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VOLATILE ORGANIC COMPOUNDS IN CHEESE PRODUCTION CHAIN (VOCHEESE)

Direttore Della Scuola: Prof.ssa Viviana Corich

Supervisore: Prof. Giovanni Bittante

Dottorando: Dott. Matteo Bergamaschi

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RIASSUNTO

Negli ultimi anni, il consumatore è diventato sempre più sensibile agli aspetti qualitativi degli alimenti, i quali sono fortemente influenzati dalle caratteristiche sensoriali come l'aroma. Diversi lavori scientifici hanno dimostrato che i composti volatili (**VOCs**) rilasciati dall'alimento sono correlati con il suo aroma e possono essere considerati come traccianti delle filiere alimentari.

Oggi, l'analisi dei VOCs richiede strumenti rapidi, sensibili, non invasivi e che abbiano bisogno dell'impiego di pochi solventi durante la preparazione del campione. E' stato dimostrato che i VOCs possono essere estratti, misurati e identificati con la Gas Cromatografia di Massa (**GC-MS**) senza pre-concentrazioni o pre-trattamenti dell'alimento da analizzare.

Gli obiettivi principali della tesi di dottorato erano di studiare la presenza di composti volatili nei prodotti lattiero-caseari. Più precisamente, questo studio aveva come obiettivi di i) qualificare e quantificare i VOCs nei prodotti lattiero-caseari, ii) esaminare la loro formazione e iii) integrare le conoscenze acquisite su questi composti attraverso tutta la filiera di produzione dalla materia prima fino al prodotto finito. Inoltre, analisi statistiche sono state utilizzate per collegare i VOCs con la caratterizzazione genetica degli animali, il sistema di allevamento e le caratteristiche individuali delle vacche (es. stadio di lattazione, ordine di parto e produzione giornaliera di latte).

L'identificazione e la quantificazione dei VOCs sono state fatte utilizzando tecniche analitiche precise, veloci e non invasive (Solid Phase Micro Extraction/Gas Chromatography-Mass Spectrometry **SPME/GC-MS** and Proton Transfer Reaction-Time of Flight-Mass Spectrometry **PTR-ToF-MS**). Per rispondere agli obiettivi generali della tesi, l'attività di ricerca è stata divisa in cinque parti connesse tra di loro.

L’obiettivo nel primo capitolo era di studiare i composti volatili presenti nello spazio di testa di campioni di formaggio. Per questo scopo, sono stati analizzati 150 formaggi stagionati per due mesi. I formaggi sono stati prodotti utilizzando una metodica di caseificazione individuale usando latte individuale di vacche di razza Bruna. Gli animali sono stati allevati in 30 aziende appartenenti a diversi sistemi di allevamento, da tradizionale (tipico della realtà montana) a moderno. In questo studio sono stati identificati 55 VOCs per ogni formaggio, classificati in diverse famiglie chimiche: acidi grassi, esteri, alcoli, aldeidi, chetoni, lattoni, terpeni e pirazine. Dai risultati emerge che il sistema di allevamento e le caratteristiche individuali delle vacche (stadio di lattazione, ordine di parto e produzione giornaliera di latte) influenzano i composti volatili. Inoltre, per testare la riproducibilità dello strumento e della metodica di caseificazione; la data di analisi cromatografica, l’ordine d’iniezione del campione nello strumento (GC), e la caldaia di caseificazione erano inclusi nel modello statistico. In molti casi, questi fattori analitici/strumentali non influenzano la quantità di VOCs rilasciata dai formaggi.

Nel secondo capitolo, il potenziale di una nuova tecnica analitica (PTR-ToF-MS) è stato approfondito per studiare, su larga scala, le caratteristiche qualitative del formaggio. Il PTR-ToF-MS dal punto di vista analitico, permette un’iniezione diretta del campione senza estrazione o pre-concentrazione, ha un breve tempo di analisi (solo pochi secondi per campione) e grande sensibilità consentendo di monitorare in tempo reale l’evoluzione dei composti volatili. L’analisi produce uno spettro molto dettagliato che può essere utile per la caratterizzazione delle qualità e della tipicità dell’alimento. In particolare, è stata analizzata l’impronta aromatica di 1,075 formaggi prodotti utilizzando latte individuale di vacche di razza Bruna allevate in 72 aziende appartenenti a diversi sistemi di allevamento. L’impronta aromatica (spettro) era caratterizzata da più di 600 picchi (variabili) per ogni formaggio. Gli spettri sono stati analizzati e dopo la rimozione degli ioni interferenti e del rumore di fondo è stato selezionato un data set costituito da 240 picchi per ogni formaggio. In seguito, basandosi sui risultati del primo contributo e sulla letteratura sono stati identificati i picchi più importanti (61) in termini quantitativi e qualitativi. Per sintetizzare la quantità di informazioni ovvero estrarre delle componenti principali (**PC**) è stata fatta un'analisi multivariata (**PCA**) a partire dai 240 picchi spettrometrici. In seguito, le PCs sono state caratterizzate sulla base delle loro correlazioni con i 240 picchi spettrometrici. Sono stati analizzati gli effetti del sistema di allevamento, dell'azienda entro sistema di allevamento, le caratteristiche individuali delle vacche (stadio di lattazione, ordine di parto e produzione di latte), e caldaia di caseificazione sulle PCs e sui 240 picchi. Dai risultati emerge che il sistema di allevamento è correlato con le PC e 57 picchi, specialmente quando le aziende come tecnica di alimentazione utilizzano il carro miscelatore (TMR) con e senza insilati nella dieta. Considerando le caratteristiche individuali delle vacche, l'effetto più significativo è lo stadio di lattazione (139 picchi), seguito dalla produzione di latte e dall'ordine di parto, con 31 e 21 picchi, rispettivamente. Infine, la caldaia di caseificazione è un effetto spesso non significativo, confermando la buona riproducibilità della micro-caseificazione utilizzata anche per lo studio di aspetti qualitativi del formaggio.

Nel terzo capitolo è stato studiato l'effetto della genetica dell'animale sui composti volatili dei formaggi. A tale scopo, sono state analizzate le componenti principali (estratte come discusso sopra nel secondo contributo) e i 240 picchi spettrometrici (PTR-ToF-MS) utilizzando un modello animale con un approccio Baesiano. Dai risultati emerge in media un'ereditabilità (h^2) del 7 % per le componenti principali, la quale è simile all'h^2 trovata per le cellule somatiche e leggermente più bassa di quella del contenuto di grasso nel latte e della produzione giornaliera di latte stimate in precedenza sugli stessi animali. E' interessante osservare che solo una piccola quantità di picchi ha una bassa h^2 (<7%). La maggior parte di essi presenta valori simili a quelli trovati per le PCs, mentre 40 picchi presentano ereditabilità simile a quella trovata per la produzione giornaliera di latte e ad altre caratteristiche qualitative del latte. La variabilità attribuita all'azienda è risultata diversa per le PCs. Questi risultati dimostrano che esiste un'interessante variabilità genetica di alcuni VOCs che potrebbe essere potenzialmente utilizzata nei programmi di miglioramento genetico.

L'obiettivo nel quarto capitolo era di studiare l'effetto della transumanza sulle caratteristiche qualitative di prodotti lattiero-caseari. Vista la grande mole di dati, questo contributo è stato diviso in due parti tra loro connesse. Nella prima parte è stata studiata l'evoluzione della qualità del latte e del formaggio, mentre nella seconda parte è stata analizzata l'evoluzione dei composti volatili dei prodotti lattiero-caseari nel processo di caseificazione.

Nella prima parte, sono state analizzate le proprietà fisiche, chimiche e tecnologiche di 11 prodotti lattiero-caseari raccolti durante la transumanza al pascolo Alpino (Malga) di vacche da latte. E' risaputo che i prodotti ottenuti durante il periodo di alpeggio possono avere un valore aggiunto dovuto alle elevate proprietà nutrizionali, salutistiche e aromatiche. Per approfondire le conoscenze finora acquisite, è stata fatta questa prova in cui è stato utilizzato il latte di massa prodotto da 148 vacche allevate giorno e notte al pascolo (1,860 m s.l.m.). Durante l'esperimento, sono state fatte 7 caseificazioni seguendo tecniche tradizionali, una ogni 2 settimane, utilizzando il latte prodotto durante la transumanza (da giugno a settembre). Sono stati raccolti per ogni caseificazione: il latte della mungitura della sera (giorno prima della caseificazione), lo stesso latte il mattino successivo (dopo il processo di scrematura naturale), la panna di affioramento, il latte della mungitura del mattino, il latte in caldaia ottenuta dalla miscela tra il latte scremato della mungitura della sera con il latte della mungitura del mattino, la cagliata, il siero, la ricotta ottenuta dal siero e il residuo della lavorazione ossia la scotta. Inoltre, la cagliata è stata usata per produrre formaggi di "Malga" che sono stati stagionati per 6 e 12 mesi. Le caratteristiche chimico-fisiche sono state misurate con una tecnologia a infrarosso. I risultati dimostrano una variazione della produzione giornaliera e composizione chimica del latte, resa in formaggio e recupero/o perdita di nutrienti nel processo di caseificazione tradizionale. In particolare, si è osservata una riduzione della produzione giornaliera di latte, grasso, proteine e lattosio del latte durante la transumanza estiva. Tuttavia, si è anche osservato un effetto positivo sulla produzione e la composizione chimica del latte del ritorno delle vacche nelle aziende di fondo valle alla fine della stagione dell'alpeggio. La resa media di formaggio in questo lavoro è risultata del 14.2%, mentre i recuperi di grasso, proteine, solidi totali ed energia sono del 85.1%, 77.8%, 49.4% e 58.1%, rispettivamente. Questi risultati sono in linea con quelli trovato in letteratura. Nella seconda parte di questo contributo, è stato misurato il contenuto di composti volatili nello spazio di testa dei campioni con la tecnica SPME/GC-MS. Dopo l'analisi, sono stati identificati 49 VOCs appartenenti alle famiglie chimiche degli alcoli, aldeidi, acidi grassi, chetoni, esteri, lattoni, terpeni e composti solforati e fenolici. Inoltre, è stata studiata l'evoluzione dei VOCs e delle loro famiglie chimiche attraverso i processi di caseificazione, di produzione della ricotta e di stagionatura del formaggio. Il confronto tra la concentrazione dei VOCs dei 4 tipi di latte (intero e scremato della sera, intero del mattino, caldaia) ha dimostrato che il processo di scrematura influenza la concentrazione di metà dei composti volatili analizzati, seguito dall'effetto della mungitura (intero della sera vs. intero del mattino) e dall'effetto del mescolamento (latte scremato della sera mescolato in parti uguali con il latte del mattino). In generale, la panna, rispetto a cagliata e ricotta, ha un maggiore contenuto di acidi grassi, terpeni e composti solforati. Inoltre, la ricotta rispetto alla cagliata ha un'elevata concentrazione di VOC, probabilmente dovuta alla maggiore temperatura utilizzata durante il processo di produzione. L'effetto del progressivo depauperamento di nutriente del latte è stato studiato attraverso il confronto tra latte in caldaia, siero e scotta. Sebbene il latte abbia un maggiore contenuto di nutrienti, il siero e la scotta hanno una maggiore concentrazione di VOC ad eccezione delle famiglie chimiche degli esteri, terpeni, composti solforati e fenolici.

Infine, l’effetto della maturazione è stato valutato attraverso il confronto tra le quantità di VOC della cagliata e dei formaggi stagionati (6 e 12 mesi). Il rilascio dei composti volatili incrementa con l’aumento del periodo di maturazione probabilmente dovuto a una maggiore attività enzimatica e microbiologica nel formaggio.

In conclusione, le tecniche analitiche di spettrometria di massa utilizzate in questo lavoro (SPME/GC-MS e PTR-ToF-MS) hanno permesso di caratterizzare i composti volatili dei prodotti lattiero-caseari in maniera efficiente. Il sistema di allevamento, le caratteristiche individuali delle vacche hanno influenzato l’impronta aromatica di formaggi individuali stagionati. In particolare, riguardo alle caratteristiche individuali degli animali il principale effetto era lo stadio di lattazione seguito da ordine di parto e produzione giornaliera di latte.

Sulla base dei fenotipi raccolti in questo lavoro è stato possibile effettuare un’analisi genetica, la quale ha dimostrato l’esistenza di un’interessante variabilità genetica connessa ai composti volatili del formaggio che potrebbe essere utile per una selezione (in)diretta delle vacche da latte sulla base di aspetti qualitativi in programmi di miglioramento genetico. Tuttavia sono necessarie altre ricerche in quest’area per esempio, nell’era della genomica, sarebbe interessante associare qualche regione specifica del genoma ai composti volatili.

L’evoluzione dei composti volatile attraverso la filiera di produzione dipende da specifici aspetti tecnologici, come l’affioramento della panna, la temperatura di coagulazione e il periodo di stagionatura. Il monitoraggio dell’impronta aromatica permette di ottenere prodotti lattiero-caseari con delle specifiche caratteristiche organolettiche utili a differenziare il prodotto sul mercato e a migliorare l’efficienza dell’intera filiera produttiva sulla base di aspetti qualitativi.

ASTRACT

In recent years, consumers have become increasingly interested in the quality aspects of food. Food quality, in turn, is strongly related to the sensory characteristics such as the flavor. Several scientific studies have shown that the Volatile Organic Compounds (VOCs) released by the food are related to the flavor and can be considered as assistive markers in the production chain.

Today, the analysis of VOCs requires fast, non-invasive, and solvent free devices. It has been shown that the VOCs can be extracted, identified, and measured with a Gas Chromatography-Mass Spectrometry (GC-MS) without any pre-concentration or pre-treatment of the food.

The main objective of this PhD thesis was to investigate the presence of volatile compounds in dairy products. More precisely, this study aimed in i) qualifying and quantifying VOCs in dairy products, ii) examining their formation and iii) integrating knowledge on VOCs by tracking their release during the whole production process from the raw materials till the final dairy product. In addition, statistical analysis was applied to link VOCs with the genetic characterization of animals, dairy system and individual cow-factors (e.g. stage of lactation, order of parity and milk yield). The identification and quantification of VOCs were performed using fast and non-invasive analytical approaches (Solid Phase Micro Extraction/Gas Chromatography-Mass Spectrometry SPME/GC-MS and Proton Transfer Reaction-Time of Flight-Mass Spectrometry PTR-ToF-MS) that can monitor the evolution of VOCs. To achieve the overall goal, the research was partitioned in four interrelated subparts as described below.

The aim of the first chapter was to study the VOCs presence in the headspace of cheese. To this purpose, 150 cheeses ripened for two months were used. The cheeses were obtained through an individual model cheese-making approach using milk from individual Brown Swiss cows. Animals reared in 30 herds belonging to different dairy systems, from traditional

(typical of the mountainous area) to modern ones. The study identified 55 VOCs classified in the chemical families of free fatty acids, esters, alcohols, aldehydes, ketones, lactones, terpenes, and pyrazines. We found that dairy system and individual cow characteristics (lactation stage, order of parity and daily milk yield) influenced the volatile compounds. In order, to test the instrument reproducibility and the model cheese-making procedure; data of GC analysis, order of injection of the sample into instrument, and vat were included in the statistical model. In many cases, these analytical factors did not affect the amount of VOCs released by cheese.

In the second chapter, the potential of a new spectrometric technique (PTR-ToF-MS) was investigated to study cheese quality traits on a large scale. The PTR-ToF-MS allows direct injection of the sample headspace without extraction or pre-concentration steps, has a shorter analysis time (only a few seconds per sample) and greater sensitivity that permit to monitor on-line the evolution of volatile compounds. The resulting spectral information can provide a very detailed description of samples, which is useful for characterizing food quality and typicality. In particular, we analyzed the volatile fingerprint of 1,075 model cheeses produced using individual milk of Brown Swiss cows reared in 72 herds of different dairy systems. The output of PTR (spectrum) was characterized by more than 600 spectrometric peaks (variables). After removing interfering ions and background noise a set of 240 peaks was selected. Further, based on the results of the first contribution and literature, 61 peaks were identified. These peaks represent the major part of the cheese flavor. To summarize the amount of information, a multivariate analysis (PCA) was applied associating principal components (PC) with the 240 spectrometric peaks. Following, we tried to characterize the PCs through the correlations between PCs and the spectrometric peaks. The effects of dairy system, herd within dairy system, individual cows characteristics (lactation stage, order of parity and milk yield), and vat used for the cheese-making on the PCs and on the 240 peaks were analyzed. Dairy system was correlated with PC and 57 spectrometric peaks, especially when the herds were using Total Mixer Ration (TMR) as feeding technique, including or not maize silage in the diets. Regarding the individual animal characteristics, the most significant effect was the stage of lactation (139 peaks), followed by milk yield and parity, with 31 and 21 peaks, respectively. Finally, the vat used for the cheese-making was not found to be significant, confirming the good reproducibility of the model cheese-making procedure used to study cheese quality aspects.

In the third chapter, the effect of cows' genetics to the VOCs of ripened cheeses was assessed. Principal components and the 240 spectrometric peaks (as described above in the second contribution) were used fitting an animal model in a Bayesian framework. On average, heritability (h^2) of 7% for PCs was found, which is similar to h^2 of somatic cell count and much lower than the h^2 of milk fat content and daily milk yield. It is interesting to note that only a small proportion of peaks showed very low h^2 (<7%). The major part of them showed values similar to those found for PCs, while forty peaks presented heritability similar to that of milk yield and other milk quality traits. The variability attributed to the herd was different for the various PC. Results suggest a potential of improvement for several cheese VOCs through genetic selection in dairy cow breeding programs.

The aim of the fourth chapter was to study the effect of summer transhumance on the quality traits of dairy products. Due to the extended work, this contribution was further splitted into two parts. In the first part, the evolution of milk and cheese quality characteristics were studied, while in the second part the evolution of VOC content of dairy products was analyzed.

For the first part, chemical characteristics and technological properties of 11 dairy products obtained during summer transhumance of cows to Alpine pastures (Malga) were analyzed.

Dairy products obtained throughout this period are known to give origin to high-value, healthier products, and extra tasty,. Bulk milk from 148 dairy cows reared day and night on Alpine pasture (1,860 m a.s.l.) was used. We performed 7 experimental cheese-making according to traditional mountain techniques, one every two weeks, using milk produced during the summer transhumance (from June to September). For each cheese-making we collected: milk from the evening milking (day before the cheese-making), the same milk the following morning (after natural creaming), the cream separated, the whole milk from the morning milking, the milk in vat obtained mixing the creamed evening milk with the whole morning milk, the fresh curd, the whey, the ricotta obtained from whey, and the residual scotta. Moreover, the curd was used to produce typical "Malga" cheese that was ripened for 6 and 12 months. The chemical characteristics were measured with infrared technology. Results highlighted variation in milk yield, milk chemical composition, cheese yield and curd recoveries and/or loss of nutrients in the traditional cheese-making. In particular, a reduction of milk yield, fat, protein and lactose contents of milk during summer transhumance was observed. Nevertheless, the return to lowland farming systems of the cows at the end of grazing season, positively affected milk yield and milk chemical composition. The average of cheese yield was 14.2%, while recoveries of fat, protein, total solids and energy were 85.1%, 77.8%, 49.4% and 58.1%, respectively. These results were in accordance to those found in the literature.

For the second part of this chapter, the VOCs content of sample headspace was measured through SPME/GC-MS. Forth nine VOCs belonging to the chemical families of alcohols, aldehydes, free fatty acids, ketones, esters, lactones, terpenes, phenolic, and sulphur compounds were detected. In addition, the evolution of VOCs and their chemical family across the cheese- and ricotta-making processing as well as during the cheese ripening period was tracked. The comparison between VOCs concentration of 4 types of milk (whole evening, creaming milk, whole morning, milk in vat) showed that the creaming process significantly affected about half of all the volatile organic compounds analyzed, followed by the effects of milking (evening milking vs. morning milking) and the mixing (creamed milk mixed with whole morning milk). In general, the cream, in contrast to curd and ricotta, showed higher content of free fatty acids, sulphurs and terpenes compounds. Moreover, in ricotta a higher VOC concentration was observed compared to the curd, probably due to the high temperature required during the ricotta process. The effect of the progressive nutrient depletion of milk was investigated by contrasts between VOC concentration of milk in the vat, whey, and scotta. Although milk contains a greater amount of nutrients, whey and scotta have shown a higher concentration of VOCs with the exceptions of esters, sulphurs, terpenes and phenolic compounds. Finally, the effect of ripening was tested by comparing the quantity of VOCs of curd and of aged cheeses (6 and 12 months). The release of volatile compounds increased with increasing ripening period in relation with the enzymatic and microbiological activity of cheese.

In summary, the spectrometric techniques (SPME/GC-MS and PTR-ToF-MS) used in this work demonstrated to be very efficient to characterize the volatile organic compounds of dairy products. The dairy system, and cow related factors affected the volatile fingerprint of ripened cheeses. Particularly, concerning the individual animal source of variation, lactation stage was the most important effect followed by the cow’s parity and the milk yield.

On the basis of phenotypes used in this work, the traits collected offered the potential for a genetic analysis to be carried out. The genetic analysis demonstrated the existence of an exploitable genetic variability of the volatile profile of cheese that might be useful for an (in)direct selection of dairy cows for cheese quality traits in breeding programs. Nevertheless, further research is needed in this area. In the era of genomics for e.g., it might be interesting to associate genomic regions to specific VOCs. This information might be useful for genomic breeding programs.

The evolution of volatile compounds across the production chain depends on specific technological aspects, such as the process of natural creaming, the temperature of coagulation, and the ripening period. The monitoring of volatile fingerprint permits to obtain dairy products with specific organoleptic characteristics useful to differentiate them on the market and to improve the supply chain efficiency on the basis of quality aspects.

GENERAL INTRODUCTION

Nowadays, there is an increasing demand of dairy products focusing in specific and high quality characteristics. Therefore, the evaluation and perception of dairy products are crucial points to determine selection and acceptance of food by consumers (Drake, 2006; Childs and Drake, 2009).

The instrumental measurement of sensory proprieties is a useful quantitative technique for assessing detailed information about quality evaluation of cheeses that can be used to differentiate them from other similar products (Tunick, 2014).

The quality of milk has a major impact on the quality of the resultant cheese, which depends on many factors that can be mainly grouped in two headings related to i) the animal as genetic component, specie, and breed (Martin et al., 2009; Bittante et al., 2015); ii) the physiological factors of the animal, such as its health, the stage of lactation, the order of parity and milk yield (Coulon et al., 2004; Cipolat-Gotet et al., 2013). Moreover, there are factors connected to the dairy system, the diet (e.g. hay, silage, compound feed) of the animals and the general herd management (Sturaro et al., 2009 and 2013).

A number of studies elucidated the relationship between milk chemical parameters and different quality aspects of cheese like sensory traits (Coulon et al., 2004). For example, several Protected Designation of Origin (PDO) cheeses are produced with an added-value chain that could be useful to help the farmers to make appropriate marketing decisions (Sturaro et al., 2013; Bovolenta et al., 2014). These products are defined by specific sensory properties which are related to a region of production, raw materials or traditional procedures of production (Bittante et al., 2011; Ojeda et al., 2015). Promoting typical products with a distinct quality may be an appropriate strategy for generating wealth and preventing the abandoning of mountain farms, which are normally less competitive and have high production costs due to unfavourable environment conditions (Sturaro et al., 2009 and 2013).

Generally, all studies conducted on quality evaluation of cheese have been performed on cheese produced by bulk milk (industrial level) and did not take into account sources of variation linked to production condition at an individual level. The reason of this is the intensive labour required for the collection of milk and the individual cheese-making procedure, as well as for the detection of volatile organic compounds (VOCs) analysis correlated to sensory traits.

To solve these problems many laboratory cheese-making procedures have been proposed and used, ranging from very simple protocols to techniques that simulate the whole industrial process of cheese-making. However, experimental cheese-making procedures also face some limitations: i) they are expensive, ii) they are time-consuming, iii) they allow for only a few replicates per day. Furthermore, most of the cheese-making studies that have been reported up to day were based on bulk milk due to practical difficulties in producing a high number of cheeses from individual milk samples. A study carry out at the individual level requires a high number of observations (animals) which implies a large number of cheeses to be analysed. Nevertheless, studies at the individual level offer the possibility to investigate the relationship between milk, cheese quality and individual sources of variations link with the animal (genetic or productive traits) or environmental factors of the production (dairy system or herd). Moreover, the individual cheese-making technology offers some advantages. For instance, the use of small quantities of milk, reduced cost and experimental time and a high number of treatments or replications per day.

The complex mixtures of VOCs released by cheese plays a key role in sensory perception and define aroma and flavor characteristics typical of each cheese variety. The complex volatile profile, its origin, its evolution in time together with the consumer preferences could be further used to improve the cheese quality. In general, it has been observed that the VOC reflects the odor and aroma of the cheeses (Izco and Torre, 2000; Agabriel et al., 2004).

The perception of the aroma and flavor of cheese is a complex physiological and psychological process that is influenced by many factors (Marilley and Casey, 2004; Le Berre et al., 2008). It is well known that perceived flavor is the result of a mixture of volatile molecules, while each one of those molecules individually do not reflect the overall odor (Le Berre et al., 2008). The VOCs are produced in different steps of production chain (Curioni and Bosset, 2002; Marilley and Casey, 2004). For example, indigenous microflora of milk, rennet, starter cultures, secondary flora and pasteurization were demonstrated to modify the quantity of volatile molecules released by cheeses (Beuvier et al., 1997; Fatma et al., 2013). Lawlor et al. (2003) found that changing the rate of acidification in the vat or changing the ripening conditions (temperature) when making cheeses influenced their flavors. The flavor of fresh dairy products is mainly the result of the activity of starter bacteria largely due to diacetyl and acetaldehyde production (Carunchia-Whetstine et al., 2006). Flavor variability has also been identified in whey products, both of intra and inter manufacture procedures (Carunchia-Whetstine et al., 2006; Drake et al., 2009). Several studies have demonstrated that flavor of whey products changes during storage, light exposure, and high temperature (Wright et al., 2009: Liew et al., 2010). Indeed, the flavor of ripened cheese depends on the interaction between starter bacteria and enzymes from the raw milk, the ripening after the rennet addition, the lipases, the proteolysis and secondary by the cheese micro flora (Fatma et al., 2013).

These volatile molecules belonging to different chemical family such as esters, alcohols, aldehydes, free fatty acids, ketones, lactones, terpenes, and pyrazine whose production is controlled by biochemical events like glycolysis (lactose), lipolysis (fat) and proteolysis (protein) along all the production chain from raw milk to cheese (Curioni and Bosset, 2002). More precisely, fat, protein and lactose are degraded into molecules with a low molecular weight that are consequently precursors of volatile aroma compounds (McSweeney and

Sousa, 2000; Smit et al., 2005). Furthermore, more than 600 volatile molecules have been identified in different cheese varieties and many of these corresponding to particular odor and aroma notes. Nevertheless, only a small proportion of these compounds are really responsible for cheese flavor (Curioni and Bosset, 2002).

Due to the importance of VOCs, there is an increasing interest in the development of simple methods for their identification and quantification. The VOCs detection requires possibly low-cost, rapid and no destructive techniques that permit to study both individual phenotypic sources of variation and environmental factors on a large number of samples in a shorter time. The available of methods to identify, to quantify and to monitor the VOCs is important to investigate quality traits in food science. Tunick (2014) reported that the research of the VOCs has been advanced by the discovery of chromatography in the mid-1940s.

The volatile organic compounds are generally analyzed by a headspace analysis gas chromatography (GC) coupled to mass spectrometry (MS), and Solid Phase Micro Extraction (SPME) is used to collect and concentrate the compounds present in the sample headspace from matrices in both solid and liquid states (Tunick et al., 2013; Padilla et al., 2014). In addition, SPME analyses may be performed at low cost with relatively simple equipment that can be automated. The GC-MS is a useful method for identifying and quantifying flavour molecules, but it does not allow establishing differences between compounds with or without odour-activity. Complementary, gas chromatography-olfactometry (GC-O) provides with extra information for investigating the pattern of odorants in terms of both their odour descriptors and activity. The GC-O technique has been used to study important aroma compounds in Cheddar cheese (Milo and Reineccius, 1997), Cantal cheese (Cornu et al., 2009), Emmentaler cheese (Preininger and Grosch, 1994), Grana Padano cheese (Moio and Addeo, 1998), and Blue cheese (Qian et al., 2001).

From a technical point of view, there is a growing interest to develop rapid and simpler methods to overcome the main limitations of GC-MS, which still remains a time-consuming procedure (Biasioli et al., 2011). Among the various possibilities proposed, for rapid identification and quantification of VOCs in cheese, has been the development of another spectrometric technique, the Proton Transfer Reaction-Time of Flight-Mass Spectrometry (PTR-ToF-MS). The PTR-ToF-MS is a relatively new technique that allows for capturing VOCs down to the part per billion ranges using only few seconds per sample (Biasioli et al., 2011). The detection is based on the reaction between VOCs with a proton affinity higher than water, hydronium ions (H_3O^+) and subsequent analysis with commercial quadrupole mass spectrometer. In comparison to other spectrometric techniques, such as gas chromatography-mass spectrometry with better identification capability, PTR-ToF-MS allows the direct injection of the air sample without pre-treatments. It also has a shorter analysis time and greater sensitivity. The resulting spectral information provides a very detailed description of samples, which is useful for characterizing food quality, origin, and typicality (Fabris et al., 2010; Galle et al., 2011). In addition, PTR spectra can be related to the sensory characterisation of cheese (Biasioli et al., 2011; Cappellin et al., 2012). Consequently, PTR-MS has found a high number of applications in chemistry, biology, and other scientific fields such as food technology and medicine (Lindinger et al., 1998).

Despite the centrality and importance of cheese VOC, potentially related to sensory proprieties, no research has yet estimated the genetic parameters of their concentration in cheese for selection purposes in breeding programs. This aspect could be mainly attributed to the difficulties in individually measuring these traits on a large number of cheeses/samples, and the lack of fast and high-throughput spectrometric techniques. Recently, some authors estimated the heritability of quality traits predicted by Fourier Transform Infrared spectrometry (FTIR) on milk samples (Bittante et al., 2014; Cecchinato et al., 2015). The genetic parameters of other quality traits have been investigated, including FA profiles (Soyeurt et al., 2007; Rutten et al., 2010; Bastin et al., 2011), protein compositions (Arnould et al., 2009), milk coagulation traits (Bittante et al., 2012), and mineral profiles (Soyeurt et al., 2009). Nevertheless, to the best of our knowledge, no comparison between genetic parameters estimated from volatile fingerprints obtained from a large number of experimental units (individual model cheeses) has been reported.

The study of quality traits like Volatile Organic Compounds of dairy products is interesting to increase the economic value of milk and to improve the cheese production chain, and to define traceability parameters on the basis of sensory characteristics.

AIMS OF THE THESIS

The main objective of this thesis was to investigate the Volatile Organic Compounds profile of dairy products across the cheese production chain. The thesis is divided in five main parts.

In the first part, the Volatile Organic Compounds detected by Solid Phase Micro Extraction/Gas Chromatography-Mass Spectrometry (SPME/GC-MS) in a selection of 150 individual model cheeses obtained from milk of Brown Swiss cows from 30 herds (5 cows from each herd) representing 5 different dairy systems is presented, studying the effect of dairy system and individual cow characteristics (Days in milk, parity and milk yield);

In the second part, the Volatile Fingerprint of a large number of individual model cheese (1,075 samples) analysed with a rapid and non-invasive spectrometric technique Proton Transfer Reaction- Time of Flight- Mass Spectrometry (PTR-ToF-MS) is reported, evaluating the effect of dairy system and individual cow factor (Days in milk, parity and milk yield);

The third part of the thesis shows the analysis of the genetic parameters of new phenotypes (Principal Components) and Spectrometric Fragments (240 PTR-Peaks) extracted by volatile fingerprint of cheeses together with variance components of herd/test date, genetic and residual components and their correlations;

In the fourth part of the thesis, the quality aspects of processed fluids, fresh products and cheeses obtained from cheese-making in a highland farm during summer transhumance is discussed;

The objective of the last part of the thesis was to study the effect of summer transhumance on Volatile Organic Compounds detected by SPME/GC-MS. Thus, the evolution of VOCs of 11 dairy products, collected from 7 cheese-making, was investigated, using milk produced in a highland temporary farm.

FIRST CHAPTER

Effects of the dairy system, herd and individual cow characteristics on the volatile organic compound profile of ripened model cheeses

M. Bergamaschi[*,†], E. Aprea[*], E. Betta[*], F. Biasioli[*], C. Cipolat-Gotet[†], A. Cecchinato[†], G. Bittante[†], and F. Gasperi[*]

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[*]Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach (FEM) Via E. Mach 1, 38010 San Michele all'Adige (TN), Italy

[†]Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell’Università 16, 35020 Legnaro (PD), Italy

ABSTRACT

The objective of this work was to study the effect of dairy system, herd within dairy system, and characteristics of individual cows (parity, days in milk and daily milk yield) on the volatile organic compound profile of model cheeses produced under controlled conditions from the milk of individual cows of the Brown Swiss breed. One hundred and fifty model cheeses were selected from a total of 1,272 produced for a wider study of the phenotypic and genetic variability of Brown Swiss cows. In our study, we selected 30 herds representing 5 different dairy systems. The cows sampled presented different milk yields (12.3-43.2 kg×d-1), stages of lactation (10-412 days in milk) and parity (1-7). A total of 55 volatile compounds were detected by solid phase microextraction gas chromatography-mass spectrometry, including 14 alcohols, 13 esters, 11 free fatty acids, 8 ketones, 4 aldehydes, 3 lactones, 1 terpene and 1 pyrazine. The most important sources of variation in the volatile organic profiles of model cheeses were dairy system (18 compounds) and days in milk (10 compounds), followed by parity (3 compounds) and milk yield (5 compounds). The model cheeses produced from the milk of tied cows reared on traditional farms had lower quantities of 3-methyl-1-butanol, 6-pentyloxan-2-one, 2-phenylethanol, and dihydrofuran-2(3H)-one than those reared in free stalls on modern farms. Of these, milk from farms using total mixed ration had higher a content of alcohols (hexan-1-ol, octan-1-ol) and esters (ethyl butanoate, ethyl pentanoate, ethyl hexanoate and ethyl octanoate) and a lower content of acetic acid than those using separate feeds. Moreover, the dairy systems that added silage to the total mixed ration produced cheeses with lower levels of volatile organic compounds, in particular alcohols (butan-1-ol, pentan-1-ol, heptan-1-ol), than those that did not. The amounts of butan-2-ol, butanoic acid, ethyl-2-methylpropanoate, ethyl-3-methylbutanoate, and 6-propyloxan-2-one increased linearly during lactation, while octan-1-ol, 3-methyl-3-buten-1-ol, 2-butoxyethanol, 6-pentyloxan-2-one, and 2,6-dimethylpyrazine showed a more complex pattern during lactation. The effect of the number of lactations (parity) was significant for octan-1-ol, butanoic acid and heptanoic acid. Lastly, octan-1-ol, 2-phenylethanol, pentanoic acid, and heptanoic acid increased with increasing daily milk yield, whereas dihydrofuran-2(3H)-one decreased. In conclusion, the volatile organic compound profile of model cheeses from the milk of individual cows was affected by dairy farming system and stage of lactation, and to smaller extent by parity and daily milk yield.

Keywords: cheese quality; lactation stage; milk yield; SPME/GC-MS; aroma.

INTRODUCTION

Dairy products are deemed acceptable by consumers mainly on the basis of their sensory qualities, with flavor playing a major role (Liggett et al., 2008; Liaw et al., 2010; Bittante et al., 2011b). Cheese flavor is determined by volatile and non-volatile compounds generated from milk fat, protein and lactose during the ripening process (Marilley and Casey, 2004; Drake et al., 2010; Le Quéré, 2011). Discovery of the origins of these molecules can provide information on both the processes and the raw materials from which the cheeses were produced (Izco and Torre, 2000). Volatile Organic Compounds (VOCs) in particular are of utmost importance in uncovering the links between production chain and consumers' acceptability ratings (Fox et al., 2004). It is also known that perceived odors/flavors are the result of a mixture of odorants and that individual components may lose their specific identity when combined with others to produce a new mixture-specific aroma (Le Berre et al., 2008). Therefore, although identification of the quality attributes of individual volatile compounds may be related to the flavor of the product, the interactions between several compounds must also be taken into account (Aprea et al., 2012).

The sensory properties of cheese are affected by several genetic, environmental and technological factors (Bittante et al., 2011b), which have increasingly raised consumers' concerns, in particular those relating to the feeding, breeding and health of animals. The chemical composition of milk, and consequently of cheese, is affected by several factors, such as dairy cow management, season, genetics, the cow's diet, parity and stage of lactation (Coulon et al., 2004; Coppa et al., 2011). It is essential to quantify the relative importance of the different factors influencing cheese production, in particular those relating to milk production conditions, in order to be able to better predict and manage cheese quality (Bittante at al., 2011a). Although the effects of farming methods and diet on milk and cheese composition have been studied (Romanzin et al., 2013; Bovolenta et al., 2014), few studies have examined the relationships between variations in the VOCs of cheese and variations among individual animals. Understanding the possible effects of environmental, managerial and individual cow factors on the characteristics of milk that could in turn affect the quality of cheese is essential (Barbano and Lynch, 2006), especially where Protected Designation of Origin (PDO) cheeses produced according to traditional processing techniques are concerned. Interesting and fundamental insights as well as practical indications can be gained by an approach that takes the entire production chain into account, that is, by studying the relationships between the characteristics of milk produced on different farms and from individual animals and the sensory characteristics of ripened cheese, in particular the VOC profile.

The development of model cheese-making procedures suitable for application to large numbers of individual samples (Cipolat-Gotet et al., 2013) offers new tools for research in this field. They appear suitable for studying the effects of herd/test date, lactation stage, parity and milk yield of cows on percentage cheese yields (fresh curd, curd solids and water retained in the curd as fractions of milk processed), daily cheese yields (expressed per cow per day of lactation), and nutrient recovery/whey loss (weight of protein, fat, total solids or energy retained in the curd as a percentage of the same nutrient in the processed milk). These data could also be used for genetic analysis, because all cheese yield and recovery traits are characterized by heritability coefficients equal to or greater than those of milk yield and composition (Bittante et al., 2013). As individual model cheese-making is not feasible at the population level, infrared (FTIR) calibrations showed to be feasible means to predict cheese yield traits from unprocessed milk samples collected for milk recording (Ferragina et al., 2013) because the predicted new phenotypes are characterized by heritability coefficients similar to those of the corresponding measured traits and the genetic correlations between the two are high (Bittante et al., 2014), allowing to be used on dairy cows of different breeds at the population level (Cecchinato et al., 2014).

If model cheese-making technology has allowed new insights to be gained, it is important to assess the feasibility of using it to improve knowledge of the factors affecting cheese quality, especially its flavor. VOCs are generally analyzed by gas chromatography (GC) coupled to mass spectrometry (MS), and solid phase microextraction (SPME) is used to collect and concentrate the compounds present in the headspace from matrices in both solid and liquid states. This method is simple, rapid sample preparation free from organic solvents, good resolution, high sensitivity and low cost, which is why SPME is now commonly used in food analysis as well as in cheese volatile compound analysis (Tunick et al., 2013; Padilla et al., 2014).

A large research project has been set up with the aim of studying the relationships between individual cow characteristics and herd environment and management. The present study is intended to contribute to our understanding of the factors affecting cheese volatile compounds potentially contributing to cheese flavor by investigating the effects of the dairy system, herds within the dairy system, and the characteristics of individual cows (days in milk, parity, milk yield) on the VOC profiles of individual model cheeses.

MATERIALS AND METHODS

Animals and milk sampling

This study is part of the "Cowplus Project", supported by the Province of Trento, whose objective is to investigate the relationships between dairy cows and cheese quality traits, and to assess the potential for genetic improvement of the dairy cattle population. The sampling procedure used in the whole project has been described in detail by Cipolat-Gotet et al. (2012) and Cecchinato et al. (2013), and the production environment by Sturaro et al.

(2013). Individual milk samples were obtained from a total of 1,272 Brown Swiss cows from 85 herds located in the Province of Trento, an Alpine area in north-eastern Italy. In the present work we analyzed a subset of 150 milk samples from 30 herds (5 cows from each herd) representing 5 different dairy systems, whose main features are summarized in Table 1.

The dairy systems in the alpine area investigated, like in many others mountainous area, vary considerably in terms of available facilities, technologies adopted, relationships with the environment, numbers and breeds of cows reared, animal welfare and productivity, and milk quality and destination (Sturaro et al., 2009). Following a research on the entire province of Trento aimed at clustering and characterizing the different dairy systems (Sturaro et al., 2013), the present study examined five dairy systems clustered mainly according type of facilities available and management practices. They ranged from the very traditional system typical of the Alps to the more modern type, very common on the plains (Table 1). The first (Traditional) system is based on small old barns with tied animals milked at the stall, calving concentrated mainly in autumn, and cows and heifers moving to highland pastures for the summer transhumance. The second (Modern) is based on larger, modern facilities, loose animals, milking parlors, year round calving and total mixed ration feeding either including (With silage) or excluding maize silage (Without silage). A variant of the very traditional system abandons seasonal organization and summer transhumance for lactating cows and adopts automatic distribution of compound feed at the manger (With AF), while an intermediate dairy system is the one with modern facilities but a traditional feeding of hay and concentrates (Hay+CF).

In the Alps, different dairy systems are often associated with different breeds of cattle, with Holstein Friesians prevalent in intensive modern farms, local and dual-purpose breeds in traditional farms, while Brown Swiss cows are reared in a range of dairy systems (Sturaro et al., 2013). The present study only investigated Brown Swiss cows to avoid confounding differences between dairy systems with different breeds.

The cows sampled differed in their daily milk yields (12.3-43.2 kg×d-1), stages of lactation (10-412 days in milk; DIM) and parity (1-7). Collection, refrigeration, transport and storage of milk samples were standardized in order to minimize differences among herds/dates. After collection, milk samples (without any preservative) were immediately refrigerated at 4°C and processed within 20 h at the Cheese-making Laboratory of the Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE) of the University of Padova (Legnaro, Padova, Italy) for fat, protein and casein percentages using a MilkoScan FT6000 apparatus (Foss Electric A/S, Hillerød, Denmark), for pH using a Crison Basic 25 electrode (Crison, Barcelona, Spain), and for cheese-making.

Model cheeses

Model cheeses were manufactured from the raw milk of individual cows according to the method described by Cipolat-Gotet et al. (2013). Briefly, 1,500 mL of milk was heated to 35 °C in a stainless steel micro-vat, supplemented with thermophilic starter culture and mixed with rennet. The resulting curd was cut, drained, shaped in wheels, pressed, salted and weighed (225±29 g). The small wheels produced were ripened at 15 °C and 85% relative humidity for the first month and at 12 °C and the same relative humidity for the second month. After 60 days the model cheeses were weighed (133±18 g) and analyzed (Table 2). The fat and protein contents of the whole cheeses after removal of the rind were measured using a FoodScan apparatus (Foss Electric A/S, Hillerød, Denmark). The pH was measured at 20 °C by inserting a Crison Basic 20 electrode (Crison, Barcelona, Spain) into each model cheese. A cylindrical sample 1.1 cm in diameter and about 3.5 cm in height was taken from the center of each model cheese and conserved at -80 °C till VOC analysis.

VOC analysis by solid phase microextraction gas chromatography-mass spectrometry

Headspace solid phase microextraction coupled with gas chromatography-mass spectrometry (SPME/GC-MS) was conducted using a modified version of the method reported in Endrizzi et al. (2012). The cheese samples were thawed and kept at room temperature (about 20 °C) for 6 h, then 3 g from each sample were placed into glass vials (20 mL, Supelco, Bellefonte, PA, USA) and gently mashed with a spatula. The vials were then capped with a PTFE/silicone septa (Supelco) and the samples equilibrated at 40 °C for 30 min. A fused silica fiber coated with 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DBV/CAR/PDMS, Supelco) was then inserted and exposed to the headspace environment for 30 min at the same temperature. Volatile compounds absorbed by the SPME fibre were desorbed at 250 °C in the injector port of a GC interfaced with a mass detector operating in an electron ionization mode (EI, internal ionization source; 70 eV) with a scan range of m/z 33-300 (GC Clarus 500, PerkinElmer, Norwalk CT, USA). Procedure phases were automatically managed using an auto-sampling system (CTC combiPAL, CTC Analysis AG, Zwingen, Switzerland). Separation was carried out on an HP-Innowax fused silica capillary column (30 m, 0.32 mm ID, 0.5 μm film thickness; Agilent Technologies, Palo Alto, CA, USA). Separation conditions were as follows: carrier gas was helium at a constant flow rate of 2 ml min-1; oven temperature programming was 40 °C for 3 min, 40-180 °C at 4 °C min-1, 180 °C for 6 min and finally 180-220 °C at 5 °C min-1. The samples (around thirteen per day) were analyzed over twelve days (date of analysis) in random order. To test the repeatability of the method, ten replicates of a reference cheese were analyzed on the same day. The observed averaged variations were 23, 14, 8, 13 and 20% respectively for acids, alcohols, esters, ketones, and aldehydes, in agreement with the literature for SPME analysis with this type of matrix (Mallia et al., 2005;

Endrizzi et al., 2012) and with other kinds of food (San Roman et al., 2014). Levels of VOCs in the samples were expressed as fractions of the total chromatography area.

Statistical analysis

Given that the distribution of all VOCs showed a strong positive skewness, data transformation was applied: the fraction of each VOC was multiplied by 106 and expressed as a natural logarithm to obtain a more Gaussian-like data distribution before statistical analysis, as it is shown by the skewness and kurtosis coefficient reported in Table 3. All transformed data that were 3 standard deviations or more outside the mean were considered outliers and excluded from the statistical analysis. The data set was analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) according to the following model:

$$Y_{ijklmnopq} = \mu + \text{dairy system}_i + \text{HTD}_j(\text{dairy system})_i + \text{DIM}_k + \text{parity}_l + \text{milk}_m + \text{vat}_n + \text{date}_o + \text{inj}_p + e_{ijklmnopq}$$

where yijklmnopq is the observed trait (55 VOCs and their 6 chemical families); μ is the overall mean; dairy systemi is the fixed effect of the i*th* dairy system (i = 1 to 5); HTD_j(dairy system)$_i$ is the random effect of the $_j$th herd/test date (j = 1 to 30) within i*th* dairy system; DIM_k (days in milk, interval from parturition to milk sampling) is the fixed effect of the k*th* class of days in milk (k = 1 to 7, class 1: ≤50 d, class 2: 51 to 100 d, class 3: 101 to 150 d, class 4: 151 to 200 d, class 5: 201 to 250 d, class 6: 251 to 300, class 7: >300 d); parity_l (number of parturitions) is the fixed effect of the l*th* parturition (l = 1 to 4 or more); milk_m (daily milk yield, $\text{kg}\times\text{d}^{-1}$) is the linear covariate of milk yield; vat_n is the fixed effect of the n*th* number of the experimental vat used for model cheese production (n = 1 to 15); date_o is the fixed effect of the o*th* day of SPME/GC-MS analysis (o = 1 to 12); inj_p is the fixed effect of the p*th* daily injection order into the instrument (p = 1 to 12); $e_{ijklmnop}$ is the residual random error term ~N (0, σ^2). Dairy system effect was tested using HTD within dairy system as the error term, while other fixed effects were tested on the residual. Orthogonal contrasts were used to test the effects of dairy system and parity.

RESULTS AND DISCUSSION

VOC profiles of individual model cheeses

The quality traits of milk and model cheeses are reported in Table 2. Average milk yields and fat, protein and casein content were 24.6 kg×d-1, and 4.3, 3.7 and 2.9%, respectively. Average fat and protein contents of these cheeses at 60 days of ripening were 38.6 and 26.8%, respectively. Milk yield showed the largest coefficient of variation and pH the smallest for both milk and cheese.

The 55 VOCs identified in the model cheeses belong to the following chemical families: 14 alcohols from C4 to C8; 13 esters from C4 to C12, mainly ethyl esters; 11 free fatty acids from C2 to C10; 8 methyl ketones from C4 to C11; 4 aldehydes from C5 to C9; 3 lactones from C4 to C10; 1 terpene; 1 pyrazine. All VOCs peak area showed a non-gaussian right-skewed distribution. The mean values of the natural logarithms of the peak area of the 55 VOCs along with their 6 summed values according to their chemical family are shown in Table 3, togheter with their skewness and kurtosis coefficients showing the almost Gaussian distribution achieved after transformation. The VOCs relate to both flavor (by retronasal perception) and odor (direct orthonasal perception), although it is well known that these sensory quality traits are the result of a mixture of compounds with the single components frequently losing their individual identity and a new mixture specific odour quality could emerge; thus the identification of the quality attribute of a single volatile compound is only indicative (Le Berre et al., 2008).

Odor-active compounds are usually identified by gas chromatography-olfactometry, although this method cannot take into account the interactions between the compounds, nor their enhancement or fusion because the individual compounds are evaluated only after having been separated. To understand food aroma as perceived by the consumer, sensory characterization of the global aroma of the food product is also necessary (Drake, 2007; Cornu et al., 2009; Thomsen et al., 2012).

All VOCs displayed low to medium variability, the coefficient of variation ranging from 3% for butanoic acid to 30% for 2-ethylhexanoic acid. However, the variability coefficients of VOCs summed according to their chemical families were much lower than those of individual VOCs (from 1% for free fatty acids to 8% for aldehydes) due to compensating patterns among the amount of individual VOCs within the same family. With respect to the low coefficients of variability, it should be remembered that the concentrations are expressed in logarithmic terms, so that in linear untransformed terms the variability is much greater and the distribution positively skewed. The volatile compound profile of model cheeses displayed a marked variability, probably also as a consequence of variability in the native microflora of raw milk from different herds and cows (Urbach, 1995; Urgeghe et al., 2012). After transformation into natural logarithms, the values of skewness and kurtosis of the peak area normalized to total chromatography area were close to zero, suggesting that they were almost normally distributed (Table 3). The effect of date of analysis was significant only for the esters and the family of other VOCs, confirming good reproducibility of the technique adopted. The four esters affected by date of analysis were ethyl-2-methylpropanoate, ethyl benzene, ethyl octanoate and ethyl decanoate, which increased significantly ($P<0.05$) over the course of the analysis period, while dihydrofuran-2(3H)-one, 6-pentyloxan-2-one and limonene (other VOCs) decreased significantly ($P<0.001$) from the first to last day of analysis (data not shown). Effects of vat and order of injection were not significant for any of the chemical

VOC families analyzed, confirming acceptable reproducibility of the procedure utilized (Table 4). The predominant chemical family of compounds identified was free fatty acids (average ln of 13.3, corresponding to a linear value of almost 60.0%). Alcohols (mean value as ln 11.8, equal to 13.3%), ketones (11.7 equal to 12.0%) and esters (10.7, equal to 4.4%) were found in intermediate amount, while aldehydes and other VOCs (both with 7.3, equal to 0.1%) were represented to a much lesser extent. Both primary and secondary alcohols were found. The primary alcohols were: butan-1-ol, pentan-1-ol, hexan-1-ol, heptan-1-ol and octan-1-ol, which may originate from the corresponding aldehydes produced from fatty acids and amino acid metabolism (Barbieri et al., 1994). They can contribute fruity, green notes to the flavor of cheese (Curioni and Bosset, 2002). The secondary alcohols were: butan-2-ol, hexan-2-ol and heptan-2-ol, which have also been reported in Parmigiano-Reggiano, Roquefort and Cheddar cheeses (Engels et al. 1997). They are important flavor components of Blue cheeses, where they are produced through reduction of methyl ketones (Curioni and Bosset, 2002). Butan-2-ol may be formed by reduction of 2,3-butandione (diacetyl) to butane-2,3-diol by the starter bacteria, and subsequent reduction to butan-2-ol by non-starter lactic acid bacteria activity during cheese ripening (Berard et al., 2007). The most abundant alcohol (taking into account the peak area), detected in all the cheese chromatograms, was 3-methyl-1-butanol formed by reduction of the aldehydes or by Strecker degradation of the amino acid leucine. This is the main volatile aromatic compound produced during cheese ripening and is observed in many types of cheeses. Similar results have also been observed in other raw milk cheeses, where this alcohol has been found in high amounts (Carbonell et al., 2002; Berard et al., 2007).

The branched-chain aldehydes 2-methylbutanal and 3-methylbutanal, likely derived from lipid oxidation, were detected in all the cheeses analyzed (Table 3). These aldehydes are often associated with malty and herbaceous flavors (Cornu et al., 2009). Aldehydes may also derive from amino acids during ripening (Strecker degradation) and in some kinds of cheeses they are considered to be the key flavor compounds (Curioni and Bosset, 2002). Liaw et al. (2010) suggested that these compounds are responsible for off-flavors in dairy products and they increase in quantity with storage or processing time. The aldehyde content in cheese is limited by redox potential, unsaturated fatty acid content and lipolysis activity (Engel et al., 1997; Ziino et al., 2005; Berard et al., 2007).

The most abundant ketones found in all samples in similar average quantities were pentan-2-one, heptan-2 one and 3-hydroxy-2-butanone (Table 3). Methyl ketones are naturally present in dairy products (Curioni and Bosset, 2002) and can also be formed by enzymatic oxidation of fatty acids to β-ketoacids with consequent decarboxylation to alkan-2-ones with the loss of one carbon atom (McSweeney and Sousa, 2000), while 3-hydroxy-2-butanone is formed through glycolysis from pyruvic acid (Marilley et al., 2004). High amounts of pentan-2-one and heptan-2-one are reported to be also present in the volatile fraction of Parmigiano-Reggiano cheese (Barbieri et al., 1994; Bellesia et al., 2003). Pentan-2-one and heptan-2-one have fruity, herbaceous flavors, characteristics of Swiss Gruyère and Camembert cheeses (Izco and Torre, 2000), while 3-hydroxy-2-butanone presents a buttery aroma (Cornu et al., 2009).

Among the esters, ethyl butanoate had the greatest area value, followed by ethyl hexanoate and ethyl acetate. A higher content of ethyl butanoate has been found in Parmigiano-Reggiano and Gruyère cheeses (Ziino et al., 2005). Esters are mainly produced by enzymatic or chemical reaction of fatty acids with alcohol (Curioni and Bosset, 2002), their concentrations usually being the limiting factor in production; they can also be formed by transesterification of partial glycerides (Ziino et al., 2005). Ethyl esters (especially ethyl butanoate and ethyl hexanoate) are considered key odorants in some cheese varieties, such as grana types (Urgeghe et al., 2012), Parmigiano-Reggiano (Barbieri et al., 1994), Canestrato

Pugliese, and Provola (Ziino et al., 2005). They have low perception thresholds and may contribute typical floral and fruity notes to cheese aromas (Liu et al., 2004) minimizing the sharpness of fatty acids and the bitterness of amine (Urgeghe et al., 2012).

The most abundant fatty acids were acetic acid, butanoic acid, and hexanoic acids (Table 3). Several authors have observed these acids in different kinds of cheeses: pasta filata (Ziino et al., 2005), Parmigiano-Reggiano (Bellesia et al., 2003) and Swiss Gruyère (Rychlik and Bosset, 2001). Butanoic acid and hexanoic acid containing four or more carbon atoms are generally produced from lipolysis of milk fat, while acetic acid results from oxidation of lactose and catabolism of alanine and serine by lactic acid bacteria (Ziino et al., 2005). Acetic acid gives rise to vinegary notes in cheese, butanoic acid to vomit and feet notes, and hexanoic acid to cheese rind (Bendall, 2001; Cornu et al., 2009).

We found a high content of 6-pentyloxan-2-one in the lactone group (Table 3). Biosynthesis of these compounds results from the activity of lipoxygenases or hydratases on unsaturated fatty acids, constituents of milk fat (Ziino et al., 2005). Lactones, also present in bovine milk (Bendall, 2001), provide peach, apricot and coconut odors in cheese. 6-pentyloxan-2-one and 6-propyloxan-2-one have also been found in Camembert, Emmental, and goat's cheese (Curioni and Bosset, 2002).

Terpenes arise from the plants constituting the animals' diets and are transferred to the milk then to the cheese. They are important in determining product origin, especially of mountain cheeses, which contain more terpenes than lowland cheeses. These compounds have been listed as odorants in Cheddar and Pecorino cheese because of their fresh, herbaceous odor (Curioni and Bosset, 2002; Acree and Arn, 2004).

McSweeney and Sousa (2000) reported 2,6-dimethylpyrazine in Swiss Gruyère, Parmigiano-Reggiano and Swiss Emmental cheeses, where it was probably formed by microorganisms;

the flavor is of burnt potato. Other authors mention the presence of an earthy odor in British farmhouse Cheddar and Camembert cheese caused by pyrazine (Curioni and Bosset, 2002).

