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Effect of different forage types on the volatile and sensory properties of bovine milk

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ABSTRACT

The effect of 3 diets (grass, grass/clover, and total mixed ration) on the volatile and sensory properties of bovine milk was assessed over an entire lactation season. Little evidence was found of direct transfer of terpenes into raw milk from the different diets, and it is likely that the monocultures of ryegrass used with and without white clover were factors as these contained very few terpenes. Evidence of direct transfer of nonterpene volatiles from forage to the subsequent raw milks was probable; however, differences in the protein carbohydrate availability and digestion in the rumen appeared to have a greater contribution to volatile profiles. Pasteurization significantly altered the volatile profiles of all milks. A direct link between the milk fatty acid content, forage, and volatile products of lipid oxidation was also evident and differences in fatty acid content of milk due to forage may also have influenced the viscosity perception of milk. Irish sensory assessors preferred pasteurized milk produced from grass-fed cows, with least preference from milk produced from total mixed ration diets. β-Carotene content was significantly higher in milks derived from grass or grass/clover and appears to have directly influenced color perception. Toluene and p-cresol are both degradation products of β-carotene and along with β-carotene were identified as potential biomarkers for milk derived from pasture. The only correlation that appeared to influence the flavor of milk as determined using ranked descriptive analysis was p-cresol. P-Cresol appears to be responsible for the barnyard aroma of milk and is also likely derived from the deamination and decarboxylation of tryptophan and tyrosine due to the higher levels of available protein in the grass and grass/clover diets. The highest levels of p-cresol were in the grass/clover diets and are likely due to the degradation of the isoflavone formononetin in the rumen, which is present in white clover swards.

Key words: milk, forage, sensory, volatile

INTRODUCTION

Products derived from cows grazing natural swards compared with those fed with preserved forages have added value among food producers and consumers because of their perceived healthiness and environmental acceptability. Bovine milk composition and flavor variations have been attributed to feed, seasonal variation, and breed (Bendall, 2001; Croissant et al., 2007; Larsen et al., 2013; Vanbergue et al., 2017). Badings and Neeter (1980) suggested that the aroma of milk is determined by many volatile compounds sometimes present in very low concentrations, some transferred from the feed, and others the result of minor conversions of milk constituents by chemical (oxidative or thermal), microbial, and enzymatic reactions. Volatile compounds in forage and feed enter milk through 2 routes: the main route is being absorbed in the digestive tract (i.e., rumen and or intestine) before diffusing into the blood and then reaching the mammary gland. The second route is the pulmonary route, where volatiles diffuse into the air and are inhaled by the cow, absorbed into the lungs, enter the blood stream, and subsequently diffuse into the mammary gland (Viallon et al., 2000). Conflicting results on the effect of different forage types on milk flavor exist. Using descriptive analysis, both Croissant et al. (2007) and Khanal et al. (2005) found significant differences in milk flavor based on diet (pasture vs. conventional TMR), but also found that differences were not perceived in consumer acceptance trials using untrained consumers. Shingfield et al. (2005) carried out descriptive sensory analysis and found that different silage and hay diets had no sensory effect on pasteurized bovine milk, whereas Moorby et al. (2009) also using descriptive sensory analysis found little effect

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on milk sensory properties based on dietary treatment (grass and red clover silages), except that boiled milk flavor increased significantly with the portion of red clover in the diet. Bertilsson and Murphy (2003) used difference testing with trained assessors and found that the sensory quality of milk differed between milks from cows fed perennial ryegrass and red and white clover silages and that the milk from red clover silages deviated more frequently from milk considered good quality milk.

Previous studies have highlighted that terpenes and carotenoids can potentially affect milk flavor directly as aromatic compounds or indirectly by acting as precursors to other volatile aromatic compounds (Martin et al., 2005; Villeneuve et al., 2013). Forage has also been shown to alter the fatty acid profile of milk and the protein content of milk (Croissant et al., 2007; Coppa et al., 2011; O'Callaghan et al., 2016b). The fatty acid composition of milk likely plays a direct role in flavor as short-chain free fatty acids ($C_{4:0}$ to $C_{10:0}$) are volatile and aromatic (Kilcawley, 2017), but also increasing levels of unsaturation in fatty acids may increase the susceptibility of oxidation and thus milk from pasture is potentially more susceptible to oxidation, despite the presence of natural antioxidants in the milk (Havemose et al., 2006). Studies have shown that cows fed on a pasture diet received more protein than cows fed on a supplement (Bendall, 2001; Coppa et al., 2011). As the pasture diet has less energy, much of the protein is broken down so that the gluconeogenic AA are used as an energy source (Mackle et al., 1999). The metabolism of branched, aromatic, and sulfur AA by rumen bacteria can result in a wide range of odor active volatile compounds (aldehydes, acids, alcohols, ketones, and phenols) that can be potentially transferred to the mammary gland (Carlson and Breeze, 1984; Calvo and de la Hoz, 1992; Villeneuve et al., 2013; Jansson et al., 2014). Heat treatment can also alter the volatile profile of bovine milk, as, for example, it can promote the decarboxylation of β-keto acids to generate methyl ketones or lactones (Forss, 1979; Hougaard et al., 2011), the degradation of β-carotene (Zepka et al., 2014), the oxidation of methanethiol to a range of sulfur compounds (Contarini et al., 1997), or the generation of Maillard reaction products (Calvo and de la Hoz, 1992).

The aim of this study was to investigate the sensory quality and aromatic properties of bovine milk (raw and pasteurized) obtained from Friesian cows over a lactation on 3 distinct feeding regimens: outdoors on a perennial ryegrass pasture, outdoors on a perennial ryegrass/white clover pasture, and indoors on TMR. This study was carried out in conjunction with a recently published study, where the experiential design, trial details, milk composition, and total fatty acid contents of the milks are provided (O'Callaghan et al., 2016b).

MATERIALS AND METHODS

Feed Samples

The grass and grass clover samples were taken using a grass clippers cutting above the root and were collected from random areas that had been grazed to get a representative sample. Representative TMR samples were taken from a bulk supply. Grass samples were denoted as "grass," grass clover samples denoted as "grass/clover," and TMR samples as "TMR." Samples were taken at 3 different time points over a season, corresponding to the 3 milk collection times (early, mid, and late lactation).

Milk Samples and Processing

Raw milk was collected from the Teagasc Moorepark dairy farm (Fermoy, Co. Cork, Ireland) from the 3 different spring-calving herds as outlined in O'Callaghan et al. (2016b) at 3 different time points of lactation (early, mid, and late). All results are averages from samples and analysis undertaken at early, mid, and late lactation. Microbial analysis was performed immediately, pasteurization was performed within 20 h, and sensory analysis within 24 h. For the purpose of this study, the different milk types are denoted as **G** for grass, **C** for grass/clover, and TMR. Where necessary, the prefix **r** is used to denote raw milk and **p** to denote pasteurized milk.

Each milk sample was homogenized [GEA Niro Soavi S.p.A. Type: NS2006H (non-aseptic)] using 2-stage homogenization at 5,000 to 150,000 kPa. The milk was then pasteurized using a Microthermics (UHT/HTST Electric Model 25HV Hybrid, Liquid Technologies, Wexford, Ireland) unit heated to 72°C and held for 15 s, then cooled to 4°C. Each milk sample were transferred at 4°C to the sterile product outlet and aseptically packed into sterile 1-L glass bottles.

Microbial Analyses

The pour plate method was used to estimate the number of viable units of microorganisms per milliliter of raw and pasteurized milk samples (total bacteria count). Dilutions from 10^0 to 10^4 of the milk sample were mixed with maximum recover diluent (Oxoid CM0733, Waltham, MA). One milliliter of each dilution was pipetted onto sterile Petri dishes using the pour plate method and left to stand and subsequently incu-

bated at 30°C for 72 h. Following incubation, colonies that developed were counted and the number of microorganisms per milliliter of the original milk sample was calculated. Analysis was performed in triplicate.

β-Carotene Analyses

The β-carotene analyses were performed as described in O'Callaghan et al. (2016a).

Milk Color Analyses

Measurements were performed according to the CIE Lab system (CIE, 1978; L, CIE lightness coordinate; a, CIE red/green color attribute; b, CIE yellow/blue color attribute), using a Minolta colorimeter (Minolta Camera, Osaka, Japan). Samples were analyzed 30 min after the exposure to air to allow the stabilization of color. Results were expressed as the average of 5 replicate measurements on the different parts of the liquid milk samples and averaged over the season.

