JARQ 52 (2), 123 - 129 (2018) https://www.jircas.go.jp

REVIEW

Effect of Pasture Intake on the Profile of Volatile Organic Compounds in Dairy Cow Milk

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Abstract

The use of pasture grazing systems offers a means to improve feed self-sufficiency and reduce labor for dairy farmers. Consumers also are interested in milk and dairy products obtained from cows on pasture-based diets. However, little is known about the difference between milk obtained from grazing cows and general milk obtained from cows on an indoor feeding system. There is almost no information about the flavor constituents, whereas many studies have been conducted on such characteristics as the fatty acid composition, conjugated linoleic acids, and carotenoids of milk from grazing cows. In this review, we describe studies that have characterized the volatile organic compounds (VOCs) in milk and our investigations on the effects of pasture intake on VOCs in milk. The VOC, 1-phytene has been used to characterize milk from grazing cows as there is a strong positive correlation between pasture intake and the concentration of 1-phytene in milk. In addition, milk from cows in a grazing system can be distinguished from that of animals in an indoor feeding system using the levels of 1-phytene, γ-dodecalactone, 2-phytene, neophytadiene, and δ-dodecalactone.

Discipline: Animal industry
Additional key words: feeding system, 1-phytene, traceability

Introduction

The use of pasture grazing systems is one of the feeding styles adopted to improve feed self-sufficiency and reduce labor for dairy farmers. The milk from grazing cows (known as “grazing milk”) is produced and sold as a special type of milk by some milk producers and dairy farmers. Consumers are becoming increasingly concerned about the sources of their food and the techniques used to raise animals for consumption. The relationship between feeding system and dairy food composition is of central importance to such concerns (Aizaki et al. 2013, Stergiadis et al. 2012). Therefore, it is important to inform consumers about the specific characteristics of milk from grazing cows. Many studies have been conducted to examine the differences in milk obtained from grazing cows and that obtained from cows fed conserved forage and concentrates. These studies have measured various characteristics such as fatty acid composition (Coppa et al. 2013, Shingfield et al. 2013), conjugated linoleic acids (Chilliard et al. 2007, Dewhurst et al. 2006, Elgersma et al. 2006), and carotenoids (Noziere et al. 2006). Regarding the volatile organic compounds (VOCs) of milk, several European groups have evaluated the plant-specific volatile compounds (Cornu et al. 2001, Mariaca et al. 1997, Tornambe et al. 2006) with the aim of identifying milk and cheese of mountain origin. VOCs are the main flavor constituents of milk, and can influence the sensory properties of milk. To date, however, no studies have evaluated the influence of the duration of grazing or pasture intake on the VOC profile of milk in Japan. Thus, an investigation of the relation between the duration of grazing or the amount of pasture intake of grazing cows might be useful for the traceability of milk and dairy products.

In this review, we first introduce a method of estimating the pasture intake of grazing cows. Then we provide an overview of studies that have investigated the characteristics of VOCs in milk from grazing cows relative to grazing period and pasture intake. Finally, we address the possibility of utilizing the VOC profile to trace the animal feeding system used in milk production.

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Received 14 February 2017; accepted 1 August 2017.

Estimating the pasture intake of cows

Dry matter intake (DMI) is an important variable for best managing the nutrition of lactating cows. However, it is more difficult and less accurate to determine the DMI of grazing cows than that of cows in confinement systems (Bargo et al. 2003). Moreover, an estimation of pasture intake (PI) is necessary for clarifying the influence of animal feed on the VOCs of milk. Using grazing behavior data, pasture intake can be expressed as the product of daily eating time (min/d), biting rate (bites/min), and bite mass (gDM/bite; Rook 2000). In particular, total daily eating time is a key constraint on DMI, and can substantially change to compensate for DMI according to forage availability (Iason et al. 1999); therefore, daily grazing time can be used as an index of foraging DMI. Grazing time can be measured visually or automatically (Forbes 1988), though visual measurement is more labor-intensive. And although devices for measuring eating time have been developed since the 1950s (Balch 1952), those devices have yet to be extensively applied, owing to issues of expense, operational difficulties, or lack of development beyond the trial product stage (Ungar and Rutter 2006).