The effects of dairy system and herds within the dairy system

Table 2 clearly shows how the dairy system can profoundly affect the daily milk yield from the same breed of cow with very similar breeding values. Farms using total mixed rations, with or without maize silage, produced on average 26.8 and 30.2 kg×d^{-1} of milk, respectively, whereas the very traditional farms produced only 19.1 kg×d^{-1}. The dairy system had a much lower effect on milk composition (fat, protein and casein content), with the greatest contents observed for farms using TMR, and also on cheese composition. The cow's diet is well known to have a major influence on milk composition but little effect on milk coagulation properties (Bittante et al., 2012); it also influences cheese composition (Lucas et al., 2006). Cheese seems to be more affected by the ratio of fat to protein in milk than by their absolute values, which have a greater effect on cheese yield than on composition (Cipolat-Gotet et al., 2013).

In the present study, we found several differences between the five dairy systems sampled with respect to the whole chemical family of volatile esters (Table 4) and 18 individual VOCs (Table 5). It is well documented that dairy systems, in particular feeding practices, affect the sensory properties of cheese (Martin et al., 2004; Tornambé et al., 2005) and its VOC content, this is especially the case with pasture-based as opposed to indoor systems (Cornu et al., 2009; Farruggia et al., 2014; Bovolenta et al., 2014). The effect is particularly pronounced in the case of typical PDO products, where modification of the raw material during processing is restricted or prohibited and where milk production conditions are underly the notion of "terroir" (Verdier-Metz et al., 2005). In this case, the final quality of the cheese is strictly dependent on the characteristics of the processed milk (Bertoni et al., 2005; Calamari et al., 2005; Endrizzi et al., 2012).

Much less is known about dairy systems using only dry feedstuffs or silages. To better understand the results of the present study, it worthwhile pointing out that no permanent dairy system in the province of Trento uses pasture or green fodder administration, and that 4 of the 5 dairy systems studied do not use silage either (Table 1). This is due to the fact that most of the milk goes into the production of Trentingrana cheese, a hard, long ripened, PDO cheese whose production specification discourage the use of pasture and green fodder, and do not allow the use of silage. Although this feeding strategy (hay and compound feed) is often more expensive, it is compensated by the much higher price paid for the milk by the cooperative dairies producing Trentingrana and other local typical cheeses (Sturaro et al., 2013) than by producers of conventional dairy products (fluid milk, yoghurt, fresh cheeses, etc.). Milk produced from cows grazed on Alpine pastures during the summer transhumance was not sampled in the present study because it is often used to produce “Malga” type cheeses directly by farmers (Bovolenta et al., 2009).

Previous studies have drawn attention on variability among the cooperative dairies on sensory characteristics of high-quality Trentingrana wheels (Bittante et al., 2011a and 2011b) reflecting the differences of the farms supplying the milk (Endrizzi et al., 2013).

Cheeses from modern farms, as opposed to traditional ones, had higher proportions of 3-methyl-1-butanol and 2-phenylethanol among the alcohols, and of 6-pentyloxan-2-one and dihydrofuran-2(3H)-one among the lactones (Table 5). Of the traditional farms, those distributing compound feed in the mangers with automatic feeders, often using more concentrates than the very traditional farms, had a different 2,6-dimethylpyrazine content to the others. In modern dairy systems, differences were observed between the VOC profiles of cheeses from farms using hay and compound feed administered in the mangers and those of cheeses from farms using total mixed rations. TMR gave rise to higher contents of two primary alcohols, hexan-1-ol and octan-1-ol, and four esters, ethyl butanoate, ethyl pentanoate, ethyl hexanoate and ethyl octanoate. On the other hand, the amount of acetic acid in the head space of the cheese samples was lower when the milk was from TMR-fed cows than when it was from conventionally fed cows, and this result could be the consequence of different phenomena. As shown in Table 1, farms adopting TMR use greater amount of concentrates resulting in lower proportion of fiber in diet DM. Often this condition leads to lower concentration of acetic acid in the rumen. At the same time the milk fat content was increased (Table 2) by TMR use, probably because of an increased de novo fat synthesis in the udder from blood acetate.

In the dairy systems using TMR, we found greater proportions of the aldehyde 3-methylbutanal, and smaller proportions of primary alcohols butan-1-ol, pentan-1-ol, hexan-1-ol, heptan-1-ol and octan-1-ol, as well as ethyl (S)-2-hydroxypropanoate, ethyl butanoate, ethyl pentanoate and limonene in those that added silage than those which did not (Table 5). Numerous aldehydes were detected in silages, milk and cheeses (Kalač et al., 2011). Toso et al., (2002) ascertained that 3-methylbutanal was by far the most prevalent aldehyde in milk produced from hay and maize silage rations. The results of the present study are in accordance with previous works that found a higher content of terpenes (such as limonene) in milk and cheese when lactating cows are fed on natural dicotyledon-rich grasses than when fed on monospecific forage or concentrate-based rations (Kalač, 2011). The addition of silage to hay and concentrates fed to lactating dairy cows has been shown to modify the characteristics of the milk (Kalač, 2011) and the ripened cheese (Verdier-Metz et al., 2005) and also the proportions of VOCs both in bulk milk (Toso et al., 2002) and in Montasio cheese (Stefanon and Procida, 2004).

All chemical VOC families were influenced by herd/test date within dairy system (Table 4), and it is very interesting to note that this effect is especially larger on ketones: the variance of HTD represented 43% of total variance, for alcohols it was 37%, while it was moderate (17 to 24%) for the other chemical families. There is little information on the effect of herd in the literature because the majority of articles published describe results obtained from statistical analyses with a single experimental herd or several farms as fixed factors.

The effect of days in milk

Of the various factors related to individual cows, stage of lactation appears to be the most important source of variation in VOC profiles: 10 compounds out of 55 quantified VOCs were affected by days in milk (Table 6). Butan-2-ol among the alcohols, butanoic acid among free fatty acids, ethyl-2-methylpropanoate and ethyl 3-methylbutanoate among the esters, and 6-pentyloxan-2-one among the lactones exhibited linear increases throughout lactation, such as cheese protein content. The quadratic patterns exhibited by 6-pentyloxan-2-one (lactone) and by 2,6-dimethylpyrazine (pyrazine), and the cubic patterns characterizing 3-methyl-3-buten-1-ol, octan-1-ol, 2-butoxyethanol (alcohols), and 6-propyloxan-2one (lactone) are more complex. Given that they are expressed as natural logarithms, the variations in the VOC profiles observed during lactation are much greater than those recorded for milk yield (decrease) and fat and protein content (increase), although their summed effects could partially explain these variations. It appears that other factors give rise to variations in VOC profiles during lactation, including: a change in the quantity and quality of feed ingested (then the rumen environment and fermentation, and the cow's digestion, absorption and metabolism); a change in the cow's energy balance (then fat mobilization/deposition, the ratio of energy to individual nutrients, and the cow's hormonal interactions); pregnancy status (and associated metabolic and physiological changes); modifications to udder secreting tissue (in relation to apoptosis and the secretion of different molecules). Cheeses produced at the end of the animal's lactation period have faster proteolysis, especially when the milk SCC content are high (Coulon et al., 2004), which may influence coagulation properties, cheese yield and sensory characteristics. Plasmin, the most important proteinase in milk, shows increased activity in late lactation. It is also possible that other modifications related to lactation stage, such as increased milk lipolysis or modification of fat composition, may have given rise to the VOC differences (Coulon et al., 2004). The effect of enzymes on VOC profile cited above could be more marked in cheeses from milk of late-lactation cows reared in modern dairy systems than traditional one because the variability of fat in dry matter (substrates) and SCS are greater (Table 2). The differences observed are also more probably due to concomitant variations in feeding practices or the health of the cow (Auldist et al., 1996). Further studies are needed to elucidate the effects of the above-cited factors on milk properties with respect to VOC content and their modification during cheese-making and ripening.

The effect of parity

Parity and/or age of the cow is an important factor affecting milk yield and quality, although it has only a small effect on milk coagulation properties (Bittante et al., 2012), model cheese yield and nutrient recovery in curd or loss in whey (Cipolat-Gotet et al., 2013).

Parity did not affect the sum of VOCs per chemical family (Table 4) and affected only a few individual VOCs in the ripened cheese (Table 7). In particular, parity in the lactating cow influenced the proportions of one alcohol and two free fatty acids. Cheeses from primiparous cows had a higher content of butanoic acid than the average content in cheeses from older cows. Cheeses from second lactations were found to have a greater proportion of octan-1-ol and a smaller proportion of heptanoic acid than cheeses from later lactations. Finally, among mature cows (three lactations and more) lower quantities of butanoic acid were observed in the cheeses from older cows (4 or more lactations) than those from younger cows (third lactation). On the other hand, the proportions of butanoic acid and heptanoic acid were found to be lower. However, the differences, although significant were much smaller than those observed during lactation. Here, too, there is little information in the literature on the effect of parity in lactating cows on the VOC profiles of cheeses.

The effect of milk yield

The VOC profile of the model cheeses tended to change somewhat with milk yield. We found that only aldehydes, as a chemical family, were affected by milk productivity (Table 4), although none of the four individual aldehydes analyzed reached statistical significance. Only 5 of the other 51 individual VOCs were affected by milk yield: 2 alcohols, 2 fatty acids, and dihydrofuran-2-(3H)-one. In fact, the peak area values for octan-1-ol, 2-phenylethanol, pentanoic acid, and heptanoic acid increased with increasing milk yield, whereas dihydrofuran-2-(3H)-one decreased. It is worth mentioning that none of these VOCs, with the exception of octan-1-ol, were contemporaneously affected by lactation stage. As lactation stage has a strong effect on milk yield, which increases at the very beginning of lactation to peak during the second month of lactation and then decline during its final stages, the two effects are sometimes confused and often, in the literature, only the former is included in the statistical models. In order to fully understand the results, it should be borne in mind that as we estimated each effect we kept the other effects constant. With respect to octan-1-ol, a concordance of sign was observed between the effects of milk yield and of days in milk, so that some overlap between the two cannot be excluded. Again, the literature offers little information and more research is needed on this topic.

CONCLUSIONS

The results of the present study provide a better understanding of the relationships between dairy farming system and individual cow characteristics and volatile compounds composition of ripened cheese produced. This is especially important in mountain territories where the abandonment of traditional farming, as well as the environmental problems, may be responsible for modification of the sensory properties of typical traditional cheeses.

The results of our study also allow us to evaluate the differences in the VOC profiles of different modern dairy systems and, in particular, the use of total mixed ration, with or without silage, and with or without the addition of water to the resulting ration. Model cheeses made from milk produced by individual cows proved to be an effective method for studying the source of variation in the volatile organic profile as a function of individual traits of the animals and dairy farming systems.

The use of the SPME/GC-MS allowed several volatile organic compounds to be determined: a total 55 VOCs were detected in the model cheeses, including 14 alcohols, 13 esters, 11 free fatty acids, 8 ketones, 4 aldehydes, 3 lactones, 1 terpene and 1 pyrazine. Dairy system, days in milk, parity and milk yield of Brown Swiss cows affect the VOC profiles of individual model cheeses. These findings could be useful in differentiating a given cheese from other products on the market by its volatile profiling, to increase the economic value of milk and even to define traceability parameters on the basis of sensory related characteristics.

Based on literature we expect that the identified compounds could play a role in the formation of flavor, but further studies are necessary to determine this relationship because the presence of a compound does not mean it plays any role in flavor, even though it has been shown to play a role with other cheeses, or its variability may result in no detectable change in flavor. Moreover, the contribution of a volatile compound to the flavor and aroma of cheese can change according with the interactions with other molecules.

Methodological sources of variation (vat, date of analysis and order of injection) were often not significant, confirming good reproducibility of the cheese-making procedure and instrumental analysis. To the best of our knowledge, the present study is the first to attempt to explain variations in the volatile organic compound profiles of cheeses produced from milk of individual cows.

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TABLE AND FIGURE

Table 1. Main features characterizing the mountain dairy systems[1] sampled

	Traditional		Modern		
	Without AF	With AF	Hay + CF	TMR	
				Without silage	With silage
Farms, n	7	5	7	5	6
Total surface (ha UAA)	20.5	17.2	25.8	22.6	36.5
Dairy cow per herd, mean	21.7	36.4	40.5	65.6	62.3
Animal condition	tied	tied	loose	loose	loose
Milking	at stalls	at stalls	parlour	parlour	parlour
Feed administration	traditional	traditional	traditional	TMR	TMR
Major forage	hay	hay	hay	hay	hay
Major concentrate	compound	compound	compound	cereal mix	cereal mix
Silage	no	no	no	no	yes
Automatic feeders at manger	no	yes	no	no	no
Summer transhumance, % cows	83	10	66	20	4
Forages DM / Total DM, %	72	57	59	50	57
Diet NDF / DM, %	49	45	43	36	42
Diet CP / DM, %	13.5	14.1	15.3	16.3	16.1

[1] AF = automatic feeders at mangers to control individually concentrate distribution; CF = compound feed distributed at mangers; Without silage = water is added in the mixer wagon to favor mixing; UAA = utilized agricultural area.

Table 2 Descriptive statistics of milk yield, milk and cheese composition traits of sampled cows according to different dairy systems[1] (mean±SD).

	Traditional (tied, hay + CF)		Modern (pens with free animals and milking parlor)		
Item	Without AF	With AF	Hay + CF	TMR	
				Without silage	With silage
Sampled cows, n	35	25	35	25	30
Milk yield, kg×d^{-1}	19.1(±6.3)	23.5(±8.4)	25.1(±7.4)	30.2(±9.7)	26.8(±6.9)
DIM, d	156(±101)	167(±112)	158(±95)	169(±100)	163(±103)
Parity	2.5(±1.1)	2.4(±1.1)	2.3(±1.0)	2.1(±1.0)	2.4(±0.9)
Milk composition:					
Fat, %	4.2(±0.6)	4.3(±1.3)	4.1(±0.9)	4.5(±1.1)	4.7(±0.9)
Protein, %	3.6(±0.4)	3.6(±0.6)	3.7(±0.4)	3.8(±0.5)	3.8(±0.4)
Casein, %	2.8(±0.3)	2.8(±0.4)	2.9(±0.3)	3.0(±0.4)	2.9(±0.3)
Fat/Protein	1.20(±0.18)	1.22(±0.46)	1.10(±0.28)	1.17(±0.16)	1.25(±0.33)
SCS[2]	2.31(±1.81)	3.31(±2.41)	3.16(±1.54)	3.17(±1.60)	3.51(±1.97)
pH	6.64(±0.09)	6.63(±0.08)	6.65(±0.08)	6.64(±0.06)	6.61(±0.08)
Cheese composition:					
Fat, %	38.6(±4.7)	37.2(±4.6)	36.7(±3.9)	39.3(±4.4)	41.0(±6.0)
Fat in DM, %	48.0(±4.1)	45.4(±4.0)	46.1(±4.9)	48.6(±4.0)	50.5(±6.6)
Protein, %	26.8(±4.4)	26.0(±4.8)	28.9(±4.1)	26.9(±3.8)	25.0(±4.5)
Total solids, %	80.3(±4.8)	81.9(±5.5)	79.8(±4.7)	80.9(±3.8)	81.2(±4.0)
pH	5.17(±0.23)	5.17(±0.12)	5.17(±0.21)	5.12(±0.11)	5.25(±0.17)

[1] AF = automatic feeders at mangers to control individually concentrate distribution; CF = compound feed distributed at mangers; Without silage = water is added in the mixer wagon to favor mixing; [2] SCS = $\log_2$(SCC/100,000)+3.

Table 3. Descriptive statistics of volatile organic compounds (VOC) detected by SPME/GC-MS on ripened individual model cheeses[1].

VOC	RT[2](min)	n	Mean	SD	P5[3]	P95[3]	Skewness	Kurtosis
Σ ALCOHOLS		150	11.8	0.5	10.9	12.5	-1.1	2.4
Butan-1-ol	8.9	150	7.8	0.9	6.4	9.3	0.1	0.1
Butan-2-ol	5.4	145	7.6	1.7	5.6	11.1	0.9	0.1
Pentan-1-ol	12.4	150	5.8	0.6	4.7	6.8	-0.7	0.5
3-Methyl-1-butanol (Isopentanol)	11.0	150	10.8	0.6	9.8	11.7	-0.6	0.7
3-Methyl-3-buten-1-ol	12.4	150	5.3	0.7	3.9	6.3	-0.8	1.5
Hexan-1-ol	15.9	150	7.8	1.2	6.1	10.1	0.5	-0.6
Hexan-2-ol	11.5	147	6.5	1.0	4.8	7.9	-0.4	-0.4
Heptan-1-ol	19.2	150	5.1	0.5	4.1	5.8	-0.2	0.3
Heptan-2-ol	14.8	150	9.3	1.1	7.5	10.8	-0.3	-0.4
Octan-1-ol	22.4	148	4.5	0.6	3.6	5.6	0.6	0.1
2-Phenoxyethanol	37.8	149	7.1	1.2	5.6	9.3	0.9	0.5
2-Phenylethanol	32.1	150	8.1	0.7	7.1	9.3	0.1	-0.5
Butane-2,3-diol	23.6	134	8.5	1.4	6.0	10.7	-0.3	-0.7
2-Butoxyethanol	17.4	150	10.2	0.8	9.0	11.6	0.3	-0.5
Σ ALDEHYDES		150	7.3	0.6	6.1	8.1	0.3	0.3
2-Methylbutanal (Butyraldehyde)	3.0	150	4.7	1.1	2.9	6.2	0.0	-0.1
3-Methylbutanal (Isovaleraldehyde)	3.1	150	5.8	0.8	4.3	7.4	0.1	0.2
Hexanal (Caproic aldehyde)	7.0	148	6.4	0.8	4.8	7.5	-0.5	-0.4
Nonanal	17.2	143	5.6	0.6	4.5	6.5	-0.1	-0.4
Σ KETONES		150	11.7	0.8	10.1	12.7	-0.9	0.6

Butan-2-one (Methylacetone)	2.8	147	7.2	1.2	5.5	9.7	0.6	0.7
3-Hydroxy-2-butanone (Acetoin)	13.5	150	10.1	1.0	8.2	11.5	-0.4	-0.5
Pentan-2-one	4.2	150	10.5	1.1	8.5	11.9	-0.7	0.1
Hexan-2-one (Propylacetone)	6.9	148	7.1	0.9	5.3	8.4	-0.7	0.2
Heptan-2-one (Butylacetone)	10.1	150	10.5	0.9	8.8	11.9	-0.5	0.2
2-Nonanone	17.1	150	8.3	1.3	6.4	10.5	0.3	-1.1
8-Nonen-2-one	18.8	146	6.4	1.3	4.7	8.6	0.2	-1.1
Undecan-2-one	23.6	114	6.7	0.9	5.5	8.4	0.5	-0.2
Σ FFA		150	13.3	0.2	13.1	13.6	-0.5	0.5
Acetic acid (Acetate)	19.9	150	12.3	0.6	11.0	13.0	-0.8	0.6
Butanoic acid (Butyric acid)	24.8	150	12.4	0.3	11.8	12.9	-0.3	-0.1
2-Methylpropanoic acid (Isobutyric acid)	23.6	148	9.3	0.8	8.1	10.5	-0.3	0.3
3-Methylbutanoic acid (Isovaleric acid)	26.6	149	9.3	1.0	7.7	10.9	0.2	0.1
Pentanoic acid (Valeric acid)	29.0	149	7.2	0.6	6.0	8.1	-0.3	0.1
Hexanoic acid (Caproic acid)	31.2	150	11.1	0.5	10.4	11.9	0.1	0.2
Heptanoic acid	34.7	149	6.2	0.9	5.1	7.8	0.4	-0.6
Octanoic acid (Caprylic acid)	36.9	148	9.3	0.5	8.6	10.2	0.5	0.8
2-Ethylhexanoic acid	33.9	111	4.8	1.4	3.1	7.4	0.8	-0.6
Nonanoic acid	39.6	150	7.5	1.2	6.0	10.1	0.8	0.0
Decanoic acid (Capric acid)	43.1	149	7.6	0.5	6.6	8.4	0.0	-0.2
Σ ESTERS		150	10.7	0.7	9.6	11.8	-0.2	-0.2
Ethyl acetate	2.6	150	8.6	1.0	6.9	10.0	-0.2	-0.2
Ethyl (S)-2-hydroxypropanoate (Lactic acid ethyl ester)	15.5	150	7.9	1.1	5.9	9.5	-0.9	1.5

Ethyl butanoate (Butyric acid ethyl ester)	5.7	150	9.9	0.8	8.4	11.1	-0.5	-0.3
Ethyl-2-methylpropanoate (Ethyl isobutyrate)	4.0	108	4.7	1.2	3.0	7.4	0.9	0.7
Ethyl 2-methylbutanoate (Ethyl 2-methylbutyrate)	6.1	102	4.7	1.1	3.1	7.0	0.8	0.4
Ethyl 3-methylbutanoate (Ethyl isovalerate)	6.5	101	5.1	1.2	3.6	7.6	1.0	0.6
Ethyl pentanoate (Ethyl valerate)	8.6	145	5.2	0.9	3.9	6.6	0.0	-0.7
Ethyl benzene	8.5	149	6.7	0.8	5.4	7.9	0.1	-0.1
Ethyl hexanoate (Ethyl caproate)	11.9	150	9.1	1.0	7.4	10.7	0.0	-0.5
Ethyl heptanoate (Heptanoic acid ethyl ester)	15.3	81	4.0	0.7	3.0	5.2	0.1	-0.1
3-Methylbutyl butyrate (Isoamyl butyrate)	12.9	147	5.0	0.7	3.9	6.2	0.3	-0.1
Ethyl octanoate (Ethyl caprylate)	18.6	150	6.5	1.0	5.0	8.2	0.1	-0.3
Ethyl decanoate (Ethyl caprate)	24.8	103	5.7	0.7	4.3	6.8	-0.5	0.1
Σ OTHERS VOC		150	7.3	0.4	6.7	7.8	0.1	-0.6
LACTONES								
Dihydrofuran-2(3H)-one (Butyrolactone)	24.3	150	5.2	0.5	4.4	6.0	0.2	0.0
6-Propyloxan-2-one (δ-octalactone)	33.5	150	5.5	0.5	4.7	6.2	-0.1	-0.2
6-Pentyloxan-2-one (δ-decalactone)	39.0	150	6.6	0.4	5.9	7.1	-0.3	-0.2
TERPENE								
Limonene	10.5	150	4.3	0.7	3.2	5.4	0.2	-0.5
PYRAZINE								
2,6-Dimethylpyrazine	15.0	133	4.7	1.0	3.5	6.6	1.0	0.2

[1] Expressed as natural logarithm of peak area normalized to total chromatography area; [2] RT = retention time; [3] P5 = 5th percentile and 95th percentile.

Table 4. Effects of dairy system, and the incidence of herd/test date within dairy system (HTD), and effects of DIM, parity, milk yield, vat, date of analysis (date) and order of injection (inj) on the sums of VOCs per chemical family variance on total variance.

	P-value		*P*-value						
Chemical family	Dairy system *P*-value	HTD[1] %	DIM	Parity	Milk yield	Vat	Date	Inj	RMSE[2]
Σ Alcohols	0.28	37	0.07	0.74	0.70	0.39	0.59	0.31	0.35
Σ Aldehydes	0.25	24	0.54	0.63	0.042	0.27	0.88	0.08	0.48
Σ Ketones	0.93	43	0.09	0.31	0.30	0.15	0.72	0.40	0.61
Σ FFA	0.40	17	0.73	0.22	0.67	0.62	0.92	0.79	0.15
Σ Esters	0.016	20	0.12	0.95	0.37	0.31	0.042	0.47	0.54
Σ Other VOC	0.07	22	0.15	0.89	0.35	0.21	0.049	0.11	0.27

[1] Herd/Test day effect expressed as proportion of variance explained by herd/test date calculated by dividing the corresponding variance component by the total variance; [2] RMSE = root mean square error.

Table 5. Dairy systems least square means (LSM) of the quantity of the volatile organic compounds (VOC) with significant orthogonal contrasts.

	Dairy system (LSM)[1]					Contrasts (*P*-value)			
Chemical family/VOC	Traditional (tied, hay + CF)		Modern (pens with free animals and milking parlor)			Traditional *vs* modern farms	Automatic feeder effect[2]	TMR effect[3]	Silage effect[4]
	Without AF (n = 35)	With AF (n = 25)	Hay + CF (n = 35)	TMR					
				Without silage (n = 25)	With silage (n = 30)				
Alcohols									
Butan-1-ol	7.88	7.65	7.42	8.45	7.33				0.003
Pentan-1-ol	5.64	5.73	5.67	6.34	5.52				0.003
3-Methyl-1-butanol	10.37	10.59	10.74	11.18	11.00	0.006			
Hexan-1-ol	7.62	7.62	7.01	8.91	7.79			0.004	0.045
Heptan-1-ol	4.99	5.03	5.01	5.46	4.88				0.014
2-Phenylethanol	7.77	7.84	7.99	8.33	8.38	0.045			
Octan-1-ol	4.30	4.59	4.10	4.99	4.32			<0.001	<0.001
Aldehydes									
3-Methylbutanal	5.57	5.91	6.05	5.48	6.21				0.035
FFA									
Acetic acid	12.26	12.14	12.57	12.00	12.24			0.037	
Esters									
Ethyl (S)-2-hydroxypropanoate	7.79	7.90	7.42	8.72	7.68				0.048
Ethyl butanoate	9.97	9.82	9.46	10.50	9.93			<0.001	0.038

Ethyl pentanoate	5.01	5.16	4.78	6.12	5.25			0.003	0.019
Ethyl hexanoate	9.05	9.10	8.50	9.69	9.13			<0.001	
Ethyl octanoate	6.57	6.85	6.07	7.03	6.60			0.004	
Lactones									
Dihydrofuran-2(3H)-one	4.99	5.18	5.23	5.43	5.21	0.042			
6-Pentyloxan-2-one	6.33	6.45	6.58	6.74	6.56	0.020			
Terpene									
Limonene	4.27	4.44	4.62	4.63	3.94				0.015
Pyrazine									
2,6-Dimethylpyrazine	4.08	5.07	4.65	4.41	4.80		0.044		

[1] Hay+CF = hay and compound feed; AF = automatic feeders; [2] Contrast between traditional farms with compound feed administered in the mangers with hay *vs* traditional farms with compound feed administered in the mangers through automatic feeders; [3] Contrast between modern farms with hay and compound feed administered in the mangers *vs* modern farms using total mixed rations; [4] Contrast between modern farms using TMR without silages *vs* TMR with silages.

Table 6. Days in milk least square means (LSM) of the quantity of the volatile organic compounds (VOC) with significant orthogonal contrasts.

Chemical family/VOC	Days in milk (LSM)							Contrasts (*P*-value)		
	≤50 (n = 20)	51-100 (n = 28)	101-150 (n = 38)	151-200 (n = 15)	201-250 (n = 12)	251-300 (n = 22)	>300 (n = 15)	Linear	Quadratic	Cubic
Alcohols										
Butan-2-ol	7.01	7.30	7.39	7.14	8.44	8.31	8.42	0.010		
3-Methyl-3-buten-1-ol	5.03	5.26	5.54	5.27	5.22	5.15	5.71			0.006
Octan-1-ol	4.66	4.29	4.46	4.44	4.45	4.72	4.20			0.007
2-Butoxyethanol	10.14	10.18	10.29	10.25	10.09	9.91	10.31			0.033
FFA										
Butanoic acid	12.57	12.54	12.43	12.23	12.38	12.30	12.24	0.002		
Esters										
Ethyl-2-methylpropanoate	3.95	4.20	4.48	4.56	4.44	4.37	5.50	0.016		
Ethyl-3-methylbutanoate	4.33	4.36	4.89	5.53	4.52	5.41	5.78	0.020		
Lactones										
6-Propyloxan-2-one	5.50	5.56	5.67	5.54	5.21	5.18	5.24	0.012		0.039
6-Pentyloxan-2-one	6.52	6.57	6.69	6.54	6.47	6.36	6.59		0.021	
Pyrazine										
2,6-Dimethylpyrazine	4.32	4.80	4.69	4.98	4.86	4.37	4.18		0.012	

Table 7. Parity least square means (LSM) of the quantity of the volatile organic compounds (VOC) with significant orthogonal contrasts.

Chemical family/VOC	Parity (LSM)				Contrasts (*P*-value)		
	1 (n = 35)	2 (n = 54)	3 (n =38)	≥ 4 (n =23)	1*vs* ≥2	2*vs* ≥3	3*vs* ≥ 4
Alcohols							
Octan-1-ol	4.43	4.64	4.31	4.45		0.028	
FFA							
Butanoic acid	12.50	12.40	12.35	12.29	0.016		0.015
Heptanoic acid	6.34	6.04	6.59	6.34		0.026	

Table 8. Linear regression (Regr[1]) with daily milk yield of the quantity of the volatile organic compounds (VOC) with a significant effect of milk yield.

Chemical family/VOC	Milk yield		
	Regr/SD	Regr/RMSE	*P*-value
Alcohols			
Octan-1-ol	0.008	0.021	0.010
2-Phenylethanol	0.015	0.057	0.016
FFA			
Pentanoic acid	0.024	0.068	0.011
Heptanoic acid	0.021	0.035	0.019
Lactone			
Dihydrofuran-2(3H)-one	-0.012	-0.063	0.011

[1] The interval unit of the regression coefficients calculated by dividing the regression coefficient of daily milk yield by the corresponding SD and root means square error (RMSE).

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SECOND CHAPTER

PTR-ToF-MS: a high-throughput and innovative method to study the influence of dairy system and cow characteristics on the volatile compound fingerprint of cheeses

M. Bergamaschi[*,†], F. Biasioli[†], L. Cappellin[†], A. Cecchinato[*], C. Cipolat-Gotet[*], A. Cornu[‡,§], F. Gasperi[†], B. Martin[‡,§], and G. Bittante[*]

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[*]Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell’Università 16, 35020 Legnaro (PD), Italy

[†]Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach (FEM) Via E. Mach 1, 38010 San Michele all'Adige (TN), Italy

[‡]INRA, UMR 1213 Herbivores, F-63122 Saint Genès Champanelle, France

[§]Clermont Université, VetAgro Sup, BP 10448, F-63000 Clermont-Ferrand, France

ABSTRACT

The aim of this work was to study the effect of dairy system and individual cow-related factors on the volatile fingerprint of a large number of individual model cheeses analyzed by Proton Transfer Reaction Time of Flight Mass Spectrometry (**PTR-ToF-MS**). A total of 1,075 model cheeses were produced using milk samples collected from individual Brown Swiss cows reared in 72 herds located in mountainous areas of Trento province (Italy). The herds belong to 5 main dairy systems ranging from traditional to modern and the cows presented different daily milk yields (24.6±7.9 kg×d^{-1}), stages of lactation (199±138 days in milk) and parities (2.7±1.8). The PTR-ToF-MS revealed 619 peaks, of which the 240 most intense were analyzed, and 61 of these were tentatively attributed to relevant volatile organic compounds on the basis of their fragmentation patterns and data from the literature. Principal component analysis (**PCA**) was used to convert the multiple responses characterizing the PTR-ToF-MS spectra into 5 synthetic variables representing 62% of the total information. These principal components were related to groups of volatile compounds tentatively attributed to different peaks and used to investigate the relationship of the volatile compound profile obtained by PTR-ToF-MS to animal and farm characteristics. Lactation stage is related to 4 principal components which brought together 52.9% of the total variance and 57.9% of the area of analyzed peaks. In particular, two principal components are positively related to peaks tentatively attributed to aldehydes and ketones, and negatively related to alcohols, esters and acids, which displayed a linear increase during lactation. The second principal component was affected by dairy system; it was higher in the modern system in which cows receive total mixed rations. The third principal component was positively related to daily milk production. In summary, we report the first application of this innovative, high throughput technique to study the effects of dairy system and individual animal factors on volatile organic compounds of model cheeses. Individual cheese-making procedures together with this spectrometric technique open new avenues for genetic selection of dairy species with respect to both milk and cheese quality.

Keywords: volatile compound fingerprint; cheese smell; PTR-ToF-MS; new phenotypes; dairy system.

INTRODUCTION

Flavor is an important driver of cheese purchase and consumption (Drake et al., 2008; Childs and Drake, 2009). Food quality assessment now requires low-cost, rapid, non-destructive testing techniques that can be applied to small samples (Rodriguez-Saona et al., 2006). Analytical tools that enable qualitative fingerprinting of a wide range of dairy products are particularly attractive, and among these there is growing interest in direct injection spectrometric techniques, particularly Proton Transfer Reaction Time of Flight Mass Spectrometry (**PTR-ToF-MS**) (Biasioli et al., 2011). PTR-ToF-MS is based on the ionisation of Volatile Organic Compounds (**VOCs**) with a proton affinity higher than water upon reaction with hydronium ions (**H_3O^+**) and subsequent detection by a high resolution time of flight mass analyzer. In particular, this apparatus is composed by: a hollow cathode discharge ion source in which H_3O^+ reagent ions are produced from water vapor used as reagent gas; a drift tube where the VOCs to be analyzed are continuously injected and ionized by proton-transfer reaction with H_3O^+ ions; and a quadruple mass analyzer where the ions are detected. Unlike other techniques with better identification capability, such as gas chromatography-mass spectrometry (**GC-MS**), PTR-ToF-MS allows direct injection of the headspace without extraction or pre-concentration steps, and has a shorter analysis time (only a few seconds per sample) and greater sensitivity. Like other headspace gas chromatographic analyses, it is also non-destructive, doesn’t require chemicals, and permits on-line monitoring of VOCs, while the resulting spectral information can provide a very detailed description of samples, which is useful for characterizing food quality and typicality (Biasioli et al., 2011; Cappellin et al., 2012a). For example, PTR-ToF-MS has applications in food science and technology, dairy products in particular, for monitoring VOC production in cheese during ripening (Aprea et al., 2007; Soukoulis et al., 2010; Fabris et al., 2010), correlating the cheese volatile profile with the sensory characterization of cheese flavor and odor (Biasioli et al., 2006), and for investigating the geographical origin and the typicality of cheese (Galle et al., 2011).

Cow management and breed, animal genetics, season, and technological factors influence milk characteristics and, as a consequence, cheese quality and sensory properties (Coulon et al., 2004; Bittante et al., 2011a and 2011b). Studies have been carried out on the influence of dairy cow feeding on the sensory properties of cheese (Martin and Coulon, 1995b; Cornu et al., 2009), but little is known about the effects of individual animal-related factors on cheese flavor.

This work was part of a large research project (Cowplus Project) aimed at developing new phenotypes of the dairy cow depicting the relationships between animal characteristics, milk quality, cheese-making aptitude and cheese sensory traits. In this project, Cipolat-Gotet et al. (2013) proposed a method to produce model cheeses from individual milk samples that has proven to be particularly useful in determining the cheese-making aptitude of milk and in allowing for the study of both individual phenotypic sources of variation (days in milk, parity, milk yield) and environmental (dairy system) factors on a large scale. The individual model cheese-manufacturing process was also used to estimate genetic parameters of the cheese-making properties of milk and of daily cheese production through direct measurement (Bittante et al., 2013) or Fourier-Transform Infrared (**FTIR**) Spectrometry prediction (Ferragina et al., 2013 and 2015; Bittante et al., 2014). Cipolat-Gotet et al. (2013) applied the model cheese-making procedure to 1,264 cows from 85 herds to study cheese yield as well as the recovery of individual milk components in the curd and also to quantify the ratios between the curd contents of fat, protein, dry matter, and energy, versus the content of the corresponding nutrient in the processed milk. These authors found a significant effect of herd and individual animal factors.

As part of the same Cowplus Project, Bergamaschi et al. (2015) showed that this individual cheese-making procedure could also be used for qualitative studies of individual cheeses. In a pilot study on a sample of 150 individual model cheeses obtained from 30 herds, they studied the VOC profile of ripened cheeses using solid phase micro extraction GC-MS (**SPME/GC-MS**) and characterized the VOC profile of ripened cheeses: 55 compounds were identified and an exploitable variability according to dairy system and individual animal characteristics was observed. Moreover, SPME/GC-MS analysis of selected cheeses offers the possibility to support PTR-ToF-MS fingerprint analysis in a large number of samples (Cappellin et al., 2012a).

The aims of this work were: i) to study the potential of PTR-ToF-MS for rapid characterization of cheeses on the basis of their volatile fingerprint, and ii) to analyze the effects of dairy system and individual cow characteristics on the volatile compounds of cheese using a large number of individual model cheeses.

MATERIALS AND METHODS

Field data

To carry out this study, also part of the Cowplus Project, a total of 1,075 Italian Brown Swiss cows reared in 72 herds located in Trento Province (Italy) were selected. Fifteen cows were chosen randomly from each herd and sampled once on the same day: the herds were sampled year round to cover all seasons and rearing conditions. The sampled cows presented different milk yields (24.6±7.9 kg×d^{-1}), stages of lactation (199±138 days in milk; DIM) and parities (2.7±1.8). The sampling procedure is described in detail by Cipolat-Gotet et al. (2012) and Cecchinato et al. (2013). The production environments, which varied in terms of production level, destination of milk, modernization of structures, and management, is discussed by Sturaro et al. (2013). The selected dairy farms of this Alpine area (825±334 m a.s.l.) utilized different dairy management strategies, with variations in facilities, feedstuff distribution, use of silages, and transhumance to temporary summer Alpine pastures.

Traditional dairy farms were those dairy systems utilizing small barns, tied animals milked at the stall, calving concentrated mainly in autumn, transhumance of cows and heifers to Alpine pastures for the summer, and feed mainly composed of hay and compound feed. These were either with or without automatic feeders at the manger (Traditional with AF, 9 farms, or Traditional without AF, 12 farms, respectively). Modern dairy farms were characterized by features of intensive systems: larger, modern facilities, loose animals, milking parlors, year round calving, use of total mixed rations (**TMR**), with or without maize silage (Modern TMR with silage, 7 farms, or Modern TMR without silage, 14 farms, respectively). Intermediate dairy systems were those farms with modern facilities but traditional feeding management composed of hay and compound feed (Modern Hay+CF, 30 farms).

Collection, refrigeration, transport, and storage of milk samples were standardized with the aim of minimizing differences among herds (corresponding to sampling dates). After collection, all milk samples (without preservatives) were immediately refrigerated (4°C), and processed into cheeses within 20 h at the Cheese-making Laboratory of the Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE) of the University of Padova (Legnaro, Padova, Italy). Fat, protein and casein percentages were determined using a MilkoScan FT6000 apparatus (Foss Electric A/S, Hillerød, Denmark). The pH was measured using a Crison Basic 25 electrode (Crison, Barcelona, Spain). Somatic cell count was obtained from the Fossomatic FC counter (Foss) then converted to SCS by logarithm transformation (Ali and Shook, 1980). Information on the cows (DIM, parity, and milk yield) was provided by the Breeders Federation of Trento Province (Trento, Italy).

Descriptive statistics of milk composition traits are summarized in Table 1. Milk composition reflected the good milk quality typical of Brown Swiss cows (Cecchinato et al., 2015).

The modern dairy systems produced more milk than the traditional systems. In particular, modern farms with TMR without silage had higher milk yields (30.0±8.4 kg×d^{-1}) than the traditional farms without automatic feeder (19.5±7.3 kg×d^{-1}). The cows reared in traditional dairy farms without automatic feeder produced milk with lower fat (4.2±0.7 %) and protein (3.7±0.4%) contents than the cows reared in the modern dairy systems with TMR and with maize silage (5.0±1.2% fat and 3.9±0.4% protein, respectively).

Cheese-making procedure

Model cheeses were manufactured using raw milk from individual cows according to the method described by Cipolat-Gotet et al. (2013) that resemble the industrial process often used to produce full fat, two-months ripened cheese. No adjustment of the procedure was applied to take into account the differences in composition of milk samples because the main aim of the project was to fully analyze the individual variability. Briefly, 1,500 mL of milk were heated, cultured, and mixed with rennet solution to a final concentration of 51.2 IU/L. The resulting curd was cut, drained, shaped in wheels, pressed, salted, and weighed. The small wheels produced for the purpose of this study were ripened for 60 days than weighed and analyzed. The fat, protein, and dry matter contents of the whole cheeses were measured using a FoodScan apparatus (Foss Electric A/S, Hillerød, Denmark). The pH was measured at 20°C by inserting a Crison Basic 20 electrode (Crison, Barcelona, Spain) into each model cheese. A cylindrical sample 1.1 cm in diameter and about 3.5 cm in height was taken from the center of each model cheese and conserved at -80°C until VOC analysis.

Descriptive statistics of the cheese compositions are presented in Table 1. The dairy system did not influenced statistically the gross composition of model cheeses even if, numerically, model cheeses from the modern dairy systems were characterized by a slightly higher level of fat and protein content than those from the traditional systems, and, within the modern dairy systems, the farms using TMR and maize silage exhibited a slightly higher fat content. In the 5 mountain dairy systems, cheese pH ranged from 5.13±0.20 (Modern Hay+CF) to 5.21±0.14 (Modern TMR with silage). Also this difference was not significant.

PTR-ToF-MS

The headspace of the 1,075 model cheeses was analyzed using a commercial PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria) and a modified version of the method described in detail by Fabris et al. (2010). Briefly, cheese samples were thawed and kept at room temperature (about 20°C) for 6 h, then 3 g sub-samples from each sample were put into glass vials (20 ml, Supelco, Bellefonte, USA) and capped by PTFE/Silicone septa (Supelco). About 75 samples chosen randomly from the 1,075 sample set were measured every day. Conditions in the drift tube were as follows: drift tube temperature 110°C, drift pressure 211 Pa, drift voltage 500 V. Internal calibration and peak extraction was performed according to a procedure described by Cappellin et al. (2011), which allowed the identification of 619 peaks and was in most cases sufficient for sum formula identification. Absolute headspace VOC concentration expressed as ppb_v (parts per billion by volume) was estimated using the formula described by Lindinger et al. (1998) with a constant reaction rate coefficient of the proton transfer reaction of 2×10^{-9} cm^3/s. This method has been shown to be viable for absolute VOC headspace concentration determination (Cappellin at al., 2012b). The use of the same reaction rate coefficient produce a systematic error which is typically negligible and the estimation accuracy can be improved if the actual reaction rate coefficient is known. The relative VOC concentrations between samples is not affected by the value of the reaction rate coefficient.

Statistical Analysis

The dataset comprised 1,075 model-cheeses (records) and 619 spectrometric peaks (variables) corresponding to the headspace concentrations of all identified peaks with a defined *m/z* value (dataset A), which total spectral intensity was 49,723,176 ppb$_v$ (Table 2). All peaks below a threshold of 1 ppb$_v$ (302 peaks) were removed from the mass spectra (dataset B) and 77 masses (42% of the total mass spectra intensity) associated with the PTR-ToF-MS ion source and water clusters (possible interference) were excluded before any statistical analysis (dataset C).

Given that the distribution of all spectra peaks showed a strong positive skewness, the data were transformed: the fraction of each peak plus one was multiplied by 10^6 and expressed as a natural logarithm to obtain a Gaussian-like data distribution. All transformed records that were 3 standard deviations or more outside the mean were considered outliers and substituted by a mean plus 3 standard deviations value before any statistical processing.

Data standardization was performed within each day of analysis (15 days) to equalize data variability resulting from the effect of this environmental factor on PTR masses; this was also confirmed by a Levene's test (data not shown). Multivariate data treatment (principal component analysis) was applied to the standardized data using Statistica 7.1 (StatSoft, Paris, France) to synthesize the information and to provide a new set of principal components (**PC**).

Dependent variables were analyzed using the mixed model procedure of SAS (SAS Inst. Inc., Cary, NC) according to the following model:

$$Y_{ijklmno} = \mu + \text{dairy system}_i + \text{HTD}_j(\text{dairy system})_i + \text{DIM}_k + \text{parity}_l + \text{milk}_m + \text{vat}_n + e_{ijklmno}$$

where $y_{ijklmno}$ is the observed trait (first five principal components and 240 spectrometric peaks); μ is the overall mean; dairy system$_i$ is the fixed effect of the *i*th dairy systems (i = 1 to 5); HTDj(dairy system)$_i$ is the random effect of the *j*th herd/test date (j = 1 to 72) within *i*th dairy systems; DIM$_k$ (days in milk, interval from calving to milk sampling) is the fixed effect of the *k*th classes of days in milk (k = 1 to 7, class 1: ≤50 d, class 2: 51 to 100 d, class 3: 101 to 150 d, class 4: 151 to 200 d, class 5: 201 to 250 d, class 6: 251 to 300, class 7: >300 d); parity$_l$ (order of parity) is the fixed effect of the *l*th parity (l = 1 to 4 or more); milk$_m$ (daily milk yield, kg×d^{-1}) is the linear covariate of daily milk production; vat$_n$ is the fixed effect of the *n*th experimental vat used for model cheese production (n = 1 to 15); and e$_{ijklmno}$ is the residual random error term ~N (0, σ^2). The effect of dairy system was tested using HTD within dairy system as the error term, while other fixed effects were tested on the residual. In addition, orthogonal contrasts were used to test the effects of dairy system and parity.

RESULTS

Volatile organic compound profiling of model cheeses analyzed by PTR-ToF-MS

A large number of peaks (619) describing volatile organic compounds (Table 2) were obtained from headspace PTR-ToF-MS analysis of individual model cheeses. Data compression was performed considering the 317 most intense peaks with spectrometric area greater than 1 ppb$_v$ which represented 99.7% of the total spectral intensity (Table 2). After elimination of 77 possibly interfering ions, 240 peaks were still available.

To aid spectra interpretation (Supplementary Table 1), the fragmentation pattern of 61 relevant compounds, representing 78.0% of the total spectral intensity of the compressed data set without interfering ions (Table 2), were retrieved from available GC-MS data on the same model cheeses (Bergamaschi et al., 2015) or from the literature (Fabris et al., 2010; Soukoulis et al., 2010; Galle et al., 2011). Where unavailable, data from the fragmentation pattern was used to identify measured peaks.

Descriptive statistics of the PTR peaks detected in the model cheeses are in the supplementary material section (Supplementary Table 2). The most intense peaks detected were at *m/z* 43.018, 43.054, 61.028, and 45.033 with a mean value in natural logarithm of 11.3, 11.1, 11.0, and 10.9, respectively (Supplementary Table 2). We attributed these spectrometric fragments to alkyl fragments, acetic acid, and ethanal.

New synthetic variables associated with cheese VOCs

Multivariate data treatment was performed to convert multiple responses (240 masses) into a small number of variables. All the correlation coefficients between the first 5 principal components and the 240 individual masses are shown in the supplementary data section (Supplementary Table 3). The 5 first principal components together explained 61.5% of the total variability of the original variables; the major positive and negative peaks of the principal components are shown in Table 3. The first principal component explained 28.3% of the total original variance. The other 4 principal components explained 10.9%, 8.6%, 7.6%, and 6.1%, respectively.

The effects of farming system

Results from an ANOVA, reported in Table 4, show the effects of dairy system, herd test/date, and individual cow-related factors on the 5 extracted principal components. The effect of individual herds within dairy system was greater for both PC3 and PC5 because the variance explained by HTD represented 53% and 48% of total variance, while for PC2, PC1 and PC4 it represented 31%, 20%, and 15%, respectively.

The second principal component differed significantly according to dairy system (Table 4): the modern dairy systems with TMR had higher values than the other 3 farming systems (Figure 1).

Moving to the analyses of individual PTR-ToF-MS, supplementary Table 4 shows that 57 out of 240 peaks were affected by the dairy system. Differences between the two traditional *vs* the three modern dairy systems were evident in only 2 of these peaks. In both cases, the cheeses from traditional farming systems had higher peaks than cheeses from modern systems. Of the traditional farms, those distributing compound feed in the mangers with automatic feeders displayed higher contents of 3 peaks (Supplementary Table 4). Of these, we identified methanol at *m/z* 33.034.

Within the modern dairy systems, the method of feedstuff distribution had a profound effect on the PTR-ToF-MS profile of model cheeses: those produced from farms using TMR were characterized by higher concentrations of 23 spectrometric peaks and lower concentrations of 12 peaks compared with farms where cows were fed conventionally (Supplementary Table 4). Of the 35 peaks affected by the use of TMR, 5 could be tentatively attributed to ethanal (*m/z* 45.033), acetic acid (*m/z* 60.021), butan-1-ol (*m/z* 75.080), and terpene fragments (*m/z* 81.070)

Among the dairy systems using TMR, those that added silages, as opposed to those adding just water to moisturize the ration and avoid de-mixing, presented significantly ($P < 0.05$) lower concentrations of 17 peaks (Supplementary Table 4). In particular, hexan-1-ol, hexan-2-ol (*m/z* 85.101), ethyl butanoate, ethyl-2-methylpropanoate, hexanoic acid (*m/z* 117.091), ethyl hexanoate, and octanoic acid (*m/z* 145.123) were successfully identified. These compounds were mainly alcohols, acids and esters. In the dairy systems without silage, we found higher concentrations of only one, unknown, peak at *m/z* 65.018 (Supplementary Table 4).

The effect of animal characteristics

The second and fourth principal components exhibited a significant ($P < 0.001$) linear increase during lactation (Table 4). The patterns along the lactation obtained by plotting least squares means of the second and fourth principal components for days in milk are presented in Figure 2, b and c respectively.

Moving to individual peaks, we found just over half the PTR-ToF-MS peaks (139 out of 240) were affected by days in milk (Supplementary Table 5). Among the 75 peaks showing linear increases throughout lactation, we identified methanethiol (*m/z* 49.011), the alcohols butan-1-ol and butan-2-ol (*m/z* 57.070; 75.080), the acids hexanoic acid (*m/z* 99.081), nonanoic acid (*m/z* 159.138) and the esters ethyl butanoate, ethyl-2-methyl propanoate (*m/z* 117.091), ethyl pentanoate, ethyl-2-methylbutanoate, ethyl-3-methylbutanoate (*m/z* 131.107). In contrast, 34 peaks showed linear decreases during lactation, and of these we identified acetonitrile (*m/z* 42.034), ethanal (*m/z* 45.033), propan-2-one (*m/z* 59.049), 3-methylbutanal, 2-methylbutanal, heptanal, nonanal, pentan-2-one (*m/z* 87.080).

The quadratic pattern was exhibited by 54 peaks, and of these ethanal (*m/z* 45.033), 2-methylbutanal, 3-methylbutanal, heptanal, nonanal, penta-2-one (m/z 69.033), hexan-1-ol, hexan-2-ol (*m/z* 85.101), phenol (*m/z* 95.049), hexanoic acid (*m/z* 99.081), ethyl butanoate, ethyl-2-methyl propanoate (*m/z* 117.091), 1-octen-3-one (*m/z* 127.112) were identified. All these substances showed a tendency to increase during the initial stage of lactation, followed by a more stable content until mid-lactation and a rapid decrease thereafter.

Finally, 12 masses showed more complex trends during lactation (cubic pattern), and among these we tentatively identified 3-methyl-1-butanol, 3-methyl-3-buten-1-ol, pentan-1-ol (*m/z* 71.086) and butan-2,3-dione (*m/z* 87.044).

Parity of cows also affected the headspace PTR-ToF-MS profile measured on individual model cheeses. In particular, it affected 22 out of 240 individual peaks compared with the previously examined sources of variation (dairy system and DIM). Cheeses from primiparous cows had higher concentrations of 7 PTR-ToF-MS peaks than cheeses from multiparous cows (Supplementary Table 6). Four of these peaks, as well as 6 peaks not affected by cows at first parity, were also different in mature cows (3rd parity) compared with older cows (4 or more parities). Of these, *m/z* 49.011 was attributed to methanethiol.

Moreover, we found that the headspace of cheeses from second-lactation cows have higher quantities of 3 masses than cheeses from cows with 3 or more calvings (Supplementary Table 6).

Cow productivity (daily milk yield corrected for DIM and parity, as well as dairy system and herd) also affected the volatile fingerprint of individual model cheeses. The third principal component increased with the increase in daily milk yield (Table 4). The concentrations of 23 peaks increased with increasing milk yield, while 8 peaks decreased (Supplementary Table 7). Among the masses that increased, we identified *m/z* 85.065 as pentanoic acid; *m/z* 86.072 as pentanal; *m/z* 87.080 as 2-methylbutanal, 3-methylbutanal, and pentan-2-one; *m/z* 101.097 as hexan-1-one, hexan-2-one, and hexanal; *m/z* 115.112 as heptan-2-one; *m/z* 129.127 as octan-1-one and *m/z* 143.143 as nonan-2-one (Supplementary Table 7).

DISCUSSION

PTR-ToF-MS data

The PTR-ToF-MS output is composed of hundreds of reciprocally related masses and the absence of a pre-separation procedure results in different compounds or fragments being retrieved as the same peak (Aprea et al., 2007; Biasioli et al., 2011; Cappellin et al., 2011). An efficient way of analyzing this kind of data is multivariate analysis (e.g. principal component) carried out in two steps: i) data compression to reduce the size of the data set without significant loss of information, and ii) identification of peaks that could help biological interpretation of the available information. The extracted principal components can be used to investigate relationships with the original variables (peaks/cheese VOCs), and the values (scores) of these factors can be treated as a new phenotype for further analysis. These synthetic indicators of VOC profiles were used to study dairy systems and sources of variation in individual dairy cows. The principal components were positively and negatively related to groups of VOCs, in many cases with same catabolic pathway, chemical family, or odor. Nevertheless, it was sometimes difficult to interpret the exhibited trends especially when the masses most related to the common components were unknown.

In agreement with results regarding the cheese-making properties of milk (Cipolat-Gotet et al., 2013 and 2014) obtained from individual model cheese manufacture, we found that the effect of vat was not significant for any of the principal components extracted, confirming the good reproducibility of the technique when utilized to study cheese quality traits (Table 4).

The effects of dairy systems and herds within the dairy system

Within farming systems, the composition of the basal diet is important for establishing the nutritional and sensory proprieties of milk and cheese (Couvreur et al., 2007; Romanzin et al., 2013; Bovolenta et al., 2014). The differences found in the VOCs of model cheeses from the 5 different dairy systems are clearly summarized by PC2 (Figure 1), which reflects milk characteristics and also the production conditions of the dairy farms. There were no significant variations in cheese gross composition according to farming system, which means that the differences in VOCs cannot be directly linked to cheese gross composition.

In this study we found no differences between the two traditional *vs* the three modern dairy systems when the PCs were analyzed (Table 4). The use of TMR in the modern farming systems resulted in greater differences in the cheese VOC profiles: the differences between the modern dairy systems with hay and compound feed administered separately (with a greater proportion of forages in the diets) and the modern systems with TMR (using more concentrates) were linked to the esters, acids and terpenes. It is well known that TMR optimizes rumen function and improves cow production efficiency in terms of dry matter intake and daily milk yield (Tafaj et al., 2007; McBeth et al., 2013). Caccamo et al. (2012) examined the effect of TMR on production and milk composition traits and found that the starch content of the ration was the most important element related to the increase in milk yield, fat, and protein contents over the cows' entire lactation.

Among the masses most positively correlated to PC2 (Table 3), we found *m/z* 117.091 (tentatively attributed to ethyl butanoate and ethyl-2-methylpropanoate) and *m/z* 145.123 (ethyl hexanoate). These compounds derive from the condensation between free fatty acids and alcohols (Curioni and Bosset, 2002) and are responsible for fruity-floral notes in cheese flavor (Frank et al., 2004; Cornu et al., 2009; Sympoura et al., 2009). Other VOCs significantly affected by TMR were terpenes, which can be transferred from the plant to the milk and have been listed as cheese odorants, having their fresh, herbaceous odor (Viallon et al., 2000; Curioni and Bosset, 2002; Carpino et al., 2004). The terpenes ingested by cows have been suspected of having an effect on rumen microflora. In particular, protein degradation and volatile fatty acid production may, due to nutrient flow out of the rumen, result in milk and cheese compositional changes, and consequently variations in sensory proprieties (De Noni and Battelli, 2008; Tornambé et al., 2008).

In our study, maize silage was the main component of the forage proportion of TMR in the modern dairy systems using silages. Several studies have shown that the use of maize silage *vs* grass generally leads to cheeses that are less valued because their flavor is less developed (Coulon et al., 2004; Hurtaud et al., 2004; Martin et al., 2004). In contrast, no significant differences were reported between cheeses made from milk produced by cows fed with hay and those from cows fed with grass silage (Verdier-Metz et al., 2005). Previous studies reported lower concentrations of alcohols from C_2 to C_6, such as hexan-1-ol and hexan-2-ol (*m/z* 85.101), in the VOC profile of milk from cows fed diets based on hay than those including maize silage (Kalač, 2011). Acids originating from plants or produced during ensiling, and alcohols formed in silage can produce various esters, such as ethyl butanoate and ethyl-2-methylpropanoate: in this work they were related to PC2 and statistically significantly influenced ($P < 0.05$) by the use of silage (*m/z* 117.091). We found other esters and acids correlated to PC2 and more significantly ($P < 0.05$) affected by the use of silage, such as ethyl hexanoate and octanoic acid (*m/z* 145.123).

