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# Portraying and tracing the impact of different production systems on the volatile organic compound composition of milk by PTR-(Quad)MS and PTR-(ToF)MS

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ABSTRACT

The aim of this study was to discover the unique volatile compositional traits of retail milk from different production systems. Forty-four retail milk samples were analyzed, including organic milk (n = 10), conventional milk (n = 14) and pasture milk (n = 20) from winter (n = 22) and summer (n = 22). Proton transfer reaction quadrupole mass spectrometry (PTR-(Quad)MS) was utilized to obtain the mass-resolved fingerprints (76 masses per sample) of volatile organic compounds (VOCs). Principal component analysis (PCA) and analysis of variance (ANOVA) were performed to evaluate the differences between the groups. The production systems were characterized by six masses, while season showed larger differences, with twenty-two masses discriminating between the milks. For 2 masses, a significant interaction of systems and seasons was observed. The chemical formula of these VOC masses were tentatively identified by Proton Transfer Reaction Time-of-Flight Mass Spectrometric (PTR-(ToF)MS). These results illustrate that the type of feed is reflected in the VOC composition of milks.



## 1. Introduction

The concept of the organic production system has been widely recognized and implemented. In 2014, the global market of organic food and drinks had reached $80 billion, while Europe took up 38%. Among these, organic dairy is one of the most important organic sectors, since it has made up about 20 percent of total organic foods & drinks since 2013 (Willer & Lernoud, 2015). To compensate for the strict rules and lower yield compared with milk from conventional dairy systems, organic milk always retailed at a higher price, i.e. approximately 30% higher than conventional milk (Marian, Chrysochou, Krystallis, & Thøgersen, 2014). Furthermore, since the EU milk quota regime came to an end in March 2015, more farmers look for added value of their products in order to differentiate from others. The combination of the higher price and the farmers looking for added value make organic milk vulnerable to fraud. In view of protection of consumers and providing a fair commercial environment, it is essential to assure the authenticity of organic milk.

Currently, in the Netherlands there is so-called 'pasture milk' on the market. It is produced by conventional dairy farms, but according to Dutch regulations, cows producing pasture milk should stay outdoors for at least 120 days per year, at least six hours a day. This is a management tool to encourage farmers to allow their cattle to roam outdoors, and dairy companies in the Netherlands pay extra price to farmers to support it. This kind of conventional-plus products draws the interest of consumers who are in favor of the idea of pasturing dairy cows. Since pasture milk is located between organic and conventional milk in terms of production management, it is relevant to consider pasture milk as well when comparing milk from the two systems.

Compared with farm milk, retail milk involves a series of processing steps, such as heating, standardization of fat content et al. During these steps, loss of VOCs that are present natively in different milks may occur. Furthermore, new VOCs may be formed as a consequence of heating, making it more difficult to find the native VOCs. These steps change the composition of milk and are likely to submerge some characteristics of organic milk. Furthermore, retail milk is a mixture of milks from different farms, which means characteristics will be more averaged and extremes levelled out. These aspects result in extra challenges for authentication. However, it is this mixture of milk that appears eventually on the shelves of the supermarkets, so authentication should preferentially be possible at this level.

Up to now, most published studies on the authentication of organic milk focused on the differences of isotope ratio (IR) (Chung, Park, Yoon, Yang, & Kim, 2014; Molkentin & Giesemann,

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2010) and fatty acids (FAs) composition (Capuano, Gravink, Boerrigter-Eenling, & van Ruth, 2015; Capuano et al., 2014; Ferreiro, Gayoso, & Rodríguez-Otero, 2015; Florence et al., 2012; Schwendel et al., 2015) between organic and conventional milk. That is because in the organic sector, cows are fed higher percentages of grass (C3 plant), while in the conventional system maize (C4 plant) is the main feed. Due to the fact that $\delta^{13}C$ of C4 plants (from −9‰ to −20‰) is higher than the $\delta^{13}C$ of C3 plants (from −21‰ to −35‰) (Badeck, Tcherkez, Nogués, Piel, & Ghashghaie, 2005; Chung et al., 2014), organic milk generally possesses a lower $\delta^{13}C$ value. With regard to the FAs composition, organic milk is richer in polyunsaturated FAs due to the higher proportion of fresh grass in the feed (Capuano et al., 2015). The methods above consider the non-volatile compounds. Compared with non-volatile compounds, volatile organic compounds (VOCs) are present in milk at lower concentrations. VOCs may however reflect the composition of the feed as well as its interaction with the rumen metabolism (Villeneuve et al., 2013). This aspect may be important for revelation of the unique traits of milk from the different production systems. Considering the global similarity of milk from different systems, a full profile of all VOCs is required to uncover small but consistent differences in characteristics.