Rutter et al. (1997) reported that when an automatic microcomputer-based system was used to record the jaw movements of grazing cattle and sheep, the overall concordance rate in the grazing time measured by manual observers and the automatic system was 91.0%. Ueda et al. (2011) used a uniaxial acceleration monitoring system that was developed as a commercially available tool to measure grazing time. The device can record the intensity of physical activity, measured as the activity level (AL) on a scale of 0-9, at 4-s intervals, and when AL_1 was used as a threshold for discriminating eating from other activities (eating ≥ AL_1), the lowest misclassification rate observed was 5.5%. Eating activity includes various dynamic movements such as biting, chewing, and neck (head) shaking. Due to these dynamic movements, high activity levels (≥AL_1) were recorded at the neck. Biting (pulling grass) is considered the most frequent movement in eating activity, and resulted in a high level of neck movement due to the resistance of living plants. Therefore, it may be possible to estimate the PI of cows using the combination of grazing time and herbage mass in a pasture before and after grazing.

Characteristics of volatile organic compounds in milk from grazing cows

Several reports on the volatile organic compounds (VOCs) in milk have shown that terpenoids are present in milk and/or cheese from grazing cows (Coppa et al. 2011, Favaro et al. 2005, Fernandez et al. 2003, Tornambe et al. 2006, Viallon et al. 2000). Terpenoids are a group of plant-specific compounds that originate almost exclusively from the secondary metabolism of plants (Tornambe et al. 2006).

We extracted VOCs from milk by steam distillation-extraction, which were analyzed using gas chromatography-mass spectrometry and the obtained profiles were compared between milk obtained during the indoor season (April) and that obtained during the grazing season (June). Among the 22 compounds identified, 1-phytene (3,7,11,15-tetramethyl-1-hexadecene), 2-phytene (3,7,11,15-tetramethyl-2-hexadecene), phytane, and neophytadiene were found at higher levels in grazing milk than in indoor milk (Fig. 1). In particular, the levels of 1-phytene and 2-phytene were found to be significantly higher ($P < 0.001$) in grazing milk. Both 1-phytene and 2-phytene are products of the hydrogenation of neophytadiene in the rumen (Urbach & Stark 1975, Urbach 1990), and classified as diterpenoids (C20). Neophytadiene is derived from phytol, which is a product of the degradation of chlorophyll a and chlorophyll b (Kräutler and Matile 1999) via hydrogenation in the rumen. Further hydrogenation of 1-phytene produces phytane (Larick et al. 1987). Urbach and Stark (1975) also isolated neophytadiene from ryegrass, and suggested that it was the precursor of 1-phytene in butterfat. Povolo et al. (2009) reported that milk and cheese samples from cows living in mountainous areas were characterized by higher amounts of 1-phytene, neophytadiene, and 2-phytene than those from cows living on the plains, as attributed to a diet richer in fresh herbage in the mountainous areas than on the plains.

Moreover, monoterpenes (C10) and sesquiterpenes (C15) were not detected in our study, as reported by several other groups. The amount or variety of these terpenoids (C10 and C15) detected from milk and dairy products appears to vary according to the altitude of the pasture (Fernandez et al. 2003), vegetation (Viallon et al. 2000, Tornambe et al. 2006, Coppa et al. 2011), and season (Tornambe et al. 2006), consequently, both were considered tracers of the feeding of animals (Viallon et al. 2000). These terpenoids are enriched in dicotyledonous plants but barely present in gramineous plants, and various dicotyledonous plants are generally more abundant in highland pastures than in lowland pastures (Mariaca et al. 1997). Terpenes are abundant in plants of the families Apiaceae, Lamiaceae, or Asteraceae in mountainous areas, whereas the terpene content is low in most Poaceae species (Tornambe et al. 2006).

One possible reason for the detection of only diterpenoids from the milk in our study might be related to the fact that these cows were fed on a gramineous meadow—the most common type of meadow in Japan. Moreover, the few quantitative changes of diterpenoids in grazing milk according to season might reflect the nature of the pasture, which was not a natural meadow with high biodiversity.