Volatile Analyses

Headspace Solid-Phase Micro Extraction of Raw and Pasteurized Milk and Feed Samples. Headspace solid-phase micro extraction (**HS-SPME**) analysis was performed as described in O'Callaghan et al. (2016a), except that 2 g of milk and 2 g of feed (G, C, and TMR) were used. All analyses were performed in triplicate.

Sorbtive Extraction of Pasteurized Milk Samples. A sorbtive extraction (**SE**) probe (Markes International Ltd., Llantrisant, UK) coated in polydimethylsiloxane, pre-conditioned for 1 h at 280°C under nitrogen, was inserted into 5 mL of milk in a 15% NaCl (wt/vol) solution in a 20-mL amber headspace vial (Apex Scientific Ltd., Maynooth, Co. Kildare, Ireland). The vial was sealed with a screw-capped silicone/polytetrafluoroethylene liner magnetic cap and agitated for 1 h at 250 rpm at 37°C in a HiSorb agitator (Markes International Ltd.). The extraction probe was removed from the sample mixture and rinsed with distilled water and dried with a lint-free cloth and inserted into an empty thermal desorption (**TD**) tube. This tube was placed in a Unity 2 TD unit (Markes International Ltd.) connected to an Agilent 7890A GC coupled with a 5977B single quadrupole MS (Agilent Technologies Ltd., Little Island, Cork, Ireland). The TD tube was initially pre-purged for 2 min using a 20 mL/min split under helium. The tube was desorbed to a cold trap (material emissions) at 110°C for 0.5 min with a 10 mL/min split, and then at 200°C for 10 min without a split. The cold trap temperature was maintained at 30°C. The trap flow was set at 50 mL/min and after a pre-trap fire purge of 2 min the trap was heated to 280°C at a rate of 100°C/s and held for 5 min without a split (total split 10:1). The flow path temperature into the GC injector was set at 160°C. A DB-5 MS (60 m × 0.25 mm × 0.25 μm) column (Agilent Technologies Ltd.) was used and the initial oven temperature was set at 35°C, held for 0.5 min, increased at 6.5°C/min to 230°C, then increased at 15°C/min to 320°C, yielding at total GC run time of 41.5 min. The carrier gas was helium held at a constant pressure of 158.579 kPa. The ion source temperature was 230°C and the interface temperature was 280°C and the MS mode was electronic ionization (70 v) with the mass range scanned between 35 and 250 amu. Compounds were identified using in-house library created in Masshunter software (Agilent Technologies Ltd.) with target and qualifier ions and linear retention indices (Vandendool and Kratz, 1963) for each compound and from combinations of authentic standards, mass spectra comparisons to the NIST 2014 mass spectral library. An auto-tune of the GCMS was carried out before the analysis to ensure optimal GC-MS performance. A set of external standards was run at the start and end of the sample set and abundances were compared with known amounts to ensure that both the SPME extraction and MS detection were performing within specifications. Analysis was performed in triplicate.

Sensory Analyses

Twenty-five naïve assessors were recruited in University College Cork, Ireland. Age range of assessors was 21 to 48 yr old. Selection criteria for assessors were availability and motivation to participate on all days of the experiment and that they were bovine milk consumers. Sensory acceptance testing was conducted as described in O'Callaghan et al. (2016a). The results presented were raw data assessed by 25 assessors each on different days at 3 separate time points (early, mid, and late lactation). Assessors used the sensory hedonic descriptors in Supplemental Table S1 (https://doi.org/10.3168/jds.2017-13141) for 3 different pasteurized milk samples (pG, pC, and pTMR). Twenty-five assessors then participated in ranking descriptive analysis (**RDA**; Richter et al., 2010) using the consensus list of sensory descriptors (Supplemental Table S1; https://doi.org/10.3168/jds.2017-13141), which was also measured on a 10-cm line scale. All samples were a presented in duplicate (Stone et al., 2012) and the results are averages of samples assessed by 10 assessors taken at 3 separate time points (early, mid, and late lactation).

Table 1. β-Carotene content (mg/kg) of the pasteurized (p) milks [grass (G), grass/clover (C), and TMR] over the lactation season[1]

Item	β-Carotene content (mg/kg)	*P*-value	Color			*P*-value
			L	a (−)	b	
pG	0.34 ± 0.02[a]	*	86.48 ± 1.96[a]	4.87 ± 0.64[a]	16.06 ± 1.36[a]	***
pC	0.26 ± 0.03[b]	*	83.73 ± 2.46[b]	4.67 ± 0.82[a]	15.85 ± 1.59[a]	***
pTMR	0.13 ± 0.00[c]	*	87.38 ± 0.25[a]	3.49 ± 0.21[b]	9.68 ± 0.86[b]	***

[a–c]Column values with different superscripts are statistically different at $P < 0.05$.

[1]The color of pasteurized milk measurements were performed according to the Commission Internationale d'Eclairage (CIE) Lab system (L = CIE lightness coordinate; a = CIE red/green color attribute; b = CIE yellow/blue color attribute) using a colorimeter.

One-way ANOVA statistical analysis: *denotes level of significance ($P \leq 0.05$); ***denotes level of significance ($P \leq 0.001$).

Statistical Analyses

Statistical analyses for data relating to sensory evaluation and volatile analysis were carried out using ANOVA partial least squares regression. Mean data were calculated with standard error of the mean; significance was denoted at $P < 0.05$. Analysis of variance partial least squares regression of sensory and volatile data was analyzed using the Unscrambler Software, version 10.3 (CAMO ASA, Trondheim, Norway). The X and Y matrix was designed so that X was the sample name(s) and Y was the volatile and sensory data. Close proximity of samples (G, C, and TMR) to sensory attributes and volatiles indicates correlation between the sample and the particular sensory attribute/volatile. The level of significance for correlation was set at $P < 0.05$. Statistical analysis for β-carotene and milk color was performed using SPSS v18.0 (IBM Statistics Inc., Armonk, NY). Data sets were analyzed for normality using the Shapiro-Wilk test and for homogeneity of variance using Levene's test. Analyses were carried out at only one time point and where normally distributed were analyzed using one-way ANOVA with post hoc Tukey test.

RESULTS

Milk Composition (Raw Milk)

Compositional results are provided in O'Callaghan et al. (2016b), who found that the main difference was that 16 fatty acids varied significantly ($P < 0.05$) depending on feeding systems.

Microbial Analyses (Raw and Pasteurized Milk)

Total bacteria count was carried out on the raw and pasteurized milk samples from each feed type (Supplemental Table S2; https://doi.org/10.3168/jds.2017-13141). As anticipated, a significant decrease ($P < 0.05$) occurred in the microbial activity after pasteurization.

β-Carotene (Pasteurized Milk)

The pG milks had significantly ($P < 0.05$) higher levels of β-carotene in comparison to both the pC and pTMR milks (Table 1), in agreement with Croissant et al. (2007). The pTMR milk contained the lowest level of β-carotene (0.13 ± 0.0 mg/kg). These concentrations are comparable with other studies (Shingfield et al., 2005; Havemose et al., 2006).

Milk Color (Pasteurized Milk)

The L (lightness), a (red/green color), and b (yellow/blue color) values were statistically different ($P < 0.001$) between the samples (pG, pC, and pTMR; Table 1). The b value was statistically ($P < 0.001$) higher in pG and pC than in pTMR. The a values were negative for all samples with a statistically ($P < 0.001$) higher value for pTMR than in pG and pC. The L value was statistically ($P < 0.05$) higher in pTMR and pG than in pC.

Volatiles Analysis (Feed, Raw, and Pasteurized Milk)

Feed Samples. Volatile analysis was carried out on the feed samples (grass, grass/clover, and TMR) by HS-SPME and were averaged for each stage of lactation. The TMR feed contained 65 volatile compounds, with the grass having 34 and the grass/clover having 49 (Table 2). Figure 1 is a pie chart showing the percentage of each chemical class identified within each feed type over lactation. It is apparent that the range of volatile chemical classes in both the grass and grass/clover feed samples were quite similar, but different to the TMR feed samples. The TMR feed samples contained more esters, acids, and phenols, and fewer ketones, aldehydes, furans, terpenes, and sulfur compounds. Thirteen esters, 6 alcohols, 6 ketones, 5 acids, 3 aldehydes, 2 phenols, 1 terpene, and 1 furan were significantly different between the feed types at $P < 0.001$; and 5 alcohols, 4 esters, 4 ketones, 2 hydrocarbons, 1 acid, 1 terpene, 1

furan, and 1 sulfur compound were significantly different between the feed types at $P < 0.05$.