The amounts of concentrates in the cows' diets may increase milk fat content, *de novo* fatty acids, and total saturated fatty acids (Borreani et al., 2013; Romanzin et al., 2013). The level of fatty acid oxidation may vary depending on the animal's diet: for instance, milk from a diet based on hay and concentrate had low-antioxidant potential (Agabriel et al., 2007; Calderon et al., 2007). The lipolysis system affected by nutritional and physiological factors plays an important role in the development of flavor and rancidity in dairy products (Chilliard et al., 2003). Furthermore, in the present study, the lower volatile compound contents in cheeses from a corn silage based diet could result from lower post-milking lipolysis activity, as previously noted by Ferlay et al. (2006).

The effect of lactation stage

The influence of lactation stage on the cheese VOC fingerprint appears to be by far the most important of the different sources of variation found in this experiment. Indeed, 4PCs that together account for 52.9% of the total original variance were affected by lactation stage (Table 4). First principal component and PC5, which together account for 34.4% of the total information, decreased across lactation (Figure 2 a and d, respectively). An opposite trend was shown by PC2 and PC4, which linearly increased during lactation (Figure 2, b and c, respectively). These variables summarize the VOC profile influenced by days in milk without losing much information because most of the peaks (75/139) also significantly increased during lactation (Supplementary Table 5). This result is in agreement with Bergamaschi et al. (2015), who studied the VOC profiles analyzed by SPME/GC-MS of a sub-set of samples from the same model cheeses discussed here. Our results concur with previous studies reporting a major influence of stage of lactation on sensory traits, with cheeses from late-lactation milk having a less pleasant odor (Coulon et al., 2004).

The ANOVA results show the compounds more significantly ($P < 0.01$) affected by stage of lactation to be alcohols, acids, esters, aldehydes and ketones. The higher concentration in cheese from late lactation milk of peaks and fragments associated with alcohols, like butan-1-ol and butan-2-ol (*m/z* 57.070; 75.080), is in agreement with previous findings regarding the flavor of raw milk and Fontina cheese (Carbonell et al., 2002; Berard et al., 2007). Primary alcohols, such as butan-1-ol, generally originate from the corresponding aldehydes, whereas butan-2-ol may be formed by the action of the starter bacteria on butan-2,3-dione to butan-2,3-diol and subsequent transformation to butan-2-ol as a consequence of non-starter lactic acid bacteria activity during cheese ripening (Berard et al., 2007). Hexanoic acid (*m/z* 99.081) also increased during lactation. This acid is generally produced from lipolysis of milk fat and has also been identified as a characteristic flavor component of Grana Padano, Parmigiano-Reggiano and Roncal cheeses (Curioni and Bosset, 2002; Bellesia et al., 2003).

A possible explanation for these positive trends in VOCs found in model cheeses could be related to the well-known effect of days in milk on the increase in milk fat and protein content during lactation (excepting the initial decrease) and on cheese gross composition (Stoop et al., 2009; Perna et al., 2014). Nevertheless, it should be pointed out that only about half of the peaks affected by days in milk showed an increase during lactation, and the majority of them showed much greater variations than those observed for milk and cheese composition (taking into account that the data are expressed as logarithm of the concentration). Moreover, many masses showed an opposite decreasing trend so that the effect of lactation stage on the PTR-ToF-MS profile is probably more complex, involving drivers other than milk and cheese composition. One possible explanation rely also on the lower coagulum drying capacity and/or syneresis and the higher soluble protein content at the end (> 200 d) of lactation (Martin and Coulon, 1995a; Ostersen et al., 1997; Bittante et al., 2015). The effect of lactation stage on VOCs could also be related to higher SCC in late lactation, sometimes accompanied by elevated levels of milk enzymes (e.g. plasmin, cathepsin), which may increase protein degradation. A reduced quantity of protein degraded, which does not affect the cheese gross composition, can originate a relatively large increase in volatile compounds (Marino et al., 2005; Mazal et al., 2007; Hand et al., 2012). Moreover, the cheeses produced with milk from animals in late lactation are also frequently described as high in moisture and with faster proteolysis and lipolysis, which products influence the texture and flavor of cheese (Coulon et al., 2004).

In addition, the energy status of the cows changes throughout lactation and significantly contributes to variations in milk fat composition because it alters different fatty acid pathways, such as *de novo* synthesis in the mammary gland and biohydrogenation in the rumen (Stoop et al., 2009). Furthermore, VOC concentration could be affected by hormonal balance during gestation, as in late lactation fetal growth causes a restriction in food intake, the mobilization of body fat reserves, a reduction in milk production, and sometimes depletion of the dairy milk components (Ostersen et al., 1997).

Other VOCs significantly ($P < 0.001$) influenced by days in milk are: ethanal (*m/z* 45.033), which may be produced through lactic acid bacteria activity from glucose via pyruvate and imparts green apple flavor notes to dairy products such as yogurt and cheese (Curioni and Bosset, 2002; Soukoulis et al., 2010); 2-methylbutanal, 3-methylbutanal (*m/z* 87.080), hexanal, and nonanal (*m/z* 69.033), which may result from lipid oxidation or degradation of amino acids (Strecker reaction) and are often associated with green grass and herbaceous aromas in cheese (Curioni and Bosset, 2002; Cornu et al., 2009; Thomsen et al., 2012); carbonyl compounds: propan-2-one (*m/z* 59.049) and pentan-2-one (*m/z* 87.080), probably produced by decarboxylation of the respective fatty acids by lactic acid bacteria (Curioni and Bosset, 2002).

Reductions in these compounds throughout lactation may be linked to elements that were not measured in our study, such as initial microbial composition and their enzymatic activity. Milk and cheese protein can biologically include active peptides which variously inhibited the amino and endo-peptidase of lactic acid bacteria (Smacchi and Gobbetti, 1998). The activity of these peptidases is very important during cheese ripening because they supply free amino acids that may be major precursors of specific flavor molecules, such as various alcohols, aldehydes, acids, esters, and sulfur compounds (Smit et al., 2005; Perna et al., 2014).

The effect of parity

Previous studies have demonstrated the importance of parity on milk composition (Kroeker et al., 1985), milk coagulation proprieties (Bittante et al., 2012), and nutrient recovery/loss in the whey of model cheeses from individual bovine and buffalo milk (Cipolat-Gotet et al., 2013 and 2014). More recently, the effect of number of lactations on the VOCs of a sub-dataset of the model cheeses considered in this work has also been investigated (Bergamaschi et al., 2015), but to our knowledge no published paper deals with the sensory properties of cheeses.

The peak *m/z* 49.011, attributed to methanethiol, known for its sulfurous note in the cheese aroma of different varieties, increased with parity (Curioni and Bosset, 2002; Bellesia et al., 2003; Cornu et al., 2009).

The effect of parity on cheese VOCs was not related to model cheese gross composition. Nevertheless, as previously reported (Erdem et al., 2010; Sánchez-Macías et al., 2013), the increase with parity of SCC (data not shown) may explain in part the VOC variations. Indeed, as already discussed above, the more intense proteolysis during ripening in the case of high SCC observed by Coulon et al. (2004) and Marino et al. (2005) may be responsible for the increasing trend of some volatile compounds. Moreover, higher SCC are usually associated with modification in other chemical compounds in milk, like soluble proteins, which modify the cheese-making ability of milk (Mazal et al., 2007; Erdem et al., 2010; Sánchez-Macías et al., 2013). Cipolat-Gotet et al. (2013) studied individual sources of variation on protein recovery of milk in curd and reported a decrease in the case of older cows.

Among the volatile compounds more significantly influenced ($P < 0.05$) by parity, we also found acids and esters. These VOCs, in particular ethyl hexanoate and octanoic acid (*m/z* 145.123), may originate from amino acid or fatty acid catabolism (McSweeney and Sousa, 2000). Acids and esters have also been reported to have an important role in the flavor of Parmigiano-Reggiano (Bellesia et al., 2003), Camembert (Curioni and Bosset, 2002) and Cantal cheese (Cornu et al., 2009). Their variation according to lactation (higher in third *vs* fourth or more parities) could be linked, at least in part, to milk composition.

The effect of milk yield

Many studies have investigated the effect of diet, stage of lactation, and farming system on milk and cheese characteristics (Coulon et al., 2004; Ferlay et al., 2006; Borreani et al., 2013), but to our knowledge, very few authors have studied the relationships between cheese VOC and daily milk yield independently from the other effects studied in this experiment.

Our findings indicate that variations in milk yield within herd, parity and DIM, influence the VOC profile of model cheeses, in agreement with previous work in this area using other analytical techniques (Bergamaschi et al., 2015).

The third principal component (Table 3) was positively related to volatile compounds belonging to two ketones, octan-1-one (*m/z* 129.127) and nonan-2-one (*m/z* 143.143), and negatively with acetonitrile (*m/z* 42.034), methanol (*m/z* 33.034) and propanoic acid (*m/z* 75.044).

The masses more significantly ($P < 0.05$) affected by milk yield corresponded to VOCs belonging to different chemical families: acids, aldehydes and ketones. Among these, we found some peaks with a lower correlation with PC3, such as hexan-1-one, hexan-2-one and hexanal (*m/z* 101.097). In contrast, propanoic acid (*m/z* 75.044) had higher correlation coefficients and characterized PC3 (Table 3). It is interesting to note that some peaks, and some principal components, were significantly affected by two or more sources of variations: e.g., 2-methylbutanal, 3-methylbutanal, penta-2-one (*m/z* 87.080) were affected by both DIM and milk yield. Moreover, ethyl hexanoate and octanoic acid (*m/z* 145.123) differed significantly according to days in milk, parity and dairy systems. The statistical model used in this experiment is supposed to segregate all these associated factors but some overlap cannot be excluded.

CONCLUSIONS

The results of the present research show PTR-ToF-MS to be a powerful technique for characterizing the volatile organic compounds of cheese, and, because of its simplicity of sample preparation and high-throughput can be applied also on a large number of samples. As this technique detects a very high number of peaks, a multivariate analysis, such as principal components analysis, is a useful method for compressing information in a few traits to be used as new phenotypes. These new synthetic indicators summarize the main trends of groups of volatile compounds, which could be related to the same catabolic pathways, chemical families or flavors.

The combination of two high-throughput techniques, like PTR-ToF-MS and the model cheese making procedure, proved to be a powerful instrument for investigating the relationships between animal characteristics, farm characteristics, milk processing, and cheese quality. This approach allowed the investigation of the effects of dairy system and individual animal characteristics on cheese volatile organic profiles at the individual level. In conclusion, high-throughput methodologies, such as model cheese production and rapid volatile compound profiling by PTR-ToF-MS, open new avenues for the investigation of the effects of farming systems and individual animal traits on the final quality of cheese, and new directions in the genetic improvement of dairy species.

Acknowledgments

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TABLE AND FIGURES

Table 1. Descriptive statistics of cow's parity, DIM and milk yield, and of milk and cheese composition.

Traits	N	Mean	SD	P5[2]	P95[2]
Parity, n	1,075	2.7	1.8	1.0	6.0
DIM, d	1,070	198	138	25	447
Milk yield, kg×d^{-1}	1,056	24.6	7.9	12.5	38.3
Milk composition					
Fat, %	1,073	4.4	0.9	3.2	5.9
Protein, %	1,073	3.8	0.4	3.1	4.5
Fat/Protein	1,073	1.18	0.25	0.86	1.58
Casein/Protein	1,071	0.769	0.018	0.750	0.787
SCS[1]	1,071	3.03	1.86	0.21	6.21
Ph	1,073	6.63	0.09	6.49	6.77
Cheese composition					
Fat, %	935	38.2	4.4	31.6	45.6
Fat in DM, %	935	47.7	4.7	40.7	54.8
Protein, %	935	27.1	4.1	20.4	33.2
Dry matter, %	935	80.1	4.7	71.8	86.9
pH	1,071	5.16	0.18	4.85	5.44

[1] SCS = $\log_2$(SCC/100,000)+3; [2] P5 = 5th percentile and P95 = 95th percentile.

Table 2. Structure of the PTR-ToF-MS data set (1,075 model cheeses from individual cows)

Dataset	Spectral peaks (n)	Spectral intensity (ppb$_v$[1])	Incidence (%) on database:		
			A	B	C
A: initial data set	619	49,723,176	100.0	-	-
B: only peaks above 1 ppb$_v$[1] threshold	317	49,594,607	99.7	100.0	-
C: without interference ions	240	28,652,992	57.6	57.8	100.0
D: only peaks tentatively identified	61	22,356,325	44.8	45.0	78.0

[1] ppb$_v$ = parts per billion by volume.

Table 3. Highest correlation coefficients between the first five principal components and the volatile compounds tentatively attributed to specific spectrometric fragments of PTR-ToF-MS

Principal component	R^2, %	Measured mass (*m/z*)	Sum formula	Theoretical mass (*m/z*)	r	Tentative identification
1	28.3	117.091	$C_6H_{13}O_2^+$	117.0910	-0.80	Ethyl butanoate, ethyl-2-methylpropanoate (ethyl isobutyrate), hexanoic acid
		127.112	$C_8H_{15}O^+$	127.1120	-0.73	1-Octen-3-one
		145.123	$C_8H_{17}O_2^+$	145.1229	-0.73	Ethyl hexanoate, octanoic acid
		99.081	$C_6H_{11}O^+$	99.0804	-0.69	Hexanoic acid
		71.086	$C_5H_{11}^+$	71.0855	-0.61	3-Methyl-1-butanol, 3-methyl-3-buten-1-ol, pentan-1-ol
2	39.2	69.070	$C_5H_9^+$	69.0698	-0.62	2-Methyl-1,3-butadiene (isoprene)
		115.112	$C_7H_{15}O^+$	115.1117	-0.59	Heptan-2-one
		59.049	$C_3H_7O^+$	59.0491	-0.59	Propan-2-one (acetone)
		87.080	$C_5H_{11}O^+$	87.0804	-0.56	2-Methylbutanal, 3-methylbutanal, pentan-2-one
		101.097	$C_6H_{13}O^+$	101.0961	-0.55	Hexan-1-one, hexan-2-one, hexanal
3	47.8	42.034	$C_2H_4N^+$	42.0330	-0.70	Acetonitrile
		33.034	CH_5O^+	33.0335	-0.69	Methanol
		129.127	$C_8H_{17}O^+$	129.1270	0.66	Octan-1-one
		75.044	$C_3H_7O_2^+$	75.0440	-0.61	Propanoic acid
		143.143	$C_9H_{19}O^+$	143.1430	0.60	Nonan-2-one

4	55.4	109.070	$C_2{}^{13}CH_{10}O_3N^+$	109.0760	-0.65	2,6-Dimethylpyrazine
		95.017	$C_2H_7O_2S^+$	95.0160	-0.52	Methyldisulfanylmethane (dimethyl disulphide)
		75.044	$C_3H_7O_2^+$	75.0440	0.40	Propanoic acid
		61.028	$C_2H_5O_2^+$	61.0284	0.31	Acetic acid and fragment of acetate ester
		33.034	CH_5O^+	33.0335	0.31	Methanol
5	61.5	60.021	$C_2H_4O_2^+$	60.0205	-0.61	Acetic acid
		103.075	$C_5H_{11}O_2^+$	103.0754	-0.58	3-Methylbutanoic acid (isovaleric acid), ethyl (S)-2-hydroxypropanoate (ethyl lactate), pentanoic acid (valeric acid)
		105.091	$C_5H_{13}O_2^+$	105.0910	0.55	1,2-Pentanediol
		87.080	$C_5H_{11}O^+$	87.0804	0.53	2-Methylbutanal, 3-methylbutanal, pentan-2-one
		131.107	$C_7H_{15}O_2^+$	131.1067	-0.49	Ethyl-2-methylbutanoate, ethyl-3-methylbutanoate (ethyl isovalerate), heptanoic acid

Table 4. *F*-values and significance from ANOVA on principal components for dairy systems, orthogonal contrasts between dairy systems, incidence of herd/test date within dairy systems (HTD), days in milk, parity, orthogonal contrasts between parity, milk yield, and vat.

	Principal Component				
Traits	1	2	3	4	5
Dairy systems	1.3	2.8*	0.7	1.2	0.5
Traditional *vs* modern farm	0.3	1.6	0.7	2.6	0.8
Automatic feeder effect[1]	0.1	2.1	0.1	0.9	0.1
TMR effect[2]	0.7	8.9*	2.1	0.5	0.2
Silage effect[3]	3.2	0.5	0.1	1.4	0.8
HTD, %[4]	20	31	53	15	48
Days in milk	4.1***	4.3***	0.6	3.1**	3.2**
Linear	6.0*	10.0**	2.2	10.0**	5.7*
Quadratic	5.6*	0.1	0.7	1.3	0.1
Cubic	1.3	0.2	0.4	0.1	2.7
Parity	4.5**	2.3	1.3	0.6	1.8
1 *vs* ≥ 2	0.7	2.1	0.1	0.4	1.8
2 *vs* ≥ 3	0.1	2.1	0.9	0.1	0.5
3 *vs* ≥ 4	0.3	3.6	0.3	0.3	0.5
Milk yield	0.1	0.1	7.4**	0.2	3.6
Regr[5]	0.046	-0.042	0.061	0.027	0.024
Vat	1.1	1.3	1.6	0.6	1.1
RMSE[6]	7.2	4.1	3.1	3.9	2.8

[1] Contrast between traditional farms with compound feed administered in the mangers with hay *vs* traditional farms with compound feed administered in the mangers through automatic feeders. [2] Contrast between modern farms with hay and compound feed administered in the mangers *vs* modern farms using TMR; [3] Contrast between modern farms using TMR without silages *vs* TMR with silages; [4] Herd/Test day effect expressed as proportion of variance explained by herd/test date calculated by dividing the corresponding variance component by the total variance; [5] Regr = Linear regression with daily milk yield of PTR-ToF-MS spectrometric peaks expressed as natural logarithm of the parts per billion by volume; [6] RMSE = root mean square error; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Figure 1. Least squares means (±SE) of the extracted second principal component scores for different dairy systems.

[1]AF = automatic feeders at mangers to control individually concentrate distribution of tied cows; [2]CF = compound feed distributed at mangers; [3]TMR = total mixed ration; [4]no silage = water is added in the mixer wagon to favor mixing.

Orthogonal contrast between modern farms with hay and compound feed administered in the mangers *vs* modern farms using total mixed rations was statistically different ($P<0.05$).

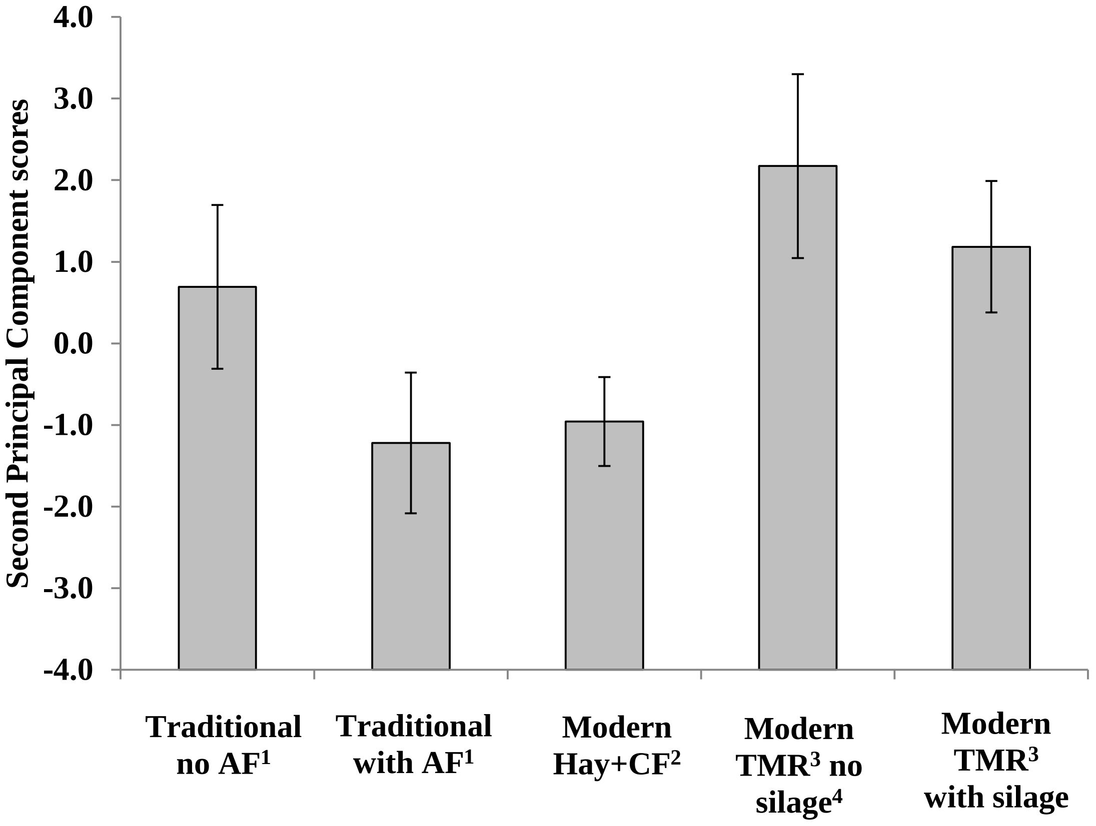
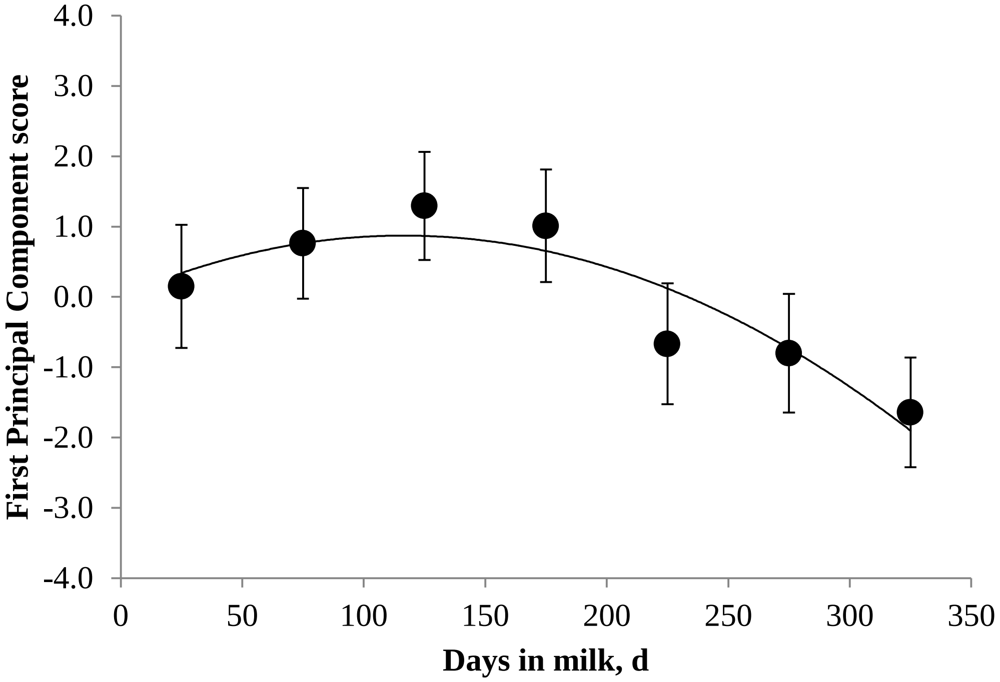
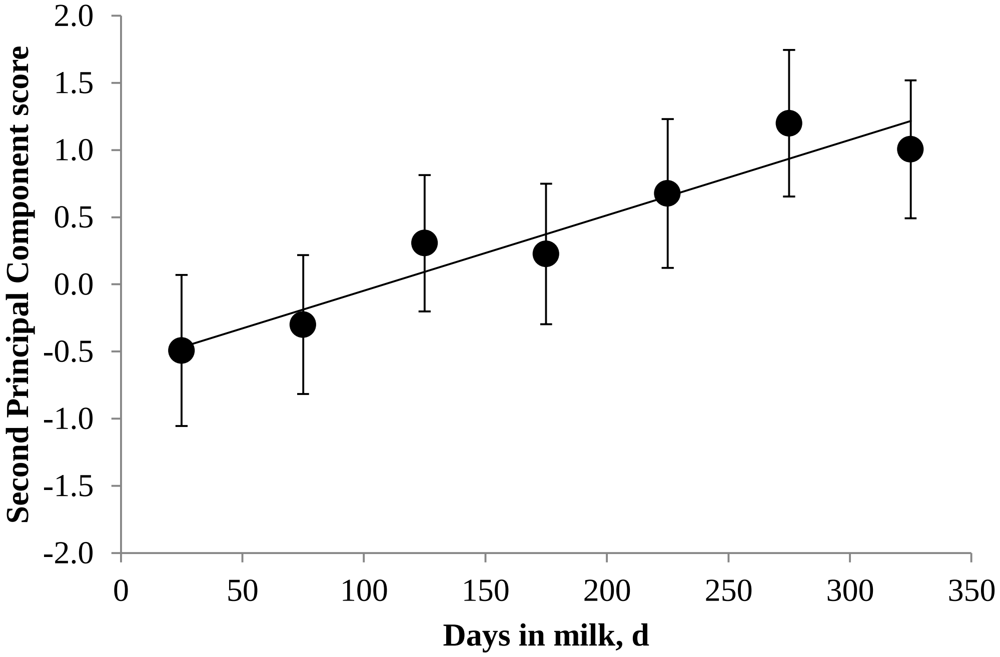


Figure 2. Least squares means (±SE) of the extracted first (**a**), second (**b**), forth (**c**) and fifth (**d**) principal component that exhibited significant (*P*<0.01) pattern along the lactation.

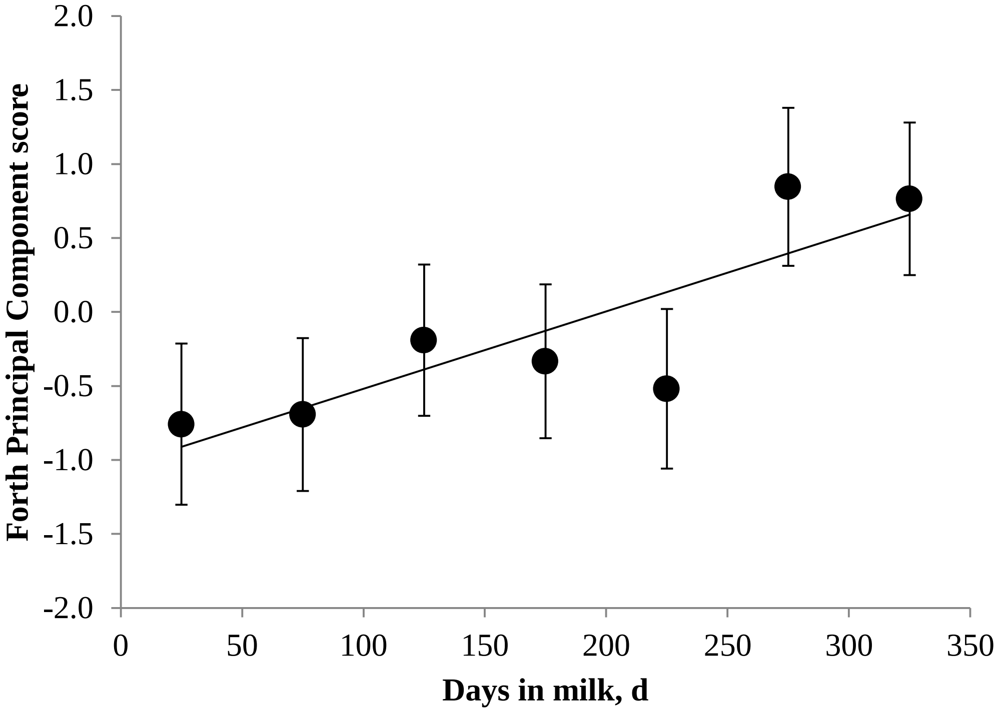
[a]



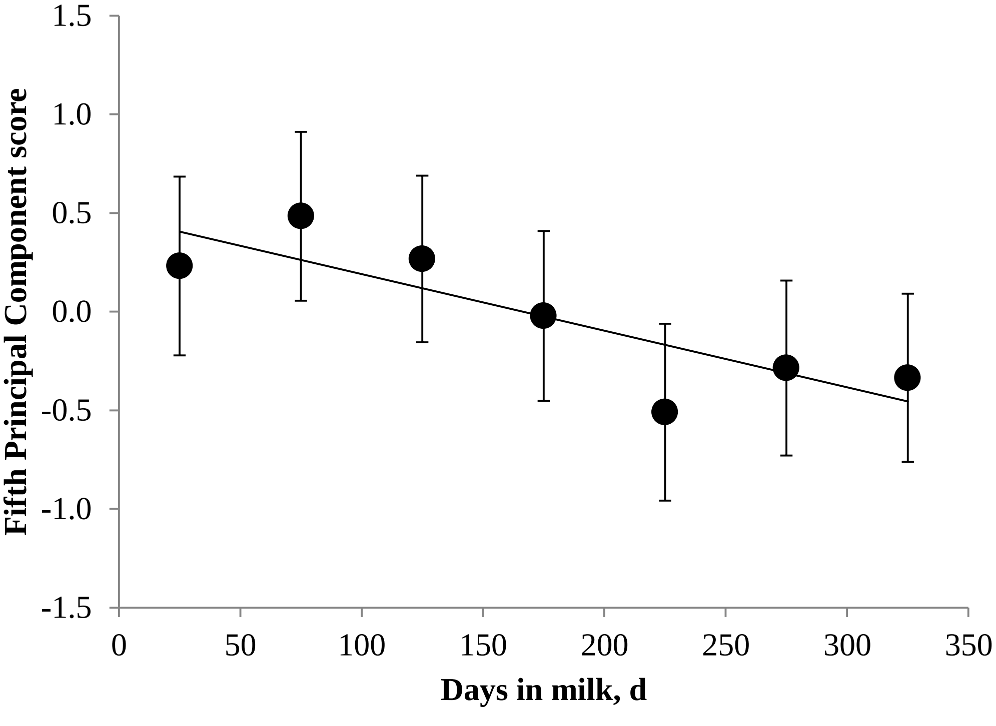
[b]



[c]


2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
Forth Principal Component score
0
50
100
150
200
250
300
350
Days in milk, d

[d]


1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
Fifth Principal Component score
0
50
100
150
200
250
300
350
Days in milk, d

Supplementary Table 1. Description of the spectrometric peaks from PTR-ToF-MS analysis of individual model cheeses.

Measured Mass *(m/z)*	Theoretical mass *(m/z)*	Chemical characteristics				VOC origin		Aromatic note in cheese	
		Tentative VOC identification	Sum formula	Chemical family	Reference	Possible origin	Reference	Odor	Reference
33.034	33.0335	Methanol	CH5O+	Alcohols	7	Lactose metabolism, methyl ketone reduction, amino acid metabolism, degradation of linoleic and linolenic acids	3		
41.039	41.0386	Alkyl fragment	C3H5+						
42.034	42.0338	Acetonitrile	C2H4N+						
43.018	43.0178	Alkyl fragment	C2H3O+						
43.054	43.0542	Alkyl fragment	C3H7+		7				
45.033	45.0335	Ethanal (acetaldehyde)	C2H5O+	Aldehydes	7			Green	3
49.011	49.0106	Methanethiol	CH5S+	Sulfurous	7	Catabolism of the amino acids (degradations)	5	Fermented cabbage	5
55.055	55.0542	Butanal	C4H7+	Aldehydes		β-oxidation of unsaturated fatty acids	9	Fruity	9
		Heptanal		Aldehydes		β-oxidation of unsaturated fatty acids	3	Lemon, herbal	8
		Alkyl fragment							
57.033	57.0335	3-Methyl-1-butanol	C3H5O+	Alcohols		Reduction of aldehydes from leucina	9	Chocolate, feet	9
		Heptan-1-ol		Alcohols		Lactose metabolism, methyl ketone reduction, amino acid metabolism, degradation of linoleic and linolenic acids	3	Green, earthy	3
		Pentan-1-ol		Alcohols		Lactose metabolism, methyl ketone reduction, amino acid metabolism, degradation of linoleic and linolenic acids	3	Balsamic	3
		Propan-1-ol		Alcohols		Lactose metabolism, methyl ketone reduction, amino acid metabolism, degradation of linoleic and linolenic acids	3	Sweet	3
		Propan-2-ol		Alcohols		Lactose metabolism, methyl ketone reduction, amino acid metabolism, degradation of linoleic and linolenic acids	3	Lactic-cheesy	8
57.070	57.0699	Alkyl fragment	C4H9+						
59.049	59.0491	Propan-2-one (acetone)	C3H7O+	Ketones	7	β-oxidation and decarboxylation of the fatty acids		Acetone	8
60.021	60.0205	Acetic acid	C2H4O2+	Acids		Metabolism of the fatty acids, lactose or citrate	6	Vinegar	3
60.053	60.0525	Propan-2-one (acetone 13C isotope)	C2[13]CH7O+	Ketones		β-oxidation and decarboxylation of the fatty acids		Acetone	8
61.028	61.0284	Acetate ester fragment	C2H5O2+						
		Acetic acid		Acids		Metabolism of the fatty acids, lactose or citrate	6	Vinegar	2
62.032	62.0318	Acetic acid	C[13]CH5O2+	Acids		Metabolism of the fatty acids, lactose or citrate	6	Vinegar	2
63.027	63.0263	Methylsulfonylmethane (dimethyl sulphide)	C2H7S+	Sulfurous		Catabolism of the amino acids (degradations)	4	Garlic-rotten	4

69.033	69.0335	2-Methylbutanal	C4H5O+	Aldehydes		Degradations of the amino acids (enzymatic or no enzymatic reactions)	3	Chocolate, chemical	3; 9
		3-Methylbutanal		Aldehydes		Degradations of the amino acids (enzymatic or no enzymatic reactions)	3	Green, malty, herbaceous	3
		Heptanal		Aldehydes		β-oxidation of unsaturated fatty acids		Fruity	8
		Nonanal		Ketones		β-oxidation of unsaturated fatty acids	9	Green	9
		Pentan-2-one		Ketones		β-oxidation and decarboxylation of the fatty acids	4	Sweet, fruity	3
69.070	69.0699	2-Methyl-1,3-butadiene (isoprene)	C5H9+	Terpenes	5	Plants	3		
71.049	71.0491	Butanoic acid	C4H7O+	Acids		Metabolism of the lactate	6; 9	Feet, vomit	9
71.086	71.0855	3-Methyl-1-butanol	C5H11+	Alcohols		Reduction of the aldehydes from leucine	15	Chocolate, feet	9
		3-Methyl-3-buten-1-ol		Alcohols				Cheese, fruity, green	3
		Pentan-1-ol		Alcohols		Lactose metabolism, methyl ketone reduction, amino acid metabolism, degradation of linoleic and linolenic acids	3	Balsamic	3
72.089	72.0889	Pentan-1-ol	C4[13]CH11+	Alcohols		Lactose metabolism, methyl ketone reduction, amino acid metabolism, degradation of linoleic and linolenic acids	3	Balsamic	3
73.065	73.0648	Butan-2-one	C4H9O+	Ketones	7	β-oxidation and decarboxylation of the fatty acids	3; 9	Butterscotch	3
		Butanal		Aldehydes	5	β-oxidation of unsaturated fatty acids	6		
74.069	74.0681	Butane-2,3-diol	C3[13]CH9O+	Alcohols		Metabolism of the citrate	6		
		Hexan-1-ol		Alcohols		Lactose metabolism, methyl ketone reduction, amino acid metabolism, degradation of linoleic and linolenic acids	3	Green	3
		Hexan-2-ol		Alcohols		Lactose metabolism, methyl ketone reduction, amino acid metabolism, degradation of linoleic and linolenic acids	3	Green	3
75.044	75.0441	Propanoic acid	C3H7O2+	Acids		Metabolism of the fatty acids	6; 9	Feet	9
		Propanoic ester							
75.080	75.081	Butan-1-ol	C4H11O+	Alcohols					
79.055	79.0542	Benzene	C6H7+	Hydrocarbons	5	Plastic/contaminations			
81.070	81.0699	Alkyl fragment (terpenes)	C6H9+	Terpenes	5	Plants	3		
83.086	83.0855	Hexanal	C6H11+	Aldehydes		β-oxidation of unsaturated fatty acids	9	Lemon, herbal	9
		Nonanal		Aldehydes		β-oxidation of unsaturated fatty acids	9	Green	9
85.065	85.0648	Pentanoic acid (valeric acid)	C5H9O+	Acids		Metabolism of the fatty acids	4	Rotten cheesy	4
85.101	85.1012	Hexan-1-ol	C6H13+	Alcohols		Lactose metabolism, methyl ketone reduction, amino acid metabolism, degradation of linoleic and linolenic acids	3	Green	3
		Hexan-2-ol		Alcohols		Lactose metabolism, methyl ketone reduction, amino acid metabolism, degradation of linoleic and linolenic acids	3	Green	3

86.071	86.0726	Pentanal	C5H10O+	Aldehydes					
87.044	87.0441	Butane-2,3-dione (diacetyl)	C4H7O2+	Ketones	7	Metabolism of the citrate	4; 9	Butter, caramel; buttery-sweet	4; 9
87.080	87.0804	2-Methylbutanal	C5H11O+	Aldehydes	1; 7	Degradations of the amino acids degradations (enzymatic or no enzymatic reactions)	4; 9	Chocolate, chemical	4; 9
		3-Methylbutanal		Aldehydes		Degradations of the amino acids (enzymatic or no enzymatic reactions)	3	Green, malty, herbaceous	3
		Pentan-2-one		Ketones	7	β-oxidation and decarboxylation of the fatty acids	3	Sweet, fruity	3
88.084	88.0838	2-Methylbutanal	C4[13]CH11O+	Aldehydes		Degradations of the amino acids (enzymatic or no enzymatic reactions)	9	Chocolate, chemical	3; 9
		3-Methylbutanal		Aldehydes		Degradations of the amino acids (enzymatic or no enzymatic reactions)	3	Green, malty, herbaceous	3
		Pentan-2-one		Ketones		β-oxidation and decarboxylation of the fatty acids	3	Sweet, fruity	3
89.060	89.0597	3-Hydroxy-2-butanone (acetoin)	C4H9O2+	Ketones	7	Metabolism of the citrate	9	Butter, caramel	9
90.063	90.0631	2-Methylpropanoic acid (isobutyric acid)	C3[13]CH9O2+	Acids		Metabolism of the fatty acids	3	Cheese, rancid, butter	3
		Butanoic acid		Acids		Metabolism of the lactate	6; 9	Feet, vomit	9
		Ethyl acetate		Esters		Esterification free fatty acids and alcohols from lactose or amino acids metabolism	3	Juicy fruit gum, apples, fruity, pineapple	3
93.069	93.0699	Methylbenzene (toluene)	C7H9+	Hydrocarbons		Plastic/contaminations	9	Plastic	9
95.017	95.0161	Methyldisulfanylmethane (dimethyl disulphide)	C2H7O2S+	Sulfurous		Catabolism of the amino acids (degradations)	4	Garlic, onion	4
95.049	95.0491	Phenol	C6H7O+	Hydrocarbons		Metabolism of the protein (casein)	6		
99.081	99.0804	Hexanoic acid	C6H11O+	Acids		Metabolism of the fatty acids	4	Sharp, goat	4
101.097	101.0597	Hexan-1-one	C6H13O+	Ketones		β-oxidation and decarboxylation of the fatty acids	3	Green	3
		Hexan-2-one		Ketones	1	β-oxidation and decarboxylation of the fatty acids			
		Hexanal		Aldehydes	5	β-oxidation of unsaturated fatty acids	9	Lemon, herbal	9
103.075	103.075	3-Methylbutanoic acid (isovaleric acid)	C5H11O2+	Acids	5	Metabolism of the protein (leucina)	3	Swiss cheese	3
		Ethyl (S)-2-hydroxypropanoate (ethyl lactate)		Esters		Esterification free fatty acids and alcohols from lactose or amino acids metabolism	3		
		Pentanoic acid (valeric acid)		Acids		Metabolism of the fatty acids	4	Rotten cheesy	4
104.079	104.079	3-Methylbutanoic acid (isovaleric acid)	C4[13]CH11O2+	Acids		Metabolism of the protein (leucina)	3	Swiss cheese	3
		Ethyl (S)-2-		Esters		Esterification free fatty acids and alcohols from lactose or amino	3		

		hydroxypropanoate (ethyl lactate)				acids metabolism			
		Pentanoic acid (valeric acid)		Acids		Metabolism of the fatty acids	4	Rotten cheesy	4
105.071	105.07	2-Phenylethanol/Styrene	C8H9+						
105.091	105.091	1,2-Pentanediol	C5H13O2+	Alcohols					
107.085	107.086	Ethylbenzene	C8H11+	Hydrocarbons	5	Plastic/contaminations		Solvent, paint	2
108.089	108.089	Ethylbenzene	C7[13]CH11+	Hydrocarbons		Plastic/contaminations		Solvent, paint	2
109.070	109.069	2,6-dimethyl pyrazine	C2[13]CH10O3N+	Pyrazines		Catabolism of the amino acids (degradations)	4	Nutty roast grain	4
115.112	115.112	Heptan-2-one	C7H15O+	Ketones	7	β-oxidation and decarboxylation of the fatty acids	4	Musty, varnish, sweet	4
116.116	116.115	Heptan-2-one	C6[13]CH15O+	Ketones		β-oxidation and decarboxylation of the fatty acids	4	Musty, varnish, sweet	4
117.091	117.091	Ethyl butanoate	C6H13O2+	Esters		Esterification free fatty acids and alcohols from lactose or amino acids metabolism	4	Fruity-melon, sweet	4
		Ethyl-2-methylpropanoate (ethyl isobutyrate)		Esters		Esterification free fatty acids and alcohols from lactose or amino acids metabolism		Fruity	
118.095	118.094	Ethyl butanoate	C5[13]CH13O2+	Esters		Esterification free fatty acids and alcohols from lactose or amino acids metabolism	4	Fruity-melon, sweet	4
		Ethyl-2-methylpropanoate (ethyl isobutyrate)		Esters		Esterification free fatty acids and alcohols from lactose or amino acids metabolism		Fruity	
		Hexanoic acid		Acids		Metabolism of the fatty acids	4	Sharp, goat	4
127.112	127.112	1-Octen-3-one	C8H15O+	Ketones	1		3	Mushroom	3
129.127	129.127	Octan-1-one	C8H17O+	Ketones					
131.107	131.107	Ethyl pentanoate (ethyl valerate)	C7H15O2+	Esters		Esterification free fatty acids and alcohols from lactose or amino acids metabolism	4	Melon, fruity, sweet	4
		Ethyl-2-methylbutanoate		Esters		Esterification free fatty acids and alcohols from lactose or amino acids metabolism	3		
		Ethyl-3-methylbutanoate (ethyl isovalerate)		Esters		Esterification free fatty acids and alcohols from lactose or amino acids metabolism	3		
		Heptanoic acid		Acids		Metabolism of the fatty acids	4	Goat cheese	4
132.109	132.11	Ethyl pentanoate (ethyl valerate)	C6[13]CH15O2+	Esters		Esterification free fatty acids and alcohols from lactose or amino acids metabolism	4	Melon, fruity, sweet	4
		Ethyl-2-methylbutanoate		Esters		Esterification free fatty acids and alcohols from lactose or amino acids metabolism	3		
		Ethyl-3-methylbutanoate (ethyl isovalerate)		Esters		Esterification free fatty acids and alcohols from lactose or amino acids metabolism	3		
		Heptanoic acid		Acids		Metabolism of the fatty acids	4	Goat cheese	4

143.143	143.143	Nonan-2-one	C9H19O+	Ketones		β-oxidation and decarboxylation of the fatty acids	9	Plastic, earthy	9
144.146	144.146	Nonan-2-one	C8[13]CH19O+	Ketones		β-oxidation and decarboxylation of the fatty acids	9	Plastic, earthy	9
145.123	145.122	Ethyl hexanoate	C8H17O2+	Esters		Esterification free fatty acids and alcohols from lactose or amino acids metabolism	3		
		Octanoic acid	C8H17O2+	Acids	5	Metabolism of the fatty acids	3	Fatty, rancid	3
146.126	146.126	Ethyl hexanoate	C7[13]CH17O2+	Esters		Esterification free fatty acids and alcohols from lactose or amino acids metabolism	3		
		Octanoic acid		Acids		Metabolism of the fatty acids	3	Fatty, rancid	3
159.138	159.138	3-Methylbutyl butanoate (isoamyl butyrate)	C9H19O2+	Esters		Esterification free fatty acids and alcohols from lactose or amino acids metabolism	3	Fruity, apricot, pear banana	3
		Nonanoic acid	C9H19O2+	Acids		Metabolism of the fatty acids	3	Goat	3

[1]Biasioli et al., 2006; [2]Cornu et al., 2009; [3]Curioni and Bosset, 2002; [4]Frank et al., 2004; [5]Galle et al., 2011; [6]McSweeney and Sousa, 2000; [7]Soukoulis et al., 2010; [8]Sympoura et al., 2009; [9]Thomsen et al., 2012.

Supplementary Table 2. Descriptive statistics of PTR-ToF-MS spectrometric peaks expressed as natural logarithm of the parts per billion by volume from individual model cheeses

m/z	Mean	SD	P5[1]	P95[1]	Skewness	Kurtosis
33.034	9.24	0.61	8.21	10.30	-0.17	0.86
34.037	5.11	0.55	4.14	6.02	-0.40	1.17
34.995	4.47	0.68	3.39	5.63	0.16	0.20
39.023	7.64	0.70	6.60	8.91	0.29	1.75
40.027	4.87	0.58	3.87	5.76	-0.20	2.18
41.039	10.35	0.46	9.62	11.14	-0.14	1.38
42.010	5.95	0.45	5.12	6.60	-0.63	0.93
42.034	8.08	0.91	6.73	9.85	0.35	-0.01
42.043	7.39	0.56	6.40	8.13	-1.13	3.42
43.018	11.25	0.42	10.55	11.95	-0.21	0.57
43.054	11.09	0.38	10.47	11.69	-0.18	1.12
43.094	5.07	0.37	4.55	5.67	0.51	2.37
44.022	7.56	0.39	6.89	8.20	-0.13	0.33
44.058	7.78	0.40	7.15	8.46	-0.05	0.87
44.980	4.64	0.66	3.38	5.58	-0.67	0.62
45.033	10.93	0.56	9.91	11.73	-0.64	1.27
46.031	5.74	0.68	4.68	6.89	0.22	1.09
46.038	7.14	0.63	6.02	8.02	-1.03	3.22
48.012	5.69	0.44	4.95	6.36	-0.42	0.42
48.053	10.03	0.82	8.51	11.06	-1.05	1.63
49.011	6.82	0.67	5.69	7.89	-0.15	0.33
49.028	5.28	0.73	3.89	6.21	-1.28	2.69
49.054	7.72	0.80	6.23	8.78	-0.81	0.67
50.000	5.11	0.85	3.55	6.29	-0.69	0.71
50.057	4.58	0.44	3.80	5.23	-0.52	0.58
51.007	4.59	0.48	3.79	5.34	-0.29	1.15
51.044	7.68	0.61	6.70	8.77	0.00	0.68
53.039	4.50	0.54	3.57	5.28	-0.23	1.50
54.034	4.24	0.59	3.22	5.25	-0.22	0.68
55.055	7.08	1.57	3.43	8.47	-1.58	1.36
56.045	5.33	0.52	4.59	6.29	0.31	1.25
56.060	5.86	0.51	4.91	6.60	-0.56	0.58
57.033	6.70	0.67	5.88	7.83	-0.41	4.86
57.070	10.55	0.66	9.66	11.96	0.98	1.36
58.041	5.72	0.89	4.23	7.12	-0.23	-0.16
58.073	7.47	0.65	6.59	8.90	1.07	1.56
59.049	10.52	0.79	9.10	11.66	-0.46	-0.26
59.329	4.34	0.51	3.41	5.07	-0.85	1.58
60.021	5.81	0.81	4.38	7.03	-0.53	0.49
60.045	5.39	0.73	4.26	6.64	-0.08	0.32
60.053	7.15	0.77	5.74	8.26	-0.45	-0.23
61.028	11.04	0.48	10.22	11.77	-0.43	0.84

61.062	7.19	0.41	6.50	7.79	-1.00	6.02
62.032	7.38	0.46	6.59	8.08	-0.37	0.52
62.068	4.40	0.31	3.87	4.92	-0.15	0.32
63.027	7.10	0.94	5.73	8.79	0.35	-0.38
63.044	8.02	0.49	7.22	8.83	-0.24	1.23
64.031	4.48	0.62	3.61	5.63	0.38	0.22
64.048	4.82	0.43	4.11	5.43	-0.82	3.02
65.018	5.37	0.72	3.95	6.26	-1.22	2.66
66.063	8.61	0.86	7.02	9.84	-0.79	0.99
67.058	5.65	0.44	4.93	6.38	-0.42	2.34
67.065	6.75	0.90	5.07	8.01	-0.72	0.63
68.067	4.29	0.33	3.74	4.81	-0.51	1.66
69.033	4.35	0.38	3.68	4.92	-0.50	1.60
69.058	4.58	0.85	3.33	6.00	0.25	-0.06
69.070	8.43	0.58	7.53	9.46	0.39	0.42
70.064	5.13	0.50	4.36	6.01	0.17	0.81
70.078	6.89	0.65	5.70	7.82	-0.51	0.43
71.049	9.50	0.60	8.61	10.60	0.38	-0.01
71.086	10.38	0.46	9.62	11.11	-0.19	0.76
72.053	6.53	0.56	5.71	7.58	0.42	-0.07
72.089	7.54	0.44	6.81	8.24	-0.15	0.76
73.027	5.50	0.67	4.23	6.35	-1.42	3.71
73.051	6.57	1.22	3.78	8.07	-1.05	1.25
73.065	9.88	0.62	8.96	11.02	0.49	0.66
74.034	4.34	0.52	3.38	5.11	-0.56	1.56
74.051	4.49	0.62	3.47	5.43	-0.26	0.64
74.069	6.85	0.60	5.97	7.93	0.53	0.73
75.027	5.47	0.96	3.78	6.93	-0.31	-0.12
75.044	8.22	0.67	7.27	9.59	0.59	0.00
75.080	7.70	1.14	5.97	9.72	0.23	-0.58
76.047	5.26	0.54	4.49	6.34	0.49	0.12
76.084	5.12	0.79	4.08	6.69	0.68	-0.15
77.060	6.43	0.68	5.34	7.55	-0.01	0.03
78.001	4.23	0.89	3.09	6.26	1.04	1.32
79.040	8.53	0.61	7.61	9.71	0.23	1.60
79.055	6.87	1.60	3.36	8.73	-1.11	0.63
79.075	6.17	0.64	5.01	7.14	-0.78	2.07
80.046	5.47	0.46	4.77	6.28	0.14	0.95
80.058	4.66	0.93	2.99	6.08	-0.31	-0.13
80.991	6.17	0.64	5.04	7.08	-0.59	0.90
81.039	4.63	0.38	4.04	5.25	-0.21	1.78
81.061	4.87	0.52	4.02	5.73	-0.15	0.74
81.070	5.37	0.46	4.60	6.00	-1.07	5.47
82.945	4.48	0.70	3.35	5.76	0.15	0.38
82.988	5.20	0.58	4.21	6.03	-0.51	0.88
83.052	4.97	0.53	4.03	5.64	-1.66	5.55
83.071	7.44	0.95	5.77	9.01	-0.10	0.51
83.086	6.13	0.69	5.21	6.86	-2.85	12.15

84.075	4.41	0.58	3.54	5.48	0.47	0.73
84.079	4.25	0.57	3.17	4.98	-1.04	2.02
84.942	4.29	0.63	3.24	5.41	-0.05	0.61
85.029	4.46	0.40	3.73	5.04	-0.56	1.28
85.065	6.60	0.60	5.72	7.68	0.51	0.45
85.101	6.66	0.55	5.84	7.65	0.33	0.49
86.072	5.00	0.71	3.83	6.09	-0.18	0.11
86.105	4.54	0.43	3.83	5.24	-0.13	1.28
87.044	7.20	0.80	6.01	8.62	0.15	0.57
87.080	10.42	1.08	8.33	11.97	-0.57	0.00
88.052	5.22	0.50	4.39	6.05	-0.22	0.70
88.084	7.61	0.94	5.84	8.98	-0.49	-0.09
89.060	10.76	0.44	10.06	11.51	0.01	-0.06
90.063	7.69	0.43	7.00	8.42	0.02	-0.07
91.051	6.52	1.46	3.23	8.36	-1.07	0.78
91.059	8.47	0.81	7.32	9.86	0.28	-0.77
92.061	6.64	0.97	5.08	8.34	0.12	-0.43
93.037	7.78	0.78	6.44	8.93	-1.19	5.03
93.069	8.96	1.61	6.64	10.95	-1.29	3.36
93.090	10.11	1.37	7.56	12.14	-0.69	0.86
93.181	4.19	0.53	3.38	4.90	0.42	2.14
93.432	4.54	0.46	3.75	5.29	-0.22	0.89
94.039	5.43	0.90	3.92	6.83	-0.34	0.34
94.074	6.64	1.35	4.92	9.59	0.77	0.04
94.095	6.92	1.37	4.51	9.17	-0.19	-0.39
95.004	4.72	0.65	3.88	5.95	0.78	0.54
95.017	5.04	0.71	3.78	6.06	-0.71	0.95
95.034	5.22	0.64	4.12	6.25	-0.26	0.16
95.049	4.98	0.56	4.17	6.19	0.72	0.94
95.081	4.93	0.67	3.93	6.37	0.50	1.09
95.096	5.44	0.98	4.05	7.28	0.39	-0.36
96.961	6.15	0.61	5.04	7.04	-0.52	0.76
97.060	4.87	0.35	4.33	5.42	-0.02	2.22
97.101	6.23	0.60	5.28	7.28	0.16	-0.13
98.105	4.43	0.47	3.67	5.19	-0.22	0.68
98.959	5.77	0.61	4.71	6.64	-0.47	0.78
99.039	5.00	0.37	4.36	5.53	-0.52	1.26
99.081	6.93	0.52	6.15	7.87	0.64	1.25
99.121	5.19	0.68	4.26	6.43	0.38	0.52
100.084	4.78	0.40	4.17	5.46	0.46	1.20
100.954	4.54	0.51	3.64	5.25	-0.64	1.34
101.060	6.36	0.33	5.81	6.86	-0.43	1.02
101.097	6.38	0.74	5.14	7.55	-0.01	-0.08
102.062	4.35	0.35	3.75	4.89	-0.42	1.30
102.099	4.52	0.50	3.69	5.30	-0.16	0.59
103.075	7.76	0.62	6.83	8.81	0.21	-0.04
104.079	5.23	0.54	4.42	6.17	0.49	0.70
105.039	4.53	0.44	3.77	5.20	-0.54	1.06

105.071	5.96	0.46	5.31	6.83	0.44	0.73
105.091	6.37	0.72	5.13	7.52	-0.02	-0.26
106.077	5.27	0.66	4.36	6.44	0.21	-0.01
106.097	4.27	0.46	3.52	4.95	-0.41	1.01
107.066	7.14	1.32	3.97	8.99	-1.07	1.28
107.085	9.06	0.89	7.39	10.38	-0.38	-0.40
108.069	4.79	0.63	3.78	5.94	0.20	0.49
108.089	6.63	0.84	5.11	7.93	-0.22	-0.56
109.070	6.30	0.70	5.32	7.51	0.28	-0.59
109.099	5.08	0.40	4.44	5.74	-0.34	1.28
110.071	4.49	0.53	3.62	5.37	-0.14	0.24
111.047	6.01	0.83	4.69	7.39	0.08	-0.56
111.080	4.55	0.40	3.90	5.16	-0.59	1.66
111.104	5.08	0.94	3.84	6.92	0.60	-0.15
111.119	4.43	0.68	3.08	5.35	-0.89	1.29
112.049	4.31	0.56	3.30	5.19	-0.49	0.89
113.029	4.64	0.48	3.82	5.39	-0.57	1.48
113.057	4.92	0.37	4.31	5.48	-0.49	1.51
113.098	4.83	0.48	4.13	5.67	0.55	1.03
115.077	5.84	0.52	5.03	6.61	-0.61	2.14
115.112	8.78	1.06	6.80	10.34	-0.41	-0.06
116.078	4.47	0.38	3.77	5.05	-0.62	1.45
116.116	6.32	0.94	4.56	7.76	-0.35	-0.14
117.047	4.87	0.63	3.70	5.76	-0.83	2.31
117.091	9.14	0.61	8.15	10.13	0.03	-0.02
118.095	6.53	0.57	5.63	7.47	0.14	-0.01
119.072	5.54	0.66	4.51	6.62	-0.03	-0.15
119.089	6.44	1.00	4.96	8.18	0.21	-0.30
119.107	6.36	0.53	5.56	7.28	0.33	-0.25
120.092	4.95	0.72	3.90	6.28	0.40	-0.08
121.068	6.43	0.68	5.53	7.73	0.62	-0.09
121.096	5.61	0.42	4.97	6.35	0.42	0.71
121.122	5.17	0.82	3.94	6.53	0.19	0.06
122.072	4.76	0.48	4.10	5.67	0.40	0.49
122.118	4.18	0.30	3.71	4.68	0.07	1.19
123.047	4.56	0.35	3.98	5.13	-0.27	1.20
123.076	4.56	0.38	3.91	5.13	-0.40	0.37
123.117	4.82	0.64	3.94	6.03	0.53	0.02
125.095	4.54	0.32	4.04	5.04	-0.50	1.80
125.132	4.39	0.45	3.69	5.14	0.13	0.39
127.073	4.49	0.38	3.80	5.05	-0.53	1.09
127.112	5.31	0.40	4.70	5.97	0.44	1.85
129.064	4.37	0.37	3.71	4.90	-0.62	1.37
129.091	4.93	0.39	4.35	5.66	0.34	0.72
129.127	4.95	0.69	3.97	6.24	0.51	-0.02
131.084	4.61	0.43	3.81	5.27	-0.67	1.59
131.107	5.75	0.66	4.87	7.11	0.88	0.78
132.109	4.29	0.40	3.72	5.03	0.66	1.29

133.073	4.45	0.37	3.84	5.01	-0.44	0.75
133.102	4.45	0.38	3.77	5.05	-0.19	0.51
133.123	5.60	0.69	4.49	6.78	0.11	-0.34
135.102	6.69	0.62	5.82	7.89	0.61	0.76
135.134	5.80	0.75	4.60	7.04	-0.01	-0.25
136.022	6.66	0.50	5.73	7.36	-0.42	-0.28
136.105	4.67	0.38	4.13	5.37	0.58	1.80
136.140	4.26	0.38	3.59	4.89	-0.31	0.97
137.024	4.70	0.44	3.88	5.31	-0.62	0.62
137.101	4.71	0.50	3.98	5.68	0.53	0.62
137.132	5.28	0.39	4.66	5.90	0.00	1.55
138.018	4.36	0.43	3.52	4.96	-0.73	1.13
139.076	4.58	0.35	3.93	5.09	-0.53	0.77
139.134	4.12	0.54	3.36	5.30	0.85	1.65
141.129	5.52	0.86	4.33	7.12	0.50	-0.28
142.131	4.26	0.57	3.40	5.31	0.21	0.44
143.115	4.69	0.55	3.79	5.68	0.21	1.01
143.143	7.11	1.19	5.44	9.24	0.42	-0.53
144.146	5.20	0.97	3.91	7.05	0.64	-0.17
145.123	7.43	0.78	6.24	8.78	0.31	-0.12
146.126	5.30	0.61	4.39	6.43	0.56	0.19
147.113	4.37	0.30	3.90	4.81	-1.11	6.04
147.134	4.37	0.32	3.81	4.86	-0.48	2.13
149.045	6.08	0.97	4.53	7.59	0.06	-0.55
149.123	5.49	0.36	4.93	6.08	0.19	0.26
150.046	4.67	0.75	3.39	5.87	-0.14	0.08
151.034	4.64	0.74	3.40	5.81	-0.16	0.21
155.144	4.26	0.39	3.64	4.88	-0.09	1.60
157.159	4.33	0.46	3.63	5.13	0.06	0.91
159.065	5.00	0.84	3.77	6.44	0.25	-0.56
159.138	4.49	0.38	3.87	5.15	0.02	0.56
161.104	4.39	0.36	3.80	4.94	-0.33	0.29
161.154	4.82	0.66	3.89	6.10	0.65	0.33
163.096	6.55	1.25	4.64	8.32	-0.44	0.48
163.131	5.72	0.47	4.97	6.56	0.35	0.54
164.100	5.30	0.85	4.08	6.68	0.32	-0.99
165.083	5.74	0.85	4.40	7.09	0.09	-0.73
166.083	4.45	0.61	3.40	5.39	-0.29	0.17
167.056	7.45	1.06	5.74	9.09	-0.08	-0.59
168.057	5.71	0.95	4.16	7.20	-0.06	-0.48
169.044	5.92	0.92	4.41	7.41	-0.09	-0.52
170.041	4.44	0.67	3.30	5.50	-0.32	0.42
171.032	4.43	0.59	3.43	5.36	-0.27	0.37
171.173	4.87	0.76	3.91	6.45	0.99	0.98
173.153	5.20	0.48	4.51	6.08	0.63	0.93
177.076	4.67	0.60	3.83	5.82	0.57	0.07
177.150	4.71	0.28	4.26	5.17	0.14	1.88
189.184	4.23	0.50	3.56	5.19	0.71	1.11

191.163	4.35	0.32	3.85	4.85	0.43	1.37
195.087	5.59	0.96	4.11	7.23	0.25	-0.48
196.088	4.48	0.64	3.43	5.60	0.13	0.21
197.074	4.64	0.64	3.64	5.78	0.22	0.09
201.184	4.34	0.41	3.71	5.04	0.14	1.37
205.186	4.29	0.31	3.81	4.77	-0.27	1.56

[1] P5 = 5th percentile and P95 = 95th percentile.