Collectively, these reports described above suggest

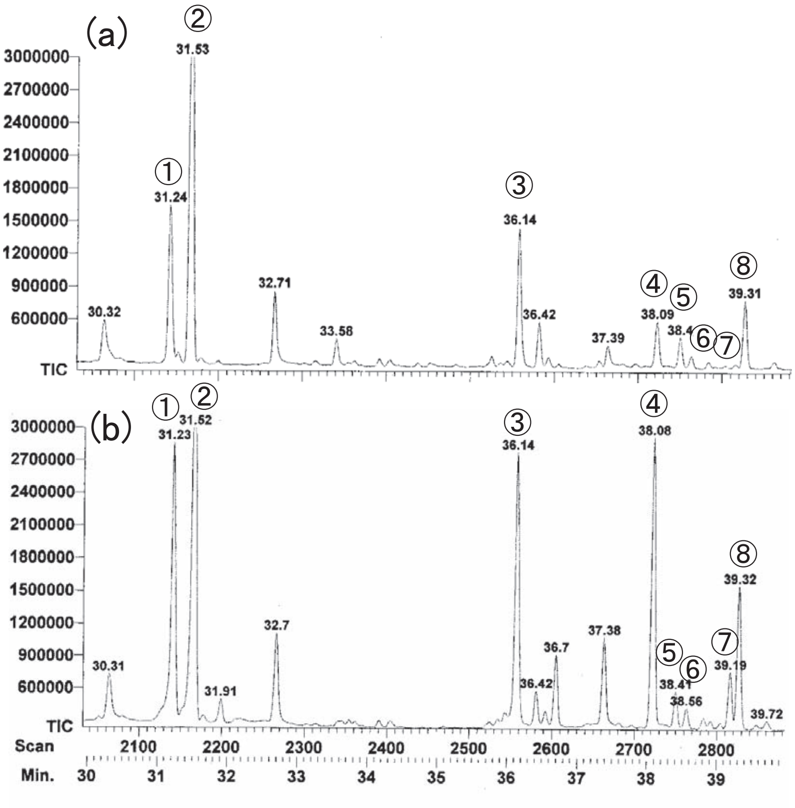

Fig. 1. Typical gas chromatography-mass spectrometry (GC-MS) profiles of volatile compounds of milk from the indoor period (a) and grazing period (b)
1. 2-tridecanone, 2. BHT (internal standard), 3. 2-pentadecanone, 4. 1-phytene, 5. octadecane, 6. phytane, 7. neophytadiene, 8. 2-phytene.

that diterpenoids are the characteristic VOCs of grazing milk from cows fed on a gramineous pasture, although all terpenoids containing monoterpenoids, sesquiterpenoids, and diterpenoids in grazing milk originate from herbage.

Effect of grazing period and pasture intake on the diterpenoids in milk

As described above, diterpenoids, especially 1-phytene and 2-phytene, are the characteristic VOCs in grazing milk. However, there have been no studies on the influence of the grazing period or PI on the VOC profile of milk in Japan. There are various grazing styles depending on the pasture area, grazing period, or amount of supplemental feed provided. An investigation of the quantitative relations between the intensity of diterpenoids in milk and the grazing period or pasture intake of grazing cows might be useful for the traceability of milk and dairy products, and could provide valuable information about the feeding system for consumers.

In a previous study, we measured the VOC profile of milk from grazing and indoor cows, and investigated the effect of the grazing period on the VOC profile (Ueda et al. 2016). Eight Holstein cows were assigned to four treatment groups according to the time spent at pasture: ID, 0 h/day; G4, 4 h/day; G8, 8 h/day; and G20, 20 h/day. Grazing treatment affected the levels of 2-tridecanone, 2-pentadecanone, γ-dodecalactone, 1-phytene, phytane, neophytadiene, and 2-phytene (Table 1). Among these compounds, the levels of 1-phytene and 2-phytene increased markedly with an increase in grazing time. However, there was no significant difference in the relative amounts of 1-phytene, 2-phytene, and neophytadiene in milk in the ID and G4 groups. As the ID cows were also fed grass silage as the forage feed along with concentrate, the grass silage might have affected the amounts of diterpenoids in the milk. However, the milk obtained from cows in a grazing system can be distinguished from that obtained using an indoor system based on the difference in the amount of 1-phytene, provided that grazing time of 8 h/day or more is used (Fig. 2).

