Meat and Muscle Biology™

Inclusion of Dry-Aged Beef Trimmings as a Quality and Flavor Enhancer for Ground Beef

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Abstract: This study evaluated the effects of dry-aged beef trimmings inclusion on quality and flavor precursors of ground beef patties. Lean and fat trim were collected from beef loins aged for 28 d using 4 different methods: wet aging (WA), dry aging (DA), dry aging in water-permeable bag (DWA), and UV-light dry aging (UDA). Trimmings were ground and incorporated with ground fresh beef top rounds and subcutaneous fat (3 d postmortem) to make patties (80% lean and 20% fat) with different formulations: fresh beef and fat (CON), fresh beef and DA fat only (DA-FAT), and mixtures of 50% fresh lean along with 30% aged lean and 20% aged fat from different aging treatments (WA, DA, DWA, and UDA). Patties were manufactured in 3 independent batches ($n = 3$) to conduct pH, cooking loss, texture analysis, lipid oxidation, 5 d aerobic display color, trained sensory evaluation, volatile compounds, and metabolomics analyses. The inclusion of aged beef trimmings did not impact the pH and cook loss of the patties ($P > 0.05$). DWA trimmings lowered chewiness compared to CON ($P < 0.05$) and induced greater product discoloration compared to all other treatments at the end display ($P < 0.05$). The addition of DA and UDA trimmings in ground beef reduced bloody flavor and promoted more volatile production compared to other treatments ($P < 0.05$). Metabolomics profiling revealed different flavor precursor profiles from the inclusion of trimmings aged differently, demonstrating that the addition of the lean trim portion influenced the flavor profile more significantly than the fat trim portion after cooking. Cooking significantly altered the metabolite profile, reducing variations between the different treatments and explaining the observed flavor changes. The results suggested that aged trimmings modify the flavor precursor profile in ground beef products. Further research to identify the impact of different cooking methods on the flavor generation potential of dry-aged trimming inclusion products would be beneficial.

Key words: dry aging, trimmings, metabolomics, volatile, sensory, value adding
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Introduction

Dry aging is a traditional process in which meat (either as a whole carcass, primal, or sub-primal) is aged without any protective barrier in a controlled environment. In recent years, the process has been regaining interest from consumers (Kim et al., 2018). This renewed interest could be attributed to the increased palatability following the application of the dry-aging process, particularly flavor, which is increasingly desired by consumers (Zhang et al., 2022). However, due to the absence of protective barriers, the process inevitably generates considerable moisture loss, requiring a product trimming process prior to obtaining consumable products. It has been reported that the overall trimming loss from the dry-aging process could reach up to 35% of the initial product weight (Lee et al., 2022), making this process expensive and wasteful.

Multiple studies have been conducted to utilize dry-aged trimmings to minimize the waste generated

from the process. Previous studies demonstrated that dry-aged trimming could be utilized as a natural flavor enhancer in both processed meat and sauces (Lee and Kim, 2021; Park et al., 2020; Xue et al., 2021). Furthermore, Xue et al. (2021) and Park and Kim (2023) reported that dry-aged trimming exerts acceptable emulsifying properties, suggesting potential for their use as functional ingredients in further processed meat products. The dry-aged trimmings often consist of a crust portion (dried meat surface trims) and a non-crust portion (lean and fat trims). Of these, only dry-aged crust portions have been explored and studied for their potential utilization and functionality. There is no information available regarding the potential usage of the non-crust portion of the dry-aged beef trimmings. While it can be speculated that the dry-aged lean and fat trim utilization would produce similar benefits to those of the crust portion, the non-crust trims are likely to have different physical properties compared to the crust portion and, therefore, might influence the product differently. Recent studies have also been incorporating alternative dry-aging methods to reduce loss and minimize microbial contamination through the utilization of dry aging in water-permeable bags (Berger et al., 2018; Li et al., 2013; Setyabrata et al., 2022b) and UV light application (Setyabrata et al., 2021b; Setyabrata et al., 2022b). These methods lead to different product qualities and hence, potentially different trimmings qualities.

Accordingly, the objective of this study was to evaluate the impact of different aged beef lean and fat trims on the meat quality and chemical properties of beef patties. Additionally, differences in flavor metabolites before and after cooking from the differently treated beef patties were also evaluated.

Materials and Methods

Sample collection and aging process

Details on the aging process can be found in our published parallel study (Setyabrata et al., 2022b). Briefly, paired beef loins (*M. Longissimus lumborum*) were collected at 5 d postmortem from a commercial processing facility and transported to Purdue University Meat Laboratory. The loins were then split into 2 equal sections (4 sections per paired loins) and randomly assigned to 4 different aging treatments (wet aging [WA], conventional dry aging [DA], dry aging in water-permeable bag [DWA] and UV-light dry aging [UDA]). All sections were aged for 28 d at 2°C, 65% relative humidity, and 0.8 m/s airflow. The UDA samples were given 2 UV-light treatments per day, each lasting for 5 min and totaling a dose of 5 J/m^2 (Philips TUV T8 UVC light, Eindhoven, the Netherlands). At the end of aging, dried surfaces were removed from all sections. The sections were then trimmed, and the lean trim (mainly consisting of *M. Multifidus dorsi*) and fat trim from each sample were collected, individually packed, and stored at −40°C until application.

Beef patties processing

A total of 3 independent batches of ground beef patties were made for the study utilizing the collected trimmings from the previously described beef loins. For each batch, trimmings from the beef loins from 4 carcasses were combined to produce ground lean and ground fat for inclusion in beef patties. The carcasses were selected to maximize the quantity of ground lean and fat trim quantity that can be utilized within each independent batch. Both lean and fat trimmings originating from the same carcasses were used within the same batch. The same carcass combinations were used for all the different aging treatments within the same batch. Trimmings were thawed at 2°C for 24 h prior to processing.

For each independent batch, fresh beef top round (*M. Semimembranosus*) and subcutaneous fat were collected at 3 d postmortem to be combined with the collected trimmings (aged lean and fat) from different aging treatments. All samples were ground through a meat grinder equipped with an 8 mm plate (M-12-FS, Torrey; Monterrey, NL, Mexico). The fresh ground meat, fresh ground fat, aged ground meat, and aged ground fat were then combined to make 80:20 (lean: fat) beef patties with 6 different formulations (Table 1). The DA fat only (DA-FAT) was added to identify the impact of only adding aged fat trimming, compared to the inclusion of both aged fat and lean trimmings. The mixture was manually mixed by hand for 5 min and re-ground for uniformity. At least 6 patties (125 g) were collected from each treatment for further analyses described below. Samples for simulated color display, cooking loss, and texture profile analysis were immediately used for the analyses. Samples not immediately used were vacuum packed individually and frozen at −80°C until analyses.

pH and cook loss measurements

The pH was measured following the method described by Nondorf and Kim (2022). A total of 3 g of samples was homogenized in 27 mL of double

Table 1. Ground beef formulation

Treatments	Formulation
Control (CON)	80% fresh beef, 20% fresh fat
Dry-aged fat (DA-FAT)	80% fresh beef, 20% DA fat
Wet-aging (WA)	50% fresh beef, 30% WA trim, 20% WA fat
Dry-aging (DA)	50% fresh beef, 30% DA trim, 20% DA fat
Dry-aging in water-permeable bag (DWA)	50% fresh beef, 30% DWA trim, 20% DWA fat
UV-light dry-aging (UDA)	50% fresh beef, 30% UDA trim, 20% UDA fat

Fresh beef: *M. Semimembranosus* collected at 3 d postmortem.

Fresh fat: Beef subcutaneous fat collected at 3 d postmortem.

distilled water. The pH was then measured using a benchtop pH meter (Sartorius Basic Meter PB-11, Sartorius AG; Goettingen, Germany) calibrated following the manufacturer's guidelines. The pH measurements were conducted in duplicate.

The cooking loss was used to measure the water-holding capacity of the samples. The cooking loss was determined by measuring the initial and final weights of the patties following cooking and was expressed as a percent loss. The patties were cooked using a double-sided clamshell griddle (Griddler GR-150, Cuisinart; Glendale, AZ, USA) to 71°C internal temperature. After cooking, the samples were allowed to rest for 10 min before being weighed. Before weighing, all samples were gently blotted using paper towels. The cook loss (%) was measured twice and calculated using the following formula: (Initial weight − Final weight)/Initial weight × 100.

