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# Assessing the fate of fatty acid esters of hydroxy fatty acids, diglycerides and monoacyldiglycerides in grilled ruminant meats marinated with unfiltered beer-based marinades

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## Abstract

Ruminant meats contain functional lipids including fatty acid esters of hydroxy fatty acids (FAHFA), diglycerides (DG), monoacyldiglycerides (MAcDG) and medium chain triglycerides (McTG) whose consumption in the normal diet can confer benefits for consumer health. However, very little is known concerning how meat processing techniques such as marination and grilling affect the quantity and quality of these functional lipids in ruminant meats. We used ultra high-performance liquid chromatography coupled to high resolution accurate mass tandem mass spectrometry (UHPLC-HRAM-MS/MS) to show how grilling following marination with either India or Wheat ale unfiltered beer-based marinades affected the quantity and quality of these functional lipids in ruminant meats. We observed MAcDG was completely degraded in grilled meats. Both unfiltered beer-based marinades retained higher ( $p < 0.05$ ) levels of FAHFA, DG and McTG in grilled meats compared to their unmarinated counterparts. Furthermore, India ale-based marinade was more effective ( $p < 0.05$ ) compared to Wheat ale-based marinade in preserving these lipids in marinated grilled beef and moose meat. Significantly, strong correlations between antioxidants, polyphenols and oxygenated terpenes present in the marinades and preserved lipid molecular species appear to suggest that antioxidants, polyphenols, and oxygenated terpenes present in the marinades could be associated with preservation of these functional lipids in the grilled meats. These findings appear to suggest that marination could preserve some of these functional lipids with India ale-based marination proving to be more effective. However, further work is required to better improve the retention of MAcDG in grilled ruminant meats. This could potentially increase consumption of FAHFA, DG, MAcDG and McTG in the diet and thereby promote consumer health.

**Keywords:** Antioxidants, Functional lipids, Grill food quality, Lipid oxidation, Polyphenols

## 1. Introduction

Application of lipids as functional ingredients in pharmaceutical and nutraceutical formulations to treat or reduce risk factors for diseases have gained widespread interests [1]. Recent interests in healthy fats as dietary sources to improve health outcomes have resulted in consumers and food scientists seeking novel combination of

functional lipid active ingredients in food formulations [2]. Monoacyldiglycerides (MAcDG), fatty acid esters of hydroxy fatty acids (FAHFA), diglycerides (DG) and medium chain triglycerides (McTG) are emerging functional lipid ingredients in the food science and health food industry [3]. MAcDG possess an acetate group at the sn-3 position of the glycerol moiety, while in McTG at least one of the three fatty acids has 6–12 carbons [4].

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Compared to these triglycerides (TG), DG are composed of only 2 fatty acyl chains esterified at either sn-1/2 or sn-1/3 positions of glycerol whereas FAHFA are composed of a fatty acid esterified to a hydroxy fatty acid [3]. Diets containing MACDG, McTG, DG and FAHFA lipids have been shown to confer beneficial effects on population health and well-being [5]. FAHFA possess anti-inflammatory and anti-diabetic properties, while MACDG has been shown to be effective in treating rheumatoid arthritis, sepsis, inflammation and asthma [3]. Consumption of dietary McTG was demonstrated to have a beneficial impact on weight management [6]. DG is reported to be an effective anti-obesity agent due to its ability to suppress abdominal and visceral fat accumulation [7]. While these lipids are present in plant-based foods such as pineapple, oats, garlic, coconut and palm kernel oils, ruminant meats including beef and moose meat constitute significant sources of FAHFA, DG, MACDG and McTG [3,4,8].

Marination and grilling are popular meat processing techniques that enhance flavor, digestibility and visual appeal of cooked ruminant meats. However, the dry conditions and high temperatures required for grilling can lead to reactions that alter nutritional and organoleptic qualities of grilled meats [9]. Increased lipid oxidation and hydrolysis which accompany grilling can result in rancidity, off-flavor, discoloration, decreased shelf-life and production of toxic compounds, which compromise grill meat quality and safety [10]. Furthermore, the presence of polyunsaturated fatty acids (PUFA) and/or ester bonds makes FAHFA, DG, MACDG and McTG susceptible to oxidative and hydrolytic degradation during grilling [11]. The use of herbs, spices and beers containing natural antioxidant compounds to protect against oxidative and hydrolytic degradation of meat lipids is well known [12,13]. The anti-radical activity of herbs, spices and beers has been attributed to mainly antioxidant, polyphenol and oxygenated terpene compounds present in culinary herbs, spices and beers which are capable of donating hydrogen atoms and/or electrons for pairing with lipid radicals produced during lipid oxidation [14]. Compared to filtered beers, unfiltered beers including session ales are richer in antioxidants and polyphenols. The polyphenol contents and antioxidant capacities have been shown to increase exponentially when unfiltered beers are infused with herbs, spices, fruits, hops and wheat during the brewing process to enhance beer flavor and consumer experience [15]. However, despite the

preponderance of marination and grilling for processing ruminant meat for consumption, not much is known concerning how marination and grilling affect the retention and quality of FAHFA, DG, MACDG and McTG in grilled foods including ruminant meats.

The objective of this study was to assess the effects of marination with beer based marinades followed by grilling on the functional lipids (FAHFA, DG, MACDG, and McTG) in ruminant meats. We hypothesized that antioxidant, phenolic and oxygenated terpene compounds present in unfiltered beer-based marinades will be effective in preserving emerging functional lipids such as FAHFA, DG, MACDG, and McTG in ruminant meat following marination and grilling. This study follows our previous work which showed moose and caribou meats as excellent sources of these functional lipids [3]. To the best of our knowledge, the current study is among the first to show the effects of marination and grilling on the levels of these lipids in beef and moose meat.

## 2. Materials and methods

### 2.1. Standards and reagents

FAHFA, DG, and McTG standards were purchased from Avanti Polar Lipids (Alabama, USA). MACDG 16:0/18:1/2:0 was obtained from Chemforce Laboratories (Alberta, Canada), and was used for MACDG quantification (0–50 µg/mL;  $R^2 \geq 0.99$ ) whereas McTG 8:0/8:0/8:0 was used for McTG quantification (0–100 µg/mL;  $R^2 \geq 0.99$ ). FAHFA working calibration solutions containing 12-POHSA, 9-POHSA, 5-POHSA and 12-PAHSA were used to generate standard curves (0–50 µg/mL;  $R^2 \geq 0.99$ ) for FAHFA quantification in grilled meats. DG calibration solutions containing 1,2-DG 18:0/20:4 and 1,3-DG 18:1/18:1 was used for DG quantification (0–100 µg/mL;  $R^2 \geq 0.999$ ). All standard solutions were prepared in chloroform [3]. Quercetin, Folin & Ciocalteu's phenol reagent and formic acid were purchased from Sigma Aldrich (Ontario, Canada). All solvents were from VWR International (Ontario, Canada) and were of HPLC grade.

### 2.2. Sample preparation

#### 2.2.1. Preparation of marinades and meat grilling

The present work focuses on FAHFA, DG, MACDG and McTG lipids in grilled meats, and builds up on our previous work on unfiltered beer-based marination effects on plasmalogens [16] and

glycerophospholipids [17] in grilled moose meat and beef. Significantly, the same batch of grilled meats was used in the present and previous related studies, and the reader is directed to these sources for detailed description of marinade preparation and meat grilling conditions [16,17]. Briefly, marinade ingredients including “herbs, spices, India ale and Wheat ale beers were purchased from supermarkets in Corner Brook, Newfoundland and Labrador, Canada. India ale contained 4.3% alcohol, water, malted barley, and hops whereas Wheat ale contained 5.2% alcohol, water, malted wheat, barley, orange, lemon, lime peel, coriander, Cascade and Willamette hops. Each marinade was formulated with 341 mL beer, 1 g oregano, 1 g parsley, 4 g mustard, 2 g salt, 8 g pepper, 1 g garlic, 25 mL virgin olive oil, 15 mL vinegar and 25 g fresh onions” [16,17]. For moose meat (M), striploin steaks (*Longissimus thoracis et lumborum*) from four (4) different animals were provided by Newfoundland and Labrador Department of Natural Resources, whilst four (4) beef (B) steaks were purchased from different local supermarkets, Corner Brook, Newfoundland, Canada. Meat samples were prepared as follows: moose steaks (0.454 kg) from each animal was cut into smaller sizes (ca. 10 g) and homogenized. Afterwards, the mixture was divided into three (3) equal portions as follows: control group (MC) corresponded to unmarinated moose meat samples only; treatment group (MW) corresponded to moose meat samples marinated with Wheat ale-based marinade while treatment group (MI) was marinated with India ale-based marinade. Taken together four replicates ( $n = 4$ ) per treatment group for moose meat were prepared for statistical considerations. Beef steaks (0.454 kg) were prepared in similar fashion as described for moose meat. Accordingly, for beef, control group (BC) contained unmarinated beef samples whereas treatment groups (BW) and (BI) corresponded to beef samples marinated with Wheat ale-based marinade and India ale-based marinade respectively. For consistency, four (4) replicates ( $n = 4$ ) per treatment group for beef were prepared. Ethics approval for this study was provided by Memorial University Animal Care Committee [18], and all experiments were performed in accordance with relevant guidelines and regulations. To prevent contamination of meat flavors, moose and beef steaks were marinated separately in closed zip lock plastic bags containing 600 mL marinade for 12 h at 4 °C. Afterwards, marinated meat samples were grilled under the following conditions: grill temperature = 200–250 °C, duration = 25 min; and internal meat temperature = 75 °C [16,17].

### 2.3. Antioxidant activity, polyphenol content and oxidation status analysis

Extraction and colorimetric measurements of antioxidant activity, polyphenol content and oxidation status of grilled meats are same as described in our previous publications [16–18]. Briefly, extraction of lipophilic antioxidants, polyphenols and pro-oxidants was performed using 0.7% v/v acidified ethanol whereas hydrophilic antioxidants, polyphenols and pro-oxidants were extracted with aqueous sodium phosphate buffer (50 Mm, pH 7.5). Total polyphenol content was measured by Folin-Ciocalteu method [19]. Antioxidant activity measurement was based on ABTS [2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)] radical cation decolorization method and the results corroborated by ferric reducing antioxidant power (FRAP) method [20,21]. The total oxidation status measurement was based on the method of Erel [22]. For statistical purposes, colorimetric measurements were performed in quadruplicates ( $n = 4$ ).

### 2.4. Extraction and analysis of FAHFA, DG MacDG and McTG

The extraction and analysis of grilled meat lipids followed the same procedure described in our previous publications [16,17]. Briefly, 2 mL chloroform/methanol (2:1, v/v) was mixed with 1 g ground grilled meat and 1 mL of 0.25% KCl [23]. The resultant mixture was centrifuged (10,000 rpm, 10 min, 0 °C), and the organic phases pooled, dried, weighed and reconstituted in 1 mL chloroform for ultra-high performance liquid chromatography/high resolution accurate mass tandem mass spectrometry (UHPLC-HRAMS/MS) analysis. Resolution of lipids was performed on an “Accucore C30 column (150 × 2 mm I.D., particle size: 2.6 µm, pore diameter: 150 Å; Thermo Fisher Scientific, ON, Canada) coupled to Dionex Ultimate 3000 ultra-high performance liquid chromatography system and Q-Exactive mass spectrometer (Thermo Fisher Scientific, ON, Canada)” [16]. UHPLC was performed as follows: solvent A = acetonitrile:water (60:40%, v/v), solvent B = isopropanol:acetonitrile:water (90:10:1%, v/v), with both mobile phases containing 10 mM ammonium formate and 0.1% formic acid. MS data was acquired in both positive and negative modes under electrospray ionization conditions, and detailed parameters for UHPLC-HRAMS/MS as well as lipidomics data processing using LipidSearch version 4.1 (Mitsui Knowledge Industry, Tokyo) are same as previously described [3,16,24]. Total FAHFA, DG, MacDG and McTG contents of

grilled meats were expressed on mg/100 g FW meat basis while lipid molecular species and fatty acid compositions (based on intact molecular species) were presented on nanomole percentage (nmol%) basis per lipid class. The differences in lipid contents and molecular species compositions between marinated and unmarinated grilled meats were calculated using equation (1):

$$\% \text{ retention / loss} = \frac{(\text{marinated} - \text{unmarinated})}{\text{unmarinated}} * 100 \quad (1)$$

While differences in lipid contents between raw and grilled meats were based on equation (2):

$$\% \text{ retention / loss} = \frac{(\text{grilled} - \text{raw})}{\text{raw}} * 100 \quad (2)$$

### 2.5. Volatile oxygenated terpenes analysis by HS-SPME-GC/MS

Volatile oxygenated terpenes including carvacrol, linalool, endo-borneol, terpinen-4-ol and terpineol in grilled meat samples (1 g) were extracted and analyzed by head space solid phase micro-extraction coupled to gas chromatography/mass spectrometry (HS-SPME-GC/MS) as previously described [18,25]. The terpenes were semi-quantified based on area counts  $\times 10^6$  of base peaks which allowed for comparison of their relative abundances in the grilled meats [25,26]. Taken together, oxygenated terpenes, antioxidant activities, polyphenol contents and oxidation status were used to rationalize preservation of FAHFA, DG, MACDG and McTG molecular species in marinated grilled meats.

