

## REVIEW

# The Noble Method in the dairy sector as a sustainable production system to improve the nutritional composition of dairy products: A review

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*Sustainable production systems in line with consumer expectations are attractive for the dairy sector. The objective of this review is to examine the benefits of an Italian method, named the Noble Method ('Metodo Nobile®'), in order to improve the nutritional properties of milk and environmental sustainability. The prohibition of silage and the use of polyphite pastures are some of the rules established by the Noble Method. The greater amount of unsaturated fatty acids and other beneficial compounds found in milk and dairy products produced by using milk from animals fed on well-managed pasture could have positive implications on consumers' health.*

**Keywords** Noble milk, Fresh forage, Nutraceutical compounds, Omega-3 polyunsaturated fatty acids, Animal welfare, Sensory properties.

## INTRODUCTION

The modern consumer is increasingly interested in dairy products derived from 'pasture-fed' livestock. This interest lies in the request for an improved finished product in terms of nutritional properties, greater animal welfare and better environmental sustainability (Hanrahan *et al.* 2018; Magan *et al.* 2021). In fact, the development of new healthy dairy products, by managing the animal feeding system, has recently started in countries such as Denmark, Norway and Switzerland (cheese and butter products with new flavours and textures) and it is a market, which is gaining more and more interest worldwide (Holmquist 2021).

Animal products provide energy and are essential nutrient sources of quality proteins, amino acids, minerals, vitamins and bioactive compounds (e.g. ruminic acid and carotenoids), which are necessary for the human diet. Indeed, milk is considered a complete food and both milk and dairy products, in a well-balanced diet, are beneficial for human nutrition (Clerfeuille *et al.* 2013; Magan *et al.* 2021). Furthermore, by implementing appropriate animal feeding strategies, important healthy components in milk such as omega-3 polyunsaturated fatty acids

(PUFA n-3), vaccenic acid (VA), ruminic acid and carotenoids can be enriched (Allothman *et al.* 2019).

Literature reports a general positive consensus about the favourable nutritional profile of milk and dairy products, in terms of unsaturated fatty acids (FAs), vitamins, polyphenols and other antioxidants, from animals fed fresh forages rather than preserved ones (e.g. silages) (Biondi *et al.* 2008; Renna *et al.* 2012c; O'Callaghan *et al.* 2016; Johansen *et al.* 2018; Corazzin *et al.* 2019; Radonjic *et al.* 2019; Formaggioni *et al.* 2020). Highly biodiverse pastures provide animals with a wider range of chemical compounds, such as terpenes, phenols, carotenoids and antioxidants, in comparison with animal fed with grass monocultures (Machado Filho *et al.* 2022). Consequently, such grazing system appears to be a valid natural strategy to improve the composition of milk products and, hence, consumers' health (Ferlay *et al.* 2017). In such pastures, Renna *et al.* (2020) found that the phenology and proximate composition of plants helped predict the fatty acid composition of milk.

In addition, grazing ruminants in a well-managed pasture can express their natural behaviour, which results in a positive animal welfare (Machado Filho *et al.* 2022; Rivero and

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Lee 2022). Grazing system is also an efficient system, which has low competition for the direct production of humane edible food crops, meeting the food security requirements needed for a rapidly growing population (Dumont *et al.* 2018). The sustainability information, such as origin of products, respect for animal welfare, lower carbon footprint and lower greenhouse gas emissions, should be effectively reported by dairy producers in order to succeed with their products in the food market (Schiano *et al.* 2020).

The Production Regulations of the Noble Method is based on this scientific evidence and provides rules for dairy chain producers with the aim of improving the nutritional characteristics of dairy products, environmental sustainability and animal welfare.

The purpose of this review is to discuss and present the Noble Method as a natural and more sustainable strategy to improve the nutritional composition of milk in order to develop healthy dairy products for consumers. Differences in the sensory properties are also addressed.

## REGULATION AND PURPOSE OF NOBLE METHOD IN THE DAIRY SECTOR

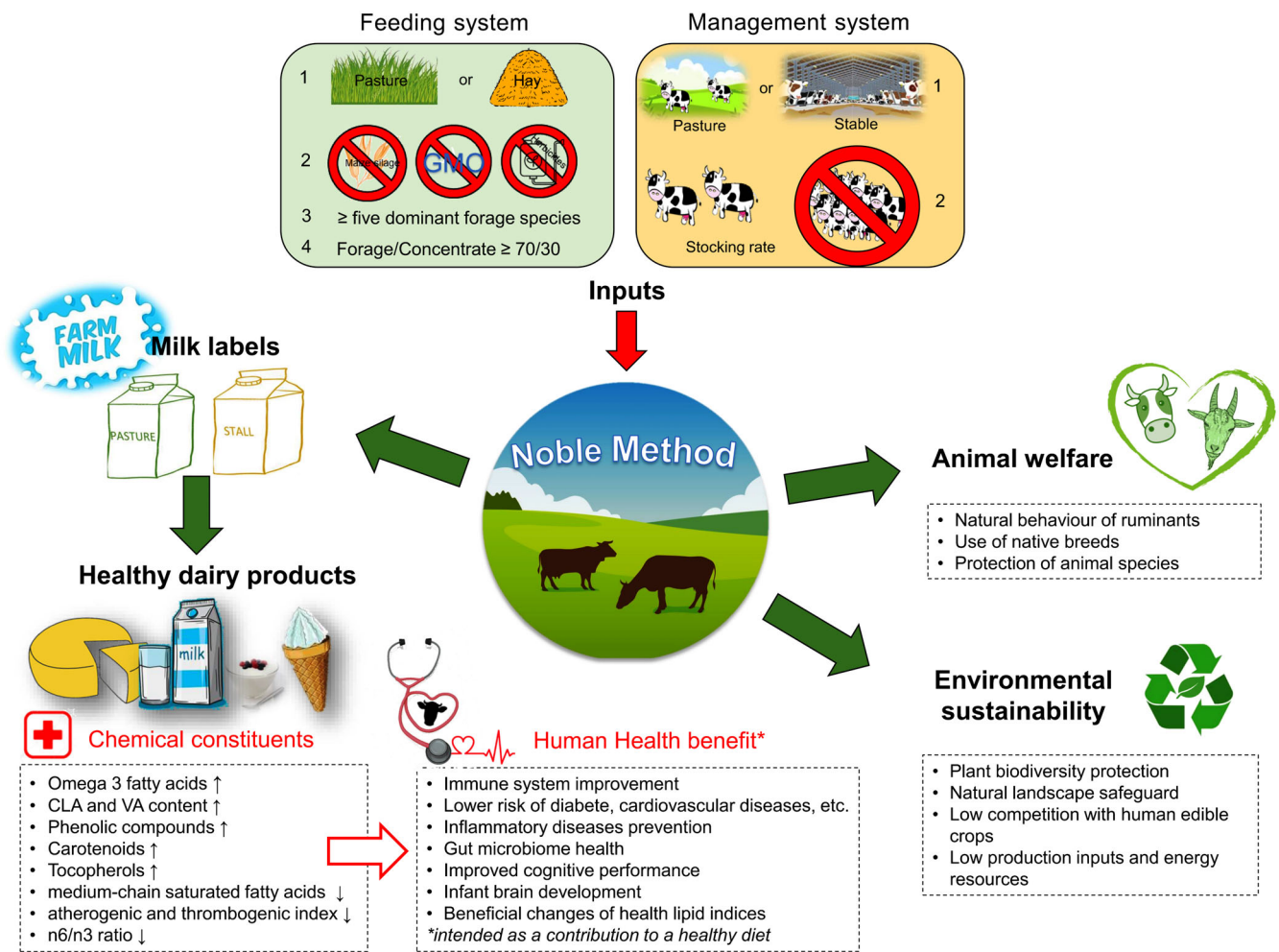
Although the Noble Method is applied to both plant and animal food chain products, this review aims to discuss this method as a new model of milk production for the dairy sector. In fact, the 'Noble milk' (Latte Nobile®) brand was created in 2011 in southern Italy, in the Campanian Apennines, with the aim of offering milk with superior nutritional and healthy characteristics by optimising the feeding and breeding system of lactating ruminants (Renna *et al.* 2015). The Noble Method has also spread to central and northern Italy, to improve the competitiveness of small mountain dairy farms, and it is currently implemented in various regions of Italy, such as Campania, Sicily, Sardinia, Lazio, Basilicata, Veneto and Piedmont (Consortium Producers 2022). The 'Metodo Nobile®' has been registered as a collective trademark by ANFoSC (Associazione Formaggi Sotto il Cielo), a nonprofit association founded 20 years ago and currently chaired by Roberto Rubino (Rubino 2014; [www.metodonobile.com](http://www.metodonobile.com)), and appears to be a promising strategy, especially for small ruminants, such as sheep and goats in farms, where the extensive system is more suitable (Ruiz *et al.* 2009).