Supplementary Table 3. Correlation coefficients between the first 5 principal components (**PC**) and 240 spectrometric peaks detected by PTR-ToF-MS in individual model cheeses.

Item	PC1	PC2	PC3	PC4	PC5
R^2, %	28.3	39.2	47.8	55.4	61.5
33.034	-0.11	-0.44	-0.69	0.31	0.02
34.037	-0.11	-0.44	-0.68	0.32	0.03
34.995	-0.11	0.02	0.08	0.06	-0.39
39.023	-0.25	-0.01	0.00	0.17	-0.34
40.027	-0.11	-0.15	-0.14	0.22	-0.28
41.039	-0.45	-0.04	0.11	0.19	-0.43
42.010	-0.65	0.03	0.00	0.23	-0.32
42.034	-0.13	-0.45	-0.70	0.34	0.24
42.043	-0.22	-0.33	-0.07	0.24	-0.33
43.018	-0.57	-0.34	-0.21	0.30	-0.33
43.054	-0.61	-0.05	0.10	0.17	-0.33
43.094	-0.65	-0.18	0.10	0.23	-0.38
44.022	-0.57	-0.37	-0.20	0.29	-0.35
44.058	-0.57	-0.03	0.13	0.16	-0.38
44.980	-0.19	-0.19	-0.19	-0.66	0.05
45.033	-0.40	0.04	0.15	-0.01	0.46
46.031	-0.31	-0.23	-0.28	0.20	0.02
46.038	-0.33	0.10	0.17	-0.03	0.43
48.012	-0.81	0.49	-0.04	0.11	0.24
48.053	-0.73	0.49	-0.10	0.12	0.33
49.011	-0.01	-0.09	0.18	-0.02	-0.36
49.028	-0.06	0.17	-0.16	0.05	0.28
49.054	-0.74	0.50	-0.10	0.12	0.33
50.000	0.72	-0.53	0.06	-0.13	-0.27
50.057	-0.77	0.53	-0.05	0.11	0.28
51.007	-0.09	-0.09	0.17	-0.03	-0.38
51.044	-0.26	-0.46	-0.59	0.26	0.00
53.039	-0.08	-0.59	-0.32	0.23	0.11
54.034	-0.15	-0.44	-0.71	0.37	0.23
55.055	-0.15	-0.41	0.04	0.20	0.04
56.045	-0.49	-0.02	0.16	-0.13	0.08
56.060	-0.24	-0.66	-0.27	0.30	-0.02
57.033	-0.55	0.01	0.15	0.19	-0.42
57.070	-0.57	0.12	0.12	0.16	-0.31
58.041	0.09	-0.74	0.43	-0.12	0.26
58.073	-0.56	0.12	0.12	0.16	-0.31
59.049	-0.12	-0.59	-0.12	0.07	0.52
59.329	-0.12	-0.56	-0.12	0.08	0.51

60.021	0.17	-0.53	0.10	0.05	-0.61
60.045	-0.26	-0.44	-0.55	0.29	0.22
60.053	-0.12	-0.59	-0.12	0.08	0.52
61.028	-0.50	-0.31	-0.23	0.31	-0.41
61.062	-0.76	0.12	-0.06	0.23	-0.22
62.032	-0.48	-0.32	-0.22	0.31	-0.43
62.068	-0.73	0.19	0.00	0.18	-0.22
63.027	-0.26	0.12	-0.19	0.08	-0.04
63.044	-0.50	-0.04	0.19	-0.04	0.36
64.031	-0.25	0.12	-0.18	0.06	-0.02
64.048	-0.46	-0.04	0.21	-0.03	0.37
65.018	-0.02	-0.26	-0.08	0.04	-0.28
66.063	-0.78	0.44	-0.07	0.10	0.31
67.058	-0.70	-0.06	0.07	0.10	0.19
67.065	-0.76	0.47	-0.08	0.09	0.31
68.067	-0.82	0.39	-0.08	0.11	0.26
69.033	-0.55	-0.41	-0.20	0.26	-0.04
69.058	-0.22	-0.38	-0.10	0.07	-0.05
69.070	-0.24	-0.62	0.32	0.00	0.17
70.064	-0.58	-0.31	-0.28	0.29	0.27
70.078	-0.03	-0.72	-0.26	0.22	0.12
71.049	-0.36	-0.39	0.15	0.04	-0.14
71.086	-0.61	0.04	0.12	0.14	-0.06
72.053	-0.37	-0.44	0.17	0.03	-0.17
72.089	-0.63	0.03	0.12	0.14	-0.07
73.027	-0.17	-0.32	0.05	0.16	-0.46
73.051	-0.25	-0.10	-0.11	-0.46	-0.08
73.065	-0.31	-0.37	-0.14	0.21	-0.22
74.034	-0.03	-0.47	-0.07	0.19	-0.57
74.051	-0.29	-0.19	-0.17	-0.42	-0.24
74.069	-0.31	-0.37	-0.14	0.21	-0.22
75.027	-0.25	-0.14	-0.27	-0.49	-0.12
75.044	-0.39	-0.40	-0.61	0.40	0.07
75.080	-0.79	0.48	-0.06	0.13	0.20
76.047	-0.44	-0.40	-0.60	0.40	0.10
76.084	-0.80	0.49	-0.03	0.12	0.18
77.060	-0.30	-0.60	-0.09	0.08	0.40
78.001	0.03	-0.05	0.02	-0.03	-0.02
79.040	-0.56	-0.34	-0.18	0.24	-0.37
79.055	0.07	-0.04	-0.13	-0.06	0.09
79.075	-0.71	0.29	-0.06	0.16	-0.04
80.046	-0.52	-0.36	-0.18	0.23	-0.38
80.058	0.10	-0.01	-0.09	-0.10	0.08
80.991	-0.09	-0.35	-0.45	0.22	0.30

81.039	-0.59	-0.30	-0.20	0.21	-0.30
81.061	-0.37	0.00	0.18	-0.11	-0.03
81.070	-0.50	-0.24	-0.17	-0.02	-0.01
82.945	-0.14	-0.45	-0.68	0.37	0.25
82.988	-0.10	-0.35	-0.44	0.24	0.30
83.052	-0.42	-0.32	-0.26	0.27	-0.04
83.071	-0.80	0.42	-0.05	0.07	0.29
83.086	-0.34	-0.36	0.20	0.11	0.12
84.075	-0.73	0.44	-0.02	0.05	0.19
84.079	-0.19	-0.09	0.06	0.03	0.11
84.942	-0.14	-0.46	-0.67	0.38	0.24
85.029	-0.66	-0.05	-0.04	-0.11	-0.01
85.065	-0.40	-0.24	0.29	0.05	-0.43
85.101	-0.60	-0.05	-0.02	0.20	0.23
86.072	0.00	-0.66	0.40	-0.10	0.18
86.105	-0.58	-0.04	-0.02	0.20	0.23
87.044	-0.08	-0.45	0.16	-0.05	0.03
87.080	-0.07	-0.56	0.35	-0.10	0.53
88.052	-0.28	-0.56	-0.08	0.16	-0.13
88.084	-0.13	-0.55	0.29	-0.06	0.55
89.060	-0.60	-0.39	-0.16	0.29	-0.21
90.063	-0.60	-0.39	-0.16	0.29	-0.21
91.051	-0.09	-0.22	-0.47	-0.38	0.00
91.059	-0.59	-0.19	-0.28	-0.54	-0.06
92.061	-0.34	-0.45	-0.68	-0.02	0.10
93.037	-0.43	0.03	-0.21	-0.32	0.04
93.069	-0.21	-0.25	-0.47	0.16	0.20
93.090	-0.75	0.40	-0.17	0.13	0.30
93.181	-0.60	0.14	-0.18	0.03	0.08
93.432	-0.76	0.27	-0.23	0.11	0.22
94.039	-0.22	-0.09	-0.14	-0.73	-0.10
94.074	-0.40	-0.23	-0.59	0.34	0.25
94.095	-0.73	0.51	-0.03	0.10	0.30
95.004	-0.19	-0.11	0.31	-0.01	-0.35
95.017	-0.17	-0.14	-0.40	-0.52	0.07
95.034	-0.26	-0.29	-0.29	-0.67	-0.15
95.049	-0.37	-0.34	-0.61	0.28	0.09
95.081	-0.30	-0.49	-0.49	0.31	0.11
95.096	-0.76	0.54	0.06	0.06	0.23
96.961	-0.17	-0.29	-0.44	0.25	0.31
97.060	-0.64	-0.20	0.04	0.10	-0.01
97.101	-0.31	-0.59	0.52	-0.04	0.34
98.105	-0.29	-0.61	0.51	-0.03	0.31
98.959	-0.17	-0.30	-0.44	0.25	0.32

99.039	-0.79	0.10	0.04	0.04	0.00
99.081	-0.69	-0.07	0.23	0.14	-0.40
99.121	-0.39	-0.11	0.32	0.07	-0.17
100.084	-0.71	-0.09	0.22	0.17	-0.38
100.954	-0.15	-0.33	-0.45	0.26	0.30
101.060	-0.73	-0.11	0.20	0.00	0.07
101.097	-0.15	-0.55	0.48	-0.11	0.43
102.062	-0.69	-0.22	0.11	0.11	-0.11
102.099	-0.21	-0.53	0.50	-0.10	0.39
103.075	-0.37	-0.16	0.27	0.08	-0.58
104.079	-0.36	-0.17	0.26	0.07	-0.60
105.039	-0.42	-0.39	-0.04	-0.58	-0.14
105.071	-0.54	-0.19	0.18	0.04	-0.27
105.091	-0.44	-0.45	0.25	-0.02	0.54
106.077	-0.20	-0.23	0.11	-0.12	-0.16
106.097	-0.43	-0.41	0.23	0.00	0.50
107.066	-0.68	-0.01	-0.01	0.23	-0.17
107.085	-0.47	0.06	0.02	-0.07	-0.06
108.069	-0.74	0.02	0.00	0.25	-0.20
108.089	-0.42	0.06	0.03	-0.08	-0.04
109.070	-0.54	-0.21	-0.17	-0.65	-0.15
109.099	-0.65	0.09	0.28	0.12	-0.11
110.071	-0.49	-0.21	-0.17	-0.67	-0.16
111.047	-0.36	-0.27	-0.24	-0.73	-0.11
111.080	-0.56	-0.09	0.02	-0.27	-0.11
111.104	-0.80	0.47	-0.03	0.14	0.25
111.119	0.07	-0.65	-0.34	0.23	-0.04
112.049	-0.34	-0.28	-0.23	-0.72	-0.11
113.029	-0.30	-0.38	-0.32	-0.64	-0.16
113.057	-0.64	-0.18	0.07	-0.26	-0.10
113.098	-0.29	-0.52	0.45	-0.05	-0.03
115.077	-0.56	-0.19	0.32	0.01	-0.05
115.112	-0.11	-0.59	0.56	-0.12	0.40
116.078	-0.48	-0.42	0.43	0.02	0.00
116.116	-0.12	-0.59	0.57	-0.12	0.40
117.047	-0.16	-0.19	-0.03	-0.05	0.02
117.091	-0.80	0.21	0.23	0.18	-0.14
118.095	-0.80	0.21	0.23	0.18	-0.14
119.072	-0.77	0.42	-0.07	0.05	0.23
119.089	-0.82	0.35	-0.18	-0.24	0.17
119.107	-0.62	0.16	0.12	-0.03	0.05
120.092	-0.82	0.38	-0.17	-0.22	0.18
121.068	-0.84	0.21	-0.08	-0.26	-0.01
121.096	-0.79	-0.05	-0.10	0.14	-0.30

121.122	-0.76	0.44	0.01	0.15	0.17
122.072	-0.84	0.22	-0.09	-0.25	-0.05
122.118	-0.80	0.24	0.02	0.23	-0.03
123.047	-0.84	0.25	-0.16	-0.23	0.03
123.076	-0.80	0.19	-0.05	-0.24	-0.01
123.117	-0.25	-0.47	0.62	-0.10	0.18
125.095	-0.82	-0.06	0.17	0.07	-0.03
125.132	-0.41	-0.31	0.56	-0.06	-0.01
127.073	-0.74	0.03	0.08	0.00	-0.09
127.112	-0.73	-0.22	0.36	0.03	-0.14
129.064	-0.75	0.00	-0.08	-0.28	-0.11
129.091	-0.50	-0.31	0.26	0.12	-0.29
129.127	-0.22	-0.48	0.66	-0.11	0.21
131.084	-0.55	-0.35	-0.17	-0.18	0.13
131.107	-0.53	0.04	0.33	0.09	-0.49
132.109	-0.53	-0.02	0.31	0.05	-0.47
133.073	-0.77	-0.18	-0.16	-0.30	-0.09
133.102	-0.64	-0.29	-0.30	0.28	0.06
133.123	-0.51	-0.35	0.48	-0.07	0.53
135.102	-0.88	0.17	0.07	0.20	-0.14
135.134	-0.80	0.41	0.03	0.11	0.19
136.022	-0.77	0.22	0.03	0.03	0.14
136.105	-0.88	0.19	0.08	0.19	-0.14
136.140	-0.78	0.41	0.07	0.11	0.16
137.024	-0.74	0.21	0.01	0.00	0.13
137.101	-0.87	0.32	-0.11	-0.21	0.10
137.132	-0.60	-0.19	-0.27	-0.09	0.04
138.018	-0.71	0.22	0.03	0.01	0.13
139.076	-0.85	0.18	-0.01	-0.21	0.04
139.134	-0.73	0.44	0.03	0.09	0.15
141.129	-0.24	-0.49	0.62	-0.11	0.19
142.131	-0.19	-0.49	0.61	-0.12	0.19
143.115	-0.45	-0.13	0.29	0.00	-0.18
143.143	-0.17	-0.46	0.60	-0.10	0.15
144.146	-0.16	-0.46	0.61	-0.11	0.16
145.123	-0.72	0.19	0.26	0.15	-0.11
146.126	-0.72	0.19	0.26	0.16	-0.11
147.113	-0.69	-0.32	0.03	0.13	-0.16
147.134	-0.73	0.04	0.27	0.04	0.04
149.045	-0.23	-0.14	-0.32	-0.74	-0.13
149.123	-0.86	0.06	-0.02	-0.15	-0.24
150.046	-0.20	-0.11	-0.32	-0.74	-0.09
151.034	-0.15	-0.13	-0.34	-0.73	-0.10
155.144	-0.63	-0.25	0.42	-0.02	-0.03

157.159	-0.37	-0.30	0.54	-0.12	0.11
159.065	-0.40	0.04	-0.12	0.04	0.04
159.138	-0.61	-0.18	0.37	0.09	-0.26
161.104	-0.70	-0.45	-0.16	-0.19	-0.10
161.154	-0.48	-0.34	0.61	-0.08	0.33
163.096	-0.40	0.02	-0.32	-0.65	0.02
163.131	-0.91	0.19	0.04	-0.02	-0.14
164.100	-0.67	0.03	-0.22	-0.58	-0.05
165.083	-0.58	-0.03	-0.27	-0.71	-0.01
166.083	-0.55	-0.03	-0.26	-0.71	0.00
167.056	-0.29	-0.23	-0.30	-0.79	-0.06
168.057	-0.28	-0.22	-0.30	-0.79	-0.07
169.044	-0.23	-0.27	-0.30	-0.79	-0.10
170.041	-0.18	-0.26	-0.29	-0.79	-0.10
171.032	-0.24	-0.31	-0.26	-0.76	-0.14
171.173	-0.23	-0.34	0.48	-0.12	0.11
173.153	-0.72	-0.01	0.34	0.09	0.11
177.076	-0.79	0.28	-0.18	-0.23	0.05
177.150	-0.88	-0.06	0.06	0.00	-0.21
189.184	-0.58	-0.13	0.49	-0.01	0.25
191.163	-0.87	0.27	0.14	0.11	-0.10
195.087	-0.77	0.39	-0.22	-0.27	0.24
196.088	-0.77	0.39	-0.19	-0.27	0.20
197.074	-0.79	0.38	-0.20	-0.28	0.21
201.184	-0.55	-0.28	0.46	-0.04	0.28
205.186	-0.88	0.06	0.18	0.10	-0.06

Supplementary Table 4. Effect of sampled dairy systems on PTR-ToF-MS spectrometric peaks expressed as natural logarithm of the parts per billion by volume (in bold the identified masses).

m/z	Dairy system[1] (LSM)					Contrasts (*P*-value)			
	Traditional (tied, hay + CF)		Modern (pens with free animals and milking parlor)						
	Without AF (n = 135)	With AF[2] (n = 180)	Hay + CF (n = 446)	TMR		Traditional *vs* modern farms	Automatic feeder effect[2]	TMR effect[3]	Silage effect[4]
				Without silage (n = 209)	With silage (n = 105)				
33.034	0.15	0.39	0.07	-0.39	-0.40		0.041		
45.033	-0.14	-0.08	-0.11	0.34	0.23			0.042	
46.038	-0.09	-0.06	-0.14	0.32	0.31			0.019	
48.012	0.02	-0.15	-0.13	0.36	0.05			0.041	
48.053	0.03	-0.15	-0.15	0.33	0.11			0.042	
49.054	0.03	-0.15	-0.15	0.34	0.11			0.038	
50.000	-0.01	0.19	0.16	-0.43	-0.01			0.021	
50.057	0.01	-0.16	-0.15	0.39	0.05			0.029	
51.044	0.12	0.36	0.08	-0.34	-0.39		0.046		
56.060	-0.04	0.25	0.10	-0.11	-0.52			0.043	
60.021	0.00	0.21	0.10	-0.32	-0.07			0.035	
63.027	0.61	0.29	-0.20	-0.29	0.11		0.010		
64.031	0.53	0.33	-0.21	-0.28	0.16		0.013		
65.018	0.33	0.37	-0.08	-0.42	0.15		0.001		0.008
67.065	0.04	-0.14	-0.14	0.34	0.10			0.035	

70.078	-0.15	0.19	0.17	-0.22	-0.33		0.025	
73.027	-0.15	0.24	0.01	-0.13	-0.06	0.034		
74.034	-0.19	0.32	0.11	-0.23	-0.27	0.017	0.015	
75.080	0.06	-0.16	-0.14	0.35	0.03		0.046	
76.084	0.03	-0.17	-0.14	0.38	0.02		0.036	
81.070	0.27	0.00	0.11	-0.09	-0.57		0.032	
84.075	-0.02	-0.15	-0.10	0.31	0.09		0.027	
84.079	0.06	0.03	-0.04	0.14	-0.23			0.027
85.101	-0.21	-0.01	-0.06	0.50	-0.46			0.001
86.105	-0.26	-0.02	-0.06	0.53	-0.44			0.001
91.051	-0.15	0.05	0.12	-0.15	-0.12		0.044	
94.095	0.05	-0.17	-0.15	0.34	0.08		0.030	
95.096	0.03	-0.22	-0.14	0.39	0.07		0.019	
97.101	-0.18	0.05	0.06	0.15	-0.29			0.024
98.105	-0.13	0.04	0.05	0.13	-0.24			0.050
111.104	-0.03	-0.16	-0.12	0.38	0.01		0.047	
111.119	-0.21	0.29	0.19	-0.22	-0.50		0.016	
113.029	-0.01	-0.04	0.12	-0.07	-0.10		0.032	
113.057	-0.01	-0.12	0.04	0.18	-0.18			0.023
115.077	-0.03	0.02	-0.01	0.18	-0.27			0.046
117.091	-0.10	0.00	-0.12	0.35	-0.15			0.050
118.095	-0.10	0.00	-0.12	0.35	-0.16			0.046
119.089	0.00	-0.18	-0.10	0.34	0.07		0.046	
119.107	-0.30	0.12	-0.12	0.14	0.49		0.050	
121.122	0.00	-0.15	-0.17	0.41	0.06		0.018	
127.073	-0.11	0.05	-0.02	0.19	-0.15			0.030
129.091	-0.17	0.10	0.03	0.15	-0.48			0.026
133.123	-0.08	-0.09	-0.02	0.31	-0.18			0.048

135.134	-0.15	-0.22	-0.10	0.42	0.13	0.034	0.022	
136.105	-0.06	-0.03	-0.11	0.31	-0.12			0.040
136.140	-0.17	-0.21	-0.11	0.46	0.11	0.023	0.014	
137.024	-0.02	-0.13	-0.07	0.25	0.08		0.036	
137.101	0.00	-0.15	-0.09	0.33	0.05		0.050	
137.132	0.29	0.09	0.09	-0.15	-0.51		0.039	
139.134	-0.03	-0.18	-0.11	0.39	-0.05		0.041	0.039
145.123	-0.12	-0.02	-0.11	0.38	-0.10			0.049
146.126	-0.12	-0.01	-0.13	0.39	-0.12			0.033
169.044	-0.05	-0.04	0.12	-0.13	0.01		0.037	
195.087	0.01	-0.17	-0.10	0.31	0.13		0.042	
196.088	0.02	-0.16	-0.10	0.31	0.10		0.040	
197.074	0.02	-0.17	-0.09	0.29	0.11		0.049	
205.186	-0.02	-0.08	-0.05	0.26	-0.16			0.029

[1] AF = automatic feeders at mangers to control individual concentrates distributions; CF= compound feed distributed at mangers; without silage = water added in the mixer wagon to enhance mixing; [2] Contrast between traditional farms with compound feed administered in the mangers with hay *vs* traditional farms with compound feed administered in the mangers through automatic feeders; [3] Contrast between modern farms with hay and compound feed administered in the mangers *vs.* modern farms using TMR; [4] Contrast between modern farms using TMR without silages *vs* TMR with silages.

Supplementary Table 5. Effect of days in milk on PTR-ToF-MS spectrometric peaks expressed as natural logarithm of the parts per billion by volume (in bold the identified masses).

	DIM (LSM)							Contrasts (*P*-value)		
	≤50	51-100	101-150	151-200	201-250	251-300	>300	Linear	Quadratic	Cubic
m/z	(n = 136)	(n = 157)	(n = 169)	(n = 142)	(n = 123)	(n = 138)	(n = 210)			
34.995	-0.05	-0.37	-0.09	0.00	0.07	0.18	0.25	<0.001		0.036
41.039	-0.06	-0.11	-0.11	-0.04	0.02	0.22	0.18	0.002		
42.010	0.00	-0.17	-0.20	-0.11	0.10	0.00	0.22	0.016	0.013	
42.034	-0.04	-0.02	-0.07	-0.06	-0.10	-0.11	-0.14	0.011		
43.054	-0.07	-0.18	-0.13	-0.15	0.07	0.23	0.23	<0.001	0.026	
43.094	-0.04	-0.16	-0.13	-0.10	0.05	0.17	0.18	0.005		
44.058	-0.07	-0.12	-0.10	-0.09	0.07	0.25	0.16	0.002		
44.980	0.29	0.19	-0.03	0.08	0.05	-0.14	-0.08	0.002		
45.033	0.27	0.25	0.01	-0.01	-0.06	-0.16	0.03	0.001	0.007	
46.038	0.27	0.26	0.05	0.03	-0.05	-0.12	0.03	0.001	0.029	
48.012	-0.07	-0.13	-0.08	-0.06	0.08	0.20	0.26	<0.001		
48.053	-0.02	-0.05	-0.04	-0.09	0.07	0.16	0.23	0.007	0.040	
49.011	-0.15	-0.18	-0.01	0.06	0.09	0.26	0.22	<0.001		
49.028	0.18	0.13	-0.01	-0.03	-0.02	-0.08	0.06		0.047	
49.054	-0.03	-0.06	-0.04	-0.08	0.07	0.16	0.23	0.005	0.045	
50.000	0.11	0.09	0.07	0.07	-0.08	-0.18	-0.22	0.001		
50.057	-0.08	-0.12	-0.06	-0.08	0.07	0.18	0.27	<0.001		
51.007	-0.17	-0.19	-0.04	0.02	0.15	0.28	0.24	<0.001		
54.034	-0.04	-0.05	-0.06	-0.08	-0.11	-0.12	-0.10	0.018		
57.033	-0.10	-0.02	-0.08	-0.05	0.09	0.19	0.11	0.018		
57.070	-0.20	-0.08	-0.07	-0.01	0.04	0.25	0.24	<0.001		

58.041	0.14	0.12	0.03	-0.01	-0.09	-0.16	-0.16	0.002		
58.073	-0.19	-0.07	-0.07	-0.01	0.04	0.24	0.22	<0.001		
59.049	0.17	0.02	-0.03	-0.08	-0.15	-0.16	-0.13	0.001		
59.329	0.19	0.04	-0.06	-0.08	-0.16	-0.15	-0.12	0.001	0.023	
60.045	0.01	0.03	-0.05	-0.08	-0.11	-0.17	-0.11	0.027		
60.053	0.18	0.02	-0.03	-0.08	-0.14	-0.17	-0.13	0.001		
61.062	-0.05	-0.17	-0.03	-0.09	0.03	0.21	0.21	0.001		
62.068	-0.05	-0.16	-0.06	-0.09	0.07	0.25	0.26	<0.001	0.046	
63.044	0.22	0.23	-0.04	-0.01	-0.01	-0.15	0.02	0.007	0.037	
64.048	0.20	0.24	-0.06	-0.05	0.02	-0.12	0.03	0.020	0.020	
66.063	-0.02	-0.03	-0.05	-0.10	0.09	0.14	0.21	0.014		
67.058	-0.12	-0.10	-0.15	-0.07	0.01	0.16	0.18	0.003		
67.065	-0.02	-0.03	-0.05	-0.09	0.10	0.15	0.21	0.014		
68.067	-0.11	-0.13	-0.08	-0.10	0.09	0.19	0.25	<0.001		
69.033	0.10	-0.13	-0.11	-0.12	0.00	-0.07	0.04		0.018	
69.058	-0.07	0.14	0.06	-0.03	-0.08	-0.14	-0.05			0.033
71.086	-0.04	-0.19	-0.15	-0.05	-0.03	0.26	0.11	0.004		0.021
72.089	-0.05	-0.18	-0.17	-0.06	-0.01	0.25	0.10	0.003		0.018
73.065	-0.14	0.00	-0.13	-0.01	0.03	0.10	0.11	0.032		
74.069	-0.14	-0.01	-0.12	-0.01	0.03	0.11	0.11	0.028		
75.080	-0.09	-0.08	-0.08	-0.04	0.06	0.18	0.25	0.001		
76.084	-0.12	-0.12	-0.10	-0.05	0.06	0.21	0.27	<0.001		
77.060	0.16	0.05	-0.06	-0.08	-0.12	-0.13	-0.10	0.011		
79.075	-0.13	-0.16	-0.01	-0.11	0.11	0.26	0.24	<0.001		
80.991	0.11	-0.12	-0.13	-0.08	-0.10	0.00	-0.01		0.017	
81.039	0.04	0.04	-0.08	-0.14	-0.01	-0.08	0.06		0.046	
81.061	0.09	0.05	-0.02	0.00	0.17	0.09	0.02			0.046
82.945	-0.02	-0.07	-0.09	-0.11	-0.12	-0.09	-0.11	0.025	0.030	

82.988	0.10	-0.13	-0.15	-0.08	-0.14	0.00	0.01		0.007	
83.071	-0.03	-0.02	-0.07	-0.09	0.10	0.14	0.21	0.018		
84.075	-0.03	-0.13	-0.06	-0.12	0.14	0.16	0.22	0.003		
84.942	-0.02	-0.05	-0.08	-0.09	-0.14	-0.11	-0.12	0.001		
85.029	0.10	-0.08	-0.11	-0.20	0.06	0.08	0.03		0.028	0.040
85.101	0.04	-0.07	-0.17	-0.15	-0.09	0.05	0.05		0.002	
86.072	0.13	0.04	0.03	-0.01	-0.04	-0.15	-0.14	0.014		
86.105	0.03	-0.08	-0.15	-0.16	-0.07	0.03	0.06		0.003	
87.044	0.01	0.12	0.06	-0.02	-0.15	-0.15	-0.08			0.043
87.080	0.10	0.08	0.02	-0.08	-0.07	-0.17	-0.10	0.014		
88.084	0.09	0.08	-0.02	-0.12	-0.08	-0.15	-0.11	0.028		
91.051	0.12	-0.03	-0.03	0.00	-0.04	-0.21	-0.16	0.023		
92.061	0.00	-0.05	-0.06	-0.08	-0.03	-0.16	-0.15	0.037		
93.090	-0.04	-0.04	-0.05	-0.13	0.06	0.15	0.21	0.010	0.042	
93.181	-0.18	-0.02	-0.07	-0.20	0.13	0.17	0.05	0.017		
93.432	-0.15	-0.13	-0.11	-0.11	0.04	0.18	0.20	<0.001		
94.095	-0.05	-0.06	-0.06	-0.08	0.07	0.14	0.25	0.003	0.049	
95.004	0.18	-0.02	-0.02	-0.07	-0.07	0.06	-0.10			0.017
95.034	0.16	0.15	-0.12	0.03	0.09	-0.23	-0.22	0.002		
95.049	-0.06	-0.08	-0.11	-0.15	-0.02	-0.08	-0.01		0.044	
95.096	-0.09	-0.09	-0.11	-0.05	0.06	0.21	0.26	<0.001		
96.961	0.06	-0.13	-0.15	-0.07	-0.11	0.03	0.03		0.019	
97.060	0.19	0.12	-0.04	-0.03	0.02	-0.01	0.04		0.031	
98.959	0.08	-0.13	-0.16	-0.08	-0.12	0.04	0.03		0.006	
99.039	0.00	-0.08	-0.14	-0.09	0.00	0.07	0.20		0.015	
99.081	-0.02	-0.14	-0.19	-0.11	0.09	0.10	0.25	0.004	0.010	
99.121	0.05	-0.08	-0.10	-0.05	-0.03	0.18	0.03			0.047
100.084	-0.01	-0.13	-0.17	-0.13	0.07	0.06	0.23	0.011	0.008	

100.954	0.08	-0.13	-0.13	-0.08	-0.13	0.02	0.02		0.013	
101.060	0.11	-0.06	-0.15	-0.08	0.06	-0.04	0.13		0.012	
102.062	0.12	-0.06	-0.09	-0.09	-0.07	-0.04	0.06		0.018	
103.075	-0.09	-0.15	-0.14	-0.05	0.13	0.05	0.10	0.021		
104.079	-0.07	-0.11	-0.11	-0.03	0.16	0.05	0.08	0.047		
108.069	-0.05	-0.15	-0.10	-0.10	0.08	0.10	0.19	0.009		
109.099	-0.07	-0.10	-0.10	-0.10	0.08	0.16	0.14	0.012		
111.047	0.14	0.09	-0.05	0.03	0.05	-0.21	-0.12	0.014		
111.080	0.04	-0.11	-0.15	-0.06	0.13	0.12	0.07			0.019
111.104	-0.10	-0.12	-0.10	-0.07	0.05	0.20	0.25	<0.001		
111.119	0.01	0.06	-0.01	-0.08	-0.14	-0.22	-0.24	<0.001		
112.049	0.14	0.13	-0.07	0.02	0.08	-0.22	-0.17	0.005		
113.029	0.16	0.16	-0.08	-0.01	0.03	-0.21	-0.19	0.002		
115.077	0.20	0.03	-0.15	-0.12	-0.04	-0.07	0.00		0.004	
116.078	0.26	-0.01	-0.12	-0.04	-0.10	-0.11	0.03		0.001	
117.047	-0.12	-0.15	-0.05	-0.08	0.09	0.11	0.16	0.005		
117.091	0.03	-0.19	-0.18	-0.17	0.01	0.14	0.31	0.001	<0.001	
118.095	0.02	-0.18	-0.18	-0.16	0.01	0.14	0.32	<0.001	<0.001	
119.072	0.05	-0.07	-0.05	-0.11	0.07	0.14	0.24	0.030	0.005	
119.107	0.02	0.04	-0.03	0.00	0.10	0.12	0.19	0.045		
120.092	0.01	-0.08	-0.08	-0.05	0.11	0.11	0.18	0.036		
121.096	-0.10	-0.14	-0.19	-0.11	0.14	0.07	0.17	0.003		
121.122	-0.13	-0.10	-0.06	-0.06	0.02	0.23	0.29	<0.001		
122.118	-0.19	-0.19	-0.09	-0.09	0.03	0.24	0.29	<0.001		
123.047	0.06	-0.07	-0.15	-0.10	0.15	0.10	0.14		0.048	0.046
125.095	0.00	-0.01	-0.11	-0.14	0.05	0.01	0.17		0.028	
127.073	-0.13	-0.10	-0.10	-0.07	0.05	0.10	0.19	0.004		
127.112	0.02	-0.05	-0.09	-0.10	0.01	0.09	0.14		0.047	

129.091	-0.01	-0.15	-0.16	-0.19	-0.07	-0.01	0.07		0.005	
131.107	-0.15	-0.20	-0.19	-0.02	0.10	0.23	0.27	<0.001		
132.109	-0.11	-0.14	-0.19	-0.03	0.10	0.15	0.19	<0.001		
133.102	-0.07	-0.05	-0.07	-0.17	-0.12	-0.05	0.07		0.021	
135.102	-0.03	-0.13	-0.19	-0.12	0.11	0.11	0.28	0.001	0.005	
135.134	-0.04	-0.15	-0.15	-0.06	0.06	0.23	0.24	<0.001	0.019	
136.022	-0.15	0.00	-0.12	-0.08	0.11	0.16	0.20	0.002		
136.105	-0.05	-0.13	-0.15	-0.15	0.11	0.11	0.27	0.001	0.008	
136.140	-0.09	-0.16	-0.12	-0.07	0.02	0.25	0.25	<0.001	0.034	
137.024	-0.10	0.00	-0.10	-0.13	0.12	0.14	0.20	0.008		
137.101	0.00	-0.07	-0.10	-0.06	0.14	0.10	0.18	0.032		
138.018	-0.16	-0.05	-0.13	-0.10	0.11	0.20	0.23	<0.001		
139.134	-0.15	-0.19	-0.09	-0.09	0.06	0.25	0.23	<0.001		
143.115	-0.22	0.03	-0.12	-0.11	0.11	0.01	0.16	0.014		
145.123	-0.09	-0.21	-0.17	-0.15	0.04	0.21	0.39	<0.001	<0.001	
146.126	-0.09	-0.20	-0.18	-0.14	0.04	0.20	0.39	<0.001	<0.001	
147.113	0.05	-0.10	-0.11	-0.19	-0.02	0.01	0.16		0.002	
147.134	-0.04	-0.10	-0.17	0.00	-0.01	0.23	0.24	0.001	0.024	
149.045	0.17	0.07	-0.02	-0.01	0.05	-0.20	-0.11	0.012		
149.123	-0.04	-0.13	-0.15	-0.09	0.21	0.14	0.13	0.008		0.021
150.046	0.17	0.06	0.01	0.00	0.07	-0.21	-0.10	0.013		
151.034	0.24	0.06	-0.03	-0.05	0.04	-0.15	-0.12	0.007		
155.144	-0.08	-0.08	-0.15	-0.06	0.10	0.13	0.17	0.010		
159.138	-0.09	-0.05	-0.12	-0.05	0.13	0.18	0.22	0.002		
163.131	-0.03	-0.14	-0.14	-0.12	0.16	0.16	0.26	0.001	0.025	
167.056	0.12	0.08	-0.04	-0.03	0.06	-0.19	-0.10	0.037		
168.057	0.13	0.08	-0.04	-0.02	0.06	-0.22	-0.10	0.024		
169.044	0.14	0.10	-0.03	-0.01	0.02	-0.24	-0.12	0.008		

170.041	0.15	0.13	-0.06	0.03	0.06	-0.22	-0.16	0.004	
171.032	0.14	0.12	0.00	0.01	0.01	-0.20	-0.14	0.006	
173.153	-0.05	-0.12	-0.09	-0.10	0.04	0.13	0.37	<0.001	0.002
177.150	-0.02	-0.12	-0.18	-0.16	0.16	0.10	0.18	0.009	0.041
191.163	-0.11	-0.14	-0.16	-0.11	0.15	0.19	0.30	<0.001	0.037
205.186	-0.07	-0.12	-0.18	-0.13	0.10	0.14	0.20	0.002	0.043

Supplementary Table 6. Effect of parity on PTR-ToF-MS spectrometric peaks expressed as natural logarithm of the parts per billion by volume (in bold the identified masses).

	Parity (LSM)				Contrasts (*P*-value)		
	1	2	3	≥ 4	1*vs* ≥2	2*vs* ≥3	3*vs* ≥ 4
m/z	(n = 298)	(n = 323)	(n =166)	(n =288)			
46.038	-0.04	0.09	0.21	0.01	0.020		0.046
48.053	0.12	0.06	0.09	-0.13			0.044
49.011	-0.02	-0.05	0.05	0.18		0.015	0.024
49.054	0.12	0.06	0.09	-0.13			0.041
50.000	-0.14	-0.02	-0.05	0.13	0.011		0.014
50.057	0.13	0.03	0.10	-0.15	0.029		0.027
51.007	-0.03	-0.06	0.09	0.16		0.005	0.011
56.060	-0.15	-0.08	0.00	-0.03	0.042		0.032
63.044	-0.07	0.01	0.22	0.00	0.024		0.010
64.048	-0.05	0.00	0.24	-0.04			0.022
67.058	0.11	-0.05	0.08	-0.19	0.018		
70.078	-0.17	-0.10	-0.02	0.02	0.028		0.009
84.075	0.17	-0.02	0.09	-0.13	0.004		0.024
94.095	0.11	0.06	0.08	-0.13			0.034
95.096	0.14	0.02	0.09	-0.14	0.020		0.026
102.062	-0.14	0.01	0.13	-0.10	0.030		
111.104	0.12	0.02	0.09	-0.16	0.028		0.030
117.047	-0.15	-0.11	0.09	0.15	0.007	0.001	0.000
123.047	0.12	0.00	0.11	-0.16	0.040		
139.134	0.10	-0.01	0.06	-0.14	0.049		
145.123	-0.09	0.00	0.18	-0.08	0.039		0.043
146.126	-0.10	0.01	0.17	-0.06	0.026		0.034

Supplementary Table 7. Linear regression (Regr) with daily milk yield (kg×d^{-1}) of PTR-ToF-MS spectrometric peaks expressed as natural logarithm of the parts per billion by volume (in bold the identified masses).

m/z	Regr	*P*-value
33.034	-0.001	0.042
40.027	-0.009	0.016
51.044	-0.003	0.029
65.018	0.004	0.040
85.065	0.011	0.042
86.072	0.014	0.032
87.080	0.013	0.015
88.084	0.011	0.022
95.034	-0.013	0.009
97.101	0.011	0.010
98.105	0.011	0.021
101.060	0.007	0.038
101.097	0.015	<0.001
102.099	0.015	<0.001
111.047	-0.012	0.022
112.049	-0.013	0.026
113.029	-0.015	0.003
115.112	0.016	0.001
116.116	0.016	0.002
123.117	0.014	0.009
129.127	0.019	<0.001
133.123	0.009	0.013
137.132	-0.003	0.041
141.129	0.013	0.011
142.131	0.015	0.006
143.143	0.015	0.007
144.146	0.015	0.006
157.159	0.014	0.007
161.154	0.013	0.003
171.173	0.013	0.012
189.184	0.009	0.027

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THIRD CHAPTER

From cow to cheese: Heritability of the flavor fingerprint of cheese investigated by direct injection mass spectrometry (PTR-ToF-MS)

M. Bergamaschi[*†], A. Cecchinato[*], F. Biasioli[†], F. Gasperi[†], B. Martin[‡§], and G. Bittante[*]

[*]Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy

[†]Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach (FEM) Via E. Mach 1, 38010 San Michele all'Adige (TN), Italy

[‡]INRA, UMR 1213 Herbivores, F-63122 Saint-Genès Champanelle, France

[§]Clermont Université, VetAgro Sup, BP 10448, F-63000 Clermont-Ferrand, France

ABSTRACT

Volatile organic compounds drive important quality traits in cheese. The aim of this work was to estimate the heritability of the volatile compound profile of cheese as detected by direct injection mass spectrometry of the headspace of model cheeses produced from milk samples obtained from individual cows. A total of 1,075 model cheeses were produced using milk samples collected from individual Brown Swiss cows. Single spectrometric peaks and their combination obtained by principal component analysis were analyzed. Genetic parameters of 240 individual spectrometric peaks and of the first 10 components (PCs) of a Principal Component Analysis (PCA) extracted from them were estimated with a Bayesian approach. The results show genetic variation in the cheese volatile fingerprint. Intra-herd heritability of the PCs was in the range of 3.6% to 10.2%, which is similar to the heritability found in the same population for milk fat, many fatty acids, SCS, and some curd firming model parameters. Results also showed genetic correlations between some PCs (-0.79 to 0.86), and also several herd-date correlations (-0.88 to 0.66), confirming the relationship between cheese flavor and dairy system. Most peaks were characterized by a high heritability value, and about one quarter displayed heritability estimates greater than the best PC (up to 21.6%). Despite the phenotypic independency of principal components from each other, residual correlations were low, but some genetic and herd correlations were high (positive or negative). The concentration in cheese of VOCs with the greater heritability presented genetic, herd and residual correlations with each other variable and generally positive. Also correlations of PCs and selected VOCs of cheeses with quality traits of milk before cheese-making were variable and often greater for genetic, intermediate for herd and smaller for residual correlations. This study indicates the existence of a link between cow genetics, milk quality and the volatile compound profile of cheese suggesting the possibility of controlling and improving the final quality of dairy products through genetic selection of cows.

Keywords: heritability, genetic correlation, cheese, volatile organic compound, flavor.

INTRODUCTION

Volatile organic compounds (**VOCs**) are important molecules determining the flavor and consequently the perceived quality of cheese (Molimard and Spinnler, 1996; Bellesia et al., 2003). It is well established that cheeses are characterized by distinct aroma profiles (Drake et al., 2008; Liggett et al., 2008) and the literature contains several studies focused on dairy systems, the cows' feeding regime, and milk quality, and their relationships with the sensory properties of cheese (Martin et al., 2005; Coppa et al., 2011; Romanzin et al., 2013).

In recent years, several instruments have been used to determine the qualitative characteristics of cheese flavor compounds (Le Quéré, 2004; Carunchia Whetstine et al., 2006; Cornu et al., 2009). Gas-chromatography combined with head space extraction has been commonly used to link VOCs with the flavor of cheese (Delgado et al., 2011; Thomsen et al., 2014; Valdivielso et al., 2016), while Bergamaschi et al. (2015a) proposed solid phase micro extraction and gas-chromatography mass spectrometry for the extraction of VOCs in individual model cheeses and found these quality traits to be affected by dairy system, herd, and the cows' parity, stage of lactation, and milk yield.

Recently, a model cheese procedure has been used to produce a large number (more than 1,000) of individual model cheeses (Cipolat-Gotet et al., 2013) that can be used to estimate the genetic parameters of cheese yields and nutrient recovery (Bittante et al., 2013a). In addition, a direct injection spectrometric technique (Proton Transfer Reaction-Time of Flight-Mass Spectrometry, **PTR-ToF-MS**) has for the first time been used to obtain the volatile fingerprints of those same model cheeses (Bergamaschi et al., 2015b). Having obtained 240 VOCs, these authors extracted principal components (**PC**) of the volatile fingerprints to obtain information on cheese flavor, and reported the effects of dairy system and individual cow characteristics on these new traits.

Despite the centrality and importance of VOCs potentially related to sensory properties, no research has been carried out to estimate the heritability and genetic correlations of their concentrations in cheese.

The objective of this study was to estimate the genetic parameters of spectrometric peaks obtained through PTR-ToF-MS and of their principal components to characterize the volatile fingerprint of model cheeses obtained from the milk of individual Brown Swiss cows.

MATERIALS AND METHODS

Field data

As part of the "Cowability-Cowplus projects", a large number of Brown Swiss cows (n = 1,075) from different herds (n = 72) located in northern Italy (Trento province) were sampled. The production environment has been previously described (Sturaro et al., 2013). On a given day, only one herd was visited and a maximum of 15 cows per herd were sampled. Detailed descriptions of the herds, the cows' characteristics, and the sampling procedure have been given in previous papers on cheese VOCs (Bergamaschi et al. 2015a and 2015b). Briefly, the milk samples without preservative were immediately refrigerated at 4°C and transferred to the Cheese-Making Laboratory of the Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE) of the University of Padova (Legnaro, Padova, Italy).

Data on individual cows and herds were provided by the Superbrown Consortium of Bolzano and Trento (Italy), while pedigree information was supplied by the Italian Brown Swiss Cattle Breeders Association (ANARB, Verona, Italy). We included cows with phenotypic records available for the investigated traits and all known ancestors. Each sampled cow had at least 4 generations of known ancestors and the pedigree file included 8,845 animals. The number of sires included was 1,326 and among them, 264 had progeny with records (2 to 80 daughters each).

Individual Cheese-making Procedure

All milk samples were processed within 20 h after collection. Details of the cheese-making procedure have been previously described by Cipolat-Gotet et al. (2013). In particular, 1,500 mL of milk was heated to 35°C in a stainless steel micro-vat, then supplemented with thermophilic starter culture, mixed with rennet, and controlled for coagulation time. The resulting curd of each vat was cut, drained, shaped in wheels, pressed, salted, weighed, sampled, and analyzed. All model cheeses were ripened for 60 days at 15 °C before being sampled for VOC analyses.

Descriptive statistics of daily milk yield, and the fat and protein content of milk from the Brown Swiss cows selected for the study, and the fat and protein content of the model cheeses are reported in Table 1.

PTR-ToF-MS Analysis

A cylindrical sample 1.1 cm in diameter and about 3.5 cm in height was taken from the center of each model cheese and kept at -80°C until VOC analysis.

The headspace of the 1,075 model cheeses was analyzed using a commercial PTR-ToF-MS 8000 instrument (Ionicon Analytik GmbH, Innsbruck, Austria) and a modified version of the method described in detail by Fabris et al. (2010). Details of the analytical procedures and peak selection are given in Bergamaschi et al. (2015b). Briefly, cheese samples were thawed and kept at room temperature (about 20°C) for 6 h, then 3 g sub-samples from each sample were put into glass vials (20 ml, Supelco, Bellefonte, USA) and capped with PTFE/Silicone septa (Supelco). About 75 samples chosen randomly from the 1,075 sample set were measured every day. Internal calibration and peak extraction were performed according to a method described by Cappellin et al. (2011), which allowed the spectrometric peaks to be identified and was, in most cases, sufficient for sum formula identification. Absolute headspace VOC concentrations expressed as parts per billion by volume (ppb_v) was estimated using the formula described by Lindinger et al. (1998) with a constant reaction rate coefficient of the proton transfer reaction of 2×10^{-9} cm^3/s. This method has been shown to be viable for determining absolute VOC headspace concentrations (Cappellin at al., 2012).

PTR-ToF-MS Data

As discussed in detail by Bergamaschi et al. (2015b), 619 peaks describing volatile organic compounds were obtained from the headspace of 1,075 individual model cheeses using PTR-ToF-MS. Data compression was performed on the 240 most important peaks after elimination of interfering ions. In addition, tentative interpretation of the spectrometric peaks was made based on the fragmentation pattern of 61 relevant compounds retrieved from available solid phase micro-extraction GC-MS data on the same model cheeses or from the literature, and representing about 80% of the total spectral intensity. The most intense peaks detected by PTR-ToF-MS were at m/z 43.018, 43.054, tentatively attributed to alkyl fragments, and 61.028 and 45.033, tentatively attributed to acetic acid and ethanol, respectively (Bergamaschi et al., 2015a and b).

Multivariate Analysis of VOCs

Multivariate data treatment (principal component analysis) was carried out on the standardized spectrometric peaks using Statistica 7.1 (StatSoft, France, Paris) in order to summarize the information and provide a new set of 10 PCs. The statistical methodology has been described in detail by Bergamaschi et al. (2015). The descriptive statistics of these 10 PCs, representing 73.6% of total variance of all VOCs, are reported in Table 1.

Heritability estimates for VOCs and their PCs

Non-genetic effects analyzed in a previous phenotypic study on the same dataset (Bergamaschi et al., 2015b) were included in the mixed models designed to estimate heritability and the genetic correlations of the VOCs and of their PCs. The model accounted for the effects of herd/sampling-processing date (72 levels), the cow’s days in milk (DIM; class 1: < 50 days, class 2: 51 to 100 days, class 3: 101 to 150 days; class 4: 151 to 200 days; class 5: 201 to 250 days; class 6: 251 to 300 days; class 7: > 300 days), and the cow’s parity (1 to 4 or more) for all traits.

Statistical inferences for the analysis of spectrometric peaks obtained from PTR-ToF-MS analysis of sample headspace (i.e., 240 peaks) were based on univariate analyses. To estimate genetic correlations between PCs, milk composition and the most important peaks (in terms of heritability estimates), we conducted a set bivariate analysis.

Each analysis was based on the following linear mixed model:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z_1h} + \mathbf{Z_2a} + \mathbf{e}$$

where $\mathbf{y}$ is a vector of records for traits 1 and 2; $\mathbf{X}$, $\mathbf{Z}_1$, and $\mathbf{Z}_2$ are appropriate incidence matrices for systematic effects in **b**, herd effects in **h**, and animal additive genetic effects in **a**, respectively; and **e** is a vector of random residuals.

Bayesian Inference

(Co)variance components and related parameters were estimated using a Bayesian approach and Markov-chain Monte Carlo methods (Sorensen and Gianola, 2002). All traits were taken as continuous variables, and their values were assumed to be sampled from the following multivariate normal distribution:

$$p(\mathbf{y}|\mathbf{b},\mathbf{h},\mathbf{a},\mathbf{R}) \sim \mathrm{MVN}(\mathbf{Xb} + \mathbf{Z_1h} + \mathbf{Z_2a}, \mathbf{I} \otimes \mathbf{R}),$$

where $\mathbf{y}, \mathbf{b}, \mathbf{h}, \mathbf{a}, \mathbf{X}, \mathbf{Z_1}$ and $\mathbf{Z_2}$ are as defined above, $\mathbf{R}$ is a 2×2 matrix of residual (co)variances, and $\mathbf{I}$ is an identity matrix of appropriate order. The data were properly ordered within the vectors, and vectors **a** and **h** contained the effects for both traits individual by individual.

In a Bayesian setting, we assumed that:

$$p(\mathbf{a}|\mathbf{G}) \sim \mathrm{MVN}(\mathbf{0}, \mathbf{A} \otimes \mathbf{G})$$

and

$$p(\mathbf{h}|\mathbf{H}) \sim \mathrm{MVN}(\mathbf{0}, \mathbf{I} \otimes \mathbf{H}),$$

where $\mathbf{G}$ is a 2×2 matrix of additive genetic (co)variances, $\mathbf{A}$ is the numerator of the Wright's relationship matrix between individuals, **H** is a 2×2 (co)variance matrix for herd effects, and $\mathbf{I}$ is the identity matrix of the same order as the number of levels of herd effects. Flat priors were assumed for the effects in $\mathbf{b}$, as well as for $\mathbf{G}$, $\mathbf{H}$, and $\mathbf{R}$. Marginal posterior distributions of unknown parameters were estimated through numerical integration by means of the Gibbs sampler

(Gelfand and Smith, 1990) implemented in the TM program (http://snp.toulouse.inra.fr/~alegarra) to obtain auto-correlated samples from the joint posterior distributions and subsequently from the marginal posterior distributions of all unknowns in the model. The lengths of the chain and of the burn-in period were assessed by visual inspection of trace plots as well as by the diagnostic tests described by Geweke (1992) and Gelman and Rubin (1992). After a preliminary run, we decided to construct a single chain consisting of 850,000 iterations and discard the first 50,000 iterations as a very conservative burn-in. Subsequently, one in every 200 successive samples was retained in order to store draws that were more loosely correlated. Thus, 4,000 samples were used to determine the posterior distributions of the unknown parameters. The lower and upper bounds of the highest 95% probability density regions for the parameters of interest were obtained from the estimated marginal densities. The posterior median was used as the point for all parameters. Auto-correlations between samples and estimates of Monte Carlo Standard Error (Geyer, 1992) were calculated. Effective sample size was evaluated using the algorithm of Geyer (1992).