Simulated color display

One patty from each treatment was collected, placed on a foam tray with a soaking pad, and overwrapped using Polyvinyl Chloride (PVC) films. The patties were then displayed under light (1800 lx, OCTRON® T8 Lamps, Osram Sylvania LTD, Canada) for 5 d. The color of the patties was evaluated daily, using a Hunter MiniScan EZ-4500L Spectrophotometer (Hunter Associates Laboratory, Inc.; Reston, VA, USA) equipped with a 25 mm (diameter) opening. Illuminant A and a standard 10° observer were used. The equipment was calibrated to black glass and white tile prior to any color measurement. The CIE L^*, a^*, and b^* color values were collected from 3 random locations on the surface of the patties. Hue angle ($\tan^{-1}(b^*/a^*)$) and Chroma ($(a^{*2} + b^{*2})^{½}$) value were calculated (King et al., 2023). All samples were vacuum packaged and frozen at −80°C until used for lipid oxidation analysis following the completion of the simulated display.

Lipid oxidation analysis

The 2-thiobarbituric acid reactive substances (TBARS) assay was conducted using the method described by Setyabrata and Kim (2019). The lipid oxidation measurement was performed on samples before and after the simulated display. Briefly, 5 g of samples was homogenized in 15 mL of double distilled water with 50 µL of butylated hydroxyl anisole. The homogenate was then mixed with 20 mM 2-thiobarbituric acid solution in 15% trichloroacetic acid solution. The mixture was mixed, heated in 80°C water bath, cooled in ice water for 10 min, and centrifuged at 2000 × *g* for 10 min. The mixture was then filtered through filter paper and the absorbance was read at 531 nm using an Epoch™ Microplate Spectrophotometer (BioTek Instrument Inc., Winooski, VT, USA). The TBARS value was expressed as mg malondialdehyde/kg meat.

Texture profile analysis

The texture profile analysis was performed on patties previously used for the cook loss measurement. Following the cooking, samples were stored at 2°C overnight prior to texture analysis. A total of 4 cores were collected from each patty ($d = 2.5$ cm) and subjected to texture analysis using TA-XT Plus Texture Analyzer (Stable Micro System Ltd., Godalming, UK). The parameter of the test was set following the description by Xue et al. (2021). The hardness (*g*), adhesiveness (*g.sec*), resilience (%), cohesiveness (%), springiness (%), and chewiness were obtained.

Descriptive sensory analysis

The sensory characteristics of the beef patties were evaluated by 6 trained panelists recruited from the Department of Animal Sciences at Purdue University (West Lafayette, IN, USA). The panelists were trained following the American Meat Science Association Research Guidelines for Cookery, Sensory Evaluation, and Instrumental Tenderness Measurements of Meat (AMSA, 2016). The panelists were trained to evaluate 10 different sensory attributes (i.e., beefy/brothy, brown roasted/grilled, bloody, metallic, rancid, umami, salty flavors notes, hardness, and cohesiveness as well as moisture sensory feelings) as described by Xue et al. (2021). The patty evaluation was conducted over 3 sessions, with the sample serving order randomized during

each session. The panelists were seated in an individual booth in a room equipped with red lighting.

The patties were cooked in a similar manner as described previously in the cooking loss measurements. Following cooking, patties were cut into 6 wedge-shaped pieces, individually placed in a sampling cup with a lid, and stored in a warmer. All samples were served to the panelist within 10 min. The panelists were supplied with unsalted crackers and water to cleanse their palate between samples. The patties were evaluated using a 15-point anchor, with 0–5 points indicating an intensity level of slight, a 6–10 point scale indicating medium intensity, and 11–15 points equivalent to strong intensity. All scores were then pooled and subjected to statistical analysis. Cooked patties samples were also collected for volatile and metabolomics analysis.

Volatile compounds analysis

Profiling of volatile compounds was conducted following the methods described by Gardner and Legako (2018). Cooked patties were minced and placed into a gas chromatography (GC) vials with 10 µL of 1,2-dichlorobenzene as an internal standard. The vials were then sealed and loaded to a Gerstel agitator (Gerstel Inc., Linthicum Heights, MD, USA) for incubation (5 min, 65°C) prior to 20 min of extraction via headspace solid-phase microextraction. The extracted compounds were then injected into a capillary column (30 m × 0.25 mm × 1.0 µm, Agilent Technologies, Inc.; Santa Clara, CA, USA), and selective ion monitoring scan mode was utilized for data collection. The compounds were identified by comparing them to an external authentic standard (Sigma-Aldrich, St. Louis, MO, USA) for validation. The volatile compounds concentrations were reported in nanograms per gram of cooked sample.

Untargeted metabolomics analysis

Metabolite extraction. Metabolomics analysis was conducted on both raw and cooked samples. Prior to metabolite extraction, samples were powdered by submerging the patties into liquid nitrogen and immediately pulverized using a blender (Waring Products, CT, USA). Then, 100 mg of the powdered meat samples was homogenized in 300 µL chloroform and 300 µL methanol using Precellys 24 tissue homogenizer (Bertin Instruments, Bretonneux, France) for the extraction as described by (Setyabrata et al., 2021a). The samples were homogenized through 3 cycles of 30 s at 6500 rpm followed by 30 s rest between the cycles. Water was then added to the homogenized mixture and centrifuged at 16,000 × *g* for 8 min. The upper layer was collected and dried for chromatographic separation.

Ultra-performance liquid chromatography–mass spectrometry. Following extraction, untargeted metabolomics analysis was conducted using the methods previously detailed by Setyabrata et al. (2022a). The samples were separated using the Agilent 1290 Infinity II UPLC system (Agilent Technologies, Palo Alto, CA, USA) equipped with a Waters Acquity HSS T3 (2.1 × 100 mm × 1.8 µm) separation column (Waters, Milford, MA, USA). The column was maintained at 40°C with the binary mobile phase flow set at 0.45 mL/min. The binary mobile phase consisted of solvent A (0.1% formic acid (v/v) in ddH2O) and solvent B (0.1% formic acid (v/v) in acetonitrile). Initial conditions of 100:0 A:B were held for 1 min, followed by a linear gradient to 70:30 over 15 min, changed to a linear gradient of 5:95 over 5 min, and 5:95 hold for 1.5 min. The sample injection volume was 5 µL.

Separated compounds were identified using Agilent MassHunter B.06 software (Agilent Technologies, Santa Clara, CA, USA), and the mass accuracy was improved by infusing Agilent Reference Mass Correction Solution (G1969-85001, Agilent Technologies; Santa Clara, CA, USA). The Agilent ProFinder (v B.08) was utilized for peak deconvolution. Peak identification was improved by applying data-dependent tandem mass spectrometer (MS/MS) collection on composite samples with 10 eV, 20 eV, and 40 eV collision energy. The metabolites were annotated using MS-DIAL software and database (http://prime.psc.riken.jp/).

Statistical analysis

This study was a randomized complete block design with the treatments (CON, DA-FAT, DA, DWA, UDA, and WA) serving as the main fixed effects and the batch serving as a both block and an experimental unit. Within each batch, the treatments were randomly assigned to patties, which served as pseudo-replicates. For the analyses subjecting the patties to the display, the storage period was also included as a fixed effect in the model. The data were analyzed using PROC GLIMMIX procedure from SAS 9.4 software (SAS Institute Inc., Cary, NC). Least-squares means for all traits were separated, and the significance level was defined at the level of $P < 0.05$. The trend was defined at the level of $0.05 \leq P \leq 0.10$.

Metabolomics analysis was conducted using Metaboanalyst 5.0 (Pang et al., 2021). The metabolites

were also analyzed using ANOVA to identify features significantly affected by the aging treatment. The Student's t-test was utilized to identify significant features between cooked and raw samples. Significance was defined at $P < 0.01$ and adjusted using the false discovery rate (FDR) method (adj $P < 0.01$). Unsupervised principal component analysis (PCA) and hierarchical clustering analysis (HCA) were performed to visualize the data. Unsupervised principal component analysis was conducted as an explorative analysis on the metabolomics data.

Results and Discussion

pH, water-holding capacity, and texture profile analyses

The pH of the beef patties was found to be similar across the treatments (Table 2). However, there was a trend ($P = 0.0876$) of increasing pH with the addition of lean and fat trim, regardless of the aging treatment applied to the trims. The increase in the pH could potentially be attributed to the higher pH of the trims. The dry-aged meat products were reported to have a pH between 5.6 and 5.7 following the aging treatment in our parallel study (Setyabrata et al., 2022b), explaining the currently observed increased pH. Park et al. (2020) and Lee et al. (2022) found that the addition of dry-aged beef crust trimmings influenced the final product pH. Park et al. (2020) reported that the pH increased with the addition of beef crust in brown sauce, while Lee et al. (2022) observed that the pH decreased with the addition of beef crust to pork patties. This highlights the potential quality variation of dry-aged beef trimmings. Hence, consideration should be given when applying dry-aged beef trimmings as it could alter final product pH and differently influence final product functionality and quality.