The dairy-based food market is broadly segmented and continues to differentiate according to the new consumers' needs. The Noble milk is part of this segmentation and is a high-value product which consumers have expressed their willingness to pay a premium to buy (Lombardi *et al.* 2016). In some Italian mountain villages, the richness of high biodiversity pasturelands allows the production of Noble milk and dairy products, which are important sources of income for the Alpine economy (Freccero 2015).

Furthermore, the increase in the proportion of grazed grass allows a reduction in milk production costs (Conboy Stephenson *et al.* 2021), with benefits for both dairy products producers and consumers. Dillon *et al.* (2005) have reported a reduction in milk production costs and capital investment in the pasture-based milk production system. In particular, the authors reported a reduction in the cost of milk production of 2.5 cents per litre for every 10% inclusion of pasture grass in the TMR diet. Hanrahan *et al.* (2018), using an internationally recognised representative database over an 8-year period (2008 to 2015) on pasture-based systems, found that each additional tonne of used dry matter pasture, on average, increased gross profit by €278 and net profit by €173 on dairy farms. Conversely, net profit reduced when the percentage of feed purchased increased. However, the same authors also reported that production costs and profits differed in consideration of the year, geographical location and dairy farm size.

The Noble Method has a production regulation which all producers must respect. Figure 1 summarises the main Noble Method Production Regulations as well as the main effects on milk and dairy products. In particular, the Noble Method prohibits the use of silage and genetically modified feedstuffs in the livestock feeding system and allows the use of pastures or grasslands with at least five different forage species. The use of hay is also allowed, but it must come from local mixed grasslands and meet certain production requirements (e.g. the prohibition on chemical herbicides and compliance with the quality standards established by the consortium, like monitoring the quality of hay through sensory analysis). The ratio between forage and concentrate must be at least 70:30. In this regard, the Noble Method Production Regulations provide for two types of labels: 'pasture' label and 'stall' label. In the 'pasture' label, the animals must be fed almost exclusively outdoors on local polyphite meadows for at least 150 days per year of grazing and the integration with concentrates cannot exceed the following daily quantities: 3 kg for cows; 1 kg for buffaloes; 0.2 kg for sheep and goats. The 'stall' label allows the use of hay in place of pasture. In order to guarantee animal performance and welfare, limits on animal density and on the maximum milk yield per lactation are also imposed (e.g. for cows, the specification states that productions must not exceed 6000 L per head; Noble Method Disciplinary 2018; Renna *et al.* 2015). Although the cow's milk production level is below twenty litres per head per day, producers who have joined the Noble Method consortium receive a price 50% higher than conventional milk market price (Rubino 2014).

Noble milk can be sold raw or pasteurised (Renna *et al.* 2015; Noble Method Disciplinary 2018). The 'Latte Nobile fresco pastorizzato' (fresh pasteurised Noble Milk) is currently packaged by the company 'Latteria Agerolese' (stamp: IT 15 / 628 CE) in glass or tetrapak package ([www](http://www)).



**Figure 1** Schematic representation of the rules of Noble Method, their effect on nutritional properties of dairy products and their contribution to human health benefits, environmental sustainability and animal welfare.

[lacompagniadellaqualita.com](http://lacompagniadellaqualita.com)). The lipid profile of raw ‘pasture’ Noble milk must contain at least 0.25 g 100 g<sup>-1</sup> fat of total conjugated linoleic acid (CLA) and 0.50 g 100 g<sup>-1</sup> fat of total n-3 FAs (Renna *et al.* 2015). Noble milk is a silage-free milk which is suitable for the production of hard cheese, since the absence of silage avoids the risk of contamination of raw milk by spores of clostridial bacteria, which can cause quality defects in aged cheese (Brändle *et al.* 2016). Some producers who have joined the Noble Method have developed several dairy products, including cheese, butter, ricotta, ice cream and yoghurt (Consortium Producers 2022; [www.lacompagniadellaqualita.com](http://www.lacompagniadellaqualita.com)). The high-end raw milk has given them the opportunity to develop healthier dairy products, with better nutritional indices, which provide a greater amount of beneficial components such as ruminic acid, VA, PUFA and carotenoids. The higher quality and reduced quantity of production could increase the price of Noble dairy products on the market in comparison with conventional ones.

