesiveness, adhesiveness, springiness, gumminess and chewiness.

### *Statistical analysis*

The statistical analysis was performed using the software R version 2.14.1. Normality of data distribution was tested by the Shapiro-Wilk test.

Assessors' responses were recorded using FIZZ software (Biosystemes, Couternon, F). Student's paired t-tests were used to evaluate differences between scores.

Cheese chemical composition, colorimetric parameters and texture were subjected to one way ANOVA with rearing system treated as fixed factor.

## Results and discussion

### *Perceived liking*

Sensory analysis results are given in Table 4.7. Consumers' scores in blind condition (Perceived liking) were 21 and 23 points for ISMC and ALP-ISMC respectively. These values are not statistically different and reveals that Montasio cheese from Italian Simmental milk is characterized by a slight to moderate good eating quality along the bi-directional LAM scale (Cardello & Schutz, 2004), independently of rearing system. These results confirm those obtained in the previous study (Romanzin *et al.*, 2013).

**Table 4.7.** Sensory analysis results

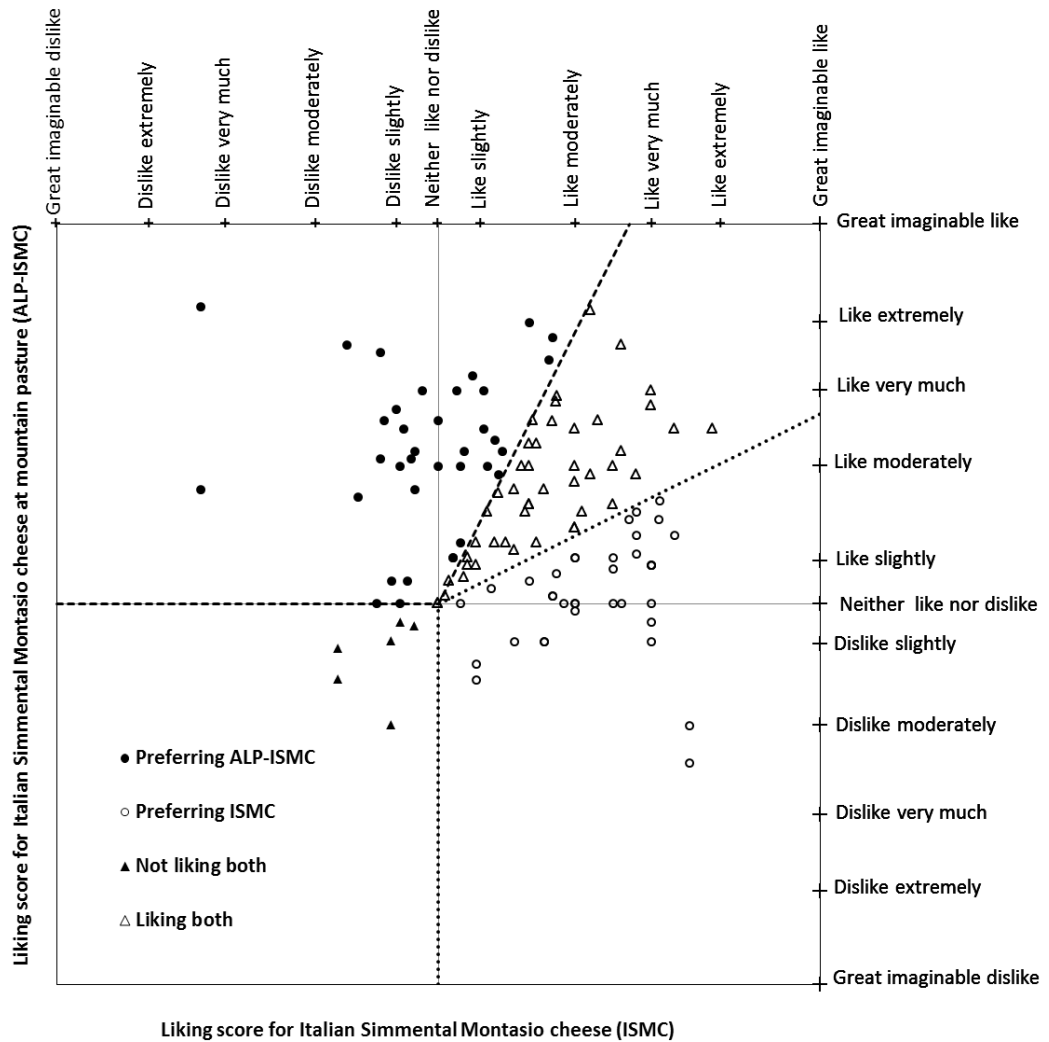
	ISMC <sup>†</sup>	ALP-ISMC <sup>†</sup>
Perceived liking (PL)	21±2.3	23±2.2
Expected liking (EL)	38±2.6 <sup>b</sup>	61±2.5 <sup>a</sup>
Actual liking (AL)	35±2.1 <sup>b</sup>	53±2.5 <sup>a</sup>
PL - EL	-17**	-38**
AL - PL	14**	30**
AL - EL	-3	-8**

<sup>†</sup>ISMC: Italian Simmental Montasio cheese; ALP-ISMC: Italian Simmental Montasio cheese at pasture;

<sup>a,b</sup>:  $P \leq 0.05$ , \*\*:  $P \leq 0.01$

However, the average evaluation does not allow to identify, if there are, clusters of consumers with different attitude towards the two types of cheese. For a better understanding of products' preference, the liking scores of individual consumers for ISMC and ALP-ISMC were plotted in Figure 4.2. The consumers amply scatter on the preference space, showing differentiate liking combinations





**Fig. 4.2.** Perceived liking of consumers for the two types of Italian Simmental Montasio cheese (ISMC). Preferring ALP-ISMC: consumers who have expressed a positive Perceived Liking for ALP-ISMC, at least twice as much than ISMC; preferring ISMC: consumers who have expressed a positive Perceived Liking for ISMC, at least twice as much than ALP-ISMC.

for the two Montasio types. Indeed, four groups of consumers can be identified based on their preferences. A first group is made by consumers (29% of respondents) who scored ALP-ISMC positively, at least twice as much than ISMC: they can be regarded as consumers preferring ALP-ISMC. This group is placed in the top, left part of the bi-dimensional liking space, defined by the dotted line in

Figure 4.2. Conversely, a second group consists of consumers (29%) who prefer the second cheese, and it is located in the bottom, right side of the scatter-plot, demarcated by the spotted line. A third cluster of consumers did not seem to prefer the intrinsic characteristics of one particular Montasio type; indeed they perceived both of them positively, giving comparable liking scores (i.e. two scores, the difference between which is lesser than the minimum value of them). Eventually, only few consumers (5%) belong to the fourth group, who scored negatively both cheeses.

In order to clarify the nature of this diverse perception, some chemical and colorimetric parameters of the experimental Montasio types were assessed and reported in Table 4.8. The ALP-ISMC had more DM (68.5 vs 66.6%) and fat content (53.9 vs 51.6% DM) than ISMC, while protein content was lower (39.1 vs 41.1% DM). In fact, the higher energy level of indoor diet reduces the milk fat concentration by decreasing the synthesis of acetic acid in the rumen (Walker *et al.*, 2004). Moreover the lower protein concentration in ALP-ISMC could be due to the low energy supply and hypoxia, which characterize the dairy cows grazing on high mountain pasture (Dovier *et al.*, 2005; Leiber *et al.*, 2006; Bovolenta *et al.* 2009). The values of ripening index were not statistically different between cheeses (12.8) and fall within the range reported for PDO Montasio by Innocente *et al.* (2002).

The number of holes and the total percentage of area occupied by holes were significantly lower in ALP-ISMC than ISMC (25.0 vs 67.1 holes/dm<sup>2</sup> and 2.51 vs 8.69% respectively). The values obtained with ISMC cheeses fall within the normal range for a Montasio cheese made from raw milk and natural starter (Innocente & Corradini, 1998), while the values of ALP-ISMC are below this range. Colorimetric parameters show that cheeses from mounting pastures were more yellow and red and less shiny than those from indoor rearing, probably as a consequence of a high carotenoid content from fresh grass (Noziere *et al.*, 2006).

