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Impact of Ultrasound-assisted Cooking and Endpoint Core Temperature on Physicochemical and Microbiological Properties, and Oxidative Stability of Beef

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ABSTRACT

This research aimed to investigate the impacts of different cooking methods (B; Boiling, US; Ultrasound-assisted slow boiling, UF; Ultrasound-assisted fast boiling) and endpoint core temperatures (ECT; 68°C, 74°C, and 80°C) on the oxidative stability, physicochemical, and microbiological properties of beef during refrigerated storage. The results demonstrated that UF application resulted in the lowest cooking loss (CL) at 74°C ECT. The US application caused a lower water activity (a_w) compared to B. The lowest oxidation-reduction potential (ORP) levels were determined in UF, whereas the US had the highest ORP levels. Ultrasound-assisted cooking did not affect pH, yeast-mold and total mesophilic aerobic bacteria (TMAB) counts. On the other hand, UF and US caused an increase in total coliform counts compared to B. According to the results of lipid hydroperoxide (LPO) and thiobarbituric acid reactive substances (TBARS), UF application was more effective in preventing lipid oxidation compared to US and B. pH, CL, ORP, hue angle (h_{ab}) and b^* values increased as the ECT increased, whereas a_w, a^*, chroma (C^*_{ab}) and browning index (*BI;* inner) values decreased. In addition, beef pieces cooked at 74°C or 80°C ECT had lower L^* values, TMAB, and total coliform counts, and higher TBARS and LPO values than those cooked at 68°C ECT. 74°C was more effective in controlling microbiological changes, whereas 68°C was a better ECT for maintaining oxidative stability. In conclusion, UF has the potential to be an effective processing technology for improving oxidative stability and physicochemical properties of beef.

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Introduction

Meat is a vital component of the human diet, providing numerous bioactive compounds such as essential amino and fatty acids (Şimşek & Kılıç, 2016). The physicochemical and nutritional quality of meat is influenced by various processing methods such as drying, packaging, smoking, freezing and cooking (Gómez et al., 2020). The cooking of meat is crucial for ensuring both deliciousness and food safety (Domínguez et al., 2015). Cooking is one of the most widely used methods to destroy foodborne pathogens, ensure microbial safety, enhance the taste and flavor of meat, and improve digestibility (Broncano et al., 2009). However, improper cooking techniques or conditions can lead to detrimental impacts such as the formation of chemical browning reactions, diminished nutritional and sensorial quality of muscle foods, aromatic polycyclic hydrocarbons (pyrene, chrysene etc.) generation and accelerated lipid oxidation (Rasinska et al., 2019; Gómez et al., 2020). Many studies on cooking techniques with high final cooking temperatures and prolonged cooking duration demonstrated increased quality losses and lipid oxidation (Serrano et al., 2007; Domínguez et al., 2015). Among different cooking techniques, the three main factors that create differences in meat quality are the applied surface temperature, core temperature, and heat transfer method (Bejerholm & Aaslyng, 2004). Whereas surface temperature plays a crucial role in determining the aroma, taste, and color of the meat, the core temperature is vital for microbial destruction and affects the textural properties of meat. The heat transfer method influences the textural and physicochemical properties and its nutritional value, which plays an important role in consumer preference. Various conventional cooking techniques such as boiling, oven-roasting and grilling are commonly used to cook meat and meat products (Campo et al., 2013; Kerth et al., 2022).

Boiling is a conventional cooking technique used to tenderize meat and enhance its flavor. Moreover, boiling can effectively inhibit pathogenic microorganisms. However, cooking meat using the boiling technique may cause negative effects such as fat and nutrient losses, discoloration, and toughening due to overboiling. For this reason, studies on innovative food processing techniques such as microwave or ultrasound-assisted cooking have gained importance to minimize the adverse changes caused by boiling on the quality characteristics of meat (Suleman et al., 2020).

Ultrasound is an innovative and environmentally friendly technique applied in meat processing for various purposes, such as cooking, extraction, emulsification, homogenization, tenderization, and preservation (Ashar et al., 2022). Ultrasound has the capability to reduce processing time and ensure food safety without compromising the quality of meat products (Zou et al., 2018). The commonly used ultrasound frequency, which can impact the physicochemical, biological and structural characteristics of meat, is 16-100 kHz with an intensity range of 10-1000 Wcm^{-2} (Ashar et al., 2022). Ultrasound are typically applied directly to the food or the cooking medium (such as water or oil) using specialized equipment. When ultrasound is implemented in a liquid medium, it generates cavitation bubbles, which are tiny gas-filled voids that form and collapse rapidly under the influence of ultrasound waves. This cavitation phenomenon produces intense localized heating, pressure changes, and microstreaming within the liquid medium. These phenomena are the causes of the alterations in properties, microstructures and chemical reactions of meat (Zhao et al., 2024). In ultrasound-assisted cooking, ultrasound waves can accelerate heat transfer within the meat, leading to shorter cooking times compared to conventional cooking techniques. Rapid and uniform heating with ultrasound-assisted cooking helps ensure that the meat reaches the required temperature for microbial inactivation, reducing the risk of foodborne illnesses and extending the shelf life of cooked meat products. Furthermore, ultrasound waves can tenderize meat by disrupting muscle fibers and breaking down connective tissue, resulting in more tender and juicy meats (Firouz et al., 2022). Recently, ultrasound technology has been effectively applied in numerous studies aimed at enhancing the quality of various meat products such as spiced beef (Zou et al., 2018), fermented sausages (de Lima Alves et al., 2020), mortadella (Cichoski et al., 2021) and meatballs (Zhao et al., 2024). However, there is no study comparing conventional boiling and ultrasound-assisted fast and slow boiling on the quality characteristics of cooked beef. Therefore, this research aimed to evaluate the effects of boiling, ultrasound-assisted fast and slow boiling, and different endpoint core temperatures on the physicochemical and microbiological properties, and the oxidative stability of beef.