Silage-free milk production has gained success over the past decade. In fact, other milk production systems were developed during that time, such as Grassmilk® in the United States and Hay Milk in Europe. Grassmilk® was launched in 2011 by Coulee Region Organic Produce Pool (CROPP) Cooperative, known as organic valley, in southwest Wisconsin, USA (Benbrook *et al.* 2018). Grassmilk is an organic milk, and the dairy products derived from it are sold under the Organic Valley brand. The cows that supply the Grassmilk are fed with fresh forages or hay, and the use of cereal crops in the flag leaf stage (preboot stage), which do not have mature seeds but only plant foliage, is also permitted. The use of mineral supplements, molasses, alfalfa pellets, sugar beets and kelp is allowed to meet the energy needs of lactating cows. As for the Noble milk, the Grassmilk is subjected to periodic analysis of FAs. Depending on the geographical area, 39 to 41 mg total n-3 FA and 26.6 to 32.8 mg total CLA per 100 g of milk must be obtained. Furthermore, the n-6/n-3 ratio must be ≤1.2. Similar to the

price of Noble milk in Italy compared with the conventional milk (Rubino 2014), in the US producers who supply Grass-milk receive a premium of 15% higher than standard prices for conventional organic milk (Davis *et al.* 2020). The Traditional Speciality Guaranteed (TSG) Hay Milk certification is applicable in the European Union and the United Kingdom and refers to the European Commission regulation 2016/304 of 2 March 2016, which provides the directives for obtaining compliance with the production specification (European Commission 2016). Cow, sheep and goat milk that meets the requirements of the specification can obtain the TSG Hay Milk certification (European Commission 2018a; 2018b). The specification provides for grazing during spring and summer seasons, and hay in winter or when pasture is not accessible. Compared with the Noble Method, the ratio between forage and concentrate is slightly higher and must be at least 75:25. Animals may not be fed by-products from the food industry except for molasses as a by-product of sugar manufacturing and dry protein feed produced during grain processing. As for Noble Method, the use of silage and GMO feed is prohibited. Moreover, the use of urea is not allowed. Furthermore, the Irish Food Board, Bord Bia, has introduced a Grass-Fed standard in Ireland, whereby cows must be fed a minimum of 90% grass or grass forage on a fresh weight basis and must spend at least 240 days a year grazing (McGuinness *et al.* 2022).

The European Commission has recently introduced novelties regarding the relationship between geographical indication brands and sustainability (European Commission 2022). The geographical indications of quality, due to their link with the territory, not only can constitute a means to reduce the environmental impact and the use of natural resources but can also be an increasingly attractive strategy for consumers, who will be encouraged to be ethically responsible about food choices. However, even in the extensive systems, a 'controlled' environment is appropriate to ensure the welfare of the animals and their host environment (Rivero and Lee 2022). The right compromise should be ensured between the production performance of the animals, where an increase in the animal performance could reduce enteric methane emissions, and the compliance with animal welfare requirements. In fact, well-defined pasture management, like that one regulated by the Noble Method, can promote biodiversity, soil conservation and environmental sustainability (Figure 1) and improve milk nutritional quality (Kuhnen *et al.* 2022; Rivero and Lee 2022). The varied heritage of grazing vegetation contributes to dairy product quality, giving them particular aromatic and nutritional features (Frecero 2015; Ravetto Enri *et al.* 2017; Claps *et al.* 2020). The biochemical complexity of different forage species, compared with the single forage crop in the animal diet (e.g. maize in monoculture), helps animal nutrition and the enteric methane emissions control, thanks to the presence of plant secondary metabolites, condensed tannins and other plant

products (Distel *et al.* 2020). In fact, in many grazing areas forage species are usually present, such as those belonging to forage legumes (birdsfoot trefoil, common vetch, purple prairie clover, sainfoin and sulla), which appear to be a potential option for the enteric methane emission control and mitigation (Aboagye and Beauchemin 2019).

The use of local grazing breeds can have positive effects on the environment, grassland biodiversity maintenance and conservation of livestock biodiversity resulting in a dairy system more accepted by consumers (Kühl *et al.* 2020; Claps *et al.* 2020). Several farms, which follow the Noble Method, are already breeding native breeds, especially for the breeding of sheep and goats, which are very well adapted to the local climate and landscape. These pasture farms could be associated with a higher level of animal welfare, although animal welfare assessment protocols also need to be implemented in extensive farms to avoid nutritional deficiencies and other animal health problems (Battini *et al.* 2021). Covering the animals' food needs with grazing, the use of local breeds and low stocking rates (low animal density) resulted in a lower use of energy and fuel resources and improved environmental sustainability (Ripoll-Bosch *et al.* 2012). However, the exclusive use of fresh fodder would not meet the metabolic needs of the ruminants in production. In fact, the Noble Method moderates but does not abolish the use of energy concentrates. Otherwise, there would be a risk of a decline in production yield and animal welfare (Knaus 2016). The high animal density of intensive animal breeding is associated with greater stress in lactating ruminants affecting animal health and longevity, milk quality and reproductive efficiency (Pulina *et al.* 2017).

On the contrary, if the pasture is not well managed, animals could be exposed to injuries or nutritional deficiencies. In fact, a one-factor animal welfare assessment (such as measuring plasma levels of cortisol and serotonin or measuring heart rate variation) does not cover all aspects of animal welfare (Machado Filho *et al.* 2022). Therefore, the use of multi-indicator evaluation protocols is recommended. Battini *et al.* (2021) reported that most animal welfare assessment studies were originally developed and conducted for intensive farming systems. Less concern was placed on the extensive system as animal welfare was considered a minor risk. The use of animal welfare assessment indicators, such as the Animal Welfare Indicators (AWIN) protocol, would be appropriate for assessing animal health status, even in extensive systems of small ruminants, in order to ensure the farming system functionality. By evaluating the sustainability of four sheep farms with different degrees of intensification, it was observed that the greater the economic indicators (e.g. in intensive farms), the lower the environmental sustainability (Ripoll-Bosch *et al.* 2012). Bach *et al.* (2008) observed variations in milk production of cows fed with the same feed. The different milk yield, ranging between 20.6 and 33.8 kg/day, was attributed to

nondietary factors such as the number of animals, number of stalls per cow, feedbunk space per cow and other management practices. However, extensive low-input farms receive lower income (Ripoll-Bosch *et al.* 2012; Kühn *et al.* 2020). In fact, the Noble Method aims to improve the quality of dairy products for consumers and the economic efficiency for producers. A litre of pasteurised Noble milk is currently sold at a price of 47% higher than the average price of a typical fresh pasteurised milk available on the market (<https://www.robertorubino.eu/prezzo-latte-nobile>). The same percentage of price increase, compared with the price of raw milk paid to milk producers, is guaranteed to Noble milk producers (Rubino 2014). A complete evaluation of the Income Over Feed Cost would be recommended to better investigate the economic convenience of the Noble Method, also in relation to particular production areas.

Surveys and focus groups conducted on a group of twenty-three dairy consumers showed that if the important sustainability attributes of dairy products are well communicated or labelled, they represent an added value for consumers who take care of ethical and environmental issues of food production (Schiano *et al.* 2020). A PCA analysis conducted on the responses of 543 people interviewed in the study of Merlino *et al.* (2022) showed that local dairy products were perceived to have higher quality. Consumers have expressed the need for greater availability of local products on the market. Focusing on quality rather than quantity, the Noble Method aims to create a value chain of dairy products (new healthy products with specific sensory features) recognising a fair price for them and the right remuneration to producers. As a local product, in addition to communication strategies of the qualities offered, it would be appropriate to increase the visibility and availability of Noble dairy products on the market.

It is worth mentioning that, due to the variability of orographic conditions and breeding systems in Italy, the regulation of the Noble Method is flexible on its rules. For example, it does not completely abolish energy concentrates and, where grazing is not possible, producers can use hay (Stall label) as a source of forage. Furthermore, the Noble Method does not specify the five different forage species, which could limit the production of Noble milk to particular areas.