No significant effect of aged beef trim inclusion was observed on cook loss measurement (Table 2). Different from the current study, Park et al. (2018), Xue et al. (2021), and Lee et al. (2022) reported that the addition of dry-aged beef trims reduced the cook loss compared to the control. The different observation could potentially be attributed to the fact that those authors utilized the dehydrated crust trim portion in a lyophilized powder form compared to the ground lean and fat trim portion utilized in the current study. It was suggested by those authors that the lyophilized meat powder could be rehydrated when exposed to moisture, thus allowing greater moisture retention following the cooking process.

The addition of aged beef trimmings to the beef patties significantly influenced the chewiness of the product ($P < 0.05$, Table 2). The current result showed that DWA had lower chewiness compared to CON ($P < 0.05$), while both DWA and CON samples were not different from DA-FAT, WA, DA, and UDA samples ($P > 0.05$). The hardness, adhesiveness, resilience, cohesion, and springiness of the beef patties samples were not impacted by the addition of differently aged beef trimmings ($P > 0.05$). The current results indicated that the addition of aged beef trimmings minimally impacts the textural properties of the final beef patties, regardless of the aging methods. The current reported results, however, were not in line with previous studies adding dry-aged beef trimmings to meat patties. While different, the results of the current study were not surprising as the aged beef trimmings were

Table 2. Impact of aged trimmings inclusion on pH, cook loss, and textural properties of ground beef patties

Treatments	pH	Cook Loss (%)	Hardness (*g*)	Adhesiveness (*g.sec*)	Resilience (%)	Cohesiveness (%)	Springiness (%)	Chewiness
CON	5.50	31.15	11467	−0.41	18.23	0.51	74.61	4374[b]
DA-FAT	5.58	32.73	10517	−0.46	17.54	0.50	75.04	3931[ab]
WA	5.61	30.01	11178	−0.90	17.76	0.50	73.21	4047[ab]
DA	5.61	30.33	11200	−0.65	17.36	0.49	75.31	4122[ab]
DWA	5.67	29.43	9481	−0.32	17.55	0.49	73.04	3397[a]
UDA	5.70	29.62	10372	−0.57	17.13	0.48	74.40	3732[ab]
SEM	0.05	1.05	617	0.21	0.38	0.01	1.04	188
P value	0.0876	0.3074	0.1853	0.2253	0.3895	0.3568	0.5392	0.0372

[a,b]Different superscript letters indicated a significant difference between the different aging methods ($P < 0.05$).

Different formulation treatments: CON (80% fresh beef + 20% fresh fat), DA-FAT (80% fresh beef + 20% DA fat), WA (50% fresh beef + 30% WA lean + 20% WA fat), DA (50% fresh beef + 30% DA lean + 20% DA fat), DWA (50% fresh beef + 30% DWA lean + 20% DWA fat), and UDA (50% fresh beef + 30% UDA lean + 20% UDA fat).

SEM: Standard Error of Means.

only minimally dehydrated from the aging process and were not further processed (lyophilized) prior to inclusion. Xue et al. (2021) reported that the addition of lyophilized dry-aged crust trimmings increased the hardness, adhesiveness, gumminess, and chewiness of beef patties. Similarly, Lee et al. (2022) found that the addition of lyophilized dry-aged beef crust trimmings increased the shear force of pork patties, indicating increased hardness. Both authors attributed the changes in texture profile to the higher protein content of the lyophilized dry-aged trimmings, allowing for stronger protein binding. As such, it could be assumed that the aged beef lean and fat trimmings had a more similar physiochemical quality and protein content to those of regular beef compared to the dehydrated dry-aged beef crust.

Color and lipid oxidation stability

Among the color measurements, hue angle (instrumental discoloration) was the only color trait significantly impacted by treatment and storage interaction ($P < 0.05$, Figure 1). The inclusion of DWA trimmings in beef patties caused greater discoloration compared to other treatments. An overall increase in hue angle value was observed across the different samples throughout the display. However, the DWA samples had a higher hue angle value on day 5 of the display compared to other treatments ($P < 0.05$). No significant interaction effect was observed for L^*, a^*, b^*, and chroma.

A significant treatment effect was observed for a^*, b^*, and chroma (Table 3). The instrumental redness (a^*) and yellowness (b^*) were observed to be higher in CON compared to UDA and DWA ($P < 0.05$), while DA-FAT, DA, and WA samples were not different from those treatments ($P > 0.05$). CON had higher chroma values compared to DWA ($P < 0.05$), which were not different compared to all other treatments ($P > 0.05$). The L^*, a^*, b^*, and chroma were impacted by storage effect, showing a significant decrease at the end of the simulated color display compared to the beginning of the display regardless of treatment ($P < 0.05$, Table 3).

The color of dry-aged beef has often been reported to be darker compared to its wet-aged counterpart due to the dehydration process (Dikeman et al., 2013; Ribeiro et al., 2021). Based on the current simulated color display results, it was found that the inclusion of aged beef trimming had a minimal impact on the initial color of the beef patties. During the display, however, the addition of DWA trimmings negatively

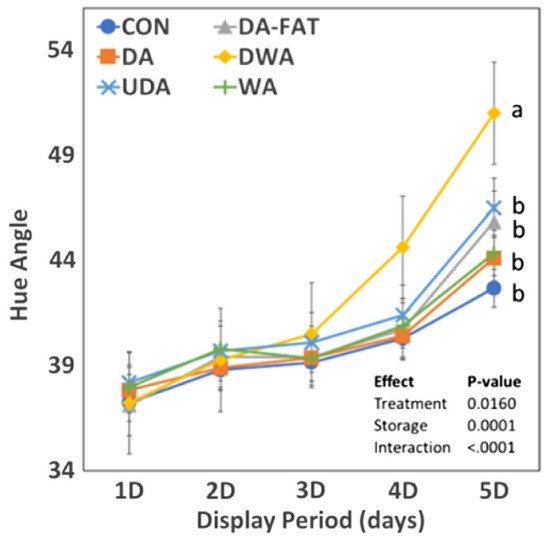

Figure 1. Impact of treated trimmings inclusion on hue angle changes of beef patties during 5 d of the display period. Different formulation treatments: CON (80% fresh beef + 20% fresh fat), DA-FAT (80% fresh beef + 20% DA fat), WA (50% fresh beef + 30% WA lean + 20% WA fat), DA (50% fresh beef + 30% DA lean + 20% DA fat), DWA (50% fresh beef + 30% DWA lean + 20% DWA fat), and UDA (50% fresh beef + 30% UDA lean + 20% UDA fat). [a,b]Different letters indicate significant differences between treatments within the same display day ($P < 0.05$).

Table 3. Impact of aged trimmings inclusion on lightness (CIE L^*), redness (CIE a^*), yellowness (CIE b^*), chroma, and TBARS of beef patties

Color Attributes	L^*	a^*	b^*	Chroma
Treatment effect				
CON	44.26	26.04^{a}	21.38^{a}	33.71^{a}
DA	45.61	24.40^{abc}	20.33^{abc}	31.78^{ab}
DA-FAT	45.59	24.23^{abc}	20.33^{abc}	31.67^{ab}
DWA	45.34	22.31^{c}	19.77^{b}	29.91^{b}
UDA	46.21	23.42^{bc}	20.20^{bc}	30.96^{ab}
WA	47.60	25.17^{ab}	21.25^{ab}	32.96^{a}
SEM	0.85	0.68	0.41	0.74
P value	0.055	0.007	0.049	0.010
Storage effect				
1 D	48.63^{a}	28.83^{a}	22.17^{a}	36.37^{a}
2 D	46.38^{b}	27.99^{a}	22.90^{a}	36.17^{a}
3 D	46.18^{b}	24.94^{b}	20.65^{b}	32.39^{b}
4 D	44.36^{c}	22.34^{b}	19.59^{b}	29.73^{b}
5 D	43.28^{c}	17.21^{c}	17.40^{c}	24.52^{c}
SEM	0.71	0.70	0.34	0.73
P value	<0.0001	<0.0001	<0.0001	<0.0001

[a–c]Different superscript letters indicated a significant difference within the same column and effect ($P < 0.05$).