Materials and Methods

Materials

The fresh *M. semimembranosus* muscles (24 h post-mortem) obtained from 1.5-2 years old cattle carcasses were used as the meat material. To create controlled conditions among replications, the post-mortem age of beef did not exceed 5 days after receipt. The pH of all muscles was measured using a portable digital pH meter (HI 9024, Hanna Instruments, Germany). The pH range of all muscles was between 5.4 and 5.6.

Sample preparation

Before experiments, the cattle muscles were trimmed to remove all visible fat and connective tissue. They were then cut into pieces (20 mm × 40 mm × 40 mm) in the direction of muscle fibers. The sliced beef pieces were randomly separated into nine groups (Table 1) for the different cooking methods (Boiling; B, ultrasound-assisted slow boiling; US, and ultrasound-assisted fast boiling; UF) and endpoint core temperatures (68°C, 74°C, and 80°C).

Table 1. Experimental groups and treatment conditions.

Cooking method (CM)	ECT
Conventional boiling; B	68°C 74°C 80°C
Ultrasound-assisted slow boiling; US	68°C 74°C 80°C
Ultrasound-assisted fast boiling; UF	68°C 74°C 80°C

ECT: Endpoint core temperature

Cooking procedures

A total of thirty beef pieces per replication in each treatment group were randomly divided into five equal portions (approximately 250 g each) for each storage day. Each portion (6 beef pieces per treatment) were put into polyamide/polyethylene (PA/PE; 80 µm) bags prior to cooking. The cooking process was carried out without vacuum application in PA/PE bags. For boiling treatment, PA/PE bags containing raw beef pieces were placed in a water bath (Nüvebath NB20, Türkiye) and cooked at different endpoint core temperatures (68°C, 74°C, and 80°C). The initial water bath temperature was 60°C. Water bath temperature was adjusted to 80°C after placing the PA/PE bags. The endpoint core temperature was monitored using a thermocouple (TK100S, Kimo Instruments, France) inserted into the geometric center of the beef pieces. An ultrasonic water bath (Model Sonorex RK103 H, Bandelin Ultrasonic Electronics, Germany) with a fixed 35 kHz frequency and 140 W intensity was used for the ultrasound treatment (Firouz et al., 2022; Nehring et al., 2023). The ultrasonic water bath was initially set to the temperatures of 60°C (US) for slow boiling and 80°C (UF) for fast boiling. For slow boiling, the ultrasonic water bath temperature was adjusted to 80°C after placing the PA/PE bags. The raw beef pieces were cooked with ultrasound-assisted until they reached the target endpoint core temperatures (68°C, 74°C, and 80°C). After that, the beef pieces were naturally cooled at sterile ambient conditions. Cooled beef pieces were vacuum-packaged using a RAMON VP280 vacuum machine (Barcelona, Spain) at a vacuum pressure of -0.85 bar in PA/PE bags (O_2 permeability rate: 10 cm^3/m^2/24 h/1 atm) and stored in a refrigerator (4°C) for 40 days. All analyses were performed two times for each replication. Water activity (a_w), color, ORP, pH, LPO and TBARS measurements were conducted in certain intervals (0, 10, 20, 30, and 40 d) during storage. Microbiological analyses were implemented on days 0, 20, and 40 throughout the storage. Furthermore, cooking loss values were calculated once for every replication on production day.

Determination of cooking loss, pH, a_w, color and ORP

The weights of beef samples were recorded before and after cooking process. The cooked beef samples were cooled at ambient temperature before being weighed. The cooking loss (CL, equation 1) was calculated as follows (López-Vargas et al., 2014);

$$CL\ (\%) = \frac{(WUB)-(WCB)}{(WUB)} \times 100 \quad (1)$$

WUB: weight of uncooked beef
WCB: weight of cooked beef

The pH measurement of beef samples was implemented as mentioned by Kılıç et al. (2016). A spear electrode connected to a portable pH meter (HI 9024, Hanna Instruments, Germany) was used to measure the pH. The a_w values of the cooked beef samples were measured at 25°C using an a_w device (Novasina LabSwift-aw, Lachen, Switzerland) according to the procedure explained by Tenderis et al. (2021).

Color measurements of cooked beef samples were carried out using a Minolta CR-200 colorimeter (Minolta Corp., Ramsey, NJ, U.S.A.) following the method described by Şimşek and Kılıç (2020). The cooked beef pieces were removed after opening the vacuum-sealed package and allowed to bloom for at least 30 min at ambient temperature before color measurement. Before measuring color values, the colorimeter was calibrated with a white reference plate (D65, L*=97.79, a*=−0.11, b*=2.69). CIE L*, a*, and b* values were obtained by measuring random locations on both the inner and outer surfaces of cooked beef samples at certain intervals throughout the storage period. Chroma (C^*_{ab}; equation 2), hue angle (h_{ab}; equation 3), and browning index (BI; equation 4) values were computed using CIE L^*, a^* and b^* values based on the following formulas (Uysal et al., 2022);

$$C^*_{ab} = (a^2 + b^2)^{1/2} \quad (2)$$

$$h_{ab} = \tan^{-1}\left(\frac{b*}{a*}\right) x\ \frac{180^\circ}{\pi} \text{ and} \quad (3)$$

$$BI = \frac{[100 \text{ x } (X-0.31)]}{0.17};\ X = \frac{(a+1.75xL)}{(5.645xL+a-3.012xb)} \quad (4)$$

A pH meter (WTW pH 3110, Germany) equipped with a redox electrode was used to measure ORP values in cooked beef samples (Tenderis et al., 2021). An ORP electrode was firmly placed into the center of the cooked beef. The smallest possible hole was made by a cutter before placing the electrode to minimize the effect of air. ORP values (mV) were recorded precisely 2 min after the electrode insertion into a sample.