Proton transfer reaction mass spectrometry (PTR-MS) is an advanced technique which allows measurement of the fingerprint of VOCs Compared with traditional gas chromatography mass spectrometry (GC–MS), PTR-MS can analyses the whole VOCs profile of the samples without sample preparation, while being sensitive and fast, and is therefore widely applied in many fields of food analysis, including dairy products analysis (Alothman, Lusk, Silcock, & Bremer, 2017; Makhoul et al., 2016; Masi, Romani, Pandolfi, Heimler, & Mancuso, 2015; Nenadis, Heenan, Tsimidou, et al., 2016; Schuhfried et al., 2016). Proton transfer reaction quadrupole mass spectrometry (PTR-(Quad)MS), one of the most widely used instruments for VOC fingerprinting, can precisely describe a VOCs profile with a mass range from 1 to 512 amu. Furthermore, PTR-MS equipped with a specially crafted high-end orthogonal acceleration reflection time of flight (ToF) shows a mass resolution of more than 6000 (up to 10,000 m/Dm), a considerably higher mass resolution than the more common PTR-(Quad)MS. This kind of higher resolution is helpful for the identification of the tentative chemical formula of the relevant masses. To our knowledge, no research has been reported on the differences in VOC composition of organic and conventional milks, especially with use of the rapid PTR-(Quad)MS and PTR-(ToF)MS. The aim of this research was to portray and explain differences in VOC composition between organic and conventional milks by PTR-(Quad)MS and PTR-(ToF)MS.

## 2. Materials and methods

### 2.1. Samples

Forty-four full fat pasteurized retail milk samples from different brands were purchased in supermarkets in the Netherlands. They included 22 samples collected in the winter period (6 organic, 8 conventional, 8 pasture milk) and 22 samples in summer (4 organic, 6 conventional, 12 pasture milk). All the samples were transferred from their original packages into 50 ml plastic centrifuge tubes, and were stored at −18 °C until analysis. Samples were defrosted at 4 °C one day before analysis.

### 2.2. PTR-(Quad)MS

PTR-MS analyses the VOC profiles of samples by utilizing a tube to extract the headspace gas from the flask. After entering, volatile compounds react with $H_3O^+$ generated from a hollow cathode ion source. Ionized volatile compounds are then analyzed by a quadrupole mass spectrometer. The high sensitivity PTR-(Quad)MS (Ionicon GmbH, Innsbruck, Austria) was used to obtain the VOC profiles of the samples. In this study, 3 ml milk sample was put into a 250 ml flask (Duran, Germany) with a plastic screw cap (Duran, Germany). The samples were equilibrated in a 35 °C water bath for 30 min. The inlet flow was 58 ml per min, and the temperature in the inlet tube and reaction chamber was 60 °C. The instrument was operated at a standard E/N (ratio of electric field strength across the reaction chamber, E, to buffer gas number density, N, within the chamber) of 105 Td (1 Td = $10^{-17}$ $cm^2$ V $mol^{-1}$). The whole range of scanning for $H3O^+$ reacted VOCs was from 20 to 160 mass-charge ratio ($m/z$), with a 0.2 s per mass dwell time. Each sample analysis was followed by a blank consisting of an empty flask. Five complete cycles were run for each sample and blank, recording the middle three cycles for samples and the last three for blanks. The average of these three cycles was calculated as the value for each replicate, and blank signal was deducted from the sample signal. The measurement for each sample was replicated 3 times independently. Masses with average concentrations higher than 0.1 ppb were considered for statistical analysis. The concentration of water cluster masses ($m/z$ 37 and 55) and $O_2$ ($m/z$ 32) were removed from the spectral data (Galle et al., 2011).

