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Research Article

Effect of *Ilex x meserveae* Aqueous Extract on the Quality of Dry-Aged Beef

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The effect of meat marinating with aqueous extract of *Ilex meserveae* dried leaves on dry-aged beef quality was investigated. Shear force, TBARS value, color parameters, fatty acid profile, and sensory properties were evaluated in beef cuts dry-aged for 21 days. The use of *Ilex meserveae* dried leaves as marinade at 0.5, 1.0, and 2.0% w/v did not affect the shear force and color parameters of dry-aged beef. The marinating of beef cuts with *Ilex meserveae* resulted in efficient prevention of lipid oxidation without impairing sensory acceptability. Aqueous extract *Ilex meserveae* can be applied as a natural ingredient in meat marinade to prevent peroxidation.

1. Introduction

Aging is a complex process that takes place during converting muscle into meat. The course of this process affects the achievement of appropriate palatability of retail and wholesale cuts. Biochemical changes that occur during aging affect the structure of intramuscular connective tissue and muscle fibers, thus shaping the culinary value of meat [1]. At present, two methods of aging are used: wet and dry. Both methods are applied to improve the quality of beef cuts. They differ in the conditions maintained during the process. Wet aging is in the packaging; that is, beef cuts are vacuum-packed and stored at refrigeration temperatures. The vast majority of beef available on the market is subjected to wet aging, because the dry-aging method is costly and time-consuming. Dry-aging must be carried out under continuously controlled conditions (temperature of 0–4°C, relative humidity of 75–85%, and airflow 0.5–2.0 m/s). This process can take several weeks or even months. Meat during this time loses up to 20% of its weight. This influences the formation of its high market price. Nevertheless, this type of meat is of great interest, and consumers are willing to pay more because of its unique overall palatability, that is, the perfect balance between tenderness, taste, and juiciness [1–5].

However, such a long period of dry-aging process and the importance of maintaining the constant process parameters raise the consumer's concern about the shelf life of final products [6]. During dry-aging, higher microbial growth was observed compared with wet-aged beef. A higher number of microorganisms in finished products may result in a further increase during storage and consequently lead to lowering product safety and deterioration of its quality [7]. Deterioration of dry-aged beef quality can also be caused by

the lipids oxidation processes, which may occur due to use of ample marbling cuts and direct exposure of meat during the dry-aging process. Adverse changes resulting in a reduction in the quality of meat products can be prevented by the introduction of substances of plant origin [8]. They may exhibit antioxidant and/or antimicrobial properties and can be introduced to food in different forms, for example powder, liquid or raw [9]. To the best of the authors' knowledge, limited scientific studies have evaluated the effect of plant-derived substances on shelf life of dry-aged beef. Therefore, the authors decided to assess the impact of *Ilex meserveae* on the physicochemical and sensory properties of dry-aged beef.

Ilex (holly) is a genus of about 480 species, but the best-known variety that is widely used is *Ilex paraguariensis St. Hilaire*. Dried leaves of this shrub are commonly used to produce yerba mate infusion, the consumption of which is recommended for a number of health benefits including potential therapeutic effect in cardiovascular disease [10]. Reports by other authors indicate the possibility of using yerba mate in meat production. *I. paraguariensis*, as a natural antioxidant, effectively reduced the oxidative changes in lipid fraction of hamburgers [11] and fermented Italian-type sausages [12] and chicken meat balls [13]. *Ilex meserveae* "Blue Angel" is characterized by a higher content of some polyphenols (e.g., chlorogenic acid and rutin) than *I. paraguariensis* [14], although information on the possibilities of its use in food processing is lacking. Hence, the authors used it in the dry-aging of beef.

2. Materials and Methods

2.1. Preparation of Marinade (IML). Leaves of *Ilex meserveae* "Blue Angel" used for this study were harvested from *Ilex* variety collection deposited at the Wrocław University of Environmental and Life Sciences herbarium. Leaves were manually picked, gently cleaned, and lyophilized. Marinades (IML) were prepared from lyophilized leaves according to the method described by Park et al. [15]. Consequently, 0.5, 1.0, and 2.0 g of grounded leaves were infused with 100 mL hot distillated water for 10 minutes on a rotary shaker. After this time, the infusion was filtered through a sterile gauze and cooled and kept for 24 hours at 4°C until use.