**Table 4.8.** Chemical composition, ripening index and colorimetric parameters

	ISMC <sup>†</sup>	ALP-ISMC <sup>†</sup>	SEM
Chemical composition:			
DM <sup>†</sup> (%)	66.6 <sup>b</sup>	68.5 <sup>a</sup>	0.28
Fat (% DM)	51.6 <sup>b</sup>	53.9 <sup>a</sup>	0.33
Protein (% DM)	41.1 <sup>a</sup>	39.1 <sup>b</sup>	0.14
WSN <sup>†</sup> (% DM)	0.57	0.51	0.012
Ripening index <sup>†</sup>	13.4	12.2	0.33
Holes (n/dm <sup>2</sup> )	67.1 <sup>A</sup>	25.0 <sup>B</sup>	0.42
Holes (% of total surface)	8.69 <sup>A</sup>	2.51 <sup>B</sup>	0.112
Colorimetric parameters:			
L*	79.5 <sup>a</sup>	76.6 <sup>b</sup>	0.37
a*	0.9 <sup>B</sup>	2.4 <sup>A</sup>	0.06
b*	14.9 <sup>B</sup>	25.0 <sup>A</sup>	0.17

<sup>†</sup> ISMC: Italian Simmental Montasio cheese; ALP-ISMC: Italian Simmental Montasio cheese at pasture, DM: dry matter, WSN: water soluble nitrogen, Ripening index: WSN/TN (x 100), TN: total nitrogen;

<sup>a,b</sup>:  $P \leq 0.05$ , <sup>A,B</sup>:  $P \leq 0.01$

The rheological properties of experimental cheeses are showed Table 4.9. Hardness, gumminess and chewiness were higher in ALP-ISMC than ISMC (75.9 vs 59.0 N, 43.2 vs 34.4 N, and 34.8 vs 28.2 N\*mm respectively). Texture of cheeses is related to a complex interaction between chemical composition and ripening parameters. The differences in water content and percentage of holes may have caused differences in the mechanical properties, hardness in particular (Innocente *et al.*, 2002; Gunasekaran & Ak, 2003). Bovolenta *et al.* (2008) in a trial on grazing cows have shown that a higher supplement level induces a higher degradation of the protein matrix in cheese that, consequently, causes lower hardness and gumminess.

**Table 4.9.** Textural profile analysis

	ISMC <sup>†</sup>	ALP-ISMC <sup>†</sup>	SEM
Hardness (N)	59.0 <sup>b</sup>	75.9 <sup>a</sup>	2.30
Cohesiveness (*100)	58.4	56.8	0.30
Adhesiveness (N*mm)	1.10	1.18	0.082
Springiness	0.82	0.81	0.003
Gumminess (N)	34.4 <sup>b</sup>	43.2 <sup>a</sup>	1.40
Chewiness (N*mm)	28.2 <sup>b</sup>	34.8 <sup>a</sup>	1.11

<sup>†</sup> ISMC: Italian Simmental Montasio cheese; ALP-ISMC: Italian Simmental Montasio cheese at pasture;

<sup>a,b</sup>:  $P \leq 0.05$

### *Expected liking*

In the second phase of the sensory test (Table 1), the respondents did not taste the cheeses and they judged them only on the basis of provided information. The Expected liking was significantly higher than the Perceived liking for both types of Montasio cheese ( $P < 0.01$ ), thus indicating that a negative disconfirmation occurred. The reason for which consumers found the cheeses worse than expected is not due to a poor eating quality of them. Indeed, both cheeses were scored, on average, in the positive half of the LAM scale after testing in blind condition, and only 5% of respondents gave negative scores for both types of cheeses (Figure 4.2). The disconfirmation rather reveals that information about the use of milk from the Italian Simmental breed, historically originated and still widespread in the region, had a positive impact on consumer expectancy about Montasio PDO cheese. The interest in pure-breed products comes from the fact that the consumer is aware that, buying them, contributes to the preservation of breed, with a significant impact on the rural economy and on the conservation of biodiversity (Gandini & Villa, 2003). For example, recovery of the Reggiana breed has depended upon the production and

promotion of “Parmigiano Reggiano delle Vacche Rosse” cheese, obtained exclusively from this breed, which is generally sold at double price compared to undifferentiated Parmigiano Reggiano cheese (Russo *et al.*, 2007; Fontanesi, 2009).

Moreover, the Expected liking was higher for pasture ( $61 \pm 2.5$ ; between “like very much” and “like extremely”) respect to indoor ( $38 \pm 2.6$ ; next to “like moderately”) rearing system of Italian Simmental cows. Consumers have high expectations for cheeses made with milk from mountain pasture, probably due to their link with naturalness, authenticity, animal welfare, healthiness and more (Corazzin *et al.*, 2010; EEA, 2010; Parente & Bovolenta, 2012).

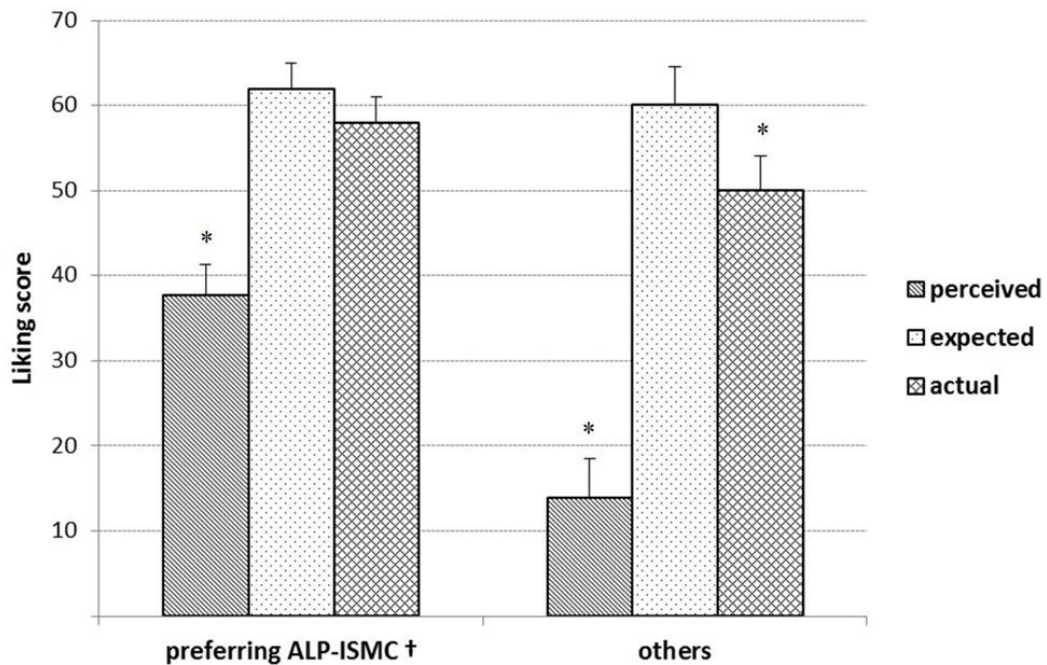
### *Actual liking*

In the third phase of their liking evaluation, consumers scored the cheeses while tasting them after having received full information about breed and farming practices (Table 4.7). The Actual liking of ISMC ( $35 \pm 2.1$  points) was similar and statistically not different from the Expected linking (i.e. “like moderately”) of a monobreed product made in the common indoor system. Thus, Actual liking of the cheese moved in the direction of the expectations and reached them, demonstrating the important effect of the information about breed, which was completely assimilated by consumers.

The ALP-ISMC received a score significantly higher than that of ISMC, equal to  $53 \pm 2.5$  points. The information had strongly improved the position on the LAM scale bringing near “like very much” for PDO Montasio obtained from Simmental cows grazing on mountain pasture.

Therefore, for both cheeses a negative disconfirmation (Perceived liking < Expected liking) and an assimilation (Actual liking > Perceived liking) occurred. However, for Alps cheese consumers assimilated 87% of expectations, which correspond to an incompletely assimilation (Actual liking < Expected liking). Also

Napolitano *et al.* (2010b) considering organic Pecorino cheese observed an incomplete assimilation of the information due to the high expectation linked to agricultural practices favorable for environment, animal welfare, and human health.



**Fig. 4.3.** Liking scores for Italian Simmental Montasio cheese at pasture (ALP-ISMC), in different condition regarding product information. †: consumers who have expressed a positive Perceived Liking for ALP-ISMC, at least twice as much than ISMC; \*: within each consumer group, difference from the expected liking  $P \leq 0.05$ .