The next section reports some studies carried out on Noble milk in order to understand the effect on the chemical, nutritional and sensory properties attributed to animals whose feeding is based on high biodiversity pastures. Literature on the Noble Method for the dairy sector still needs to be deepened. Therefore, this study has also considered and discussed the beneficial effects of milk and dairy products obtained from animals managed with an extensive breeding method, which can be compared with the breeding method regulated by the Noble Method.

## NOBLE METHOD TO OPTIMISE THE QUALITY OF MILK AND DAIRY PRODUCTS

Dietary guidelines for proper human nutrition recommend at least three servings of dairy foods a day (Hess *et al.* 2020). Milk, yogurt and other dairy products are an important group of foods that have long been known to promote well-balanced nutrition. Worldwide, about 43% of raw milk is consumed as drinking milk and about 25% for making cheese. Cheese production is expected to grow at 1.2% per capita until 2028 (Al-Attabi *et al.* 2021). Dairy products provide essential nutrients more efficiently than other rearing systems and are a valuable source of minerals, fats, amino acids and vitamins, which help people meet the recommended daily intake of essential nutrients (Haug *et al.* 2007; Britt *et al.* 2018). Milk proteins bring important nutritional and functional properties in human diet, possessing a high biological value, good digestibility and absorption (Rafiq *et al.* 2016). Milk proteins also exhibit mineral carrier properties, which can help in nutrient transport, bioavailability and absorption (Hoolihan 2004; Delshadian *et al.* 2018). Bioactive peptides with important health benefits (*e.g.* antihypertensive, antimicrobial and immunomodulatory activity) are released from gastrointestinal digestion of milk proteins (Madureira *et al.* 2010). Milk is a good source of calcium and phosphorus, and dairy products cover about 30–60% of the daily calcium requirement in a typical European diet (Welch *et al.* 2009). Dairy products are also the main dietary source of ruminic acid, a CLA found in ruminant lipids and milk fat, associated with human health for immune function and protection against some diseases such as cancer, obesity and diabetes (Yang *et al.* 2015; Ferlay *et al.* 2017).

Although several factors (animal breed, genetics, lactation stage and milking technology) can affect the concentrations of the different components of milk which are important for human nutrition, animal diet, especially for the effect on milk FAs, is among the most naturally controllable, as well as a simple tool to improve environmental and economic sustainability and animal welfare. In fact, changes in feeding strategies lead to changes in rumen activity and mammary biosynthesis processes, which affect the chemical and sensory properties of milk and dairy products as well as the production of enteric methane emissions by ruminants (Alothman *et al.* 2019; Ni *et al.* 2020).

### Fatty acids and health implications

Milk fat has a complex composition, consisting of over four hundred different FAs of varying length and degree of unsaturation (Lindmark Månsson 2008). Dairy fat possesses a distinctive flavour, and it is almost the exclusive source of some important health-related FAs in human diet, such as ruminic acid, branched-chain FAs and some short-chain FAs (*e.g.* butyric acid; Gómez-Cortés *et al.* 2018).

With the growing demand for healthy and ‘natural’ foods, several studies have focused on the opportunity to improve the quality of milk fat by managing the animal feeding system. Animal nutrition is the most investigated factor to improve the fat composition of dairy products (Schwendel *et al.* 2015).

De novo synthesis in the mammary gland, animal diet and metabolised adipose tissue represent the main sources of origin of ruminant milk FAs (Ungerfeld *et al.* 2019). In general, short (C:4-C:10) and medium (C12:0, C14:0 and C16:0) chain FAs are synthesised by mammary gland metabolism (Ferlay *et al.* 2017). Preformed FAs (>C16) of dietary origin undergo ruminal metabolism (lipolysis and subsequent biohydrogenation in the rumen) as well as fermentable carbohydrates which are fermented by rumen microbial community, producing volatile FAs (mainly acetic, propionic and butyric acid) that can promote lipid synthesis in the mammary gland. Despite animal diet induced changes in the rumen ecosystem, it has been reported that changes in milk fatty acid composition in response to the diet are accompanied by tissue-specific alterations in the expression of one or more lipogenic genes (Shingfield *et al.* 2013); hence, the formulation of animal diet to exploit this advantage is increasingly being considered.

Biohydrogenation of unsaturated FAs, like linoleic acid, occurs in rumen, passing through the formation of numerous cis and trans isomers of 18:1 (e.g. oleic acid and VA) and of conjugated 18:2 (e.g. CLA), leading also to the production of saturated FAs, like stearic acid (Doreau *et al.* 2010). However, stearic acid can be desaturated in the mammary gland, thus improving the fatty acid profile of milk (Tudisco *et al.* 2019). Therefore, the CLA isomers can result both from rumen synthesis through the incomplete biohydrogenation of linoleic acid thanks to the action of rumen bacteria, and from endogenous synthesis in the mammary gland starting from the precursors of biohydrogenation. Rumenic acid (c9, t11-18:2) is the most health-related isomer and the main CLA-isomer found in milk (Delmonte *et al.* 2005). The different CLA isomers have different biological activity in humans, as reviewed by Ferlay *et al.* (2017), despite the clinical and epidemiological studies conducted on humans are still scarce. Ferlay *et al.* (2017) have also deepened the formation pathway of isomers of CLA in ruminants. Rumenic acid can be synthesised from vaccenic acid (t11-18:1, VA) by the enzyme delta-9-desaturase (Griinari *et al.* 2000). It is estimated that more than half of rumenic acid present in milk comes from the desaturation of VA in the mammary gland. Furthermore, VA can also be converted to rumenic acid in human tissue (Turpeinen *et al.* 2002). An updated review, addressed the bioactive role of some FAs present in milk fat, reports that rumenic acid and VA are useful in chronic inflammatory disease prevention (Gómez-Cortés *et al.* 2018). It has also been reported the important role of branched-chain FAs and butyric acid for human health.

Branched-chain FAs have a positive effect on the infants’ gut microbiome. Butyric acid is a source of energy for intestinal epithelial cells and promotes positive intestinal bacteria health (Gómez-Cortés *et al.* 2018).

A grass-based diet can improve the lipid profile of milk (Biondi *et al.* 2008; Renna *et al.* 2012b; 2012c; Corazzin *et al.* 2019). There is good agreement on the positive role that fresh forage plays with respect to silage, or grazing rather than an indoor conventional system, on the fatty acid composition of milk fat (White *et al.* 2001; Couvreur *et al.* 2006; Corazzin *et al.* 2019). Furthermore, the cultivar type and phenological stage of the forage crops showed an important contribution (Chilliard *et al.* 2001; Cabiddu *et al.* 2003; 2004; Johansen *et al.* 2018; Radonjic *et al.* 2019; Renna *et al.* 2020).

Riuzzi *et al.* (2021) proposed the fatty acid profile as marker to discriminate the source of forage in animal feeding system (maize silage, hay and permanent pasture). The profile of FAs and terpene compounds was used as a tracer for Asiago d’Allevio PDO cheeses based on their production season (Renna *et al.* 2012a). Asiago cheese produced from milk collected in the summer grazing season had a different fatty acid and terpene profile than Asiago cheese produced from milk from the autumn and winter indoor seasons. Furthermore, terpene compounds helped discriminate cheese from the early versus the late summer grazing season.