Determination of TBARS and LPO values

TBARS of cooked beef samples was evaluated using the extraction procedure as outlined by Kılıç et al. (2014). Beef samples (2 g) were homogenized (15 s, 13500 rpm) in 12 mL of TCA extraction solution (0.1% propylgallate (PG), 0.1% ethylenediaminetetraacetic acid, disodium salt (EDTA), and 7.5% trichloroacetic acid (TCA)). The homogenates were filtered using filter paper (Whatman no:1). The filtrate (1.0 mL) was mixed with thiobarbituric acid (1.0 mL, 0.02 M) and incubated at 100°C for 40 min. The mixture was cooled (25°C) and centrifuged (10 min, 4,200 rpm, 4°C). The absorbance of the supernatant was determined at 532 nm using a spectrophotometer (T80 UV/VIS, PG Instruments, England). The TBARS results were expressed as µmol MDA/kg of meat. A calibration curve was prepared from 1,1,3,3-tetraethoxypropane (TEP).

The procedure described by Kılıç et al. (2014) was performed to determine the LPO levels. According to this procedure, cooked beef sample (0.5 g) was mixed with 5 mL of chloroform/methanol (1:1) and homogenized for 30 s. NaCl (3.08 mL; 0.5%) solution was added to this mixture and vortexed for 30 s. Then, this mixture was centrifuged at 2000 rpm for 10 min to separate the mixture into two phases. The lower phase (2 mL) was mixed with 1.33 mL of cold chloroform/methanol (1:1), and then vortexed. Ammonium thiocyanate (25 µL; 4.38 M) and iron (II) chloride (25 µL; 18 mM) were added to the mixture to determine lipid hydroperoxides. The mixture was kept at ambient temperature for 20 min, and then the absorbance was measured at 500 nm. A calibration curve was formed by using cumene hydroperoxide. LPO results were expressed as µmol LPO/kg of meat.

Microbiological analyses

The microbiological analyses were implemented using the procedures stated by Uysal et al. (2022). Plate Count Agar (PCA, Merck, Germany), Potato Dextrose Agar (PDA, Merck, Germany) and Eosin Methylene-blue Lactose Sucrose Agar (EMB, Merck, Germany) were used as selective agar mediums for total mesophilic aerobic bacteria (TMAB), yeast-mold and total coliform counts, respectively. The plates for TMAB, total coliforms and yeast-mold were counted following an incubation at 30°C for 24-48 h, 37°C for 24-48 h and 25°C for 72-120 h, respectively. The microbiological results were expressed as log cfu/g of meat.

Statistical analysis

The experiments were designed as a completely randomized block design as two replications. All analyses were repeated in duplicate. The statistical model to evaluate the collected data of ORP, pH, a_w, color, TBARS, and LPO was implemented using a 3 × 3 × 5 factorial design, which included three factors: cooking method, endpoint core temperature, and storage time. To evaluate the data from microbiological analyses, 3 × 3 × 3 factorial design was applied. For cooking loss determination, the data were analyzed with a 3×3 factorial design. All collected data were analyzed using analysis of variance (ANOVA) with the generalized linear mixed model (GLMM) of Minitab 19.2.0 (Minitab Statistical Software, USA). The main effects used in the fixed model were cooking methods (B, US and UF), endpoint core temperatures (68°C, 74°C and 80°C) and storage times (0, 10, 20, 30 and 40 days). The replicates were assigned as a random effect. All main effects and responsible interactions were evaluated. Non-significant interactions were not included in the model. For significant interactions ($p<0.05$), distinctions between means were assessed by Tukey's Multiple Comparison Test. The results are presented as mean and SEM (standard error of the mean).