However, as shown in Figure 4.3, the assimilation of product information has not been consistent among consumers. Those who have shown a preference for the sensory properties of ALP-ISMC completely assimilated the information on the grazing rearing system. On the other hand, for consumers who do not prefer ALP-ISMC (the set of consumers who prefer ISMC and the neutral ones) the assimilation was not complete, revealing that for them both information and

intrinsic properties play a significant role in the actual liking of the pasture-derived cheese.

The study provide useful insights in order to increase the added value of dairy products of small-scale traditional farms, where husbandry is often based on local breeds, use of grasslands, and where production costs tend to be higher.

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### **4.3. Volatile compounds and sensory properties of Montasio cheese made from milk of Italian Simmental cows grazing on alpine pastures**

As part of CRITA project entitled: “Formaggio Montasio DOP-PDM: una sperimentazione a supporto”, and in collaboration with AAFVG, during the summer 2013 have been performed in Malga Montasio an experimental trial.

The aim of work was to evaluate the effects of different management techniques to Italian Simmental cows grazing on alpine pastures on the aromatic and sensory properties of Montasio PDO-PDM cheese. In particular in 2013 were tested two supplement levels and two pastures of different botanical composition.

The results has been submitted to an international journal for publication as: Volatile compounds and sensory properties of Montasio cheese made from milk of Italian Simmental cows grazing on alpine pastures (Bovolenta S., Romanzin A., Corazzin M., Gasperi F., Biasoli F., Piasentier E.).

# **Volatile compounds and sensory properties of Montasio cheese made from milk of Italian Simmental cows grazing on alpine pastures<sup>(\*)</sup>**

## **ABSTRACT**

Montasio is one of the most important Protected Designation of Origin cheese produced in North-East Italy both in lowland and mountain. This product has been investigated in terms of its chemical composition, texture, and microbiological characterization. Few data have been reported on the volatile profile and sensory properties, particularly for mountain cheeses. The study was aimed to characterize the volatile fraction of Montasio produced from milk of Italian Simmental cows grazing on alpine pastures. In addition, chemical, physical and sensory analysis was carried out. Seventy two Italian Simmental cows grazing on two alpine pastures, that differ for botanical composition, receiving two different levels of supplement (3.0 vs 1.5 kg/head/d). Sixty-two volatile compounds, including alcohols (11), aldehydes (6), ketones (13), esters (5), hydrocarbons (3), organic acids (7), phenolic compounds (4), monoterpenes (7), sesquiterpenes (1), sulfur compounds (2), and others volatiles (3) were detected. The trained assessors described the experimental cheeses with an intense color, a small and evenly distributed eyes, an intense odour and flavour of milk - sour milk and cow and a tender and creamy texture. No effect was recorded for the supplement level. Pasture type has an effect on the volatile fraction, allowing to distinguish the cheeses, without evident effects on the organoleptic perception of assessors.

<sup>(\*)</sup>This chapter has been submitted to *Journal of Dairy Science* for publication as:

**Volatile compounds and sensory properties of Montasio cheese made from milk of Italian Simmental cows grazing on alpine pastures.** S. Bovolenta, A. Romanzin\*, M. Corazzin, F. Gasperi, F. Biasoli and E. Piasentier

**Keywords:** Montasio cheese, Simmental cows, mountain pasture, volatile compounds, sensory properties

## INTRODUCTION

The Alps are characterized by a great variety of environments and socio-economic situations that have created the conditions for the production of a large number of dairy products, strongly linked to local resources utilization and traditions (Hauwuy et al., 2006; Boni and Seidl, 2012; Sturaro et al., 2013). In particular, the use of forages with a great diversity of plant species and of processing techniques able to maintain milk characteristics, originate dairy products with distinct organoleptic properties (Coulon et al., 2004; Martin et al., 2005).

Sensory properties are recognized as one of the most important criteria determining consumer choice. It is generally accepted that the volatile compounds profile is a kind of fingerprint able to characterize dairy products and defining their links to the area and methodology of production. Cheese odor and flavor, in fact, are the result of an equilibrium between volatile and non-volatile compounds, originated from fat, protein and carbohydrate of milk (Fox and Wallace, 1997; McSweeney and Sousa, 2000).

Many studies have been reported the volatile profile of several cheeses, such as Grana Padano (Moio and Addeo, 1998), Parmigiano Reggiano (Careri et al., 1994; Bellesia et al., 2003), Cheddar (Arora et al., 1995; Shakeel-ur-Rehman et al., 2000; Frank et al., 2004), Gruyère (Mallia et al., 2005), Emmental (Dirinck and De Winne, 1999), Torta del Casar (Delgado et al., 2010) and Fontina (Berard et al., 2007).

A large variety of volatile compounds contribute to cheese aroma and odor. The compounds with low detection thresholds are often those that contribute the most to the aroma (d'Acampora Zellner et al., 2008) therefore the relationship between perceived intensity and concentration changes from different compounds (Thomsen et al., 2012). The sensory science offers consolidated techniques for the determination of the characteristics perceived by senses and to study the consumer behaviours. In particular, discriminant or descriptive analysis can be used for this purpose (Drake, 2007).

Montasio is one of the most important Protected Designation of Origin (PDO) cheese produced in North-East Italy. It is a semi-hard and semi-cooked cheese made from raw or thermized cow milk. Over the years, this product has been investigated in terms of its chemical composition (Innocente et al., 2002), texture (Innocente et al., 2000; Maifreni et al., 2002), and microbiological characterization (Marino et al., 2003) but few data have been reported on the volatile profile and sensory properties of Montasio cheese (Innocente et al., 2013).

Although Montasio cheese takes its name from a mountain plateau, it is produced largely in lowlands. Recently, in order to valorize the mountain cheese and to link it to the breed more present in this area, the production of PDO Montasio was combined with two additional labels: "*Prodotto della Montagna*" (Mountain Product) and "*Solo di Pezzata Rossa Italiana*" (Only Italian Simmental breed) (Romanzin et al., 2013).

Therefore, this study aimed to characterize the volatile fraction of Montasio cheese produced from milk of Italian Simmental cows grazing on different alpine pastures and different levels of concentrate supplementation. On the same cheeses a descriptive sensory analysis was carried out.

## MATERIALS AND METHODS

### ***Experimental design and treatments***

The experiment was conducted in an alpine farm (*Malga Montasio*, Udine, Italy; latitude 46°24'45''N, longitude 13°25'53''E; altitude 1500-1800 m). For the experiment were selected two pastures, with different vegetation types, grazed at the same phenological stage (flowering period of gramineae). First pasture (P1; 1500 m asl) was characterized by alpine species of rich pasture like *Poa alpina*, *Phleum alpinum*, *Festuca pratensis*, *Trifolium repens*, *Trifolium pratense* and *Leontodon hispidus*. Second pasture (P2; 1700 m asl) was characterized by alpine species of poor pasture like *Sesleria caerulea*, *Festuca rubra*, *Carex sempervirens*, *Potentilla crantzii* and *Lotus alpinus*.

Seventy two Italian Simmental cows were allowed to pasture day and night and milked twice daily. Cows were assigned to two experimental groups, according to their mean performance characteristics recorded during a two week preliminary period (average  $\pm$  standard deviation): for milk yield ( $16.9 \pm 3.3$  kg/d), stage of lactation ( $192.8 \pm 64.0$  d in milk) and somatic cells counts ( $130,000 \pm 47,000$  cells/ml). During a 15-days preliminary period cows were given access to the same homogeneous pasture and received 2.2 kg/head/d in average of supplement.

During the grazing periods, at milking, 36 cows (Group High) were supplemented with 3 kg/head/d in average of concentrate (based on maize, barley, beet pulp, soy and wheat). The other 36 cows (Group Low) were supplemented with 1.5 kg/head/d in average of concentrate.