Different formulation treatments: CON (80% fresh beef + 20% fresh fat), DA-FAT (80% fresh beef + 20% DA fat), WA (50% fresh beef + 30% WA lean + 20% WA fat), DA (50% fresh beef + 30% DA lean + 20% DA fat), DWA (50% fresh beef + 30% DWA lean + 20% DWA fat), and UDA (50% fresh beef + 30% UDA lean + 20% UDA fat).

SEM: Standard Error of Means.

affected the color of the beef patties, exhibiting rapid color degradation and accelerated discoloration during the display. Similar results were previously reported by Setyabrata et al. (2022b), in which the authors found that DWA samples had greater hue angle and visual discoloration by day 5 of the display compared to all other aging methods. The authors speculated that the utilization of dry-aged bags hindered the dehydration process, inducing more oxidation and thus leading to lower color stability during display. Thus, it could be postulated that the inclusion of DWA beef trimmings impacted the overall observed color of the beef patties and potentially exacerbated the discoloration rate of the patties during retail display. The current results differ when compared to other studies utilizing dry-aged beef crust trimmings, in which the additional trimmings significantly reduce the lightness of the final product (Lee et al., 2022; Xue et al., 2021). It was reported that the addition of lyophilized dry-aged beef crust trimmings caused significant darkening in the final products, mainly because the crust powder had darker color and reduced surface moisture as the dehydrated powder retained more moisture (Lee et al., 2022; Xue et al., 2021). Similar to the current study, Xue et al. (2021) reported a decrease in a^* following dry-aged crust inclusion in beef patties; however, Lee et al. (2022) reported a conflicting impact on the a^*, showing an increase with dry-aged crust inclusion in pork patties. This result suggests the need to further evaluate the impact of aged trim inclusion on final product color quality.

Only storage effect was found to be affecting the lipid oxidation ($P < 0.05$, Figure 2). No significant treatment and treatment × storage interaction effects were observed. A greater increase in lipid oxidation was observed in all samples following the display ($P < 0.05$), regardless of treatments applied. Our findings are in agreement with previous studies, observing minimal differences in the extent of lipid oxidation from different aging treatments based on TBARS measurement (Setyabrata et al., 2022b; Xue et al., 2021).

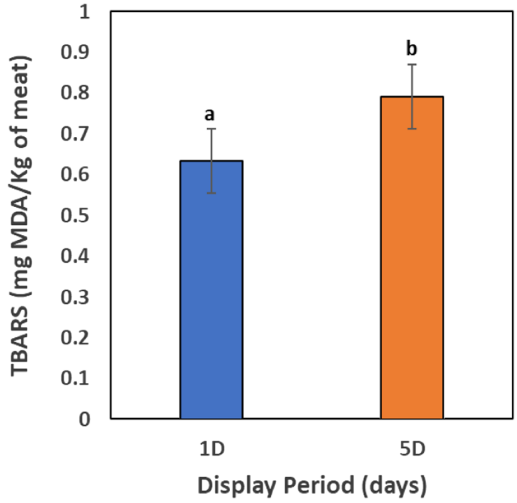

Figure 2. Impact of treated trimmings inclusion on lipid oxidation (TBARS) of beef patties before and after 5 d display. Different formulation treatments: CON (80% fresh beef + 20% fresh fat), DA-FAT (80% fresh beef + 20% DA fat), WA (50% fresh beef + 30% WA lean + 20% WA fat), DA (50% fresh beef + 30% DA lean + 20% DA fat), DWA (50% fresh beef + 30% DWA lean + 20% DWA fat), and UDA (50% fresh beef + 30% UDA lean + 20% UDA fat). [a,b]Different letters indicate significant differences within the different display periods ($P < 0.05$).

Trained sensory panel and volatile compound analysis

Trained panel sensory evaluation revealed that the addition of DA and UDA trims decreased the perceived bloody flavor in the final beef patties compared to CON, DA-FAT, and WA patties ($P < 0.05$, Table 4). The bloody flavor score for DWA patties was not different compared to all treatments ($P > 0.05$). Similarly, there was a tendency ($P = 0.087$) that the addition of DA and UDA trims reduced the metal flavor of the patties compared to other treatments. No significant impact was observed on beefy, brown roasted, rancid, umami, salty, hardness, cohesiveness, and moisture sensory attributes from the different beef patties.

It is to our surprise that the beef patties' flavor was not impacted by the addition of the aged beef trimmings. Previous reports by Park et al. (2018), Xue et al. (2021), and Lee et al. (2022) found that the addition of dry-aged beef crust trimmings significantly improved the flavor scores by the trained panel. Xue et al. (2021) further reported that the addition of lyophilized dry-aged beef crust trimming significantly increased the brown roasted flavor and exhibited a strong trend of increasing umami flavor. Those authors suggested that the increased flavor was due to the increased concentration of the flavor precursor in the crust portion as a result of the dehydration process during dry aging. In the current study, the crust trim portion was not utilized in patty manufacturing. It is possible that the aged beef lean and fat trimmings minimally concentrated the flavor precursor compared to the crust portion as they were not immediately exposed to the environment, thus limiting the dehydration and flavor-enhancing ability.

The addition of DA trimmings decreased the bloody and tended to reduce the metal flavor of the patties ($P = 0.087$); however, only the addition of lean trim decreased the off flavors as the sole addition of aged fat trim in DA-FAT samples did not lead to the

Table 4. Impact of aged trimmings inclusion on sensory properties of ground beef patties

Treatments	Beefy	Brown Roasted	Bloody	Metal	Rancid	Umami	Salty	Hardness	Cohesiveness	Moisture
CON	9.74	9.74	2.93[b]	3.19	1.93	7.84	1.39	4.67	7.10	4.52
DA-FAT	9.43	10.07	2.87[b]	3.03	2.07	7.71	1.30	4.79	6.88	4.86
WA	9.79	10.02	3.02[b]	3.11	1.76	8.31	1.45	4.93	7.14	4.58
DA	9.79	10.00	2.36[a]	2.45	1.79	8.10	1.48	5.12	6.95	4.62
DWA	10.17	10.52	2.62[ab]	2.67	2.02	8.55	1.44	4.81	7.07	4.80
UDA	9.40	9.81	2.31[a]	2.37	1.79	7.89	1.38	5.19	7.05	4.40
SEM	0.41	0.43	0.38	0.38	0.19	0.59	0.14	0.49	0.70	0.56
P value	0.3160	0.2939	0.0172	0.0870	0.7738	0.3195	0.8048	0.1954	0.9771	0.8400

[a,b]Different superscript letters indicated a significant difference between the different aging methods ($P < 0.05$).

Different formulation treatments: CON (80% fresh beef + 20% fresh fat), DA-FAT (80% fresh beef + 20% DA fat), WA (50% fresh beef + 30% WA lean + 20% WA fat), DA (50% fresh beef + 30% DA lean + 20% DA fat), DWA (50% fresh beef + 30% DWA lean + 20% DWA fat), and UDA (50% fresh beef + 30% UDA lean + 20% UDA fat).

SEM: Standard Error of Means.

same result. The presence of metallic and bloody notes is often attributed to the increase in myoglobin and heme iron concentration in the meat product (Yancey et al., 2006), indicating that the metallic and bloody flavor is mainly governed by the lean portion of the meat. Interestingly, the current findings showed that DA and UDA trimming significantly reduced the metallic flavor, followed by DWA, while the addition of WA trimming did not impact the flavor. Previous reports by Setyabrata et al. (2021a) suggested that the dry-aging process might cause modification to myoglobin or heme iron, reducing the metallic flavor despite increasing the concentration of those compounds. It is possible that the modification through dry aging mitigated and reduced the development of these flavors, although further studies are still required to confirm this speculation.

The different addition of aged beef lean and fat trim only minimally influenced the volatile profile of the beef patties (Table 5). A total of 69 volatile compounds were detected. Of the detected volatile compounds, only 2-acetyl pyrrole was found to be affected by the addition of aged beef trimmings, exhibiting greater concentration with the addition of DA lean and fat trims compared to all other treatments ($P < 0.05$), except UDA where they were not different ($P > 0.05$). Three compounds tend to increase with the addition of the DA and UDA-aged beef trimmings (2-pentanone [$P = 0.0925$], 2-heptanone [$P = 0.0627$], and furfural [$P = 0.0919$]). Of the 4 affected compounds, 3 were found to be from the ketone group and one from the furan group.