Results and Discussions

Cooking Loss

The cooking method (CM), endpoint core temperature (ECT), and their interaction (CMxECT) had an impact ($p<0.05$) on the cooking loss (Figure 1). Cooking loss is primarily influenced by moisture evaporation and protein denaturation during thermal processing. Studies have consistently shown that higher cooking temperatures cause greater cooking loss due to increased protein shrinkage and moisture expulsion (Tornberg, 2005; Pang et al., 2021). Moreover, Klinhom et al. (2017) reported that cooking loss depends on mass transfer during thermal processing. Therefore, different cooking methods result in varying losses due to differences in heat transfer rates and exposure times. In the present research, CM x ECT interaction demonstrated that cooking loss was higher in UF compared with both US and B at 68°C ECT, whereas the methods of US or B resulted in a higher cooking loss compared to UF at 74°C ECT ($p<0.05$). At 68°C ECT, UF exhibited a greater cooking loss than B or US, possibly due to the higher initial temperature applied for fast cooking. Similarly, Aaslyng et al. (2003) observed that at 60°C ECT, the fast-heating rate (190°C oven temperature) resulted in higher cooking loss compared to a slower heating rate (90°C oven temperature). On the other hand, at 74°C ECT, UF displayed a lower cooking loss ($p<0.05$). This situation may be due to the shorter cooking time associated with ultrasound-assisted fast cooking. Another possible reason is that the cavitation effect produced by ultrasound can change the structure of muscle proteins by disrupting the myofibrils. This process can create small spaces with uniform and ordered network structures among myofibrillar proteins, allowing them to retain more moisture (Aaslyng et al., 2003; Zhao et al., 2024). Likewise, Zhao et al. (2024) demonstrated that ultrasound-assisted cooking improved the cooking yield of pork meatballs. In the present research, at 80°C ECT, there was no difference in cooking loss among cooking methods. Moreover, the cooking loss was significantly increased ($p<0.05$) with the increasing ECT in all cooking methods. The cooking losses were comparable among methods at 80°C ECT, likely because the protein denaturation and structural changes plateau at high temperatures, limiting further differences among methods. Aaslyng et al. (2003) observed that the differences in cooking loss became smaller as the endpoint internal temperature increased, and at 80°C, the cooking procedure no longer had an effect. These results align with Tornberg (2005), who noted that protein denaturation begins at 40°C and accelerates with increasing temperature, peaking around 90°C. Similarly, numerous researchers have reported an increase in cooking loss of various meat types with increased endpoint core temperatures (García-Segovia et al., 2007; Smith et al., 2011; Cauble et al., 2021). Furthermore, Adhikari et al. (2004) noted that cooking losses increased linearly with higher time and temperature.

pH, a_w, ORP and color

Study results (Table 2) indicated that pH values were influenced ($p<0.01$) by ECT and storage time (ST). In contrast, CM had no significant impact on pH values of beef. Likewise, previous studies conducted on pork meatballs and broiler meat revealed that there were no significant differences in pH values among ultrasound-assisted cooking and water-bath cooking treatments (Ashar et al., 2022; Zhao et al., 2024). According to the present study results, pH values significantly increased with the increasing ECT ($p<0.01$). Similarly, Huang et al. (2011) observed that pH values in pork consistently increased as internal temperatures increased. Researchers explained that pH changes in meat during heating were likely due to the dynamic balance of acid-base groups at the surface of sarcoplasmic proteins, which degrade as the temperature increases. In addition, Khan et al. (2019) revealed that cooking causes an increase in pH due to protein degradation, resulting from the divergence of bonds involving hydroxyl, sulfhydryl, and imidazole groups. In the present study, pH values of cooked meats gradually decreased during storage ($p<0.01$). Likewise, Yingyuad et al. (2006) observed a gradual decrease in pH values of vacuum-packed grilled pork during storage. The primary cause of pH reduction in vacuum-packed meat products is the growth of lactic acid bacteria, which leads to the production of lactic acid (Fernández-López et al., 2008).

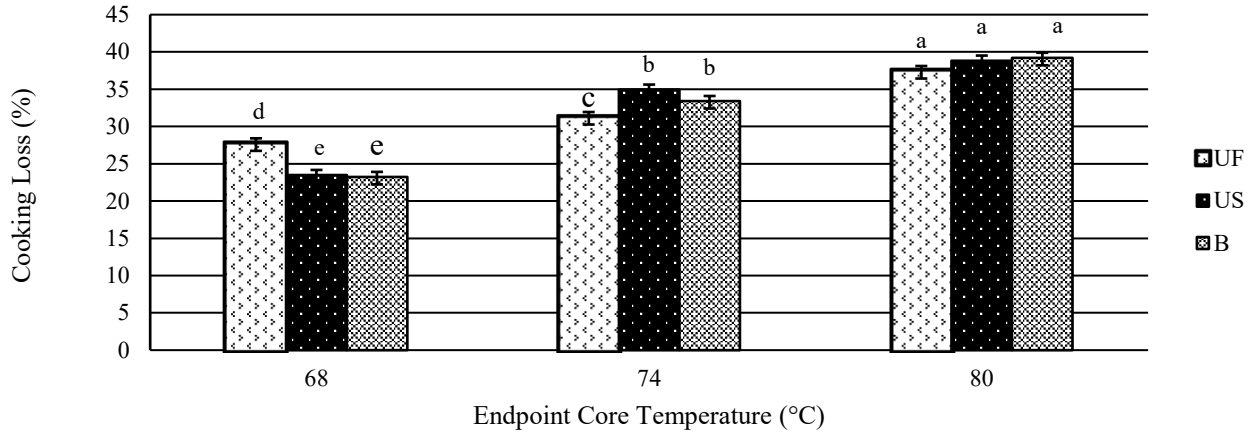

Figure 1. Influences of cooking methods and endpoint core temperatures on cooking loss values of beef.
The results were expressed as mean values with standard error bars. [a-c]Means with different superscript letters in the figure indicate statistically significant differences at ($p<0.05$).