2.2. Preparation of Beef Samples. Twenty Simmental beef cattle (at the age of 14 (±1) months) kept in a private farm on the Lower Silesia (Poland), average weight 530 ± 20 kg, were transported to a local slaughterhouse (Buków, Poland). After slaughter (at 48 h postmortem), samples of longissimus lumborum muscle ($n = 20$) were taken from retail butchery (Świdnica, Poland). The meat samples taken were transported to the Department of Functional Food Products Development (Wrocław University of Environmental and Life Sciences, Poland) and stored at 4°C for 24 hours. Following the chilling process, beef cuts were manually portioned to samples weighted about 600 ± 50 g each. Samples were divided into four groups (5 pieces per group) and prepared by dipping in marinades with different concentration (0, 0.5, 1.0, and 2.0% w/v) of *Ilex x m.* leaves. The IML were added in the amount of 5% relative to the weight of the meat. The marinating process lasted 24 hours at 4°C in vacuum bags (PA/PE). Afterwards, the samples were unpacked and removed from the IML and dry-aged for three weeks at 4°C with 75% relative humidity. All analyses planned in the experiment were carried out directly after aging process.

2.3. Instrumental Texture Analyses: Shear Force. After the aging period, the beef samples were cut into 2.5 cm steaks, wrapped in aluminum foil, and cooked in a convection oven at 200°C until an internal temperature of 72°C was reached. Next, after cooling to ambient temperature, from each steak, at least five cubes of 1 cm width and height were prepared. The cubes were sheared perpendicular to the course of the muscle fibers using Zwick/Roell 169 Z010 testing machine (Zwick Testing Machines Ltd., Leominster Herefordshire, UK) at crosshead speed of 0.5 mm/s, working distance 15 to 20 mm, and trigger force 0.2 N.

2.4. Instrumental Color Measurement. Instrumental color measurement of fresh surface was conducted similarly to the method described by Biffin, Smith, Bush, Collins, & Hopkins [16]. Color of the beef steaks was measured after 40 min of blooming (6 ± 1°C) by using a Konica Minolta Chroma Meters CR-400 (Osaka, Japan). Color was expressed by L* (lightness), a* (redness), and b* (yellowness) parameters in CIE Lab system. The chroma meter was set at D65 illuminant and 10° standard observer. Additionally, the hue [tan −1 (b*/a*)2] and chroma [(a*2 + b*2) 0.5] were calculated. Before each measurement, the colorimeter was calibrated against a standard white tile ($Y = 94.2$; $x = 0.313$; $y = 0.320$). Data in the paper represented an average value out five measurements.

2.5. TBARS Assay. The effect of *Ilex meserveae* marinade on lipid oxidation of the dry-aged beef was measured by thiobarbituric acid reactive substances (TBARS) method described by Luciano et al. [17] with slight modifications. For that, 0.5 g of ground dry-aged beef was homogenized with 10 mL of 10% trichloroacetic acid (Chempur, Piekary Slaskie, Poland). Homogenates were centrifuged for 10 min (Sigma 3K30; Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany) at room temperature and 4000 rpm. Afterwards, homogenates were filtered through filter paper (Whatman number 1) to the clean conical flasks. Two mL of clear filtrates were vortexed with 2 mL of 0.02 M aqueous 2-thiobarbituric acid (Sigma-Aldrich, St. Louis, MO, USA) in screw cap glass tubes and next, incubated at 100°C in a water bath (Julabo TW12, Julabo Inc., Allentown, USA). The incubation was stopped after 40 min and tubes were cooled on ice. The absorbance of color complex of 2-thiobarbituric acid (TBA) and minor lipid oxidation products was measured at 532 nm by spectrophotometer (Rayleigh UV-1800, Beijing Rayleigh Analytical Instrument Corp., China). The TBARS values were estimated by standard curve with 1,1,3,3-tetraethoxypropane (Sigma-Aldrich) and expressed as mg of malonaldehyde (MDA) per kg of sample.