Ueda et al. (2011) measured herbage mass in a pasture before and after grazing, and also measured the time each cow spent eating. A simple regression analysis of PI relative to each diterpenoid identified revealed a strong correlation between the amount of 1-phytene in the milk and PI (Fig. 3; r = 0.807, $P < 0.001$). This finding indicated that the intent

Table 1. Composition and mean levels of volatile compounds of milk. (% of BHT peak area) †

LRI §	Compound	Identification method ¶			Treatment ‡ ID (n = 8)	G4 (n = 8)	G8 (n = 8)	G20 (n = 8)
					Means of level (% of BHT peak area)			
	Aldehydes							
798	hexanal	S	L		2.95	1.04	2.76	3.32
1004	octanal	S	L	AC	1.60	1.60	1.65	2.36
1105	nonanal	S	L	BC	42.00	21.57	36.00	38.15
1166	2-nonenal		L	AC	0.15	0.00	0.07	0.23
1617	tetradecanal		L	A	0.66	0.40	0.19	0.22
1819	hexadecanal		L	A	1.26	1.00	1.11	1.45
	Ketones							
885	2-heptanone	S	L	A	44.32	34.83	40.57	41.55
992	2-octanone	S	L	A	1.05	0.49	1.03	1.91
1091	2-nonanone	S	L	B	28.49	22.91	27.51	27.82
1296	2-undecanone	S	L	B	27.71	16.52	21.96	23.18
1497	2-tridecanone	S	L		25.39	13.03 *	17.11	18.18
1699	2-pentadecanone		L		21.40	9.68 **	12.74 *	12.37 *
	Acids							
1071	heptanoic acid	S	L		0.44	0.18	0.62	0.45
1364	decanoic acid	S	L		3.37	11.16	13.97	13.26
1569	dodecanoic acid	S	L		3.70	10.31	12.40	12.52
1761	tetradecanoic acid	S	L		14.65	11.78	12.26	19.16
1964	hexadecanoic acid	S	L	A	4.48	0.87	3.14	11.20
	Lactones							
910	γ-butyrolactone	S	L	A	2.14	3.49	4.05	4.60
1488	γ-decalactone	S	L	A	0.96	0.54	1.20	1.29
1507	δ-decalactone	S	L	C	2.30	2.62	3.31	3.26
1690	γ-dodecalactone	S	L	C	2.57	1.24 **	1.31 **	1.70 *
1720	δ-dodecalactone	S	L	C	8.47	12.62	10.74	8.16
	Hydrocarbones							
1600	hexadecane	S	L	A	0.85	0.47	0.58	0.64
1800	octadecane	S	L	A	1.95	1.53	1.46	2.12
	Diterpenoides							
1784	1-phytene			AC	28.44	36.72	50.26 ***	65.38 ***
1807	phytane	S	L	AC	0.94	0.45 *	0.64	0.74
1838	neophyadiene		L	AC	1.53	1.43	1.84	2.85 *
1843	2-phytene		L	C	8.81	11.78	11.88	17.66 ***

Means between ID and each grazing treatment with different superscripts differ (*, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$). Modified data from Ueda et al. (2016)

† Level expressed by GC-FID peak area relative to that of 600 ng of tert-buthylhydroxytoluene in 100 mL of milk as internal standard.

‡ ID, indoor and non grazing; G4, 4 h/d spent at pasture; G8, 8 h/d spent at pasture; G20, 20 h/d spent at pasture.

§ LRI (linear retention index) was calculated using a series of standard *n*-alkanes (C7-C20).

¶ Identification by authentic compounds (S), library (L), or other published data (A, King et al.(1993); B, Moio et al. (1996); C, Watanabe et al. (2008)).

of 1-phytene in grazing milk reflects the amount of grass consumed by cows feeding on a pasture. Viallon et al. (2000) reported that monoterpenes could only be detected in milk at 8 h after ingestion of terpene-rich forage, and sesquiterpenes appeared in milk at 32 h after ingestion. This suggests that terpenoids in forage might move into the milk with few metabolic changes in the body of the cow.