### ***Cheese manufacture***

The experimental cheeses were produced from whole and raw milk from two consecutive milkings. The evening milk, cooled to 8 °C, was pooled with milk from the morning. Cheese makings were performed for three consecutive days in accordance with the product specification of PDO Montasio. In the vat, milk was heated to 32 °C and then was added 10 g/kg of natural starter (micro-flora naturally present in raw milk and cultured in appropriate conditions for a day). After 20 min of incubation was added the rennet (Clerici Sacco, 96% chymosin). The curd was cut to obtain pellets of size comparable to grain of rice and it was cooked at 45 °C for 20 min. The curd was then blended out fire for 30 min and left to stand, then, after draining the whey, it was placed in molds and pressed. Fresh cheeses were then placed in brine for 48 h (18% salt concentration). Cheese shape was cylindrical, about 6 kg weight and 70 mm height. Cheeses were ripened for 60 days in a ripening cellar with controlled temperature (12 °C) and humidity (85%) until sampled for analysis.

### ***Chemical and rheological analysis***

Cheese samples were analyzed for dry matter (DM), fat and protein contents according to AOAC (2000) methods.

A spectrophotometer (Minolta CM2600d) was used for measuring colorimetric parameters: lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ).

The rheological properties of cheeses were evaluated with a Texture Analyser (TA Plus, Lloyd Instruments, UK) using the procedure described by Bourne (1978) and modified by Gunasekaran and Ak (2003). Texture Profile Analysis (TPA) was applied on the cylinders of cheese (20 mm in diameter and 20 mm height) compressed axially in two consecutive cycles, with a deformation of 50% of the original height and applying a force of 100 mm/min. The rheological parameters



analyzed were: hardness, cohesiveness, adhesiveness, springiness, gumminess and chewiness.

### ***Head-space analysis by SPME/GC-MS***

Samples were analyzed according SPME (Solid Phase Microextraction) GC-MS technique based on the procedure described in Endrizzi et al. (2012).

Briefly, 3 g of grated cheese were placed in glass vials (20 mL, Supelco, Bellefonte, PA, USA) adding 4 mL bi-distilled water, 50 µl of a solution of three internal standards with purity not lower than 99% (4-methyl-2-pentanone 0.0509 g/L, Aldrich, Milan, Italy; ethyl heptanoate 0.06 g/L, Aldrich, Milan, Italy; and isobutyric acid 20.021 g/L, Fluka, Milan, Italy) and a magnetic stir bar before capping with PTFE/Silicone septa (Supelco, Bellefonte, PA, USA).

Cheese samples were equilibrated at 40 °C under stirring (750 rpm) for 30 minutes and then a 2 cm fused silica fiber coated with divinylbenzene / carboxen / polydimethylsiloxane 50/30 µm (DBV/CAR/PDMS, Supelco, Bellefonte, PA, USA) was introduced and exposed to the headspace environment for 30 minutes. Volatile compounds concentrated on the SPME fiber were desorbed at 250 °C in the injector port of a GC interfaced with a mass detector which operates in electron ionization mode (EI, internal ionization source; 70 eV) with a scan range from m/z 30–300 (GC Clarus 500, PerkinElmer, Norwalk CT, USA). Procedure phases were automatically managed by using a self-sampling system (CTC combiPAL, CTC Analysis AG, Zwingen, CH). Separation was achieved on a HP-Innowax fused-silica capillary column (30 m, 0.32 mm ID, 0.5 µm film thickness; Agilent Technologies, Palo Alto, CA, USA). The GC oven temperature program consisted in 40 °C for 3 min, 40-180 °C at 4 °C/min, 180 °C for 6 min, 180-220 °C at 5 °C/min. Helium was used as carrier gas with a constant column flow rate of 2 mL/min. Compounds identification was based on mass spectra matching with the standard NIST-98/Wiley library and retention indices (RI) of authentic

reference standards compared with those found in literature. The method allowed the separation and semi-quantification of 64 compounds. To test the repeatability of the method, we analyzed 8 replicates of a reference sample prepared by homogenizing different cheese samples from the trial. The observed max variation (5%, 13 %, 7 %, 13%, 12% and 13% respectively for the classes of acids, alcohols, esters, ketones, aldehydes and sulfur compounds), are in agreement with the literature for SPME analysis with this type of matrix (Barbieri et al., 1994; Bellesia et al., 2003). Every sample was measured in triplicate.

### ***Descriptive sensory analysis***

A ten-member panel was trained according to specific procedures for the sensory evaluation of hard and semi-hard cheese (ISO 22935/1,2,3 2009) and experienced in quantitative descriptive analysis of typical semi-hard cheeses. During training, the panel developed a profile protocol for a quantitative descriptive method containing 26 attributes related to visual appearance (6 attributes), odor (5), texture (4), taste (6) and flavour (5) (Table 4.10). The panel was presented firstly cheeses having sensory characteristics extremes for each term representing the anchors of each scale and secondly more common semi-hard cheeses, and through panel consensus, scores were assigned to these cheeses. Through training and group discussion, panel variability was minimized. Sensory profiling was performed in a laboratory built according to the UNI-ISO 8589 standard. The panel rated the intensity of each sensory attribute on a 100 mm unstructured scale, anchored at each end. Six cheeses (1 for each production day and for each of the 2 supplement levels) produced from P1 and a standard cheese were assessed by trained judges 3 times in 3 successive sessions using randomized block design in order to minimize carry over effects. The same procedure was followed in order to evaluate the cheeses produced from P2. The standard cheese was use in order to allow the comparison between the 2

pastures. Samples were presented at room temperature ( $20\text{ }^{\circ}\text{C} \pm 2$ ) as small fingers, wrapped in aluminium foil, measuring 15 x 15 mm in thickness and 50 mm long. Samples were identified using random three digit numerical code.

### ***Statistical analysis***

The statistical analysis was performed using SPSS for Windows (version 7.5.21, Inc 1989-1997). Normality of data distribution and homogeneity of variance were tested using Kolmogorov-Smirnoff and Levene test respectively. Milk and cheese data were subjected to two-way ANOVA with supplement level (High, Low) and pasture (P1, P2) treated as fixed factors. Sensory data were analyzed using mixed model where supplement level and pasture were considered as fixed effects, and judge as random effect. When an ordinal interaction from the perspective of a factor was significant, the main effect of the same factor was discussed (Keppel, 1973). Data were also processed by Principal Component Analysis (PCA) carried out using The Unscrambler X version 10.2 (Camo Software AS, Oslo, Norway). Data were weighted with  $1/\text{SD}$  and the full cross-validation method was used.

**Table 4.10.** List of sensory attributes measured by the expert panel

<b>Visual appearance</b>	Instruction: look the image of sample (*) or the real sample (**) and evaluate the following attributes
Eyes, diameter	Average diameter of the eyes*
Eyes, shape regularity	Regularity in the shape of eyes*
Eyes, distribution regularity	Regularity in the distribution of eyes in the surface*
Color intensity	Intensity of yellow competent in the surface colour **
Rind height	Height of the ring**
Elasticity	The rapidity of recovering initial thickness after a deforming pressure with the thumb
<b>Odour (By Smelling)</b>	Smell the sample opening the sealed pot and after breaking of sample block into 2 parts and evaluate the following attribute
Milk	The odour associated with fresh cream
Sour Milk	The odour associated with sour milk products (not fat yogurt)
Cow	The odour associate with cattle (straw layer from cattle )
Ammonia	The odour associated with ammonia (fresh milk aromatized with 500 mg/l of NH <sub>4</sub> OH)
Herbage	The odour associated with the freshly cut green herbage
<b>Texture</b>	Instruction: Manipulate the sample (manual texture) or chew it (oral texture) and evaluate the following attribute:
Tenderness	The force requires to bite with molar the sample cube (first bite)
Granules	The degree of the consistency of particles in the mouth after 8 acts of chewing
Creaminess	The ability of sample to create a cream during chewing
Adhesivity	The force required to unstick from teeth the chewed cheese (after 8 acts of chewing)
<b>Taste</b>	Instruction: taste the sample and evaluate the following attribute
Sweet	The basic taste sensation related to fructose added in ricotta cheese (30 g/kg)
Salty	The basic taste sensation related to sodium chloride added in ricotta (6 g/kg)
Umami	chemical feeling factor related to specific peptides and nucleotides (Monosodium Glutamate, 1% in water)
Acid	The basic taste sensation related to lactic acid added in ricotta (5 g/kg)
Bitter	The basic taste sensation related to caffeine added in ricotta (0,2 g/kg)
Pungent	The burning sensation of tongue and mouth surface related to capsaicin added in ricotta cheese (20 mg / kg)
<b>Flavour (by tasting)</b>	Instruction: taste the sample and evaluate the following attribute
Milk	The flavour associated with fresh cream/ milk (pasteurized cream 35 % fat)
Sour Milk	The flavour associated with sour milk products (not fat natural yogurt)
Cow	The flavour associate with cattle (straw layer from cattle )
Ammonia	The flavour associated with ammonia (fresh milk aromatized with 500 mg/kg of NH <sub>4</sub> OH)
Herbage	The flavour associated with the freshly cut green herbage