The fatty acid profile of milk of grazing ruminants, compared with silage-based diet, is more favourable for the consumers’ health. Compared with a TMR confined-based feeding, the grass-based diet led to obtaining milk with less medium-chain saturated FAs (such as 12:0, 14:0 e 16:0), and a higher content of  $\alpha$ -linolenic acid (n-3), VA, rumenic acid, iso and anteiso branched-chain FAs (Patel *et al.* 2013; Rego *et al.* 2016; Alothman *et al.* 2019; Gebreyowhans *et al.* 2019). Increased consumption of medium-chain saturated FAs has been associated with an increased risk of hypercholesterolemia; on the contrary, the consumption of unsaturated FAs can help reduce LDL cholesterol (McPherson and Spiller 2020). Moreover, Simopoulos (2011) reported an inadequate consumption of omega-3 FAs in Western populations, with an n-6/n-3 ratio of 10–20 / 1 in human diet, in which the recommended ratio should be at least less than 4. Renna *et al.* (2015) reported, in pasture Noble milk, values range from 0.29 (winter) to 1.71 (summer) g 100 g<sup>-1</sup> fat and from 0.60 (winter) to 1.99 (summer) g 100 g<sup>-1</sup> fat for CLA and n-3 FA, respectively. Benbrook *et al.* (2018) collected milk samples for 3 years from cows fed almost exclusively with fresh grass and compared their results with nonorganic and organic milk. They found a significant difference in terms of FAs. In particular, ‘grass-milk’ had the more favourable omega-6/omega-3 (n-6/n-3) ratio of 0.95, compared with the ratio of 5.77 and 2.28, respectively, for nonorganic and organic milk. Grassmilk also had a higher content of rumenic acid and

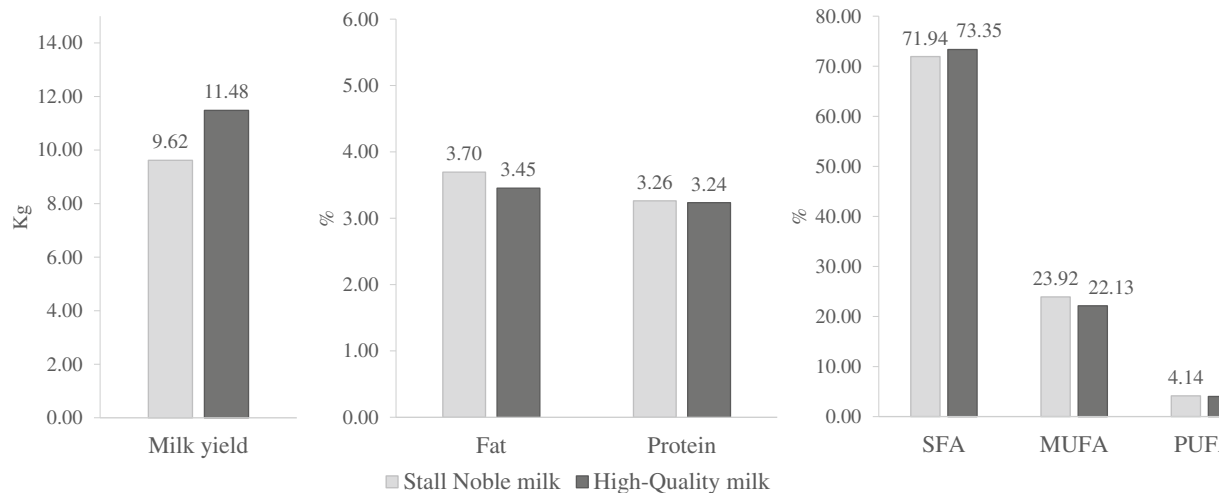
eicosapentaenoic acid. A low n-6/n-3 ratio can help reduce the risk of cardiovascular and other metabolic diseases (Benbrook *et al.* 2018). In addition, it has also been shown that omega-3 and omega-6 FAs, as well as their ratio, influence the cognitive performance of children and adults (Sheppard and Cheatham 2018) and brain development of infants (Shahidi and Ambigaipalan 2018).

Figure 2 reports the results obtained from a study conducted on lactating cattle homogeneous for genetic type (Italian Friesian), parity, lactation stage and milk production but coming from two different farming systems (Roncoroni *et al.* 2014). In the Noble farming system, cows were fed with a ratio between forage and concentrate of 70:30 and hay as a forage source (according to the 'stall' label of the Noble Method), while in High-Quality milk system, the ratio between forage and concentrate was 60:40 and maize silage was used as a forage source. High-Quality milk is a drinking milk, which must comply with precise parameters established by the Italian law (Legge n.169/1989). Indeed, it must undergo High Temperature Short Time pasteurisation (HTST, e.g. 72 °C for no less than 15 seconds) since the heat treatment (like pasteurised Noble milk) must occur within 48 hours from milking, having a minimum percentage of undenatured whey proteins after pasteurisation of 15.5%. Moreover, the processed raw milk must have been produced in Italy. Slightly higher milk yield was reported in the High-Quality milk, while the 'stall' Noble milk showed a higher fat content and a trend in healthier fatty acid profile. The total monounsaturated (MUFA) and polyunsaturated (PUFA) FAs showed a higher content in 'stall' Noble milk, while saturated FAs (SFA) showed a lower content (Roncoroni *et al.* 2014). The lower quantity of energy concentrates in the cow's diet reared according to the Noble Method led to a lower milk yield. A ration with a higher quantity of concentrates is usually used indoors in intensive farming in order to increase production yield (Knaus 2016). The use of pasture rather than preserved fodder results in a greater increase in terms of unsaturated FAs, rumenic acid and VA. Therefore, 'pasture' Noble milk may offer a more favourable nutritional composition. In fact, interesting findings on lipid components of 'pasture' Noble milk were reported by Lombardi *et al.* (2014), which revealed differences in the nutritional composition of bovine Noble milk according to seasonality. In particular, the content of n-3 FAs and rumenic acid was higher in Noble milk produced in summer and spring than the content produced in winter. This trend has been associated with greater availability of grazing in warm seasons, since the botanical composition of pasturelands and the nutritional value of forages vary according to the growing season (Gorlier *et al.* 2012). Furthermore, as summarised in Figure 3, the study compared Noble milk produced in the summer with High-Quality milk produced in the same season. Noble milk had more than double CLA and n-3 compared with commercial

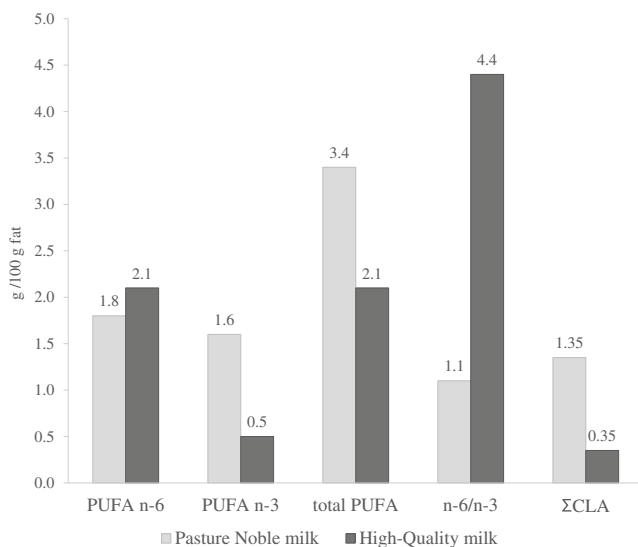
High-Quality milk, as well as the most favourable n-6/n-3 ratio.