Volatiles in Raw Milk. The volatiles in the raw milk samples were also analyzed by HS-SPME. In total, 40 volatile compounds were identified in the rG, rC, and rTMR milk samples (Table 3). Twenty-two volatile compounds identified in the feed samples were also found in the raw milk samples. Only 12 volatiles were present in each feed type and in each raw milk (acetic acid, butanoic acid, hexanoic acid, heptanal, nonanal, acetone, 2-heptanone, 1-pentanol, 1-octen-3-ol, toluene, m-xylene, and p-xylene). Thirteen volatile compounds were found in all raw milk samples (octanoic acid, nonanoic acid, n-decanoic acid, 2-ethyl-hexanoic acid, pentanal, 3-methyl butanal, decanal, methyl isobutyl ketone, ethyl benzene, ethyl ether, dimethyl sulfide, dimethyl sulfone, and β-pinene), but were not present in the corresponding feed samples. In addition some other compounds were present in some specific feeds but not in the associated milks (1-octanol, hexanal, and 2-nonanone). Only 6 of the 40 volatile compounds detected in the raw milk samples were statistically ($P < 0.001$) different (Table 2) based on diet (acetic acid, hexanal, 2-butanone, 1-pentanol, dimethyl sulfone, and toluene). Acetic acid, 1-pentanol, and toluene were highest in the rG milks and lowest in the rTMR milks.

Volatiles in Pasteurized Milk. The pasteurized milk samples were also analyzed by HS-SPME using the same extraction and chromatography conditions. Thirty-six volatile compounds were identified in the pasteurized milk samples (pG, pC, and pTMR) and 32 (Table 3) were common to the raw milk samples. However, only 21 compounds were present in each raw and corresponding pasteurized milk sample. Some compounds present in the raw milk samples were not present in their corresponding pasteurized milk samples, suggesting alteration by heat treatment. Only 1-octen-3-ol was present in all the raw milk samples, but absent in all the pasteurized milk samples; other volatiles (benzaldehyde, 2-methyl-butanal, 2-ethyl-hexanoic acid, butanoic acid, butyl acetate, 1-hexanol, 1-octanol 3-methyl-butanol, β-pinene, and 2-pentyl furan) were also absent, but only for some pasteurized milk feed types. Other compounds were present in pasteurized milks but absent in the raw milks (n-decanoic acid, 2-methyl butanal, benzeneacetaldehyde, 2-nonanone, butyl acetate, ethyl ether, 3-octanone, o-xylene, 1-octanol, and γ-butrolactone). Statistical differences based on diet were evident; pentanal, heptanal, 1-pentanol, dimethyl sulfone, γ-butyrolactone, toluene, and β-pinene at $P < 0.001$ and benzeneacetaldehyde, 2-butanone, and methyl isobutyl ketone at $P < 0.05$ (Table 3). Figure 2 is a partial least squares regression plot of the average volatiles over a season in the raw and pasteurized milk samples as determined by HS-SPME. This plot highlights differences the volatile profile between the raw milks (rG, rC, and rTMR) very effectively, but also highlights the effect of pasteurization, as both the rG and rC milks are clearly separated from the pG and pC milks. It is apparent from Figure 2 that in relation to the volatiles deemed statistically different by partial least squares regression (1-pentanol, toluene, dimethyl sulfone, and 2-butanone) some observations can be made. The association of 1-pentanol is greater with the pC and pG milks than the rC and rG milks, and that dimethyl sulfone has a greater association with the rG and rC than the pG and pC milks. Also, 2-butanone has a greater association with rTMR than with pTMR, highlighting direct effects of pasteurization on these volatile compounds.

We also investigated the volatiles in the pasteurized milk samples using an alternate extraction technique, SE. The SE technique uses polydimethylsiloxane as an absorbent and is more applicable in general to less polar compounds and has significantly greater absorbent capacity than a SPME fiber. In addition, we included a salting out stage to aid recovery of polar compounds. The selectivity of both extraction techniques was quite different. The SE technique identified 38 volatiles in the pasteurized milk samples (Table 4), where the HS-SPME technique identified 36, and only 14 volatiles were common to both extraction techniques. Additional aldehydes, ketones, lactones, alcohols, furans, esters, a hydrocarbon, and a terpene were identified using the SE technique. Most volatiles identified using SE were present in all the pasteurized milk samples.

Sensory Analysis (Pasteurized Milk)

The use of sensory acceptance testing in conjunction with RDA facilitated the analysis of the samples and replication, and these rapid sensory methods provided a general sensory observable trend. Figure 3 highlights the hedonic sensory analysis of pasteurized milks over lactation. pG milk scored higher for every attribute, but was statistically ($P < 0.05$) highest for "overall acceptability," "liking of texture," and "liking of flavor." The pC milk scored lowest for all attributes except for "liking of texture," but only differed statistically ($P < 0.05$) to the pTMR milk for "liking of flavor" and "overall acceptability." The panel consisted of Irish consumers of milk, who would be most familiar with milk produced from grass-fed milk and therefore this result may not be that surprising. Incorporation of larger numbers of panelists including members more familiar with milk derived from pTMR may provide alternate results. Supplemental Figure S1 (https://doi.org/10.3168/jds.2017-13141) represents a radar plot of the

Table 2. The volatile compounds, identified by headspace solid-phase micro extraction GC-MS analysis of the fed samples (grass, grass/clover, and TMR); values indicate area values for each compound