Table 2. pH, a_w and ORP values of cooked beef samples according to cooking methods, endpoint core temperatures and storage time.

	pH	ORP	a_w
Cooking Method (CM, n=60)			
B	5.58[a]	-66.88[b]	0.940[a]
US	5.59[a]	-62.63[a]	0.937[b]
UF	5.58[a]	-77.68[c]	0.938[ab]
SEM	0.02	1.62	0.001
Significance	NS	**	*
Endpoint Core Temperature (°C; ECT, n=60)			
68	5.52[c]	-87.89[c]	0.940[a]
74	5.59[b]	-69.19[b]	0.939[ab]
80	5.65[a]	-50.11[a]	0.937[b]
SEM	0.02	1.62	0.001
Significance	**	**	*
Storage Time (Day; ST, n=36)			
0	5.66[a]	-106.25[e]	0.942[a]
10	5.63[b]	-77.29[d]	0.940[ab]
20	5.61[b]	-62.98[c]	0.936[c]
30	5.52[c]	-56.45[b]	0.938[bc]
40	5.51[c]	-42.35[a]	0.936[c]
SEM	0.02	1.67	0.001
Significance	**	**	**
CM×ECT	**	**	*
CM×ST	NS	**	*
ECT×ST	**	**	NS
CM×ECT×ST	NS	**	NS

B: Boiling, US: Ultrasound-assisted slow boiling, UF: Ultrasound-assisted fast boiling, SEM: Standard error of the mean, *NS:* Not significant, [a-e]Means with different superscript letters in the same column indicate statistically significant differences at (**p<0.01) and (*p<0.05).

The CM, ECT and ST significantly affected the ORP values of cooked meats (p<0.01). ORP measurements (Table 2) revealed that the lowest ORP levels were determined in UF, whereas US had the highest ORP levels (p<0.01). In addition, increasing ECT caused an increase in ORP levels of cooked beef pieces (p<0.01). Regardless of CM and ECT, ORP levels of cooked beef pieces gradually increased during storage (p<0.01). ORP is a key indicator for tracking chemical reactions and biological processes that lead to oxidation. ORP values indicate the relative tendency of a system to either gain or lose electrons (Latoch & Stasiak, 2015). Ignatova et al. (2010) reported that ORP is affected by environmental factors such as dissolved oxygen, temperature, and pH. Claus and Jeong (2017) indicated that lower ORP values are associated with higher pH. On the other hand, researchers noted that the endpoint internal cooking temperature did not affect ORP values in cooked ground turkey breasts (Claus & Jeong, 2017). Zhang et al. (2022) reported that the ORP values increased steadily in heat-processed beef with increasing storage time. Moreover, Latoch and Stasiak (2015) noted that alterations in ORP levels could result from increased lipid oxidation during storage.

In the present study, a_w values (Table 2) were influenced by CM, ECT (p<0.05) and ST (p<0.01). Comparisons of CM revealed that US had a lower a_w value compared to B (p<0.05). There were no a_w value differences in B versus UF; and US versus UF. Bao et al. (2022) stated that the use of ultrasound resulted in reduced a_w values. Researchers have attributed this phenomenon to ultrasound generating air turbulence at the air-product interface, which enhances moisture removal from the surface. In addition, Leães et al. (2020) observed that long-term ultrasound application (20 min) was more effective in reducing a_w compared to short-term application (10 min). Researchers generally did not find a significant difference in a_w between the short-term ultrasound application and the untreated group. Regarding ECT application, beef cooked at 80°C ECT presented a lower a_w value than that cooked at 68°C ECT (p<0.05). Furthermore, although a_w values gradually decreased during the first 20 days of storage (p<0.01), a_w values were quite stable during the rest of the storage period. Pandey et al. (2014) noted that shami kebab samples processed at high temperatures exhibited lower a_w, which was attributed to the effective removal of moisture.

Table 3 and Table 4 illustrated the effects of different cooking methods and endpoint core temperatures on the color properties of cooked beef. Color measurements demonstrated that L^*, b^*, h_{ab} and *BI* values (p<0.05) obtained from cooked beef's inner surface and a^*, b^*, C^*_{ab}, h_{ab} and *BI* values (p<0.01) obtained from cooked beef's outer surface were affected by CM. Moreover, all color parameters obtained from both the inner and outer surfaces of cooked beef pieces were affected by ECT and ST (p<0.05). On the other hand, ECT did not affect *BI* values of cooked beef's outer surface.

As far as cooking methods are considered, color measurements revealed that the lower L^* value and the higher *BI* and b^* values obtained from cooked beef's inner surface were determined in UF compared to B (p<0.05). Ultrasonic cavitation causes a decrease in L^* value, and an increase in *BI* value by stimulating the formation of free radicals and leading to enzymatic browning (Zhao et al., 2024). In addition, the higher a^*, b^*, C^*_{ab} and *BI* values obtained from cooked beef's outer surface were determined in UF compared to B (p<0.01).

Table 3. Colour values (inner surface) of cooked beef samples according to cooking methods, endpoint core temperatures and storage time.