Compared with milk of Cilentana breed goats fed indoor with alfalfa hay and 500 g/head of concentrate, the goats with free access to pasture (as a hay substitute) produced milk with a higher quantity of rumenic acid, associated with the influence of grazing on the increased expression of the enzyme stearoyl-CoA desaturase (Tudisco *et al.* 2019).

Coppa *et al.* (2012) studied the evolution of the fatty acid profile of cows' milk from a hay-based to pasture-based animal diet, considering both a rapid (from zero grazing to full grazing in three days) and progressive (from zero grazing to full grazing in sixteen days) diet transition. The authors reported no significant differences in milk production, fat and protein yield during the investigated period. The differences in the amount of C18:2n-6 and C18:3n-3 PUFA became significant after five days for both progressive and rapid transition to pasture, while C18:1n-9 MUFA (oleic acid), total CLA isomers and VA FAs became significant after three days for rapid and five days for progressive transition to pasture. Such FAs are all increased in milk of grazing cows. This was due to the increased intake of fresh fodder in the cows' diet. The value of CLA became constant after 16 days and 24 days depending on the rapid or progressive diet transition. As grazing intake increased in cows' diet, the n-6/n-3 ratio, the atherogenicity and thrombogenicity indices decreased. Moreover, the authors reported a peak of butyrate after three days from the addition of pasture, probably due to rumen fermentation due to the higher quantity of carbohydrates in the fresh forage. No difference in the gross composition of milk of sheep fed on polyphyte pasture (composed mainly of *Lolium perenne*, *Dactylis glomerata* and *Trifolium repens*) compared to those fed indoors was reported (De Renobales *et al.* 2012). On the contrary, the authors reported a significant increase in milk yield of 30% in grazing animals and an increase in PUFA, more than three times CLA (15 to 50 µg/g fat) and a reduction in the atherogenicity index of milk. Biondi *et al.* (2008) studied the evolution of FAs in milk of ten Comisana ewes whose diet changed from stall to pasture-based diet. After one month of stall feeding based on hay, straw and concentrate, the ewes were brought to pasture and the milk samples were analysed for 23 days starting on the last day of stall feeding. Myristic and palmitic acids significantly decreased, while VA increased and  $\alpha$ -linolenic acid tripled from the third day following the transition to pasture. Rumenic acid approximately tripled at the end of the investigated period. Similar results were found by Renna *et al.* (2012c) in the milk of ten Valdostana goats. Furthermore, the authors reported a two times lower n-6/n-3 ratio at the end of the investigated period. The high content of  $\alpha$ -linolenic acid in the fresh fodder had a positive linear correlation with the rumenic acid and the VA found in the milk fat of grazing cows (Yamaguchi *et al.* 2017), and low



**Figure 2** Average of milk production (left), fat and protein (center) and fatty acids profile analysed by Milkoscan (right) of “stall” Noble milk compared to High-Quality milk (adapted from Roncoroni *et al.* (2014)). High-Quality milk is a drinking milk which can only undergo an HTST pasteurisation treatment and must have, after the heat treatment, a percentage of undenatured whey proteins  $\geq 15.5\%$  (Italian law n.169, 1989).



**Figure 3** Average of omega-6 polyunsaturated fatty acids (PUFA n-6), omega-3 polyunsaturated fatty acids (PUFA n-3), total PUFA, n-6/n-3 ratio and total conjugated linoleic acid (in g per 100 g fatty acids methyl esters) of “pasture” Noble milk compared with High-Quality milk (adapted from Lombardi *et al.* (2014)).

supplementation of barley and beet pulp-based energy concentrate did not affect the levels of rumenic acid in the milk fat of grazing cows (Renna *et al.* 2010). The Noble Method supplies low levels of concentrate in animal diet, necessary to meet the metabolic needs of lactating ruminants.

The application of grazing can improve some nutritional indices of butter and cheese products. For example, the increase in fresh grass improved the nutritional value of butter in terms of reducing the atherogenic index, due to the

minor content of lauric (12:0), myristic (14:0) and palmitic (16:0) acid (Couvreur *et al.* 2006). In their review, Shingfield *et al.* (2013) summarised the effect of forage species, conservation method and herbage maturity on the quantity of FAs in the milk. Palmitic acid is among the saturated FAs the most influenced by the diet in the different ruminant species, obtaining reductions of about 30% in grazing animals. Generally, cheese and other dairy products made from milk obtained from ruminants fed with fresh fodder also exhibited a better nutritional composition (Uzun *et al.* 2018; Corazzin *et al.* 2019; Formaggioni *et al.* 2020).

### Minor compounds and health implications

Several studies have dealt with the enhancement of dairy products in tocopherols, carotenoids, phenolic compounds and other antioxidant compounds through the management of ruminant feeding system (La Terra *et al.* 2010; Cabiddu *et al.* 2019; Gutiérrez-Peña *et al.* 2021).

The development of carotenoid-enriched dairy products is an area of study, which is gaining interest. Carotenoids include more than a thousand natural pigments, which are not de novo synthesisable by humans; hence, a diet is the only source of intake (Conboy Stephenson *et al.* 2021).  $\beta$ -carotene acts as provitamin A following enzymatic breakdown during metabolism, resulting in the formation of retinoids in humans (Rodríguez-Concepcion *et al.* 2018). Since carotenoids are fat-soluble compounds, eating them from dairy products could improve bioavailability (Conboy Stephenson *et al.* 2021).

Forage plants allow ruminants to accumulate carotenoids in milk, which will then be transferred to dairy products contributing to their nutritional and sensory properties (Noziere *et al.* 2006). Fresh fodder is the preferred source of



carotenoids for the ruminants' diet. Villar *et al.* (2021) reported significantly higher zeaxanthin and  $\beta$ -cryptoxanthin content in pasture cows' milk. Compared with conventional maize silage-based indoor feeding system, pasture-fed livestock produced higher levels of lutein,  $\beta$ -carotene, vitamins A and E in milk and cheese (Martin *et al.* 2002; Cabiddu *et al.* 2019). Greater amounts of  $\beta$ -carotene were also found in sweet cream butter obtained from pasture-fed cows compared to those fed in stall with maize silage, grass silage and concentrates in an approximately 1:1:2 ratio (5.16 and 2.27 mg kg<sup>-1</sup> of butter, respectively; O'Callaghan *et al.* 2016). The greatest influence was found in ruminants grazing rich-forb and leguminous grasslands. The phenological stage of the cultivar and its technological treatment also affects the nutritional value of forage. The content of carotenes and carotenoids in forages decreases according to the age of plant, the advancement of the phenological stage and the forage conservation method (e.g. ensiling or drying). Considering different cattle breeds (Holstein, Brown Swiss and Modicana cows) and grazing seasons (spring and autumn), it was found that the content of  $\alpha$ -tocopherol and  $\beta$ -carotene in milk was greater in spring (16.2 and 9.7 mg kg<sup>-1</sup> of milk fat, respectively) than in autumn (11.2 and 0.8 mg kg<sup>-1</sup> of milk fat, respectively) with no significant differences among the various breeds (Marino *et al.* 2012). This trend was mainly attributed to the different botanical composition and quality of the pasture depending on the grazing season.