### 2.3. PTR-(ToF)MS

Since the classes of compounds in each kind of milk are the same, four organic, four pasture, and four conventional milk samples, including winter and summer milks were selected from previous 44 samples and analyzed by PTR-(ToF)MS (Ionicon GmbH, Innsbruck, Austria) to identify the chemical formula for each mass tentatively. These samples were selected according to the results of previous quadrupole analysis to representatively cover the variability. Regardless different concentrations, the types of compounds in different milks are consistent. The headspace measurement conditions were identical to those for the PTR-(Quad)MS analysis. The temperature of the inlet was 61 °C and the reaction chamber was operated at a 1000 V drift voltage, a 3.80 mbar drift pressure, and an E/N of 135 Td (1 Td = $10^{-17}$ $Vcm^2$). Scanning was operated for 1 s, ranging from 17 to 507 $m/z$. A blank was measured before each sample. Each replicate was scanned 60 times, which resulted in an analysis time of 1 min per sample. Three replicates of each sample were analyzed and the spectra subsequently averaged.

### 2.4. Statistical analysis

The whole dataset was auto scaled to eliminate the effect of the different intensity range of each mass. Principal component analysis (PCA) was performed using Pirouette 4.5 (Infometrix, WA, USA) to evaluate the overall characteristics of the dataset. After that, one-way ANOVA was applied to the PTR-(Quad)MS data set to obtain the significant differences between milks derived from different systems and the differences between milk in summer and winter. In order to control family wise error of multiple comparison, Benjamini-Hochberg (BH) adjustment (Benjamini & Hochberg, 1995)was applied. The P values from ANOVA were assessed and adjusted to maintain the false discovery rate (FDR) at 0.05. Subsequently, Fisher's least significant difference (LSD) tests were applied to determine significant differences between the individual groups. Two-way ANOVA was applied to consider the production system and season factors simultaneously, and also examine their interactions. The variance component ratio (square sum of variance of certain factor/total square sum of variance) of each factor in the two-way ANOVA was calculated. The ratio value

represents the contribution of each factor to the total observed variance. A higher ratio indicates a greater influence of a particular factor (Zhao, Guo, Wei, & Zhang, 2012), in this study either the production system or the seasonal factor.

## 3. Results and discussion

Forty-four samples of organic, pasture and conventional milks were collected and analyzed by PTR-(Quad)MS. The mass range 27–160 amu was considered for data analysis after removing *m*/*z* 32, 37 and 55. The average mass spectra are shown in Fig. 1. Masses *m*/*z* 59 was the predominant mass for all samples, at least 6 times higher concentrations than the other masses. However, the concentrations of this mass do not present any significant differences, neither for the production systems nor for the seasons. Obviously the compounds' concentrations are influenced by other factors. Furthermore, retail samples are expected to show less variation due to the mixing of milks as well as through the industrial procedures, such as pasteurizing, fat standardizing and storing, which narrow the gap between different milk (Gandy et al., 2008; Karatapanis, Badeka, Riganakos, Savvaidis, & Kontominas, 2006).

Statistical analysis on the full profile was carried out to search for the small but consistent differences between the milks of the three production systems. All spectral data were subjected to PCA. A PCA scores plot is presented in Fig. 2, which shows the grouping trend of the organic samples versus the others. The first three PCs explain 39% of total variance. The relatively low explained variance reflects the multidirectional differences between organic and conventional milk, which cannot be illustrated completely by one or two components. Random variance within and among the groups also contributes to the relatively low variance explanation. In the PCA scores plot, conventional

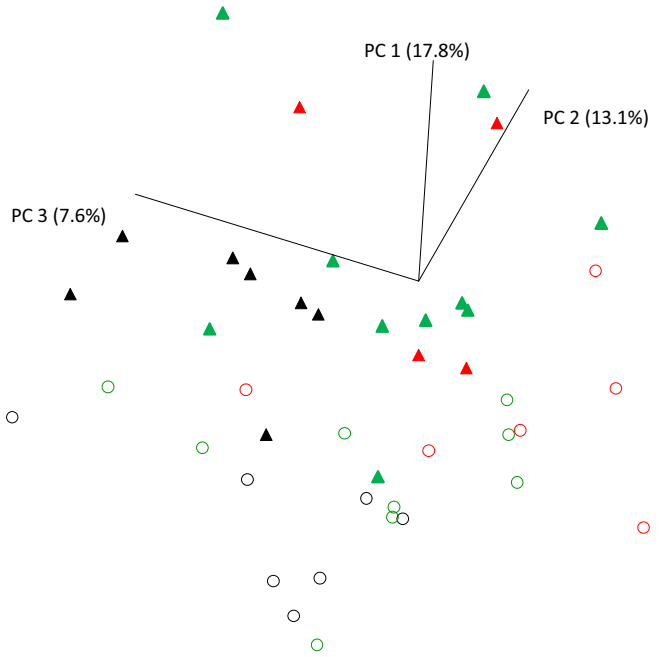


**Fig. 2.** Scores plot of first three dimensions of PCA on the mass spectral data of VOCs of organic (red), pasture (green) and conventional (black) milk in winter (open circles) and summer (solid triangle). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