### 2.6. Statistical analysis

For all statistical analysis, XLSTAT (Premium Version, Addinsoft, NY, USA) was used. Lipid concentrations in grilled meat samples are presented as mean  $\pm$  standard error (SE). One-way analysis of variance (ANOVA) based on a general linear model (GLM) was used to determine if there were significant differences between FAHFA, DG, MACDG and McTG levels in marinated and unmarinated grilled meat samples. In this model, marination treatment groups (MC, MW, MI, BC, BW, and BI) were treated as fixed effect whereas treatment replications ( $n = 4$  per treatment group) were considered as random effect. A similar model was applied to antioxidant activities, phenolic contents, oxidation status and

oxygenated terpene contents of grilled meat samples [27]. Least square means were compared using Fisher's Least Significant Difference (LSD) test, and differences between means were deemed significant when  $p \leq 0.05$ . Associations between lipid molecular species and treatment groups were modelled using principal component analysis (PCA). Pearson's correlation coefficients ( $r$ ) were used as measures of strength of relationships between lipid molecular species and antioxidant activities, phenolic contents, oxygenated terpene contents and oxidation status of grilled meats [28].

## 3. Results and discussion

### 3.1. Grilling completely degraded the MACDG content in grilled meat

MACDG has been reported in plant and animal sources including camelina, larvae of *Eurosta solidaginis*, caribou and moose meats [3]. However, MACDG contents in cooked foods are generally very limited in the literature. One of the most interesting finding in this paper is that when we marinated and grilled moose meat the MACDG was completely degraded. These findings suggest loss of these lipids possibly by thermal, oxidative and/or non-oxidative degradation during grilling [29]. Degradation of meat lipids during cooking is a ubiquitous process involving oxidation, dehydration, decarboxylation, hydrolysis of ester bonds, double bond conjugation, polymerization, dehydrocyclization, aromatization, dehydrogenation, and degradation by carbon-carbon cleavage, which could compromise the quality, safety and shelf life of cooked meat [11,30]. Further work is required to determine the process responsible for the complete loss of MACDG in grilled foods, which further limits their intake in the diet.

### 3.2. Marination effects on grilled meats functional lipids

#### 3.2.1. FAHFA content and composition in grilled ruminant meat

The fat content of grilled meat samples in current study have been reported in our related publications [16–18]. Briefly, total fat of grilled beef was  $3.92 \pm 0.56$  g/100 g FW compared to  $0.68 \pm 0.08$  g/100 g FW in grilled moose. To evaluate effects of beer-based marination on grilled meats, the FAHFA content and composition of India ale-based marinated moose and beef (MI and BI), Wheat ale-based marinated grilled moose and beef (MW and BW),

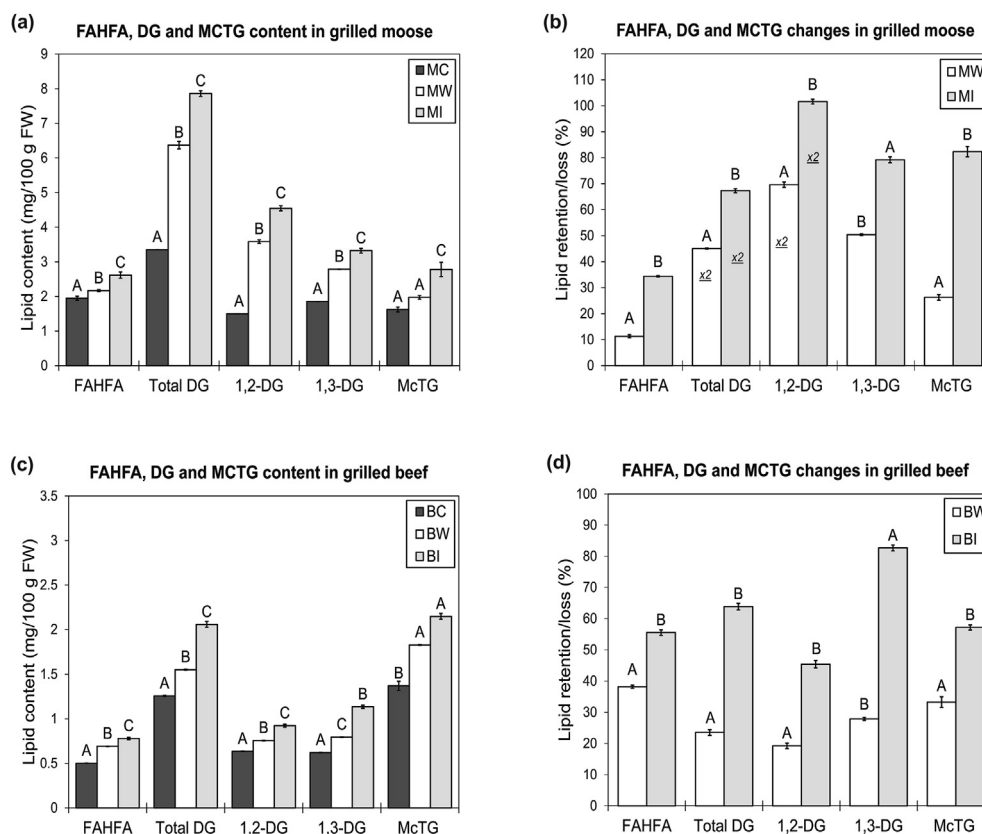


Fig. 1. Marination effects on FAHFA, DG, and MCTG content of grilled meats. Values in bar charts represent mean  $\pm$  standard errors. Means with different letters are significantly different at LSD ( $p < 0.05$ ;  $n = 4$ ). a, c) FAHFA, DG and MCTG content (mg/100 g FW) of grilled moose meat and beef, respectively. b, d) Retention levels (%) of FAHFA, DG and MCTG in grilled moose and beef, respectively. Total DG = 1,2-DG + 1,3-DG. [BC, MC] = unmarinated beef and moose; [BI, MI] = India ale-based marinated beef and moose; [BW, MW] = Wheat ale-based marinated beef and moose. FAHFA = Fatty acid esters of hydroxy fatty acids; DG = Diglycerides; MCTG = Medium chain triglycerides.

were compared with unmarinated grilled moose and beef (MC and BC) samples, as is shown in Fig. 1. Unfiltered beer-based marination had a beneficial effect on FAHFA in grilled meats. In moose, FAHFA content was higher ( $p < 0.05$ ) in marinated grilled moose meats (MW and MI) compared to unmarinated counterpart (Fig. 1a). The corresponding differences were positive (11–34%) in Fig. 1b, which appears to suggest the retention of FAHFA lipids by marination against degradative processes during grilling [31]. Antioxidant compounds in culinary herbs, spices and beers have been shown to suppress oxidative degradation of lipids when used to marinate meats before cooking [32]. Antioxidants including phenolics acids, hydrocinnamic acids, oxygenated terpenes and vitamin C (ascorbate) in culinary herbs and spices inhibit lipid oxidative degradation by donating electrons or hydrogen atoms for pairing with lipid radicals formed during lipid oxidation reactions in meat [14]. Furthermore, FAHFA retention levels in MI were higher ( $p < 0.05$ ) compared to MW suggesting superior preservation

of FAHFA in moose meat by India ale-based marinade (34.5%) compared to Wheat-ale based marinade during grilling (11.3%) as shown in Fig. 1b. Significantly, while the two beer-based marinades under evaluation were formulated from the same type and quantities of antioxidant-rich herbs and spices, they differed in the type of unfiltered session ale beers used for marinade preparation (see section 2.2.1). India ale is a dark beer containing melanoidins which are derived from kilned malts, and is a potent antioxidant compound with health advantages [33]. In contrast, Wheat ale beer contains antioxidants including limonoids and vitamin C (ascorbate) from infusion of orange, lemon and lime extracts during brewing to enhance beer taste and consumer experience [34]. Thus, it is conjectured that the combination of herbs, spices and India-ale beer produced a marinade with superior phenolic content more effective at preserving FAHFA lipids in moose meat against oxidative degradative processes during grilling compared to Wheat-ale based marinade [18]. It is important to point out that while

FAHFA contents of grilled moose meats (marinated and unmarinated) were generally lower compared to the FAHFA content (3.67 mg/100 g FW) reported for raw moose meat in our previous work [3], marination mitigated degradation of FAHFA in grilled moose meats. In marinated grilled moose meats, the loss due to grilling (calculated using eqn. (2)) in MI (−28.7%) and MW (−41.0%) were lower than in MC (−47.0%), which appears to suggest beneficial effect of beer-based marination on retention of FAHFA in grilled moose meat. However, further work is needed to increase retention of FAHFA in moose meat against degradation during grilling.

The FAHFA contents of grilled beef are shown in Fig. 1c, and were lower ( $p < 0.05$ ) compared to FAHFA in grilled moose meat. The disparity in FAHFA levels between beef and moose meat to beer-based marination and grilling could be due to differences in intramuscular fat composition between beef and moose meat which result from genetics and diets among other factors [35,36].

Moose are free-ranging herbivores and produce meat with superior nutritional quality including high protein to fat ratio and superior fatty acid content [37]. By contrast, most commercial beef cattle are raised on high-grain/low-forage rations which produce beef with superior marbling quality preferred by consumers. This leads to reduced protein/fat content of beef compared to grass fed animals including moose [38]. Unfiltered beer-based marination showed similar preserving effect on FAHFA content of grilled beef. In grilled beef, we demonstrated that India ale-based marinade was more effective compared to Wheat ale-based marinade for preserving FAHFA (Fig. 1c). The FAHFA content of BI and BW were higher ( $p < 0.05$ ) compared to BU which demonstrate retention of FAHFA in beef by marination. The calculated differences were positive in BI (55%) and BW (38%) which suggests superior preservation of FAHFA in beef by India ale-based marinade compared to Wheat ale-based marinade against degradation during grilling (Fig. 1d).