Compound	CAS no.[1]	LRI[1]	Grass	Grass/clover	TMR	*P*-value
Ester						
Ethyl acetate	141–78–6	559	0.00E+00	9.85E+05	6.74E+07	***
Ethyl propionate	105–37–3	709	1.05E+05	0.00E+00	7.41E+06	***
n-Propyl acetate	109–60–4	712	1.43E+04	8.57E+02	1.54E+07	***
Ethyl butanoate	105–54–4	800	2.08E+05	3.58E+04	1.75E+08	***
Methyl valerate	624–24–8	822	5.51E+03	7.21E+04	7.16E+06	NS
Butyl acetate	123–86–4	812	0.00E+00	0.00E+00	8.90E+06	***
1-Butanol, 3-methyl-, acetate	123–92–2	874	4.71E+05	0.00E+00	1.72E+07	***
Propyl butanoate	105–66–8	890	0.00E+00	0.00E+00	8.44E+07	NS
Pentyl acetate	628–63–7	911	0.00E+00	0.00E+00	1.16E+06	***
Ethyl pentanoate	539–82–2	898	7.20E+05	0.00E+00	5.79E+07	***
Methyl hexanoate	106–70–7	922	0.00E+00	3.93E+06	2.68E+07	***
Pentyl propionoate	624–54–4	967	0.00E+00	0.00E+00	7.77E+06	*
Butyl butanoate	109–21–7	993	0.00E+00	0.00E+00	5.15E+07	*
Ethyl hexanoate	123–66–0	996	2.59E+05	0.00E+00	5.94E+08	***
Butyl isovalerate	109–19–3	1,044	0.00E+00	0.00E+00	8.74E+05	*
Propyl hexanoate	626–77–7	1,091	0.00E+00	0.00E+00	2.55E+08	***
Ethyl octanoate	106–32–1	1,191	2.49E+05	1.66E+05	1.69E+07	*
Isopentyl hexanoate	2198–61–0	1,245	0.00E+00	0.00E+00	9.78E+06	***
Pentyl hexanoate	540–07–8	1,282	0.00E+00	0.00E+00	7.54E+06	NS
Hexyl hexanoate	6378–65–0	1,380	0.00E+00	0.00E+00	9.24E+06	***
Alcohol						
Ethanol	64–17–5	<500	3.07E+07	4.51E+06	8.90E+07	NS
1-Butanol	71–36–3	599	0.00E+00	0.00E+00	6.99E+06	NS
2-Butanol R	14898–79–4	<500	0.00E+00	0.00E+00	2.21E+05	NS
1-Penten-3-ol	616–25–1	650	1.34E+07	1.05E+07	2.46E+05	***
3-Methyl-1-butanol	123–51–3	733	6.24E+07	4.86E+06	3.39E+07	*
2-Methyl-1-butanol	1565–80–6	737	1.60E+07	1.13E+06	1.01E+07	***
1-Pentanol	71–41–0	764	5.07E+06	5.05E+06	8.80E+05	*
2-Penten-1-ol, (Z)-	1576–95–0	766	2.01E+06	3.59E+06	0.00E+00	***
2-Furanmethanol	98–00–0	862	4.24E+05	8.43E+05	3.89E+06	NS
2-Hexen-1-ol, (E)-	928–95–0	849	3.76E+05	3.14E+07	0.00E+00	*
3-Hexen-1-ol, (Z)-	928–96–1	855	2.49E+07	2.27E+07	0.00E+00	***
1-Hexanol	111–27–3	867	2.22E+07	1.13E+08	1.11E+07	*
1-Octen-3-ol	3391–86–4	978	1.75E+06	1.29E+08	1.38E+07	*
3-Octanol	589–98–0	995	7.80E+06	1.16E+08	2.18E+06	***
Phenylethyl alcohol	60–12–8	1,119	4.86E+06	1.97E+06	5.03E+07	***
Ketone						
Acetone	67–64–1	<500	2.62E+05	6.88E+07	1.72E+07	***
2-Butanone	78–93–3	527	9.35E+03	1.07E+08	3.26E+07	***
2-Pentanone	107–87–9	663	9.38E+06	1.78E+06	1.88E+07	*
2,3-Butanedione	431–03–8	640	0.00E+00	0.00E+00	8.72E+04	NS
3-Pentanone	96–22–0	691	8.29E+06	1.62E+07	1.46E+06	***
Acetoin	513–86–0	735	0.00E+00	3.14E+05	3.05E+06	*
2-Hexanone	591–78–6	788	2.32E+04	5.21E+04	0.00E+00	*
2-Heptanone	110–43–0	889	1.28E+06	4.00E+05	7.86E+06	***
3-Octanone	106–68–3	984	5.58E+07	5.90E+08	1.36E+07	***
8-Nonen-2-one	5009–32–5	1,121	1.74E+04	0.00E+00	0.00E+00	***
2-Nonanone	821–55–6	1,131	8.26E+05	1.64E+05	0.00E+00	*
2-Undecanone	112–12–9	1,289	1.83E+05	2.37E+04	8.40E+05	NS
Aldehyde						
2-Methyl butanal	96–17–3	609	9.78E+04	9.90E+04	6.04E+06	NS
Hexanal	66–25–1	801	0.00E+00	5.42E+06	0.00E+00	***
Heptanal	111–71–7	902	1.19E+04	2.96E+06	2.88E+05	NS
Benzaldehyde	100–52–7	897	1.29E+06	1.99E+06	1.05E+07	NS
Benzeneacetaldehyde	122–78–1	998	0.00E+00	4.01E+05	6.15E+06	***
Nonanal	124–19–6	1,100	6.75E+05	1.59E+06	5.62E+06	***
Acid						
Acetic acid	64–19–7	<500	8.54E+06	3.79E+06	1.63E+08	***
Butanoic acid	107–92–6	828	7.45E+04	1.10E+04	1.88E+07	***
3-Methyl butanoic acid	503–74–2	858	3.62E+03	0.00E+00	1.44E+07	***
2-Methyl butanoic acid	116–53–0	871	0.00E+00	0.00E+00	4.35E+06	***
Hexanoic acid	142–62–1	971	9.43E+05	4.26E+06	4.06E+07	***
Heptanoic acid	503–74–2	858	0.00E+00	0.00E+00	3.74E+06	*
2-Methyl propanoic acid	116–53–0	871	0.00E+00	0.00E+00	4.76E+05	NS

Continued

Table 2 (Continued). The volatile compounds, identified by headspace solid-phase micro extraction GC-MS analysis of the fed samples (grass, grass/clover, and TMR); values indicate area values for each compound

Compound	CAS no.[1]	LRI[1]	Grass	Grass/clover	TMR	*P*-value
Hydrocarbon						
Toluene	108–88–3	768	3.19E+05	5.01E+04	4.38E+05	NS
Ethylbenzene	100–41–4	864	5.23E+05	0.00E+00	1.82E+06	*
m-Xylene	108–38–3	874	1.53E+06	1.93E+06	1.34E+06	NS
p-Xylene	106–42–3	897	3.28E+05	2.87E+04	2.77E+04	NS
Benzene, 1,3-bis (1,1-dimethyl)	1014–60–4	1,251	1.02E+06	0.00E+00	1.57E+06	*
Phenolic						
Phenol	108–95–2	976	1.31E+04	1.20E+04	1.73E+06	NS
4-Ethyl-phenol	123–07–9	1,162	2.19E+06	1.34E+05	3.22E+07	***
Creosol (4-methylguaiacol)	93–51–6	1,192	0.00E+00	0.00E+00	6.40E+05	***
Terpene						
L-α-Terpineol	98–55–5	1,002	1.80E+04	0.00E+00	4.60E+05	***
β-Myrcene	123–35–3	988	1.26E+05	1.54E+04	0.00E+00	*
Z-β-Ocimene	3338–55–4	1,035	7.68E+06	3.76E+05	0.00E+00	NS
trans-β-Ocimene	3779–61–1	1,046	3.93E+06	1.90E+05	0.00E+00	NS
Furan						
2-Ethyl furan	3208–16–0	702	2.65E+05	1.91E+07	7.48E+06	***
2-Pentyl-furan	3777–69–3	990	3.24E+05	9.74E+06	1.27E+07	*
Sulfur						
Dimethyl disulfide	624–92–0	745	3.56E+04	3.26E+03	7.20E+04	*

[1]CAS no. = Chemical Abstracts Service number. LRI = linear retention index.

One-way ANOVA statistical analysis: * and *** denote significant differences at $P < 0.05$ and $P < 0.001$, respectively.

average ranked descriptive sensory analysis of the pG, pC, and pTMR milks over lactation. Distinct differences were evident between each milk based on forage type, with "color," "barnyard aroma," and "viscosity" significantly ($P < 0.05$) different between the samples averaged over lactation. The pG milk scored highest for "color" and "viscosity" and pC milk for "barnyard aroma." It would be interesting to compare results of the RDA used in this study with quantitative descriptive sensory analysis to determine if greater differences could be observed.

DISCUSSION

O'Callaghan et al. (2016b) found some differences in the composition of milks from each of the diets in this study. A main difference was that 16 fatty acids varied significantly ($P < 0.05$) depending on feeding systems. Polyunsaturated fatty acids were significantly higher ($P < 0.05$) in rG and rC milks than rTMR milks; however, some potentially important differences existed that may affect oxidative rancidity. In relation to the PUFA present at the greatest concentrations, linoleic acid was significantly higher in rTMR milks than in rG or rC milks and linoleic acid and CLA were significantly higher in rG and rC than in rTMR milks, palmitic acid was significantly ($P < 0.05$) higher in rTMR than in rG or rC milks, and no statistical difference was evident between the rG, rC, and rTMR milks for oleic acid (O'Callaghan et al., 2016b).

Twenty-two volatile compounds were identified in the feed and in the raw milk samples, with 12 volatiles present in each feed type and in each raw milk (acetic acid, butanoic acid, hexanoic acid, heptanal, nonanal, acetone, 2-heptanone, 1-pentanol, 1-octen-3-ol, toluene, m-xylene, and p-xylene). It is possible that at least some of these compounds were transferred directly from the feed to the raw milk either by the pulmonary or digestive route, as highlighted by Contarini et al. (1997) and Valero et al. (2001). However, Bugaud et al. (2001a) stated that it is difficult to get correlations between nonterpene volatiles in the feed and in the subsequent milk because of the metabolic activity of microbial populations in the rumen and in the milk. Losses of volatiles may also occur due to excretion or due to accumulation in other tissues (Bertilsson and Murphy, 2003). Contarini et al. (1997) highlighted that acetone can originate directly from feed. It is well established that toluene is a product of β-carotene degradation in the rumen (Villeneuve et al., 2013). Acetic acid is primarily a product of carbohydrate metabolism (Kilcawley, 2017); with both butanoic and hexanoic acids synthesized de novo by the mammary gland, the presence of free fatty acids in milk is due to incomplete esterification in the mammary gland before lipid creation or lipolysis in the milk during storage (Villeneuve et al., 2013; O'Callaghan et al., 2016b). Heptanal and nonanal are primary products, and 2-heptanone, 1-pentanol, 1-octen-3-ol are secondary products of lipid oxidation (or also possibly β-ketoacid