2.6. Fatty Acids Analysis. Lipids for fatty acids analysis were extracted by Folch, Lees, and Stanley [18] method using methylene chloride and methanol (2 : 1) as an extraction solvent. Up to 50 mg of fat, 5 mL of 0.5 M NaOH in methanol, 3 ml of 14% BF 3 in methanol, and water pebbles were added. The sample was then placed for 30 min in water bath preheated to 70°C. After cooling to room temperature, 6 mL of hexane 95% HPLC purity and 1 mL of saturated water solution of NaCl was added. Then, the upper layer was collected, filtered through $MgSO_4$ into a heart flask, and evaporated to dryness. The methyl esters of fatty acids were then dissolved in 1.5 mL of hexane 95% HPLC purity and placed in vials for gas chromatography. Content of vials (1 μl) was automatically injected into GC-MS system (Agilent 6890 N Series, 5973 MS) equipped with chromatograph column DB-225MS (60 m, 250 μm; 0.25 μm). Helium was used as a carrier gas (He), and split injection ratio was set on 1 : 100. Temperatures were set at 280°C for injector and 160°C for column. Oven temperature was programmed at 140°C for 5 min and then increased linearly with 4°C per minute to 240°C. Fatty acids were identified by the comparison of their retention times with standards and presented in this paper as mg per 100 g of muscle.

2.7. Sensory Evaluation. The sensory acceptance test was performed by 17 assessors (2 sessions, day after day), whom we informed about the principles of meat sensory analysis. The analysis was conducted in accordance with the Polish Standard PN-ISO 4121 : 1998 [19]. After 21 days of aging, the beef samples were cut into 2.5 cm steaks, wrapped in aluminum foil, and cooked in a convection oven at 200°C until an internal temperature 72°C was reached. After cooking, 1 cm × 1 cm cubes were placed on plates with three-digit codes and served. The sensory evaluation was performed in the sensory test room under white light. Assessors were asked to determinate the degree of acceptance of the following hedonic descriptors: overall appearance, smell, taste, color, hardness, and juiciness using a nine-point rating scale of acceptance (1, dislike extremely, to 9, like extremely [20]). The sensory evaluation was carried out on two production batches.

2.8. Statistical Analysis. Collected data of two production batches were analyzed statistically with TIBCO Statistica, version 13.3 (TIBCO Software Inc., USA) by one-way analysis of variance (ANOVA). Duncan's multiple range test ($P < 0.05$) was conducted after the homogeneity of variance had been confirmed. The experimental factor was the concentration of *Ilex x meserveae* extract in beef marinades. The data are presented as mean ± s.e. (standard error) and P value.

3. Results and Discussion

Beef is a raw material rich in myoglobin and is also characterized by a high content of unsaturated fatty acids, which can be subjected to oxidative changes during maturation. Oxidation products can reduce the useful value of meat, that is, functional and sensory properties, nutritional value, and texture [21,22]. Moreover, products of lipid oxidation may lead to DNA damage and mutagenic and carcinogenic changes in cells [23]. Technological procedures such as cooling or packaging may only limit the initiated process of lipids oxidation [24]; therefore, it is so important to monitor its progress during storage of meat. The degree of peroxidation of muscle lipids is most commonly determined by the content of substances reacting with 2-thiobarbituric acid [1]. The addition of plant-derived substances, for example, extracted from leaves of green tea, may extend the shelf life of raw beef patties by limitation of lipid oxidation process [25]. The results of own studies indicated that the addition of *Ilex meserveae* dried leaves to marinade effectively ($P = 0.001$) reduced lipid oxidation during beef aging (Table 1).

Recent studies have shown that extracts from herbs and spices and other botanicals were effective as natural antioxidants when added to meat products [26–29]. In the own studies, the strongest protection of secondary lipid oxidation products (TBARS) formulation was indicated in beef samples marinated with the highest concentration of plant additive (2% w/v of IML, 0.68 mg MDA/kg). A similar, dose-dependent, but not significant, effect against lipid oxidation was observed by Jongberg, Tørngren, and Skibsted [30] in pork chops prepared with commercial green tea and maté extracts stored for seven days in high-oxygen atmosphere packaging. Moreover, previous studies of Racanicci, Danielsen, and Skibsted [13] support the results of present study that *Ilex* varieties addition to meat marinades resulted in effective protection against the peroxidation. In their work, the addition of water extractable antioxidants of yerba mate limited lipids and vitamin E oxidation in precooked meat balls made from chicken breast during 10 days of chilled storage. Ferreira, Sampaio, Torres, and Bastos-Markowicz [11] have also demonstrated an effective antioxidant action of yerba mate (*Ilex paraguariensis St. Hilaire*) added as a natural antioxidant to beef burgers. For these studies, the addition of a much smaller amount of the plant substance (0.1%) was enough to prevent lipid oxidation. Inhibition of oxidation processes at the lowest dose was associated with the use of ethanol extract with a higher concentration of active phytochemicals compared with the use of the same amount of water extract. In this regard, previous studies reported that the use of solvents with different polarity in the extraction procedure may have a significant effect on phytochemicals content and antioxidant activity of the obtained extract [31, 32].