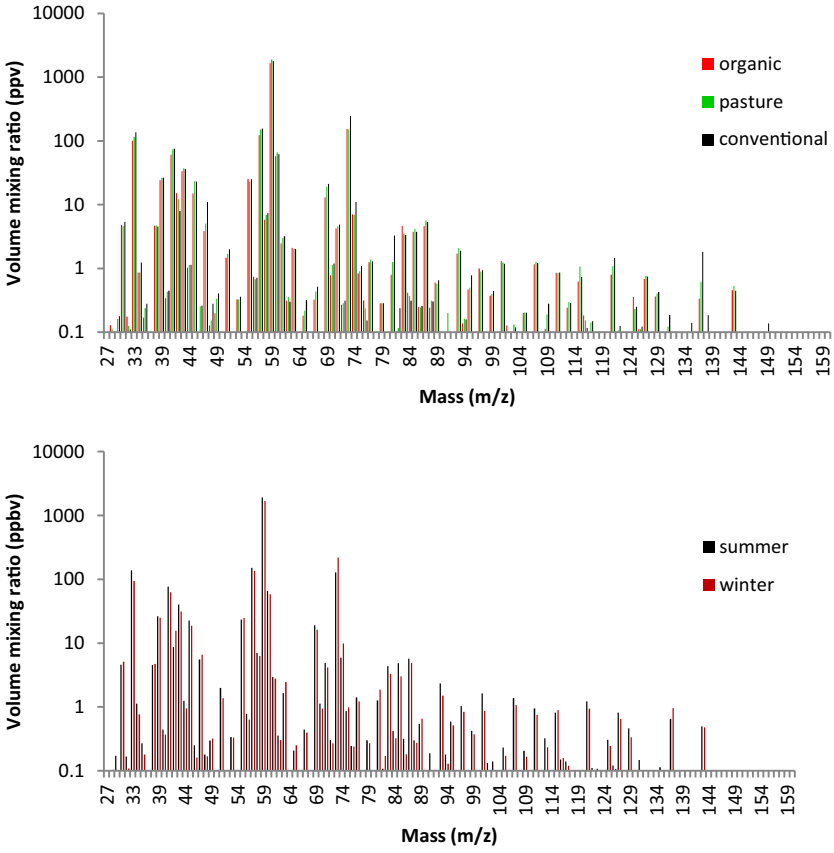


**Fig. 1.** PTR-(Quad)MS spectra: Average VOC spectra of organic, pasture, and conventional milks (upper); Average spectra of all winter and summer milks (lower).

samples are distributed over the left part while the organic samples distribute in the right part, except for one sample. Pasture samples are mixed with both organic and conventional milk and are located relatively close to the demarcation line between organic and conventional milks. Season is shown to have a large influence on the VOC composition as well. In the PCA scores plot, the winter and summer samples are mainly located at the lower and upper side of the plot, respectively. Combining the information on the seasons and production systems, it is interesting to find that pasture milk in summer is close to organic milk, while pasture milk in winter is more similar to conventional milk. This may be due to the requirements for pasture milk, which dictates a particular quantum of grazing. Pasture milk farmers will have cows outside during summer when the grass is abundant and temperature outside is acceptable, which explains the similarity of summer pasture milk and organic milk VOC composition. After spending the minimum time on pasture, a part of farmers would manage their cows in a more conventional way to increase yields (Cederberg & Mattsson, 2000). If cows are not grazing at all in winter, this milk can still be sold as pasture milk, as long as the cows fulfil the grazing requirements over the whole year. Since the grassland of pasture milk is also cultivated in conventional ways, the similarity of summer pasture milk with organic milk indicates that the characteristics of VOCs in organic milk are more related to the specific feed type (fresh grass) instead of the organic management of the grass. In winter time, cows in the 'pasture milk system' will be managed equally to those in the conventional systems, which explains the similarity with the conventional milk in winter time. In order to search for the relevant masses contributing to the differences between groups, ANOVA was performed for each mass, taking the production systems and the seasons into account.