The observed volatile results corroborate with the trained sensory panel observation in this study, exhibiting that the addition of aged beef trims minimally influenced the flavor profile of the beef patties. However, the results indicated that the DA and UDA process might significantly alter the lean and fat trims, generating a greater potential to alter and improve the final product flavor when added to beef patties. The identified compounds, 2-acetyl pyrrole, 2-pentanone, 2-heptanone, and furfural, were previously found to produce a positive flavor attribute in meat products, producing nutty, sweet, citrus, and almond odor, respectively (Sohail et al., 2022). While those compounds could positively impact the flavor of the final products, the concentration of the compounds might not be enough to induce the expected aroma. It is possible that the detection threshold of those compounds was higher and therefore did not meaningfully impact the flavor perceived by the sensory panelist. Further study to identify the flavor generation following different cooking techniques might be beneficial to understand and maximize the flavor generation process.

Metabolomics profile analysis

The addition of different aged beef lean and fat trims induced a distinct impact on the metabolomics composition of the samples before and after that cooking process. In the raw samples, PCA and HCA results (Figure 3) exhibited that the CON, DA-FAT, DA, and UDA patties had a more similar metabolite profile compared to the WA and DWA patties (significant metabolites presented in Table S1). The PCA and HCA of the cooked patties (Figure 4), however, revealed a different pattern. The analyses showed a clear clustering of WA, DA, DWA, and UDA treatments from the CON and DA-FAT treatments (significant metabolites presented in Table S2). The observation indicated that the aging process potentially alters the initial flavor precursor presence within the raw products. While different, however, the aged trims

Table 5. Impact of aged trimmings inclusion on volatile compounds of beef patties

Volatile Compound Name (ng/g Sample)	CON	DA-FAT	WA	DA	DWA	UDA	SEM	*P* Value
Pyrazines								
Methyl-pyrazine	0.194	0.322	0.271	0.342	0.253	0.251	0.044	0.2738
2,5-Dimethylpyrazine	0.470	0.771	0.596	0.744	0.606	0.596	0.098	0.3363
Trimethylpyrazine	0.008	0.024	0.019	0.024	0.015	0.014	0.006	0.3916
2-Ethyl-3,5/6-dimethylpyrazine	0.488	0.798	0.607	0.703	0.629	0.642	0.096	0.3906
Strecker aldehydes								
2-Methylbutanal	2.337	3.536	3.329	3.803	2.857	2.938	0.514	0.4298
3-Methylbutanal	2.845	6.874	3.882	9.393	5.380	5.130	2.426	0.5055
Phenylacetaldehyde	0.686	1.306	1.021	1.275	0.958	0.938	0.188	0.2576
Methional	2.393	5.065	3.749	4.577	3.659	3.439	0.800	0.3073
Benzaldehyde	3.473	7.649	6.166	7.046	4.745	4.719	1.396	0.3280
n-Aldehydes								
Acetaldehyde	3.432	10.150	8.098	10.313	4.004	5.555	2.284	0.1961
Butanal	1.427	2.241	2.266	2.454	1.688	2.022	0.370	0.3967
Pentanal	14.180	10.332	33.178	14.641	8.141	12.624	8.161	0.2593
Hexanal	98.737	103.360	223.650	116.180	84.845	103.890	47.660	0.2766
Heptanal	21.629	12.391	25.374	12.328	8.904	13.710	5.727	0.2071
Octanal	5.591	5.342	9.315	4.992	3.912	4.858	1.745	0.2932
Nonanal	7.838	11.219	14.275	11.098	8.631	8.650	2.607	0.5410
Decanal	1.629	1.966	2.146	5.307	1.405	4.016	1.712	0.5514
Dodecanal	1.153	14.963	6.207	17.220	4.641	5.937	5.507	0.3240
2,4-Decadienal	0.480	3.117	3.368	3.113	0.410	1.489	1.367	0.4515
2-Undecenal	0.241	2.451	2.909	1.690	1.877	1.479	0.929	0.4721
Ketones								
2-Propanone	10.655	15.382	29.192	27.134	8.479	19.452	8.156	0.3948
2,3-Butanedione	6.698	8.193	12.918	9.347	5.225	10.950	3.646	0.4328
2-Butanone	2.555	3.178	9.577	8.417	1.415	5.715	3.046	0.2223
2-Pentanone	0.177	0.325	0.369	0.700	0.245	0.654	0.172	0.0925
3-Hydroxy-2-butanone	36.337	31.327	60.582	51.874	23.568	59.215	20.549	0.4070
2-Heptanone	1.089	4.887	2.729	8.572	1.556	8.391	2.115	0.0627
2-Acetyl pyrrole	1.287[a]	7.165[a]	7.651[a]	15.685[b]	7.200[a]	8.095[ab]	2.531	0.0421
Alcohols								
Ethanol	1.216	3.394	2.966	1.376	1.366	2.250	0.667	0.1625
1-Penten-3-ol	32.010	9.520	26.812	16.090	8.331	17.101	9.480	0.3384
1-Pentanol	7.356	4.640	15.976	5.787	5.174	5.903	3.715	0.2256
2,3-Butanediol	0.709	0.868	1.665	1.245	0.902	1.639	0.426	0.3302
1-Hexanol	2.325	1.753	3.633	1.795	1.652	2.024	0.723	0.2914
1-Octen-3-ol	4.740	4.220	6.562	3.848	3.420	3.381	1.091	0.3090
1-Octanol	1.317	1.438	2.087	1.407	1.275	1.133	0.340	0.4678
Sulfur-containing								
Methanethiol	5.610	13.333	13.959	14.789	5.704	10.594	3.719	0.3170
Dimethyl sulfide	1.874	2.972	5.313	3.939	1.382	2.978	1.424	0.4079
Carbon disulfide	74.084	372.960	261.640	338.830	210.590	221.910	83.729	0.2315
Diallyl sulfide	0.502	0.775	0.668	0.710	0.636	0.682	0.100	0.5433
3-Methyl-thiophene	0.526	0.840	0.850	0.880	0.719	0.786	0.123	0.3868
Carboxylic acids								
Acetic acid	11.264	23.928	46.268	33.652	17.233	20.512	9.207	0.1769
Butanoic acid	1.215	1.175	2.993	1.508	1.388	1.214	0.637	0.3434
Hexanoic acid	1.741	1.550	2.867	2.326	1.421	2.308	0.761	0.5239
Heptanoic acid	1.962	3.445	1.724	2.179	2.592	2.530	0.619	0.4179
Octanoic acid	3.196	11.508	7.388	13.380	7.307	10.653	2.774	0.2096
Nonanoic acid	1.388	2.210	2.112	2.433	1.852	1.902	0.381	0.5145

Table 5. (*Continued*)

Volatile Compound Name (ng/g Sample)	CON	DA-FAT	WA	DA	DWA	UDA	SEM	*P* Value
Butanoic acid, methyl ester	0.055	0.047	0.106	0.094	0.050	0.095	0.034	0.4316
Hexanoic acid, methyl ester	0.334	0.460	0.469	0.434	0.372	0.388	0.064	0.6061
Heptanoic acid, methyl ester	0.025	0.044	0.050	0.067	0.037	0.038	0.012	0.1670
Octanoic acid, methyl ester	0.543	0.911	0.736	0.844	0.724	0.741	0.118	0.3962
Nonanoic acid, methyl ester	1.142	1.807	1.395	1.628	1.429	1.800	0.240	0.3770
Hydrocarbons								
Benzene	0.098	0.036	0.188	0.082	0.035	0.085	0.058	0.2396
Toluene	1.202	1.827	2.021	1.953	1.408	1.675	0.374	0.5600
1-Octene	8.186	4.616	19.175	6.401	5.393	6.958	4.797	0.2213
Octane	6.840	6.932	23.364	17.297	8.391	19.145	7.242	0.2214
Ethyl benzene	2.382	3.264	3.579	3.523	2.734	3.084	0.660	0.7220
p-Xylene	5.569	9.306	16.171	12.724	7.664	10.026	3.631	0.3299
Styrene	2.430	3.331	3.651	3.599	2.795	3.147	0.673	0.7206
Nonane	2.408	1.609	3.735	1.512	1.143	1.761	0.774	0.2750
Alpha-pinene	0.625	0.868	0.788	0.825	0.717	0.788	0.120	0.7370
Decane	6.260	8.178	13.715	13.301	6.458	8.648	3.323	0.3722
D-limonene	1.106	1.183	1.383	1.239	0.913	1.119	0.264	0.7891
Tetradecane	0.712	0.413	1.194	0.558	0.300	0.614	0.397	0.5702
Furans								
Furfural	0.078	0.188	0.111	0.318	0.060	0.143	0.060	0.0919
2-Furanmethanol	0.453	0.560	1.744	0.863	0.588	0.743	0.386	0.2425
2-Furancarboxaldehyde	1.072	1.689	1.309	1.508	1.350	1.337	0.203	0.4341
2-Pentyl furan	0.852	1.121	1.146	1.117	0.836	0.903	0.169	0.5973
Furan, 2-methyldithio	3.572	16.924	4.084	10.582	11.723	18.939	7.216	0.5858
Others								
Butyrolactone	2.464	2.326	3.524	1.253	0.982	1.624	0.917	0.4374
Triacetin	1.451	3.019	2.243	2.196	6.115	1.992	1.793	0.5219

[a,b]Different superscript letters indicated a significant difference between the different aging methods ($P < 0.05$).