decarboxylation in the case of 2-heptanone; Calvo and de la Hoz, 1992; Moio et al., 1993; Valero et al., 2001; Vazquez-Landaverde et al., 2005). Both m-xylene and p-xylene may be the result of carotenoid degradation, namely β-carotene degradation in the rumen (Zepka et al., 2014) or possibly directly transferred from feed (Buchin et al., 1998). Other volatiles were present in the feed and corresponding raw milk samples, but only for specific samples. In general, herbage-based diets have a higher protein to readily digestible carbohydrate ratio, and thus contain more volatiles from AA metabolism (Mackle et al., 1999; Coppa et al., 2011) because the pasture diet has less energy. The metabolism of branched, aromatic, and sulfur AA by rumen bacterial can result in a wide range of odor active volatile compounds (aldehydes, acids, alcohols, ketones, and phenols) that can be potentially transferred to the mammary gland (Carlson and Breeze, 1984; Calvo and de la Hoz, 1992; Villeneuve et al., 2013; Jansson et al., 2014). The TMR feed contained a large amount of esters presumably due to the presence of alcohols derived from carbohydrate fermentation and short-chain free fatty acids, and thus butyl acetate may have been transferred directly from the feed due to its absence in the other feeds and milks. Some volatiles were present in some specific feeds but not in the associated raw milks, suggesting that these compounds were likely produced either in the rumen or in the milk, or concentrated in the milk.

Only 6 of the 40 volatile compounds detected in the raw milk samples were statistically ($P < 0.001$) different based on diet (acetic acid, hexanal, 2-butanone, 1-pentanol, dimethyl sulfone, and toluene). Acetic acid, 1-pentanol, and toluene were highest in the rG milks and lowest in the rTMR milks. As previously stated, acetic acid is primarily a product of carbohydrate metabolism (Kilcawley, 2017). Levels were highest in rG and rC milks, yet levels of lactose were similar in all milks (O'Callaghan et al., 2016b) and acetic acid levels were highest in the TMR feed. However, acetic acid can also be produced from the metabolism of AA (Ganesan and Weimer, 2007) and this may also be a factor due to the higher levels of available protein in the milks from grass and grass/clover. Acetic acid is also utilized in the biosynthesis of β-ketoacids in the mammary gland (Mottram et al., 1996) and this may also account for some of the differences in these milks. 1-Pentanol is derived from reduction of pentanal (Villeneuve et al., 2013), which is derived from the oxidation of arachidonic acid (C20:4 n-6; Romeu-Nadal et al., 2004), which was highest pG and pC milk (O'Callaghan et al., 2016b). Toluene was highest in rG and rC milks and derived from β-carotene, which was also higher in pG and pC milks. It is well established that β-carotene is at higher levels in fresh than in conserved forage (Croissant et

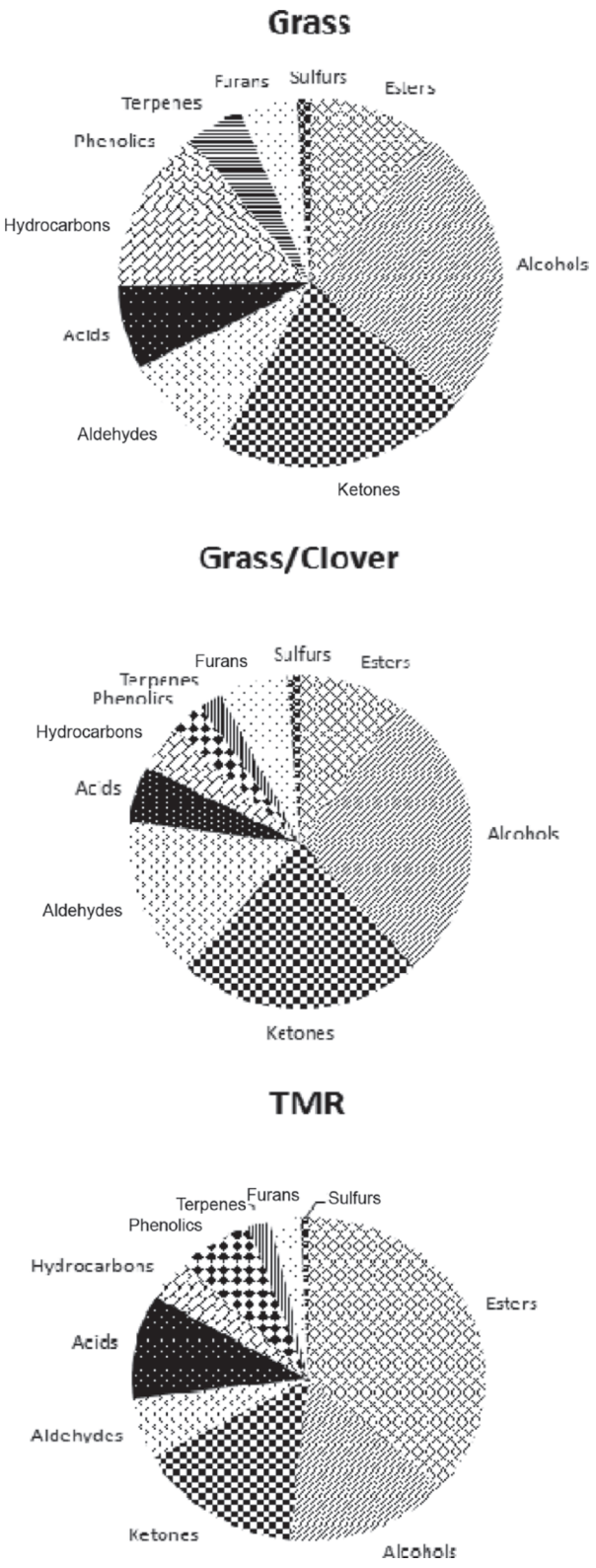

Figure 1. Pie charts showing the percentage of different chemical classes (esters, alcohols, ketones, aldehydes, acids, alcohols, aldehydes, phenols, hydrocarbons, furans, terpenes, and sulfur compounds) identified within each feed type (grass, grass/clover, and TMR) over the whole lactation season.

al., 2007; Coppa et al., 2011). Hexanal and 2-butanone were highest in the rTMR milks and lowest in rG milks. Hexanal is a primary product of lipid oxidation and mostly associated with the degradation of oleic and linoleic acid (Vazquez-Landaverde et al., 2005), and thus higher levels can be directly related to higher levels of linoleic acid in the rTMR milk (O'Callaghan et al., 2016b). 2-Butanone is thought to be transferred directly from the feed (Valero et al., 2001), as levels were higher in the grass/clover feed than in the TMR feed; however, metabolism of carbohydrate in the rumen or milk is also likely a factor.

Table 3. Relationship between cow feeding regimen [grass (G), grass/clover (C), and TMR] and the raw (r) and pasteurized (p) milk volatile compounds, identified by headspace solid-phase micro extraction GC-MS analysis; values indicate area values for each compound