### 3.1. Effect of the production system

Significant differences (BH adjusted $p < 0.05$) between different production systems were observed for six masses (Table 1). Generally, organic milks showed higher abundances of these discriminating VOCs than milks from the other production systems. It is important to note that these specific masses are present at relatively low concentrations. Three of the significantly different masses were present at volume mixing ratios lower than 1 ppbv, whereas mass $m/z$ 69, was the most abundant discriminating mass with a level of 18 ppbv (mean value of all the samples). This is still relatively low when compared with the predominant mass $m/z$ 59, for which a volume mixing ratio of 1797 ppbv was observed. It illustrates a fact that the major volatile compounds reflect the principal profile of the milk, while some less predominant compounds show the traits of milk derived from different systems. In order to examine the significant differences between the three different systems, post hoc Fisher's LSD tests were subsequently applied.

All these six masses revealed significant differences in concentration between organic and conventional milk. In contrast, three masses showed significantly different concentrations between conventional milk and pasture milk, whereas four masses were different between organic and pasture milk. Therefore, in general, pasture milk shows more similarity to conventional milk. These results are in line with those reported by Capuano et al. (2015), who studied the FA and triglyceride (TAG) profile of organic milk, conventional milk and pasture milk. The FA and TAG profiles of pasture milk and conventional milk were also highly similar. They concluded that this phenomenon was observed for the following reasons: (1) the flexible rules for pasture milk are not enough to generate differences; (2) the mixture of milks into retail milk can conceal the small differences between these two kinds of milk, since rules only specify the total outdoors time instead of the exact period, and the mixture in retail milk could possibly consist of indoors milk and outdoors milk at the same time; (3) some conventional cows could also graze outside or be fed with fresh grass.

To identify the tentative chemical formula of the characteristic ions, PTR-(ToF)MS analysis was carried out. Thanks to the higher resolution, the exact mass value could be measured after which the compounds were further tentatively identified with the help of related references (Table 2).

Masses $m/z$ 67, 69 and 70 showed significantly higher concentrations in organic milk. From the PTR-(ToF)MS it appears that these masses represent terpenes or fragments of terpenes. Terpenes are a class of compounds which are specifically present in plants (Tornambé et al., 2006) and can remain stable during metabolism in the cow (Ueda, Asakuma, Miyaji, & Akiyama, 2015). The relationship between the concentration of terpenes and milk production systems has been reported before by Abilleira et al. (2011). Combined with the results in our research, these compounds have the potential to be characteristic traits or markers for organic milk, or at least for outdoor grazing.

Based on the results above, it can be concluded that, with the help of PTR-(ToF)MS, the characteristic and discriminating VOCs for the production systems have been elucidated and tentatively identified.

### 3.2. Effect of the seasons

Twenty-two masses showed significant differences between the summer and winter milks (Tables 2 and 3), i.e. considerably more than the number of masses showing differences between the production systems (six masses). This is probably related to the greater differences in feeding strategies between winter and summer. During winter, there are higher proportions of silage, hay and grain fed, while in summer, more fresh grass is available for feeding (Biolatto et al., 2007). Actually most of the cows are fed with silage in winter in the Netherlands because of the shortage of fresh grass. In winter time, the main difference between organic and conventional/pasture cow feed is that the latter will contain more concentrates and the silage will comprise a higher proportion of maize. This leads to some differences, but they are smaller than

**Table 1**
Masses showing significant differences in abundance for organic, pasture, and conventional milks analyzed by PTR-(Quad)MS: mean volume mixing ratios (ppbv), standard deviation, and p value (ANOVA)[a].

| Mass ($m/z$) | Organic (n = 10) | Pasture (n = 20) | Conventional (n = 14) | P value[b] |
|---|---|---|---|---|
| 47 | 11 ± 9[b] | 5 ± 3[a] | 4 ± 2[a] | 0.03 |
| 48 | 0.3 ± 0.2[b] | 0.2 ± 0.1[a] | 0.1 ± 0.1[a] | 0.03 |
| 67 | 0.5 ± 0.1[b] | 0.4 ± 0.1[a] | 0.3 ± 0.1[a] | 0.03 |
| 69 | 21 ± 6[b] | 19 ± 6[a] | 13 ± 3[a] | 0.03 |
| 70 | 1.2 ± 0.3[b] | 1.1 ± 0.4[b] | 0.8 ± 0.2[a] | <0.01 |
| 109 | 0.3 ± 0.1[c] | 0.2 ± 0.1[b] | 0.1 ± 0.1[a] | <0.01 |

[a] Different letters in the same row indicate significant differences (Fisher's LSD test, $p < 0.05$).
[b] Adjusted according to Benjamini-Hochberg (BH).