In milk of goats fed on polyphyte pasture compared to those fed in the stable with a ration based on silage and hay, a content of about 35% higher total phenolic compounds has been reported (Chávez-Servín *et al.* 2018). Moreover, such an increment was retained in unpasteurised milk, whey and cheese derivatives. Similar results were published in a previous study, which reported a higher content of total phenolic compounds and antioxidant activity in unpasteurised goat's milk soft cheese obtained from grazing animals (Hilario *et al.* 2010). The higher quantity of these compounds with antioxidant properties also improved the cheese oxidative stability, which is desirable since in dairy products there is a higher quantity of unsaturated FAs (Martin *et al.* 2002; Cabiddu *et al.* 2019). De Renobales *et al.* (2012) reported an increase in the antioxidant activity of sheep milk with the inclusion of polyphyte pasture in an indoor diet. The Trolox equivalent antioxidant capacity value of milk increased by about 7% compared with the nonpasture diet. The highest amount of some antioxidants, such as tocopherols,  $\beta$ -carotene, lutein and zeaxanthin in milk, can also slow down the oxidation of proteins, delaying the formation of dityrosine (Havemose *et al.* 2004). Hence, these antioxidant compounds could also reduce the susceptibility to oxidation of dairy products richer in unsaturated FAs to the benefit of the sensory properties of the product and consumers' health.

## Sensory properties and consumer's choice implications

Although health and ethical aspects, such as animal welfare and the environmental impact of dairy production, are part of the recent concerns of consumers and producers, sensory properties remain an important factor, which influence consumers' purchasing choices and product recognition (Kühn *et al.* 2006; Delgado-Pertíñez and Horcada 2021). This section discusses how the differences among the animal diet affect the physical, flavour and sensory properties of the dairy products and how they are accepted by consumers.

Lombardi *et al.* (2014) used two different untrained panels of about 120 people for sensory tests in order to verify the ability of consumers to perceive the differences between a Piemonte Noble milk and conventional milk available on the market. For this test, a Noble pasteurised milk and samples of a High-Quality milk (commercial milk sold in Piedmont region) were compared. The Noble milk was found to have a higher intensity in taste, freshness and flavour and the 88% of the testers correctly identified it. Khanal *et al.* (2005) used a blinded sensory panel to assess the consumer acceptability of milk and cheddar cheese enriched with ruminic acid, employing an open panel of about 80 consumers, who met in three different sessions. The content of ruminic acid ranged from 0.52 to 1.69 g/100 g of fatty acid methyl esters in milk and from 0.47 to 1.46 g/100 g of fatty acid methyl esters in cheese depending on diet (preserved forages and concentrates in 1:1 ratio versus pasture, respectively). The results showed no significant difference in terms of acceptability for consumption of dairy products enriched with ruminic acid compared with conventional ones. In this study, it has also been reported that consumers would pay a higher price for milk, which offers greater nutritional benefits. A proper communication is needed on the benefits that these products offer in terms of human, animal and environmental health as well as a chain traceability (Britt *et al.* 2018; Stampa *et al.* 2020).

Raw Noble milk from grazing goats scored higher for 'grassy' and 'sweet aromatic' sensory descriptors compared with raw milk from indoor hay-fed goats (Balivo *et al.* 2023). These differences were also found instrumentally by electronic nose. Romanzin *et al.* (2013) differentiated the mountain PDO Montasio cheese in terms of physical properties, comparing Simmental cows' milk obtained from a mountain pasture-fed system or an indoor hay-based system. Pasture cheeses exhibited higher values of instrumental texture parameters and greater yellowness indicated by a more positive b\* value. Similarly, O'Callaghan *et al.* (2016) found a higher value of the b\* colour index (yellowness) in butter obtained from pasture milk. The colour was found to be the parameter that allowed a rapid discrimination of Noble cows' milk from conventional cows' milk (Renna *et al.* 2014). It was reported that cheeses from cows grazing a mountain pasture were less

elastic and more resistant to deformation than those one obtained from cows fed on a valley pasture (Bugaud *et al.* 2001b). This difference was attributed to the lower amount of saturated FAs and the greater proteolytic activity of plasmin in the mountain group. De Renobales *et al.* (2012) reported a resistance to compression at room temperature of sheep milk curd fed on pasture of 83.56 g compared with the value of 91.69 g for sheep curd fed in stables with no pasture. The modification of milk fatty acid profile, caused by the pasture-based diet compared with an indoor diet based on maize silage and concentrates, also showed differences in the physical properties of sweet cream butter (O'Callaghan *et al.* 2016). Butter obtained from pasture milk had lower hardness values at room temperature and a lower onset crystallisation temperature (about 1.5 °C lower), due to the highest quantity of unsaturated FAs and consequently a lower amount of saturated FAs, such as myristic and palmitic acid. In fact, Couvreur *et al.* (2006) reported that the modifications in the composition of milk fat due to the animal diet were responsible for the decrease in final melting temperature and solid fat content in butter, resulting in a lower firmness in the mouth. From their findings, the authors have also defined the spreadability index as a ratio between palmitic (SFA) and oleic acid (MUFA).

In a recent study, Manzocchi *et al.* (2021) evaluated the sensory differences between milk and uncooked cheese (Cantal-type cheese) from cows fed with forage belonging to the same grassland but integrated as fresh or preserved (hay and silage) both indoors and outdoors (grazing). Fresh grass-fed cows' milk cheese (both grazed and in the stable) was perceived by panellists creamier, yellower and more intense in the barnyard and dry fruit/nuts odour. On the contrary, cheese derived from preserved forage exhibited a more lactic odour and a lower colour intensity. Also in this case, the higher creaminess was attributed to the higher quantity of unsaturated FAs in milk fat due to green forage rather than preserved (Villeneuve *et al.* 2013), which reduced the melting temperature of milk fat.

A grass-based diet increased the yellow colour of the cheese (Faulkner *et al.* 2018) due to the higher level of  $\beta$ -carotene in milk. Among ruminants, only bovines accumulate high amounts of carotenoids, due to the lower synthesis efficiency of vitamin A (Noziere *et al.* 2006). Therefore, the differences in yellowness of dairy products obtained from grazing-fed animals may not be evident in other ruminant species.