\* evaluated on a photo of a 10 cm x 5 cm sector of inner surface of cheese (by scanner)

\*\* evaluated on the cheese sample

## RESULTS AND DISCUSSION

### *Chemical composition and rheological proprieties*

As shown in Table 4.11 the pasture composition and the supplement level did not significantly affect the chemical composition and the color of experimental cheeses. However, there are some differences in protein content, although not significant ( $P = 0.07$  for both experimental thesis). Bovolenta et al. (2008), in a trial on Brown cows grazing on alpine pasture and fed two different levels of supplementation (4.8 vs 2.4 kg/OM/d), observed no difference in chemical composition of the cheeses. Instead, in a later work Bovolenta et al. (2009) point out that a greater difference (4.8 vs 1.6 kg/OM/d) determines significant changes in dry matter, fat and protein content.

Few differences were found on the texture parameters of the cheeses (Table 4.12), that are closely linked to the chemical composition (Innocente et al., 2002) and cheese making techniques (Mainfreni et al., 2002; Lucey et al., 2003).

**Table 4.11.** Chemical composition and colour of cheeses (n=12)

	Supplement level		Pasture		SEM	Significance		
	High	Low	P1 <sup>1</sup>	P2 <sup>2</sup>		SL <sup>3</sup>	P <sup>4</sup>	SL×P
<i>Chemical composition</i>								
DM <sup>5</sup> , %	66.29	66.88	66.30	66.88	0.210	ns	ns	ns
Fat, %DM	53.05	53.32	52.66	53.71	0.377	ns	ns	ns
Protein, %DM	41.27	39.65	41.26	39.66	0.395	ns	ns	ns
pH	5.30	5.27	5.32	5.25	0.021	ns	ns	ns
<i>Colorimetric parameters</i>								
L*	77.2	77.9	77.2	77.8	0.25	ns	ns	ns
a*	2.0	1.9	2.0	1.9	0.07	ns	ns	ns
b*	23.0	23.0	22.8	23.1	0.25	ns	ns	ns

<sup>1</sup>P1: rich pasture; <sup>2</sup>P2: poor pasture; <sup>3</sup>SL: supplement level; <sup>4</sup>P: pasture; <sup>5</sup>DM: dry matter; \*\*: P<0.01; \*: P<0.05; ns: P>0.05.

**Table 4.12.** Cheese texture analysis, TPA test (n=12)

	Supplement level		Pasture		SEM	Significance		
	High	Low	P1 <sup>1</sup>	P2 <sup>2</sup>		SL <sup>3</sup>	P <sup>4</sup>	SLxP
Hardness, N	52.6	51.3	48.7	55.1	1.24	ns	*	ns
Cohesiveness	0.58	0.57	0.58	0.57	0.004	ns	ns	ns
Adhesiveness, N*mm	0.85	0.79	0.72	0.92	0.063	ns	ns	ns
Gumminess, N	30.7	29.1	28.5	31.3	0.86	ns	ns	ns
Chewiness, N*mm	25.6	24.0	24.0	25.6	0.73	ns	ns	ns
Springiness	0.83	0.82	0.84	0.82	0.001	*	**	*

<sup>1</sup>P1: rich pasture; <sup>2</sup>P2: poor pasture; <sup>3</sup>SL: supplement level; <sup>4</sup>P: pasture; \*\*: P<0.01; \*: P<0.05; ns: P>0.05.

### ***Volatile compounds by SPME-GC-MS***

The effects of supplement levels and pasture type on volatile fraction of Montasio cheese samples were evaluated by SPME/GC-MS analysis. The concentrations of volatile compounds, including alcohols (11), aldehydes (6), ketones (13), esters (5), hydrocarbons (3), organic acids (7), phenolic compounds (4), monoterpenes (7), sesquiterpenes (1), sulfur compounds (2), and others volatiles (3) are given in Table 4.13. A total of 62 volatiles were detected. The largest chemical families were represented by organic acids and esters in terms of quantity and by ketones and alcohols in terms of number of volatile compounds detected.

Dovier et al. (2005), in a study carried out to compare similar cheeses made with milk from two rearing systems (mountain pasture vs indoor lowland) and two periods (July vs September), showed 111 volatile compounds, belonging to 13 classes: esters (28), alcohols (25), acids (13), ketones (10), hydrocarbons (7), aldehydes (5), terpenes (5), sulphur compounds (4), alkyl benzenes (3), nitrogen compounds (3), furans (2), benzene derivatives (1) and unknown compounds (5). Surprisingly, some authors have reported on the cheese Montasio only a few volatile compounds. Polentarutti et al. (2001) in 60-d-old Montasio cheeses identified 6 short-chain fatty acids, 2 primary alcohols, and 3 methyl-ketones.

Innocente et al. (2013) in Montasio cheese from 60 to 365 d of ripening, have detected 11 compounds (5 fatty acids, 3 alcohols, 2 ketones, and 1 ester).

Alcohols can give alcoholic and floral note. In total, 11 different alcohols were identified in 60 d ripened Montasio cheeses. 2-butanol and 3-methyl-1-butanol were the most abundant alcohols in all samples. 2-Butanol was identified in large amounts and is formed as follows: diacetyl is reduced to acetoin by bacterial enzymes from raw milk, thereafter to 2,3-butanediol, to 2-butanone, and finally to 2-butanol (Bontinis et al., 2012). 3-Methyl-1-butanol may be formed by reduction of 3-methyl-1-butanal through Strecker degradation of Leucine (Urbach, 1995; McSweeney and Sousa, 2000). This compound was found to be higher in P1 than P2 ( $P = 0.08$ ). Total alcohols were similar among different experimental thesis. Innocente et al. (2013) in Montasio cheese from 60 to 365 d of ripening, have verified that the alcohols were the most significant contributors to the volatile profile of the cheeses.

Aldehydes, although they are represented by six compounds, are not very abundant in the experimental cheeses. In effect, these are transitory compounds and do not accumulate in the cheese, because it quickly transform into alcohols or the corresponding acids (Hayaloglu et al., 2013). For this reason, a low level of aldehydes indicated a good ripening of the cheeses, while a high concentration of aldehydes may cause off-flavors (Moio and Addeo, 1998). 3-methylbutanal, which provides malt, oil, or butter aroma to cheese (Serhan et al., 2010), was mainly detected in cheeses. They is formed by degradation of Leucine and is found as potent odor compound in different cheese varieties (Curioni and Bosset, 2002; Hayaloglu et al., 2013). Pasture type has caused significant differences between some of the aldehyde detected. In particular, acetaldehyde, hexanal, nonanal, and benzaldehyde were significantly higher in P2.