**Table 2**
Masses showing significant differences in abundance between winter and summer milks analyzed by PTR-(Quad)MS: mean volume mixing ratios (ppbv), standard deviation, and p value (ANOVA).

| Mass ($m/z$) | Summer (n = 22) | Winter (n = 22) | P value[a] |
|---|---|---|---|
| 33 | 130 ± 50 | 90 ± 30 | <0.01 |
| 35 | 0.3 ± 0.1 | 0.2 ± 0.1 | 0.02 |
| 42 | 9 ± 4 | 16 ± 6 | <0.01 |
| 43 | 40 ± 7 | 31 ± 6 | <0.01 |
| 44 | 1.2 ± 0.3 | 1.0 ± 0.2 | <0.01 |
| 51 | 2.0 ± 0.6 | 1.4 ± 0.5 | <0.01 |
| 63 | 2 ± 1 | 2 ± 1 | 0.05 |
| 71 | 5 ± 1 | 4 ± 1 | 0.02 |
| 73 | 130 ± 60 | 220 ± 110 | 0.01 |
| 74 | 6 ± 3 | 10 ± 5 | 0.01 |
| 84 | 0.4 ± 0.1 | 0.3 ± 0.1 | 0.05 |
| 85 | 4.9 ± 0.7 | 3.0 ± 0.4 | <0.01 |
| 86 | 0.3 ± 0.1 | 0.2 ± 0.0 | <0.01 |
| 87 | 6 ± 1 | 5 ± 1 | 0.05 |
| 93 | 2.3 ± 0.5 | 1.5 ± 0.4 | <0.01 |
| 94 | 0.2 ± 0.1 | 0.1 ± 0.1 | 0.02 |
| 101 | 1.6 ± 0.5 | 0.9 ± 0.5 | <0.01 |
| 107 | 1.4 ± 0.3 | 1.1 ± 0.2 | <0.01 |
| 111 | 1.0 ± 0.2 | 0.8 ± 0.2 | <0.01 |
| 113 | 0.3 ± 0.1 | 0.2 ± 0.1 | <0.01 |
| 127 | 0.8 ± 0.1 | 0.7 ± 0.1 | <0.01 |
| 129 | 0.5 ± 0.1 | 0.3 ± 0.1 | <0.01 |

[a] Adjusted according to Benjamini-Hochberg (BH).

the impact of the difference between fresh grass (summer feed) and silage (winter feed) on the VOC composition of the milk.

Among the 22 significant differing masses, there are 14 masses with concentrations higher than 1ppbv, tentatively identified as 10 different compounds (Table 3), excluding fragments and isotopologues. Data on cow's milk are not always available, therefore, also data on other dairy products are evaluated. The compound 2-butanone (mass $m/z$ 73) is the major compound showing significant differences between summer and winter milks. Since the concentration of 2-butanone is higher in milk derived from silage and lower in the milk derived from fresh grass, it reflects the higher usage of silage during winter (Croissant, Washburn, Dean, & Drake, 2007). Rios, Fernández-García, Mínguez-Mosquera, and Pérez-Gálvez (2008) reported that toluene (mass $m/z$ 93) and benzaldehyde (mass $m/z$ 107) could be generated by the degradation of carotenoids. In our research, the higher concentrations of toluene and benzaldehyde could be explained by the larger amount of carotenoids in fresh grass during summer time, with similar results by Sivadier, Ratel, and Engel (2010). According to previous researches, the concentration of hexanone (mass $m/z$ 101) in cheese decreased when silage was part of the ruminant diet (M Bergamaschi, Aprea, et al., 2015;; Muñoz, Ortigosa, Torre, & Izco, 2003). Butenal (mass $m/z$ 71) is also evidenced as diet tracer of pasture fed lambs (Sivadier, Ratel, Bouvier, & Engel, 2008; Sivadier et al., 2010), but Vasta and Priolo (2006) reported these compounds could be derived from the oxidation of lipid, which could also have happened during retail milk processing and storage. In this case, given the higher concentrations of butenal in summer milk in the study, no conclusion can be drawn for the relationship between these compounds and summer diet. There are two additional compounds which show significantly higher concentrations in winter milk acetonitrile ($m/z$ 42) and dimethyl-sulfide ($m/z$ 63). Since the winter diet comprises higher contents of concentrates (Heck, van Valenberg, Dijkstra, & van Hooijdonk, 2009), the concentrations of crude protein, casein, true protein and whey protein in winter milk are significantly higher than the