Across-herd heritability was computed as:

$$\mathrm{h}^2_{\mathrm{AH}} = \frac{\sigma^2_{\mathrm{a}}}{\sigma^2_{\mathrm{a}} + \sigma^2_{\mathrm{h}} + \sigma^2_{\mathrm{e}}}$$

where σ^2_{a}, σ^2_{h}, and σ^2_{e} are additive genetic, herd-date and residual variances, respectively.

Intra-herd heritability was computed as:

$$\mathrm{h}^2_{\mathrm{IH}} = \frac{\sigma^2_{\mathrm{a}}}{\sigma^2_{\mathrm{a}} + \sigma^2_{\mathrm{e}}}$$

where σ^2_{a} and σ^2_{e} are additive genetic and residual variances, respectively. Inference on additive genetic variation and heritability were based on the marginal posterior density estimated using pooled samples of all Gibbs chains obtained in the multivariate analyses.

The additive genetic correlations (r_a) were computed as:

$$r_a = \frac{\sigma_{\mathrm{a1,a2}}}{\sigma_{\mathrm{a1}} \cdot \sigma_{\mathrm{a2}}}$$

where $\sigma_{a1,a2}$ was the additive genetic covariance between traits 1 and 2, and σ_{a1} and σ_{a2} were the additive genetic standard deviations for traits 1 and 2, respectively.

The herd-date correlations (r_h) were computed as:

$$r_h = \frac{\sigma_{h1,h2}}{\sigma_{h1} \cdot \sigma_{h2}}$$

where $\sigma_{h1,h2}$ was the herd-date covariance between traits 1 and 2, and σ_{h1} and σ_{h2} were the herd-date standard deviations for traits 1 and 2, respectively.

The residual correlations (r_e) were computed as:

$$r_e = \frac{\sigma_{e1,e2}}{\sigma_{e1} \cdot \sigma_{e2}}$$

where $\sigma_{e1,e2}$ was the residual covariance between traits 1 and 2, and σ_{e1} and σ_{e2} were the residual standard deviations for traits 1 and 2, respectively.

RESULTS AND DISCUSSION

Variance Components and Heritability of PCs extracted from Volatile Profiling of cheese

The proportions of variance explained by the first 10 PCs are reported in Table 1. A list of the most correlated tentatively identified individual VOCs of each PC can be found in Supplementary Table S1.

Estimates of the marginal posterior densities for the additive genetic, herd-date and residual variances are given in Table 2. The genetic variance of each PC was always smaller than the herd-date variance, while the latter was smaller than the residual variance in all but two cases: PC3 had greater herd-date variance than residual variance, while in PC5 these two components were of the same magnitude. These data depict different proportions of the main sources of variation in individual PCs (genetic, herd and individual/residual components).

All 10 principal components extracted from the volatile fingerprint of the model cheeses were found to be heritable (Table 2). Across-herd heritability of PCs ranged from 2.4% to 8.6%, while intra-herd heritability ranged from 3.6% to 10.2%. The marginal posterior distribution of the intra-herd heritability of the 10 PCs extracted from the volatile fingerprint of the cheeses are illustrated in Figure 1 a and b.

Notably, the two most important PCs, which explain about 40% of overall variability in the volatile fingerprint of cheeses, were characterized by similar heritabilities (h^2_{IH}: 8.4% in PC1 and 8.5% in PC2). It worth noting that PC1 was not affected by dairy system, while PC2 was greater in the cheese samples from milk produced by cows reared in modern facilities and fed on total mixed rations, whether with or without silage (Bergamaschi et al., 2015b). Both displayed variation during lactation, but in opposite directions: PC1 decreased curvilinearly, while PC2 increased linearly.

The third principal component was characterized by slightly lower intra-herd heritability than PC1 and PC2 (7.1%), and a much lower across-herd heritability (3.3%) due to the strong effect of herd-date on this component of cheese flavor (Table 2). It worth noting that this sizeable environmental variability is not caused by the dairy system but by large variability among individual herds within each dairy system (Bergamaschi et al., 2015b). This PC, which explains almost 10% of the total volatile fingerprint variation, was also found to increase with increasing daily milk yield of the cow, but was not affected by parity and DIM.

The fourth PC, explaining almost 8% of the total cheese volatile fingerprint, was the most heritable (h^2_{AH} = 8.6% and h^2_{IH} = 10.2%). This PC was not much affected by dairy system or individual herd, but was found to increase during lactation (Bergamaschi et al., 2015b).

Of the other PCs, PC5, PC6 and PC9 were characterized by low heritability (< than 6%), while the heritability values of PC7, PC8 and PC10 (7.1 to 9.8%) were intermediate between those of PC1 and PC2 and those of PC4 (Table 2).

To our knowledge, this study offers the first heritability estimates of phenotypes summarizing the cheese volatile profile.

As no other data are available in the literature, it would be of great interest to compare the heritability of the PCs of the volatile fingerprint of model cheeses, excluding the three less heritable PCs (PC5, PC6 and PC9), with the heritability of other traits studied in the same project from the same cows, or at the population level with the same breed and in the same area. The heritability estimate of daily milk yield (Bittante et al., 2013a; Cecchinato et al., 2014) in individual cows was about double of the cheese PC while the same estimate obtained by Cecchinato et al. (2015a) at the population level, was similar to the PC with the highest heritability. Regarding milk quality, the fat content and SCS were similar to the PC of the cheese volatile fingerprint, while milk protein, casein, casein number, lactose, and urea were much more heritable at both the experimental and population levels. The very detailed fatty acid profile of the milk samples yielded heritability estimates in the same range as the PC of the volatile fingerprint of cheeses obtained from the same milk, with the exception of the saturated odd-numbered fatty acids and a few others (Pegolo et al., 2015).

As for the cheese-making process, the traditional milk coagulation properties were also much more heritable than the PC of the cheese volatile fingerprint, with the exception of curd firmness recorded 45 min after rennet addition (Cecchinato et al., 2013). Curd firming modeling (Bittante et al., 2013b) applied to the same milk samples yielded heritability coefficients greater than those of the PC of the cheese volatile fingerprint for rennet coagulation time and for the curd firming instant rate constant, and of the same size for potential curd firmness and of the syneresis instant rate constant (Cecchinato et al., 2015b).

The technological traits (three cheese yields and four milk nutrient recoveries in the curd) measured in the fresh model cheeses were also much more heritable than the PC of the volatile fingerprint of the same model cheeses after 2 months of ripening (Bittante et al., 2013a). The same traits predicted by Fourier transform infrared spectrometry using the calibration proposed by Ferragina et al. (2013) in the same milk samples (Bittante et al., 2014) and at the population level (Cecchinato et al., 2015a) were characterized by heritability values of the same size.

Summarizing these results, the PC of the volatile fingerprints of cheese obtained from the PTR-ToF-MS procedure can be said to be not only heritable, but to also have heritability values similar to several milk quality traits (fat content, many fatty acid contents, SCS) and some coagulation properties (potential curd firmness and the syneresis instant rate constant) that are actively selected or for which genetic selection has been proposed.

Phenotypic, Genetic, Herd-date and Residual Correlations among PCs

Figure 4 reports the phenotypic, genetic, herd-date and residual correlations among the 10 first PCs of the cheese volatile fingerprint.

As expected for principal components, the phenotypic correlations among them were always close to zero (-0.02 to 0.02), and the zero value was always included in their HPD95% (data not shown). However, this phenotypic independency is often the result of additive genetic, herd-date and residual correlations very different from zero and opposite in sign.

As an example, the first three PCs, which together explain about half the variability of all the 240 spectrometric peaks obtained with PTR-ToF-MS, are negatively correlated with each other from the genetic point of view (Figure 4), yet PC2 and PC3 were positively correlated for herd-date but had a negative residual correlation. Other relevant additive genetic correlations were found between PC3 and PC6 (positive), and between PC10 and both PC5 and PC7 (negative). Many relevant herd-date correlations were found among the 10 PCs while the residual correlations were generally much smaller (Figure 4). Comparisons with the literature are not possible and these correlations need further research to ascertain their importance and the meaning of these new traits, especially in relation to the sensorial properties of the cheeses. Moreover, the various herd and animal factors affecting the PCs (see Bergamaschi et al., 2015b) and their correlations with milk quality traits could further help in understanding the characterization of cheese flavor.

Variance Components and Heritability of Individual Spectrometric Peaks of the Volatile Fingerprint of Cheese

A univariate Bayesian animal model was applied to each of the 240 individual spectrometric peaks obtained with PTR-ToF-MS. The variance components and heritability estimates are listed in Supplementary Table S2. Figure 2 shows the distribution of the heritability values of the individual peaks related to the VOCs of the cheese samples. Only a few peaks are characterized by negligible heritability (6 peaks < 3.5%). Slightly more than one third yielded values similar to the three PCs of the volatile fingerprint characterized by lower heritability, about one third of the values were similar to the other 7 PCs, and about one sixth were characterized by heritability values greater than the more heritable PCs (PC4).

It can be seen from Table 4 that there is a tendency towards a decrease in concentration with increasing heritability (note that the concentration is expressed on a logarithmic scale). Sometimes, compounds with the lower concentration are characterized by a proportional increase in instrumental error and then a decrease in their heritability is expected. This is not true for the spectrometric peaks examined in the present study, even though a large number of peaks with low concentrations (< 1ppb$_v$) were not included in the study. This is an indirect indication of the great potential of the PTR-ToF-MS technique in evaluating the volatile fingerprint of cheese.

Confirmation of the large variability in the results is also evident in Figure 3, which shows the relationships between VOC heritability and both their mass and the concentration expressed as an average of each VOC (Figure 3 a and b), the standard deviation and variability coefficient (Figure 3 c and d). Only the phenotypic standard deviation of the concentration seems to affect VOC heritability.

Tables 4 and 5 list the 10 VOCs with the greatest heritability among the VOCs tentatively identified or not identified, respectively. These lists confirm that several individual spectrometric peaks are characterized by heritability values of the same magnitude as milk yield, some milk quality traits (Othmane et al., 2002; Rosati and Van Vleck, 2002) and also some technological parameters (Ikonen et al., 2004).

It is interesting to note that some peaks were related to principal components and corresponded with specific odors and aromas detected in many cheese varieties (Bellesia et al., 2003; Cornu et al., 2009). For instance, among the masses most positively correlated with PCs, Bergamaschi et al. (2015b) found *m/z* 117.091 and *m/z* 145.123, and their isotopes *m/z* 118.095 and 146.126, which in this study had heritabilities of 12% and 13%, respectively. These peaks have been tentatively attributed to ethyl butanoate, ethyl-2-methylpropanoate and ethyl hexanoate (Bergamaschi et al., 2015b). Esters may originate from the interaction between FFA and alcohols produced by microorganisms, and are responsible for fruity-floral notes in cheese aroma (Cornu et al., 2009). In addition, the peak with *m/z* 95.017 associated with methyldisulfanylmethane had a heritability of 12.5% and characterized PC4 (Table 5). This sulfur compound may be derived from diet or formed from the amino acid methionine released during cheese ripening (McSweeney and Sousa, 2000). We found an exploitable heritability (20.6%) of *m/z* 81.070 associated with terpene fragments. Terpenes are degradation products of carotenoids (Carpino et al., 2004) and have been listed as cheese odorants (Abilleira et al., 2011), giving a fresh, green odor (Horne et al., 2005). It is well known that volatile terpene in cheese could be used as a biomarker of area of production, and type and phenological stage of forage (Viallon et al., 1999; Cornu et al., 2005). In this work, we found that these molecules are also affected by animal genetics (Table 5).

These peaks could be the first to be studied in terms of effects on the flavor and acceptability of cheese, because they exhibited exploitable genetic variation.

Our results also open up a new field of research on potential indirect methods for animal phenotyping (for example through infrared spectrometry) or genotyping (candidate gene associations and genomic selection).

Phenotypic, Genetic, Herd-date, and Residual Correlations among VOCs

Figure 4 shows that, differently from PCs, the 10 VOCs characterized by the greatest heritability estimates presented variable but generally positive correlations with each other. The residual correlations often moderate to high and positive. Only residual correlations of peaks relative to methanethiol (theoretical mass *m/z* 49.011) and methyldisulfanylmethane (theoretical mass *m/z* 95.017) presented very low residual correlations with the other 8 PCs. These two VOCs are also those that showed some negative genetic and herd correlations with the others, which are generally positively correlated with each other. As discussed above, the microorganism are considered to be the key agents of the production of these volatile compounds cheese ripened.

Individual VOCs concentration is a quantitative analysis, so it appears logical that an increase of the global quantity of VOCs in the cheese could results in an increase of the major part of individual VOCs, end especially of those with the greatest concentration, and thus in positive phenotypic correlations with each other. It seems from Figure 4 that the genetic or herd factors causing an increase of the global quantity of cheese odorants could be responsible also of the positive genetic and herd correlations among the majority of individual VOCs.

Probably methanethiol and methyldisulfanylmethane could be determinate by different genetic and/or herd pathways from those characterizing the global quantity of cheese odorants and this could explain the residual independency respect to other VOCs. The reason of the negative genetic and herd correlations need to be investigated in future research.

The difference respect to the correlations previously observed among PCs is due to the fact that, being by definition PCs phenotypically independent from each other, the genetic, herd and residual correlations could represent a signal of different and often opposite direction of genetic, herd and residual correlations between PCs resulting in their phenotypic independency. In this way correlations between PCs seem more a qualitative picture while individual VOCs are more quantitative items.

Phenotypic, Genetic, Herd-date, and Residual Correlations of PCs and VOCs with Milk Quality traits

The last analysis carried out was the estimation of dependency of cheese VOCs and their PCs from the quality traits of milk used for cheese-making. Figure 5 depicts the presence of positive and negative correlation coefficients that are small at residual level, low to moderate at herd level and low to high at genetic level.

In this case it is not evident the presence of some PCs or VOCs with pattern clearly different from the others, neither in the case of methanethiol and methyldisulfanylmethane. What it is more evident in this case is that some quality trait of milk is characterized by a more evident effect on the 10 VOCs characterized by the greater heritability. In particular, milk content of fat, protein, casein, and total solids seems generally correlated positively with all VOCs, meaning that genetic factors leading to more concentrated milk seems responsible also of an increase of VOCs content in the cheese obtained.

Very different is the pattern of casein number (ratio between casein and total protein). In this case all genetic correlations are negative and, with two exception, highly negative. As both numerator and denominator of the ratio are generally positively correlated with VOCs concentration, this could means that genetic factors responsible of an increase of caseins inferior respect to that of total protein could be responsible of a decrease of VOCs concentration. The possible role of whey protein on these traits should be studied in future research.

It appears also, from Figure 5, that also milk pH and SCS could exert some negative effect on cheese VOCs, and also in this case future research appear to be useful.

CONCLUSIONS

In this work we report the first estimation of the genetic parameters of the cheese volatile profile in a cattle population.

The results from this study show the heritability estimates of the 10 principal components extracted from 240 peaks obtained with PTR-ToF-MS to be low, although 7 of them were found to be similar to other milk traits, like fat content, many fatty acid percentages, SCS, and some curd firming model parameters.

Variability due to herd-date was very different for the different PCs. Although the phenotypic correlations among the 10 PCs were, as expected, close to zero, some genetic correlations, for example several herd-date correlations, were sizeable. The residual correlations were generally lower.

Only a small proportion of the individual spectrometric peaks had very low heritability. The large majority of peaks were characterized by heritability values similar to those obtained for the

PCs, while about one sixth of them had higher heritability values similar to those of daily milk yield and several other milk and cheese traits.

The concentration in cheese PCs and of VOCs with the greater heritability presented genetic, herd and residual correlations with each other variable and generally positive. Also correlations of PCs and selected VOCs of cheeses with quality traits of milk before cheese-making were variable and often greater for genetic, intermediate for herd and smaller for residual correlations.

Our results demonstrate the existence of genetic variability in factors related to cheese volatile profiles potentially useful for improving the flavor of cheese. This study opens new avenues for characterizing the relationships between PCs or individual peaks and the sensory profile of cheese, and for the study of the potential for indirect prediction òof the phenotypic (milk quality traits and/or infrared spectrometry on milk) and/or genetic values (candidate gene associations and genomic selection) of the animals.

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TABLE AND FIGURES

Table 1. Descriptive statistics of milk production, cheese composition and the first principal components characterizing the volatile compounds fingerprint of 1,075 individual model cheeses analyzed by PTR-ToF-MS.

Traits	Mean	CV, %
Milk yield, kg×d^{-1}	24.6	32.1
Milk composition:		
Fat, %	4.4	20.5
Protein, %	3.8	10.5
Cheese composition:		
Fat, %	38.2	11.5
Protein, %	27.1	15.1
Cheese volatile fingerprint (cumulative R^2):		
PC 1	0.283	-
PC 2	0.392	-
PC 3	0.478	-
PC 4	0.554	-
PC 5	0.615	-
PC 6	0.652	-
PC 7	0.678	-
PC 8	0.701	-
PC 9	0.720	-
PC10	0.736	-

Table 2. Features of marginal posterior densities of additive genetic (σ^2_A), herd-date (σ^2_H), and residual (σ^2_E), variances, and across-herd (h^2_{AH}), and intra-herd (h^2_{IH}) heritabilities for principal components derived from volatile fingerprint of 1,075 individual model cheeses analyzed by PTR-ToF-MS[1].

Traits	σ^2_A		σ^2_H		σ^2_E		h^2_{AH}		h^2_{IH}	
	Mean	HPD95%	Mean	HPD95%	Mean	HPD95%	Mean	HPD95%	Mean	HPD95%
PC 1	4.49	0.46; 11.70	15.14	9.79; 22.59	48.81	41.85; 55.09	0.065	0.01; 0.17	0.084	0.01; 0.21
PC 2	1.48	0.09; 4.04	9.12	6.21; 13.24	15.95	13.53; 18.04	0.056	0.01; 0.15	0.085	0.01; 0.22
PC 3	0.71	0.05; 1.90	11.77	8.22; 16.60	9.31	8.09; 10.44	0.033	0.01; 0.08	0.071	0.01; 0.18
PC 4	1.62	0.15; 3.96	2.80	1.69; 4.37	14.27	12.05; 16.28	0.086	0.01; 0.21	0.102	0.01; 0.24
PC 5	0.43	0.02; 1.27	7.30	5.08; 10.43	7.21	6.32; 8.04	0.029	0.01; 0.09	0.057	0.01; 0.16
PC 6	0.22	0.01; 0.73	2.93	1.97; 4.23	5.97	5.36; 6.59	0.024	0.01; 0.08	0.036	0.01; 0.11
PC 7	0.47	0.04; 1.27	1.61	1.06; 2.39	4.50	3.77; 5.11	0.071	0.01; 0.19	0.095	0.01; 0.25
PC 8	0.48	0.06; 1.14	0.64	0.36; 1.04	4.43	3.79; 5.04	0.086	0.01; 0.20	0.098	0.01; 0.23
PC 9	0.21	0.01; 0.65	0.47	0.25; 0.77	3.86	3.40; 4.30	0.047	0.01; 0.16	0.053	0.01; 0.14
PC10	0.30	0.01; 0.86	0.47	0.26; 0.76	3.08	2.56; 3.50	0.078	0.01; 0.22	0.089	0.01; 0.25

[1] Mean = mean of the marginal posterior density of the parameter; HPD95% = lower and upper bound of the 95% highest posterior density region.

Table 3. Average concentration and estimates of phenotypic (σ_P), residual (σ_E) and genetic (σ_G) standard deviations and of heritability (h^2) for 240 spectrometric peaks from PTR-ToF-MS analysis of 1,075 individual model cheeses from Brown Swiss cows.

h^2, %	Peaks, no.	Concentrations		Average of the SD			Heritability (h^2)			
		ppb$_v$[2]	CV, %[3]	σ_P	σ_E	σ_G	Mean	SD	Min	Max
<2	0	-	-	-	-	-	-	-	-	-
2-4	11	6.26	29.1	1.507	0.874	0.166	0.035	0.002	0.032	0.039
4-6	60	6.25	28.3	1.491	0.823	0.188	0.050	0.005	0.041	0.059
6-8	69	6.05	31.4	1.501	0.864	0.234	0.068	0.006	0.060	0.079
8-10	38	5.30	20.5	1.529	0.817	0.253	0.087	0.005	0.080	0.097
10-12	22	5.19	22.1	1.522	0.795	0.276	0.108	0.006	0.101	0.119
12-14	20	6.73	27.8	1.584	0.725	0.280	0.130	0.006	0.120	0.139
14-16	13	5.28	12.3	1.612	0.795	0.328	0.146	0.005	0.140	0.157
16-18	4	4.91	5.2	1.644	0.788	0.358	0.172	0.008	0.161	0.179
18-20	1	5.17	-	1.646	0.721	0.352	0.192	-	0.192	0.192
>20	2	5.33	1.2	1.702	0.666	0.344	0.211	0.007	0.206	0.216
All	240	5.90	28.5	1.523	0.822	0.239	0.082	0.035	0.032	0.216

[1] Mean value of each peak of the classes; [2] Data expressed as natural logarithm of part per billion by volume; [3] Coefficient of variation of the mean value of each peak of the classes.

Table 4. Spectrometric peaks with highest heritability (h^2) with tentative of identification of volatile compounds from PTR-ToF-MS analysis of 1,075 model cheeses; their SD phenotypic (σ_P), residual (σ_E), and genetic (σ_G).

						SD			
Measured Mass (*m/z*)	Theoretical Mass (*m/z*)	Tentative identification	Sum formula	ppb$_v$[1]	CV, %	σ_P	σ_E	σ_G	h^2
49.011	49.0106	Methanethiol	CH_5O^+	6.82	9.8	1.609	0.800	0.302	0.125
57.033	57.0699	3-Methyl-1-butanol	$C_3H_5O^+$	6.70	10.0	1.638	0.805	0.317	0.134
75.080	75.0810	Butan-1-ol, pentan-1-ol, heptan-1-ol	$C_4H_{11}O^+$	7.70	14.7	1.619	0.763	0.302	0.136
81.070	81.0699	Alkyl fragment (terpenes)	$C_6H_9^+$	5.37	8.6	1.701	0.669	0.340	0.206
83.086	83.0855	Hexanal, nonanal	$C_6H_{11}^+$	6.13	11.3	1.611	0.825	0.333	0.140
95.017	95.0161	Methyldisulfanylmethane	$C_2H_7O_2S^+$	5.04	14.1	1.632	0.847	0.370	0.161
117.091	117.0910	Ethyl butanoate, ethyl-2-methylpropanoate	$C_6H_{13}O_2^+$	9.14	6.6	1.600	0.780	0.295	0.125
118.095	118.0940	Ethyl butanoate, ethyl-2-methylpropanoate	$C5^{[13]}CH_{13}O_2^+$	6.53	8.7	1.604	0.775	0.298	0.129
145.123	145.1220	Ethyl hexanoate, octanoic acid	$C_8H_{17}O_2^+$	7.43	10.5	1.577	0.777	0.304	0.133
146.126	146.1260	Ethyl hexanoate, octanoic acid	$C_7^{[13]}CH_{17}O_2^+$	5.30	11.5	1.583	0.774	0.310	0.138

[1] Data expressed as natural logarithm of part per billion by volume.

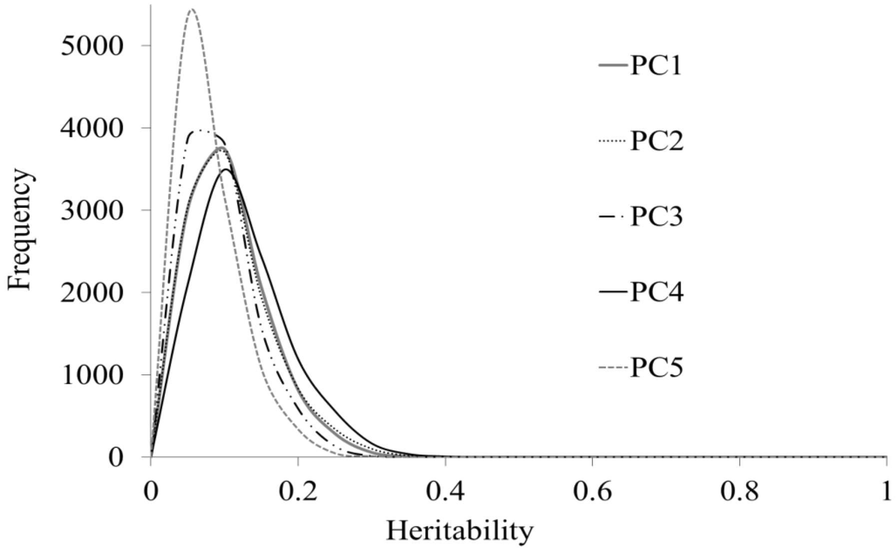
Table 5. Not identified spectrometric peaks with highest heritability (h^2) from PTR-ToF-MS analysis of 1,075 model cheeses; their SD phenotypic (σ_P), residual (σ_E), and genetic (σ_G).

			SD			
m/z	ppb$_v$[1]	CV, %	σ_P	σ_E	σ_G	h^2
50.056	4.58	9.6	1.63	0.754	0.313	0.147
93.431	4.54	10.0	1.655	0.759	0.354	0.179
95.095	5.44	18.1	1.631	0.766	0.331	0.157
111.104	5.08	18.5	1.624	0.774	0.322	0.147
120.092	4.95	14.5	1.638	0.797	0.363	0.172
121.122	5.17	15.8	1.646	0.721	0.352	0.192
136.140	4.26	8.9	1.627	0.759	0.319	0.150
137.132	5.28	7.3	1.702	0.663	0.348	0.216
171.173	4.87	15.7	1.491	0.905	0.384	0.152
173.153	5.20	9.3	1.61	0.825	0.345	0.149

[1] Data expressed as natural logarithm of part per billion by volume.

Figure 1. Marginal posterior distributions of the intra-herd heritability for principal components (PC1-PC5) [**a**] and (PC6-PC10) [**b**] derived from volatile fingerprint of 1,075 individual model cheeses analyzed by PTR-ToF-MS.

[a]



[b]

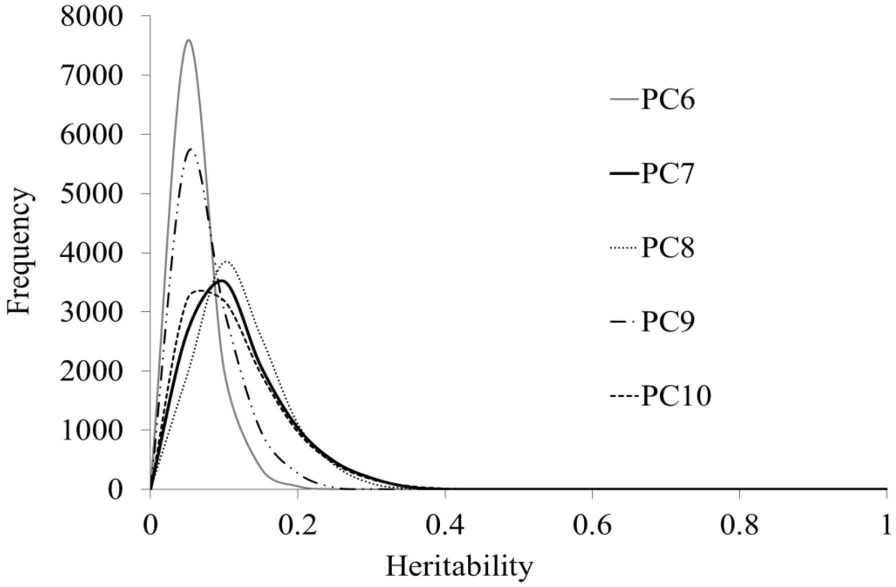


Figure 2. Frequency of heritability of 240 spectrometric peaks detected by PTR-ToF-MS analysis of 1,075 individual model cheeses.

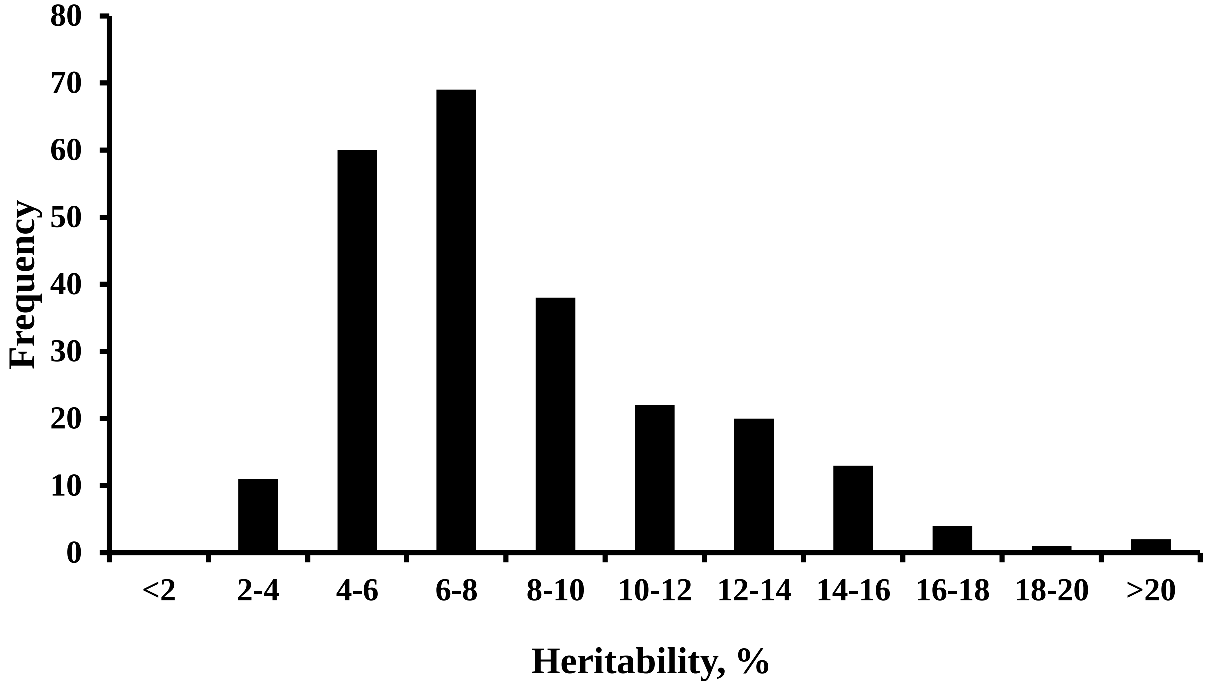


Figure 3. Distribution of heritability of 240 spectrometric peaks detected by PTR-ToF-MS analysis of 1,075 individual model cheeses according to mass of the volatile compound **[a]**, the concentration in the cheese sample **[b]**, the phenotypic standard deviation of the concentration **[c]**, and the coefficient of variation of concentration **[d]**.

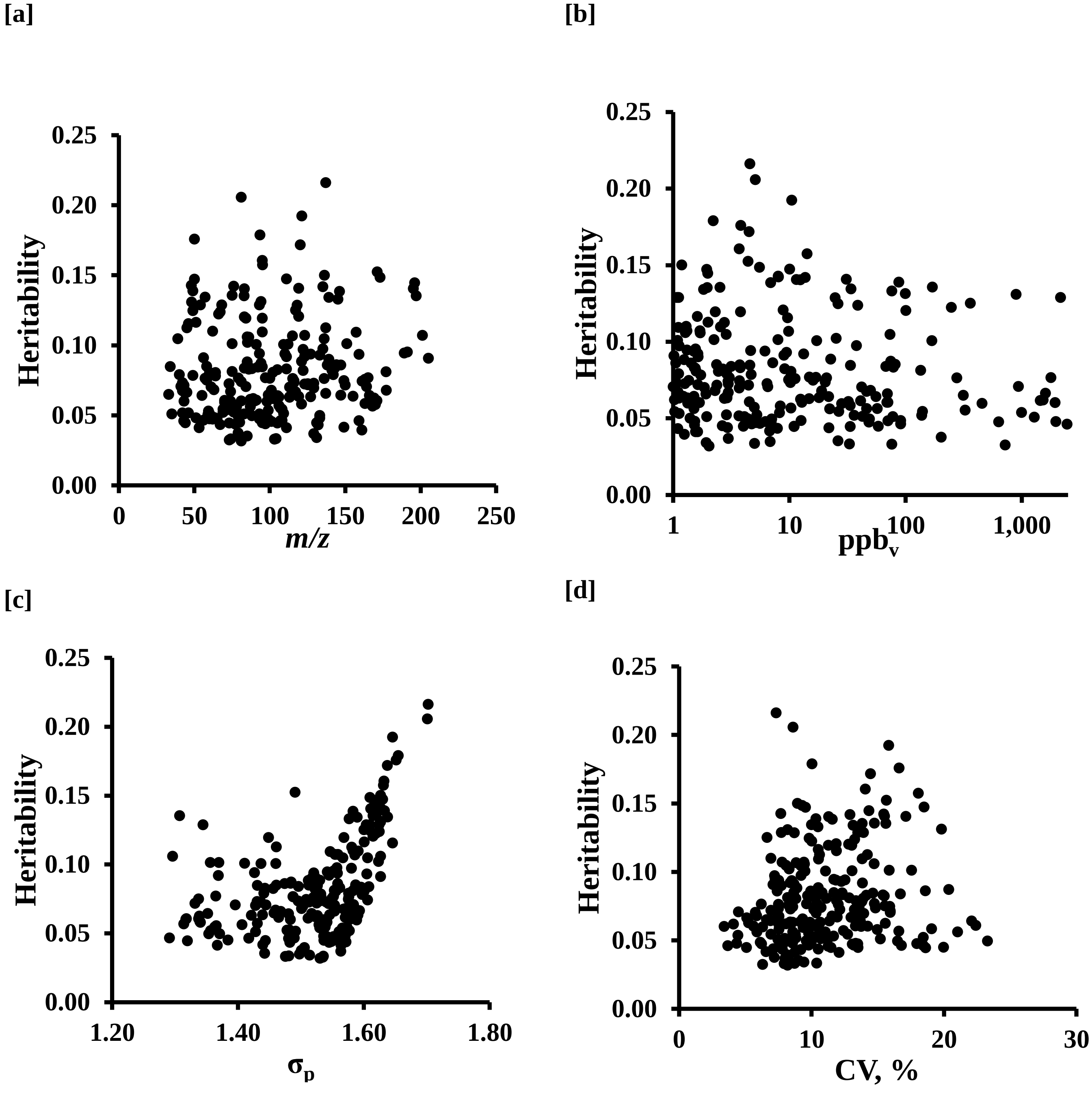
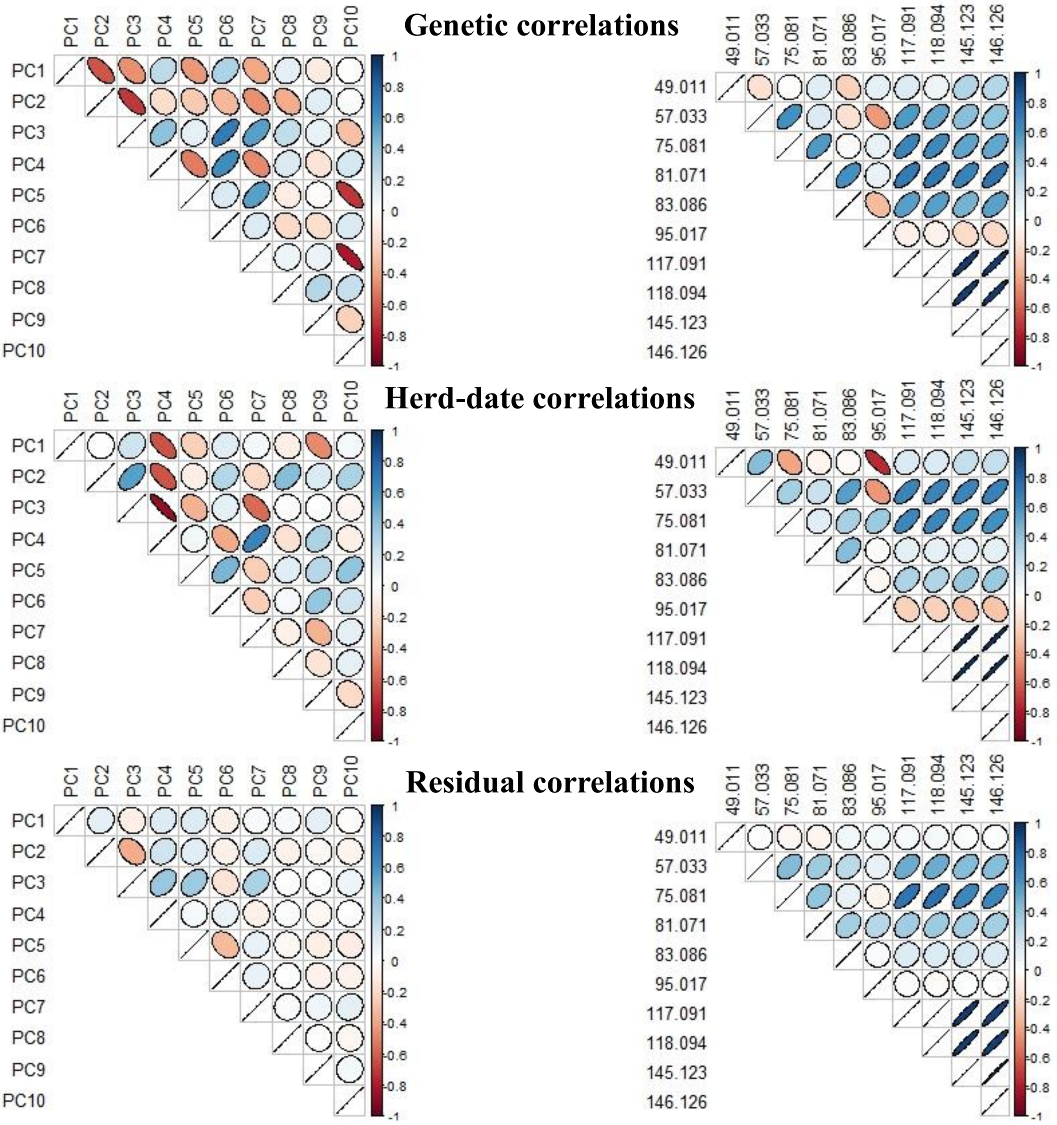


Figure 4. Genetic, herd and residual correlations among the first 10 principal components (left triangles) and among the 10 individual identified VOCs (right triangles) with the highest heritability (from uncolored circles, no correlation, to thin dark colored ovals, high correlations; negative correlations: reddish ovals from top left to bottom right; positive correlations: blueish ovals from top right to bottom left).



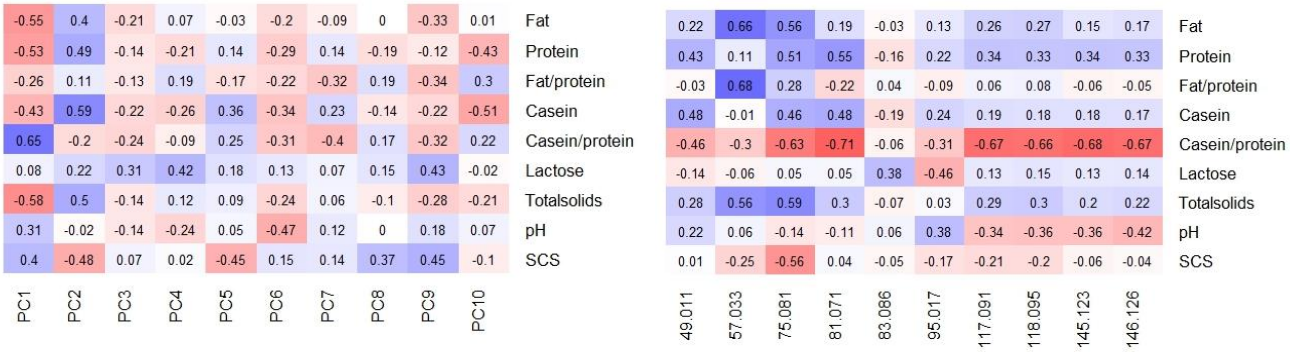
Genetic correlations

Herd-date correlations

Residual correlations

Figure 5. Genetic, herd and residual correlations between the first 10 principal components (left squares) and milk quality traits and between the 10 individual identified VOCs (right squares) with the highest heritability coefficients and milk quality traits (from bright red color for the highly negative correlations to uncolored cells for uncorrelated traits, to bright blue color for the highly positive positive correlations).

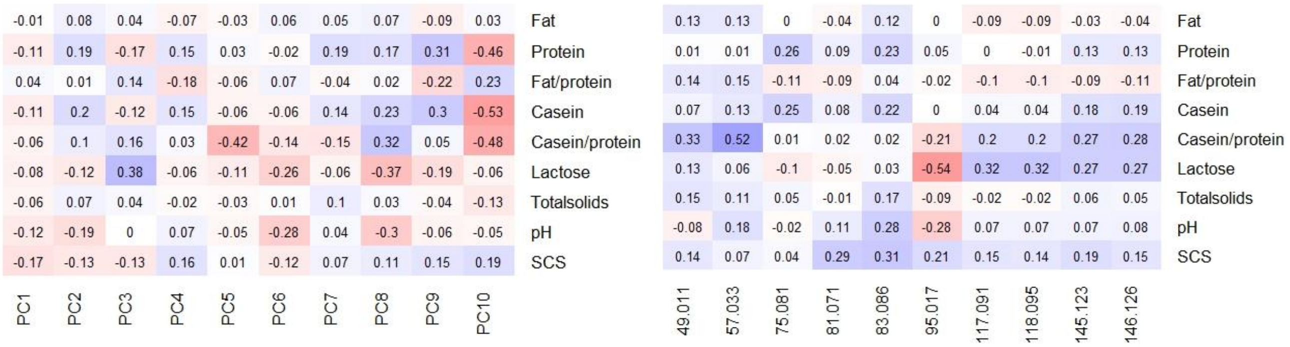
Genetic correlations



PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	
-0.55	0.4	-0.21	0.07	-0.03	-0.2	-0.09	0	-0.33	0.01	Fat
-0.53	0.49	-0.14	-0.21	0.14	-0.29	0.14	-0.19	-0.12	-0.43	Protein
-0.26	0.11	-0.13	0.19	-0.17	-0.22	-0.32	0.19	-0.34	0.3	Fat/protein
-0.43	0.59	-0.22	-0.26	0.36	-0.34	0.23	-0.14	-0.22	-0.51	Casein
0.65	-0.2	-0.24	-0.09	0.25	-0.31	-0.4	0.17	-0.32	0.22	Casein/protein
0.08	0.22	0.31	0.42	0.18	0.13	0.07	0.15	0.43	-0.02	Lactose
-0.58	0.5	-0.14	0.12	0.09	-0.24	0.06	-0.1	-0.28	-0.21	Totalsolids
0.31	-0.02	-0.14	-0.24	0.05	-0.47	0.12	0	0.18	0.07	pH
0.4	-0.48	0.07	0.02	-0.45	0.15	0.14	0.37	0.45	-0.1	SCS

49.011	57.033	75.081	81.071	83.086	95.017	117.091	118.095	145.123	146.126	
0.22	0.66	0.56	0.19	-0.03	0.13	0.26	0.27	0.15	0.17	Fat
0.43	0.11	0.51	0.55	-0.16	0.22	0.34	0.33	0.34	0.33	Protein
-0.03	0.68	0.28	-0.22	0.04	-0.09	0.06	0.08	-0.06	-0.05	Fat/protein
0.48	-0.01	0.46	0.48	-0.19	0.24	0.19	0.18	0.18	0.17	Casein
-0.46	-0.3	-0.63	-0.71	-0.06	-0.31	-0.67	-0.66	-0.68	-0.67	Casein/protein
-0.14	-0.06	0.05	0.05	0.38	-0.46	0.13	0.15	0.13	0.14	Lactose
0.28	0.56	0.59	0.3	-0.07	0.03	0.29	0.3	0.2	0.22	Totalsolids
0.22	0.06	-0.14	-0.11	0.06	0.38	-0.34	-0.36	-0.36	-0.42	pH
0.01	-0.25	-0.56	0.04	-0.05	-0.17	-0.21	-0.2	-0.06	-0.04	SCS

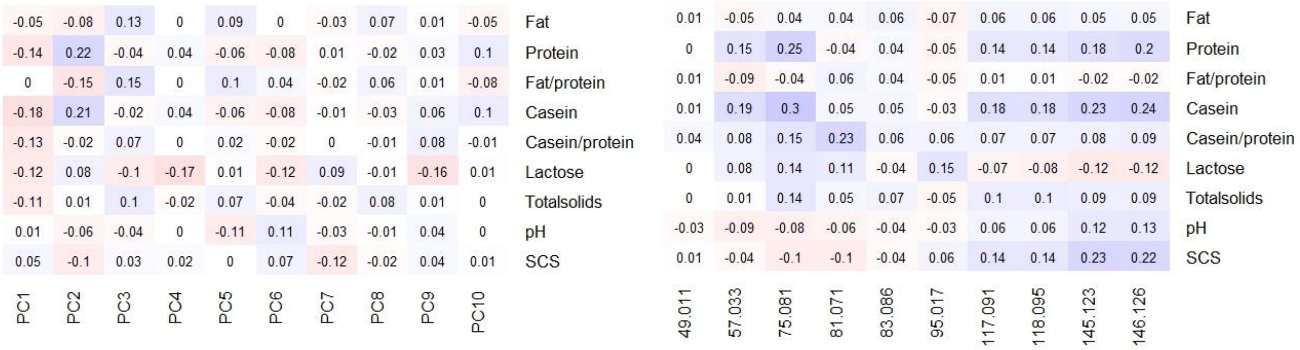
Herd-date correlations



PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	
-0.01	0.08	0.04	-0.07	-0.03	0.06	0.05	0.07	-0.09	0.03	Fat
-0.11	0.19	-0.17	0.15	0.03	-0.02	0.19	0.17	0.31	-0.46	Protein
0.04	0.01	0.14	-0.18	-0.06	0.07	-0.04	0.02	-0.22	0.23	Fat/protein
-0.11	0.2	-0.12	0.15	-0.06	-0.06	0.14	0.23	0.3	-0.53	Casein
-0.06	0.1	0.16	0.03	-0.42	-0.14	-0.15	0.32	0.05	-0.48	Casein/protein
-0.08	-0.12	0.38	-0.06	-0.11	-0.26	-0.06	-0.37	-0.19	-0.06	Lactose
-0.06	0.07	0.04	-0.02	-0.03	0.01	0.1	0.03	-0.04	-0.13	Totalsolids
-0.12	-0.19	0	0.07	-0.05	-0.28	0.04	-0.3	-0.06	-0.05	pH
-0.17	-0.13	-0.13	0.16	0.01	-0.12	0.07	0.11	0.15	0.19	SCS

49.011	57.033	75.081	81.071	83.086	95.017	117.091	118.095	145.123	146.126	
0.13	0.13	0	-0.04	0.12	0	-0.09	-0.09	-0.03	-0.04	Fat
0.01	0.01	0.26	0.09	0.23	0.05	0	-0.01	0.13	0.13	Protein
0.14	0.15	-0.11	-0.09	0.04	-0.02	-0.1	-0.1	-0.09	-0.11	Fat/protein
0.07	0.13	0.25	0.08	0.22	0	0.04	0.04	0.18	0.19	Casein
0.33	0.52	0.01	0.02	0.02	-0.21	0.2	0.2	0.27	0.28	Casein/protein
0.13	0.06	-0.1	-0.05	0.03	-0.54	0.32	0.32	0.27	0.27	Lactose
0.15	0.11	0.05	-0.01	0.17	-0.09	-0.02	-0.02	0.06	0.05	Totalsolids
-0.08	0.18	-0.02	0.11	0.28	-0.28	0.07	0.07	0.07	0.08	pH
0.14	0.07	0.04	0.29	0.31	0.21	0.15	0.14	0.19	0.15	SCS

Residual correlations



PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	
-0.05	-0.08	0.13	0	0.09	0	-0.03	0.07	0.01	-0.05	Fat
-0.14	0.22	-0.04	0.04	-0.06	-0.08	0.01	-0.02	0.03	0.1	Protein
0	-0.15	0.15	0	0.1	0.04	-0.02	0.06	0.01	-0.08	Fat/protein
-0.18	0.21	-0.02	0.04	-0.06	-0.08	-0.01	-0.03	0.06	0.1	Casein
-0.13	-0.02	0.07	0	0.02	-0.02	0	-0.01	0.08	-0.01	Casein/protein
-0.12	0.08	-0.1	-0.17	0.01	-0.12	0.09	-0.01	-0.16	0.01	Lactose
-0.11	0.01	0.1	-0.02	0.07	-0.04	-0.02	0.08	0.01	0	Totalsolids
0.01	-0.06	-0.04	0	-0.11	0.11	-0.03	-0.01	0.04	0	pH
0.05	-0.1	0.03	0.02	0	0.07	-0.12	-0.02	0.04	0.01	SCS

49.011	57.033	75.081	81.071	83.086	95.017	117.091	118.095	145.123	146.126	
0.01	-0.05	0.04	0.04	0.06	-0.07	0.06	0.06	0.05	0.05	Fat
0	0.15	0.25	-0.04	0.04	-0.05	0.14	0.14	0.18	0.2	Protein
0.01	-0.09	-0.04	0.06	0.04	-0.05	0.01	0.01	-0.02	-0.02	Fat/protein
0.01	0.19	0.3	0.05	0.05	-0.03	0.18	0.18	0.23	0.24	Casein
0.04	0.08	0.15	0.23	0.06	0.06	0.07	0.07	0.08	0.09	Casein/protein
0	0.08	0.14	0.11	-0.04	0.15	-0.07	-0.08	-0.12	-0.12	Lactose
0	0.01	0.14	0.05	0.07	-0.05	0.1	0.1	0.09	0.09	Totalsolids
-0.03	-0.09	-0.08	-0.06	-0.04	-0.03	0.06	0.06	0.12	0.13	pH
0.01	-0.04	-0.1	-0.1	-0.04	0.06	0.14	0.14	0.23	0.22	SCS

Supplementary Table S1. Highest correlation coefficients between the first 10 principal components and the volatile compounds tentatively attributed to specific spectrometric fragments of PTR-ToF-MS spectra.

PC	Measured mass (*m/z*)	Sum formula	Theoretical mass (*m/z*)	r	Tentative identification
1	117.091	$C_6H_{13}O_2^+$	117.0910	-0.80	Ethyl butanoate, ethyl-2-methylpropanoate (ethyl isobutyrate), hexanoic acid
	127.112	$C_8H_{15}O^+$	127.1120	-0.73	1-Octen-3-one
	145.123	$C_8H_{17}O_2^+$	145.1229	-0.73	Ethyl hexanoate, octanoic acid
	99.081	$C_6H_{11}O^+$	99.0804	-0.69	Hexanoic acid
	71.086	$C_5H_{11}^+$	71.0855	-0.61	3-Methyl-1-butanol, 3-methyl-3-buten-1-ol, pentan-1-ol
2	69.070	$C_5H_9^+$	69.0698	-0.62	2-Methyl-1,3-butadiene (isoprene)
	115.112	$C_7H_{15}O^+$	115.1117	-0.59	Heptan-2-one
	59.049	$C_3H_7O^+$	59.0491	-0.59	Propan-2-one (acetone)
	87.080	$C_5H_{11}O^+$	87.0804	-0.56	2-Methylbutanal, 3-methylbutanal, pentan-2-one
	101.097	$C_6H_{13}O^+$	101.0961	-0.55	Hexan-1-one, hexan-2-one, hexanal
3	42.034	$C_2H_4N^+$	42.0330	-0.70	Acetonitrile
	33.034	CH_5O^+	33.0335	-0.69	Methanol
	129.127	$C_8H_{17}O^+$	129.1270	0.66	Octan-1-one
	75.044	$C_3H_7O_2^+$	75.0440	-0.61	Propanoic acid
	143.143	$C_9H_{19}O^+$	143.1430	0.60	Nonan-2-one

4	109.070	$C_2{}^{13}CH_{10}O_3N^+$	109.0760	-0.65	2,6-Dimethylpyrazine
	95.017	$C_2H_7O_2S^+$	95.0160	-0.52	Methyldisulfanylmethane (dimethyl disulphide)
	75.044	$C_3H_7O_2^+$	75.0440	0.40	Propanoic acid
	61.028	$C_2H_5O_2^+$	61.0284	0.31	Acetic acid and fragment of acetate ester
	33.034	CH_5O^+	33.0335	0.31	Methanol
5	60.021	$C_2H_4O_2^+$	60.0205	-0.61	Acetic acid
	103.075	$C_5H_{11}O_2^+$	103.0754	-0.58	3-Methylbutanoic acid (isovaleric acid), ethyl (S)-2-hydroxypropanoate (ethyl lactate), pentanoic acid (valeric acid)
	105.091	$C_5H_{13}O_2^+$	105.0910	0.55	1,2-Pentanediol
	87.080	$C_5H_{11}O^+$	87.0804	0.53	2-Methylbutanal, 3-methylbutanal, pentan-2-one
	131.107	$C_7H_{15}O_2^+$	131.1067	-0.49	Ethyl-2-methylbutanoate, ethyl-3-methylbutanoate (ethyl isovalerate), heptanoic acid
6	71.049	$C_4H_7O^+$	71.0491	0.57	Butanoic acid
	41.039	$C_3H_5^+$	41.0386	-0.56	Alkyl fragment
	57.070	$C_4H_9^+$	57.0699	-0.46	Alkyl fragment
	90.063	$C_3{}^{[13]}CH_9O_2^+$	90.0631	0.41	2-Methylpropanoic acid (isobutyric acid), butanoic acid, ethyl acetate
	89.060	$C_4H_9O_2^+$	89.0597	0.41	3-Hydroxy-2-butanone (acetoin)
7	144.146	$C_8{}^{[13]}CH_{19}O^+$	144.1460	0.43	Nonan-2-one
	143.143	$C_9H_{19}O^+$	143.1430	0.42	Nonan-2-one
	45.033	$C_2H_5O^+$	45.0335	-0.38	Ethanal (acetaldehyde)
	41.039	$C_3H_5^+$	41.0386	-0.38	Alkyl fragment
	59.049	$C_3H_7O^+$	59.0491	-0.32	Propan-2-one (acetone)

8	105.071	C_8H_{9+}	105.0700	-0.32	2-Phenylethanol/styrene
	143.143	$C_9H_{19}O^+$	143.1430	0.24	Nonan-2-one
	55.055	$C_4H_7^+$	55.0542	0.20	Butanal, heptanal, alkyl fragment
	61.028	$C_2H_5O_2^+$	61.0284	0.19	Acetic acid and fragment of acetate ester
	132.109	$C_6{}^{[13]}CH_{15}O_2^+$	132.1100	-0.18	Ethyl-2-methylbutanoate, ethyl-3-methylbutanoate (ethyl isovalerate), heptanoic acid
9	55.055	$C_4H_7^+$	55.0542	0.32	Butanal, heptanal, alkyl fragment
	71.086	$C_5H_{11}^+$	71.0855	-0.29	3-Methyl-1-butanol, 3-methyl-3-buten-1-ol, pentan-1-ol
	49.011	CH_5S^+	49.0106	-0.25	Methanethiol
	73.065	$C_4H_9O^+$	73.0648	0.25	Butan-2-one, butanal
	74.069	$C_3{}^{[13]}CH_9O^+$	73.0648	0.22	Butane-2,3-diol
10	45.033	$C_2H_5O^+$	45.0335	0.39	Ethanal (acetaldehyde)
	49.011	CH_5S^+	49.0106	-0.23	Methanethiol
	132.109	$C_6{}^{[13]}CH_{15}O_2^+$	132.1100	-0.21	Ethyl-2-methylbutanoate, ethyl-3-methylbutanoate (ethyl isovalerate), heptanoic acid
	69.070	$C_5H_9^+$	69.0698	-0.18	2-Methyl-1,3-butadiene (isoprene)
	99.081	$C_6H_{11}O^+$	99.0804	0.16	Hexanoic acid

Supplementary Table S2. Average concentrations of spectrometric peaks and coefficient of variation (CV, %) from PTR-ToF-MS analysis of 1,075 cheese samples, together with their phenotypic (σ_p), residual (σ_e), genetic (σ_g) and heritability (h^2) SD.