Different formulation treatments: CON (80% fresh beef + 20% fresh fat), DA-FAT (80% fresh beef + 20% DA fat), WA (50% fresh beef + 30% WA lean + 20% WA fat), DA (50% fresh beef + 30% DA lean + 20% DA fat), DWA (50% fresh beef + 30% DWA lean + 20% DWA fat), and UDA (50% fresh beef + 30% UDA lean + 20% UDA fat).

SEM: Standard Error of Means.

potentially went through a similar reaction as the final cooked products showed a similar metabolomics and flavor profile regardless of the aging method applied. It was previously reported that different dry-heat cookery strongly influenced the flavor production, thus affecting the perceived final meat flavor (Vierck et al., 2021). As such, it could be speculated that while the different aging methods alter the presence of the precursors, the cooking process applied might not promote the desired reaction to maximize flavor production, resulting in a product with similar flavor quality. Furthermore, while different, it is possible that the abundance of the flavor precursor might be too low to influence the flavor generation process, limiting the volatile compounds and subsequently flavor development. This postulation, however, needs to be confirmed.

A clear clustering between raw and cooked samples was observed through PCA and HCA (Figure 5), showing a clear impact of cooking on the metabolite profile of the beef patties, regardless of the formulations. It could be observed from the PCA results that cooking decreased the variation of metabolites between treatments, generating products with more similar metabolites when compared to the raw samples. A total of 21 metabolites were identified through MS/MS match and found to be significantly influenced by the cooking process (FDR < 0.01, Table S3), regardless of the formulation. Of those, 10 metabolites were found to be more abundant in the raw patties and can be loosely grouped into nucleotides (adenosine diphosphate and hypoxanthine), amino acids/peptides (leucine and leucylleucine), and lipids (acetamidomethylpropyl acetate, glycyrrhetic acid, harringtonine, hydroxyquinazolinyl acetamide, gelomulide N, and phosphocholine). Eleven metabolites were observed to be greater in cooked patties

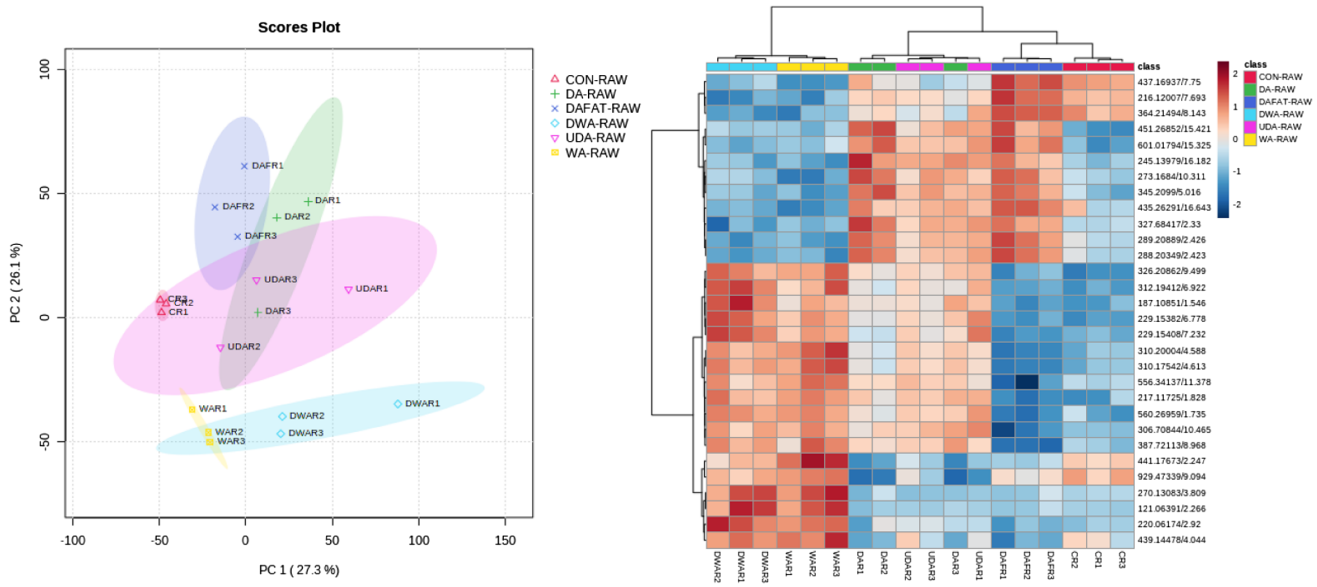

Figure 3. Principal component analysis (PCA) and hierarchical clustering analysis (HCA) of metabolites from raw beef patties made with different formulations. Different formulation treatments: CON (80% fresh beef + 20% fresh fat), DA-FAT (80% fresh beef + 20% DA fat), WA (50% fresh beef + 30% WA lean + 20% WA fat), DA (50% fresh beef + 30% DA lean + 20% DA fat), DWA (50% fresh beef + 30% DWA lean + 20% DWA fat), and UDA (50% fresh beef + 30% UDA lean + 20% UDA fat).

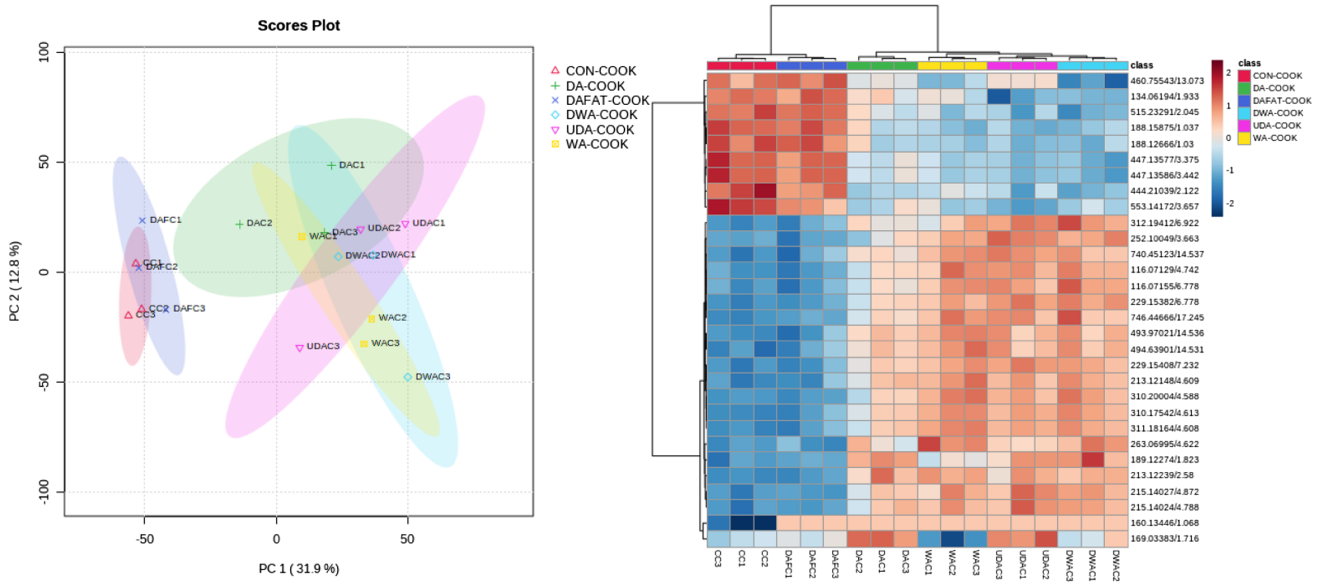

Figure 4. Principal component analysis (PCA) and hierarchical clustering analysis (HCA) of metabolites from cooked beef patties made with different formulations. Different formulation treatments: CON (80% fresh beef + 20% fresh fat), DA-FAT (80% fresh beef + 20% DA fat), WA (50% fresh beef + 30% WA lean + 20% WA fat), DA (50% fresh beef + 30% DA lean + 20% DA fat), DWA (50% fresh beef + 30% DWA lean + 20% DWA fat), and UDA (50% fresh beef + 30% UDA lean + 20% UDA fat).