**Table 3**
Tentative identification of VOCs by PTR-(ToF)MS.

| Mass measured in PTR-(Quad)MS ($m/z$) | Mass measured in PTR-(ToF)MS | Protonated chemical formula | Tentative identification | Reference[a] |
|---|---|---|---|---|
| 33 | 33.034 | $CH_4OH^+$ | Methanol | 1 |
| 35 | 35.037 | $CH_4^{18}OH^+$ | Methanol | 1 |
| 42 | 42.034 | $C_2H_4N^+$ | Acetonitrile | 1 |
| 43 | 43.054 | $C_3H_6H^+$ | Alkyl fragment | 1, 2 |
| | 43.017 | $C_2H_2OH^+$ | Ester fragment | 2 |
| 44 | 44.021 | $^{13}CCH_2OH^+$ | Ester fragment | 2 |
| 47 | 47.012 | $CH_2O_2H^+$ | Formic acid | 2 |
| | 47.049 | $C_2H_6OH^+$ | Ethanol | 3 |
| 48 | 48.052 | $^{13}CCH_6OH^+$ | Ethanol | 3 |
| 51 | 51.044 | NI[b] | | – |
| 63 | 63.025 | $C_2H_6SH^+$ | Dimethyl-sulfide | 1, 4 |
| 65 | 65.060 | $C_2H_8O_2H^+$ | Hydrated ethanol | 5 |
| 67 | 67.054 | $C_5H_6H^+$ | Pentenal fragment(terpene) | 2 |
| 69 | 69.070 | $C_5H_8H^+$ | 2-Methyl-1,3-butadiene(isoprene) | 1 |
| 70 | 70.073 | $^{13}CC_4H_8H^+$ | Isoprene | 1 |
| 71 | 71.049 | $C_4H_6OH^+$ | Butenal | 2, 3, 6 |
| | 71.085 | $C_5H_{10}H^+$ | 3-Methyl-1-butanol | 3 |
| 73 | 73.065 | $C_4H_8OH^+$ | 2-Butanone | 3, 4, 6, 7 |
| 74 | 74.067 | $^{13}CC_3H_8OH^+$ | 2-Butanone | 3, 4, 6, 7 |
| 84 | 84.088 | $^{13}CC_5H_{10}H^+$ | Hexanal fragment | 4, 7 |
| 85 | 85.100 | $C_6H_{12}H^+$ | Hexene | 7 |
| 86 | 86.104 | $^{13}CC_5H_{12}H^+$ | Hexene | 7 |
| 87 | 87.079 | $C_5H_{10}OH^+$ | Pentanone/pentanal | 4, 7 |
| 93 | 93.070 | $C_7H_8H^+$ | Toluene | 7 |
| 94 | 94.073 | $^{13}CC_6H_8H^+$ | Toluene | 7 |
| 101 | 101.094 | $C_6H_{12}OH^+$ | Hexanal/hexanone | 3, 4, 5, 7, 9 |
| 107 | 107.050 | $C_7H_6OH^+$ | Benzaldehyde | 4 |
| 109 | 109.071 | $C_8H_{12}H^+$ | 2,6-Dimethyl pyrazine | 8 |
| 111 | 111.081 | $C_7H_{10}OH^+$ | 2,4-Heptadienal | 4 |
| 113 | 113.095 | $C_7H_{12}OH^+$ | Heptenal | 4, 7 |
| 127 | 127.112 | $C_8H_{14}OH^+$ | 1-Octen-3-one | 4 |
| 129 | 129.127 | $C_8H_{16}OH^+$ | Octanone | 6 |

[a] 1. Galle et al. (2011); 2. Buhr, van Ruth, and Delahunty (2002); 3. Valero, Villamiel, Miralles, Sanz, and Martínez-Castro (2001); 4. Villeneuve et al. (2013); 5. Veronika et al. (2014); 6. Routray and Mishra (2011); 7. Pereda et al. (2008); 8. Bergamaschi, Aprea, et al., 2015; Bergamaschi, Biasioli et al., 2015; 9. Muñoz et al. (2003);
[b] NI: not identified.

concentrations in summer milk (Křížová, Hanuš, Roubal, Kučera, & Hadrová, 2013). The higher protein content in the diet may result in elevated concentrations of compounds derived from protein degradation (Forss, 1979), such as the compounds mentioned, i.e. acetonitrile and dimethyl sulfide (Muñoz et al., 2003).