	Inner Surface					
	*L**	*a**	*b**	C^*_{ab}	h_{ab}	*BI*
Cooking Method (CM, n=60)						
B	57.98^{a}	15.18^{a}	2.87^{c}	15.59^{a}	13.03^{b}	23.19^{b}
US	57.31^{ab}	15.02^{a}	3.17^{b}	15.52^{a}	14.33^{a}	23.84^{ab}
UF	57.00^{b}	14.86^{a}	3.47^{a}	15.32^{a}	13.95^{ab}	24.48^{a}
SEM	0.39	0.22	0.07	0.23	0.34	0.34
Significance	*	NS	**	NS	*	*
Endpoint Core Temperature (°C; ECT, n=60)						
68	58.15^{a}	20.44^{a}	1.83^{c}	20.54^{a}	5.23^{c}	27.28^{a}
74	57.05^{b}	13.99^{b}	3.40^{b}	14.42^{b}	13.85^{b}	23.34^{b}
80	57.09^{b}	10.62^{c}	4.27^{a}	11.47^{c}	22.23^{a}	20.90^{c}
SEM	0.39	0.22	0.07	0.23	0.34	0.34
Significance	*	**	**	**	**	**
Storage Time (Day; ST, n=36)						
0	55.51^{c}	16.85^{a}	3.89^{a}	17.46^{a}	15.02^{a}	28.24^{a}
10	55.54^{c}	15.81^{b}	3.44^{b}	16.32^{b}	14.01^{ab}	26.07^{b}
20	57.31^{b}	15.06^{b}	3.07^{c}	15.49^{bc}	13.28^{b}	23.64^{c}
30	58.87^{a}	14.24^{c}	2.86^{cd}	14.63^{c}	13.07^{b}	21.69^{d}
40	59.92^{a}	13.12^{d}	2.58^{d}	13.49^{d}	13.46^{ab}	19.55^{e}
SEM	0.46	0.28	0.08	0.29	0.44	0.43
Significance	**	**	**	**	*	**
CM×ECT	*	**	**	**	**	*
CM×ST	NS	*	*	*	NS	NS
ECT×ST	NS	NS	*	NS	NS	NS
CM×ECT×ST	NS	NS	**	NS	*	NS

B: Boiling, US: Ultrasound-assisted slow boiling, UF: Ultrasound-assisted fast boiling, C^*_{ab}: Chroma, h_{ab}: hue angle, *BI*: browning index, SEM: Standard error of the mean, *NS:* Not significant, $^{a-e}$Means with different superscript letters in the same column indicate statistically significant differences at (**p<0.01) and (*p<0.05).

Table 4. Colour values (outer surface) of cooked beef samples according to cooking methods, endpoint core temperatures and storage time.

	Outer Surface					
	*L**	*a**	*b**	C^*_{ab}	h_{ab}	*BI*
Cooking Method (CM, n=60)						
B	44.14^{a}	7.76^{b}	5.89^{c}	9.88^{b}	39.09^{b}	27.11^{b}
US	43.72^{a}	7.29^{c}	6.36^{b}	9.81^{b}	42.00^{a}	28.27^{b}
UF	44.42^{a}	8.74^{a}	7.03^{a}	11.34^{a}	39.20^{b}	31.98^{a}
SEM	0.56	0.18	0.11	0.15	0.87	0.64
Significance	NS	**	**	**	**	**
Endpoint Core Temperature (°C; ECT, n=60)						
68	45.90^{a}	9.99^{a}	5.84^{b}	11.63^{a}	30.65^{c}	29.40^{a}
74	43.46^{b}	7.61^{b}	6.66^{a}	10.18^{b}	41.44^{b}	29.86^{a}
80	42.92^{b}	6.18^{c}	6.78^{a}	9.22^{c}	48.19^{a}	28.10^{a}
SEM	0.56	0.18	0.11	0.15	0.87	0.64
Significance	**	**	**	**	**	NS
Storage Time (Day; ST, n=36)						
0	42.85^{b}	9.90^{a}	7.25^{ab}	12.41^{a}	36.46^{c}	35.79^{a}
10	41.18^{b}	8.51^{b}	7.42^{a}	11.41^{b}	41.76^{ab}	34.86^{a}
20	42.46^{b}	7.81^{bc}	6.83^{b}	10.50^{c}	42.21^{a}	30.82^{b}
30	46.75^{a}	7.15^{c}	5.69^{c}	9.27^{d}	39.93^{b}	23.73^{c}
40	47.21^{a}	6.27^{d}	4.95^{d}	8.13^{e}	40.12^{b}	20.39^{d}
SEM	0.63	0.21	0.15	0.20	0.98	0.83
Significance	**	**	**	**	**	**
CM×ECT	*	NS	**	**	*	**
CM×ST	*	**	*	*	**	NS
ECT×ST	NS	*	NS	NS	**	NS
CM×ECT×ST	NS	**	*	**	NS	**

B: Boiling, US: Ultrasound-assisted slow boiling, UF: Ultrasound-assisted fast boiling, C^*_{ab}: Chroma, h_{ab}: hue angle, *BI*: browning index, SEM: Standard error of the mean, *NS:* Not significant, $^{a-e}$Means with different superscript letters in the same column indicate statistically significant differences at (**p<0.01) and (*p<0.05).

Table 5. LPO (µmol LPO/kg) and TBARS (µmol MDA/kg) values of cooked beef samples according to cooking methods, endpoint core temperatures and storage time.

	LPO	TBARS
Cooking Method (CM, n=60)		
B	55.81[a]	4.31[a]
US	53.22[b]	4.11[b]
UF	48.99[c]	3.65[c]
SEM	0.76	0.05
Significance	**	**
Endpoint Core Temperature (°C; ECT, n=60)		
68	48.90[b]	3.80[b]
74	55.02[a]	4.16[a]
80	54.11[a]	4.12[a]
SEM	0.76	0.05
Significance	**	**
Storage Time (Day; ST, n=36)		
0	27.46[e]	2.03[e]
10	41.23[d]	3.08[d]
20	52.25[c]	3.86[c]
30	64.79[b]	4.95[b]
40	77.65[a]	6.22[a]
SEM	0.83	0.07
Significance	**	**
CMxECT	NS	NS
CMxST	**	**
ECTxST	*	NS
CMxECTxST	NS	NS

B: Boiling, US: Ultrasound-assisted slow boiling, UF: Ultrasound-assisted fast boiling, LPO: Lipid hydroperoxide, TBARS: Thiobarbituric acid reactive substances, SEM: Standard error of the mean, *NS:* Not significant, [a-e]Means with different superscript letters in the same column indicate statistically significant differences at (**p<0.01) and (*p<0.05).