**Table 4.13.** Volatiles in experimental cheeses by SPME/GC-MS analysis ( $\mu\text{g}/\text{kg}$ )

Volatile	RI <sup>1</sup>	Detection method <sup>2</sup>	Supplement level		Pasture type		SEM	Significance <sup>5</sup>		
			High	Low	P1 <sup>3</sup>	P2 <sup>4</sup>		SL	P	SLxP
<b>Alcohols</b>										
2-Butanol	1032	RI, MS	200.66	229.28	99.30	330.64	72.182	ns	ns	ns
2-Pentanol	1149	RI, MS	34.74	35.43	40.07	30.10	4.308	ns	ns	ns
1-Butanol	1174	RI, MS	1.33	1.99	1.41	1.92	0.606	ns	ns	ns
4-methyl 2-pentanol	1190	RI, MS	14.53	15.69	19.21	11.01	2.985	ns	ns	ns
3-Methyl-1-butanol	1227	RI, MS	126.96	142.51	208.40	61.07	36.901	ns	ns	ns
1-Pentanol	1268	RI, MS	5.17	5.65	5.67	5.16	0.551	ns	ns	ns
2-Heptanol	1331	RI, MS	23.98	26.14	23.61	26.51	3.779	ns	ns	ns
Hexanol	1363	RI, MS	2.37	3.07	2.66	2.78	0.324	ns	ns	ns
2-Ethyl Hexanol	1496	RI, MS	1.73	1.67	1.53	1.88	0.063	ns	*	ns
2-nonanol	1526	RI, MS	15.20	12.11	9.77	17.54	2.038	ns	ns	ns
2-phenyl-2-propanol	1763	RI, MS	49.43	48.83	46.63	51.63	4.183	ns	ns	ns
Sum of alcohols			476.11	522.36	458.25	540.23	89.264	ns	ns	ns
<b>Aldehydes</b>										
Acetaldehyde	706	RI, MS	13.47	17.69	11.46	19.71	1.299	ns	*	ns
2-Methyl Butanal	917	RI, MS	13.13	11.49	16.79	7.83	2.624	ns	ns	ns
3-Methyl Butanal	921	RI, MS	21.78	24.08	34.46	11.39	6.433	ns	ns	ns
Hexanal	1098	RI, MS	17.18	19.04	15.05	21.17	1.211	ns	*	ns
Nonanal	1396	RI, MS	2.86	3.16	2.57	3.45	0.139	ns	*	*
Benzaldehyde	1528	RI, MS	3.69	4.41	2.97	5.13	0.054	**	**	**
Sum of aldehydes			72.12	79.87	83.30	68.69	9.578	ns	ns	ns
<b>Ketones</b>										
2-Propanone	822	RI, MS	58.85	46.38	39.11	66.12	3.136	ns	**	ns
2-Butanone	906	RI, MS	129.61	48.18	25.24	152.56	38.777	ns	ns	ns
2-Pentanone	982	RI, MS	28.27	19.54	25.71	22.10	3.506	ns	ns	ns
Diacetyl	986	RI, MS	51.39	31.59	33.98	48.99	3.823	*	ns	ns
3-Heptanone	1164	RI, MS	3.47	4.13	3.89	3.71	0.194	ns	ns	ns
2-Heptanone	1196	RI, MS	45.94	36.86	23.43	59.37	7.938	ns	*	ns
Acetoin	1300	RI, MS	32.30	44.18	48.85	27.63	8.019	ns	ns	ns
2-Nonanone	1392	RI, MS	19.63	17.73	12.92	24.44	4.100	ns	ns	ns
2-undecanone	1602	RI, MS	15.87	11.84	6.72	20.99	2.304	ns	*	ns
Butyrolactone	1630	RI, MS	8.70	9.00	6.35	11.35	0.494	ns	**	ns
Acetophenone	1655	RI, MS	2.56	2.70	2.60	2.66	0.102	ns	ns	ns
Dimethyl Sulfone	1901	RI, MS	7.09	9.30	11.45	4.94	1.022	ns	*	ns
delta Octalactone	2196	RI, MS	5.18	5.71	5.82	5.07	0.186	ns	ns	ns
Sum of ketones			408.86	287.12	246.06	449.92	35.347	ns	*	*
<b>Esters</b>										
Ethyl Acetate	893	RI, MS	81.11	79.94	64.30	96.75	15.304	ns	ns	ns
Isopropyl Isobutyrate	968	RI, MS	138.77	132.66	141.33	130.11	2.254	ns	*	ns
Ethyl Butyrate	1046	RI, MS	610.27	595.44	546.89	659.12	164.888	ns	ns	ns
Ethyl Hexanoate	1244	RI, MS	222.38	198.17	196.66	223.89	60.735	ns	ns	ns
Ethyl Octanoate	1438	RI, MS	25.74	25.02	24.55	26.22	6.172	ns	ns	ns
Sum of esters			1078.26	1031.23	973.42	1136.08	246.877	ns	ns	ns
<b>Hydrocarbons</b>										
2-octene	839	RI, MS	68.67	58.03	58.97	67.73	4.646	ns	ns	ns
Toluene	1049	RI, MS	276.60	311.40	367.60	220.30	14.665	ns	**	ns
m-xylene	1134	RI, MS	17.00	10.65	11.69	15.96	2.145	ns	ns	ns
Sum of hydrocarbons			362.17	380.08	468.26	303.99	14.848	ns	**	ns

<sup>1</sup>RI: Retention Index; <sup>2</sup>MS: Mass Spectrometry; <sup>3</sup>P1: rich pasture; <sup>4</sup>P2: poor pasture; <sup>5</sup>SL: Supplement level, P: Pasture; \*\*: P<0.01; \*: P<0.05; ns: P>0.05.



**Table 4.13 (continued).** Volatiles in experimental cheeses by SPME/GC-MS analysis ( $\mu\text{g}/\text{kg}$ )

Volatile	RI <sup>1</sup>	Detection method <sup>2</sup>	Supplement level		Pasture type		SEM	Significance <sup>5</sup>		
			High	Low	P1 <sup>3</sup>	P2 <sup>4</sup>		SL	P	SLxP
<b>Organic acids</b>										
Acetic Acid	1477	RI, MS	71182.84	80786.73	70073.12	81896.45	3805.192	ns	ns	ns
Butyric Acid	1718	RI, MS	38206.25	43935.03	44333.85	37807.44	2298.74	ns	ns	ns
Hexanoic Acid	1921	RI, MS	27479.77	28095.15	29685.90	25889.01	1313.00	ns	ns	ns
Octanoic Acid	2152	RI, MS	11274.70	11303.15	11930.53	10647.32	350.776	ns	ns	ns
Nonanoic Acid	2455	RI, MS	3400.94	4017.89	5484.90	1933.94	305.43	ns	**	ns
Decanoic Acid	2599	RI, MS	4257.76	4252.07	8509.82	0	285.855	ns	**	ns
methyl 2-hydroxy-4-methyl-valerate	2693	RI, MS	14.75	14.98	18.37	11.35	3.602	ns	ns	ns
Sum of organic acids			155817.0	172405.0	170036.5	158185.5	7261.19	ns	ns	ns
<b>Phenolic compounds</b>										
Phenol	2016	RI, MS	5.06	5.28	5.91	4.40	0.124	ns	**	ns
4-methyl phenol (p-cresol)	2093	RI, MS	6.53	6.59	5.69	7.42	0.323	ns	*	ns
3-Methyl phenol (m-cresol)	2101	RI, MS	6.35	7.18	7.55	5.99	0.250	ns	*	ns
4-allyl phenol	2108	RI, MS	6.04	7.46	3.75	9.76	0.338	ns	**	ns
Sum of phenolic compounds			23.98	26.49	22.90	27.56	0.503	*	**	ns
<b>Monoterpenes</b>										
a-Pinene	1022	RI, MS	46.08	41.60	28.12	59.55	3.905	ns	**	ns
Camphene	1071	RI, MS	10.75	13.36	6.22	17.89	1.674	ns	**	ns
beta-pinene	1108	RI, MS	45.45	44.73	35.28	54.90	5.909	ns	ns	ns
Sabinene	1125	RI, MS	11.41	8.98	9.97	10.42	0.662	ns	ns	ns
Limonene	1203	RI, MS	26.21	22.42	15.60	33.02	2.618	ns	*	ns
1,8-Cineol	1217	RI, MS	5.78	7.62	1.22	12.18	1.084	ns	**	ns
8-Hydroxylinalool	1332	RI, MS	15.05	14.53	12.42	17.16	1.275	ns	ns	ns
Sum of monoterpenes			160.73	153.23	108.84	205.13	11.817	ns	**	ns
<b>Sesquiterpenes</b>										
Caryophyllene	1597	RI, MS	12.72	12.35	8.41	16.65	0.751	ns	**	*
<b>Sulfur compounds</b>										
Carbon Disulfide	734	RI, MS	26.04	21.69	21.16	26.56	2.699	ns	ns	ns
Dimethyl Sulfide	745	RI, MS	11.41	10.87	10.48	11.80	0.663	ns	ns	ns
Methanethiol	-	MS	4.63	5.57	4.96	5.24	0.304	ns	ns	*
Sum of sulfur compounds			42.08	38.13	36.60	43.60	3.254	ns	ns	ns
<b>Others</b>										
1-methyl-2-diethyl-amine	814	RI, MS	7.59	8.66	7.18	9.07	2.292	ns	ns	ns
Diethylmethylamine	849	RI, MS	2.92	3.74	0.75	5.92	2.173	ns	ns	ns

<sup>1</sup>RI: Retention Index; <sup>2</sup>MS: Mass Spectrometry; <sup>3</sup>P1: rich pasture; <sup>4</sup>P2: poor pasture; <sup>5</sup>SL: Supplement level, P: Pasture; \*\*: P<0.01; \*: P<0.05; ns: P>0.05.