The effect of beef marinating with *Ilex meservae* on the results of instrumental texture analysis is shown in Table 1. The shear force values ranged from 18.8 to 21.4 N. No significant differences ($P > 0.05$) in shear force were found between the samples of dry-aged beef. However, there was a numerical difference between the shear force values of beef samples marinated with 1% and 2% concentration of IML and samples marinated without IML. Values of shear force obtained in this study are in agreement with those presented by O'Sullivan, Cruz-Romero, and Kerry [33], who obtained values of 22.6 N at 21 d. In the own study, the lack of

Table 1: Effect of *Ilex meserveae* on TBARS and shear force value of dry-aged beef.

	Ilex x meserveae concentration				
	0.0%	0.5%	1.0%	2.0%	*P* value
TBARS (mg MDA/kg)	$1.41^{bc} \pm 0.08$	$1.77^{c} \pm 0.25$	$1.30^{b} \pm 0.05$	$0.68^{a} \pm 0.02$	0.001
Shear force (N)	$19.1^{a} \pm 1.62$	$21.4^{a} \pm 1.93$	$18.6^{a} \pm 0.94$	$18.8^{a} \pm 0.81$	0.948

a-c mean values in row with different superscripts are significantly different at $P < 0.05$.

Table 2: Effect of *Ilex meserveae* on color parameters of dry-aged beef.

	Ilex x meserveae concentration				
	0.0%	0.5%	1.0%	2.0%	*P* value
L*	25.7 ± 0.84	24.8 ± 0.49	25.7 ± 0.55	25.2 ± 0.53	0.700
a*	12.4 ± 0.36	11.4 ± 0.6	11.5 ± 0.55	12.6 ± 0.34	0.186
b*	1.50 ± 0.28	1.41 ± 0.23	1.94 ± 0.21	1.52 ± 0.17	0.360
Chroma	12.5 ± 0.39	11.5 ± 0.62	11.7 ± 0.54	12.7 ± 0.36	0.225
Hue	6.65 ± 1.11	6.71 ± 0.87	9.75 ± 1.31	6.68 ± 0.64	0.092

Table 3: Effect of *Ilex meserveae* on fatty acid profile (mg/100 g muscle) of dry-aged beef.

	Ilex x meserveae concentration				
	0.0%	0.5%	1.0%	2.0%	*P* value
C14:0	23.1 ± 0.11	22.8 ± 0.16	24.6 ± 0.18	7.95 ± 0.27	0.788
C14:1	$4.57^{a} \pm 0.01$	$6.05^{a} \pm 0.00$	$8.26^{b} \pm 0.05$	$7.95^{b} \pm 0.06$	0.001
C16:0	382.0 ± 0.22	388.1 ± 0.95	402.5 ± 1.06	395.1 ± 0.94	0.805
C16:1	47.9 ± 0.33	47.9 ± 0.43	54.3 ± 0.51	53.0 ± 0.71	0.940
C18:0	298.5 ± 1.09	300.5 ± 1.02	294.5 ± 0.73	277.3 ± 0.96	0.778
C18:1n9	550.6 ± 1.12	567.6 ± 1.24	570.4 ± 1.09	548.8 ± 1.35	0.968
C18:2	247.5 ± 0.55	259.3 ± 1.01	234.3 ± 1.19	255.8 ± 1.29	0.792
C18:3	53.9 ± 0.45	61.8 ± 0.59	49.1 ± 0.68	57.4 ± 0.72	0.876
C20:3	25.8 ± 0.12	23.8 ± 0.11	25.8 ± 0.41	26.0 ± 0.21	0.985
C20:4	128.0 ± 0.77	128.5 ± 0.7	116.2 ± 1.04	121.6 ± 0.87	0.919
C20:5	40.0 ± 0.48	47.2 ± 0.52	34.4 ± 0.84	43.3 ± 0.66	0.855
∑SFA	703.6	711.5	721.7	693.2	
∑MUFA	603.1	621.5	632.9	609.8	
∑PUFA	495.2	520.7	459.8	504.2	