			SD			Heritability	
m/z	ppb$_v$[1]	CV, %	σ_p	σ_e	σ_g	h^2	SD h^2
33.034	9.24	6.6	1.463	0.429	0.113	0.065	0.009
34.037	5.11	10.8	1.461	0.407	0.124	0.085	0.010
34.995	4.47	15.2	1.480	0.887	0.206	0.051	0.032
39.023	7.64	9.2	1.606	0.826	0.282	0.105	0.053
40.027	4.87	11.9	1.576	0.855	0.251	0.079	0.045
41.039	10.35	4.5	1.584	0.776	0.214	0.071	0.032
42.010	5.95	7.5	1.496	0.894	0.251	0.073	0.042
42.034	8.08	11.2	1.356	0.308	0.072	0.052	0.004
42.043	7.39	7.5	1.568	0.851	0.229	0.067	0.036
43.018	11.25	3.7	1.553	0.855	0.188	0.046	0.026
43.054	11.09	3.4	1.529	0.864	0.219	0.060	0.034
43.094	5.07	7.2	1.544	0.869	0.241	0.071	0.041
44.022	7.56	5.1	1.551	0.855	0.185	0.045	0.025
44.058	7.78	5.2	1.554	0.831	0.221	0.066	0.034
44.980	4.64	14.2	1.461	0.928	0.330	0.113	0.060
45.033	10.93	5.1	1.593	0.765	0.204	0.066	0.031
46.031	5.74	11.9	1.646	0.739	0.267	0.115	0.045
46.038	7.14	8.8	1.577	0.774	0.181	0.052	0.025
48.012	5.69	7.7	1.624	0.763	0.311	0.143	0.049
48.053	10.03	8.2	1.627	0.741	0.287	0.131	0.044
49.011	6.82	9.8	1.609	0.800	0.302	0.125	0.051
49.028	5.28	13.8	1.532	0.896	0.262	0.078	0.051
49.054	7.72	10.3	1.633	0.737	0.296	0.139	0.045
50.000	5.11	16.6	1.652	0.750	0.346	0.176	0.053
50.057	4.58	9.6	1.630	0.754	0.313	0.147	0.050
51.007	4.59	10.5	1.601	0.801	0.290	0.116	0.050
51.044	7.68	7.9	1.538	0.577	0.130	0.048	0.013
53.039	4.50	12.1	1.564	0.702	0.145	0.041	0.017
54.034	4.24	13.9	1.344	0.255	0.098	0.129	0.005
55.055	7.08	22.1	1.480	0.915	0.239	0.064	0.040
56.045	5.33	9.8	1.291	0.957	0.212	0.047	0.037
56.060	5.86	8.7	1.627	0.704	0.223	0.091	0.032
57.033	6.70	10.0	1.638	0.805	0.317	0.134	0.058
57.070	10.55	6.2	1.576	0.753	0.217	0.077	0.033
58.041	5.72	15.5	1.549	0.839	0.239	0.075	0.039
58.073	7.47	8.7	1.587	0.747	0.228	0.085	0.035
59.049	10.52	7.5	1.571	0.696	0.161	0.051	0.020
59.329	4.34	11.7	1.572	0.699	0.165	0.053	0.022
60.021	5.81	13.9	1.556	0.867	0.257	0.081	0.048
60.045	5.39	13.5	1.552	0.600	0.134	0.047	0.014
60.053	7.15	10.7	1.571	0.696	0.162	0.051	0.021
61.028	11.04	4.4	1.539	0.867	0.194	0.048	0.027

61.062	7.19	5.8	1.553	0.840	0.231	0.070	0.036
62.032	7.38	6.2	1.541	0.867	0.193	0.047	0.027
62.068	4.40	6.9	1.583	0.828	0.291	0.110	0.049
63.027	7.10	13.3	1.578	0.594	0.161	0.068	0.018
63.044	8.02	6.1	1.565	0.812	0.183	0.048	0.026
64.031	4.48	13.7	1.597	0.618	0.180	0.078	0.022
64.048	4.82	8.8	1.601	0.794	0.235	0.080	0.040
65.018	5.37	13.4	1.536	0.866	0.194	0.048	0.026
66.063	8.61	10.0	1.619	0.766	0.286	0.122	0.044
67.058	5.65	7.8	1.483	0.896	0.191	0.043	0.030
67.065	6.75	13.3	1.624	0.767	0.288	0.124	0.044
68.067	4.29	7.7	1.610	0.794	0.305	0.129	0.050
69.033	4.35	8.8	1.574	0.799	0.191	0.054	0.027
69.058	4.58	18.5	1.478	0.924	0.217	0.052	0.035
69.070	8.43	6.9	1.539	0.871	0.209	0.054	0.031
70.064	5.13	9.8	1.588	0.674	0.171	0.061	0.021
70.078	6.89	9.4	1.589	0.756	0.190	0.059	0.025
71.049	9.50	6.3	1.536	0.888	0.224	0.060	0.038
71.086	10.38	4.5	1.530	0.835	0.199	0.054	0.029
72.053	6.53	8.6	1.529	0.892	0.217	0.056	0.038
72.089	7.54	5.9	1.539	0.829	0.202	0.056	0.030
73.027	5.50	12.2	1.513	0.908	0.254	0.072	0.050
73.051	6.57	18.6	1.320	0.981	0.212	0.044	0.036
73.065	9.88	6.3	1.535	0.838	0.154	0.032	0.019
74.034	4.34	12.0	1.553	0.863	0.231	0.067	0.037
74.051	4.49	13.8	1.317	0.970	0.245	0.060	0.044
74.069	6.85	8.7	1.536	0.839	0.155	0.033	0.020
75.027	5.47	17.6	1.370	0.954	0.320	0.101	0.060
75.044	8.22	8.2	1.524	0.490	0.146	0.081	0.013
75.080	7.70	14.8	1.619	0.763	0.302	0.136	0.048
76.047	5.26	10.3	1.482	0.469	0.110	0.052	0.009
76.084	5.12	15.5	1.616	0.769	0.313	0.142	0.052
77.060	6.43	10.5	1.572	0.760	0.163	0.044	0.021
78.001	4.23	21.0	1.406	0.944	0.230	0.056	0.037
79.040	8.53	7.2	1.501	0.900	0.178	0.038	0.024
79.055	6.87	23.3	1.371	0.964	0.220	0.050	0.038
79.075	6.17	10.4	1.552	0.835	0.241	0.077	0.039
80.046	5.47	8.4	1.498	0.895	0.170	0.035	0.024
80.058	4.66	20.0	1.384	0.958	0.208	0.045	0.034
80.991	6.17	10.3	1.577	0.783	0.198	0.060	0.028
81.039	4.63	8.2	1.530	0.843	0.153	0.032	0.019
81.061	4.87	10.7	1.606	0.683	0.193	0.074	0.028
81.070	5.37	8.6	1.701	0.669	0.340	0.206	0.056
82.945	4.48	15.6	1.307	0.220	0.087	0.135	0.004
82.988	5.20	11.2	1.561	0.789	0.183	0.051	0.025
83.052	4.97	10.7	1.608	0.798	0.241	0.084	0.036
83.071	7.44	12.8	1.615	0.787	0.291	0.120	0.046
83.086	6.13	11.3	1.611	0.825	0.333	0.140	0.056
84.075	4.41	13.1	1.568	0.839	0.309	0.119	0.052

84.079	4.25	13.4	1.444	0.938	0.258	0.070	0.046
84.942	4.29	14.7	1.296	0.220	0.076	0.106	0.003
85.029	4.46	8.9	1.512	0.910	0.283	0.088	0.051
85.065	6.60	9.1	1.442	0.924	0.177	0.035	0.025
85.101	6.66	8.3	1.624	0.725	0.245	0.102	0.038
86.072	5.00	14.1	1.562	0.829	0.249	0.083	0.041
86.105	4.54	9.5	1.627	0.728	0.250	0.106	0.041
87.044	7.20	11.0	1.532	0.890	0.217	0.056	0.036
87.080	10.42	10.4	1.571	0.791	0.203	0.061	0.029
88.052	5.22	9.6	1.568	0.840	0.192	0.050	0.029
88.084	7.61	12.4	1.596	0.789	0.238	0.083	0.035
89.060	10.76	4.1	1.581	0.818	0.210	0.062	0.031
90.063	7.69	5.6	1.579	0.818	0.207	0.060	0.030
91.051	6.52	22.4	1.512	0.906	0.231	0.061	0.039
91.059	8.47	9.5	1.460	0.936	0.313	0.101	0.057
92.061	6.64	14.6	1.526	0.492	0.150	0.085	0.016
93.037	7.78	10.0	1.428	0.947	0.220	0.051	0.037
93.069	8.96	18.0	1.569	0.716	0.160	0.048	0.022
93.090	10.11	13.5	1.624	0.764	0.294	0.129	0.048
93.181	4.19	12.5	1.550	0.871	0.281	0.094	0.050
93.432	4.54	10.0	1.655	0.759	0.354	0.179	0.062
94.039	5.43	16.5	1.354	0.971	0.221	0.049	0.038
94.074	6.64	20.4	1.484	0.437	0.135	0.087	0.013
94.095	6.92	19.8	1.626	0.767	0.298	0.131	0.046
95.004	4.72	13.8	1.591	0.567	0.199	0.110	0.024
95.017	5.04	14.1	1.632	0.847	0.370	0.161	0.064
95.034	5.22	12.3	1.431	0.942	0.286	0.085	0.055
95.049	4.98	11.3	1.448	0.367	0.135	0.119	0.012
95.081	4.93	13.5	1.444	0.430	0.093	0.045	0.007
95.096	5.44	18.1	1.631	0.766	0.331	0.157	0.055
96.961	6.15	10.0	1.560	0.793	0.179	0.048	0.024
97.060	4.87	7.1	1.548	0.773	0.166	0.044	0.021
97.101	6.23	9.6	1.525	0.901	0.260	0.077	0.045
98.105	4.43	10.5	1.483	0.919	0.232	0.060	0.039
98.959	5.77	10.5	1.566	0.783	0.186	0.054	0.026
99.039	5.00	7.4	1.457	0.922	0.242	0.065	0.043
99.081	6.93	7.6	1.465	0.908	0.232	0.061	0.040
99.121	5.19	13.1	1.566	0.815	0.181	0.047	0.025
100.084	4.78	8.5	1.487	0.903	0.260	0.077	0.044
100.954	4.54	11.3	1.556	0.788	0.172	0.046	0.022
101.060	6.36	5.3	1.526	0.884	0.229	0.063	0.037
101.097	6.38	11.6	1.571	0.825	0.222	0.068	0.034
102.062	4.35	8.1	1.522	0.897	0.230	0.062	0.038
102.099	4.52	11.1	1.583	0.830	0.246	0.081	0.038
103.075	7.76	8.0	1.475	0.902	0.167	0.033	0.023
104.079	5.23	10.4	1.481	0.896	0.167	0.033	0.023
105.039	4.53	9.7	1.443	0.942	0.283	0.083	0.056
105.071	5.96	7.7	1.568	0.817	0.176	0.045	0.025
105.091	6.37	11.4	1.590	0.799	0.209	0.064	0.030

106.077	5.27	12.4	1.431	0.945	0.232	0.057	0.040
106.097	4.27	10.7	1.591	0.806	0.209	0.063	0.030
107.066	7.14	18.5	1.480	0.903	0.199	0.046	0.030
107.085	9.06	9.8	1.365	0.959	0.232	0.055	0.041
108.069	4.79	13.2	1.489	0.889	0.196	0.046	0.029
108.089	6.63	12.7	1.363	0.960	0.230	0.054	0.040
109.070	6.30	11.1	1.436	0.941	0.314	0.101	0.062
109.099	5.08	8.0	1.491	0.900	0.210	0.051	0.034
110.071	4.49	11.8	1.426	0.945	0.304	0.094	0.057
111.047	6.01	13.9	1.369	0.956	0.304	0.092	0.058
111.080	4.55	8.7	1.367	0.960	0.199	0.041	0.032
111.104	5.08	18.5	1.624	0.774	0.322	0.147	0.053
111.119	4.43	15.4	1.587	0.616	0.186	0.083	0.024
112.049	4.31	13.1	1.411	0.946	0.317	0.101	0.063
113.029	4.64	10.4	1.428	0.947	0.260	0.070	0.046
113.057	4.92	7.5	1.421	0.944	0.245	0.063	0.044
113.098	4.83	9.9	1.546	0.886	0.230	0.063	0.037
115.077	5.84	8.8	1.586	0.864	0.299	0.107	0.055
115.112	8.78	12.0	1.532	0.883	0.254	0.076	0.044
116.078	4.47	8.6	1.575	0.845	0.240	0.075	0.039
116.116	6.32	14.8	1.531	0.884	0.249	0.074	0.044
117.047	4.87	13.0	1.460	0.909	0.243	0.067	0.043
117.091	9.14	6.7	1.600	0.780	0.295	0.125	0.050
118.095	6.53	8.7	1.604	0.775	0.298	0.129	0.051
119.072	5.54	11.9	1.615	0.792	0.293	0.121	0.046
119.089	6.44	15.5	1.621	0.815	0.330	0.141	0.054
119.107	6.36	8.4	1.574	0.639	0.166	0.063	0.020
120.092	4.95	14.5	1.638	0.797	0.363	0.172	0.062
121.068	6.43	10.5	1.530	0.883	0.275	0.089	0.049
121.096	5.61	7.5	1.542	0.853	0.212	0.058	0.034
121.122	5.17	15.8	1.646	0.721	0.352	0.192	0.058
122.072	4.76	10.1	1.523	0.888	0.265	0.082	0.046
122.118	4.18	7.2	1.581	0.791	0.260	0.097	0.047
123.047	4.56	7.8	1.559	0.864	0.299	0.107	0.055
123.076	4.56	8.4	1.545	0.880	0.280	0.092	0.049
123.117	4.82	13.2	1.437	0.944	0.264	0.072	0.047
125.095	4.54	7.0	1.525	0.894	0.249	0.072	0.042
125.132	4.39	10.2	1.540	0.900	0.252	0.073	0.044
127.073	4.49	8.6	1.439	0.934	0.243	0.063	0.042
127.112	5.31	7.5	1.520	0.900	0.289	0.094	0.053
129.064	4.37	8.4	1.430	0.937	0.263	0.073	0.048
129.091	4.93	8.0	1.564	0.802	0.157	0.037	0.020
129.127	4.95	13.9	1.503	0.913	0.250	0.070	0.044
131.084	4.61	9.4	1.514	0.881	0.166	0.034	0.023
131.107	5.75	11.5	1.537	0.697	0.151	0.045	0.019
132.109	4.29	9.2	1.545	0.707	0.150	0.043	0.019
133.073	4.45	8.4	1.487	0.909	0.209	0.050	0.034
133.102	4.45	8.6	1.571	0.693	0.156	0.048	0.020
133.123	5.60	12.3	1.605	0.826	0.264	0.093	0.041

135.102	6.69	9.2	1.557	0.848	0.279	0.097	0.046
135.134	5.80	12.9	1.622	0.755	0.307	0.142	0.047
136.022	6.66	7.5	1.488	0.896	0.258	0.076	0.046
136.105	4.67	8.1	1.567	0.842	0.288	0.105	0.048
136.140	4.26	8.9	1.627	0.759	0.319	0.150	0.052
137.024	4.70	9.3	1.468	0.907	0.240	0.066	0.042
137.101	4.71	10.6	1.581	0.837	0.298	0.112	0.052
137.132	5.28	7.3	1.702	0.663	0.348	0.216	0.060
138.018	4.36	9.9	1.474	0.902	0.277	0.086	0.053
139.076	4.58	7.7	1.518	0.895	0.282	0.090	0.050
139.134	4.12	13.1	1.590	0.821	0.323	0.134	0.059
141.129	5.52	15.5	1.456	0.939	0.281	0.082	0.052
142.131	4.26	13.4	1.441	0.942	0.276	0.079	0.052
143.115	4.69	11.7	1.513	0.891	0.271	0.085	0.050
143.143	7.11	16.7	1.496	0.924	0.280	0.084	0.052
144.146	5.20	18.6	1.484	0.928	0.285	0.086	0.054
145.123	7.43	10.5	1.577	0.777	0.304	0.133	0.054
146.126	5.30	11.6	1.583	0.774	0.310	0.138	0.055
147.113	4.37	6.9	1.517	0.893	0.234	0.064	0.042
147.134	4.37	7.4	1.558	0.831	0.255	0.086	0.042
149.045	6.08	15.9	1.337	0.964	0.274	0.075	0.055
149.123	5.49	6.6	1.439	0.906	0.189	0.042	0.030
150.046	4.67	16.0	1.332	0.965	0.268	0.072	0.052
151.034	4.64	15.9	1.356	0.950	0.319	0.101	0.063
155.144	4.26	9.2	1.464	0.935	0.244	0.064	0.044
157.159	4.33	10.5	1.546	0.897	0.314	0.109	0.062
159.065	5.00	16.8	1.417	0.943	0.208	0.046	0.032
159.138	4.49	8.5	1.558	0.877	0.282	0.094	0.053
161.104	4.39	8.2	1.506	0.891	0.181	0.039	0.027
161.154	4.82	13.6	1.508	0.917	0.260	0.074	0.046
163.096	6.55	19.1	1.340	0.969	0.241	0.058	0.042
163.131	5.72	8.3	1.520	0.862	0.247	0.076	0.043
164.100	5.30	15.9	1.396	0.950	0.262	0.070	0.049
165.083	5.74	14.8	1.365	0.960	0.277	0.077	0.052
166.083	4.45	13.7	1.352	0.965	0.253	0.064	0.047
167.056	7.45	14.2	1.318	0.972	0.246	0.060	0.045
168.057	5.71	16.6	1.314	0.973	0.238	0.057	0.043
169.044	5.92	15.6	1.339	0.967	0.250	0.062	0.046
170.041	4.44	15.0	1.340	0.966	0.239	0.058	0.045
171.032	4.43	13.3	1.337	0.967	0.245	0.060	0.045
171.173	4.87	15.7	1.491	0.905	0.384	0.152	0.077
173.153	5.20	9.3	1.610	0.825	0.345	0.149	0.060
177.076	4.67	12.8	1.553	0.868	0.258	0.081	0.046
177.150	4.71	5.9	1.501	0.888	0.240	0.068	0.042
189.184	4.23	11.7	1.542	0.901	0.291	0.094	0.053
191.163	4.35	7.3	1.552	0.831	0.270	0.095	0.046
195.087	5.59	17.1	1.627	0.805	0.326	0.141	0.056
196.088	4.48	14.3	1.618	0.819	0.337	0.145	0.058
197.074	4.64	13.8	1.615	0.821	0.325	0.135	0.056

201.184	4.34	9.4		1.554	0.901	0.312		0.107	0.053
205.186	4.29	7.1		1.523	0.880	0.278		0.091	0.053

[1] Data expressed as natural logarithm of part per billion by volume.

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FOURTH CHAPTER

Cheese-making in highland pastures: Milk technological properties, cream, cheese and ricotta yields, milk nutrient recovery, and product composition

M. Bergamaschi[*†], **C. Cipolat-Gotet**[*], **G. Stocco**[*], **C. Valorz**[‡], **I. Bazzoli**[‡], **E. Sturaro**[1,*], **M. Ramanzin**[*], and **G. Bittante**[*]

[*] Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell’Università 16, 35020 Legnaro (PD), Italy

[†] Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach (FEM) Via E. Mach 1, 38010 San Michele all'Adige (TN), Italy

[‡] Breeders Federation of Trento Province, via delle Bettine, 40, 38100 Trento, Italy

ABSTRACT

Summer transhumance of dairy cows to high Alpine pastures is still practiced in many mountainous areas throughout Europe. It is important for many permanent dairy farms because the use of high altitude pastures increases milk production while high priced typical local dairy products often boost farm income. The practice contributes to landscape preservation, attracts tourism, and helps maintain traditional cultural values. As traditional cheese- and ricotta-making procedures in Alpine pastures are central to this dairy system, the objective of this study was to characterize the quality and efficiency of products and their relationships with the quality and availability of grass during the grazing season. The milk from 148 lactating cows of different breeds from 12 permanent farms reared day and night on Alpine pastures on a temporary farm in the Eastern Italian Alps (Juribello, Trento, 1,860 m asl) was processed every two weeks during the summer (7 cheese makings from late June to early September). During each processing, 11 dairy products (four types of milk, two byproducts, three fresh products and 2 ripened cheeses) were sampled, stored and analyzed. In addition, 8 subsamples of fresh forage from the pasture used by the cows were collected, pooled and analyzed. At the beginning of the pasturing season the cows were 233±90 days in milk, had 2.4±1.7 parities and produced 23.6±5.7 kg×d^{-1} milk. The milk yield decreased with the move from permanent to temporary farms (-21%) and during the entire summer transhumance (-14%), but partly recovered after the cows returned to the permanent farms (+10%). Similar patterns were observed for the daily yields of fat, protein, casein, lactose, and energy as there were no large variations in the quality of the milk, with the exception of the first period of Alpine pasture. The SCS of milk increased during transhumance, but this resulted from a concentration of cells in a lower quantity of milk rather than an increase in the total number of cells ejected daily from the udder. There was a strong quadratic trend in availability of forage, as both fresh and dry matter weight per Ha, with a maximum in late July. The quality of forage also varied during the summer with a worsening of chemical composition. The evening milk (before and after natural creaming), the whole morning milk, and the mixed vat milk had different chemical compositions, traditional coagulation properties, and curd firming modeling parameters. These variations over the pasture season were similar to the residual variations with respect to chemical composition, and much lower with respect to coagulation and curd firming traits. There were much larger variations in cream, cheese and ricotta yields (expressed as fresh weight, solid weight or retained water as a percentage of the processed milk), and nutrient recoveries (expressed as the weight of fat, protein, solids and energy of the dairy product as a percentage of the weight of the same nutrient in processed milk) during the pasture season, only partly explained by variations in milk composition. The protein content of forage correlated with some of the coagulation and curd firming traits, the ether extract of forage was positively correlated with milk fat content and cheese yields, and fiber fractions of forage were unfavorably correlated with some of the chemical and technological traits. Variations in the chemical compositions of the 11 dairy products during the grazing season were similar or lower than the residual variations. Traditional cheese- and ricotta-making procedures shewed a good efficiency allowing to obtain, in average cream, cheese and ricotta yields of 6.3%, 14.2%, and 4.9%, respectively, and an overall recovery of almost 100% of milkfat, 88% of milk protein, and 60% of total milk solids.

Keywords: transhumance, pasture, milk, cheese-making.

INTRODUCTION

In addition to their economic function, mountain farms have an important role in many countries in terms of the preservation of landscape, conservation of historical traditions, and production of typical local products (Penati et al., 2011; Haddaway et al., 2013; Battaglini et al., 2014). One of the most distinctive and important traditional practices for both landscape preservation and typical local product production is the summer transhumance of cows and small ruminants from permanent farms in the valleys to temporary farms in highland areas to exploit the Alpine pastures. This tradition still has particular significance in many mountainous areas of several European countries, especially the Alps region (Sturaro et al., 2013), where the making of typical cheeses on Alpine pastures allows the maximum benefits to be obtained from milk production. The quality of dairy products is closely connected with production conditions and the feeding strategies adopted (Bovolenta et al., 2009), while their fatty acid profiles are generally favorable to human health, as they are characterized by a high content of unsaturated fatty acids and conjugated linoleic acid, which seem to have anti-tumor and immuno-modulatory effects (Dewhurst et al. 2006; Ferlay et al., 2006; Coppa et al., 2011).

Some studies have shown that the botanical composition of pasture and the type of diet the cow is fed have an effect on the quality of milk and cheese (Agabriel et al., 1999; Ferlay et al., 2006; Romanzin et al., 2013). The importance of pasture as a key factor in the traceability and quality of dairy products has also been demonstrated (Buchin et al., 2006; Martin et al., 2005 and 2009).

However, few studies have been carried out on the evolution of the technological properties of milk during Alpine summer pasturing and the efficiency of traditional cheese production practices on temporary farms. Moreover, no information is available on byproducts of traditional cheese making, like cream for butter production, and ricotta from whey processing.

In light of this, a study on production efficiency and the relationships between dairy products derived from cheese and ricotta making in term of their physico-chemical composition is of particular interest. The aim of this study was to investigate: i) the quality and technological properties of milk produced; ii) cream, cheese and ricotta yields from traditional cheese-making procedures; iii) the qualitative characteristics of dairy products derived from cheese and ricotta making during the course of summer pasturing on a temporary highland farm; and iv) the relationships between the efficiency of the whole cheese-making process and the quantity and quality of available forage.

MATERIALS AND METHODS

Environmental Conditions and Pasture Characteristics

The study was conducted from June to September on a temporary summer highland farm (1,860 m a.s.l.) located in the north-eastern part of the Italian Alps (Malga Juribello, Trento, Italy).

The climate at the site is characterized by long, cold winters and warm, wet summers. The pasture is a typical *Nardetum alpigenum* association, which has replaced the native woodland and shrub land as a result of continuous human and animal action (Orlandi et al., 2000).

The feeding strategy was pasture-based and the cows were free to graze day and night. The pastures covered a total of 180 Ha, and the herd was moved to different areas of the pasture according to grass availability, without a rigid rotation.

Grass Availability and Composition

Every two weeks on the day before experimental cheese-making, the grass available in the pasture sections grazed by cows was sampled by cutting the grass in 8 sites of 1×1 m at an approximate height of 3 cm from the ground. Samples were weighed and a fixed proportion of each was pooled into a composite sample of the 8 sites, representing the grass available to the cows on a given date. The proportions varied on the different sampling dates to obtain composite samples weighing about 2 kg.

The composite samples were frozen and stored at -20°C until chemical analysis at the laboratory of the Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE) of the University of Padova (Legnaro, Italy). The content of dry matter, crude protein, ether extract, neutral detergent fiber, acid detergent fiber, acid detergent lignin, and ash of the composite samples were analyzed in duplicate using the official AOAC method (2000) and the Van Soest et al. (1991) procedure.

During the experiment, the grazing cows were given a supplement of compound feed (5.0±1.5 kg×d^{-1}) distributed in the milking parlor according to milk yield (17.9±4.5 kg×d^{-1}). The fat content of the compound feed was higher during the later part of summer grazing than during the earlier part to take into account the decrease in the digestibility of the grass. Both compound feeds were sampled and analyzed in duplicate.

Animals and Milking

A total of 148 dairy cows were moved to Malga Juribello from 12 permanent farms in the province of Trento. The main characteristics of these farms and cows are given in Table 1. The farms represented mainly traditional farming systems (Sturaro et al., 2013), with tie stalls and feeding mainly based on hay and compound feeds, although also few farms and cows from modern dairy systems were present. Cow breeds were mainly Brown Swiss (75.0%), dual purpose Simmental (18.2%), and crossbreeds, with only a few head of Rendena and Holstein-Friesian. The mean and standard deviation of the cows' DIM, parity, and milk yield on their move to the summer pastures are shown in Table 1. This table clearly shows that the animals were prevalently multiparous lactating cows in the second stage of lactation. Some of the permanent farms produced milk for traditional cheese making according to European Union Protected Designation of Origin

(**PDO**) criteria, while others produced milk for processing into fresh cheese, fluid milk and other dairy products.

Malga Juribello's modern milking parlor, like many others on temporary summer farms in the area, was housed in the old barn where used the past the cows were kept tied overnight. The cows were milked twice daily at roughly 12 h intervals (4.00 a.m. and 4.00 p.m.).

Cheese making, ricotta making, and Collection of Samples

Malga Juribello has a small dairy which was used for making experimental cheeses every two weeks according to the following procedure. Raw milk (250 L) from the evening milking was collected in an open tank and maintained at about 15°C overnight to permit partial spontaneous fat creaming. The following morning, the cream was removed from the surface and the creamed milk transferred to the vat and mixed with the freshly collected morning whole milk (250 L). The cream, creamed milk, and mixed milk in the vat were sampled. The mixed milk was heated in the vat to 27°C and inoculated with 250 g of yogurt composed of pasteurized milk, *Streptococcus thermophilus* and *Lactobacillus bulgaricus*. After about one hour of incubation, 25 g of commercial rennet [NATUREN extra 1,030 NB, 1,030 international milk clotting units (**IMCU**) × g^{-1}] diluted in 550 mL of water (final rennet dilution = 51.5 IMCU × L^{-1} of milk) was added to the milk. Clotting time was about 15 min and detected visually. Firm coagulum was manually cut (with a lira) into pellets the size of rice grains. After cutting, the curd in the vat was turned to facilitate draining, then cooked at 44-45°C for 15 min. Finally, the curd was separated and sampled then put into cylindrical molds (30 cm diameter x 12 cm height) and pressed for 20 h during which time it was turned 2-3 times to facilitate draining. The following day, the fresh cheeses were salted by placing in brine (16% NaCl) for 80 h. They were then ripened for 6 and 12 months in a ripening cellar until analysis. The temperature in the cellar was about 12°C, the relative humidity 85%.

The whey remaining from cheese making was sampled and used to produce ricotta. Briefly, 200 L of whey was transferred into a smaller vat and heated to 90 °C, then 750 mL of wine vinegar was added to catalyze the thermo-acid coagulation. The ricotta was removed from the vat, weighed, sampled, and placed in molds to allow it to cool and the ‘scotta’ (residual liquid) to drain. The scotta was also sampled. The cream, curd, whey, ricotta, and scotta samples, as well as the milk samples, were stored in a freezer (- 20°C) until analysis.

Milk and Cheese Analysis

Analyses of the chemical and physical traits were performed at DAFNAE’s milk laboratory. Fat, protein, and casein percentages were determined by infrared analysis (MilkoScan FT2, Foss Electric A/S, Hillerød, Denmark). Somatic cell counts were obtained with a Fossomatic FC automatic cell counter (Foss) then converted to SCS by logarithmic transformation (Ali and Shook, 1980). The pH was measured with a Crison Basic 25 electrode (Crison Instruments SA, Barcelona, Spain). The chemical composition of the curd, ricotta, and cheeses was measured using a FoodScan (Foss, Hillerød, Denmark); cheeses were analyzed at 6 and 12 months of ripening.

Milk Coagulation Properties (MCP), Yields, and Nutrient Recoveries of Cheese, Ricotta, and Cream

In this study we used the traditional parameters of milk coagulation properties (**RCT**, $\mathbf{k_{20}}$, $\mathbf{a_{30}}$) reported by McMahon and Brown (1982), and the MCP, curd firming, and syneresis modelling parameters ($\mathbf{RCT_{eq}}$, $\mathbf{CF_P}$, $\mathbf{k_{CF}}$, $\mathbf{k_{SR}}$, $\mathbf{CF_{max}}$, $\mathbf{T_{max}}$) proposed by Bittante (2011) and Bittante et al. (2013).

Cheese yields and nutrient recoveries (**REC**) were obtained according to the procedure described in detail by Cipolat-Gotet et al. (2013) for model cheese making. The yields and nutrient recoveries of the ricotta (**RIC**) and cream (**CRE**) were similarly obtained.

Statistical Analysis

Statistical analysis was performed using SAS (SAS Inst. Inc., Cary, NC). All the dairy product physico-chemical composition and grass chemical composition data were processed using mixed model analyses of variance according to the following model:

$$Y_{ijk} = \mu + \text{dairy product}_i + \text{cheese-making date}_j + e_{ijk}$$

where y_{ijk} is the measured physico-chemical traits of the dairy product; μ is the overall mean; dairy product$_i$ is the fixed effect of the *i*th dairy product (i = 1 to 11); cheese-making date$_j$ is the random effect of the *j*th day of cheese making (k = 1 to 7); e_{ijk} is the residual random error term $\sim N(0, \sigma^2)$.

In addition, orthogonal contrasts were used to compare the LSMs of the different products obtained and the seasonal variations in yields and nutrient recoveries.

RESULTS AND DISCUSSION

Alpine Pasture Productivity and Quality

The availability of grass in the Alpine pasture, expressed as fresh grass, increased greatly from the beginning of the grazing season in mid-June (1.99 t/Ha) until August (3.82 t/Ha), and decreased thereafter to a very low level (1.91 t/Ha) at the end of the grazing season in mid-September (Figure 1). The evolution of available dry matter followed a similar, although less marked, pattern (Figure 1), confirming the results obtained by Bovolenta et al. (1998, 2002).

The chemical compositions and nutritional values of grass and the supplementary compound feeds are provided in Table 2. The quality of grass varied considerably during the grazing season, with increases in DM (24.3 to 34.1%), NDF (43.4 to 50.8 % DM), ADF (21.9 to 26.6 % DM), and ADL (3.2 to 5.3 % DM) and a decrease in CP (16.8 to 11.4 % DM) (Figure 2).

Milk yield and Quality before, during and after Summer Transhumance to Alpine pastures

Table 3 reports the variations in daily yield and quality traits of milk produced before, during and after summer transhumance. We noted a strong reduction (-21 %) in milk yield after moving from permanent farms to summer pastures, which could be due to increased stress in the animals in reaction to very different environmental and feeding conditions. Milk yield during the grazing season was almost constant during the first phase (July) but further decreased by about 15% during the second phase (August); this pattern is consistent with the effects of advancing pregnancy. We observed a positive effect on milk yield of returning to the permanent farms (+10 %).

A very similar pattern was observed by Leiber et al. (2006) in Brown Swiss cows moved to high altitude pastures in Switzerland: -27% of energy-corrected milk yield after moving from lowland to highland pastures, and -10% during summer pasturing, although they did not observe production recovery after the cows returned to lowland pastures. Zendri et al. (2016a *Submitted*) monitored milk production on 15 temporary farms in the same area as the present study and observed a smaller negative effect (-10%) of moving lactating cows to summer pastures, but a greater decrease in milk yield (-43%) during the grazing season and a higher recovery (+26%) after return to the permanent farms. The differences between this and the pattern observed in the present study can be partly explained by the different breed compositions of the herds. Most of the cows in the present study were Brown Swiss and dual-purpose breeds, which in Zendri et al.'s (2016a *Submitted*) study exhibited a lower negative effect of summer transhumance on milk yield than the Holstein Friesians.

Considerable variations in milk quality were also found during the trial (Table 3). In particular, there was a marked increase in milk fat content after the cows were moved to the summer pastures, concomitant with a substantial reduction in milk yield, resulting in a much smaller decrease in daily milk fat yield. On the other hand, summer transhumance had little effect on the milk protein and casein content, with daily production decreasing at a rate similar to that observed for milk yield. An increase in milk fat content and relative stability of the milk protein and casein contents were also observed by Leiber et al. (2006), but not by Zendri et al. (2016a).

Lactose content of milk decreased at the beginning of summer pasturing concomitant with an increase in SCS. Decrease of lactose content and the increase of SCS after moving to summer temporary farms were observed also in the survey led by Zendri et al. (2016a), while Leiber et al.

(2006) found only a decrease in lactose without the parallel increase in SCS. In this study, the increased SCS was probably mainly due to a concentration of somatic cells as a result of the decrease in daily milk yield rather than to an increase in the incidence of sub-clinical mastitis, since the daily excretion of somatic cells with milk (SCY) increased only marginally during summer transhumance.

Taking the observed variations in qualitative traits together, the energy content of milk was estimated (NRC, 2001) to increase by about 5% soon after arrival at the Alpine pasture and subsequently to return to the previous values (Table 3), while the daily output of milk energy decreased by 18% after moving the cows to Alpine pastures, and by a further 20% during summer grazing, to recover by 14% after returning to the permanent farms (Table 3).

The values of the milk quality traits recorded during the trial were compared with those measured over the previous 8 years (Figure 3). Lactose and casein contents were similar, while fat content was smaller and protein content greater during the trial than in the previous years.

Milk Composition, Coagulation time, Curd firming, and Syneresis during Cheese making

The traditional procedure used on temporary Alpine farms (*malga* in Italian) to produce *malga* cheeses involves naturally creaming the milk obtained at the evening milking by leaving it overnight, then partially skimming it the following morning and mixing it in the cheese vat with the whole milk obtained at the morning milking. The chemical composition of these four types of milk and the variations recorded between different cheese-making dates are presented in Table 4.

Gravity separation of the cream from the evening milk (creaming effect) not only reduces the milk’s fat (-38%) and total solid (-10%) contents, but slightly concentrates its casein (+4%) and lactose (+3%) contents. In addition, the main effect of creaming is a sizeable reduction in the somatic cell content of the evening milk, on both logarithmic (SCS: -70%) and linear (SCC: -83%) scales. A high somatic cell content can reduce cheese yield and increase lipolysis and proteolysis activity in whole milk and yogurt (Politis and Ng-Kwai-Hang, 1988), although natural creaming is also used in the production of hard cheeses from raw milk as a way of removing anti-dairy microbes, especially clostridia, that can cause late inflation of cheeses (Bertoni et al., 2001; Caplan et al., 2013; Feligni et al., 2014).

The milk from the morning milking had a higher content of fat (+6%), lactose (1.5%), total solids (+2%), and especially somatic cells (SCS: +25%; SCC: +88%) than the milk obtained the previous evening. Mixing the whole milk of the morning with the creamed milk of the previous evening resulted in a significant deviation from the expected values (average of the two milks) only for SCS (+29%), but this deviation is only apparent due to the logarithmic nature of the trait: in fact, the expected value in logarithmic terms is 3.66, very close to the observed value of 3.59.

The lactodynamographic properties of milk are also presented in Table 4. Natural creaming had no effect on traditional coagulation properties and on the modeling parameters of curd firming and syneresis. Stocco et al. (2015) also observed a very small effect of natural creaming on lactodynamographic traits in milk destined for the production of Grana Padano cheese. It is worth noting that morning milk, in contrast to evening milk, was characterized by an accelerated pattern of coagulation curd firming and syneresis, leading to lower potential curd firmness at infinite time, and lower measured maximum curd firmness, even if attained earlier. Mixing creamed evening milk with morning milk was unfavorable for curd firmness (Table 4) as this resulted in a mixture in the cheese-vat intermediate between the two components in terms of coagulation time and k_{20}, but lower in terms of a_{30} (Table 4). Curd firming modeling revealed that both instant rate constants (curd firming and syneresis) were greater than the average of the two components and more similar to those of the whole morning milk, which explains the observed lower potential and maximum curd firmness.

Comparison between the root mean squares of cheese-making date and of error of all traits is shown in Table 4. This reveals that the variability brought about by advancing grazing season is similar to that characterizing residual variability in the case of all milk quality traits (SCS excepted), while it is about half in the case of traditional milk coagulation properties and even lower for curd firming modeling parameters.

On average, rennet coagulation time was good (measured both as a single point and on the basis of curd firming modeling), and was shorter than that of milk from Brown Swiss cows reared in permanent mountain farms in the same province (Cecchinato et al., 2013). The other two traditional traits (k_{20} and a_{30}) were also more favorable for milk obtained during summer transhumance. In a trial under similar Alpine conditions, Bovolenta et al. (2009) recorded less favorable traditional coagulation properties than in the present study, but they found that these traits depended on the quantity of compound feed administered to cows on Alpine pastures. A more analytical approach based on modeling all the information from the lacto-dynamograph revealed potential curd firmness and the instant rate constant of curd firming to be similar in milk from Alpine pastures (present study) and milk from permanent farms (Bittante et al., 2015). The syneresis instant rate constant differed, however, and was much slower in milk from the Alpine pastures, which explains the greater maximum curd firmness attained, even over similar time intervals.

Cheese- and ricotta-making Yields and Nutrient Recoveries

Cream, cheese, and ricotta yields obtained according to traditional cheese-making procedures on the temporary summer Alpine farm are given in Table 5 together with the nutrient recoveries of each phase. We found average cream, cheese, and ricotta yields of 6.3%, 14.2%, and 4.9%, respectively. Quantitative data obtained from Alpine pastures are very scarce, but these results are comparable to those obtained, albeit in different conditions, by Martin et al. (2009) and Cipolat-Gotet et al. (2013) for cheese making, and by Pintado et al. (2001) for ricotta making.

As shown in Figure 4a, the patterns during summer transhumance were towards a slight increase for cheese yield (linear) and ricotta yield (cubic), and a decrease (cubic) for cream-yield.

Variation in yields over the summer transhumance was due to in part to the observed changes in milk composition (especially fat and casein content, Figure 3) consequent to advancing lactation stage, as well as changes of season (Verdier-Metz et al., 1998; Zendri et al., 2016a *Submitted*). Water retention in fresh products (cream, curd and ricotta) is also an important component of yields, and especially of ricotta (Table 5). However, the yields of the different products depend first of all on the protein and fat content of milk (Verdier-Metz et al., 2001) and on the recovery of milk nutrients in dairy products compared with the loss in wasted effluents (whey and scotta).

Milk nutrient recoveries in cream, curd, and ricotta are also shown in Table 5. Natural creaming yields cream with a 38% milk fat content representing about 10% of milk solids and 18% of milk energy. No information on the efficiency of natural creaming in traditional Alpine cheese making is available.

During the second stage of processing, partially skimmed milk is used to produce fresh cheese containing about 85% of fat and 78% of proteins, representing almost 50% of total solids and 58% of processed milk energy. Little specific information is available on these technological traits in *malga* cheese production, although more data are available on cheese quality traits (Buchin et al., 1999; Bovolenta et al., 2009; Hurtaud et al., 2009). Taking into account the partial defatting of milk, the above recovery rates of fresh cheese may be considered normal as they are similar to those obtained in the lowlands under very different conditions with milk from cows of Alpine breeds (Verdier et al., 1995; Martin et al., 2009; Cecchinato and Bittante, 2015 *Accepted*).

Seasonal variations in fat and protein recoveries in cheese contributed to the total solids and energy recoveries, which exhibited an almost linear upward trend during the summer grazing season (Table 5 and Figure 4b). Although there are very few experimentally measured cheese-making technological parameters for mountain cheeses, predictions of cheese yields (fresh and solid cheese yields, and cheese water retention) and nutrient recoveries in curd (fat, protein, total solids, and energy) have been made through Fourier transform infrared spectral analysis (Ferragina et al., 2013, 2015) of milk collected for recordings on permanent mountain farms (Cecchinato et al., 2015) and temporary summer Alpine farms (Zendri et al., 2016b *Submitted*). In both cases, the predicted average values of the various traits, and the pattern during summer transhumance in the latter case were very similar to the values measured in the present study, confirming the good prediction ability of FTIR calibrations.

In the third stage, production of ricotta from milk whey processing, the fresh product yield (5%) was about three quarters dependent on water retention and one quarter dependent on solids (Table 5). This last figure represented almost all the milk fat present in whey (lost during cheese making) and almost half the protein (mainly whey proteins). On the whole, total solids recovered in ricotta were 14% of whey solids and 23% of whey energy (Table 5). No information is available on ricotta production on temporary summer Alpine farms. The fluctuations in these traits in ricotta making recorded during summer transhumance are shown in Figure 4b.

Relationships between Grass Quantity and Quality and Milk Technological Properties

Variations in milk composition and technological properties during summer grazing also depend on contemporaneous variation in the quantity and quality of the available grass (Buchin et al., 1999; Collomb et al., 2002; Gorlier et al., 2012). Although the present study was not designed to quantify fresh forage intake and composition, it was possible to get some indication of the relationship between the cows' feeding regime and their production levels by examining Pearson correlations between the quantity and quality of forage available in the grazed area and, firstly, composition, coagulation, curd firming and syneresis (Table 6), and, secondly, cheese and ricotta yields and nutrient recoveries (Table 7).

Regarding milk composition, there was a tendency towards an unfavorable relationship between the quantity of forage dry matter available in the Alpine pastures grazed by the cows and the milk fat and total solids contents (Table 6). The quality of forage, too, affected milk composition, as there was a strong positive correlation between the lipid content of grass and that of milk and of milk total solids. MCPs were highly affected by grass quality as they were all unfavorably related to fiber content (NDF), while coagulation time was favorably related to grass protein content. Moving on to cheese yield, it appears there may be a negative relationship between the quantity of dry matter available in the pasture and cheese yield expressed in terms of fresh cheese, cheese dry matter or moisture retained in the cheese (Table 7). This does not seem to be due to the recovery efficiency of the various nutrients in cheese but rather to a possible reduction in milk fat and total solids content (Table 6). The correlation between the lipid content of pasture and of milk seems also to be the main explanation for the link between the ether extract content of grass and the three cheese yields examined (Table 7). The yields and nutrient recoveries of ricotta did not seem to be correlated with the quantity and quality of available grass. There appears to be no information on these matters in the literature.

Flow of Milk Nutrients to different Dairy Products and Byproducts

To obtain a clear picture of the entire traditional cheese- and ricotta-making procedures adopted by temporary summer farms on Alpine pastures, the chemical compositions of the processed fluids (vat milk, whey and scotta) of the fresh products (cream, curd, and ricotta) and ripened cheeses (after 6 or 12 months of ripening) are reported in Table 8. Schematization of the whole flow process from milk to dairy products and byproducts through the different stages in terms of total weight, milk fat, milk protein, and total solids is shown in Figure 5 (5a, 5b, 5c, and 5d, respectively).

At the end of the process, the saleable products obtained (which include cream and ricotta) represent about 20% of milk weight, the other 80% being the final byproduct (scotta). Moving from fresh weight to nutrient recovery, it is clear that cheese making has a much more important role. The final recovery of milk fat is close to 100% with about two thirds retained in *malga* cheese and one third in cream and ricotta. In the case of milk protein, the proportion lost in the scotta is about one eighth as almost 80% is retained in *malga* cheese and 10% in ricotta. The presence of most of the lactose and minerals in the scotta reduced the overall total solids recovery to about 60%, with almost 50% represented by *malga* cheese and the remainder by cream and ricotta.

Cheeses produced according to traditional procedures from raw milk obtained from cows grazing on Alpine pastures are easily distinguishable from the industrial products of the lowlands, especially in terms of their fatty acid profile (Collomb et al., 2002; Coppa et al., 2011), color, and flavor (Carpino et al., 2004). There is still significant production of *malga* cheeses in-situ on temporary summer farms even now. According to Zendri et al. (2013), summer pastures utilized by dairy cows account for two thirds of all grazed Alpine pastures, and about one third of temporary summer farms still produce cheese and other dairy products at an average altitude of 1,661±235 m asl. Evidence of the importance of in-situ cheese and ricotta making in the Alps comes from the fact that many of these temporary farms are involved in agritourism through direct sales of their own products as well as meals made using these products (Zendri et al., 2013).

CONCLUSIONS

This work explored the links between the efficiency of traditional cheese and ricotta making and the quality of dairy products obtained during the grazing season on temporary highland farms. To our knowledge, this is the first time that cheese- and ricotta-making processes during summer transhumance have been characterized by collecting different dairy products (milk, cream, whey, scotta, ricotta, curd and ripened cheese).

The results reported in this experiment suggest sizeable variations in the chemical compositions and nutrient recoveries of cheese, ricotta, and cream during summer transhumance. In particular, we confirmed the decrease in milk yield from cows after moving to the Alpine pastures and during the summer grazing season, probably due to nutrient imbalance, but we also found partial recovery of productive functions after returning to the lowland permanent farms. Monitoring of technological characteristics of milk during summer grazing revealed variations that partly correlated with the quantity and quality of forage available in the areas grazed by the cows. The traditional cheese- and ricotta-making procedures used on the temporary highland farms during summer was characterized in terms of cream, cheese and ricotta yields, and nutrient recoveries across the various stages. The overall process could be considered efficient, as it allows recovery of about 20% of milk weight, 60% of milk total solids, 88% of milk protein, and almost 100% of milk fat in the fresh products. Moreover, transhumance practices maximize resource exploitation through grazing and support traditional activities. Indeed, milk produced on temporary highland farms can be used to produce typical cheeses and ricotta with an added-value linked to terroir and historical production traditions. These findings may be relevant for greater appreciation of the quality of dairy products from upland farms operating extensive farming systems.

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TABLE AND FIGURES

Table 1. Descriptive statistics of permanent farms and of production traits of cows (mean±SD) at the beginning of Alpine pasture season.

Permanent farm (n)	Altitude (m a.s.l.)	Housing	TMR	Cow (n)	Breed[2]	DIM (d)	Parity (n)	Milk yield ($kg \times d^{-1}$)	Milk for PDO
1	980	Loose	no	8	BS	345±105	2.0±1.6	22.8±2.7	yes
2	892	Tied	no	20	BS; HF; CB; SI	206±75	1.9±1.2	22.2±5.5	no
3	688	Loose	no	40	BS	245±75	2.0±1.2	27.5±4.3	yes
4	178	Tied	no	9	BS; CB; SI	265±114	2.1±2.5	20.9±4.2	yes
5	695	Loose	yes	12	BS; SI; RE	191±29	2.0±1.2	22.9±5.0	no
6	774	Tied	no	7	BS	238±20	2.7±1.3	27.2±1.6	yes
7	1,030	Loose	no	9	BS; HF	283±93	1.5±2.1	25.5±4.7	no
8	150	Tied	yes	25	BS	218±102	2.9±1.7	20.0±6.3	no
9[1]	669-1,343	Tied	no	18	BS; HF; CB; SI; RE	210±99	3.5±1.6	22.8±6.7	yes
Total	798±366	-	-	148	-	233±90	2.4±1.7	23.6±5.7	-

[1] This is a cluster composed by 4 small herds; [2] BS = Brown Swiss; HF = Holstein-Friesian; CB = Crossbreed; SI = Simmental; RE = Rendena.

Table 2. Descriptive statistics and ANOVA of forage mass and chemical composition, sampled every two weeks during summer transhumance (n = 7), and descriptive statistics of compound feeds used during the trial.

Item	Pasture		Summer variation (*P*-value)[1]			Root means square		Compound feed[2]	
	Mean	SD	Linear	Quadratic	Cubic	Cheese-making date	Error	First period	Second period
Grass mass (t DM×Ha^{-1})	0.93	0.34	***	***	***	0.51	0.01	-	-
DM of grass mass, %	29.54	3.89	***	***	***	5.50	0.06	-	-
Grazed grass (%DM):									
CP	12.65	2.00	***	***	***	2.83	0.33	14.27	14.72
Ether extract	2.53	0.55	***	**	***	0.78	0.07	1.89	3.67
NDF	49.04	3.37	***	***	***	4.77	0.50	26.16	25.64
ADF	25.36	2.15	***	Ns	***	3.04	0.36	9.93	9.14
ADL	4.52	0.88	***	Ns	***	1.24	0.10	1.51	1.66
Ash	7.71	0.56	***	Ns	**	0.79	0.16	8.97	8.09

[1] **P < 0.01; ***P < 0.001; ns = not significant; [2] Compound feed = the first period was from late June to early August, while the second period was from late August to early September.

Table 3. Individual milk recording data before, during and after summer transhumance to alpine pasture.

	Permanent farm	Summer Alpine pasture (temporary farm):				Permanent farm
Traits	June	Early July	Late July	Early August	Late August	September
Cows, n	148	148	148	148	148	148
Lactating cows, n	148	148	148	130	119	109
Productive traits:						
Milk yield, kg×d^{-1}	23.6±5.7	18.7±5.2	18.5±5.4	17.7±4.9	16.0±4.9	17.6±6.3
Fat, kg×d^{-1}	0.89±0.23	0.78±0.18	0.71±0.19	0.68±0.17	0.59±0.15	0.67±0.26
Protein, kg×d^{-1}	0.87±0.22	0.69±0.17	0.67±0.19	0.66±0.16	0.59±0.18	0.67±0.21
Lactose, kg×d^{-1}	1.16±0.29	0.89±0.26	0.89±0.27	0.85±0.25	0.74±0.24	0.84±0.31
Energy, MJ×d^{-1}	73.9±17.4	60.9±14.3	57.6±15.7	55.6±13.3	48.7±13.2	55.3±18.9
SCY[1], U×d^{-1}	30.9±1.7	31.2±1.4	31.2±1.5	31.3±1.5	30.9±1.5	30.7±1.7
Milk composition:						
Fat, %	3.82±0.60	4.29±0.67	3.90±0.55	3.94±0.80	3.80±0.63	3.86±0.76
Protein, %	3.72±0.37	3.74±0.32	3.64±0.35	3.77±0.43	3.75±0.41	3.87±0.52
Fat/Protein	1.04±0.19	1.15±0.16	1.07±0.13	1.05±0.19	1.02±0.14	1.01±0.21
Casein, %	2.91±0.30	2.94±0.25	2.83±0.26	2.95±0.32	2.90±0.32	2.98±0.41
Casein/Protein	0.79±0.01	0.79±0.01	0.78±0.01	0.78±0.01	0.77±0.01	0.77±0.02
Lactose, %	4.90±0.18	4.73±0.23	4.81±0.20	4.83±0.22	4.59±0.23	4.75±0.37
Energy, MJ×kg^{-1}	3.15±0.26	3.30±0.30	3.14±0.26	3.19±0.37	3.10±0.31	3.17±0.35
SCS[2], U×mL^{-1}	2.81±1.66	3.47±1.43	3.49±1.48	3.59±1.55	3.48±1.60	3.08±1.73

[1] SCY = $\log_2$(SCC×Milk yield×1,000); [2] SCS = $\log_2$(SCC/100,000)+3.

Table 4. Composition and technological traits of bulk milk used for cheese-making during summer transhumance.

	Milk (LSM)				Contrast (*F*-value)[1]			Root means square	
Traits	Evening whole (*A*)	Evening after creaming (*B*)	Morning whole (*C*)	Vat (mixture) (*D*)	Creaming effect[2] *A* vs. *B*	Milking effect[3] *A* vs. *C*	Mixing effect[4] *D* vs. *(B+C)*	Cheese-making date	Error
Milk composition:									
Fat, %	4.08	2.53	4.31	3.37	212.9^{***}	4.5^{*}	0.3	0.35	0.34
Protein, %	3.68	3.72	3.68	3.70	4.3	0.1	0.1	0.07	0.06
Casein, %	2.70	2.81	2.70	2.76	19.4^{***}	0.1	0.2	0.06	0.08
Lactose, %	4.79	4.94	4.86	4.89	31.7^{***}	6.6^{**}	0.2	0.08	0.09
Total solids, %	13.28	12.00	13.55	12.73	148.2^{***}	6.5^{**}	0.2	0.38	0.34
pH	6.54	6.53	6.52	6.51	2.2	5.9^{**}	3.3	0.02	0.02
SCS[5], U×mL^{-1}	3.62	1.05	4.53	3.59	313.7^{***}	37.9^{***}	40.0^{***}	0.04	0.47
MCP[6]:									
RCT, min	17.2	17.5	14.9	16.9	0.5	23.2^{***}	2.8	0.84	1.53
k_{20}, min	3.27	3.53	3.24	3.64	2.5	0.1	3.2	0.28	0.57
a_{30}, mm	47.7	46.2	44.1	40.5	0.6	3.1	7.0^{**}	3.14	6.59
CF_t modeling[7]:									
RCT_{eq}, min	17.9	18.5	15.8	17.8	1.7	19.5^{***}	0.9	0.94	1.55
CF_P, mm	65.5	63.7	57.4	52.0	0.4	7.7^{**}	24.7^{***}	2.54	9.49
k_{CF}, min^{-1}	12.1	13.8	17.1	17.0	1.3	11.7^{**}	10.4^{**}	0.00	4.70
k_{SR} , min^{-1}	0.43	0.59	0.88	0.92	1.5	11.5^{**}	12.5^{***}	0.00	0.43
CF_{max}, mm	57.5	55.7	49.4	44.0	0.4	7.7^{**}	24.7^{***}	2.55	9.49
t_{max}, min	45.0	43.7	38.5	38.2	0.2	6.2^{*}	7.4^{**}	1.60	8.50

[1] $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; [2]Contrast between the whole milk of the evening milking vs. the same milk the following morning after natural creaming overnight; [3]Contrast between the whole milk of the evening milking vs. the whole milk of the morning milking; [4]Contrast between milk collected from the vat (mixture of the creamed evening milk and the whole morning milk) vs. the mean of the creamed evening milk and the whole morning milk; [5]SCS = $\log_2$(SCC/100,000)+3; [6]MCP = milk coagulation proprieties; RCT = rennet coagulation time of samples coagulating within 45 min from enzyme addition; k_{20} = curd-firming time of samples reaching 20 mm of firmness within 45 min from enzyme addition; a_{30} = curd firmness 30 min after enzyme addition; [7]CF_t modeling = modeling of the 180 curd firmness observations collected for each milk sample; CF_P =

potential asymptotical curd firmness in absence of syneresis; k_{CF} = curd firming instant rate constant; k_{SR} = curd syneresis instant rate constant; RCT_{eq} = rennet coagulation time estimated trough individual curd firming equation; CF_{max} = maximum curd firmness value; t_{max} = time at CF_{max}.

Table 5. ANOVA for yields, nutrient and energy recoveries of cheese- and ricotta-making observed during summer transhumance.