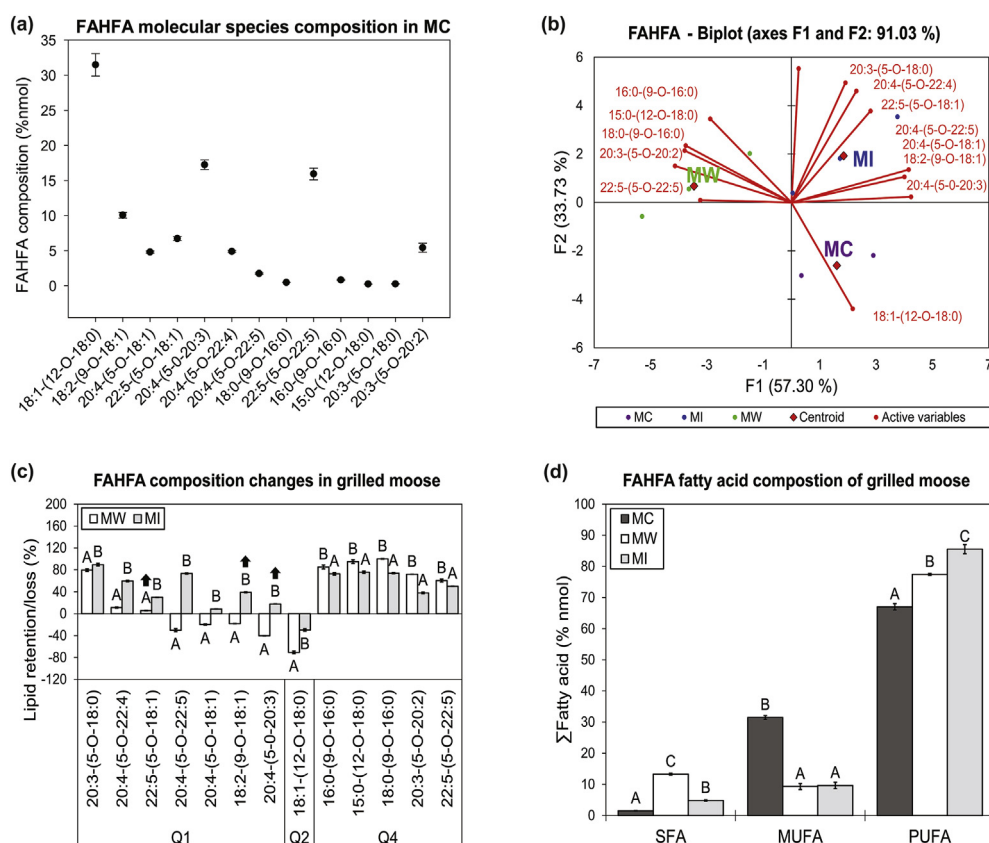


Fig. 2. Marination effects on FAHFA molecular species in grilled moose meat. Values in bar charts and dot plot represent mean  $\pm$  standard errors. Means with different letters are significantly different at LSD ( $p < 0.05$ ;  $n = 4$ ). a) FAHFA species profile. b) Principal component analysis showing clustering of FAHFA species. c) FAHFA retention levels (%). d) FAHFA fatty acid composition. [MC] = unmarinated moose; [MI] = India ale-based marinated moose; [MW] = Wheat ale-based marinated moose; FAHFA = Fatty acid esters of hydroxy fatty acids; PUFA = polyunsaturated fatty acids; MUFA = monounsaturated fatty acids; SFA = saturated fatty acids.

To evaluate how marination affected the quality of FAHFA in grilled meats, we investigated the molecular species and fatty acid compositions of FAHFA in grilled moose meat and beef (Fig. 2 and 3). FAHFA molecular species profile in grilled moose was predominated by unsaturated fatty acid (UFA) enriched species including 18:1-(12-O-18:0), 20:4-(5-O-20:3), 22:5-(5-O-22:5) and 18:2-(9-O-18:1), which constituted 75% of FAHFA composition (Fig. 2a) [3]. Following PCA, 12 out of 13 FAHFA species clustered with marinated moose samples in Q-1 and Q-4 of the PCA biplot based on their concentrations and degrees of unsaturation (Fig. 2b). These species were generally preserved by marination against degradation in grilled moose meat as evidenced by their positive retention levels as shown in Fig. 2c [39]. Among these, 7 PUFA enriched FAHFA species grouped MI samples in Q-1 of PCA biplot and were characterized by higher ( $p < 0.05$ ) retention levels in MI (9–89%) compared to MW samples (–40 to 79) possibly due to superior suppression of oxidative degradation by antioxidants present in India ale-based marinade

during grilling (Fig. 2c,d) [18]. Furthermore, these preserved species showed stronger positive correlations with antioxidants ( $TAA^{ABTS}$ :  $r = 0.19–0.93^{**}$ ,  $p < 0.05$  or  $0.01$ ;  $TAA^{FRAP}$ :  $r = 0.33–0.95^{**}$ ,  $p < 0.05$  or  $0.01$ ), polyphenols (TPC:  $r = 0.32–0.97^{**}$ ), and oxygenated terpenes ( $\sum OT$ :  $r = 0.31–0.91^{**}$ ) present in India ale-based marinade compared to corresponding correlations in Wheat ale-based marinade (Table 1). Significantly, these retentions were accompanied by significant reduction in oxidation levels of MI samples as evidenced by negative correlations between the retained species in MI and total oxidation status (TOS:  $r = -0.58$  to  $-0.93^{**}$ ), which appears to suggest a relationship between suppression of oxidative degradation of these PUFA enriched FAHFA species and antioxidant compounds present in India ale-based marinades [18,25]. In contrast, the 5 remaining mainly saturated fatty acid (SFA) enriched FAHFA species including 16:0-(9-O-16:0), 15:0-(12-O-18:0), 18:0-(9-O-16:0), 20:3-(5-O-20:2) and 22:5-(5-O-22:5) clustered MW samples in Q-4 and showed higher retention levels in MW with corresponding higher

Table 1. Pearson's correlation coefficients showing relationships between antioxidant activity, phenolic content, oxygenated terpenes, oxidation status and preserved FAHFA molecular species in grilled meats.

| Moose            | MI Samples   |         |         |              |           | MW Samples   |         |         |              |           |
|------------------|--------------|---------|---------|--------------|-----------|--------------|---------|---------|--------------|-----------|
| FAHFA            | $TAA^{ABTS}$ | TPC     | TOS     | $TAA^{FRAP}$ | $\sum OT$ | $TAA^{ABTS}$ | TPC     | TOS     | $TAA^{FRAP}$ | $\sum OT$ |
| 18:2-(9-O-18:1)  | 0.19         | 0.32    | –0.58   | 0.33         | 0.31      | –0.66        | –0.63   | 0.70*   | –0.62        | –0.29     |
| 20:4-(5-O-18:1)  | 0.56         | 0.67    | –0.83** | 0.66         | 0.63      | –0.80**      | –0.78*  | 0.82**  | –0.77*       | –0.48     |
| 22:5-(5-O-18:1)  | 0.74*        | 0.82**  | –0.91** | 0.81**       | 0.78*     | 0.24         | 0.28    | –0.16   | 0.29         | 0.61      |
| 20:4-(5-O-20:3)  | 0.49         | 0.60    | –0.78*  | 0.60         | 0.57      | –0.93**      | –0.91** | 0.93*   | –0.91**      | –0.68     |
| 20:4-(5-O-22:4)  | 0.79*        | 0.86**  | –0.91** | 0.85**       | 0.81**    | 0.60         | 0.63    | –0.53   | 0.64         | 0.84**    |
| 20:4-(5-O-22:5)  | 0.84**       | 0.90**  | –0.92** | 0.88**       | 0.85**    | –0.67        | –0.65   | 0.71*   | –0.64        | –0.31     |
| 18:0-(9-O-16:0)  | 1.00**       | 1.00**  | –0.87** | 0.97**       | 0.93**    | 0.99**       | 1.00**  | –0.98** | 1.00**       | 0.87**    |
| 22:5-(5-O-22:5)  | 0.51         | 0.39    | –0.05   | 0.36         | 0.34      | 0.64         | 0.60    | –0.65   | 0.60         | 0.29      |
| 16:0-(9-O-16:0)  | 0.93**       | 0.97**  | –0.94** | 0.95**       | 0.91**    | 0.95**       | 0.96**  | –0.90** | 0.97**       | 0.97**    |
| 15:0-(12-O-18:0) | 0.94**       | 0.7**   | –0.92** | 0.95**       | 0.91**    | 0.98**       | 0.98**  | –0.99** | 0.98**       | 0.80**    |
| 20:3-(5-O-18:0)  | 0.93**       | 0.97**  | –0.93** | 0.95**       | 0.91**    | 0.91**       | 0.92**  | –0.83** | 0.93**       | 0.98**    |
| 20:3-(5-O-20:2)  | 0.74*        | 0.64    | –0.33   | 0.62         | 0.59      | 0.96**       | 0.96**  | –0.96** | 0.95**       | 0.76*     |
| Beef             | BI Samples   |         |         |              |           | BW Samples   |         |         |              |           |
| FAHFA            | $TAA^{ABTS}$ | TPC     | TOS     | $TAA^b$      | $\sum OT$ | $TAA^{ABTS}$ | TPC     | TOS     | $TAA^{FRAP}$ | $\sum OT$ |
| 18:1-(12-O-18:0) | 0.87**       | 0.77*   | –0.74*  | 0.85**       | 0.76*     | 0.87**       | 0.71*   | –0.73*  | 0.84**       | 0.74*     |
| 20:4-(5-O-20:3)  | 0.98**       | 0.93**  | –0.94** | 0.90**       | 0.99**    | 0.98**       | 0.96**  | –0.95** | 0.99**       | 0.96**    |
| 18:0-(9-O-16:0)  | 0.68         | 0.76*   | –0.79*  | 0.62         | 0.78*     | –0.96**      | –0.96** | 0.90**  | –0.93**      | –0.95**   |
| 20:3-(5-O-18:0)  | 0.66         | 0.77*   | –0.79*  | 0.68         | 0.69      | 0.52         | 0.52    | –0.39   | 0.39         | 0.50      |
| 18:2-(9-O-18:1)  | –0.92**      | –0.92** | 0.97**  | –0.95**      | –0.90**   | 0.95**       | 0.93**  | –0.79*  | 0.81**       | 0.95**    |
| 20:4-(5-O-18:1)  | –0.97**      | –0.99** | 0.97**  | –0.92**      | –0.97**   | 0.56         | 0.64    | –0.66   | 0.62         | 0.57      |
| 22:5-(5-O-18:1)  | –0.96**      | –0.95** | 0.97**  | –0.98**      | –0.93**   | 0.47         | 0.55    | –0.42   | 0.39         | 0.45      |
| 20:4-(5-O-22:4)  | 0.17         | 0.19    | –0.05   | 0.04         | 0.20      | 0.46         | 0.45    | –0.26   | 0.29         | 0.48      |
| 20:4-(5-O-22:5)  | –0.89**      | –0.89** | 0.82**  | –0.81**      | –0.88**   | 0.85**       | 0.85**  | –0.85** | 0.83**       | 0.84**    |
| 22:5-(5-O-22:5)  | –0.69        | –0.71*  | 0.81**  | –0.77        | –0.68     | 0.51         | 0.51    | –0.32   | 0.35         | 0.53      |
| 16:0-(9-O-16:0)  | 0.37         | 0.50    | –0.59   | 0.61         | 0.30      | 0.98**       | 0.99**  | –0.94** | 0.95**       | 0.97**    |
| 20:3-(5-O-20:2)  | 0.87**       | 0.92**  | –0.97** | 0.90**       | 0.89**    | 0.98**       | 0.99**  | –0.94** | 0.95**       | 0.97**    |

Values with \*: significant correlation ( $p < 0.05$ ); \*\*: significant correlation ( $p < 0.01$ ). [BC, MC] = Unmarinated beef and moose; [BI, MI] = India ale-based marinated beef and moose; [BW, MW] = Wheat ale-based marinated beef and moose.  $\sum OT$  = Total oxygenated terpenes (linalool + endo-borneol + terpinen-4-ol + terpineol + carvacrol + carvacrol isomer-1 + carvacrol isomer-2); TAA = Total antioxidant activity; TPC = Total phenolic content; TOS = Total oxidant status.  $TAA^{ABTS}$  = ABTS antioxidant activity;  $TAA^{FRAP}$  = FRAP antioxidant activity; FAHFA = Fatty acid hydroxy fatty acid.

positive correlations with antioxidants ( $TAA^{ABTS}$ :  $r = 0.64–0.99^{**}$ ,  $p < 0.05$  or  $0.01$ ;  $TAA^{FRAP}$ :  $r = 0.60–1.00^{**}$ ,  $p < 0.05$  or  $0.01$ ), polyphenols (TPC:  $r = 0.60–1.00^{**}$ ), and oxygenated terpenes ( $\sum OT$ :  $r = 0.29–0.98^{**}$ ) along with reduced oxidation status in MW samples as shown by significant negative correlations with TOS ( $r = -0.65$  to  $-0.99^{**}$ ) in Table 1. Taken together, these results demonstrate higher retention of SFA enriched FAHFA species in grilled moose meat by Wheat ale-based marination compared to India ale-based marinades (Fig. 2d). It is important to point out that the TAA, TPC and TOS values used to rationalize retention of FAHFA species in grilled moose in current study are provided in our previous publication [18]. The present results for FAHFA in marinated grilled moose are in good agreement with these values, which showed that higher ( $p < 0.05$ ) TPC in MI compared to MW samples.