			Raw milk sample				Pasteurized milk sample			
Compound	CAS no.[1]	LRI[1]	rG	rC	rTMR	*P*-value	pG	pC	pTMR	*P*-value
Acid										
Acetic acid	64–19–7	626	9.73E+05	4.90E+05	2.72E+05	***	1.91E+05	4.13E+05	3.03E+05	NS
Butanoic acid	107–92–6	771	4.62E+05	9.12E+05	3.36E+05	NS	2.50E+04	3.31E+05	0.00E+00	NS
Hexanoic acid	142–62–1	968	2.64E+06	3.22E+06	2.28E+06	NS	1.95E+05	2.35E+05	1.06E+05	NS
Octanoic acid	124–07–2	1,257	1.29E+06	1.71E+06	1.18E+06	NS	1.33E+05	1.42E+05	1.99E+05	NS
Nonanoic acid	112–05–0	1,352	3.83E+05	5.43E+05	1.75E+05	NS	1.37E+05	1.21E+05	6.73E+04	NS
n-Decanoic acid	334–48–5	1,451	5.00E+05	6.90E+05	7.10E+05	NS	2.13E+04	0.00E+00	0.00E+00	NS
2-Ethyl-hexanoic acid	149–57–5	1,204	3.36E+04	4.59E+04	9.97E+03	NS	1.37E+04	0.00E+00	1.02E+04	NS
Aldehyde										
Pentanal	110–62–3	701	2.75E+05	6.11E+05	2.38E+05	NS	8.34E+05	1.04E+06	9.13E+04	***
2-Methyl butanal	5.8024	659	0.00E+00	0.00E+00	1.81E+04	NS	7.10E+03	8.20E+03	0.00E+00	NS
3-Methyl-butanal	5.688	678	1.23E+04	1.33E+05	5.60E+04	NS	3.87E+04	3.19E+04	3.31E+04	NS
Hexanal	66–25–1	803	5.45E+05	1.13E+06	7.77E+06	***	1.63E+05	3.89E+05	2.17E+05	NS
Heptanal	111–71–7	904	2.69E+05	3.48E+05	3.65E+05	NS	1.99E+05	2.68E+05	8.96E+04	***
Benzaldehyde	100–52–7	971	2.95E+05	0.00E+00	0.00E+00	NS	0.00E+00	0.00E+00	0.00E+00	NS
Octanal	124–13–0	1,006	2.18E+04	1.89E+04	2.60E+04	NS	2.51E+04	2.89E+04	9.45E+03	NS
Nonanal	124–19–6	1,206	2.75E+05	2.65E+05	2.53E+05	NS	1.61E+05	1.76E+05	9.94E+04	NS
Decanal	112–31–2	1,307	6.86E+04	2.78E+04	2.19E+04	NS	3.03E+04	1.21E+04	2.02E+04	NS
Benzeneacetaldehyde	15.5629	1,051	0.00E+00	0.00E+00	0.00E+00	NS	7.42E+03	4.51E+04	0.00E+00	*
Ketone										
Acetone	67–64–1	<500	1.42E+07	1.97E+07	1.38E+07	NS	1.37E+07	1.86E+07	1.10E+07	NS
2-Butanone	78–93–3	587	0.00E+00	2.46E+06	9.21E+06	***	0.00E+00	2.06E+06	7.52E+06	*
Methyl isobutyl ketone	108–10–1	737	3.47E+04	1.49E+04	1.58E+04	NS	4.86E+04	8.52E+04	1.08E+04	*
2-Heptanone	110–43–0	891	3.14E+04	4.23E+04	7.23E+04	NS	9.84E+04	1.02E+05	9.68E+04	NS
3-Octanone	106–68–3	987	0.00E+00	9.06E+03	1.18E+04	NS	8.06E+03	4.80E+03	7.76E+03	NS
2-Nonanone	821–55–6	1,092	0.00E+00	0.00E+00	8.03E+03	NS	9.91E+03	6.06E+03	6.78E+03	NS
Alcohol										
1-Pentanol	71–41–0	767	5.58E+05	4.79E+05	1.18E+05	***	9.70E+05	1.62E+06	1.63E+05	***
3-Methyl-1-butanol	123–51–3	768	0.00E+00	0.00E+00	1.16E+04	NS	0.00E+00	0.00E+00	0.00E+00	NS
1-Hexanol	10.513	869	0.00E+00	2.15E+04	2.28E+04	NS	0.00E+00	0.00E+00	1.95E+04	NS
2-Ethyl-1-hexanol	104–76–7	1,029	6.00E+04	2.47E+04	3.20E+04	NS	5.13E+04	1.99E+04	2.08E+04	NS
1-Octanol	111–87–5	1,077	0.00E+00	2.81E+03	0.00E+00	NS	0.00E+00	0.00E+00	7.89E+02	NS
1-Octen-3-ol	3391–86–4	994	3.51E+05	2.16E+05	5.19E+04	NS	0.00E+00	0.00E+00	0.00E+00	NS
Ester										
Ethyl benzene	10.333	862	6.79E+05	8.05E+05	5.30E+05	NS	3.64E+05	5.05E+05	4.51E+05	NS
Butyl acetate	8.963	812	0.00E+00	0.00E+00	7.65E+05	NS	1.45E+06	1.49E+06	0.00E+00	NS
Ethyl ether	4.0487	501	9.40E+04	3.01E+04	0.00E+00	NS	6.19E+04	0.00E+00	3.32E+04	NS
Sulfur										
Dimethyl sulfide	75–18–3	508	5.96E+06	5.54E+06	4.61E+06	NS	3.55E+06	3.46E+06	1.84E+06	NS
Dimethyl sulfone	67–71–0	919	2.63E+05	3.62E+05	1.68E+04	***	1.65E+05	1.58E+05	6.45E+03	***
Lactones										
γ-Butyrolactone	96–48–0	895	0.00E+00	0.00E+00	0.00E+00	NS	0.00E+00	0.00E+00	1.14E+04	***
Hydrocarbon										
Toluene	108–88–3	770	6.90E+06	4.67E+06	2.05E+05	***	5.03E+06	4.08E+06	1.81E+05	***
o-Xylene	10.5998	872	0.00E+00	1.58E+06	1.82E+06	NS	1.10E+06	1.45E+06	1.69E+06	NS
m-Xylene	10.628	873	6.60E+06	8.22E+06	5.89E+06	NS	5.14E+06	9.39E+06	5.66E+06	NS
p-Xylene	10.6343	874	2.74E+05	4.42E+05	6.26E+05	NS	4.14E+05	3.39E+05	5.12E+05	NS
Terpene										
β-Pinene	13.7438	985	1.91E+04	2.13E+04	1.61E+04	NS	1.55E+04	1.95E+04	0.00E+00	***
Furan										
2-Pentyl-furan	13.8957	990	0.00E+00	0.00E+00	4.63E+03	NS	0.00E+00	0.00E+00	0.00E+00	NS

[1]CAS no. = Chemical Abstracts Service number. LRI = linear retention index.

One-way ANOVA statistical analysis: * and *** denote significant differences at $P < 0.05$ and $P < 0.001$, respectively.