These results clarified that the intensity of 1-phytene in milk increased with grazing period per day, and if the grazing period is 8 h/day or more, grazing milk can be distinguished from indoor milk by the difference in the amount of 1-phytene in the milk. Moreover, the 1-phytene content in

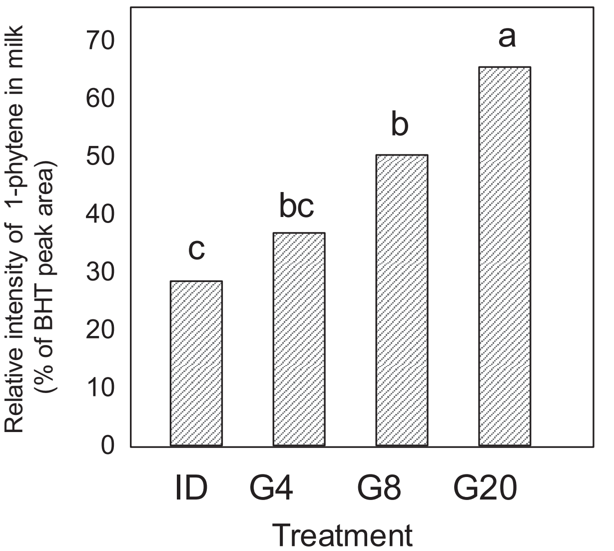

Fig. 2. Relative intensity of 1-phytene in milk of each grazing treatment
ID, indoor and non-grazing; G4, 4 h/d spent at pasture; G8, 8 h/d spent at pasture; G20, 20 h/d spent at pasture. a,b,c, mean values with different letters are significantly different (P < 0.05).

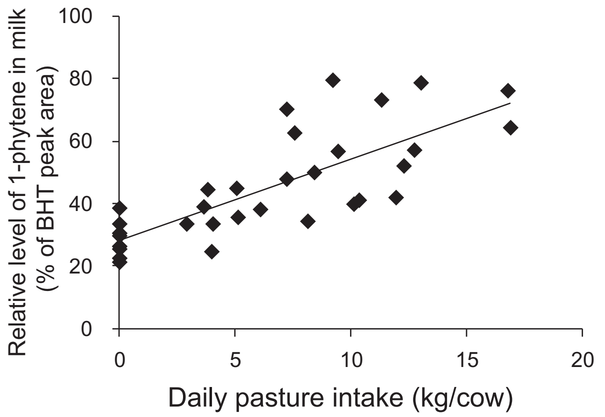

Fig. 3. Correlation between the relative intensity of 1-phytene in milk and daily pasture intake (r = 0.807, P < 0.001)

milk could be a useful quantitative marker of the PI of grazing cows, given the significantly positive correlation between the amount of 1-phytene in milk and daily PI.

Distinguishing between feeding systems using volatile organic compound profiles of milk

Several studies have examined the traceability of milk products and the ability to discriminate among feeding systems using the VOC profiles of milk. Toso et al. (2002) reported that it was possible to distinguish diet compositions using nine volatile compounds of milk. Coppa et al. (2011) reported that the VOCs of milk were affected by feeding and grazing systems, and that a multivariate analysis of VOCs provided reliable discrimination among the feeding systems. Fernandez et al. (2003) showed that sesquiterpenes (δ-elemene, β-bourbonene, β-caryophyllene, β-chamigrene, γ-cadinene, and β-sesquiphellandrene) in milk could be used to distinguish between two dairy production areas, based on both geographical origin and the seasonal herd management pattern.

Ueda et al. (2016) could not distinguish between a grazing time of 4 h/day and an indoor (non-grazing) system using only the content of 1-phytene; however, a strong correlation was found between 1-phytene in milk and PI (as described above). Therefore, we used a discriminant analysis on the VOC content of milk from cows under an indoor (n = 20) or grazing (n = 14 for 4 h/day, n = 8 for 8 h/day, and n = 20 for 20 h/day) system (Ueda et al. unpublished data). In a partial least-squares regression analysis, we used PI (kg/day) as the response variable (Y-axis) and all 28 VOCs as the explanatory variables (X-axis). Variable importance for projection scores (in Fig. 4) showed that 10 VOCs (including diterpenoids) made a positive contribution, and that 18