The FAHFA molecular species composition in grilled beef was predominated by SFA enriched 18:1-(12-O-18:0), 18:0-(9-O-16:0), 16:0-(9-O-16:0) and 20:3-(5-O-20:2) species as shown in Fig. 3a [40].

Significantly, beef FAHFA composition contrasts with grilled moose meat composition which was predominated by PUFA enriched FAHFA species, and is in line with differences in intramuscular fat composition between beef cattle and moose alluded to previously [35]. PCA successfully clustered grilled beef samples in distinct quadrants of the PCA biplot (Fig. 3b). Eleven (11) mainly PUFA enriched FAHFA species were grouped with BW in positive quadrants of component 1 (F1) of the biplot. The retention levels of these species were positive and higher ( $p < 0.05$ ) in BW samples (21–99%) compared to BI (–81–31%), which appears to suggest greater retention of PUFA enriched FAHFA species in beef by antioxidants in Wheat ale-based marinade compared to India ale-based marinade against degradation during grilling (Fig. 3c–d) [11]. Furthermore, the preserved PUFA species were more strongly positively correlated with antioxidants ( $TAA^{ABTS}$ :  $r = 0.46–0.98$ ,  $p < 0.05$  or  $0.01$ ;  $TAA^{FRAP}$ :  $r = 0.29–0.99^{**}$ ,  $p < 0.05$  or  $0.01$ ), polyphenols (TPC:  $r = 0.45–0.99^{**}$ ), and oxygenated

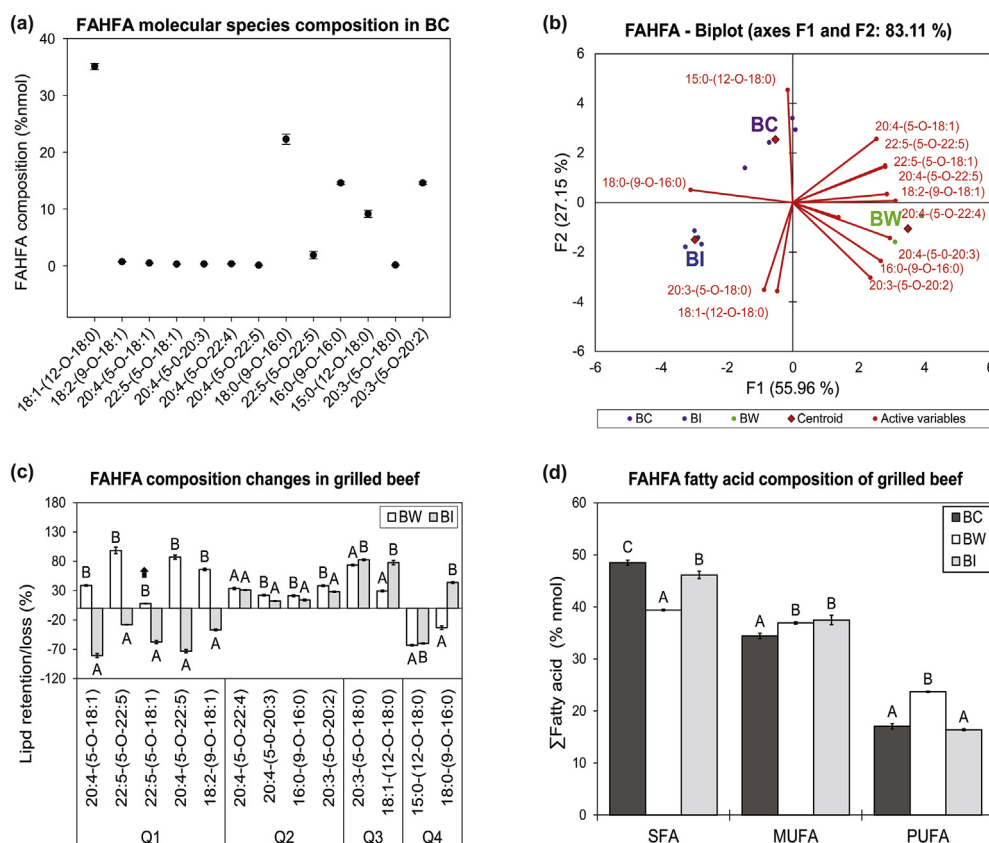


Fig. 3. Marination effects on FAHFA molecular species in grilled beef. Values in bar charts and dot plot represent mean  $\pm$  standard errors. Means with different letters are significantly different at LSD ( $p < 0.05$ ;  $n = 4$ ). a) FAHFA species profile. b) Principal component analysis showing clustering of FAHFA species. c) FAHFA retention levels (%). d) FAHFA fatty acid composition. [BC] = unmarinated grilled beef; [BI] = India ale-based marinated beef; [BW] = Wheat ale-based marinated beef; FAHFA = Fatty acid esters of hydroxy fatty acids; PUFA = polyunsaturated fatty acids; MUFA = monounsaturated fatty acids; SFA = saturated fatty acids.

terpenes ( $\sum\text{OT}$ :  $r = 0.45\text{--}0.97^{**}$ ) present in Wheat ale-based marinade, and negatively correlated with TOS ( $r = -0.26$  to  $-0.95^{**}$ ) of BW compared to the corresponding correlations in BI samples as shown in Table 1 [41]. In contrast, 4 SFA enriched FAHFA species clustered in Q-3 and Q-4 of biplot with BI and BC respectively (Fig. 3b). Among these species, 18:1-(12-O-18:0), 18:0-(9-O-16:0) and 20:3-(5-O-18:0) which made up 57% of FAHFA composition in grilled beef showed higher ( $p < 0.05$ ) retention levels in BI (44–83%) compared to BW samples (–33–74%) as shown in Fig. 3c. These retentions were accompanied by stronger ( $p < 0.05$ ) correlations between retained SFA enriched species and  $\text{TAA}^{\text{ABTS}}$  ( $r = 0.66\text{--}0.87^{**}$ ,  $p < 0.05$  or  $0.01$ ),  $\text{TAA}^{\text{FRAP}}$  ( $r = 0.62\text{--}0.85^{**}$ ,  $p < 0.05$  or  $0.01$ ), TPC ( $r = 0.76^{*}\text{--}0.77^{*}$ ),  $\sum\text{OT}$  ( $r = 0.69\text{--}0.78^{*}$ ) and TOS ( $r = -0.74^{*}$  to  $-0.79^{*}$ ) of BI samples compared to BW (Table 1). It is important to point out that while total FAHFA content was higher ( $p < 0.05$ ) in BI (0.78 mg/100 g FW) compared to BW (0.69 mg/100 g FW), Wheat ale-based marination preserved 11 FAHFA species compared to only 7 species retained by India ale-based marinade. The retention of greater number of FAHFA species in beef by Wheat ale marination against oxidative degradation during grilling appears to be in good accordance with higher ( $p < 0.05$ ) TAA, TPC and corresponding lower TOS of BI compared to BW reported in our previous work [18]. These associations appear to suggest antioxidants, polyphenols and oxygenated terpenes present in the marinades could be significantly related to preservation of FAHFA species in grilled beef [42]. However, while these correlations show associations between antioxidants, polyphenols and oxygenated terpenes present in the marinades and suppression of FAHFA degradation in the grilled meats, they do not explain cause or effects of marination; but rather suggest the existence of relationships, and as such further work is required to fully investigate the scope of the relationships.

In summary, the results indicate that the unfiltered beer-based marinades effectively preserved ( $p < 0.05$ ) FAHFA in beef and moose meat against oxidative degradation during grilling compared to unmarinated counterparts. In moose, marination preserved mainly SFA and PUFA enriched FAHFA species whereas MUFA and PUFA enriched species were retained in beef against degradation during grilling (Figs. 2d and 3d). Significantly, marination did not preserve MUFA enriched FAHFA species in grilled moose. Furthermore, SFA enriched FAHFA species were not preserved ( $p < 0.05$ ) by marination in grilled beef. Consumption of MUFA and PUFA enriched FAHFA has been demonstrated in human,

cell and animal studies to be beneficial in reducing inflammation associated with ulcerative colitis and chronic low-grade inflammation in obese patients with type 2 diabetics [43]. Furthermore, SFA enriched FAHFA species have been found to be effective in treating type 2 diabetes [40]. In view of these associated health benefits, preservation of FAHFA in grilled ruminant meats by marination could increase consumption of FAHFA lipid species as part of the normal diet, which could potentially contribute to reducing the risks for developing diabetes and inflammatory diseases while safeguarding the nutritional quality of grilled ruminant meat. However, further work needed to improve the effectiveness of the marinades for preserving all FAHFA species in grilled beef and moose meat.

### 3.2.2. DG content and composition in grilled ruminant meat

Dietary DG are less likely to be stored as body fat compared to triglycerides and have been shown to reduce body weight accumulation [44]. In this study, we demonstrate unfiltered beer-based marination preserved DG in ruminant meats against degradation during grilling (Fig. 1). In moose meat, total DG was higher ( $p < 0.05$ ) in MI and MW compared to MC (Fig. 1a). The calculated retention levels was significantly higher in MI (134%) compared to MW (90%) as shown in Fig. 1b, which suggests greater preservation of DG by India ale-based marination compared to Wheat ale-based marination against oxidative degradative processes during grilling [25,41]. The trend in beef was similar in that the total DG content was higher ( $p < 0.05$ ) in marinated grilled beef (BW and BI) compared to BU (Fig. 1c), along with superior ( $p < 0.05$ ) retention levels obtained when beef was marinated with India-ale based marinade (64%) compared with Wheat ale-based marinade (24%) as shown in Fig. 1d. Viegas et al. (2012) observed similar antioxidant effect on suppression of oxidation and HCA formation when dark beers in combination with herbs and spices were used to marinate beef [12]. DG were demonstrated in clinical studies to suppress obesity and post prandial hyperlipidemia which increase risk factors for diabetes and cardiovascular diseases in human subjects [45,46]. As such, preservation of DG in beef and moose meat by marination against degradation during grilling could improve its consumption in the diet. Naturally occurring DG is composed of 1,2-DG and 1,3-DG isoforms. The more stable 1,3-DG are formed by isomerization of 1,2-DG whilst 1,2-DG are produced from incomplete biosynthesis of TG and/or from lipolysis of TG [47]. Consistent with total DG distributions, 1,2-DG

and 1,3-DG were higher ( $p < 0.05$ ) in the marinated grilled meats compared to unmarinated counterparts (see Fig. 1a and c). Furthermore, the calculated retention levels followed the trends similar to DG content in marinated meats with significantly higher positive retention levels in India ale-based marinated compared to wheat ale-based marinated grilled meats (Fig. 1b, d). Taken together, these results appear to suggest that unfiltered beer based marination could be used to protect 1,2- and 1,3-DG against oxidative degradation during grilling of beef and moose meat. Of nutritional interest is the fate of 1,3-DG in grilled meat since they are more effective compared to 1,2-DG isomers at lowering post-prandial serum TG and obesity when present in the diet, which are known risk factors for developing diabetes and cardiovascular diseases [44]. Significantly, both marinades evaluated in current study were effective for preserving the nutritionally beneficial 1,3-DG isomers in grilled ruminant meats against degradation.