Item	Mean	SD	Summer variation (Contrast *F*-value)[1]			Root means square	
			Linear	Quadratic	Cubic	Cheese-making date	Error
Cream yields, %							
Fresh cream	6.32	1.56	67.8***	4.4	79.6***	2.68	0.49
Cream solids	2.27	0.57	48.0***	22.5***	64.4***	0.98	0.18
Cream water	4.06	1.03	69.6***	0.3	76.9***	1.76	0.33
Cream recoveries, %							
CRE_{FAT}	38.2	5.9	3.4	6.3*	43.6***	9.91	2.25
$\text{CRE}_{\text{PROTEIN}}$	-1.1	1.9	0.1	0.1	0.1	0.87	2.27
$\text{CRE}_{\text{SOLIDS}}$	9.8	2.7	3.9	3.9	11.1**	4.02	1.74
$\text{CRE}_{\text{ENERGY}}$	17.6	3.8	6.4*	7.1*	22.3***	6.12	1.91
Cheese yields, %							
Fresh cheese	14.22	0.78	17.9***	0.1	2.8	1.21	0.46
Cheese solids	6.33	0.27	14.8**	0.1	2.0	0.42	0.16
Cheese water	7.89	0.54	13.8**	0.1	2.3	0.79	0.36
Cheese recoveries, %							
REC_{FAT}	85.1	1.7	0.3	7.4*	2.1	2.63	1.06
$\text{REC}_{\text{PROTEIN}}$	77.8	0.9	1.4	5.5*	10.0**	1.34	0.64
$\text{REC}_{\text{SOLIDS}}$	49.4	1.9	0.6	1.3	6.7*	2.51	1.59
$\text{REC}_{\text{ENERGY}}$	58.1	1.3	0.5	0.3	4.0	1.32	1.22
Ricotta yields, %							
Fresh ricotta	4.97	0.72	31.9***	30.3***	64.7***	1.21	0.24
Ricotta solids	1.22	0.18	15.4**	7.5*	72.5***	0.30	0.06
Ricotta water	3.75	0.57	28.3***	30.1***	45.4***	0.94	0.21
Ricotta recoveries, %							
RIC_{FAT}	99.0	1.3	34.0***	69.5***	41.8***	5.46	1.88
$\text{RIC}_{\text{PROTEIN}}$	45.0	2.5	15.3**	12.8**	9.3*	3.93	1.34
$\text{RIC}_{\text{SOLIDS}}$	14.1	3.7	7.4*	0.8	2.8	5.11	2.63
$\text{RIC}_{\text{ENERGY}}$	22.8	3.5	7.6*	0.3	8.2*	5.46	1.88

[1] $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

Table 6. Pearson correlations between quantity and quality of the grass available in the Alpine pasture and the milk composition, coagulation and curd firming properties.

	Grass available t/Ha:	Grass composition (%DM)[1]:		
	DM	CP	E.E.[4]	NDF
Milk composition (%):				
Fat	-0.72	0.36	0.91***	-0.19
Protein	-0.52	0.43	0.17	-0.64
Lactose	0.14	0.3	-0.24	-0.42
Total solids	-0.71	0.56	0.73	-0.54
SCS[2]	0.63	-0.02	0.01	0.36
MCP[3]:				
RCT, min	0.68	-0.89**	-0.51	0.79*
k_{20}, min	0.48	-0.58	-0.03	0.78*
a_{30}, mm	-0.47	0.63	0.15	-0.88**
CF_t modeling[4]:				
RCT_{eq}, min	0.67	-0.93**	-0.36	0.88**
CF_P, mm	-0.66	0.65	0.15	-0.90**
k_{CF}, min^{-1}	0.39	-0.76*	-0.08	0.95***
k_{SR} , min^{-1}	0.37	-0.65	0.02	0.87**
CF_{max}, mm	-0.44	0.65	0.15	-0.91**
t_{max}, min	-0.44	0.74*	0.14	-0.95**

[1] $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; [2] SCS = $\log_2$(SCC/100,000)+3; [3] MCP = milk coagulation proprieties; RCT = rennet coagulation time of samples coagulating within 45 min from enzyme addition; k_{20} = curd-firming time of samples reaching 20 mm of firmness within 45 min from enzyme addition; a_{30} = curd firmness 30 min after enzyme addition; [4] CF_t modeling = modeling of the 180 curd firmness observations collected for each milk sample; CF_P = potential asymptotical curd firmness in absence of syneresis; k_{CF} = curd firming instant rate constant; k_{SR} = curd syneresis instant rate constant; RCT_{eq} = rennet coagulation time estimated trough individual curd firming equation; CF_{max} = maximum curd firmness value; t_{max} = time at CF_{max}; [4] Ether extract.

Table 7. Pearson correlations between quantity and quality of the grass available in the Alpine pasture and the technological properties of cheese- and ricotta-making in the temporary summer farm.

	Grass available t/Ha:	Grass composition (%DM)[1]:		
	DM	CP	E.E.[2]	NDF
Cheese yields (%):				
Fresh cheese	-0.71	0.44	0.92**	-0.42
Cheese solids	-0.73	0.43	0.93**	-0.47
Cheese water	-0.69	0.42	0.92**	-0.37
Cheese recoveries, %				
REC_{FAT}	0.33	0.31	-0.49	-0.13
$REC_{PROTEIN}$	-0.25	-0.35	0.61	0.35
REC_{SOLIDS}	-0.18	-0.31	0.57	0.40
REC_{ENERGY}	-0.32	-0.06	0.71	0.35
Ricotta yields, %				
Fresh ricotta	0.05	-0.25	0.31	0.44
Ricotta solids	-0.19	-0.29	0.55	0.41
Ricotta water	0.13	-0.22	0.23	0.43
Ricotta recoveries, %				
RIC_{FAT}	-0.17	0.19	-0.31	-0.20
$RIC_{PROTEIN}$	0.05	0.45	0.39	-0.05
RIC_{SOLIDS}	-0.21	0.38	0.62	-0.17
RIC_{ENERGY}	-0.31	0.18	0.68	-0.05

[1] **$P < 0.01$; [2] Ether extract.

Table 8. Composition of processed fluids, fresh products and cheeses obtained from cheese- and ricotta-making during summer transhumance.

	pH	Fat, %	Protein, %	Lactose, %	Total solids, %	SCS[2], U×mL^{-1}	Salt, %
Processed fluids (LSM):							
vat milk (A)	6.51	3.37	3.70	4.89	12.7	3.59	-
whey (B)	6.31	0.59	0.95	4.79	7.4	2.59	-
scotta (C)	5.70	0.01	0.56	5.05	6.7	0.08	-
Contrasts (F-value)[1]:							
vat milk vs. whey (A vs. B)	10.8^{***}	$5,684.4^{***}$	$29,546.6^{***}$	2.9	$3,297.8^{***}$	46.8^{***}	-
whey vs. scotta (B vs. C)	98.3^{***}	246.8^{***}	592.6^{***}	19.7^{***}	52.9^{***}	329.1^{***}	-
Root means square:							
cheese-making date	0.123	0.104	0.049	0.145	0.195	0.431	-
error	0.199	0.120	0.052	0.192	0.300	0.472	-
Fresh products (LSM):							
cream (D)	6.51	28.1	3.2	-	36.3	-	-
curd (E)	6.59	20.4	20.2	-	44.4	-	-
ricotta (F)	6.14	8.7	8.8	-	24.7	-	-
Contrasts (F-value)[1]:							
cream vs. curd (D vs. E)	1.2	164.5^{***}	$4,849.4^{***}$	-	164.8^{***}	-	-
curd vs. ricotta (E vs. F)	69.9^{***}	362.3^{***}	$2,141.5^{***}$	-	953.4^{***}	-	-
Root means square:							
cheese-making date	0.090	0.647	0.441	-	0.772	-	-
error	0.175	1.955	0.791	-	2.039	-	-
Cheeses (LSM):							
curd (E)	6.59	20.4	20.2	-	44.4	-	0.88
cheese ripened 6 mo (G)	5.49	29.4	29.9	-	66.0	-	2.02
cheese ripened 12 mo (H)	5.83	30.1	32.2	-	69.1	-	2.15
Contrasts (F-value)[1]:							
curd vs. cheeses (E vs. G+H)	935.3^{***}	$1,906.2^{***}$	$2,744.2^{***}$	-	$4,313.6^{***}$	-	$3,058.2^{***}$
6 mo vs. 12 mo ripening (G vs. H)	49.5^{***}	4.3^{*}	51.7^{***}	-	32.5^{***}	-	13.8^{***}
Root means square:							
cheese-making date	0.097	0.292	0.690	-	0.278	-	0.017
error	0.088	0.622	0.598	-	1.021	-	0.063

[1] $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; [2] $SCS = \log_2(SCC/100{,}000)+3$.

Figure 1. Availability (Mean±SD) of fresh grass and grass dry matter (t/Ha) in the areas of the Alpine summer pasture grazed by the dairy cows used for experimental cheese- and ricotta-making.

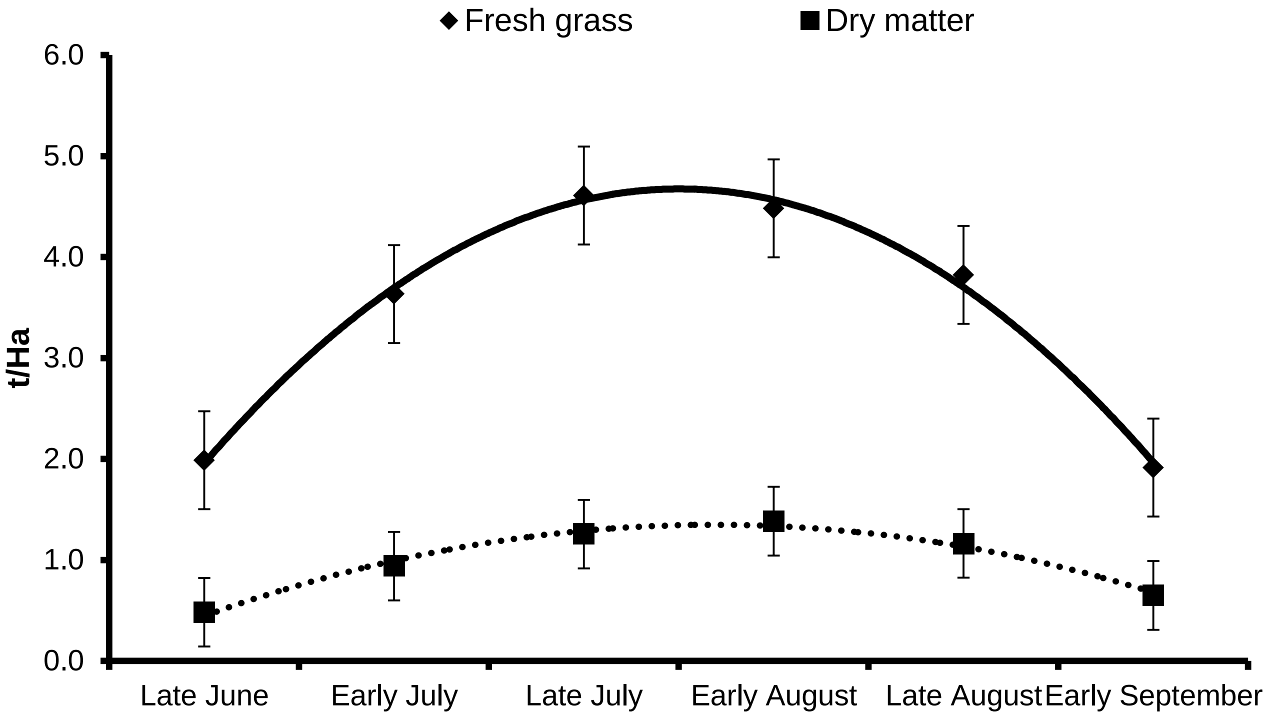


Figure 2. Average content of dry matter (DM, % of fresh wt), crude protein (CP; %DM), ether extract (FAT, %DM), neutral detergent fiber (NDF, %DM) and acid detergent fiber (ADF, %DM) of grass in the areas of the Alpine summer pasture grazed by the dairy cows used for experimental cheese- and ricotta-making.

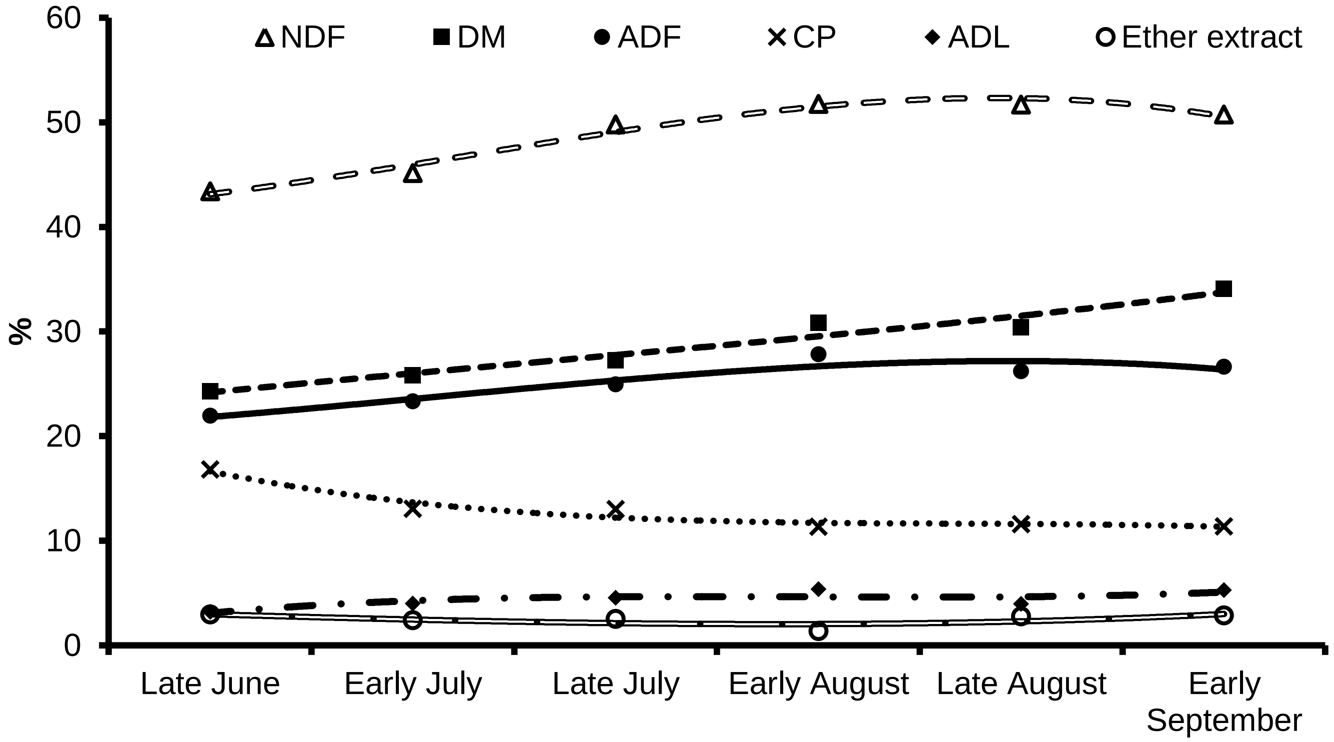


Figure 3. Evolution of milk composition during summer pasture in the 8 years before trial (the lines represent the mean content and the grey area the interval between mean+SD and mean-SD for each chemical component) and in the year of the trial (individual point indicators for each component) according to the milk recording data carried out in the temporary summer farm.

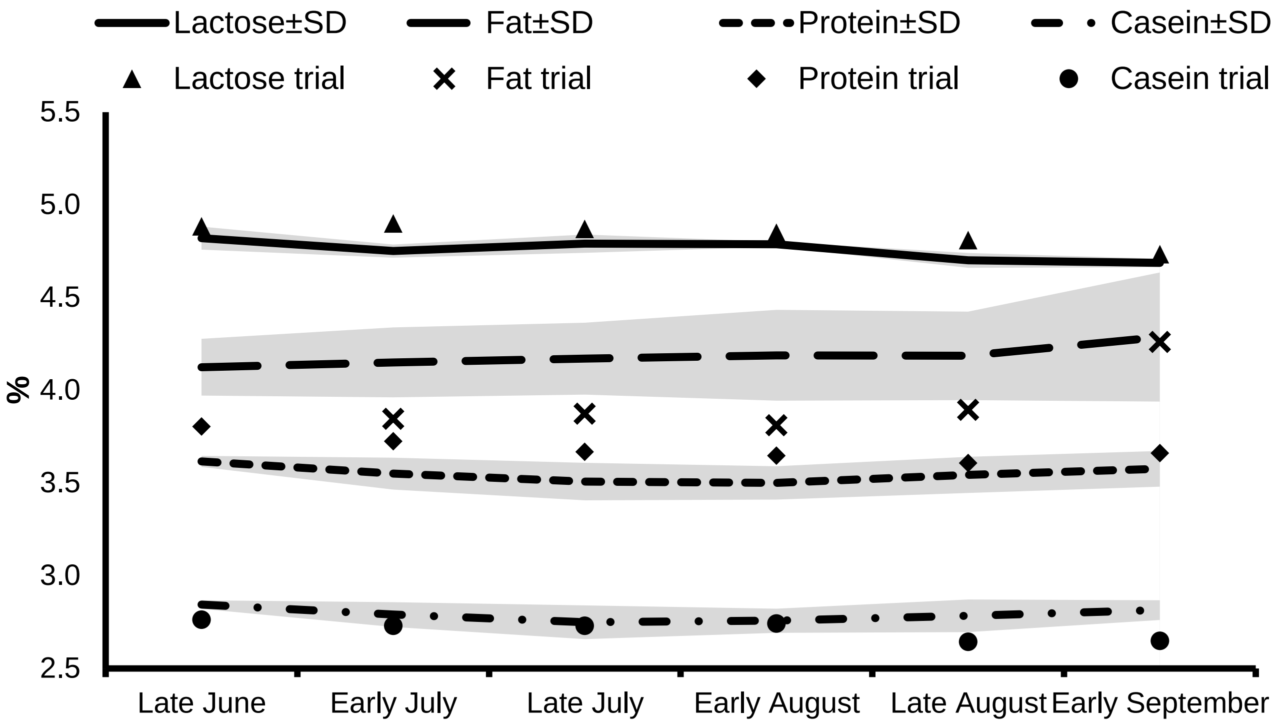
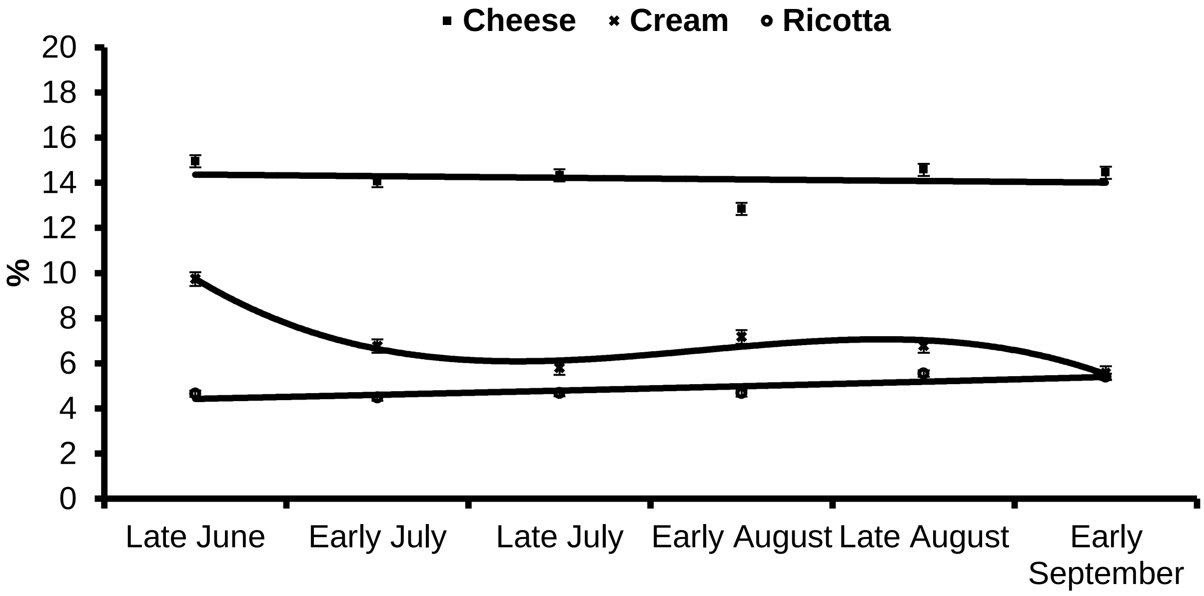


Figure 4. Evolution (LSM±SE) of yields [**a**], energy and total solids recoveries in cheese (REC), cream (CRE), and ricotta (RIC) [**b**] during summer pasture.

[a]



[b]

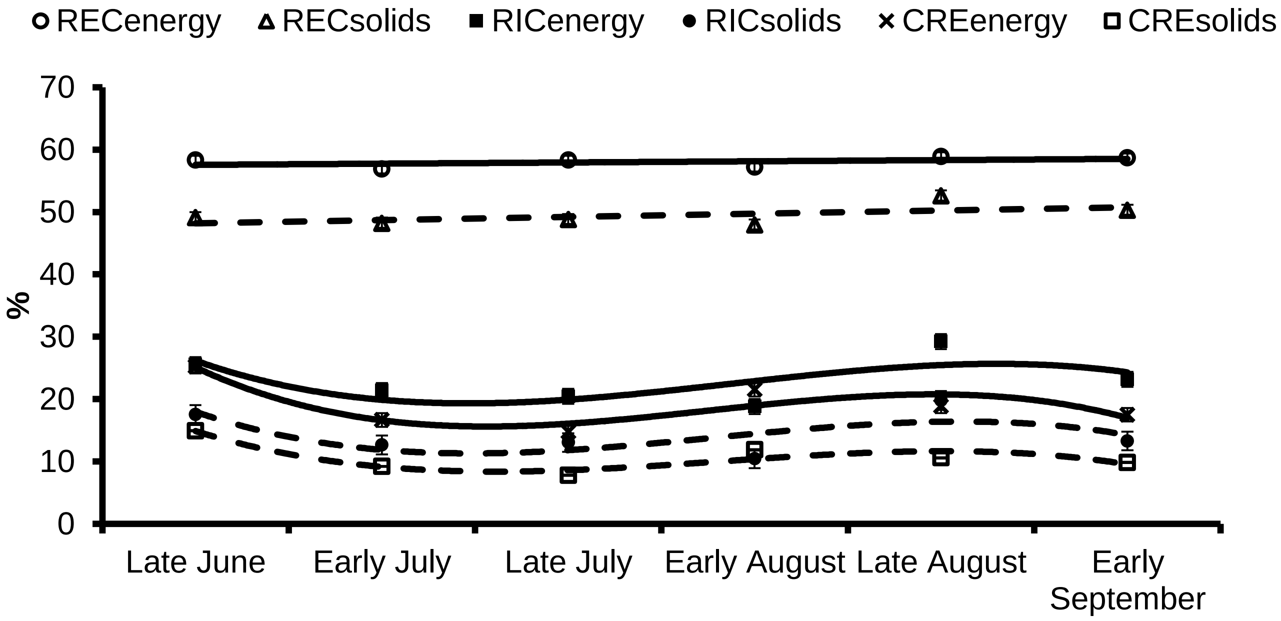


Figure 5. Flow of average weight [**a**], fat [**b**], protein [**c**] and total solids [**d**] of different dairy products and by-products obtained from creaming, cheese- and ricotta-making.

[a]

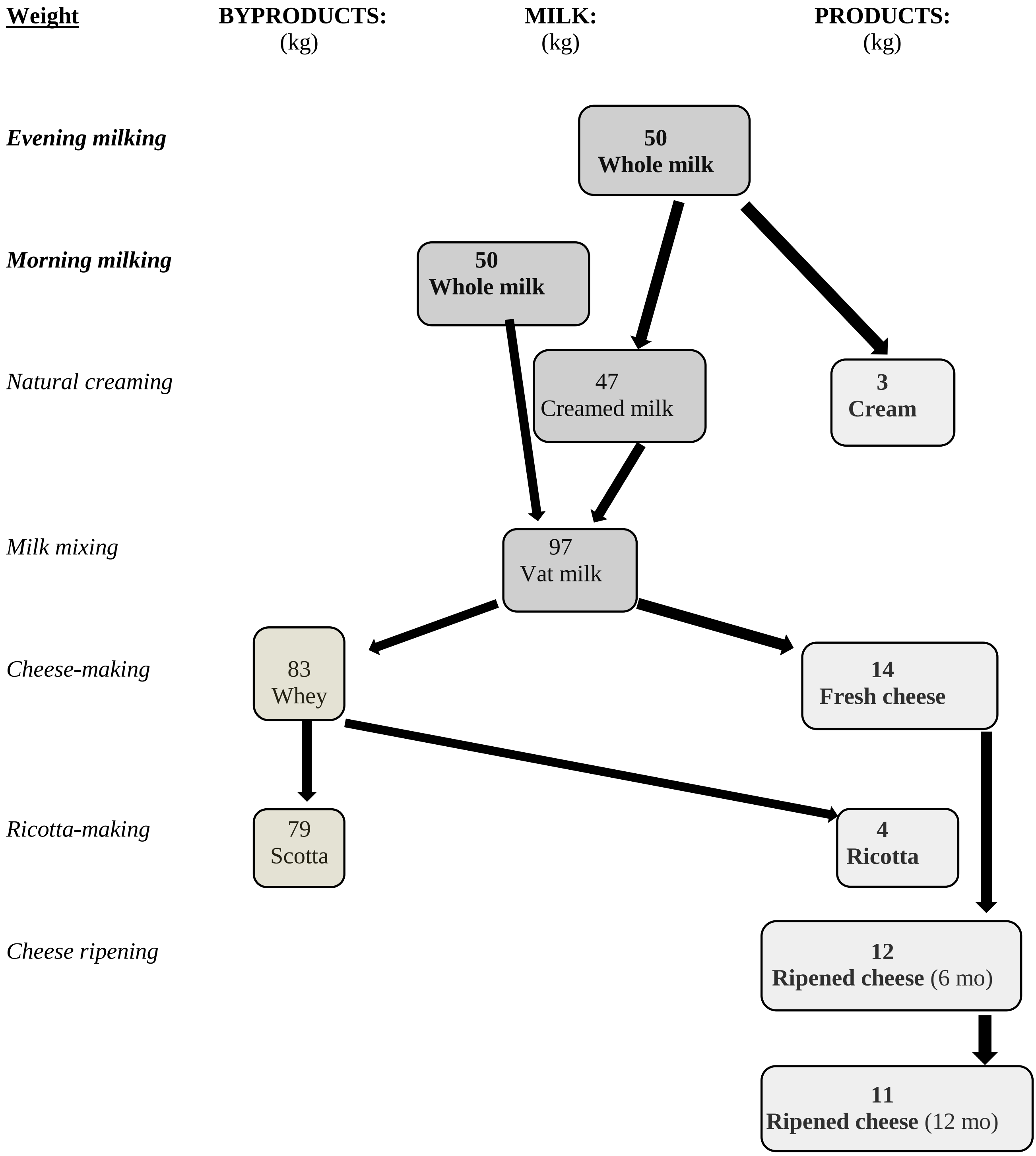


Figure 5 [b].

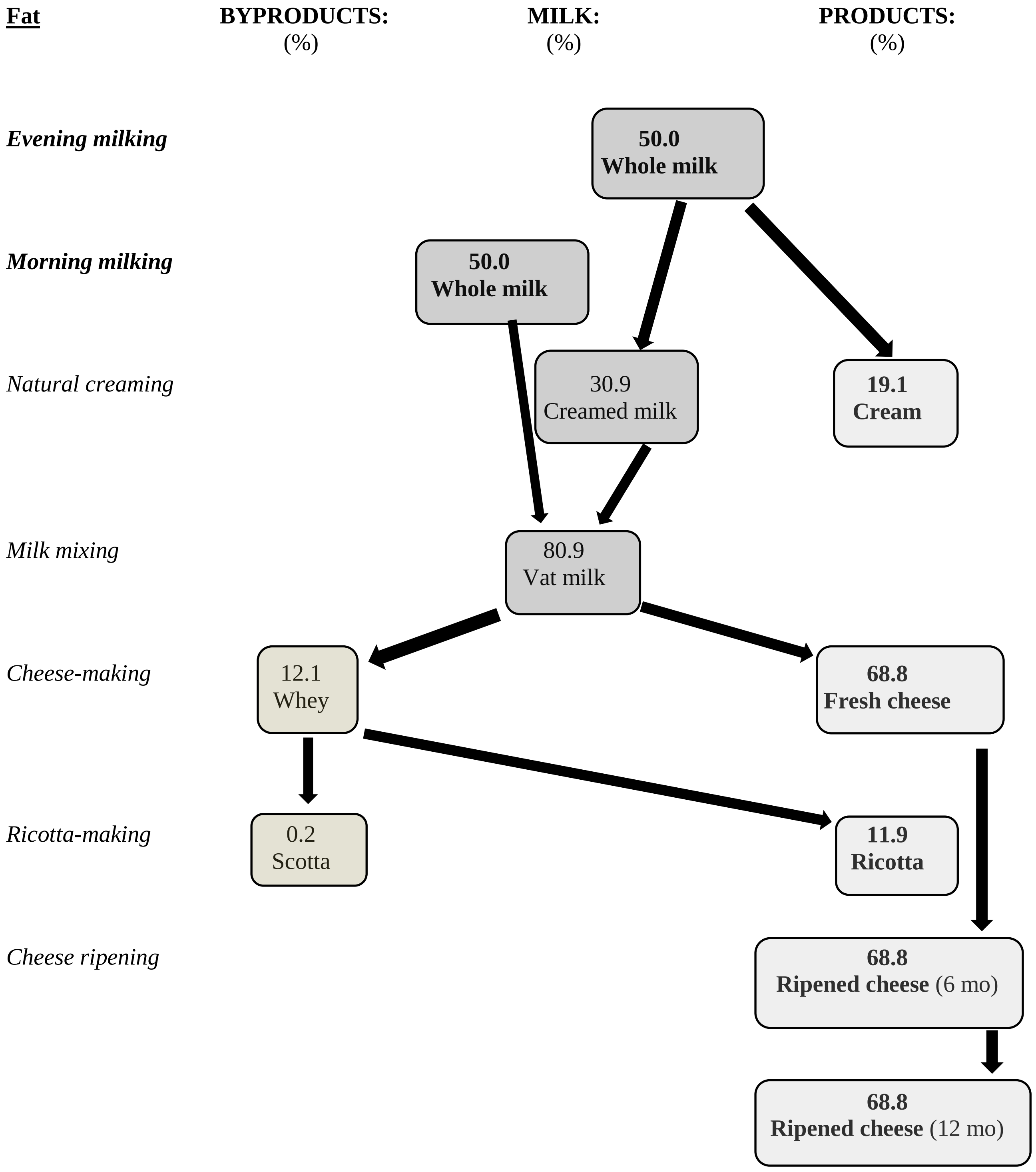

Fat
BYPRODUCTS: (%)
MILK: (%)
PRODUCTS: (%)
Evening milking
50.0 Whole milk
Morning milking
50.0 Whole milk
Natural creaming
30.9 Creamed milk
19.1 Cream
Milk mixing
80.9 Vat milk
Cheese-making
12.1 Whey
68.8 Fresh cheese
Ricotta-making
0.2 Scotta
11.9 Ricotta
Cheese ripening
68.8 Ripened cheese (6 mo)
68.8 Ripened cheese (12 mo)

Figure 5 [c].

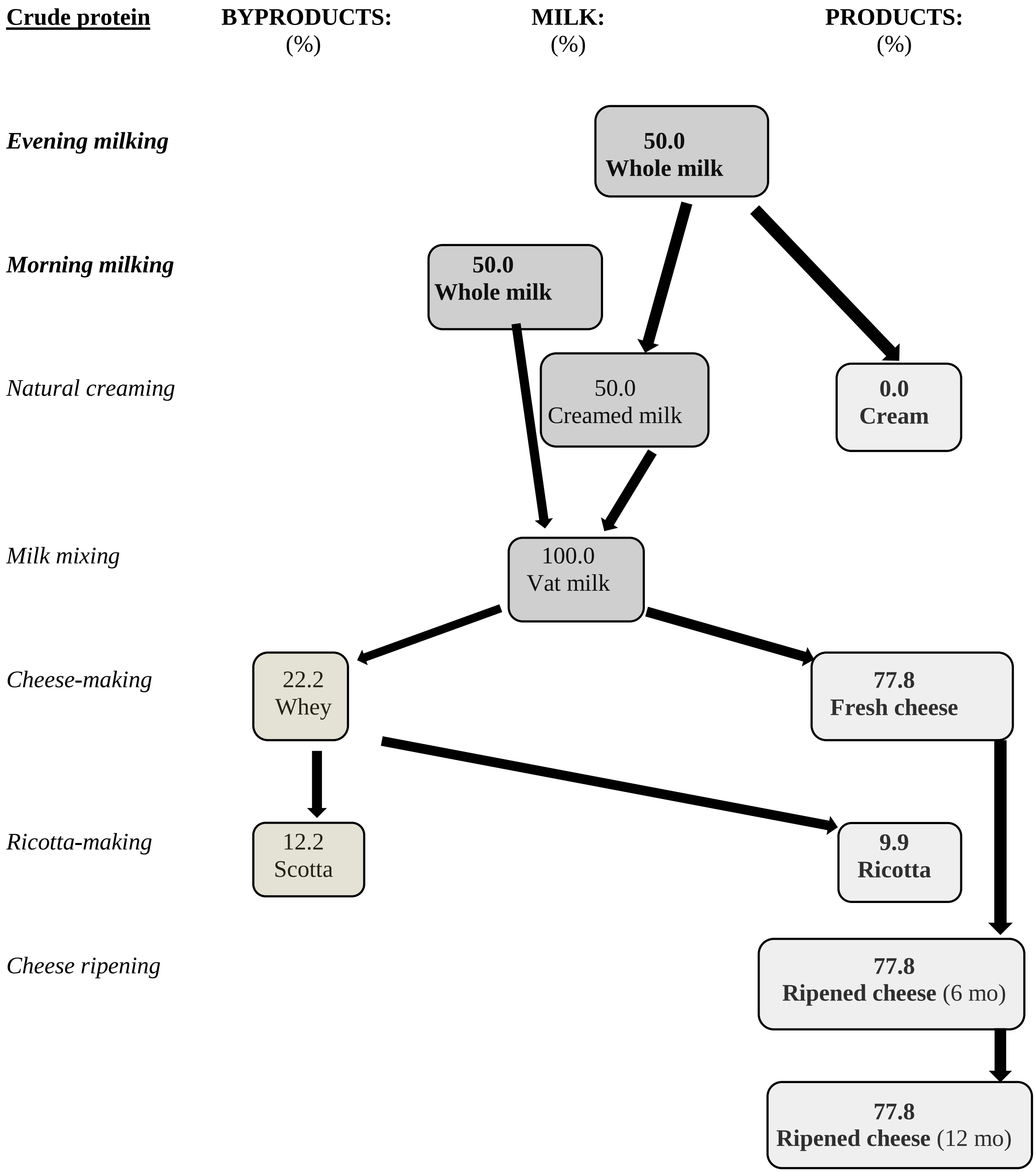
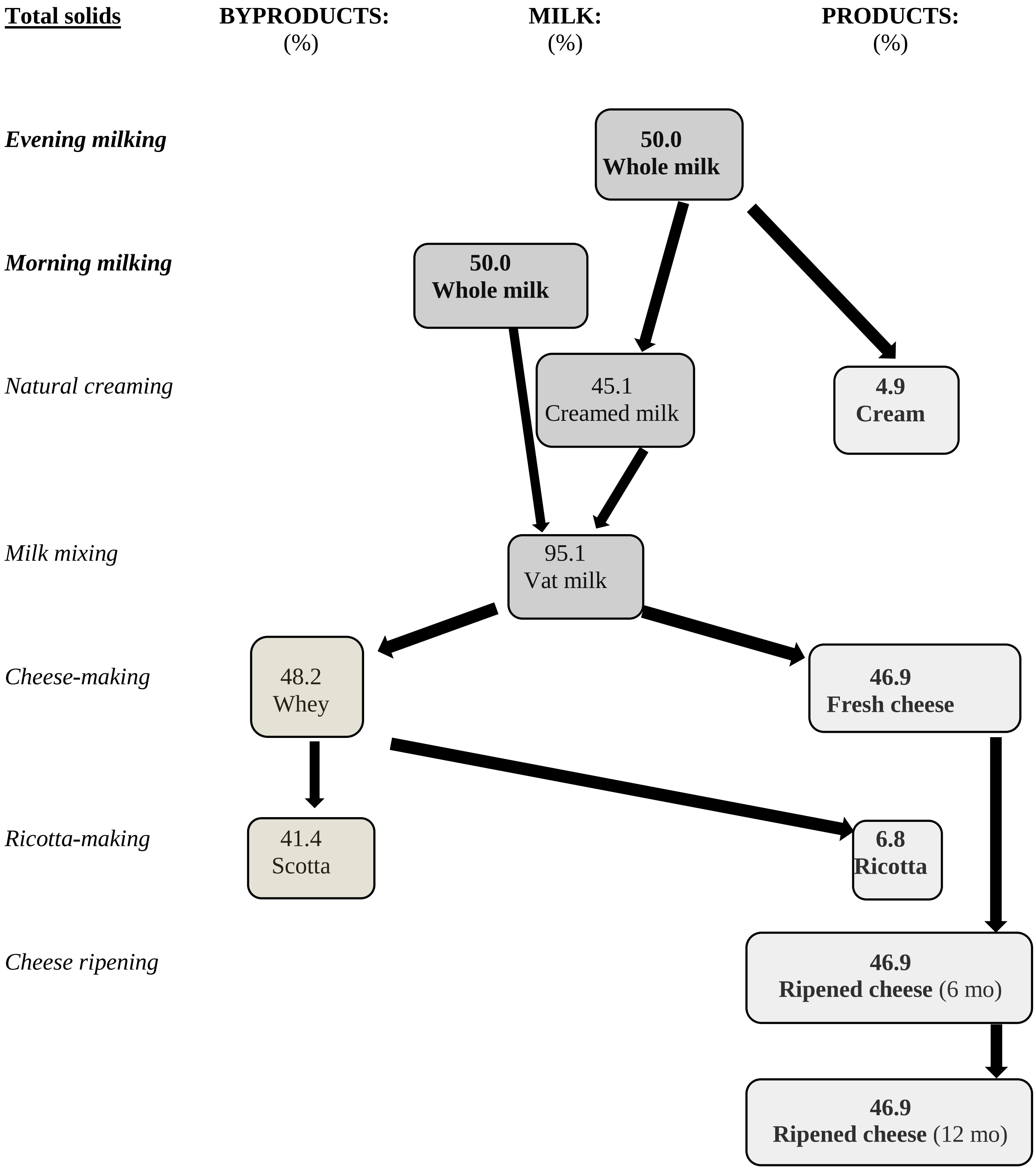

Crude protein
BYPRODUCTS:
(%)
MILK:
(%)
PRODUCTS:
(%)
Evening milking
Morning milking
Natural creaming
Milk mixing
Cheese-making
Ricotta-making
Cheese ripening
50.0
Whole milk
50.0
Whole milk
50.0
Creamed milk
0.0
Cream
100.0
Vat milk
22.2
Whey
77.8
Fresh cheese
12.2
Scotta
9.9
Ricotta
77.8
Ripened cheese (6 mo)
77.8
Ripened cheese (12 mo)

Figure 5 [d].


Total solids
BYPRODUCTS: (%)
MILK: (%)
PRODUCTS: (%)
Evening milking
Morning milking
Natural creaming
Milk mixing
Cheese-making
Ricotta-making
Cheese ripening
50.0 Whole milk
50.0 Whole milk
45.1 Creamed milk
4.9 Cream
95.1 Vat milk
48.2 Whey
46.9 Fresh cheese
41.4 Scotta
6.8 Ricotta
46.9 Ripened cheese (6 mo)
46.9 Ripened cheese (12 mo)

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FIFTH CHAPTER

From cow to cheese: Flavor fingerprint of milk, cream, curd, whey, ricotta, scotta, and ripened cheese obtained during summer Alpine pasture

M. Bergamaschi[*,†], F. Gasperi[†], and G. Bittante[*]

[*]Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE), University of Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy

[†]Department of Food Quality and Nutrition, Research and Innovation Centre, Fondazione Edmund Mach (FEM) Via E. Mach 1, 38010 San Michele all'Adige (TN), Italy

ABSTRACT

The objectives of this study were the characterization of volatile organic compounds and the evaluation of their fate in different dairy products derived from cheese-making and ricotta-making using milk of cows reared in a highland area. For this purpose, a group of 148 cows were grazed on pasture from June to September day and night. Samples were collected from experimental cheese-making performed every 2 wk through pasture season. Volatile organic compounds content in milk, cream, whey, ricotta, scotta, fresh and ripened cheeses for 6 and 12 months were determined by a solid phase micro extraction/gas chromatography-mass spectrometric. Forty-nine molecules were identified and belonging to the following chemical families: alcohols (13), aldehydes (9), esters (8), free fatty acids (6), ketones (5), lactones (2), sulphurs (2), terpenes (2), phenol (1) and benzene (1). We investigated the evolution of volatile compounds and their chemical family across the cheese-making and ricotta-making processing and during cheese ripening. The results showed that the concentration of volatile compounds differ significantly among dairy products. The comparison between volatile compounds of 4 types of milk (whole evening, creamed milk, whole morning, milk in vat) showed that the creaming process affect about half of all the volatile organic compounds analyzed, followed by the effects of milking (evening milking vs. morning milking) and the mixing (creamed milk mixed with whole morning milk). Generally, the cream, in contrast to fresh cheese and ricotta, shown higher content of free fatty acids, sulphurs and terpenes compounds, while ricotta shown higher volatile compounds concentration than fresh cheese, probably due to the high temperature during processing. The effect of the progressive nutrient depletion of milk was investigated by contrasts between volatile compounds concentration of milk in the vat, of whey, and of scotta. Although the greater amount of nutrients in milk; whey and scotta have shown a higher concentration of VOCs with the exceptions of esters, sulphurs, terpenes and phenolic compounds. Finally, the effect of ripening was tested by comparison between the quantity of VOCs of fresh cheese and of aged cheeses (6 and 12 months). The release of volatile compounds increased dramatically with increasing ripening period in relation with the enzymatic and microbiological activity of cheese. The evolution of volatile compounds depends by specific technological aspects such as natural creaming, temperature of coagulation, and ripening period. We conclude that the monitoring of volatile molecules permits to obtain dairy products with appropriate organoleptic characteristics useful to improve of cheese production chain.

Keywords: pasture, milk, cheese, SPME/GC-MS, flavor.

INTRODUCTION

The sensory quality of food is useful in the market for discriminating different products as well as for explaining consumer preferences (Drake et al., 2008). The Volatile Organic Compounds (**VOCs**) are descriptors of the sensory quality of the food related to the flavor. Different mass spectrometric techniques are used for the detection of VOCs. The mass spectrometric (**GC-MS**) permits the qualitative and quantitative determination of molecules responsible of flavor (Tunick et al., 2014).

Several works have been published on the effect of animal diets, type of grass and botanical composition of pastures on VOCs and sensory proprieties of milk and cheese (Agabriel et al., 2004; Carpino et al., 2004; Bovolenta et al., 2014). Grazing of cows in pastures provides milk with a series of odour-active compounds that could be responsible for the specific odor and aroma of pasture-milk (Carpino et al., 2004). Differences in the sensory traits of cheeses obtained using milk either from animals grazing on highland or lowland pastures have been reported (Martin et al., 2005; Coppa et al., 2011). Therefore, the type of a dairy system is strongly related to the final dairy product, that is milk and thereby cheese. Moreover, it has been shown that cheese quality is also likely to be influenced by milk pasteurization (Martin et al., 2004). Thus, recent studies have been focused in quantifying the effect of different dairy systems, individual animal characteristics, farm altitude and quantity of concentrate in the diet, on VOCs of the cheese (Bovolenta et al., 2014; Bergamaschi et al., 2015a)..

Concerning the volatile profile of cream and ricotta, as well as of by-products of the cheese-making process (like whey and scotta), the literature is limited. Moreover, no study thus far was focused on the flux and changes of individual VOCs during the cheese-making process.

The aim of this work was an integrated research on the volatile fingerprint of the cheese, following the whole process from milk till the final product, i.e. the (ripened) cheese and its fresh products and by-products. More precisely, the objectives of our study were i) to investigate the effect on milk VOCs of creaming (whole milk vs. creamed milk), milking (evening milking vs. morning milking) and mixing in vat; ii) to characterize the volatile fingerprint of the fresh products obtained from cheese-making process (cream, curd, ricotta); iii) to study the VOCs of the fresh products and by-products during the cheese-making process (milk in vat, whey and scotta); iv) to detect the volatile fingerprint during cheese ripening; v) to estimate the flux of VOCs during cheese-making process (from milk to ripened cheese).

MATERIALS AND METHODS

Animals and Milk

The study was carried out from June to September in an Alpine temporary summer farm (Malga Juribello, Paneveggio - Pale di San Martino Natural Park, Trento, Italy 1,860 m a.s.l.) equipped for the processing of milk obtained from the Alpine pasture to produce the traditional “malga” cheese. A total of 148 cows belonging to different genetic types were grazing on highland pasture day and night. As discussed in detail by (Bergamaschi et al., 2016 *Submitted*), the production traits of the cows were milk yield: 23.6±5.7 kg×d^{-1}, DIM: 233±90 d in milk; parity: 2.4±1.7. The cows were milked twice daily. The feeding strategy was pasture-based supplemented with a compound feed accordingly to milk production. Compound feed included mixture of corn, wheat barn, soybean meal and molasses of sugarcane given twice daily during the milking.

Cheese-making and Ricotta-making manufactured

A total of 7 cheese-making sessions were performed using the bulk milk collected every 2 weeks during the summer pasture. The cheeses and ricotta were manufactured at Malga Juribello according to procedure descripted in detail by (Bergamaschi et al., 2016 *Submitted*). Briefly, the raw whole milk from the evening milking was collected in an open flat tank to permit the natural creaming overnight. The following morning the cream was separated from milk, and the creamed milk was transferred into a vat and mixed with the freshly collected morning whole milk. A sample of whole evening milk, of cream, of creamed evening milk, of whole morning milk and of mixed milk in the cheese vat was collected during preliminary operations of cheese-making.

In the vat, milk was heated (27°C), inoculated with natural starter, and rennetted (51.5 **IMCU** × L^{-1} of milk). The resulting curd was cut, turned to facilitate draining, and cooked (at 44-45°C). After that, the curd was put in moulds (30 cm diameter × 12 cm height) pressed, and salted. Moreover, the fresh cheeses were ripened for 6 and 12 mo in a ripening cellar until analysis. The fresh cheese, and the cheeses ripened for 6 and 12 mo were sampled for analyses.

After curd removing, the whey was used to produce ricotta. Briefly, the whey was transferred into a smaller vat and heated to 90 °C, and then it was added with vinegar to catalyze the coagulation. The resulting ricotta cheese was separated, weighted, and placed into mould (20 cm diameter × 15 cm height) to allow the draining and cooling. The whey, ricotta and resulting residual "scotta" were also sampled for analyses.

In total, 11 dairy products 4 types of milk, cream, curd (fresh cheese), whey, ricotta, scotta and 6 mo and 12 mo ripened cheeses) were sampled during each of the 7 cheese-making sessions realized during summer every two weeks.

Analysis of Quality Traits

Analyses of chemical traits reported in Table 1 were performed by Department of Agronomy, Food, Natural Resources, Animals and Environment (DAFNAE) of the University of Padova (Legnaro, Padova, Italy). Fat, protein and lactose percentages were assessed using a MilkoScan FT2 apparatus (Foss Electric A/S, Hillerød, Denmark), while the chemical composition of ricotta and cheeses were measured using a FoodScan (Foss, Hillerød, Denmark). Regarding the cheeses the analyses were assessed at the end of ripening period (6 and 12 mo) on a sample per cheese with the ring removed. In addition, a slice of cheese (about 18 × 8 × 4 cm) taken from the center of cheese was vacuum-packed and conserved at -20 °C until VOCs analysis.

Analysis of Volatile Organic Compounds

Volatile Organic Compounds were analyzed by solid-phase micro extraction (SPME) and GC-MS technique at Fondazione Edmund Mach (San Michele all'Adige, Trento, Italy). For the extraction of VOCs the method used was a modified version of procedure described by Bovolenta et al. (2014). Briefly, 3 g of each of the 11 products (evening whole milk, creamed milk, morning whole milk, milk in vat, cream, fresh cheese, ricotta, whey, scotta, grated 6 mo and 12 mo ripened cheese) sampled at each cheese-making session were placed in glass vials (20 mL, Supelco, Bellefonte, PA) adding 2 g of sodium chloride (Aldrich, Milan, Italy), 4 mL of bi-distilled water, 0.050 mL of a solution of one internal standard with purity not lower than 99% (4-methyl-pentan-2-one = 0.0049 mg/mL, Aldrich), and a magnetic stir bar before capping with polytetrafluoroethylene/silicone septa (Supelco). Each sample was measured in triplicate. Volatile compounds were concentrated on the SPME fiber (50/30 µm divinylbenzene/carboxen/polydimethylsiloxane, DBV/CAR/PDMS, Supelco). After extraction samples were desorbed in the injector port of GC which was at 250°C interfaced with a mass detector which operates in electron ionization mode (internal ionization source; 70 eV) with a scan range from 30 to 300 *m/z* (GC Clarus 500, PerkinElmer, Norwalk, CT). Samples were injected using an auto sampler (CTC combiPAL, CTC Analysis AG, Zwingen, Switzerland). Then, the separation was achieved on a HP-Innowax fused-silica capillary column (30 m, 0.32 mm internal diameter, 0.5 µm film thickness; Agilent Technologies, Palo Alto, CA). The oven temperature was programmed at 40°C for 3 min, 40 to 180°C at 4°C/min, 180°C for 6 min, and 180 to 220°C at 5°C/min. Carrier gas was helium with a constant column flow rate of 2 mL/min. The compounds were identified by the comparison of their mass spectra with the standard Wiley and NIST MS library (McLafferty, 2006). Results were expressed as µg/kg equivalent to the internal standard. Additionally, two replicates of one cheese of the trial were analyzed per each of the 19 days of analysis to evaluate the instrumental repeatability. The averaged variations of the alcohols, esters, ketones, acids and aldehydes families were 18.1%, 23.1%, 18.4%, 33.0%, and 15.2%, respectively.

These results were in agreement with the literature for SPME analysis (Endrizzi et al., 2012; Bergamaschi et al., 2015a).

Statistical Analysis

The concentration in µg/kg of each VOC plus 1.0 was expressed as a natural logarithm to obtain a Gaussian-like data distribution before any data treatment. After transformation the data of VOCs in the 11 dairy products analyzed per each cheese-making session were processed by ANOVA using the PROC MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) following the statistical model:

$$Y_{ijk} = \mu + \text{dairy product}_i + \text{date}_j + e_{ijk}$$

where y_{ijk} is the VOCs content, their chemical family and their sum; µ is the overall mean; dairy product_i is the fixed effect of the *i*th dairy product (i = 1 to 11); date_j is the random effect of the *j*th cheese-making session (k = 1 to 7); e_{ijk} is the residual random error term ~N (0, σ^2). In addition, orthogonal contrasts were used to evaluate the effect of milk treatments (milking, creaming, mixing), the differences among the fresh products, the nutrient depletion of milk, and ripening period of cheese.

RESULTS AND DISCUSSION

Volatile Organic Compounds

A total of 49 molecules were identified by (SPME) and GC-MS on at least one of the dairy products analyzed. These compounds belonging to the following chemical classes: alcohols (13), aldehydes (9), esters (8), free fatty acids (6), ketones (5), lactones (2), sulphurs (2), terpenes (2), phenol (1) and benzene (1). The variability of volatile compounds among 11 dairy products was in most cases statistically different.

The milk samples contained a much smaller content of VOCs than other dairy product and also the number of identified compounds was lower: 8 of the 13 alcohols, 8 of the 9 aldehydes, 6 of the 8 esters, 5 of the 6 FFA, 4 of the 5 ketones, and all the “other” 8 VOCs. The majority of the compounds identified were found also by Marsili (1999) and Tunick et al. (2015), while some others (e.g., acetone, 2-undecanone) found by Contarini and Povolo (2002) were not found in our study. Moreover, milk flavor was affected by milking, creaming and mixing before cheese-making.

The differences between Morning and Evening Milk on VOCs

As shown in Table 2 the morning evening milking yielded milk with a similar overall content of volatile compound and also a similar content of different chemical classes of compounds, with the only exception of the smaller contents of ketones in the morning milk than in the evening one.

Moving to individual VOCs, more than half (21 out of 34 VOCs) were affected by milking, even if only a dozen showed relevant differences. Among these, it worth noting the greater content of the compounds with 8 carbon chains (octan-1-ol, octanal, ethyl octanoate, octanoic acid) and of methylsulfonylmethane in the morning whole milk, and of 2-phenoxyethanol, 2-methylpropanal, butan-2-one, methylthiomethane, 6-propyloxan-2-one, and of both limonene and α-pinene in the evening whole milk.

The effect of milking was studied rarely. Martin et al. (2009) observed that milking influence milk composition and quality aspects, including the volatile profile of the final product. No research, the authors are aware of, studied directly the differences between morning and evening milk in relation to its flavor profile.

In the interpretation of these differences it should be taken into account that the milking were done at 12 hours of interval (4.00 a.m. and 4.00 p.m.), that the cows were outside and free to graze day and night and that the compound feed was given to individual cows during milking, half in the morning and half in the evening. Thus, these differences may not be explained by different diurnal and nocturnal milking intervals, that was found affecting lipoprotein lipase activity (Gobbetti et al., 1996; Chilliard et al., 2003).

The differences in VOCs profile, beyond the difference in environmental light and temperature, could be related to circadian rhythms of the cows and to different pattern in ingested forage quantity and quality and rumen fermentation and nutrient absorption.

The effect of Creaming on Milk VOCs

As shown in Table 2, the flavor of milk was characterized by 34 VOCs belonging to alcohols (7), aldehydes (7) esters (6), FFA (4), ketones (3) and other compounds (7). The most abundant compounds identified were ethyl butanoate (69.0 µg/kg) and ethyl hexanoate (14.1 µg/kg).

Overall, the evening milk after natural overnight creaming presented a smaller content of volatile compounds than soon after milking before creaming (Table 2). This pattern was not observed for all the chemical classes of VOCs. In fact, the decrease regarded the quantitatively most important classes of esters and "other" compounds, while alcohols and aldehydes increased, and FFA and ketones were not affected as a whole.

Also within chemical classes the pattern was different among individual VOCs: and 26 on 34 VOCs were influenced by creaming process. Among the 8 alcohols, after creaming, milk showed a relevant increase of the content of 3-methyl-2-buten-1-ol, pentan-1-ol, hexan-1-ol, and 1-octen-3-ol), but a strong decrease of the heavier 2-phenoxyethanol and of octan-1-ol.

Among the 8 aldehydes: the oct-2-enal was identified and quantified only after creaming; hexanal, heptanal, octanal increased strongly; while 2-methylpropanal and decanal decreased. Among the 5 FFA, hexanoic and octanoic acids decreased and nonanoic acid more than doubled after natural overnight creaming; of the 4 identified ketones butan-2-one decreased and heptan-3-

one and nonan-2-one increased. All the other VOCs decreased after creaming with the only exception of dihydrofuran-2(3H)-one and of 3-methylphenol.

The factors explaining the differences between creamed and whole evening milk could be related: to the time (overnight) spent for natural creaming and related metabolic activities in milk (Gatti et al., 2014); and to the concentration and separation in cream of fat, somatic cells and microorganisms present in the milk (Imhof, R., and J. O. Bosset. 1994; Fedele et al., 2005). Previous researches have shown that also the sensory proprieties of milk are depended by the milk fat content (Phillips et al., 1995; Frøst et al., 2001).

The effect of Mixing Creamed and Whole Milk in the Vat on VOCs content

In our study, overall VOC content of milk mixture obtained in the vat from evening creamed milk and whole morning milk was not different from intermediate expected value. This was true for all the chemical classes of VOCs, with the only exceptions of alcohols (Table 2). Among individual VOCs, 14 out of 34 compounds showed vat values different from expected. Taking into consideration that the average of the concentration of two linear values do not correspond to the average of their log transformation, it should be noted that the vat milk showed a relevant increase of the content of pentan-1-ol, hexan-1-ol, and of ethyl acetate, and a decrease of 2-ethylhexanol, 2-phenoxyethanol, and of nonanoic acid.

These variations were never studied before and could be due: to the time interval from the sampling of the two milks (in creaming tank and milk parlor tank) and of their mix in the vat; to the stirring and partial oxygenation; and to the increase of the temperature (Barrefors et al., 1995; Timmons et al., 2001).

Seasonal Variation of VOCs content of Milk during Summer Alpine Pasture

The overall quantity of VOCs showed a small variability from a cheese-making date to the other (2.2% of total variance). The sums of the chemical classes of VOCs were characterized by a low to moderate variability (the maximum was showed by ketones with 38.8%). The pattern of individual VOCs was very different from each other's going from almost null values up to 92.2% of limonene (Table 2). The analysis of seasonal patterns is beyond the scope of the present study, but the effect of season on terpenes profile was reported previously by other authors, which observed different herbage intake, digestive and metabolic process of animals (Fedele et al., 2005). Other authors noted a transfer of several terpenes from feed to milk after different period of grazing (Tornambé et al., 2006; Viallon et al., 2000). In addition, these differences might be explained by different botanical composition of pasture across summer season (Martin et al., 2005; De Noni and Battelli, 2008). The variation of quantity and quality of forage available for the cows on the pastures were discussed in the previous study on the same summer farm and animals (Bergamaschi et al., 2016 *Submitted*).