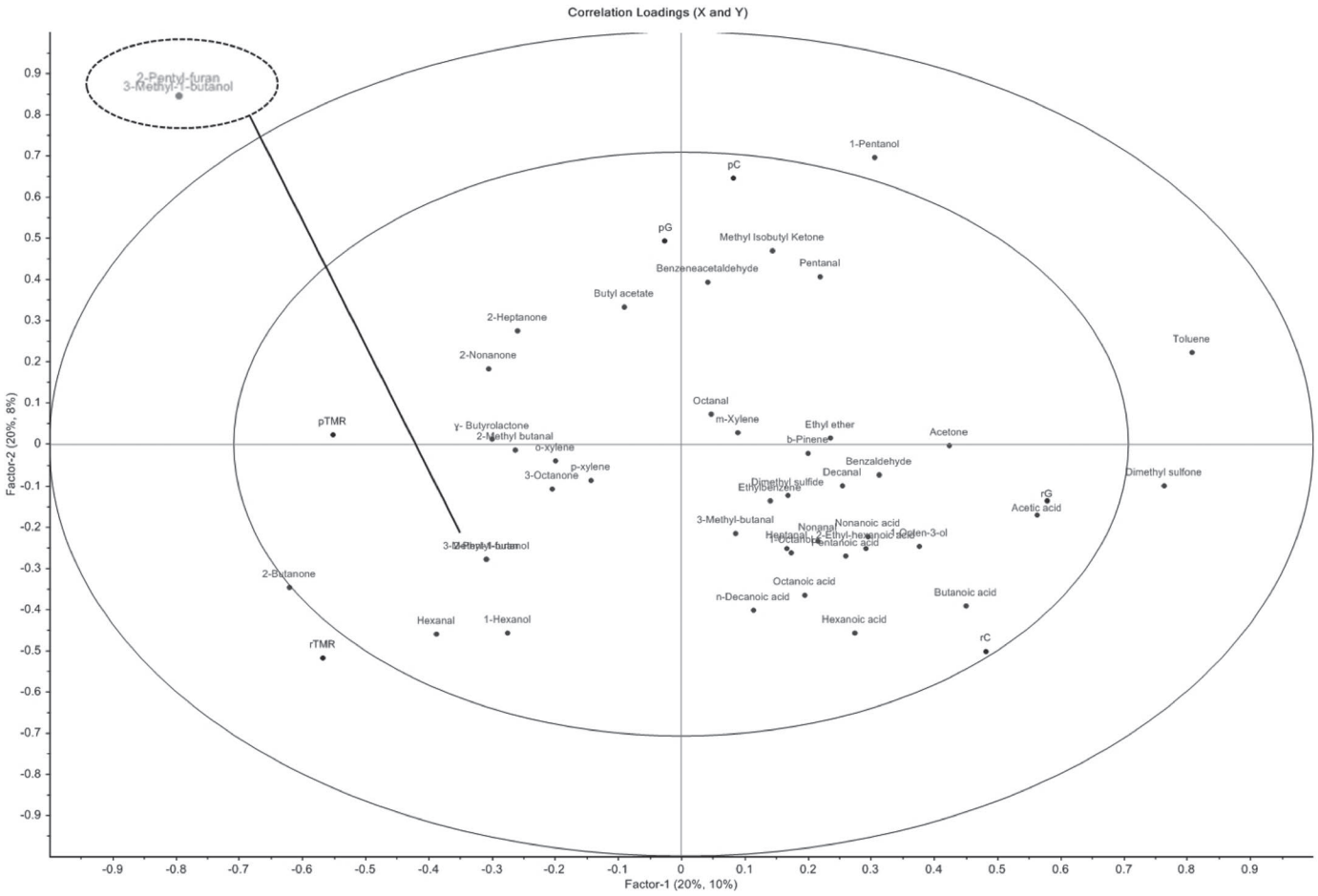

Figure 2. Multivariate data analysis partial least squares regression plot of headspace solid-phase micro extraction results for raw and pasteurized milk samples of different feeding systems (grass, grass/clover, and TMR) over the lactation season. The different milk types are denoted as G for grass, C for grass/clover, and TMR. The prefix r is used to denote raw milk and p to denote pasteurized milk.

Some compounds present in the raw milk samples were not present in their corresponding pasteurized milk samples, suggesting losses or changes due to heat treatment. However, metabolic and enzymatic reactions that occur during raw milk storage before pasteurization may also be responsible for losses of compounds after pasteurization (Calvo and de la Hoz, 1992; Contarini et al., 1997). Other compounds were present in pasteurized milks but absent in the raw milks and it is well established that certain volatiles can increase in milk after heat treatment, for example, ketones and lactones (Calvo and de la Hoz, 1992). Lactones are formed from thermal breakdown of δ- and γ-hydroxyacids (Dimick et al., 1969). Products of Strecker degradation (Contarini et al., 1997), sulfur compounds (Vazquez-Landaverde et al., 2005), degradation of β-carotene to toluene, xylenes, and other compounds are all thought to increase after heat treatment (Zepka et al., 2014). It has been suggested that some esters, such as ethyl acetate, are formed by heat-catalyzed esterification reactions (Vazquez Landaverde et al., 2005). Forss (1979) also suggested the autoxidation of SFA is promoted by heat treatment. Obviously the degree and extent of heating governs product formation, but it appears the activation energy required may not need to be that high.

As stated, statistical differences based on diet were also evident (pentanal, heptanal, 1-pentanol, dimethyl sulfone, γ-butyrolactone, toluene, and β-pinene at $P < 0.001$ and benzeneacetaldehyde, 2-butanone, and methyl isobutyl ketone at $P < 0.05$) in the pasteurized milks as determined by HS-SPME (Table 3). Pentanal, heptanal, and 1-pentanol are all products of lipid oxidation and were higher in pG and pC than in pTMR milks, which follows the same trends for raw milk based on fatty acid content. Methyl isobutyl ketone is also likely a product of lipid oxidation but was not present in raw milk. Toluene also follows the same trend for raw milk. Dimethyl sulfone is a product of methionine degradation (Vazquez-Landaverde et al., 2005; Villeneuve et al., 2013) and may be higher in pG and pC than pTMR

Table 4. Relationship between cow feeding regimen [grass (G), grass/clover (C), and TMR] and the pasteurized milk (p) volatile compounds, identified by sorbtive extraction GC-MS analysis; values indicate area values for each compound

Target compound	CAS no.[1]	LRI[1]	pG	pC	pTMR	*P*-value
Acid						
Acetic acid	64–19–7	592	2.79E+07	2.64E+07	4.03E+07	NS
Butanoic acid	107–92–6	769	1.24E+06	1.43E+06	2.18E+06	*
Hexanoic acid	142–62–1	967	2.28E+06	0.00E+00	1.48E+06	NS
Octanoic acid	124–07–2	1,163	1.30E+07	7.50E+06	9.72E+06	NS
Nonanoic acid	112–05–0	1,259	9.08E+06	7.60E+06	8.84E+06	NS
n-Decanoic acid	334–48–5	1,360	7.62E+07	7.76E+07	6.92E+07	NS
Aldehyde						
Pentanal	110–62–3	700	9.51E+05	5.80E+05	3.09E+05	*
Hexanal	66–25–1	800	2.50E+06	2.19E+06	3.60E+06	NS
Heptanal	111–71–7	902	1.80E+06	1.66E+06	1.87E+06	NS
Octanal	124–13–0	1,003	2.69E+06	2.90E+06	3.19E+06	NS
Nonanal	124–19–6	1,104	1.57E+07	1.62E+07	1.82E+07	NS
2-Nonenal, (E)-	18829–56–6	1,161	6.74E+05	1.48E+05	3.89E+05	NS
Decanal	112–31–2	1,206	6.28E+06	8.78E+06	7.39E+06	NS
2-Decenal, (Z)-	2497–25–8	1,264	7.23E+05	4.04E+05	1.03E+06	NS
2-Undecenal	2463–77–6	1,364	2.40E+05	0.00E+00	9.38E+05	NS
Dodecanal	112–54–9	1,405	0.00E+00	4.19E+05	2.76E+05	NS
Furan						
Unidentified hydroxy-2(5)H-furanone	78508–96–0	753	2.68E+06	2.92E+06	4.74E+06	*
Furfural	98–01–1	832	7.97E+06	7.93E+06	1.37E+07	NS
5-Hydroxymethylfurfural	67–47–0	1,224	1.53E+07	1.45E+07	2.76E+07	NS
2-Furanmethanol	98–00–0	850	1.28E+08	1.45E+08	2.37E+08	*
5-Methyl-2-furanmethanol	3857–25–8	949	4.17E+05	3.64E+05	2.14E+05	NS
Ketone						
2-Heptanone	110–43–0	888	3.05E+05	2.25E+05	7.73E+04	NS
Acetophenone	98–86–2	1,071	9.65E+05	1.31E+06	1.24E+06	NS
2-Pentadecanone	2345–28–0	1,698	2.48E+06	2.26E+06	1.95E+06	NS
Lactone						
γ-Crotonolactone	497–23–4	909	5.04E+06	5.39E+06	7.66E+06	NS
σ-Valerolactone (isomer)	542–28–9	1,055	1.26E+06	1.45E+06	3.14E+05	NS
σ-Decalactone	705–86–2	1,501	3.71E+06	4.84E+06	5.01E+06	NS
σ-Dodecalactone	713–95–1	1,719	3.90E+06	4.66E+06	4.67E+06	NS
Alcohol						
Isomaltol	3420–59–5	979	1.77E+06	7.49E+05	2.53E+06	NS
1-Phenylethanol	98–85–1	1,063	4.34E+05	5.77E+05	1.24E+05	*
1-Octanol	111–87–5	1,068	4.61E+05	4.62E+05	3.26E+05	NS
1-Tetradecanol	112–72–1	1,678	9.96E+05	1.07E+06	1.01E+06	NS
1-Hexadecanol	36653–82–4	1,881	6.66E+06	5.24E+06	3.40E+06	***
1-Octadecanol	112–92–5	<2,000	6.73E+06	5.68E+06	3.59E+06	***
Ester						
Methyl hexadecanoate	112–39–0	1,922	5.21E+05	2.12E+05	2.19E+05	NS
Isopropyl palmitate	142–91–6	<2,000	1.85E+06	1.29E+06	2.70E+06	NS
Hydrocarbon						
p-Cresol	106–44–5	1,070	7.54E+05	8.75E+05	2.03E+05	***
Terpene						
Squalene	111–02–4	<2,000	3.54E+07	2.94E+07	2.96E+07	NS

[1]CAS no. = Chemical Abstracts Service number. LRI = linear retention index.

One-way ANOVA statistical analysis: * and *** denote significant differences at $P < 0.05$ and $P < 0.001$, respectively.

milks due to higher concentrations of more digestible proteins, in agreement with other studies (Toso et al., 2002; Coppa et al., 2011). Benzeneacetaldehyde is also product of protein metabolism, thus higher levels in pG and pC may also be due to degradation in the rumen (Kilcawley, 2017). 2-Butanone also follows the same trends as the raw milk. β-Pinene is most likely derived directly from forage, but concentrations are quite low in pG and pC and absent in pTMR. γ-Butyrolactone is likely formed from hydroxy acids during pasteurization.