The nutritional quality of DG is derived mainly from structural differences of DG lipids and not based on fatty acid composition [3,44]. 1,2-DG molecular species profile in grilled moose meat was composed of 15 species predominated by C18 linked 24:0/18:1, 18:1/18:1, and 16:0/18:2 species which together made up 70% of the composition as shown in Fig. 4a [3]. Beer based marination preserved 13 1,2-DG molecular species against degradation during grilling of moose meat (Fig. 4b). Specifically, consistent with higher total 1,2-DG content in MI compared to MW, India ale-based marination retained 9 species in grilled moose meat amongst which 24:0/18:1, 18:0/20:0, 18:0/22:5, 16:0/18:2, 18:1/18:3 and 18:0/20:4 showed higher ( $p < 0.05$ ) retention levels in MI (10–83%) compared to MW (–21–41%) (Fig. 4b). Taken together, the results indicate superior preservation of 1,2-DG composition in moose meat against oxidative degradation during grilling by India-ale based marinade compared to Wheat ale-based marinade (Fig. 4c, d). This superior retention in India-ale based marinade was accompanied by higher correlations with antioxidants ( $TAA^{ABTS}$ :  $r = 0.59–0.99^{**}$ ,  $p < 0.05$  or  $0.01$ ;  $TAA^{FRAP}$ :  $r = 0.73^{*}–0.97^{**}$ ,  $p < 0.05$  or  $0.01$ ), polyphenols (TPC:  $r = 0.58–0.99^{**}$ ), oxygenated terpenes ( $\sum OT$ :  $r = 0.83^{**}–0.97^{**}$ ) and oxidation status (TOS:  $r = -0.87^{**}$  to  $-0.61$ ) in MI compared to MW (Table 2), suggesting strong relationships between the preserved 1,2-DG species and anti-radical components present in India-ale based marinade [48]. Furthermore, a similar trend was observed for 11 1,2-DG species retained in MW (Fig. 4b), where correlations between the preserved species and  $TAA^{ABTS/FRAP}$

(0.50–1.00<sup>\*\*</sup>,  $p < 0.05$  or  $0.01$ ) TPC (0.56–1.00<sup>\*\*</sup>),  $\sum OT$  (0.47–0.95<sup>\*\*</sup>) and TOS (–0.99<sup>\*\*</sup> to –0.53) were in good accordance with suppression of oxidation degradation of these lipids by antioxidants, polyphenols and oxygenated terpenes in Wheat ale- and India ale-based marinades as shown in Table 2 [16]. The 1,3-DG profile in grilled moose meat was composed mainly of 16:0/18:1, 18:0/18:1, 20:0/18:1 and 18:0/16:0 (Fig. 4e) [3]. The distribution of 1,3-DG molecular species in marinated grilled moose mirrored the trend observed for 1,2-DG species. India ale-based marination retained 9 1,3-DG species including the 3 most abundant 1,3-DG species moose as evidenced by positive calculated retention levels (4–94%), which suggest their preservation against degradation during grilling (Fig. 4f). In contrast, 11 1,3-DG species were preserved by Wheat ale-based marination (9–99%) against degradation during grilling of moose samples (Fig. 4f). It is important to point out that whilst 16:0/18:1, 18:0/18:1 and 18:0/16:0 were also retained in MW, the calculated retention levels were generally higher in MI (72–31%) compared to MW (39–9%), which is in line with greater proportion of 1,3-DG composition preserved in MI compared to MW (Fig. 4g, h). As alluded to previously, preservation of lipid species in marinated grilled moose meat against degradation could be due to the anti-radical action of antioxidant compounds present in the marinades [25]. Accordingly, we observed positive correlations between retained 1,3-DG species and antioxidants ( $TAA^{ABTS/FRAP}$ :  $r = 0.08–0.99^{**}$ ,  $p < 0.05$  or  $0.01$ ), polyphenols (TPC:  $r = 0.18–1.00^{**}$ ), oxygenated terpenes ( $\sum OT$ :  $r = 0.03–0.90$ ) present in the marinades and negative correlations with oxidation status (TOS:  $r = -0.99^{**}$  to  $-0.04$ ), which appear to suggest associations between preservation of 1,3-DG molecular species in moose meat and antioxidants, polyphenols, and oxygenated terpenes present in the marinades (Table 2) [11].

The 1,2-DG molecular species profile in grilled beef was dominated by same C18 linked species (24:0/18:1, 18:1/18:1 and 16:0/18:2) as observed in grilled moose (Fig. 5a). However, the effect of marination on 1,2-DG molecular species distribution was more widespread in beef compared to grilled moose, with greater number of 1,2-DG species preserved by India ale-based marination compared Wheat ale-based marination (Fig. 5b). These differences could be due to variations in intramuscular fat compositions between beef and moose meat as alluded to previously [35]. India ale-based marination preserved 10 1,2-DG species in grilled beef against oxidative degradation as evidenced by positive retention levels in BI (8–99%). Seven (7) of the preserved species including 18:0/

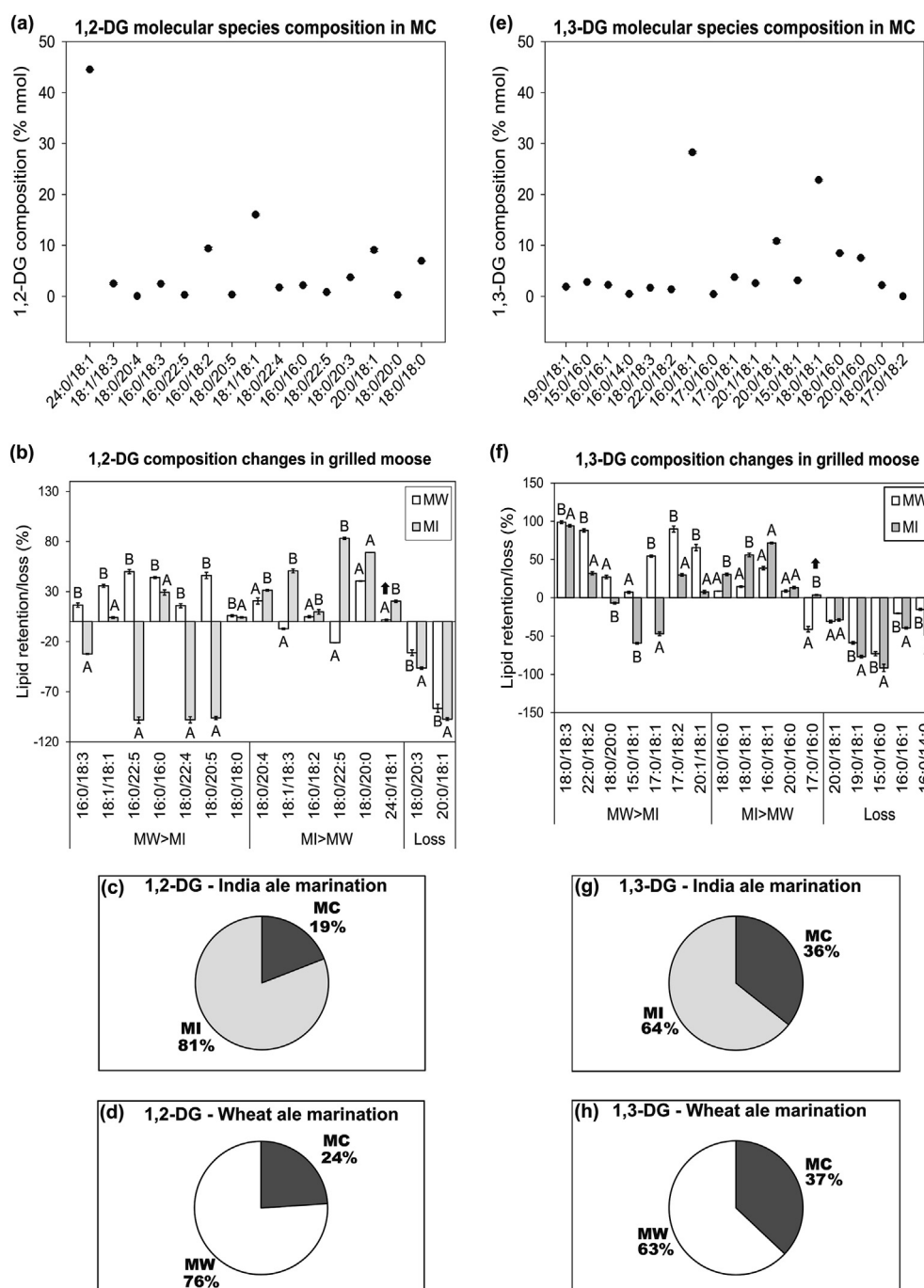


Fig. 4. Marination effects on DG molecular species in grilled moose meat. Values in bar charts and dot plot represent mean  $\pm$  standard errors. Means with different letters are significantly different at LSD ( $p < 0.05$ ;  $n = 4$ ). a, e) 1,2-DG and 1,3-DG species profile of grilled moose meat, respectively. b, f) 1,2-DG and 1,3-DG retention levels (%), respectively. c-d) Relative composition (%) of 1,2-DG in marinated and unmarinated grilled moose. g-h) Relative composition (%) of 1,3-DG in marinated and unmarinated grilled moose. [MC] = unmarinated moose; [MI] = India ale-based marinated moose; [MW] = Wheat ale-based marinated moose. DG = Diglycerides.

18:0, 18:0/20:3, 20:0/18:1, 18:1/18:1, 16:0/18:2, 18:0/20:4, and 18:0/20:0 were characterized by higher ( $p < 0.05$ ) retention levels in BI samples (8–99%) compared to BW (–67–95%). This trend is consistent with greater preservation of 1,2-DG in beef by antioxidants present in India ale-based marination

compared with Wheat ale-based marination against degradation during grilling (see Fig. 5b, 5c-d) [12]. Furthermore, correlation analysis showed stronger ( $p < 0.05$ ) positive associations between the preserved species and antioxidants ( $TAA^{ABTS}$ ;  $r = 0.58–0.98^{**}$ ,  $p < 0.05$  or  $0.01$ ;  $TAA^{FRAP}$ :

Table 2. Pearson's correlation coefficients showing relationships between antioxidant activity, phenolic content, oxygenated terpenes, oxidation status and preserved DG molecular species in grilled moose meat.