Ketones are highly represented in different dairy products. They were the most numerous volatiles in the experimental cheeses. The major ketones were 2-butanone, 2-propanone, diacetyl, 2-heptanone, and 3-hydroxy-2-butanone (acetoin). Among them, 2-propanone and 2-heptanone, were significantly more present in P2, while only diacetyl differ between different supplementations. Among others ketones, 2-undecanone and butyrolactone were higher in P2 conversely dimethyl sulfone was higher in P1. The sum of ketones was significantly higher in P2. Ketones have a low perception threshold and, in particular, diacetyl and acetoin are known to confer buttery notes to cheeses (Molimard and Spinnler, 1996; Moio and Addeo, 1998).

Esters were the second most abundant chemical group detected. In particular, the ethyl esters were the main esters and ethyl butyrate represent more than 50% of the total. Esters may contribute to sweet, floral and fruity notes of cheese flavor, although generally the perception threshold of these compounds is low (Molimard and Spinnler, 1996). They contribute to cheese flavor by reducing the sharpness of fatty acids and the bitterness of amines (Pinho et al., 2003). Regarding the experimental factors were not detected significant differences, with the exception of the isopropyl isobutyrate, which is slightly higher in P1.

Three hydrocarbons were identified: 2-octene, toluene, and m-xylene. Among these, the toluene is the result of the most present and significantly higher in P1 respect to P2. This compound, which originates from milk or the solvent used for the volatile analysis (Molimard and Spinnler, 1996), was detected also in other studies (Delgado et al., 2011; Bontinis et al., 2012; Hayaloglu et al., 2013).

The organic acids were found at high concentrations in the experimental cheeses. Acetic, butyric, hexanoic, and octanoic acids were the most abundant volatiles detected. For these compounds no significantly differences was highlighted. Instead, an effect of pasture type was found on nonanoic and decanoic acids concentrations. The organic acids are not only aroma compounds

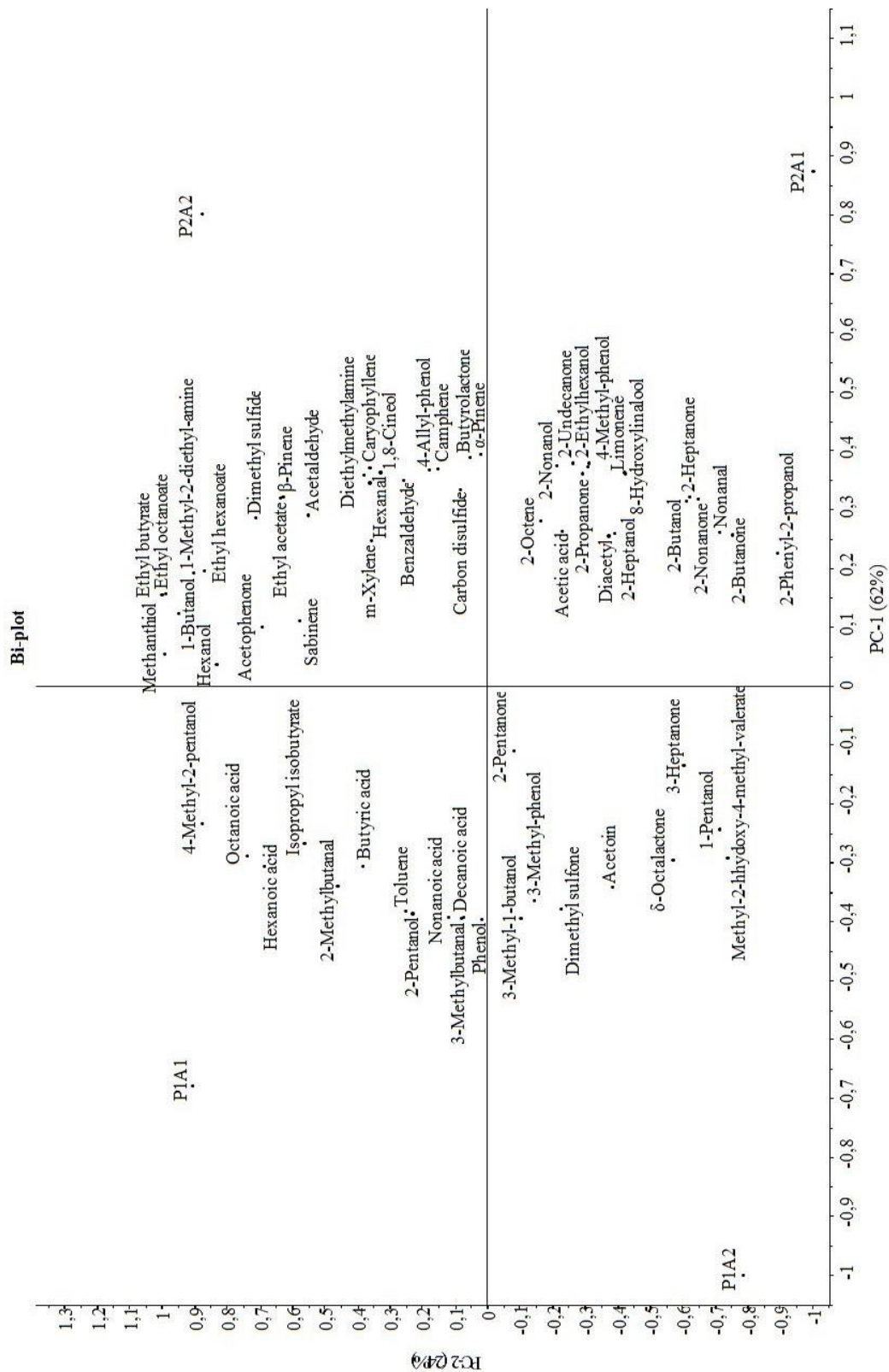
by themselves, but also they are precursors of other aroma compounds, such as methyl ketones, alcohols, lactones, aldehydes, and esters (Collins et al., 2003). Three biochemical pathways are important in the formation of carboxylic acid: proteolysis, lipolysis, and lactose fermentation (McSweeney and Sousa, 2000). Due to their low aroma thresholds, short and medium-chain carboxylic acids are considered to be important contributors to the flavour profile in a wide variety of cheeses (Pinho et al., 2003; Tavaría et al., 2004; Kraggerud et al., 2008) and each has a characteristic note. For example, acetic acid has a typical vinegary odor, sour and sharp, butyric acid has a rancid cheese-like odor and hexanoic acid is perceived as a goat-like odor (Curioni and Bosset, 2002).

Phenolic compounds are present in the experimental cheeses in very low quantities, just above the detection limit. Four compounds were detected: phenol, phenol 4-methyl phenol (p-cresol), 3-methyl phenol (m-cresol) and 4-allyl-phenol. They appear to make a positive contribution to aroma at about threshold concentration, but tend towards an unpleasant note as their concentration raise. The sensory quality ranges from sharp, medicinal, sweet to smoky, plastic and unpleasant sheep-yard notes (Curioni and Bosset, 2002; Majcher et al., 2011). 4-Methyl-phenol originates from the microbial breakdown of tyrosine (Parliment et al., 1982). p-Cresol was listed as a main odorant of Cheddar and thought to be responsible for its cowy-barny note (Suriyaphan et al., 2001).

Terpenes are important compounds in alpine cheeses (Bosset et al., 1999; Bugaud et al., 2001; Bovolenta et al., 2005). This aroma compounds originate from the plants of the pastures, then transferred to the milk of grazing cows and finally to the cheese (Mariaca et al., 1997). In all Montasio cheeses produced from milk of grazing cows, 7 monoterpenes and 1 sesquiterpene were detected. The most important monoterpenes are  $\alpha$ -pinene,  $\beta$ -pinene, and limonene.  $\alpha$ -Pinene and limonene, together with camphene and 1,8-cineol, results also significantly greater in P2 respect to P1. The sum of monoterpenes detected in

the cheese produced from P2 were double than those of P1, while no difference was detected between cheeses made with different levels of supplementation. Even caryophyllene, unique sesquiterpene found in cheeses, appeared twice in P2 compared to P1. Terpenes are known as important compounds for determining the geographical origin of a cheese type nonetheless their importance in the formation of cheese flavour remains controversial (Curioni and Bosset, 2002).