VOC profile of Fresh Products (cream, cheese and ricotta)

The overall concentrations of VOCs were much greater in fresh products than in milk (Table 3), and also very different among them: on a linear scale concentration was 78 µg/kg for vat milk, 854 µg/kg for cream, 572 µg/kg for fresh cheese and 2,724 µg/kg for ricotta, despite the fact that this is more a byproduct obtained from the whey after collecting the cream and after cheese-making process. Esters and FFA were the two chemical classes more represented in fresh dairy products. From the qualitative point of view the VOC profile of fresh dairy products is very similar to those of milks: the same 8 alcohols were present in both categories of dairy products; in the case of aldehydes 3-methylbutanal is found in cream, but not in milk, fresh cheese and ricotta; ethyl lactate appears only in ricotta samples; acetic acid in cream and ricotta samples; and pentan-2-one only in ricotta samples.

Quantitatively the concentration of VOCs of the different chemical classes in cream is roughly intermediate respect to the other two dairy products, with the relevant exception of all volatile FFA, acetic acid excluded, much more present in the flavor of cream (Table 3). In the case of aldehydes, in cream a good concentration of 3-methylbutanal was found (not present in the other two dairy products) while oct-2-enal was not found (present in the other products). All the other VOCs were found in greater concentration in cream than in fresh cheese and ricotta, with the exception of dihydrofuran-2(3H)-one.

The VOC profile of ricotta is very different from fresh cheese (Table 3). Some alcohols (particularly 3-methylbutan-1-ol, hexan-1-ol, and 1-octen-3-ol) and their sum were much greater in ricotta; but 2-ethylhexanol was less represented in ricotta. On the contrary, the content of volatile aldehydes expressed in µg/kg is about 150 times greater in ricotta than in fresh cheese. All individual aldehydes presented much greater concentration in ricotta, and particularly hexanal, heptanal, octanal, and nonanal. These VOCs may be originated from β-oxidation of unsaturated fatty acids or degradations of the amino acid (McSweeney and Sousa, 2000; Marilley and Casey, 2004) and have been associated with lemon and green notes in dairy products (Cornu et al., 2009).

The opposite phenomenon was recorded for esters, which were much lower in ricotta than in the fresh cheese. This was because of the almost disappearing of ethyl butanoate, ethyl hexanoate, ethyl octanoate, and ethyl decanoate, not compensated by the relevant increase of ethyl acetate (Table 3). The volatile FFA of ricotta was much less concentrated than the corresponding compounds of cream, but not much different respect to those of fresh cheese. All volatile ketones were much more concentrated in the air around ricotta samples than around the fresh cheese. In the case of other VOCs differences in concentration were not great, with the exception of methylsulfonylmethane, much more concentrated in ricotta, and of dihydrofuran-2(3H)-one in fresh cheese.

A possible interpretation of the differences among the three fresh products, it should be considered the order of the three steps of processing and the conditions. The cream is separated from the whole evening milk after a natural creaming with mild change in acidity and temperature. The fresh cheese is obtained in the second step after moderate acidification and heating and an enzymatic treatment. Lastly, ricotta is obtained after intense acidification and, particularly, heating of the whey.

The effect of Nutrients Depletion from Milk in Vat to Scotta on VOCs profile of Processed Fluids

The progressive depletion of milk nutritional components, especially fat and protein, during cheese (whey) and ricotta (scotta) production affects VOC belonging to different chemical classes.

Despite curd and ricotta up taking, the overall concentration of VOCs increased moving from milk in vat, to whey, and especially to scotta (Table 4). This last byproduct presented a VOCs concentration, on a linear scale, more than double (+136%) respect to the previous byproduct (whey). From the qualitative point of view, the volatile profile of whey is characterized by the same VOCs of milk, while scotta presented two esters (ethyl lactate and butyl butanoate) in very low concentration, not present in milk and whey.

The three fluid products/byproducts were characterized by very different profile of VOCs. The alcohols were more concentrated in both byproducts, particularly in the case of 3-methylbutan-1-ol, of 2-ethylhexanol, and of octan-1-ol (especially in scotta). In the meantime, pentan-1-ol, hexan-1-ol, and 1-octen-3-ol were much lower in scotta than in whey. Regarding volatile aldehydes, the concentrations were generally increasing moving from milk to whey and to scotta. In the case of the major part of esters the pattern is opposite (decreasing from milk to whey and to scotta) with some exceptions (ethyl acetate and ethyl decanoate, much higher in scotta). The pattern of volatile FFA and ketones moving from a fluid to the other along the processing chain is similar to what observed for aldehydes, with an increase from milk to whey and to scotta. Lastly, in the case of other VOCs, the opposite, decreasing, pattern was observed (like for majority of esters), arriving to the disappearance (or at least to values below detection limit) of limonene in both dairy byproducts.

Also for the comparison of VOCs among the dairy fluids along the processing chain, no results are present in the scientific literature, the authors are aware of. Esters, especially with few atoms of carbon, are considered important aroma compounds in milk and cheese with their fruity notes (Cornu et al., 2009; Villeneuve et al., 2013). Higher concentration of these compounds in milk could be associated to positive relationship between the aroma compounds and the proteins (serum albumin, casein) which have been previously investigated (Fares et al., 1998). Our finding could be also explained by the different ability of volatile compounds to diffuse through the food matrix. In fact, their molecular weight and molecular size are primarily factor determining diffusion (Goubet et al., 1998).

Flavor variability and quality in whey has been also evaluated (Mahajan et al., 2004; Gallardo-Escamilla et al., 2005). These authors reported different flavors in whey made from different types of cheeses and also flavor variability within the same cheese type.

Free fatty acids with six or more atoms of carbons can be formed through the action of native milk or bacterial lipases on triglycerides (Chilliard et al., 2003; Mahajan et al., 2004). Moreover, short-chain FFA have been identified in many foods including liquid whey (Whetstine et al., 2003; Mahajan et al., 2004). In according to Gallardo-Escamilla et al. (2005) starter culture used during production process of the dairy products can vary flavor and related compounds. These authors suggested an important effect of several VOC including acetic acid to predict fruity odor in dairy products. The butan-2-one was described as responsible of sheep fat and chocolate notes in dairy flavor (Cornu et al., 2009), while nonan-2-one has been reported to be important flavor compounds in fermented milk (Gallardo-Escamilla et al., 2005) and cheese (Thomsen et al., 2012). Accumulation of some volatile compounds in dairy products with a low pH (higher acidity) such as scotta in this trial was reported by Imhof and Bosset, (1994).

The effect of Ripening on Cheese VOCs

Ripening of Malga cheese caused a tremendous change of flavor profile, with an increase of overall linear VOCs concentration equal to 22 and 31 times the concentration of fresh cheese in 6 months and 12 months ripening, respectively (Table 5). Also from the qualitative point of view, the ripening caused an increase of the number of individual VOCs identified in cheese. Respect to samples from fresh cheese, samples from ripened cheese presented (Table 5): 5 more alcohols with high concentration (butan-1-ol, butan-2-ol, pentan-2-ol, hexan-2-ol, and heptan-2-ol); one aldehyde (3-methylbutanal); 2 esters (ethyl lactate and ethyl butanoate); and one FFA (acetic acid). On the other side, two VOCs identified in fresh cheese are no more detectable in ripened cheeses (nonenal and dihydrofuran-2(3H)-one).

Moreover, all the sums of VOCs of different chemical classes and 37 out of 38 individual VOCs present in both fresh and ripened cheeses were characterized by a much greater concentration in ripened cheeses. The only exception was the methylthiomethane (Table 5).

Esters and FFA were the chemical families present at the highest concentration in all the three dairy products. In particular, we found higher amount of ethyl butanoate, ethyl hexanoate, acetic acid and butanoic acid. This is in agreement with Bellesia et al. (2003) that reported ethyl butanoate and ethyl hexanoate in grana type's cheeses. Moreover, acids may be produced from lipolysis of milk fat or from oxidation by LAB (McSweeney and Sousa, 2000). Acetic acid and butanoic acid have typical vinegar and feet notes, whereas esters may contribute fruity aroma in cheese (Cornu et al., 2009).

The presence of microorganisms in curds and the activity of milk native enzymes, especially proteases and lipases, could be the major responsible of these variations (Gatti et al., 2014). Alcohols, such as 3-methyl-1-butanol, responsible for the fresh cheese note, was found in rennet-curd cheese during early ripening (Moio et al., 1993). The milk constituent (fat and protein) retained in the curd can be transform by LAB enzymes (e. g. proteinases, peptidases, esterases) in cheese flavor compounds and aroma precursors (Smit et al., 2005).

As reported above, the higher concentration of esters in cheeses may be attributed to the enzymatic action of microorganisms during ripening (Gobbetti et al., 1996). In a review, Gatti et al.

(2014) reported that more than one-third of cheese proteins are hydrolyzed by synergic activity of plasmin, cathepsins, and the long ripening times.

Several factors have been shown to affect FFA concentration in cheese during ripening, including animal diets, individual cow characteristics (Agabriel et al., 2004; Bovolenta et al., 2014; Bergamaschi et al., 2015a), and milk storage conditions (Endrizzi et al., 2012). In addition, some authors found that FFA profile of cheese fat varied according to processing temperature and aging time (Nudda et al., 2005). Volatile differences may partly derive from differences in FA profile of herbage consumed by cows and botanical composition of pastures (Martin et al., 2005; Coppa et al., 2011). Comparing cheese samples from wheels ripened for 6 vs 12 months we observed an increase with ripening of overall VOCs concentration much smaller than that observed from fresh cheese to 6 months cheese, and also a large variability among and within chemical classes of VOCs (Table 5).

The sum of concentrations of the 13 volatile alcohols identified increased from 6 to 12 mo ripening, and this was due to the increase of 5 compounds of this class (particularly of butan-1-ol, 3-methyl-2-buten-1-ol, and heptan-2-ol), while one compound decreased (1-octen-3-ol) and 7 showed minor variations. The sums of concentrations of the 9 aldehydes decreased with advanced ripening because only one of them increased (3-methylbutanal) and 5 decreased (especially hexanalheptanaloctanal, and oct-2-enal). Sum of the 8 esters was not much different in 6 and 12 month cheeses despite almost all increased, because of the strong decrease of butyl-butanoate and the stability of ethyl-lactate. The sum of FFA increased together with 4 of the 6 compound of this class, while acetic acid remained almost unchanged and only nonanoic acid decreased. Variations of the 5 ketones were not much important as only pentan-2-one and heptan-3-one increased. Lastly, all the other 8 VOCs increased with the very important exceptions of α-pinene and especially of limonene that decreased.

Concerning the monoterpenes isolated in the headspace of ripened cheese of this experiment (α-pinene and limonene), these compounds were the main terpenes found in PDO cheese produced in Italian Alpine regions (De Noni and Battelli, 2008). They are metabolites of many plants from highland pastures (Buchin et al., 1999; De Noni and Battelli, 2008) and may be used as bio-markers (Favaro et al., 2005). Seasonal variations of these molecules have been previously reported by Abilleira et al. (2010).

Quantitative Evolution of VOCs along the Cheese- and Ricotta making chain

The temptative quantification of volatile organic compounds along the cheese- and ricotta-making chain expressed for 100 kg of milk processed is summarized in Table 6. The product weights were obtained from the weighing of all the 11 products /by-products controlled and sampled during the 7 cheese- and ricotta-making experimental sessions realized from late June to early September, as described in a previous study. The quantity of VOCs was estimated multiplying the weight of the product obtained from 100kg of processed milk and the VOCs concentrations from Tables 2, 3, 4 and 5, transformed in linear scale. It is beyond the scope of this study to analyze in details the quantitative balance of each VOC during the whole processing, but the data of table 6 highlight the complex fate of odorants in milk and milk products allowing to estimate the partition of each substance in different products, the disappearance and especially the appearance of the different VOCs as a result of milk native enzymatic actions, microbiological activity, physical treatments and interactions among these factors.

Results highlighted a partition of VOCs between evening whole milk, creamed milk and cream, while other compounds remain constant during creaming process. More precisely, the 60-65% of the quantity of 2-phenoxyethanol, octan-1-ol, 2-methylpropanal, nonenal, decanal, ethyl acetate, octanoic acid, methylthiomethane, toluene, and α-pinene in the evening whole milk was transfer to creamed milk, while the 30-35 % of the concentration of these VOCs characterized the volatile profile of the cream. It is well known that fat content of dairy products has an impact on volatile compounds and consequently on the flavor release (Cadwallader and Singh, 2009). The presence of fat in the food matrix can solubilize hydrophobic compounds. Nevertheless, only a small proportion of these volatile molecules are allowed into the headspace, thus increasing the sensory threshold and influence the flavor release (Kim et al., 2011; Shepard et al., 2013). This difference was confirmed by Relkin et al. (2004) that shown a higher sensory thresholds in oil than water. This may explain why alcohols and aldehydes compounds were more prevalent in creamed milk than evening whole milk. The cream in comparison to whole milk and creamed milk have higher concentration of butanoic acid, which is a flavor-active compound (Singh et al., 2003), derived from lactose fermentation (Zhang et al., 2009). As discussed above in this paragraphs, 3-methyl-2-buten-1-ol, 3 methylbutan-1-ol, heptan-2-one, nonan-2-one, dihydrofuran-2(3H)-one seems to be quite constant over the creaming process especially between evening whole milk and creamed milk (Table 6).

We found a quantitative transfer of VOCs of milk in vat to curd (fresh cheese) and whey. Approximately, the 25-30% of 1-octen-3-ol, 2-phenoxyethanol, octan-1-ol, and butanoic acid was retained in the fresh cheese, while the 70-75% was lost in the whey (Table 6). Another interesting step of cheese production chain is the evolution of aroma profile during ricotta-making. In general, we found an increase of FFA, esters and ketones in scotta in comparison to whey and ricotta. Of these chemical families, the most significant increases were that of ketone butan-2-one, which found to be 29 times higher in scotta than whey. These differences of proportions, as discuss above, could be due to the heat treatment of the product before coagulation or the lactic acid fermentation (Shepard et al., 2013). In addition, ricotta shown a higher concentration of aldehydes hexanal, octanal and decanal derived from β-oxidation of unsaturated fatty acids (McSweeney and Sousa, 2000). This finding suggests a higher proportion of medium-chain fatty acids (C_6-C_8) compared with long- and short-chain FA in the whey resulted from raw milk used in this experiment (Chilliard et al., 2009).

CONCLUSIONS

For the first time, VOCs content in whole milk from the evening milking, creamed milk, whole milk from the morning milking, cream, fresh cheese, whey, ricotta, scotta, and ripened cheeses analyzed by SPME/GC-MS were qualitatively and quantitatively analyzed.

In this trial, carried out in Alpine pasture, molecules belonging to different chemical family: alcohols, aldehydes, esters, free fatty acids, ketones, lactones, sulphurs, terpenes, phenols and benzenes were identified.

The data regarding the sums, in mg, of all VOCs of the 11 controlled products/byproducts obtained from processing of 100 kg of whole milk (Figure 1), represent the relevant changes of overall quantity of odorants characterizing the different steps/operations of the cheese- and ricotta-making process. The natural creaming overnight caused a small decrease of overall VOCs quantity (the sum of cream and creamed milk is about 4% lower than the quantity of VOCs in the initial whole milk). The transfer, mixing and heating of evening creamed milk and morning whole milk in the cheese-vat caused a further decrease of overall VOCs quantity (-13%). On the contrary, at the end of cheese-making the quantity of VOCs of the whey was 8% more than the quantity in the vat milk before starter and rennet additions and the fresh cheese produced was characterized by a VOCs quantity almost twice than those in the milk vat, so that cheese-making, summing whey and cheese odorants, caused a relevant increase of overall VOCs quantity (+198%).

Moving to the ricotta-making step, it is possible to see from Figure 1 that the quantity of odorants in the residual scotta is about twice the quantity contained in whey before its heating and acidification, ant that contained in ricotta is about 1.5 times the initial quantity. Summing scotta and ricotta, the overall quantity of VOCs is strongly increased (+252%).

The third step, cheese ripening, caused a dramatic change of overall quantity of VOCs that increased 11 times (+1,019%) after 6 months and 16 times (+1,548%) after 12 months of ripening (Figure 1).

Particularly, the main volatile compounds isolated from the headspace were: ethyl butanoate and ethyl hexanoate from milk and fresh cheese; hexanoic acid, ethyl butanoate and butanoic acid from cream; hexanal, heptanal and octanal from ricotta; hexan-1-ol and hexanal from whey; 3-methylbutan-1-ol from scotta; and butan-2-ol and butanoic acid from cheeses.

The evolution of volatile compounds depends by specific technological aspects of cheese-making and ricotta-making processing such as natural creaming, type of coagulation, temperature of coagulation, and ripening period. The transfer of VOCs change in quantity and type with the cheese-process and for this reason also the corresponding flavor profile can change.

We conclude that the summer transhumance influence the quality of dairy products within the same cheese-making process. The evaluation of volatile profile permits to obtain products with optimized organoleptic characteristics useful to improve of production chain. This study established distinctive volatile compounds can be used as markers of product and process. To our knowledge, this is the first study that reported the monitoring of volatile compounds between 11 dairy products collected from the same cheese-making process. Further studies will be necessary to evaluate the evolution of volatile organic compounds during the summer transhumance.

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TABLE AND FIGURES

Table 1. Descriptive statistics of chemical composition of sampled dairy products (n=7) during summer transhumance.

Dairy products	Fat, %		Protein, %		Lactose, %	
	Mean	SD	Mean	SD	Mean	SD
Processed fluids:						
Evening whole milk	4.08	0.53	3.68	0.09	4.79	0.12
Evening creamed milk	2.53	0.29	3.72	0.08	4.94	0.12
Morning whole milk	4.31	0.7	3.68	0.08	4.86	0.1
Milk in vat (Mix)[1]	3.37	0.25	3.7	0.09	4.89	0.13
Whey	0.59	0.09	0.95	0.07	4.79	0.31
Scotta	0.01	0.01	0.56	0.03	5.05	0.23
Fresh products:						
Cream	28.1	2.97	3.22	0.22	3.43	0.13
Fresh cheese	20.4	0.69	20.2	0.93	-	-
Ricotta	8.85	1.86	8.79	1.21	-	-
Ripened cheese:						
Cheese (6 mo)	29.4	0.55	29.9	0.76	-	-
Cheese (12 mo)	30.1	0.78	32.2	0.89	-	-

[1] It is obtained mixing the evening creamed milk with the morning whole milk in equal part.

Table 2. Effect of natural creaming, milking, mixing, and incidence of cheese-making day (date) on VOC and their chemical family expressed as natural logarithm of concentration (µg×kg^{-1}).

Chemical family/VOC	Milk LSM:				Contrast (*F*-value)[1]			Date (%)[5]	RMSE[6]
	Evening Whole	Evening creamed	Morning whole	Mixture (Vat)	Creaming effect[2]	Milking effect[3]	Mixing effect[4]		
	(A)	*(B)*	*(C)*	*(D)*	*A* vs. *B*	*A* vs. *C*	*D* vs. *(B+C)*		
∑ **VOC**	**4.61**	**4.23**	**4.65**	**4.36**	**11.1****	**0.1**	**0.7**	**2.2**	**0.37**
∑ **Alcohols**	**1.85**	**2.04**	**1.89**	**2.44**	**7.0****	**0.4**	**24.2*****	**31.3**	**0.24**
3-Methyl-2-buten-1-ol	0.15	0.19	0.18	0.18	14.1^{***}	8.6^{*}	1.1	47.6	0.03
3-Methylbutan-1-ol	0.26	0.29	0.33	0.37	0.4	1.8	1.9	10.0	0.05
Pentan-1-ol	1.39	1.63	1.45	1.89	24.8^{***}	1.5	74.3^{***}	39.8	0.15
Hexan-1-ol	0.44	0.84	0.36	0.84	36.0^{***}	1.4	17.9^{***}	46.7	0.21
1-Octen-3-ol	0.10	0.30	0.14	0.25	76.9^{***}	3.5	2.1	54.7	0.07
2-Ethylhexanol	0.56	0.57	0.55	0.50	1.1	0.3	26.9^{***}	81.6	0.05
2-Phenoxyethanol	0.25	0.14	0.19	0.12	49.5^{***}	16.7^{***}	9.3^{**}	12.5	0.05
Octan-1-ol	0.18	0.14	0.23	0.16	10.3^{**}	11.8^{***}	4.4^{*}	25.8	0.04
∑ **Aldehydes**	**1.65**	**2.20**	**1.56**	**1.94**	**28.6*****	**0.8**	**0.4**	**27.3**	**0.33**
2-Methylpropanal	0.96	0.85	0.43	0.71	10.4^{**}	239.1^{***}	5.6^{*}	60.7	0.11
Benzaldehyde	0.23	0.24	0.16	0.14	0.2	5.2^{*}	4.9^{*}	1.2	0.10
Hexanal	0.70	1.48	0.77	1.03	33.5^{***}	0.3	0.8	41.3	0.38
Heptanal	0.66	1.05	0.54	0.81	18.5^{***}	1.5	0.1	57.7	0.28
Octanal	0.18	0.47	0.40	0.43	28.0^{***}	15.9^{***}	0.1	41.3	0.18
Oct-2-enal	-	0.18	0.09	0.13	-	-	0.4	60.0	0.06
Nonenal	0.31	0.40	0.23	0.23	3.5	3.7	5.7^{*}	30.1	0.14
Decanal	0.37	0.23	0.38	0.30	57.9^{***}	0.4	0.2	25.0	0.06
∑ **Esters**	**3.78**	**3.14**	**3.97**	**3.43**	**3.8**	**0.4**	**0.2**	**0.0**	**1.04**
Ethyl acetate	0.74	0.47	0.76	0.84	8.8^{**}	0.1	8.9^{**}	6.3	0.27
Ethyl butanote	4.22	3.14	4.05	3.44	92.4^{***}	2.2	2.6	32.9	0.33
Ethyl hexanoate	2.71	1.89	2.49	2.12	19.3^{***}	1.4	0.2	1.1	0.53
Ethyl octanoate	1.31	0.77	1.13	0.99	33.1^{***}	3.9	0.3	5.7	0.28
Ethyl decanoate	0.81	0.49	0.62	0.65	26.6	8.3^{**}	2.9	45.8	0.18
Ethyl dodecanoate	0.40	0.29	0.39	0.35	15.9^{***}	0.1	0.1	36.9	0.09
∑ **FFA**	**2.40**	**2.34**	**2.56**	**2.49**	**1.3**	**0.2**	**0.1**	**0.2**	**0.47**
Butanoic acid	0.79	0.64	0.73	0.73	2.0	0.3	0.3	3.9	0.31
Hexanoic acid	1.48	1.20	1.62	1.45	6.8^{*}	1.6	0.2	8.7	0.34
Octanoic acid	1.59	1.33	1.79	1.62	7.9^{**}	4.5^{*}	0.6	27.3	0.29

Nonanoic acid	0.31	0.70	0.43	0.36	58.1^{***}	5.3^{*}	22.8^{***}	10.9	0.16
Decanoic acid	1.24	1.24	1.42	1.32	0.1	7.6^{**}	0.1	42.9	0.21
∑ Ketones	**1.33**	**1.33**	**1.07**	**1.21**	**0.1**	**23.9^{***}**	**0.1**	**38.8**	**0.17**
Butan-2-one	0.96	0.84	0.62	0.71	5.2^{*}	39.9^{***}	0.2	38.8	0.17
Heptan-2-one	0.36	0.42	0.31	0.37	2.3	1.4	0.1	26.0	0.12
Heptan-3-one	0.50	0.58	0.46	0.53	33.2^{***}	12.3^{***}	1.7	22.3	0.04
Nonan-2-one	0.16	0.21	0.17	0.17	14.5^{***}	0.1	2.3	42.2	0.04
∑ Others	**2.87**	**2.44**	**2.87**	**2.66**	**29.9^{***}**	**0.1**	**0.1**	**35.4**	**0.25**
Methylsulfonylmethane	1.19	0.86	1.44	0.94	22.7^{***}	11.3^{**}	9.5^{**}	56.7	0.21
Methylthiomethane	1.29	1.16	0.53	0.96	25.5^{***}	872.5^{***}	28.5^{***}	80.4	0.08
Dihydrofuran-2(3H)-one	0.19	0.19	0.22	0.18	0.5	10.7^{**}	9.4^{**}	21.2	0.02
3-Methylphenol	0.05	0.06	0.05	0.06	1.0	0.1	0.1	14.8	0.02
Toluene	2.44	2.07	2.59	2.35	72.9^{***}	12.1^{***}	0.3	77.4	0.14
6-Propyloxan-2-one	0.37	0.16	0.26	0.21	70.1^{***}	19.2^{***}	0.1	37.5	0.08
Limonene	0.33	0.23	0.26	0.22	25.9^{***}	12.3^{***}	2.1	92.4	0.06
α-Pinene	0.28	0.20	0.15	0.19	31.6^{***}	92.4^{***}	0.9	84.0	0.05

[1] $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; [2] Contrast between whole milk from the evening milking vs. evening creamed milk; [3] Contrast between whole milk from the evening milking vs. whole milk from the morning milking; [4] Contrast between milk collected from the vat vs. the mean of the milk from morning milking and evening creamed milk; [5] The incidence of cheese-making day is calculated dividing the variance of cheese-making on total variance; [6] RMSE= root means square error.

Table 3. Quantity and significant orthogonal contrasts between VOC and their chemical family expressed as natural logarithm of concentration (µg×kg^{-1}) of cream, curd (fresh cheese) and ricotta and incidence of cheese-making day (date).

Chemical family/VOC	LSM			Contrasts (*F*-value)[1]		Date (%)[2]	RMSE[3]
	Cream (*A*)	Fresh cheese (*B*)	Ricotta (*C*)	*A* vs. *(B+C)*	*(B* vs. *C)*		
∑ **VOC**	**6.75**	**6.35**	**7.91**	**47.5^{***}**	**50.3^{***}**	**11.9**	**0.53**
∑ **Alcohols**	**3.50**	**3.50**	**4.90**	**46.4^{***}**	**141.6^{***}**	0.0	**0.38**
3-Methyl-2-buten-1-ol	0.56	0.40	0.45	19.7^{***}	2.1	47.9	0.11
3-Methylbutan-1-ol	2.14	1.65	4.29	40.9^{***}	335.3^{***}	45.1	0.44
Pentan-1-ol	2.77	2.52	3.10	0.1	23.9^{***}	4.2	0.38
Hexan-1-ol	1.30	2.49	3.14	106.8^{***}	14.3^{***}	11.1	0.55
1-Octen-3-ol	0.61	0.55	2.65	201.3^{***}	671.1^{***}	20.8	0.26
2-Ethylhexanol	1.34	1.45	0.82	25.1^{***}	174.7^{***}	47.7	0.15
2-Phenoxyethanol	0.71	0.16	0.57	39.7^{***}	37.9^{***}	0.0	0.20
Octan-1-ol	0.78	0.38	0.86	14.1^{***}	97.6^{***}	46.8	0.15
∑ **Aldehydes**	**3.39**	**2.74**	**7.75**	**251.3^{***}**	**$1,366.8^{***}$**	**26.1**	**0.44**
2-Methylpropanal	1.72	0.73	3.84	33.8^{***}	748.9^{***}	22.8	0.36
3-Methylbutanal	2.50	-	-	-	-	-	0.15
Benzaldehyde	0.70	1.19	2.62	164.7^{***}	156.5^{***}	24.1	0.33
Hexanal	1.04	1.76	7.45	846.2^{***}	$1,498.6^{***}$	37.7	0.45
Heptanal	1.13	1.62	6.05	576.1^{***}	$1,176.5^{***}$	37.5	0.41
Octanal	0.64	0.77	4.27	257.2^{***}	642.2^{***}	36.9	0.44
Oct-2-enal	-	0.12	2.95	-	822.8^{***}	40.7	0.32
Nonenal	0.41	0.72	3.17	157.5^{***}	294.2^{***}	18.3	0.44
Decanal	1.04	0.59	1.27	3.5	106.2^{***}	44.9	0.21
∑ **Esters**	**5.03**	**6.44**	**4.24**	**1.8**	**67.4^{***}**	**0.0**	**0.87**
Ethyl acetate	2.08	0.84	4.17	10.3^{**}	465.8^{***}	5.6	0.49
Ethyl lactate	-	-	0.68	-	-	-	0.06
Ethyl butanoate	4.45	6.42	0.60	62.6^{***}	$1,726.6^{***}$	20.2	0.44
Ethyl hexanoate	3.86	5.25	0.76	41.2^{***}	818.1^{***}	21.4	0.49
Ethyl octanoate	2.37	3.78	0.37	4.5^{*}	398.1^{***}	6.6	0.51
Ethyl decanoate	1.13	2.73	0.45	14.9	239.5^{***}	20.5	0.41
Ethyl dodecanoate	0.56	0.99	0.38	10.3^{**}	166.8^{***}	1.5	0.14
∑ **FFA**	**5.36**	**3.34**	**3.71**	**189.6^{***}**	**5.7^{*}**	**34.8**	**0.50**
Acetic acid	0.90	-	3.29	-	-	-	0.21
Butanoic Acid	3.91	1.27	1.21	368.3^{***}	0.1	43.7	0.51
Hexanoic Acid	4.71	2.39	2.44	276.5^{***}	0.1	44.2	0.51
Octanoic Acid	3.52	2.37	1.81	205.5^{***}	22.1^{***}	26.8	0.37
Nonanoic Acid	1.24	0.64	0.91	33.0^{***}	8.1^{**}	4.2	0.29
Decanoic Acid	2.35	1.78	1.73	46.9^{***}	0.2	20.9	0.33

∑ **Ketones**	**1.78**	**1.55**	**4.21**	**397.4*****	**1,723.2*****	**42.3**	**0.21**
Butan-2-one	1.71	0.87	3.82	48.6***	788.8***	23.9	0.34
Pentan-2-one	-	-	2.96	-	-	-	0.06
Heptan-2-one	1.36	1.09	3.53	367.7***	1,819.1***	47.8	0.19
Heptan-3-one	0.52	0.57	0.72	35.4***	37.8***	14.8	0.08
Nonan-2-one	0.85	0.72	2.62	217.5***	878.1***	30.5	0.20
∑ **Others**	**4.74**	**3.56**	**3.42**	**223.4*****	**2.3**	**39.9**	**0.31**
Methylsulfonylmethane	3.53	0.53	2.39	197.1***	113.8***	37.5	0.50
Methylthiomethane	2.66	1.15	1.37	397.4***	7.6**	56.5	0.26
Dihydrofuran-2(3H)-one	0.56	1.21	0.55	36.9***	107.6***	17.5	0.19
3-Methylphenol	0.28	0.07	0.15	74.8***	13.4***	37.9	0.07
Toluene	4.09	3.33	2.78	331.5***	70.2***	71.6	0.21
6-Propyloxan-2-one	0.66	0.40	0.60	8.4**	9.2**	13.3	0.20
Limonene	1.21	0.78	0.64	26.2***	1.3	75.6	0.36
α-Pinene	1.01	0.66	0.50	32.3***	3.1	58.9	0.27

[1] $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; [2]The incidence of cheese-making day is calculated dividing the variance of cheese-making on total variance; [3]RMSE= root means square error.

Table 4. Quantity and significant orthogonal contrast between VOC and their chemical family expressed as natural logarithm of concentration (μg×kg^{-1}) of milk in vat, whey and scotta and incidence of cheese-making day (date).

Chemical family/VOC	LSM			Contrasts (*F*-value)^1		Date (%)^2	RMSE^3
	Milk in vat *(A)*	Whey *(B)*	Scotta *(C)*	*A vs (B+C)*	*B vs C*		
∑ **VOC**	**4.44**	**4.51**	**5.37**	**28.1*****	**63.9*****	**30.5**	**0.35**
∑ **Alcohols**	**2.43**	**3.29**	**3.53**	**26.2*****	**1.2**	**0.0**	**0.72**
3-Methyl-2-buten-1-ol	0.18	0.24	0.12	0.1	98.4***	52.0	0.04
3-Methylbutan-1-ol	0.41	1.28	3.32	175.7***	143.5***	52.2	0.51
Pentan-1-ol	1.89	1.94	0.41	77.2	266.7***	42.5	0.30
Hexan-1-ol	0.84	2.41	0.53	8.0**	46.7***	16.4	0.81
1-Octen-3-ol	0.25	1.06	0.36	48.8***	82.6	42.3	0.25
2-Ethylhexanol	0.50	0.75	1.46	2,472.0***	2,423.7***	78.5	0.05
2-Phenoxyethanol	0.12	0.12	0.17	7.9**	45.6***	35.5	0.02
Octan-1-ol	0.16	0.21	0.56	279.7***	524.6***	45.3	0.05
∑ **Aldehydes**	**1.94**	**2.54**	**3.82**	**67.5*****	**53.8*****	**48.2**	**0.56**
2-Methylpropanal	0.71	0.66	2.87	147.2***	481.0***	22.3	0.32
3-Methylbutanal	-	0.62	-	-	-	84.1	0.23
Benzaldehyde	0.14	0.25	1.25	64.1***	116.6***	27.6	0.27
Hexanal	1.01	1.94	2.08	18.1***	0.2	24.5	0.83
Heptanal	0.81	1.21	2.46	63.6***	64.9***	62.2	0.47
Octanal	0.42	0.29	1.48	58.6***	288.4***	28.1	0.22
Oct-2-enal	0.13	0.39	0.41	40.8***	0.2	53.8	0.15
Nonenal	0.23	1.00	0.84	46.8***	1.7	53.4	0.37
Decanal	0.30	0.31	0.89	227.1***	634.8***	27.2	0.07
∑ **Esters**	**3.43**	**1.97**	**3.33**	**17.1*****	**39.8*****	**4.7**	**0.70**
Ethyl acetate	0.85	0.85	3.18	95.9***	278.7***	18.5	0.42
Ethyl lactate	-	-	0.21	-	-	-	0.03
Butyl butanoate	-	-	0.07	-	-	-	0.03
Ethyl butanoate	3.43	1.41	0.32	1,351.3***	203.8***	33.1	0.24
Ethyl hexanoate	2.12	1.39	0.72	365.6***	104.2***	43.3	0.20
Ethyl octanoate	1.96	1.71	0.37	64.0***	99.8***	36.7	0.20
Ethyl decanoate	0.65	0.43	0.90	0.1	36.1***	15.5	0.24
Ethyl dodecanoate	0.35	0.16	0.11	132.6***	6.4*	38.2	0.07
∑ **FFA**	**2.49**	**3.00**	**4.24**	**309.4*****	**283.6*****	**48.7**	**0.24**
Acetic acid	-	-	2.29	-	-	81.5	0.07
Butanoic Acid	0.73	0.69	1.17	13.9***	63.4***	45.7	0.19
Hexanoic Acid	1.45	1.89	2.59	167.6***	100.8***	39.7	0.23
Octanoic Acid	1.62	2.14	3.28	343.3***	292.6***	40.3	0.22
Nonanoic Acid	0.36	0.39	1.04	113.3***	277.1***	17.0	0.13
Decanoic Acid	1.32	1.67	2.91	275.5***	346.5***	67.6	0.22
∑ **Ketones**	**0.85**	**1.32**	**2.72**	**645.2*****	**692.0*****	**22.3**	**0.17**
Butan-2-one	0.71	0.50	2.85	204.0***	937.1***	28.4	0.25

Heptan-2-one	0.37	0.81	2.43	649.7***	786.6***	33.9	0.18
Heptan-3-one	0.53	0.82	1.14	1,179.8***	437.2***	26.0	0.05
Nonan-2-one	0.17	0.35	1.01	301.7***	373.0***	37.5	0.11
∑ **Others**	**2.66**	**1.83**	**1.37**	**402.4*****	**55.8*****	**25.8**	**0.20**
Methylsulfonylmethane	0.96	0.69	0.57	47.2***	5.1*	48.3	0.15
Methylthiomethane	0.96	0.51	0.56	415.8***	3.1***	62.7	0.08
Dihydrofuran-2(3H)-one	0.18	0.13	0.15	56.5***	9.5**	46.0	0.02
3-Methylphenol	0.06	0.04	0.04	23.7***	0.6	40.0	0.01
Toluene	2.35	1.38	0.63	695.6***	154.7***	51.8	0.19
6-Propyloxan-2-one	0.21	0.24	0.27	15.9***	4.6*	66.2	0.04
Limonene	0.22	-	-	-	-	35.1	0.15
α-Pinene	0.19	0.13	0.07	61.8***	25.0***	67.2	0.04

$^{1*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ^{2}The incidence of cheese-making day is calculated dividing the variance of cheese-making on total variance; ^{3}RMSE= root means square error.

Table 5. Quantity and orthogonal contrast between VOC and their chemical family expressed as natural logarithm of concentration (µg×kg^{-1}) of fresh cheese and ripened cheese and incidence of cheese-making day (date).

Chemical family/VOC	LSM			Contrasts (*F*-value)^{1}		Date (%)^{2}	RMSE^{3}
	Fresh cheese *(A)*	Ripened cheese					
		6 mo *(B)*	12 mo *(C)*	*A* vs. *(B+C)*	*B* vs. *C*		
∑ VOC	**6.35**	**9.43**	**9.79**	**360.1*****	**4.3***	**28.1**	**0.56**
∑ Alcohols	**3.50**	**7.99**	**8.44**	**615.9*****	**4.2***	**22.3**	**0.71**
Butan-1-ol	-	5.09	6.19	-	157.7***	91.0	0.27
Butan-2-ol	-	7.66	7.77	-	1.7	92.6	0.27
3-Methyl-2-buten-1-ol	0.41	4.41	5.83	3,019.3***	211.8***	18.9	0.32
3-Methylbutan-1-ol	1.70	6.09	6.41	681.2***	2.6	39.1	0.59
Pentan-1-ol	2.52	3.03	3.09	46.1***	0.5	51.8	0.29
Pentan-2-ol	-	5.88	5.70	-	1.5	38.2	0.47
Hexan-1-ol	2.49	4.63	5.17	178.4***	6.4*	1.2	0.67
Hexan-2-ol	-	2.27	2.95	-	120.7***	83.0	0.19
Heptan-2-ol	-	5.41	6.36	-	174.2***	52.0	0.22
1-Octen-3-ol	0.55	1.71	0.95	173.2***	120.2***	19.3	0.22
2-Ethylhexanol	1.45	2.61	2.16	210.8***	33.8	72.4	0.23
2-Phenoxyethanol	0.16	2.99	2.44	382.0***	11.8	20.0	0.45
Octan-1-ol	0.38	1.66	1.30	533.2***	44.5	66.4	0.17
∑ Aldehydes	**2.74**	**5.69**	**5.24**	**449.3*****	**8.8****	**41.5**	**0.48**
2-Methylpropanal	0.71	4.96	4.87	599.5***	0.2	52.5	0.62
3-Methylbutanal	-	2.47	2.97	-	23.3***	69.2	0.32
Hexanal	1.81	3.74	2.34	142.1***	154.5***	53.9	0.35
Benzaldehyde	1.20	1.85	2.08	39.1***	2.9	19.1	0.40
Heptanal	1.62	3.75	2.87	161.8***	33.3***	23.4	0.50
Oct-2-enal	0.12	1.17	0.26	56.5***	96.9***	12.7	0.29
Octanal	0.77	2.73	2.12	176.4***	16.7***	15.4	0.45
Nonenal	0.75	-	-	-	-	96.5	0.03
Decanal	0.60	2.01	1.64	353.4***	22.4***	36.0	0.23
∑ Esters	**6.44**	**7.75**	**8.15**	**32.5*****	**1.7**	**5.6**	**0.99**
Ethyl acetate	0.85	3.98	4.70	1,252.0***	41.6***	37.3	0.35
Ethyl lactate	-	3.36	3.36	-	0.1	53.0	0.33
Butyl butanoate	-	3.78	2.89	-	30.1***	79.9	0.51
Ethyl butyrate	6.48	6.83	7.42	20.4***	13.3***	55.0	0.51
Ethyl hexanoate	5.27	6.93	7.50	287.9***	19.0***	50.0	0.41
Ethyl octanoate	3.80	5.12	5.65	215.5***	19.9***	51.1	0.37
Ethyl decanoate	2.73	4.00	4.75	317.1***	54.3***	44.4	0.32

Ethyl dodecanoate	0.97	2.55	3.16	913.2^{***}	78.4^{***}	47.6	0.21
∑ FFA	**3.34**	**8.56**	**8.97**	$\mathbf{1,730.1}^{***}$	$\mathbf{7.3}^{**}$	**35.3**	**0.49**
Acetic Acid	-	7.12	7.20	-	0.2	25.3	0.54
Butanoic Acid	1.29	7.88	8.33	$1,742.8^{***}$	5.9^{*}	37.8	0.60
Hexanoic Acid	2.40	6.94	7.50	$1,242.7^{***}$	13.9^{***}	44.1	0.49
Octanoic Acid	2.38	5.21	5.67	$1,731.9^{***}$	30.2^{***}	49.0	0.27
Nonanoic Acid	0.62	3.89	3.10	746.1^{***}	42.4^{***}	49.7	0.38
Decanoic Acid	1.78	3.92	4.31	990.4^{***}	20.7^{***}	24.6	0.28
∑ Ketones	**1.55**	**4.85**	**5.13**	$\mathbf{251.1}^{***}$	**1.3**	**4.8**	**0.81**
Butan-2-one	0.87	4.93	4.76	662.3^{***}	0.8	64.7	0.57
Pentan-2-one	0.01	3.94	4.55	235.9^{***}	55.3^{***}	74.3	0.25
Heptan-2-one	1.09	4.32	4.43	$1,452.1^{***}$	1.1	52.7	0.32
Heptan-3-one	0.57	0.82	1.01	41.2^{***}	9.1^{**}	31.3	0.20
Nonan-2-one	0.72	3.23	3.43	832.6^{***}	3.9	54.5	0.33
∑ Others	**3.56**	**5.91**	**5.02**	$\mathbf{97.3}^{***}$	**5.9**	**9.6**	**0.72**
Methylsulfonylmethane	0.53	1.91	2.25	146.8^{***}	5.2^{*}	27.0	0.43
Methylthiomethane	1.15	0.64	1.55	0.37	66.1^{***}	62.0	0.33
Dihydrofuran-2(3H)-one	1.21	-	-	-	-	23.0	0.09
3-Methylphenol	0.07	1.12	1.33	$1,118.4^{***}$	32.6^{***}	50.3	0.12
Toluene	3.33	4.23	4.73	177.7^{***}	23.2^{***}	45.0	0.32
6-Propyloxan-2-one	0.41	2.70	2.96	$3,042.4^{***}$	28.9^{**}	40.7	0.16
α-Pinene	0.70	2.07	1.62	77.9^{***}	8.3^{**}	15.1	0.44
Limonene	0.83	5.93	3.15	485.5^{***}	218.5^{***}	25.7	0.57

[1]$^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; [2]The incidence of cheese-making is calculated dividing the variance of cheese-making on total variance; [3]RMSE= root means square error.

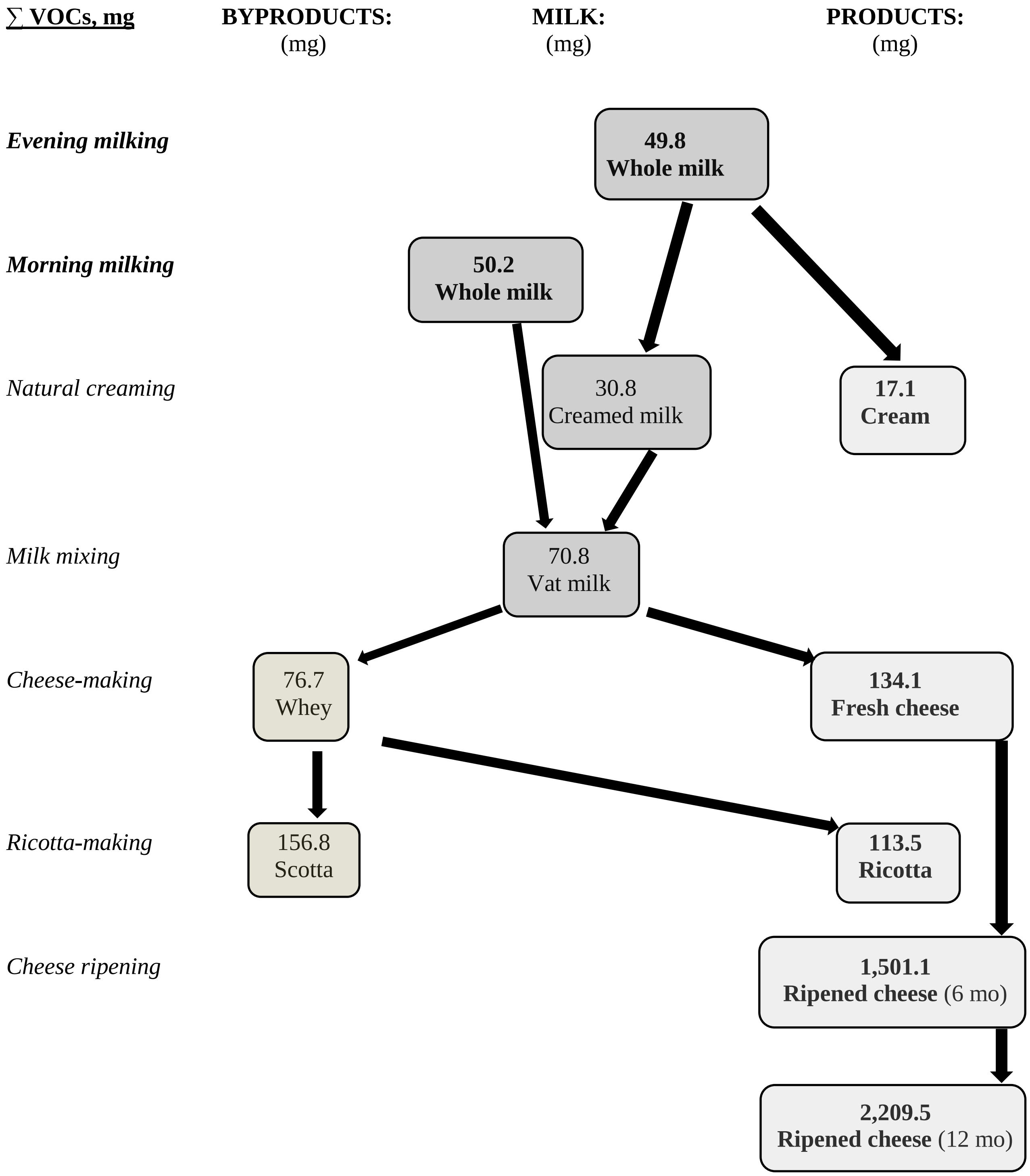
Table 6. Quantity (mg) of volatile organic compounds (VOC) in the milk, fresh and ripened dairy products and fluid by-products obtained from 100 kg of milk along the cheese- and ricotta-making chain.

	Milk:				Fresh products:			Byproducts:		Ripened cheese:	
Item[1]	Evening whole	Creamed	Morning whole	Vat (Mix)[2]	Cream	Fresh cheese	Ricotta	Whey	Scotta	6 mo	12 mo
Product weight, kg	**50**	**47**	**50**	**97**	**3**	**13.8**	**4.2**	**83.2**	**79**	**12**	**11.4**
∑ VOC, mg	**5.46**	**3.38**	**5.5**	**7.76**	**1.87**	**14.7**	**12.44**	**8.41**	**17.19**	**164.51**	**242.16**
∑ Alcohols	**0.271**	**0.348**	**0.284**	**0.892**	**0.1**	**0.482**	**0.614**	**3.065**	**2.738**	**48.963**	**68.43**
Butan-1-ol	-	-	-	-	-	-	-	-	-	3.025	7.599
Butan-2-ol	-	-	-	-	-	-	-	-	-	35.175	38.14
3-Methyl-2-buten-1-ol	0.008	0.01	0.01	0.019	0.002	0.007	0.002	0.023	0.01	1.03	4.273
3-Methylbutan-1-ol	0.015	0.016	0.02	0.053	0.03	0.059	0.336	0.677	2.261	7.742	9.281
Pentan-1-ol	0.155	0.2	0.165	0.553	0.048	0.161	0.103	0.631	0.041	0.277	0.25
Pentan-2-ol	-	-	-	-	-	-	-	-	-	4.441	4.52
Hexan-1-ol	0.028	0.071	0.022	0.15	0.008	0.208	0.11	2.225	0.056	1.576	2.265
Hexan-2-ol	-	-	-	-	-	-	-	-	-	0.112	0.237
Heptan-2-ol	-	-	-	-	-	-	-	-	-	2.7	7.274
1-Octen-3-ol	0.005	0.017	0.008	0.028	0.003	0.01	0.062	0.194	0.035	0.059	0.019
2-Ethylhexanol	0.038	0.037	0.037	0.063	0.009	0.047	0.005	0.093	0.262	0.187	0.092
2-Phenoxyethanol	0.014	0.007	0.01	0.013	0.003	0.002	0.004	0.01	0.014	0.254	0.155
Octan-1-ol	0.01	0.007	0.013	0.017	0.004	0.006	0.006	0.019	0.059	0.055	0.033
∑ Aldehydes	**0.232**	**0.421**	**0.195**	**0.618**	**0.104**	**0.221**	**10.65**	**2.209**	**3.679**	**4.132**	**2.692**
2-Methylpropanal	0.084	0.065	0.027	0.103	0.015	0.017	0.218	0.095	1.485	2.752	2.119
3-Methylbutanal	-	-	-	-	0.061	-	-	0.085	-	0.145	0.258
Hexanal	0.063	0.197	0.059	0.224	0.008	0.077	7.823	1.703	0.593	0.563	0.125
Benzaldehyde	0.014	0.013	0.008	0.015	0.004	0.033	0.059	0.024	0.249	0.069	0.1
Heptanal	0.046	0.114	0.038	0.164	0.007	0.069	2.127	0.528	0.889	0.629	0.202
Oct-2-enal	-	0.01	0.005	0.013	-	0.002	0.088	0.047	0.04	0.03	0.004
Octanal	0.011	0.032	0.026	0.053	0.003	0.021	0.358	0.034	0.279	0.197	0.09
Nonenal	0.02	0.024	0.013	0.026	0.002	0.015	0.118	0.314	0.11	-	-
Decanal	0.023	0.013	0.024	0.034	0.006	0.012	0.011	0.031	0.113	0.081	0.053

∑ **Esters**	**3.402**	**1.48**	**3.363**	**3.538**	**0.511**	**12.986**	**0.327**	**0.7**	**2.369**	**32.277**	**54.162**
Ethyl acetate	0.058	0.028	0.065	0.141	0.027	0.018	0.308	0.143	2.087	0.716	1.426
Ethyl lactate	-	-	-	-	-	-	0.004	-	0.021	0.366	0.362
Butyl butanoate	-	-	-	-	-	-	-	-	0.006	0.796	0.52
Ethyl butanoate	3.44	1.214	3.012	2.999	0.284	10.189	0.004	0.283	0.03	14.599	26.995
Ethyl hexanoate	0.789	0.314	0.698	0.747	0.159	3.296	0.005	0.261	0.084	13.424	22.753
Ethyl octanoate	0.144	0.061	0.107	0.173	0.033	0.714	0.002	0.124	0.016	2.201	3.489
Ethyl decanoate	0.068	0.03	0.048	0.102	0.007	0.211	0.003	0.048	0.117	0.674	1.487
Ethyl dodecanoate	0.025	0.016	0.024	0.041	0.002	0.024	0.002	0.015	0.009	0.148	0.268
∑ **FFA**	**0.565**	**0.461**	**0.68**	**1.168**	**0.757**	**0.427**	**0.212**	**1.69**	**5.553**	**69.969**	**111.47**
Acetic acid	-	-	-	-	0.004	-	0.14	-	0.716	17.585	17.375
Butanoic acid	0.073	0.044	0.056	0.121	0.221	0.044	0.012	0.089	0.177	37.743	66.826
Hexanoic acid	0.197	0.115	0.212	0.332	0.384	0.185	0.049	0.513	0.991	13.691	23.48
Octanoic acid	0.208	0.143	0.258	0.421	0.11	0.143	0.023	0.654	2.058	2.304	3.492
Nonanoic acid	0.019	0.051	0.027	0.042	0.008	0.013	0.007	0.042	0.147	0.65	0.327
Decanoic acid	0.126	0.121	0.163	0.291	0.033	0.07	0.02	0.393	1.465	0.63	0.87
∑ **Ketones**	**0.142**	**0.133**	**0.101**	**0.23**	**0.03**	**0.074**	**0.501**	**0.292**	**2.613**	**4.225**	**4.433**
Butan-2-one	0.084	0.064	0.046	0.103	0.014	0.021	0.211	0.055	1.463	2.642	1.998
Pentan-2-one	-	-	-	-	-	0.001	0.087	-	-	0.643	1.226
Heptan-2-one	0.022	0.025	0.019	0.046	0.009	0.028	0.143	0.109	0.842	0.965	1.132
Heptan-3-one	0.033	0.037	0.029	0.068	0.002	0.011	0.004	0.107	0.167	0.017	0.02
Nonan-2-one	0.009	0.011	0.009	0.018	0.004	0.015	0.056	0.035	0.141	0.316	0.397
∑ **Others**	**0.849**	**0.532**	**0.873**	**1.317**	**0.369**	**0.511**	**0.133**	**0.453**	**0.239**	**5.554**	**2.082**
Methylsulfonylmethane	0.119	0.07	0.176	0.159	0.129	0.01	0.051	0.085	0.062	0.076	0.127
Methylthiomethane	0.136	0.106	0.035	0.16	0.043	0.036	0.013	0.057	0.06	0.01	0.053
Dihydrofuran-2(3H)-one	0.011	0.01	0.012	0.02	0.002	0.033	0.003	0.012	0.013	-	-
3-Methylphenol	0.003	0.003	0.003	0.006	0.001	0.001	0.001	0.003	0.003	0.025	0.032
Toluene	0.545	0.335	0.65	0.949	0.19	0.406	0.067	0.257	0.075	0.846	1.418
6-Propyloxan-2-one	0.023	0.008	0.015	0.023	0.003	0.007	0.004	0.022	0.024	0.172	0.214
Limonene	0.021	0.012	0.017	0.026	0.012	0.021	0.005	0.03	0.023	4.907	0.366
α-Pinene	0.017	0.011	0.008	0.02	0.007	0.015	0.003	0.012	0.006	0.108	0.049

[1] The data of volatile organic compounds (VOC) and their chemical families are expressed in mg; [2] It is obtained mixing the evening creamed milk with the morning whole milk in equal part.

Figure 1. Flow of average content (mg) of Volatile Organic Compounds (VOCs) in the headspace of different dairy products and by-products obtained from creaming, cheese- and ricotta-making.



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GENERAL CONCLUSIONS

The main objective of this thesis was to investigate the Volatile Organic Compounds profile of dairy products across the cheese production chain.

Volatile Organic Compounds were analysed with two spectrometric techniques: Solid Phase Micro Extraction/Gas Chromatography-Mass Spectrometry (SPME/GC-MS) and Proton Transfer Reaction-Time of Flight-Mass Spectrometry (PTR-ToF-MS). The complementary of these two instruments permits the identification and measurement of volatile profile from a high number of samples/cheeses and their relation to individual animal traits (days in milk, parity and milk yield) opening new prospective in the breeding program on the basis of sensory traits.

The dairy system, and cow related factors affected the volatile fingerprint of ripened cheeses. The most significant effect within farming systems was found to be related to the use of Total Mixer Ration, a technique of distributing feedstuff to cows that seems to have a high impact on cheese volatiles. Concerning individual animal source of variation lactation stage was the most important effect followed by order of parity and daily milk yield.

On the basis of the phenotypes collected in this work, it was possible to carry out a genetic analysis at a population level. The results demonstrated the existence of an exploitable genetic variability of the volatile profile of cheese. Moreover, it would be interesting to see future studies focusing on the exploitation of the genomic background of the traits presented in this study.

The evolution of volatile organic compounds across the grazing season of the cows in a highland farm depends by specific technological aspects of cheese-making process. In this work of thesis, we found an effect of creaming, temperature of coagulation, and ripening period on the concentration of volatile organic compounds of milk, cream, curd, whey, ricotta, scotta and ripened cheese.

In summary, the monitoring of volatile fingerprint permits to obtain dairy products with specific organoleptic characteristics useful to differentiate them on the market and to improve cheese production chain. Moreover, the estimate of genetic parameters could be useful for an indirect selection of dairy species for cheese quality traits.