| Species   | MI Samples          |         |         |                     |         | MW Samples          |         |         |                     |         |
|-----------|---------------------|---------|---------|---------------------|---------|---------------------|---------|---------|---------------------|---------|
|           | TAA <sup>ABTS</sup> | TPC     | TOS     | TAA <sup>FRAP</sup> | ∑OT     | TAA <sup>ABTS</sup> | TPC     | TOS     | TAA <sup>FRAP</sup> | ∑OT     |
| 24:0/18:1 | 0.99**              | 0.99**  | −0.87** | 0.95**              | 0.89**  | 0.99**              | 0.99**  | −0.99** | 0.99**              | 0.86**  |
| 18:1/18:3 | 0.95**              | 0.94**  | −0.78*  | 0.92**              | 0.90**  | −0.40               | −0.45   | 0.53    | −0.46               | −0.40   |
| 18:0/20:4 | 0.59                | 0.58    | −0.61   | 0.73*               | 0.83**  | 0.56                | 0.54    | −0.53   | 0.55                | 0.48    |
| 16:0/18:3 | −0.99**             | −1.00** | 0.88**  | −0.96**             | −0.91** | 0.98**              | 0.98**  | −0.96** | 0.98**              | 0.85**  |
| 16:0/22:5 | −0.98**             | −1.00** | 0.90**  | −0.97**             | −0.93** | 0.99**              | 0.98**  | −0.95** | 0.98**              | 0.86**  |
| 16:0/18:2 | 0.72*               | 0.80**  | −0.88** | 0.78*               | 0.73**  | 0.50                | 0.56    | −0.66   | 0.56                | 0.47    |
| 18:0/20:5 | −0.96**             | −0.98** | 0.90**  | −0.97**             | −0.91** | 0.93**              | 0.93**  | −0.88** | 0.92**              | 0.81**  |
| 18:1/18:1 | 0.54                | 0.63    | −0.68   | 0.71*               | 0.72*   | 0.98**              | 1.00**  | 0.99**  | 1.00**              | 0.88**  |
| 18:0/22:4 | −0.98**             | −1.00** | 0.90**  | −0.97**             | 0.93**  | 0.94**              | 0.92**  | −0.87** | 0.92**              | 0.80**  |
| 16:0/16:0 | 0.96**              | 0.98**  | −0.87** | 0.98**              | 0.95**  | 0.99**              | 1.00**  | 0.98**  | 1.00**              | 0.87**  |
| 18:0/22:5 | 0.97**              | 0.98**  | −0.82** | 0.99**              | 0.97**  | −0.97**             | −0.98** | 0.98**  | −0.99**             | −0.86** |
| 18:0/20:0 | 0.93**              | 0.94**  | −0.79*  | 0.96**              | 0.94**  | 0.74*               | 0.78*   | −0.65   | 0.77*               | 0.95**  |
| 18:0/18:0 | 0.81**              | 0.87**  | −0.89** | 0.88**              | 0.85**  | 0.97**              | 0.98*   | −0.98** | 0.98**              | 0.85**  |
| 1,3-DG    | TAA <sup>ABTS</sup> | TPC     | TOS     | TAA <sup>FRAP</sup> | ∑OT     | TAA <sup>ABTS</sup> | TPC     | TOS     | TAA <sup>FRAP</sup> | ∑OT     |
| 18:0/18:3 | 0.29                | 0.25    | −0.04   | 0.42                | 0.52    | 0.98**              | 0.99**  | −0.99** | 0.98**              | 0.82**  |
| 22:0/18:2 | 0.64                | 0.63    | −0.58   | 0.58                | 0.52    | 0.90**              | 0.90**  | −0.95** | 0.89**              | 0.63    |
| 16:0/18:1 | 0.54                | 0.57    | −0.42   | 0.70*               | 0.76*   | 0.21                | 0.18    | −0.09   | 0.23                | 0.41    |
| 17:0/16:0 | 0.96**              | 0.95**  | −0.82** | 0.89**              | 0.84**  | 0.18                | 0.20    | −0.19   | 0.14                | 0.03    |
| 17:0/18:1 | −0.99**             | −1.00** | 0.88*   | −0.98**             | −0.94** | 0.71*               | 0.76*   | −0.86** | 0.74*               | 0.52    |
| 20:1/18:1 | 0.14                | 0.23    | −0.43   | 0.19                | 0.14    | 0.94**              | 0.97**  | −0.98** | 0.96**              | 0.82**  |
| 15:0/18:1 | −0.97**             | 0.97**  | −0.80** | −0.93**             | −0.91** | 0.08                | 0.18    | −0.12   | 0.17                | 0.41    |
| 18:0/18:1 | 0.99**              | 1.00**  | −0.90** | 0.95**              | 0.90**  | 0.98**              | 0.99**  | −0.98** | 0.98**              | 0.84**  |
| 18:0/16:0 | 0.98**              | 0.99**  | −0.92** | 0.95**              | 0.89**  | 0.98**              | 1.00**  | −0.99** | 0.99**              | 0.87**  |
| 20:0/16:0 | 0.94**              | 0.93**  | −0.80** | 0.85**              | 0.80**  | 0.92**              | 0.93**  | −0.90** | 0.90**              | 0.78*   |
| 18:0/20:0 | −0.38               | −0.39   | 0.54    | −0.36               | −0.02   | 0.96**              | 0.98**  | −0.90** | 0.94**              | 0.88**  |
| 17:0/18:2 | 0.60                | 0.68    | −0.77** | 0.64                | 0.60    | 0.92**              | 0.86**  | 0.93**  | 0.94**              | 0.88**  |

Values with \*: significant correlation ( $p < 0.05$ ); \*\*: significant correlation ( $p < 0.01$ ). [BC, MC] = unmarinated beef and moose; [BI, MI] = India ale-based marinated beef and moose; [BW, MW] = Wheat ale-based marinated beef and moose. ∑OT = Total oxygenated terpenes (linalool + endo-borneol + terpinen-4-ol + terpineol + carvacrol + carvacrol isomer-1+ carvacrol isomer-2); TAA = Total antioxidant activity; TPC = Total phenolic content; TOS = Total oxidant status. TAA<sup>ABTS</sup> = ABTS antioxidant activity; TAA<sup>FRAP</sup> = FRAP antioxidant activity; DG = Diglycerides.

$r = 0.35$ – $0.95^{**}$ ,  $p < 0.05$  or  $0.01$ ), polyphenols (TPC:  $r = 0.49$ – $1.00^{**}$ ), and oxygenated terpenes (∑OT:  $r = 0.69$ – $0.99^{**}$ ) in India ale-based marinade compared to Wheat ale-based marinade, which were accompanied by negative correlations with the total oxidation status (TOS:  $r = -0.98$  to  $0.46$ ), and appear to suggest that antioxidants, polyphenols, and oxygenated terpenes present in the marinade could be associated with suppression of oxidative degradation of these species in grilled beef (Table 3) [12,25]. By contrast, of the 8 1,2-DG species retained when Wheat ale-based marinade was used to marinate beef before grilling, only 3 species including 15:0/16:0, 16:0/14:0 and 18:0/18:3 showed significantly higher retention levels in BW (7–70%) compared to BI (−36–27%) samples (Fig. 5b). Furthermore, correspondingly higher correlation values for TAA, TPC, ∑OT and TOS in BW samples (Fig. 5b and Table 3), appear to suggest associations between the retained species and antioxidants, polyphenols, and oxygenated terpenes present in this marinade [41]. Taken together, greater retention

of the most abundant 1,2-DG species in beef by India ale-based marinade is line with the higher total 1,2-DG content of BI compared to BW, which suggest India ale based marinade is more effective for preserving 1,2-DG in beef against oxidative degradation during grilling.

The 1,3-DG profile in grilled beef was similar to the composition in grilled moose which was predominated by 16:0/18:1 and 18:0/18:1 (Fig. 5e). Furthermore, a similar trend was observed for marination effects on 1,3-DG distribution in grilled beef where 12 out of 17 1,3-DG species were preserved against degradation during grilling by beer based marination (Fig. 5f). Eight (8) of the 1,3-DG species were retained in BW samples, whereas 10 species were preserved in BI (Fig. 5f). In BI, the retention levels of 9 of the preserved 1,3-DG species including the most abundant species 16:0/18:1 and 20:1/18:1, 17:0/18:2, 16:0/18:1, 22:0/18:2, 17:0/16:0, 16:0/16:1, 18:0/20:0, 17:0/18:1 were positive and higher ( $p < 0.05$ ) in BI samples (11–93%) when compared to BW samples (−48–65%) as shown in Fig. 5f. The retention of these species was

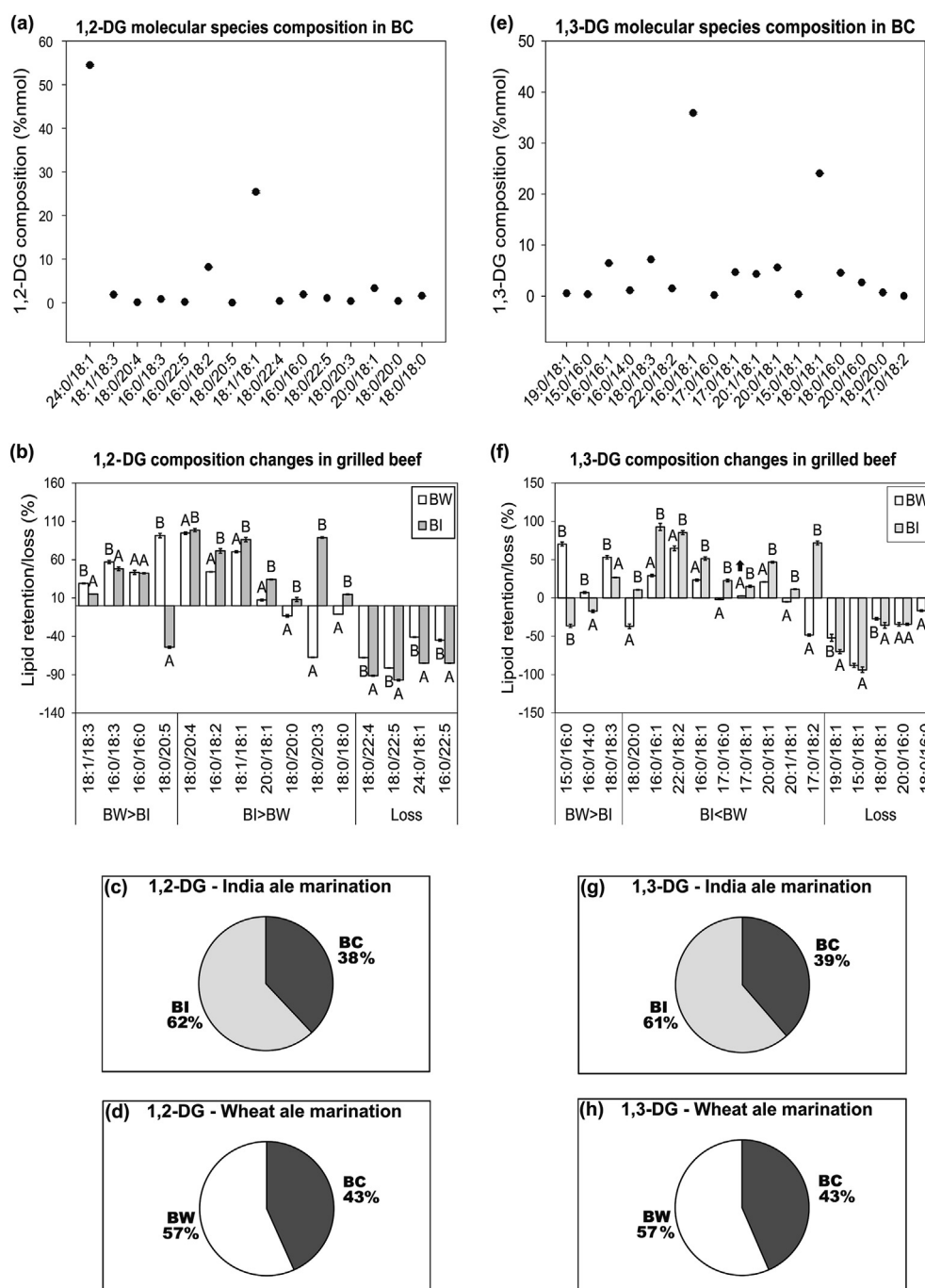


Fig. 5. Marination effects on DG molecular species in grilled beef. Values in bar charts and dot plot represent mean  $\pm$  standard errors. Means with different letters are significantly different at LSD ( $p < 0.05$ ;  $n = 4$ ). a, e) 1,2-DG and 1,3-DG species profile of grilled beef, respectively. b, f) 1,2-DG and 1,3-DG retention levels (%), respectively. c-d) Relative composition (%) of 1,2-DG in marinated and unmarinated grilled beef. g-h) Relative composition (%) of 1,3-DG in marinated and unmarinated grilled beef. [BC] = unmarinated beef; [BI] = India ale-based marinated beef; [BW] = Wheat ale-based marinated beef. DG = Diglycerides.

accompanied by higher TAA<sup>ABTS/FRAP</sup>, TPC,  $\sum$ OT and TOS values in BI samples compared to BW (Table 3), which suggest a relationship between the preserved 1,3-DG species and antioxidants, polyphenols, and oxygenated terpenes in India ale-based

marinade and suppression of oxidative degradation of 1,3-DG molecular species [32]. By contrast, 16:0/14:0, 15:0/16:0 and 18:0/18:3 were characterized by superior ( $p < 0.05$ ) retention levels in BW (7–70%) compared to BI (–37–27%) (Fig. 5f). Complementing

Table 3. Pearson's correlation coefficients showing relationships between antioxidant activity, phenolic content, oxygenated terpenes, oxidation status and preserved DG molecular species in grilled beef.