Three sulfur compounds are detected in experimental cheeses: carbon disulfide, dimethyl sulfide and methanethiol. They essentially originate from methionine degradation and result from cleavage of a bond between carbon and sulfur by a methionine-demethylase (Yvon and Rijnen, 2001). These components are described as having strong garlic and very ripe cheese odors generally associated with odour notes of cooked cabbage, broccoli or cauliflower (Kubickova and Grosch, 1998). Furthermore, their perception thresholds are low and consequently they are involved in the final aroma of mould-ripened cheeses. Methanethiol derives from methionine and may be a precursor for further sulfur compounds such as dimethyldisulphide and dimethyltrisulphide via oxidative reactions. Methanethiol, as well as dimethyl sulphide, appears to be one of the characteristic flavour compounds of Camembert and Cheddar (Curioni and Bosset, 2002).



**Figure 4.4.** Principal Component Analysis of volatile compounds.

Figure 4.4 shows the first two components of PCA. The samples were well separated by type of pasture, while it was not a clear separation of samples according to supplement level. P1 was characterized by: 3-methyl-1-butanol, 3-methylbutanal, phenol, nonanoic and decanoic acids, while P2 was characterized by:  $\alpha$ -pinene, butyrolactone, 2-undecanone, 2-propanone and p-cresol.

### ***Sensory panel evaluation***

Table 4.14 shows the results of the descriptive sensory evaluation. The panelist described the experimental cheeses with an intense color, a small and evenly distributed eyes, an intense odour and flavour of milk - sour milk and cow and a tender and creamy texture.

The supplement level did not affect any of the attributes examined. Perhaps, a supplement level of 3 kg/head/d, supposedly to below the 20% of the diet intake, are not enough at changing the sensory properties of pasture cheeses. Lodi et al. (2005), in a study on Bitto cheese produced with milk of grazing cows and goats, show that different feeding (supplement levels: 2.4 vs 0 kg/head/d) did not influence the microbiological, chemical and sensory profile of cheese.

The pasture composition has little effect on cow attribute in odor and flavor, probably it was related to some volatile compounds (e.g. p-cresol). Therefore, the assessors did not confirm the differences between pastures found with the volatile compounds analysis.

**Table 4.14** Sensory panel evaluations of cheeses (n=12)<sup>1</sup>

	Supplement level		Pasture		SEM	Significance		
	High	Low	P1 <sup>2</sup>	P2 <sup>3</sup>		SL <sup>4</sup>	P <sup>5</sup>	SL×P
<i>Visual appearance</i>								
Colour intensity	58.8	54.3	62.4	50.6	1.60	ns	*	ns
Rind height	54.4	53.4	52.9	54.9	1.66	ns	ns	ns
Eyes, distribution regularity	46.5	39.9	41.0	45.4	1.93	ns	ns	ns
Eyes, diameter	41.4	29.1	35.1	35.4	1.41	ns	ns	*
Eyes, shape regularity	44.9	48.9	46.2	47.6	2.04	ns	ns	ns
Elasticity	47.4	49.5	50.6	46.3	1.51	ns	ns	*
<i>Odour</i>								
Milk	36.7	38.3	37.9	37.2	1.47	ns	ns	*
Cow	30.6	31.4	27.2	34.9	1.88	ns	*	ns
Sour milk	42.3	44.2	43.6	42.9	1.48	ns	ns	ns
Ammonia	16.8	18.2	18.2	16.8	1.49	ns	ns	ns
Herbage	17.2	16.3	14.9	18.6	1.68	ns	ns	ns
<i>Taste</i>								
Sweet	39.1	37.7	36.8	40.0	1.15	ns	ns	ns
Salty	37.9	40.5	40.1	38.3	1.02	ns	ns	ns
Umami	31.7	34.4	31.5	34.6	1.39	ns	ns	ns
Acid	17.6	17.7	16.9	18.4	1.46	ns	ns	ns
Bitter	16.4	16.4	17.3	15.6	1.22	ns	ns	ns
Pungent	12.7	14.0	12.6	14.0	1.24	ns	ns	ns
<i>Flavour</i>								
Milk	32.2	33.8	33.3	32.7	1.36	ns	ns	ns
Cow	27.7	28.9	24.6	32.0	1.69	ns	**	ns
Sour milk	37.2	35.5	35.6	37.2	1.48	ns	ns	ns
Ammonia	16.3	16.7	15.4	17.5	1.59	ns	ns	ns
Herbage	16.0	15.8	14.5	17.3	1.58	ns	ns	ns
<i>Texture</i>								
Tenderness	52.8	54.9	55.5	52.2	1.33	ns	ns	ns
Adhesivity	38.4	36.7	31.6	43.4	1.54	ns	ns	ns
Creaminess	45.1	44.5	38.4	51.2	1.70	ns	*	ns
Granules	30.7	32.9	35.1	28.5	1.23	ns	*	ns

<sup>1</sup>anchors on a 100 mm unstructured scale; <sup>2</sup>P1: rich pasture; <sup>3</sup>P2: poor pasture; <sup>4</sup>SL: supplement level; <sup>5</sup>P: pasture; \*\*: P<0.01; \*: P<0.05; ns: P>0.05.

## CONCLUSIONS

The study allow to characterize Montasio cheese “Mountain Product” through a volatile and sensory profiles. The large number of volatile compounds identified on the mountain product was probably due to the cows diet (fresh grass from alpine pastures) and to the traditional cheesemaking techniques utilized (raw milk and natural starter). Concentrate supplementation, needed to cover the feeding requirements of grazing cows, had no effect neither on volatile compounds nor on sensory properties of the cheeses. Pasture type, instead, if from one side allows to distinguish experimental cheeses on the basis of volatile profile, on the other hand does not have a detectable effect by trained assessors.

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## 5. CONCLUSIONS

A closer link between the typical dairy products, the local traditions and the mountain areas, can be a useful tool to reverse the negative trend of the alpine animal husbandry. In Friuli Venezia Giulia Region, as a result of a recent agreement between “*Consorzio di Tutela del Formaggio Montasio*” and “*Associazione Nazionale Allevatori Pezzata Rossa Italiana*” and the inclusion in the product specification of Montasio of the opportunity to utilize the label PDM (Mountain Product), has been proposed the Montasio PDO-PDM “*Solo di Pezzata Rossa Italiana*”. This cheese combines the only Friulian PDO, the regional mountain area and the main cattle breed reared in this area.

The interest for this new dairy product, and the potential positive impact that its valorization can lead to dairy farming in regional mountain area, have materialized in a project aimed to study and promote this cheese, in collaboration with “*Centro di Ricerca e Innovazione Tecnologica in Agricoltura*” (CRITA) and “*Associazione Allevatori del Friuli Venezia Giulia*” (AAFVG).

The thesis has allowed to evaluate the potential production context and to provide a scientific and technical contribute for the production of cheese. Among the potential producers presents on the territory have been identified two dairies in order to ensure a production from winter (Dairy of Ugovizza) and from summer (Malga Montasio). These producers are well rooted in the territory and seem to be able to ensure a production fairly constant over time.

The comparison of Montasio cheeses produced from Simmental cows reared indoor or grazing on alpine pasture has shown the differences in chemical composition, nutritional proprieties, color and texture. The product is generally liked by the potential consumers. However, this liking can be further enhanced if it is linked to a correct information about the product origin that can highlights its peculiarities such as the strong link with the territory of production (use of

local fodder, use of local cattle breed, maintenance of traditions and alpine landscape).

As regards to the alp cheese, it is seen as a moderate supplement with concentrates does not affect the sensory characteristics of product. The pasture type has an effect limited to the volatile fraction without detectable effects on the organoleptic perception of trained assessors. Other results of these trials are currently being processed and will help to complete this first analysis of the product.

It will be necessary in the future to assess the variability factors of product quality in relation to technological and microbiological aspects. Last but not least, even with the help of data concerning the environmental and social sustainability of farms, it will be useful to set an appropriate marketing campaign to support the product in order to ensure economic sustainability for producers.