Wang et al. (2019) reported an increase in a^* and b^* values in ultrasound-assisted fried meatballs. Furthermore, US had higher h_{ab} values obtained from both the inner and outer surfaces of cooked beef pieces compared to those cooked with the B method (p<0.05). There were no differences among cooking methods in terms of L^* values of the outer surface and a^* and C^*_{ab} values of the inner surface.

Comparisons of color results concerning different ECT applications indicated that a^* and C^*_{ab} values obtained from both the inner and outer surfaces and *BI* values of the inner surface significantly decreased with the increasing ECT (p<0.01). Similarly, previous studies revealed that a^* values of meat products significantly decreased as endpoint core temperature increased (Lien et al., 2002; Sen et al., 2014; Cauble et al., 2021). The reduction in the redness (a^*) values of cooked meats is related to the myoglobin denaturation that occurs in fresh meats during cooking (Rincon et al., 2015). On the other hand, h_{ab} values of both inner and outer surfaces and b^* values of the inner surface increased with increasing ECT (p<0.01). Likewise, Mancini et al. (2005) and Sen et al. (2014) observed a linear increase in h_{ab} values of pork and mutton chops with increasing endpoint internal temperatures. Torun et al. (2023) noted that higher cooking temperatures increased b^* values in beef. Furthermore, beef cooked at 74°C or 80°C ECT had lower L^* values in both inner and outer surfaces than that cooked at 68°C ECT (p<0.05). Yancey et al. (2011) revealed a slight decline in L^* values of beef steaks with increasing internal cooked temperatures. Torun et al. (2023) found a similar result for L^* value changes in beef due to the internal cooked temperatures. There were no *BI* value differences in the outer surface among different ECT applications.

Regarding the storage time, a^*, b^*, C^*_{ab}, and *BI* values obtained in both inner and outer surfaces gradually decreased during the storage period (p<0.01). These results are in line with the reports of Zhang et al. (2021), who found that color values decreased in spiced beef during cold storage. Molins et al. (1987) similarly observed a decline in color values throughout the storage period. Although L^* values obtained on the outer surface were quite stable during the first 20 days, these values increased on day 30 (p<0.01), and did not change during the rest of the storage. Moreover, L^* values determined on the inner surface of cooked beef were quite stable during the first 10 days, this value gradually increased on days 20 and 30 (p<0.01), and it did not change during the rest of the storage. In addition, h_{ab} value determined on the outer surface increased on day 10 (p<0.01), whereas the values of h_{ab} did not change during the rest of the storage. On the other hand, h_{ab} values obtained from the inner surface did not generally change during the whole storage period.

TBARS and LPO

TBARS and LPO results (Table 5) demonstrated that CM, ECT and ST had an evident influence on TBARS and LPO values of cooked beef pieces (p<0.01). Regarding CM, the lowest TBARS and LPO values were obtained in the samples cooked with UF method, whereas the samples cooked with B method had the highest TBARS and LPO values (p<0.01). Furthermore, TBARS and LPO values obtained in the samples cooked with US method remained between the values obtained in the samples cooked with UF and those cooked with B method (p<0.01). Similarly, Cichoski et al. (2015) reported a reduction in the oxidation

of ultrasound-treated sausages. In addition, Zhang et al. (2021) noted that ultrasound-assisted cooking effectively reduced the degree of lipid oxidation during cold storage, thereby helping to prevent the formation of undesired flavors in spiced beef. da Silva et al. (2020) revealed that the application of ultrasonic-assisted cooking resulted in lower peroxide index, conjugated diene levels, and TBARS levels throughout storage compared to control. Moreover, Ashar et al. (2022) showed that broiler meat cooked at 50°C in an ultrasonic bath had a lower TBARS level compared to broiler meat cooked at 72°C in a water bath and cooked at 60°C, 70°C and 80°C in an ultrasonic bath. Researchers also reported that broiler meat cooked at 72ºC in a water bath and 60°C and 70°C in an ultrasonic bath had the same TBARS levels. In contrast, other studies have indicated that ultrasound-assisted cooking increases lipid oxidation levels in meat products (Zou et al., 2018; Zhang et al., 2020). Considering TBARS and LPO values according to ECT, beef cooked at 74°C or 80°C ECT had higher TBARS and LPO values than those cooked at 68°C ECT ($p<0.01$). On the other hand, there was no difference in terms of TBARS and LPO values between beef cooked at 74°C and 80°C ECT. As far as considering ST, both TBARS and LPO values increased as the storage time increased ($p<0.01$). Likewise, Spanier and Miller (1996) observed that lipid oxidation increased with higher endpoint cooking temperatures. The cooking temperature, method, and duration influence the production of free radicals, which may result in lipid oxidation in meat (Schwartz et al., 2022). Sen et al. (2014) noted that the endpoint cooking temperatures did not significantly affect lipid oxidation, whereas the storage duration had a significant impact on the degree of lipid oxidation.