| Species   | BI Samples          |         |         |                     |         | BW Samples          |         |         |                     |         |
|-----------|---------------------|---------|---------|---------------------|---------|---------------------|---------|---------|---------------------|---------|
|           | TAA <sup>ABTS</sup> | TPC     | TOS     | TAA <sup>FRAP</sup> | ∑OT     | TAA <sup>ABTS</sup> | TPC     | TOS     | TAA <sup>FRAP</sup> | ∑OT     |
| 1,2-DG    |                     |         |         |                     |         |                     |         |         |                     |         |
| 18:/18:3  | 0.98**              | 0.99**  | −0.97** | 0.92**              | 0.99**  | 0.99**              | 1.00**  | −0.94** | 0.94**              | 0.99**  |
| 18:0/20:4 | 0.98**              | 1.00**  | −0.97** | 0.93**              | 0.99**  | 0.99**              | 1.00**  | −0.93** | 0.94**              | 0.98**  |
| 16:0/18:3 | 0.95**              | 0.93**  | −0.90** | 0.83**              | 0.99**  | 1.00**              | 1.00**  | −0.92** | 0.94**              | −0.99** |
| 16:0/18:2 | 0.97**              | 1.00**  | −0.98** | 0.94**              | 0.96**  | 0.97**              | 0.99**  | −0.95** | 0.94**              | 0.97**  |
| 18:0/20:5 | −0.72*              | −0.77*  | 0.81**  | −0.74*              | −0.72*  | 0.94**              | 0.93**  | −0.84** | 0.88**              | 0.93**  |
| 18:1/18:1 | 0.98**              | 1.00**  | −0.97** | 0.93**              | 0.98**  | 0.99**              | 1.00**  | −0.93** | 0.94**              | 0.99**  |
| 16:0/16:0 | 0.97**              | 0.99**  | −0.96** | 0.94**              | 0.95**  | 0.99**              | 0.99**  | −0.92** | 0.94**              | 0.98**  |
| 18:0/20:3 | 0.96**              | 0.99**  | −0.96** | 0.95**              | 0.94**  | −0.98**             | −1.00** | 0.94**  | −0.94**             | −0.98** |
| 20:0/18:1 | 0.98**              | 0.98**  | −0.96** | 0.90**              | 0.99**  | 0.74*               | 0.81**  | −0.84** | 0.78*               | 0.75*   |
| 18:0/20:0 | 0.58                | 0.49    | −0.46   | 0.35                | 0.69    | −0.84**             | −0.79*  | 0.68    | −0.73*              | −0.83** |
| 18:0/18:0 | 0.97**              | 1.00**  | −0.98** | 0.94**              | 0.96**  | −1.00**             | −0.99** | 0.91**  | −0.93**             | −0.99** |
| 1,3-DG    |                     |         |         |                     |         |                     |         |         |                     |         |
| 15:0/16:0 | −0.32               | −0.36   | 0.30    | −0.41               | −0.23   | 0.76*               | 0.80**  | −0.79*  | 0.74*               | 0.64    |
| 16:0/16:1 | 0.97**              | 1.00**  | −0.98** | 0.94**              | 0.97**  | 0.98**              | 0.99**  | −0.95** | 0.94**              | 0.97**  |
| 16:0/14:0 | −0.88**             | −0.91** | 0.86**  | −0.87**             | −0.86** | 0.60                | 0.65    | −0.70*  | 0.63                | 0.61    |
| 18:0/18:3 | 0.99**              | 0.99**  | −0.96** | 0.92**              | 0.99**  | 0.99**              | 1.00**  | −0.94** | 0.94**              | 0.98**  |
| 22:0/18:2 | 0.97**              | 1.00**  | −0.96** | 0.92**              | 0.99**  | 0.99**              | 0.99**  | −0.91** | 0.93**              | 0.99**  |
| 16:0/18:1 | 0.97**              | 0.99**  | −0.94** | 0.93**              | 0.95**  | 0.84**              | 0.78*   | −0.65   | 0.72*               | 0.83**  |
| 17:0/16:0 | 0.75*               | 0.79*   | −0.73*  | 0.80**              | 0.66    | −0.15               | −0.22   | 0.32    | −0.22               | −0.16   |
| 17:0/18:1 | 0.99**              | 0.97**  | −0.91** | 0.91**              | 0.98**  | 0.31                | 0.35    | −0.27   | 0.31                | 0.23    |
| 20:1/18:1 | 0.76*               | 0.63    | −0.89** | 0.85**              | 0.65    | −0.94**             | −0.92** | 0.82**  | −0.86**             | −0.93** |
| 20:0/18:1 | 0.98**              | 0.99**  | −0.96** | 0.92**              | 0.99**  | 0.96**              | 0.99**  | −0.95** | 0.93**              | 0.96**  |
| 18:0/20:0 | 0.97**              | 0.97**  | −0.91** | 0.93**              | 0.93**  | −0.99**             | −0.99** | 0.90**  | −0.93**             | −0.99** |
| 17:0/18:2 | 0.98**              | 1.00**  | −0.97** | 0.94**              | 0.98**  | −0.99**             | −1.00** | 0.94**  | −0.94**             | −0.99** |

Values with \*: significant correlation ( $p < 0.05$ ); \*\*: significant correlation ( $p < 0.01$ ). [BC, MC] = unmarinated beef and moose; [BI, MI] = India ale-based marinated beef and moose; [BW, MW] = Wheat ale-based marinated beef and moose. ∑OT = Total oxygenated terpenes (linalool + endo-borneol + terpinen-4-ol + terpineol + carvacrol + carvacrol isomer-1 + carvacrol isomer-2); TAA = Total antioxidant activity; TPC = Total phenolic content; TOS = Total oxidant status; TAA<sup>ABTS</sup> = ABTS antioxidant activity; TAA<sup>FRAP</sup> = FRAP antioxidant activity; DG = Diglycerides.

these retention values, correlation analysis showed that 1,3-DG species with higher retention levels in BW samples were more positively correlated with antioxidants (TAA<sup>ABTS</sup>:  $r = 0.60$ – $0.99$ \*\*,  $p < 0.05$  or  $0.01$ ; TAA<sup>FRAP</sup>:  $r = 0.63$ – $0.98$ \*\*,  $p < 0.05$  or  $0.01$ ), polyphenols (TPC:  $r = 0.65$ – $1.00$ \*\*) oxygenated terpenes (∑OT:  $r = 0.61$ – $0.98$ \*\*) present in Wheat ale-based marinade, and negatively correlated with oxidation status (TOS:  $r = -0.94$ \*\* to  $-0.70$ \*) of BW samples (Fig. 5f and Table 3), which appear to suggest that the preservation of these species may be associated with antioxidants, polyphenols, and oxygenated terpenes in Wheat ale-based marinade [17]. Taken together, the results indicate that India ale-based marinade was more effective for preserving the quantity and quality of DG lipids in beef against oxidative degradation during grilling (Fig. 5g–h). As alluded to previously, these correlations by themselves do not wholly explain the cause of preservation of DG species in marinated beef samples, and only suggest a strong relationship between preservation of 1,3-DG molecular species in marinated grilled beef and suppression of lipid oxidative degradation in marinated grilled beef [32]. As such, further work

which is currently beyond the scope of this study will be required to elucidate the nature of these relationships, as well as the mechanisms underlying the retention of these species by components present in the unfiltered beer-based marinades.

Collectively, marination with both wheat and India ale unfiltered beer-based marinades appear to be effective for preserving DG in beef and moose meat against degradation during grilling, particularly the nutritionally beneficial 1,3-DG isomers. Increasing the access to DG (particularly 1,3-DG) in the diet through consumption of marinated grilled meats could have beneficial implications in managing postprandial lipidemia and obesity which are known risk factors for developing cardiovascular disease and type 2 diabetes among the population.

### 3.2.3. McTG content and composition in grilled ruminant meats

McTG are less widely distributed in ruminant meats compared to long chain triglycerides. However, they have gained widespread interest owing to their role in lowering body weight, decreasing metabolic syndrome, abdominal obesity and

inflammations [49,50]. Treatment of moose meat with marinades composed of unfiltered session ales, herbs and spices preserved McTG in moose meat against degradation during grilling (Fig. 1a). McTG content was higher ( $p < 0.05$ ) in marinated moose meat (MW and MI) compared to MU which suggests retention of McTG by marination against oxidative degradative processes during grilling. Furthermore, the calculated differences were higher in MI (82%) compared to MW (26%) which is in line with superior suppression of lipid oxidation when India ale-based marinade was used to marinate moose meat before grilling as shown in Fig. 1b [12,17]. The McTG molecular species composition in grilled moose was composed of 9 species which was predominated by 10:0/12:0/14:0, 8:0/12:0/12:0 and 10:0/12:0/12:0 (Fig. 6a). In line with the higher McTG content of MI compared to MW samples, India ale-based marination retained 6 McTG species compared to 5 species preserved by Wheat ale-based marination (Fig. 6b). The retention levels of 5 of these species including 16:0/12:0/14:0, 8:0/18:1/

18:1, 10:0/12:0/12:0, 7:0/16:0/18:1 and 10:0/12:0/14:0 were higher ( $p < 0.05$ ) in MI samples (97–21%) compared to MW (87% to –10%) along with strong correlations with antioxidants ( $r = 0.87^{***}$ – $0.99^{**}$ ), polyphenols ( $0.92^{**}$ – $1.00^{**}$ ), and oxygenated terpenes ( $r = 0.80^{**}$ – $0.95^{**}$ ) present in the India ale-based marinades (Fig. 6b and Table 4) [14].

The trend in grilled beef was similar and showed total MCTG content was preserved against degradation by beer-based marination during grilling of beef (see Fig. 1c). McTG content was higher ( $p < 0.05$ ) in marinated grilled beef (BW and BI) compared to BC and was characterized by higher ( $p < 0.05$ ) retention level in BI (57%) compared to BW (33%), which appears to suggest preservation of McTG in marinated beef during grilling (Fig. 1d) [17]. The molecular species profile of grilled beef was predominated by 16:0/8:0/18:1, 16:0/10:0/18:1 and 16:0/12:0/14:0 (Fig. 6c). Fifteen (15) out of the 18 McTG species in beef were preserved in marinated beef against oxidative degradation during grilling. India ale-based marinade preserved 13 species

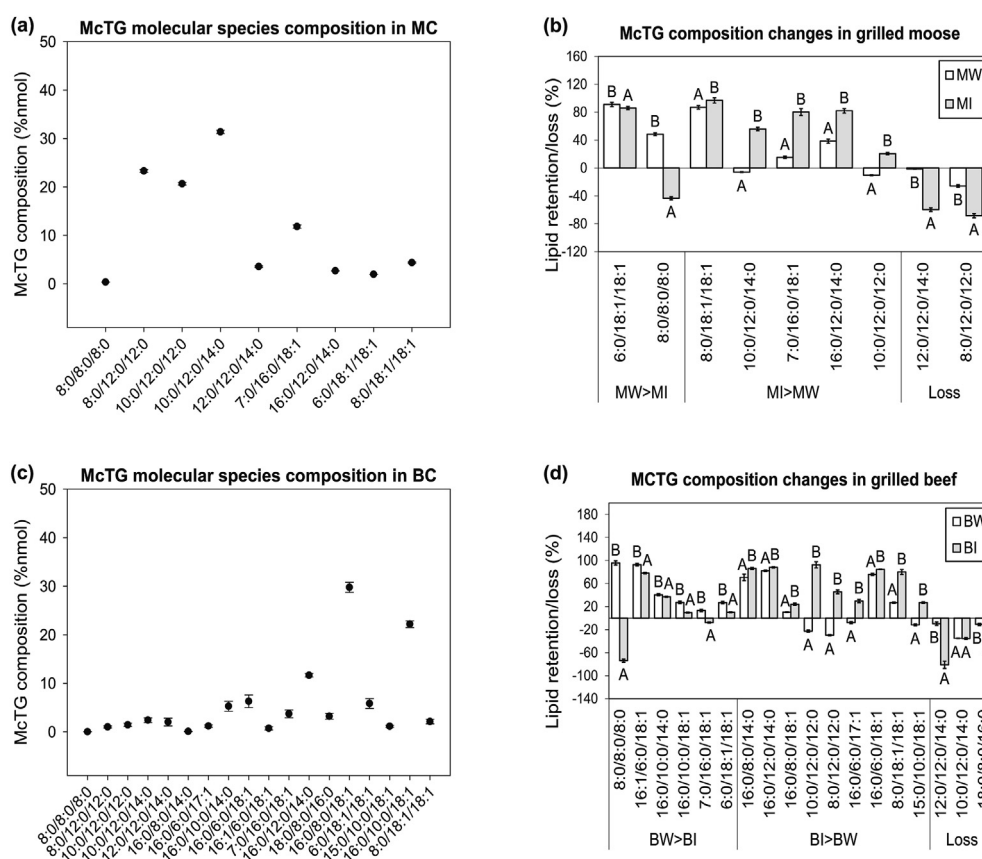


Fig. 6. Marination effects on MCTG molecular species in grilled meats. Values in bar charts and dot plot represent mean  $\pm$  standard errors. Means with different letters are significantly different at LSD ( $p < 0.05$ ;  $n = 4$ ). a–c) MCTG species profile of grilled moose meat and beef, respectively. b–d) MCTG retention levels (%) in grilled moose meat and beef, respectively. [BC, MC] = unmarinated beef and moose; [BI, MI] = India ale-based marinated beef and moose; [BW, MW] = Wheat ale-based marinated beef and moose. MCTG = Medium chain triglycerides.