and predominantly belonged to nucleotides (5'-S-methylthioadenosine, adenine, adenosine monophosphate, anabasamine, hypoxanthine, and NAD), followed by amino acids/peptides (cysteinylglycine, glutathione, and methionine) and vitamin (niacinamide). Interestingly, more umami-related compounds were found to be greater in abundance in the cooked samples compared to the raw samples. Greater abundance of 5'-S-methylthioadenosine, adenosine monophosphate, and glutathione were measured in cooked samples, indicating a potentially greater umami taste in the product (Toldrá and Flores, 2010). It is possible that the cooking further degraded ADP into the adenosine compounds and promoted the flavor through

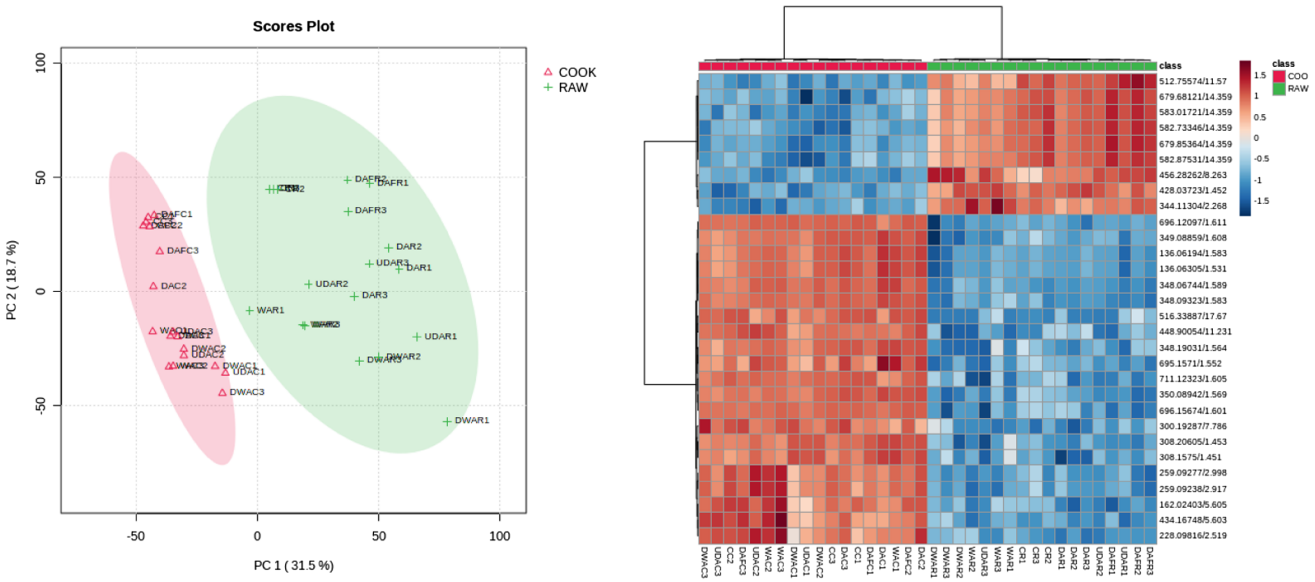

Figure 5. Principal component analysis (PCA) and hierarchical clustering analysis (HCA) of metabolites from raw and cooked beef patties made with different formulations. Different formulation treatments: CON (80% fresh beef + 20% fresh fat), DA-FAT (80% fresh beef + 20% DA fat), WA (50% fresh beef + 30% WA lean + 20% WA fat), DA (50% fresh beef + 30% DA lean + 20% DA fat), DWA (50% fresh beef + 30% DWA lean + 20% DWA fat), and UDA (50% fresh beef + 30% UDA lean + 20% UDA fat).

direct production of the adenosine compounds. Additionally, the degradation of ATP also leads to the liberation of ribose sugar that could further react with Maillard reaction products to generate a desirable meat aroma (Moloney and McGee, 2023). The greater abundance of meat flavor-related compounds such as cysteinylglycine and methionine were also observed in the cooked samples. Both cysteine and methionine have been known to participate in the Maillard reaction with ribose sugar to produce volatile compounds often related to meat flavor (Ramalingam et al., 2019).

Metabolomic profiling also indicated that the inclusion of lean trim resulted in different metabolomics profiles in the patties and potentially greater flavor alteration (Figure 6). The PCA revealed a clear separation between CON, DA-FAT, and DA in raw samples, exhibiting a different metabolite composition across the samples. However, CON samples and DA-FAT samples clustered together following the cooking process compared to DA samples. These showed that while initially different, the CON and DA-FAT samples were more likely to undergo similar reactions during the cooking and generated a more identical product compared to DA samples. Similarly, HCA results also indicated that CON had more similarities to DA-FAT when compared to DA samples. A total of 8 metabolites were identified through MS/MS match and found to be significantly influenced by the lean and fat formulation (FDR < 0.01, Table S4). Seven of the metabolites were predominantly greater in DA samples and could be grouped into amino acids (L-5-oxoproline and proline), nucleotides (isonicotinic acid and naphthalenyl pyrimidinylamine), and lipids (isopropenyl napthalenone, hydroxy quinazolinone, and propionyl carnitine). The DA-FAT samples were found to have a greater abundance of tyrosine, and none was observed to be present in greater abundance in CON samples.

This observation indicates that the lean trim portions might have a greater responsibility in driving the generation of flavor compared to the fat trim portion alone. It is possible that the greater abundance of compounds with flavor potentials in DA samples, such as isonicotinic acid, L-5-oxoproline, and proline, promoted more flavor compound production through the Maillard reaction in the beef patties, leading to greater flavor improvement in the samples. This finding is in line with the observed trained sensory panel results, showing lower bloody and metal flavor in the DA patties compared to CON and DA-FAT patties. It is possible that while limited, the flavor generated from these compounds is enough to mask the bloody and metal flavor perceived from the samples, thus potentially improving the product acceptability. Similar findings were previously reported by Jiang and Bratcher (2016) and Hicks et al. (2023) in which those authors found that different lean sources impacted the flavor precursor profile of the final ground beef patties, thus influencing the final product flavor.

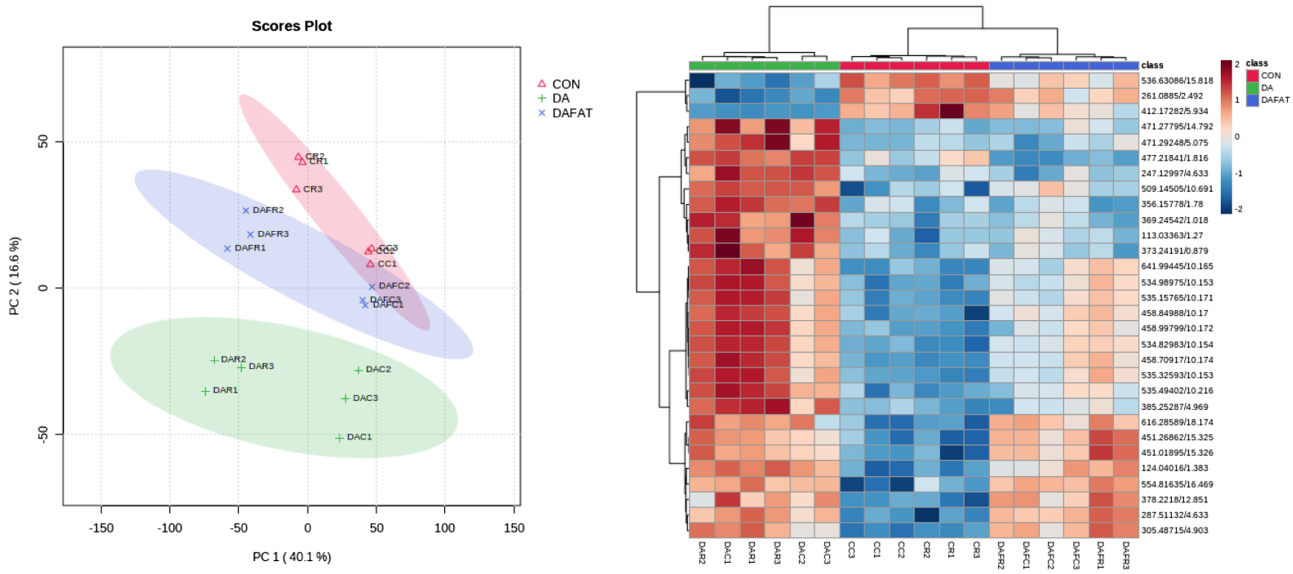

Figure 6. Principal component analysis (PCA) and hierarchical clustering analysis (HCA) of metabolites from CON, DA, and DA-FAT raw and cooked beef patties. Different formulation treatments: CON (80% fresh beef + 20% fresh fat), DA-FAT (80% fresh beef + 20% DA fat), and DA (50% fresh beef + 30% DA lean + 20% DA fat).