The SE extraction identified different volatiles due to differences in the selectivity of the techniques used. Most volatiles identified using SE were present in all the pasteurized milk samples. Only hexanoic acid and 2-undecanal were present in pG and pTMR milks, but absent in pC milks, and dodecanal was absent in pG milk but present in pC and pTMR milks. Three compounds, 1-hexadecanol, 1-octadecanol, and p-cresol, were significantly different based on forage type ($P < 0.001$). Both alcohols were at highest levels in the

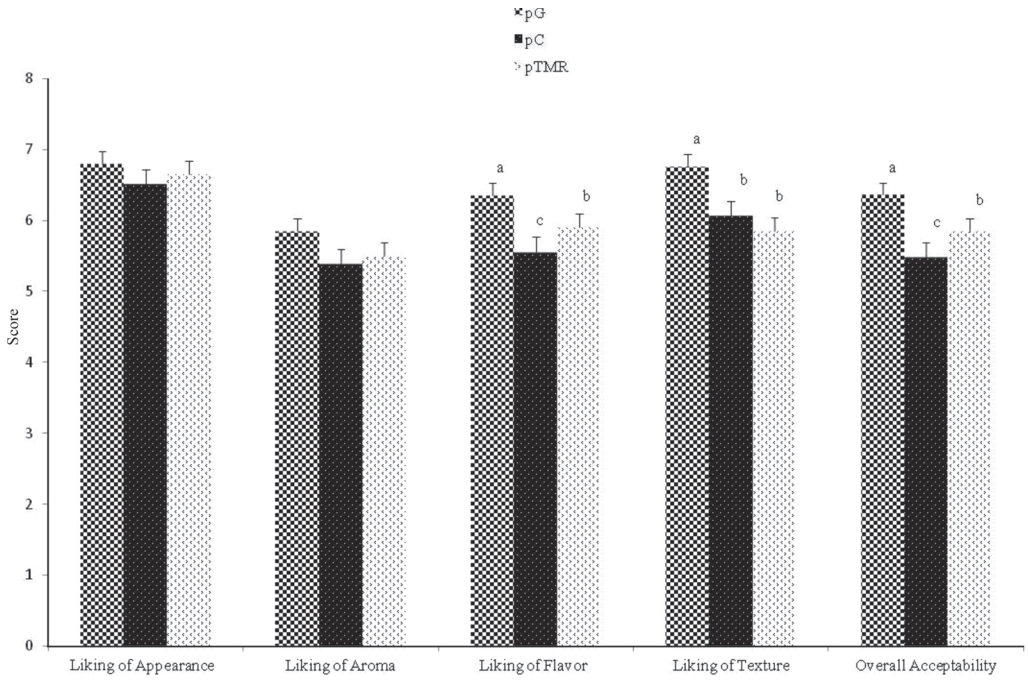

Figure 3. Hedonic sensory analysis of pasteurized milk derived from different feeding systems of grass (G), grass/clover (C), and TMR (p denotes pasteurized milk). The 3 milk samples were assessed by naïve Irish assessors (n = 25) familiar with milk using blind replicates in a full balanced block design, where assessors evaluated all samples in duplicate. Results expressed are averages of milk from early, mid, and late lactation; thus, 150 repetitions/sensory observations were made for each product. The error bars represent standard mean error within replicates. Columns with different letters (a–c) for each attribute are statistically different ($P < 0.05$).

pG milks and lowest in pTMR milks, whereas p-cresol was highest in the pC milks and lowest in the pTMR milks. The alcohols are likely products of secondary lipid oxidation (Bugaud et al., 2001b), but p-cresol can be formed from a range of different sources. As the protein content is higher in pasture than in TMR, deamination and decarboxylation of AA are thought to occur to a greater extent in the rumen of cows fed pasture (Mackle et al., 1999; Coppa et al., 2011), and it has been established that phenolic compounds such as p-cresol result from the degradation of tryptophan and tyrosine (Carlson and Breeze, 1984). However, p-cresol may also be produced from the degradation of β-carotene by a series of cyclization and oxidation reactions (Ueno et al., 2004). The higher levels in pC milks are likely due to the degradation of formononetin, an isoflavone that primarily occurs in leguminous plants such as clover (Zepka et al., 2014; Kilic and Lindsay, 2005). Five volatile compounds were significantly different based on forage at ($P < 0.05$; butanoic acid, pentanal, hydroxy-2(5)H-furanone, 2-furanmethanol, and 1-phenylethanol). Butanoic acid, hydroxy-2(5)H-furanone, and 2-furanmethanol were highest in pTMR milks and lowest in pG milks. Bugaud et al. (2001b) highlighted that furans in dairy products maybe a result of Maillard reactions between an AA and a sugar, or from oxidation of PUFA, thus levels may be higher in pTMR milks due to more available carbohydrate in the TMR feed or linked to differences in the fatty acid profile of the milk. Butanoic acid levels were higher in TMR feed, but not high or identified by HS-SPME in rTMR or pTMR milks, although this could be due to limitations of SPME (mainly fiber divinylbenzene/carboxen/polydimethylsiloxane) analysis for very polar compounds. As mentioned, butanoic acid is also synthesized de nova by the mammary gland, which may be influenced by the feed (Yayota et al., 2013). Pentanal was highest in pG and lowest in pTMR milks and this correlates well with HS-SPME data and the fatty acid content of the milks. 1-Phenylethanol was highest in pC milks and lowest in pTMR milks and is a product of the metabolism of the aromatic AA phenylalanine (Lee and Richard, 1984) and thus may be higher in the pC and pG milks due to the greater protein content (Mackle et al., 1999; Coppa et al., 2011).

In terms of sensory analysis, "color," "barnyard aroma," and "viscosity" were significantly ($P < 0.05$) different based on forage as determined by RDA. The difference in "color" is correlated with β-carotene content and b value, which corresponds to yellow/blue attribute. Martin et al. (2005) summarized that dairy products produced from pasture have a higher yellow intensity. It is difficult to discern a direct link between "viscosity" and the different forage milk samples, although it

may be associated with differences in the fatty acid content of the pG milk to the other pasteurized milks. Palmitic and oleic acids are the principal saturated and unsaturated fatty acids in dairy products with high and low melting points, respectively. The ratio of oleic acid to palmitic acid has previously been used as an index of hardness in butter and cheese (Martin et al., 2005). In this case the ratio of oleic to palmitic acid is lower in pG and highest in pTMR milks (O'Callaghan et al., 2016b). Barnyard aroma or flavor has been associated with p-cresol in dairy products (Moio et al., 1993; Kilic and Lindsay, 2005), and Khanal et al. (2005) associated barnyard flavor in milk with cows grazing on pasture. The major alkylphenol present in ruminant milk is p-cresol; although numerous potential sources of p-cresol exist, it seems most likely that a direct link between p-cresol levels in rC milk as detected by SE and barnyard aroma exists due to the degradation of the isoflavone formononetin in white clover (Kilic and Lindsay, 2005).

CONCLUSIONS

Pasteurization altered the volatile profile of all milks, with losses of some compounds and the development or augmentation of others. Sensory assessors preferred pasteurized milk produced from grass-fed cows, and had the least preference for milk produced from cows fed TMR over the season. This may be due to the fact that the assessors were Irish and thus used to milk derived from grass fed cows. Tentative associations between feed and some nonterpene volatiles appear to highlight a potential direct transfer to milk. Little or no evidence existed in relation to the transfer of terpenes from feed to milk, possibly due in part to the nature of the forage used in the trials. Some products of lipid oxidation were evident and were likely related to fatty acid content as influenced by forage type. However, none of these affected the sensory perception of milk as determined by RDA. Other differences in volatiles based on diet appear to relate to the protein/carbohydrate availability and subsequent digestion in the rumen. β-Carotene appears to have directly influenced perceived milk color with higher levels in milk derived from grass and grass/clover. Degradation of β-carotene in the rumen to toluene and in part to p-cresol was likely responsible for higher levels of these volatiles in milk from grass and grass/clover and could be used with β-carotene as potential biomarkers for milk derived from pasture. Higher levels of p-cresol in pC and pG milks are also likely due to the metabolism of aromatic AA in the rumen; however, the highest levels in pC are very likely due to the degradation of an isoflavone found in white clover also in the rumen. p-Cresol appears to have directly affected the sensory properties of milk as it is most likely the source of barnyard aroma in these milks.

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