Table 4. Pearson's correlation coefficients showing relationships between antioxidant activity, phenolic content, oxygenated terpenes, oxidation status and preserved MCTG molecular species in grilled meats.

| Moose          |  | MI Samples          |         |         |                     |         | MW Samples          |         |         |                     |         |
|----------------|--|---------------------|---------|---------|---------------------|---------|---------------------|---------|---------|---------------------|---------|
| MCTG           |  | TAA <sup>ABTS</sup> | TPC     | TOS     | TAA <sup>FRAP</sup> | ΣOT     | TAA <sup>ABTS</sup> | TPC     | TOS     | TAA <sup>FRAP</sup> | ΣOT     |
| 8:0/8:0/8:0    |  | −0.93**             | −0.92** | 0.81    | −0.85**             | −0.80** | 0.53                | 0.41    | −0.52   | 0.59                | 0.44    |
| 10:0/12:0/12:0 |  | 0.91**              | 0.92**  | −0.85** | 0.87**              | 0.80**  | −0.80**             | −0.79*  | 0.84**  | −0.77*              | −0.49   |
| 10:0/12:0/14:0 |  | 0.97*               | 0.98**  | −0.88** | 0.96**              | 0.92**  | −0.60               | −0.58   | 0.46    | −0.61               | −0.76*  |
| 7:0/16:0/18:1  |  | 0.86**              | 0.88**  | −0.78*  | 0.83**              | 0.81**  | 0.96**              | 0.95**  | −0.90** | 0.96**              | 0.89**  |
| 16:0/12:0/14:0 |  | 0.99**              | 1.00**  | −0.85** | 0.98**              | 0.95**  | 0.96**              | 0.95**  | 0.89**  | 0.96**              | 0.89**  |
| 6:0/18:1/18:1  |  | 0.99**              | 1.00**  | −0.88*  | 0.98**              | 0.94**  | 0.99**              | 1.00**  | −0.96** | 1.00                | 0.90**  |
| 8:0/18:1/18:1  |  | 0.99**              | 1.00**  | −0.87** | 0.96**              | 0.92**  | 0.99**              | 0.98**  | −0.94** | 0.98**              | 0.87**  |
| Beef           |  | BI Samples          |         |         |                     |         | BW Samples          |         |         |                     |         |
| MCTG           |  | TAA <sup>ABTS</sup> | TPC     | TOS     | TAA <sup>FRAP</sup> | ΣOT     | TAA <sup>ABTS</sup> | TPC     | TOS     | TAA <sup>FRAP</sup> | ΣOT     |
| 8:0/8:0/8:0    |  | −0.53               | −0.57   | 0.61    | 0.52                | 0.55    | 0.99**              | 1.00**  | 0.93**  | 0.94**              | 0.99**  |
| 8:0/12:0/12:0  |  | 0.67                | 0.73*   | −0.76*  | 0.56                | 0.80**  | −0.83**             | −0.78*  | 0.60    | 0.72*               | −0.76*  |
| 10:0/12:0/12:0 |  | 0.95**              | 0.98**  | −0.95** | 0.88**              | 0.97**  | −0.99**             | −0.99** | 0.96**  | −0.97**             | −0.98** |
| 16:0/8:0/14:0  |  | 0.98**              | 1.00**  | −0.97** | 0.93**              | 0.98**  | 0.84**              | 0.72*   | −0.61   | 0.73*               | 0.66    |
| 16:0/6:0/17:1  |  | 0.56                | 0.38    | −0.61   | 0.70*               | 0.74**  | −0.08               | −0.08   | 0.07    | −0.07               | −0.08   |
| 16:0/10:0/14:0 |  | 0.96**              | 0.97**  | −0.96** | 0.95**              | 0.92**  | 0.99**              | 1.00**  | −0.94** | 0.95**              | 0.98**  |
| 16:0/6:0/18:1  |  | 0.98**              | 1.00**  | −0.97** | 0.93**              | 0.98**  | 0.98**              | 1.00**  | −0.94   | 0.94**              | 0.98**  |
| 16:1/6:0/18:1  |  | 0.89**              | 0.90*   | −0.86** | 0.89**              | 0.83**  | 0.99**              | 1.00**  | 0.94**  | 0.95**              | 0.99**  |
| 7:0/16:0/18:1  |  | −0.98**             | −0.98** | 0.94**  | −0.90**             | −0.99   | 0.95**              | 0.95**  | −0.96** | 0.95**              | 0.97**  |
| 16:0/12:0/14:0 |  | 0.95**              | 0.98**  | −0.96** | 0.91**              | 0.96**  | 0.94**              | 0.97**  | −0.94** | 0.92**              | 0.95**  |
| 16:0/8:0/18:1  |  | 1.00**              | 0.99**  | −0.96** | 0.94**              | 1.00**  | 0.97**              | 0.98**  | −0.90** | 0.91**              | 0.98**  |
| 6:0/18:1/18:1  |  | −0.94**             | −0.99** | 0.98**  | −0.93**             | −0.94** | 0.86**              | 0.90**  | −0.79*  | 0.80**              | 0.83**  |
| 15:0/10:0/18:1 |  | 0.71*               | 0.86**  | −0.75*  | 0.81**              | 0.70*   | −0.49               | −0.49   | 0.45    | −0.46               | −0.48   |
| 16:0/10:0/18:1 |  | −0.91**             | −0.94** | 0.88**  | −0.93**             | −0.83** | 0.67                | 0.61    | −0.42   | 0.54                | 0.60    |
| 8:0/18:1/18:1  |  | 0.97**              | 1.00**  | −0.98** | 0.93**              | 0.98**  | 0.99**              | 1.00**  | −0.95** | 0.96**              | 0.98**  |

Values with \*: significant correlation ( $p < 0.05$ ); \*\*: significant correlation ( $p < 0.01$ ). [BC, MC] = unmarinated beef and moose; [BI, MI] = India ale-based marinated beef and moose; [BW, MW] = Wheat ale-based marinated beef and moose. ΣOT = Total oxygenated terpenes (linalool + endo-borneol + terpinen-4-ol + terpineol + carvacrol + carvacrol isomer-1 + carvacrol isomer-2); TAA = Total antioxidant activity; TPC = Total phenolic content; TOS = Total oxidant status; TAA<sup>ABTS</sup> = ABTS antioxidant activity; TAA<sup>FRAP</sup> = FRAP antioxidant activity; MCTG = Medium chain triglycerides.

compared to 11 species retained by Wheat ale-based marinade as evidenced by positive calculated retention levels in the marinated grilled beef samples (Fig. 6d). The retention of these species in marinated grilled beef was accompanied by positive correlations with antioxidants, polyphenols, oxygenated terpenes present in the marinades and negative correlations with TOS (Table 4). These results appear to suggest a relationship between suppression of degradation of these MCTG species and antioxidants in the beer-based marinades evaluated in the current study [11]. Furthermore, the results indicate that unfiltered beer-based marination could be an effective precooking technique to preserve MCTG against degradation during grilling of beef and moose meat.

In the present work, we attributed preservation of FAHFA, DG and MCTG molecular species in grilled meats to anti-radical action of antioxidant compounds present in the beer-based marinades evaluated [18]. The marinades used in this study were composed of antioxidant-rich herbs, spices, and unfiltered session beers. The beers were drafted from barley grains containing catechin, gallic acid

and ferulic acid which have antioxidant and anti-cancer properties [15]. The antioxidant activity of the marinades is also attributed to herbs, spices and olive oil used to formulate the marinades, which contained polyphenols and oxygenated terpenes with anti-radical properties. Gingerol, geraniol, shogaol, and linalool are responsible for the antioxidant activity of ginger, while garlic, onion, mustard and parsley contain quercetin, kaempferol, vitamin E, and luteolin as major antioxidant compounds [14]. Furthermore, oregano contains caffeic acid, *p*-coumaric acid, carvacrol, terpineol, and terpinen-4-ol with known antioxidant properties [14]. Olive oil is rich in polyphenols including cinnamic acid, homovanillic acid and oleuropein responsible for its antioxidant, anti-inflammatory and anti-microbial bioactivities [51]. Available evidence demonstrate that herbs and spices either alone or in combination with beers are able to suppress lipid oxidation in meat, which was in agreement with the preservation of FAHFA, DG and MCTG lipids species observed in this study [12,32,48]. However, these antioxidants were not effective in preserving MCTG from degradation in grilled ruminant meats.

#### 4. Conclusions

Moose meat and beef contain FAHFA, DG, MACDG and McTG which confer health benefits when consumed as part of the normal diet. We have demonstrated that grilling completely degrades MACDG in beef and moose meat regardless of the unfiltered beer-based marinade used prior to grilling. India ale-marination was more effective for retaining FAHFA, DG and McTG compared to Wheat ale-based marinade. FAHFA was retained in both meats regardless of the marinade used prior to grilling. Both the SFA and PUFA linked FAHFA in moose were retained by both marinades, while the MUFA linked FAHFAs were not retained. Interestingly, the MUFA linked FAHFAs were retained in beef regardless of the marinade used, but the SFA enriched FAHFAs were degraded following marination and grilling. This indicated FAHFA retention varied with meat type and kind of unfiltered beer-based marinade used. The nutritionally important 1,3-DG isomers and McTG were retained in both moose and beef following marination and grilling with either India or wheat ale based unfiltered beer marinades. The successful retention of these functional lipids in either beef or moose meat following marination and grilling were highly associated with the oxygenated terpenes, total phenolics, and antioxidant activity of the unfiltered beer-based marinades.

This work demonstrates for the first time the effects of marination and grilling on the fate of DG, FAHFAs and MCTG in grilled ruminant meats. It also demonstrated that the fate of these functional lipids during grilling is dependent on the meat type and kind of unfiltered beer-based marinade used. FAHFA, DG (particularly the 1,3- DG isomers) and McTG are emerging classes of functional lipids with interesting health benefits associated with the treatment or management of asthma, arthritis, obesity, post prandial lipidemia, diabetes, and inflammatory based illnesses. Ruminant meats are suitable sources of these functional lipids in the diet and marination with either session ale-based marinades could be beneficial in enhancing their retention in grilled meat. However, attention should be paid to the kind of meat and type of marinades used. Further work needs to be done to better understand what is accounting for the variation in retention of the different classes of these functional lipids across meat type and marinades. This information would be useful to further optimize beer-based marinade formulations and applications in improving the nutritional and functional quality of grilled food.

#### Author contributions

Raymond H. Thomas: Conceptualization, Methodology, Supervision, Funding acquisitions. Thu H. Pham, Evan Wheeler, Nicole Walsh, Charles F. Manful: Investigation and validation. Charles F. Manful, Thu H. Pham: Formal analysis, Data curation. Charles F. Manful: Writing - original draft. Thu H. Pham, Muhammad Nadeem, Oludoyin A. Adegun, Nicole Walsh: Review and editing. All authors edited and approved the final manuscript.

#### Conflict of interest

The authors declare no conflict of interest.

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