Microbiological properties of cooked beef

Microbiological analysis results (Table 6) showed that TMAB counts were influenced by ECT and ST ($p<0.01$) but not CM. On the other hand, CM, ECT and ST had no significant impact on yeast-mold counts of cooked beef. Moreover, CM, ST ($p<0.01$) and ECT ($p<0.05$) significantly changed the total coliform counts of cooked beef. Following the present study results, there were no TMAB and yeast-mold count differences among different cooking methods. On the other hand, the cooking methods of US or UF had a higher total coliform count in comparison to B method ($p<0.01$). Additionally, the highest coliform count was detected in the samples cooked with US method. da Silva et al. (2020) reported that the ultrasonic-assisted cooking treatment had similar mesophilic and psychrotrophic bacteria counts compared to control. Previous studies have reported conflicting results regarding the antimicrobial effects of ultrasound application (Piyasena et al., 2003; Cichoski et al., 2015; Piñon et al., 2020). Piñon et al. (2020) observed an increase in the numbers of psychrotrophic, mesophilic, lactic acid bacteria, and *Staphylococcus aureus* in chicken breasts after the ultrasonication process. Sams and Feria (1991) suggested that the increase in microbial counts was attributed to the release of nutrients from meat undergoing ultrasonication. Conversely, other studies have highlighted a positive effect of ultrasound on microbial reduction (Piyasena et al., 2003; Cichoski et al., 2015). The main theory on how ultrasound affects the viability of microorganisms is based on the phenomenon of cavitation, which can disrupt the system and lead to microbial cell rupture (da Silva et al., 2020).

Table 6. Microbiological analysis results (log cfu/g) of cooked beef samples according to cooking methods, endpoint core temperatures and storage time.

	TMAB	Yeast and Mold	Total Coliforms
Cooking Method (CM, n=36)			
B	4.85[a]	< 1	2.61[c]
US	4.93[a]	< 1	3.42[a]
UF	5.00[a]	< 1	3.00[b]
SEM	0.49	0.06	0.18
Significance	NS	NS	**
Endpoint Core Temperature (°C; ECT, n=36)			
68	5.27[a]	< 1	3.25[a]
74	4.85[b]	< 1	2.94[b]
80	4.67[b]	< 1	2.83[b]
SEM	0.49	0.06	0.18
Significance	**	NS	*
Storage Time (Day; ST, n=36)			
0	< 1[c]	< 1	< 1[c]
20	6.83[b]	< 1	4.01[b]
40	7.47[a]	< 1	4.66[a]
SEM	0.49	0.06	0.18
Significance	**	NS	**
CMxECT	NS	NS	NS
CMxST	NS	NS	**
ECTxST	NS	NS	NS
CMxECTxST	NS	NS	NS

B: Boiling, US: Ultrasound-assisted slow boiling, UF: Ultrasound-assisted fast boiling, TMAB: Total mesophilic aerobic bacteria, SEM: Standard error of the mean, *NS:* Not significant, [a-c]Means with different superscript letters in the same column indicate statistically significant differences at (**$p<0.01$) and (*$p<0.05$).

Considering the microbiological analysis results regarding ECT, beef cooked at 74°C or 80°C ECT had lower TMAB and total coliform counts compared to those cooked at 68°C ECT ($p<0.05$). Conversely, no significant difference in TMAB and total coliform counts was observed between 74°C and 80°C ECT applications. Regarding ST, TMAB and total coliform counts gradually increased throughout the storage ($p<0.01$). Yeast-mold count results according to CM, ECT and ST were determined below the detection limits. Sen et al. (2014) stated that initial aerobic plate counts were lower at higher endpoint cooking temperatures. A similar result was demonstrated by Torun et al. (2023) who noted that the lower cooking temperatures resulted in higher microbial counts.

Conclusion

The study results indicated that ultrasound-assisted cooking positively influenced the oxidative stability of cooked beef. Based on the results of TBARS and LPO assessments, UF demonstrated a greater ability to delay lipid oxidation. The cooking loss values of US and B samples at the endpoint core temperature of 68°C were lower than those of UF samples. On the other hand, B or US at 74°C ECT increased cooking loss values compared to UF. US exhibited lower a_w values compared to B. Ultrasound-assisted cooking had no significant impact on pH. Regarding the color values of the outer surface, there were no differences in L^* values among cooking methods. On the other hand, the higher a^*, b^*, C^*_{ab} and BI values obtained from the outer surfaces of cooked beef were determined in UF compared to B. UF samples exhibited lower L^* values and higher BI and b^* values on the inner surface compared to B. Moreover, US samples displayed higher h_{ab} values on both the inner and outer surfaces of cooked beef than those of B. No differences in a^* and C^*_{ab} values were observed on the inner surfaces of cooked beef among different cooking methods. There were no significant differences in TMAB and yeast-mold counts among the cooking methods, however, coliform counts increased with ultrasound-assisted cooking. pH, CL, ORP, hue angle (h_{ab}) and b^* values increased as the ECT increased, whereas a_w, a^*, chroma (C^*_{ab}) and browning index (BI; inner) values decreased. In addition, beef cooked at 74°C or 80°C ECT had lower L^* values, TMAB, and total coliform counts, and higher TBARS and LPO values than those cooked at 68°C ECT. There were no differences in terms of TMAB and total coliform counts, as well as L^*, TBARS and LPO values between beef pieces cooked at 74°C and 80°C ECT. Therefore, ultrasound-assisted fast boiling (UF) could be an effective processing technology for enhancing the oxidative stability and physicochemical properties of beef.

Declarations

Author Contribution Statement

Dilara Aydın: Investigation, Data collection, Formal analysis. Birol Kılıç: Methodology, Conceptualization, Supervision, Review and editing. Azim Şimşek: Methodology, Conceptualization, Data collection, Formal analysis, Writing original draft, Review and editing.

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Conflict of Interest

The authors do not have any conflict of interest.

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