### 3.3. Interactions of production systems and seasons

Two-way ANOVA was performed to investigate the interaction of systems and seasons. According to the results of two-way ANOVA, the concentrations of four masses were significantly influenced by both systems and seasons, i.e. methanol (*m*/*z* 35), acetonitrile (*m*/*z* 42), isoprene (*m*/*z* 70) and pentanone/pentanal (*m*/*z* 87). The contribution of the production system and seasonal factors were subsequently evaluated. System percentages of the total square sum of variance of these four masses amounted 45, 39, 68 and 47% respectively. Similarly for the seasonal effect, percentages were 54, 60, 21 and 40% respectively. The contribution of the production system was more pronounced for isoprene (*m*/*z* 70).

The masses above showed no significant interactions of the two factors though. Among all 132 masses, two masses presented significant production system x season interactions. Formic acid/ethanol (*m*/*z* 47) and hydrated ethanol (*m*/*z* 65) showed significant different effects of the systems during summer and winter. Their abundances are shown in Fig. 3. During winter, the measured concentrations were significantly higher for organic milk, whereas in summer no significant influence of the systems was observed ($P > 0.05$). This is probably due to different feeding strategies in summer and winter seasons. In summer more similarities in grass feeding may exist, either by grazing or supplied in the farm. In winter time in conventional farms the concentrate proportion may be higher and the grass proportion in silage may be lower than in organic farms resulting in these consistent differences in VOC concentrations. The two masses appear to be unique markers for organic milk in winter time, but are not useful for substantiation or discrimination of organic summer milks.

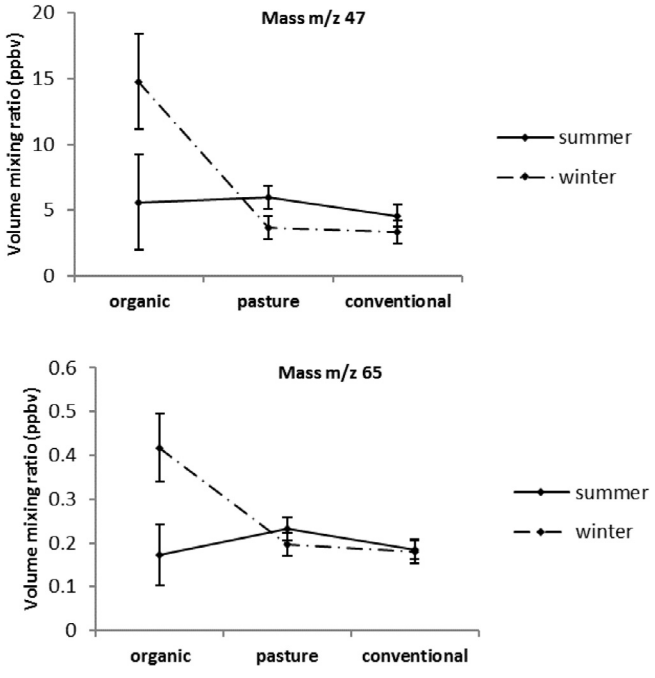


**Fig. 3.** Masses identified in PTR-(Quad)MS analysis showing significant interactions of the production system factor and the seasonal factor.

## 4. Conclusion

The study has demonstrated the different volatile profiles of organic, pasture and conventional milk during winter and summer. Terpenes and their fragments appear important markers for organic milks. Organic and conventional milks show substantial differences in VOC composition, whereas pasture milks show an intermediate pattern, being more similar to organic in summer time and more similar to conventional in winter time, reflecting their seasonally changing feed strategy. Organic milks differ more distinct in VOC composition from conventional and pasture milks in winter time due to the more distinct feeding regimes. The VOC markers are useful for future development of methods for the authentication of organic milks.

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