Other important attributes, reported in the literature and linked to animal fed with fresh forage, are volatile organic compounds (VOCs), which are responsible for the aroma of dairy products. The transfer of volatile compounds from diet to milk can occur through the respiratory tract (inhalation) or ingestion. VOCs in milk could indirectly arise from some non-volatile precursors induced by the diet such as carotenoids,

PUFA and proteins (Kilcawley *et al.* 2018; Clarke *et al.* 2020). Thus, VOCs can present several pathways of formation. For example, p-cresol in milk can derive from the metabolism of isoflavones in feed (Clarke *et al.* 2020) and the catabolism of unsaturated FAs leads to the formation of straight-chain aldehydes and alcohols (Collins *et al.* 2003). Linear aldehydes have been associated with 'green grass-like' odour (Moio *et al.* 1993), as well as some primary and secondary alcohols (Curioni and Bosset 2002). Toluene and p-cresol are among the main VOCs proposed in the literature as chemical markers of pasture dairy products. They can result from  $\beta$ -carotene, which is greater in pasture milk (O'Callaghan *et al.* 2017; Faulkner *et al.* 2018). p-Cresol can also derive from the metabolism of aromatic amino acids, and in one study, a greater activity of plasmin was found in milk from mountain pasture cows (Bugaud *et al.* 2001a).

Using olfactometry techniques, Clarke *et al.* (2022) compared the aroma of raw bovine milk obtained from cows fed outdoors on perennial ryegrass versus milk from cows fed indoors on TMR, which comprised a mixture of grass silage, maize silage and concentrates. The authors reported that the abundance of thirteen VOCs varied by diet and 30% of VOCs affected the aroma perception of raw milk samples. The panel perceived odours such as roasted, fresh, floral, sweet and cheesy for grazing milk associated with aroma-active volatiles such as hexanal, hydrocinnamic acid and 2-phenylethanol, and pungent, cabbage, buttery and caramel odours associated with 2,3-butanedione (diacetyl) and  $\gamma$ -nonalactone for indoor milk. Several differences were found, for example, esters and alcohols were reported in greater level in pasture cheeses compared with silage cheeses leading to a greater intensity of herbaceous, fruity and sweet odours (Formaggioni *et al.* 2020). Some sesquiterpenes have been identified in dairy products from pasture-fed animals (Renna *et al.* 2012a; Valdivielso *et al.* 2017). Two terpenes,  $\alpha$ -terpineol and  $\beta$ -caryophyllene, have only been identified in goat's milk and cheese from goats bred in native pasture (Sant'Ana *et al.* 2019). Similarly, in the Noble milk produced by goats raised on pasture compared to that of goats raised in stable, Balivo *et al.* (2023) reported that terpene compounds were the chemical class that qualitatively differentiates 'pasture' Noble milk. Mono and sesquiterpenes are volatile compounds transferred directly from plants grazed by ruminants to milk, by both the oral and respiratory route (Székelyhidi *et al.* 2020; Faccia *et al.* 2022). Although terpenes are more abundant in cheeses derived from mountain and pasture milk (e.g. Alpine regions; Lombardi *et al.* 2008; Altomonte *et al.* 2019; Moran *et al.* 2019), their contribution to the odour of dairy products is still controversial because they may contribute to the aroma of cheese with floral, woody and spicy notes. Cifuni *et al.* (2022) reported a variation of milk terpene profile according to changes in pastureland altitude (from 700 m to 2200 m above sea level). Depending on the pasture altitude, the authors also obtained a correct classification of 98% of the milk samples

using C14:0 iso, C17:0 anteiso, C18:1 trans-11 (VA) and C18:2 trans-9, trans-12 FAs as discriminant variables in leave-one-out cross-validation statistical analysis.

The profile of VOCs can provide a fingerprint of milk of animals fed with different diets, also in relation to the botanical composition of the pasture and its location. Such differences could help discriminate and certify the authenticity of Noble milk and, hence, subsequently dairy products. By changing the intake of different forage species in goats' diet, a variation of the terpene profile in milk was obtained (Fedele *et al.* 2004). It was reported the content of terpenes varied qualitatively and quantitatively in different pastures (Peiretti *et al.* 2020).

Although a distinctive aroma for milk obtained from animals fed with fresh grass is reported, the link with specific volatile compounds has not yet been well understood. Khanal *et al.* (2005) reported that trained panellists did not find differences between the sensory attributes in the cheddar cheeses (cows' milk fed with pasture versus cows' milk fed with maize silage and hay), except for the intensity of the barn descriptor, which scored greater in pasture milk. By contrast, O'Callaghan *et al.* (2016) reported no significant differences in barn odour in butter obtained from cows fed on a pasture diet versus a stall diet based on maize silage, grass silage and concentrates in an approximately 1:1:2 ratio. The same authors reported that some ketones (2-butanone) were more present in dairy butters obtained from milk of indoor-fed cows, while some alcohols (1-pentanol), terpenes ( $\beta$ -pinene) and toluene were significantly more abundant in butter obtained from milk of grazing cows (O'Callaghan *et al.* 2016). In another study on milk and mozzarella di Bufala Campana DOP cheese, it has been found that the use of fresh forage (sorghum) enhances the grassy notes of milk and induces few changes in the VOC profile of Mozzarella cheese, but nonetheless detectable by sensory analysis (Sacchi *et al.* 2020). In this case, it was found a higher content of toluene and some aldehydes in the milk obtained from buffaloes fed with fresh fodder.

Summarising the differences in milk and dairy product sensory properties due to animals fed on pasture, it can be reported that the greater presence of carotenoids influences the colour of bovine dairy products. The greater biodiversity and phenology of pasture forage species influences the profile of volatile compounds of raw milk, such as the quantity and type of terpene compounds while the higher presence of unsaturated FAs affects the physical and thermal properties of dairy products. Such differences could have important implications for future research on Noble milk and dairy products, like ensuring authenticity to protect consumers from the risk of fraud.

## CONCLUSION

The optimisation of animal diet through well-defined feeding and breeding strategies, as regulated by the Noble Method,

represents an interesting opportunity for the dairy sector. The animal feeding strategy specified by the Noble Method is based on approaches aimed at favouring optimal nutritional properties of dairy products rather than maximising the yield, taking care of animal welfare and environmental sustainability. Such practices, especially if regulated by a Production Specification, have a good reputation among consumers of dairy products. The greater amount of some compounds in dairy products such as PUFA, VA, CLA, carotenoids and phenolic compounds would improve the health of the dairy consumer. The healthier fatty acid profile and the reduction of medium-chain saturated FAs improve some lipid quality indices such as the thrombogenic index and atherogenic index. Carotenoids and polyphenols are responsible for a greater antioxidant activity.

Sensory differences due to the animal diet have also been reported in terms of colour, texture and flavour in milk and dairy products. Such differences could help in the authentication of Noble milk and dairy product to protect producers and consumers from the risk of fraud.

However, despite the promising results of such studies, the Noble milk and dairy products literature is still lacking and needs to be extended.

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## AUTHOR CONTRIBUTIONS

**Andrea Balivo:** Data curation; visualization; writing – original draft; writing – review and editing. **Raffaele Sacchi:** Funding acquisition; project administration; supervision. **Alessandro Genovese:** Conceptualization; supervision; visualization; writing – review and editing.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

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