a-b mean values in row with different superscripts are significantly different at $P < 0.05$; ∑SFA: sum of saturated fatty acids; ∑MUFA: sum of monounsaturated fatty acids; ∑PUFA: sum of polyunsaturated fatty acids.

differences between shear forces of treatments was confirmed by results of texture sensory perception. The degree of hardness acceptation evaluated by assessors was the same for all meat samples. Similar results were obtained for sensory evaluation of color; that is, the evaluators did not indicate the differences between the assessed beef samples as reflected in the results of the instrumental color measurement (Table 2).

Color is the main feature that determines the purchase of meat; pale or dark meat is unacceptable to most consumers. The color of raw meat is mainly determined by the content of pigments (myoglobin and hemoglobin), physical structure, and light scattering properties of the muscle [34, 35]. One of the challenges for the application of plant origin additives in meat products is finding the right dose to get the desired effect without losing meat's organoleptic traits (e.g., taste, color, and odors) [36]. In the own studies, L*, a*, and b* parameters, chroma, and hue were estimated. Addition of *Ilex meserveae* dried leaves to beef marinade did not affect ($P > 0.05$) the CIE L*a*b* parameters of color and at the same time did not deteriorate color perception in the sensory evaluation. A similar effect was reported by Ferreira, Sampaio, Torres, and Markowicz-Bastos [11], who introduced ethanolic extract of yerba mate (*Ilex paraguariensis St. Hilaire*) to beef burgers. Even high doses of plant extract did not affect the evaluation of the hamburger color.

Regarding the fatty acid profile of dry-aged beef (Table 3), no significant ($P < 0.05$) effect of used marinade was found, with the exception of myristoleic acid (C14:1) content. Composition of saturated fatty acids (SFAs), monounsaturated fatty acid (MUFAs), and polyunsaturated fatty acids (PUFAs) was similar in all treatments. Moreover, Ferreira, Sampaio, Torres, and Markowicz-Bastos [11] indicated that yerba mate (*Ilex paraguariensis St. Hilaire*) ethanolic extract had no effect on saturated fatty acid and unsaturated fatty acids the day after production of beef burgers or even after 90 days of storage. Results of own study might be related to the period of aging process. According to the results presented by Utama et al. [37], the changes in fatty acid composition only take place after 40 days of beef aging; that is, extension of the aging period decreased the

proportion of MUFA and n3 PUFA. Lack of changes in fatty acid profile observed in the own experiment could be also explained by the use of good manufacture practices during preparation of beef cuts and the low temperature during storage [11].

4. Conclusions

Aqueous extracts of *Ilex meserveae* dried leaves were effective as a marinade component in prevention of lipid oxidation during dry-aging of beef. This effect was observed when 1% concentration of plant material was added to beef marinade. There were no significant differences in values of instrumental measurement of color and shear force. Furthermore, *Ilex meserveae* marinade did not result in deterioration of sensory acceptability, thus suggesting that *Ilex meserveae* should be considered as a novel natural ingredient to prevent rancidity of the final products. However, the effectiveness of other types of extracts (e.g., ethanolic) needs to be determined in further studies.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Anna Marietta Salejda conceptualized the study, performed data curation, was responsible for funding acquisition, developed methodology, performed project administration, supervised the study, and wrote the original draft. Łukasz Bobak carried out the formal analysis. Anna Marietta Salejda, Aleksandra Szmaja, Łukasz Bobak, Anna Zwyrzykowska-Wodzińska, and Anna Fudali carried out the investigation. Przemysław Bąbelewski and Maciej Bienkiewicz provided the resources. Grażyna Krasnowska was responsible for writing, review, and editing.

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