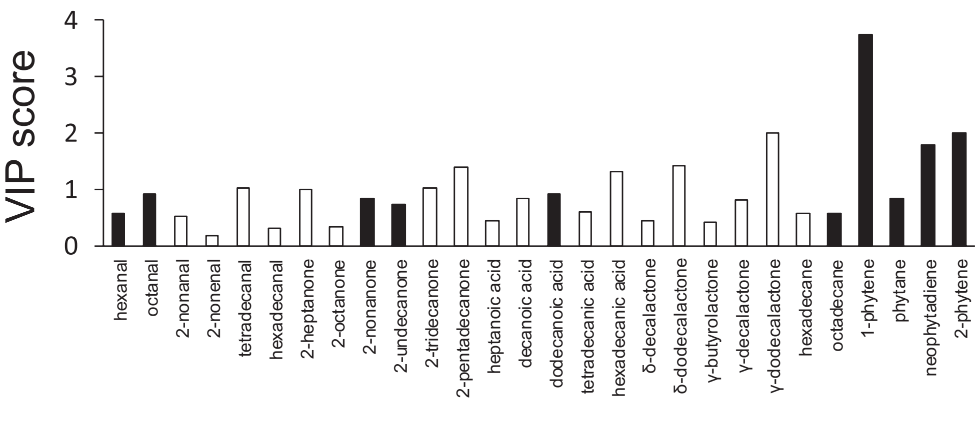

Fig. 4. Partial least-squares regression to identify factors with high variable importance for projection (VIP) scores of volatile compounds for pasture intake
Relationships between volatile compounds and herbage intake are shown as positive (■) or negative (□).

Table 2. Results of discriminant analysis for raw milk samples from indoor or grazing cows, using volatile compounds as classification criteria

Discriminant variables in the model	Milk samples in the correct group		Milk samples misplaced		% Correctly classified
	Indoor §	Grazing §	Indoor	Grazing	
1-phytene	20	32	10	-	83.87
1-phytene, γ-dodecalactone	19	37	5	1	90.32
1-phytene, γ-dodecalactone, 2-phytene	20	36	6	-	90.32
1-phytene, γ-dodecalactone, 2-phytene, noephytadiene	20	36	6	-	90.32
1-phytene, γ-dodecalactone, 2-phytene, noephytadiene, δ-dodecalactone	19	39	3	1	93.55
1-phytene, γ-dodecalactone, 2-phytene, noephytadiene, δ-dodecalactone, 2-pentadecanone	19	37	5	1	90.32

§ A total of 62 cows' data were used for discriminant analysis; Indoor (n = 20), Grazing (4 h/day (n = 14), 8 h/day (n = 8), and 20h/day (n = 20)).

VOCs (including lactones) made a negative contribution to PI. The most important VOC was 1-phytene (+3.747), followed by γ-dodecalactone (–1.989), 2-phytene (+1.982), neophytadiene (–1.792), δ-dodecalactone (–1.418), and 2-pentadecanone (–1.394). These VOCs were selected as covariates for a discriminant analysis of milk samples from indoor or grazing cows (Table 2). The rate of correct classification was 93.55% when 1-phytene, γ-dodecalactone, 2-phytene, neophytadiene, and δ-dodecalactone were used for the discriminant analysis, although it was 83.87% when only 1-phytene was used.

Diets that induce propionate metabolism in the rumen cause the formation of sweet-flavored compounds γ-dodecalactone and γ-dodec-cis-6-enolactone (Urbach 1990). Stark et al. (1978) also reported a marked increase in the γ-dodecalactone potential when cows were transferred from a pasture diet to that with hay and crushed oats. Keen (1998) showed that a European-type diet consisting largely of concentrates (with both starch and fatty acid precursors, i.e., grain, meals, among others) promoted the formation of γ-lactone precursors in the rumen. In addition, Ueda et al. (2014) reported that milk from cows fed corn silage had a high content of lactones. Thus, it seems that the amount of concentrate or corn silage in the feed affects the content of lactones in the milk.

From these results and reports, it can be concluded that an indoor system could be distinguished from a grazing system using the quantities of 1-phytene, γ-dodecalactone, 2-phytene, neophytadiene, and δ-dodecalactone in milk, although distinguishing between milk from an indoor system and 4 h-grazing was impossible when using only diterpenoids.

Conclusion

Studies on VOCs in milk revealed that 1-phytene derived from chlorophyll in pasture plants was the characteristic VOC in the milk of grazing cows. In addition, a strong positive correlation was identified between the amount of 1-phytene in milk and PI. These results show that 1-phytene is a useful marker for estimating PI in cows. Furthermore, it is possible to distinguish products from cows in a grazing or indoor feeding system by quantifying the appropriate combination of diterpenoids from the pasture and the content of lactones from the concentrate. Thus, VOCs in milk are of value for tracing the origin of milk and dairy products.

As VOCs affect the flavor of foods, their effect on the palatability of milk remains a matter for further study.

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