Conclusion

The results showed that the addition of dry-aged lean and fat trims minimally impacted the overall quality attributes of ground beef patty. However, the addition of DA and UDA trims decreased bloody characteristics, tended to reduce metallic flavor, and promoted greater volatile production compared to the addition of WA and DWA beef trims. Metabolomics profiling revealed that different aging methods lead to different availability of flavor precursors in raw samples, although it did not immediately translate to the formation of different flavors determined by the trained panel evaluation and metabolomics profiling of the cooked patties. Metabolomics analysis also demonstrated that the addition of a lean trim portion has a greater impact on the final meat flavor profile compared to a fat trim portion. The findings indicated that the non-crust lean and fat trim portion has a distinct quality from the crust trimming portion. Therefore, further optimization of the utilization methods for these trimmings is necessary to maximize the benefits. In particular, a titration of different levels of inclusion of dry-aging trimmings would be warranted to identify the optimal combination ratio of the trimmings for practical impacts. Further studies to identify the physiochemical and functional properties of the trims will be also beneficial to identify best practice strategies for utilizing non-crust dry-aged beef trimmings. Additionally, studies to determine the impact of different cookery methods might be important to maximize dry-aging flavor development.

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Supplementary

Table S1. Significantly impacted metabolites from raw beef patties made with different formulations ($P < 0.01$, FDR < 0.01)

Mz/RT	Adduct type	Matching	Metabolite name corrected	Highest Concentration
162.11252/1.523	[M+H]+	MS-MS	Carnitine	CON
218.1385/3.117	[M+H]+	MS-MS	Propionylcarnitine	CON
240.09982/5.314	[M+Na]+	MS-MS	securinine	CON
146.16336/0.571	[M+H]+	MS-MS	Spermidine	CON
518.32117/20.382	[M+Na]+	MS-MS	Acetamido-methylpropyl-trimethyl-oxohexadecahydropentalenophenanthrenyl-acetate	DA
100.0744/4.523	[M+H]+	MS-MS	2-Piperidone	DA
184.07422/20.807	[M]+	MS-MS	Phosphocholine	DA
594.34076/18.49	[M+Na]+	MS-MS	Veratrosine	DA
132.10289/4.713	[M+H]+	MS-MS	Leucine	DAFAT
245.18292/10.239	[M+H]+	MS-MS	Leucylleucine	DAFAT
146.05917/6.612	[M+H]+	MS-MS	3-Formylindole	DWA
121.06391/2.266	[M+H]+	MS-MS	Phenylacetaldehyde	DWA
86.09607/0.571	[M+H]+	MS-MS	Piperidine	DWA
116.07155/6.778	[M+H]+	MS-MS	Proline	DWA
160.13446/1.068	[M+H]+	MS-MS	5-Aminovaleric acid betaine	UDA
124.04016/1.383	[M+H]+	MS-MS	Isonicotinic acid	WA

Table S2. Significantly impacted metabolites from cook beef patties made with different formulations ($P < 0.01$, FDR < 0.01)

Mz/RT	Adduct type	Matching	Metabolite name corrected	Highest Concentration
162.11183/4.453	[M+H]+	MS-MS	D-Carnitine	CON
144.1021/1.294	[M+H]+	MS-MS	Proline betaine	CON
182.08194/3.9	[M+H]+	MS-MS	Tyrosine	CON
104.10654/0.743	[M]+	MS-MS	Choline	DA
460.25073/14.942	[M+Na]+	MS-MS	Bullatine B	DAFAT
349.0556/2.02	[M+H]+	MS-MS	Inosine-5'-monophosphate	DWA
124.04016/1.383	[M+H]+	MS-MS	Isonicotinic acid	DWA
722.44397/17.779	[M+H]+	MS-MS	Khasianine	DWA
585.32306/12.421	[M+H]+	MS-MS	lappaconitine	DWA
121.06391/2.266	[M+H]+	MS-MS	Phenylacetaldehyde	DWA
116.07155/6.778	[M+H]+	MS-MS	Proline	DWA
205.09795/6.611	[M+H]+	MS-MS	TRYPTOPHAN	DWA
160.13446/1.068	[M+H]+	MS-MS	5-Aminovaleric acid betaine	UDA
116.07129/4.742	[M+H]+	MS-MS	Proline	UDA
218.13754/1.749	[M+H]+	MS-MS	Propionylcarnitine	UDA
257.11627/4.296	[M+H]+	MS-MS	Pterostilbene	UDA
241.15627/3.484	[M+Na]+	MS-MS	Isopropenyl-dimethyl-hexahydro-naphthalenone	WA
137.04796/1.822	[M+H]+	MS-MS	Hypoxanthine	WA
130.04974/1.656	[M+H]+	MS-MS	L-5-Oxoproline	WA
86.09607/0.571	[M+H]+	MS-MS	Piperidine	WA

Table S3. Significantly impacted metabolites from raw and cooked beef patties made with different formulations ($P < 0.01$, $FDR < 0.01$)

Mz/RT	Adduct type	Matching	Metabolite name corrected	Highest Concentration
298.09402/7.487	[M+H]+	MS-MS	5'-S-Methylthioadenosine	Cook
136.06194/1.583	[M+H]+	MS-MS	Adenine	Cook
136.06219/7.489	[M+H]+	MS-MS	ADENINE	Cook
348.06744/1.589	[M+H]+	MS-MS	Adenosine Monophosphate	Cook
254.16431/4.622	[M+H]+	MS-MS	Anabasamine	Cook
179.04893/1.422	[M+H]+	MS-MS	Cysteinylglycine	Cook
308.09146/1.42	[M+H]+	MS-MS	Glutathione	Cook
137.0448/1.533	[M+H]+	MS-MS	Hypoxanthine	Cook
150.05898/12.563	[M+H]+	MS-MS	Methionine	Cook
664.11615/3.243	[M+H]+	MS-MS	NAD	Cook
123.05711/3.241	[M+H]+	MS-MS	Niacinamide	Cook
496.33612/21.854	[M+H]+	MS-MS	Acetamidomethylpropyl acetate	Raw
428.03683/1.524	[M+H]+	MS-MS	Adenosine_Diphosphate	Raw
471.35284/12.742	[M+H]+	MS-MS	glycyrrhetic acid	Raw
532.258/7.717	[M+H]+	MS-MS	harringtonine	Raw
137.04388/4.658	[M+H]+	MS-MS	Hypoxanthine	Raw
132.10318/7.147	[M+H]+	MS-MS	Leucine	Raw
245.18292/10.239	[M+H]+	MS-MS	Leucylleucine	Raw
747.35327/9.511	[M+Na]+	MS-MS	Hydroxyquinazolinyl acetamide	Raw
415.20993/20.21	[M-H2O+H]+	MS-MS	Gelomulide N	Raw
184.07422/20.807	[M]+	MS-MS	Phosphocholine	Raw

Table S4. Significantly impacted metabolites from CON, DA and DA-FAT raw and cooked beef patties formulations ($P < 0.01$, $FDR < 0.01$)

Mz/RT	Adduct type	Matching	Metabolite name corrected	Highest Concentration
241.15627/3.484	[M+Na]+	MS-MS	Isopropenyl naphthalenone	DA
271.13794/4.426	[M+H]+	MS-MS	Hydroxy quinazolinone	DA
124.04016/1.383	[M+H]+	MS-MS	Isonicotinic acid	DA
130.04974/1.656	[M+H]+	MS-MS	L-5-Oxoproline	DA
276.12497/13.95	[M+H]+	MS-MS	Naphthalenyl pyrimidinylamine	DA
116.07155/6.778	[M+H]+	MS-MS	Proline	DA
218.13754/1.749	[M+H]+	MS-MS	Propionylcarnitine	DA
182.08194/3.9	[M+H]+	MS-MS	Tyrosine	DA-FAT