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Research Article

Changes in physicochemical, microbiological and molecular properties of beef during tray and microwave drying affected by salting

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Abstract

The effects of salting pretreatment with NaCl (100%) or partial replacement of NaCl (50%) with KCl (50%), for sodium reduction, on protein denaturation, DNA fragmentation, heme-iron stability, proximate composition, water activity and color of beef were investigated during microwave drying (MD) and tray drying (TD). One of the most significant concerns about applied novel technologies is effects of these processes on the meat molecular structure in terms of detection of adulteration. Therefore, the primary aim of this study is to analyse the DNA integrity and protein denaturation of dried beef. Results indicated that water activity values of all the dried beef was below 0.30 which is safe for microbial growth at room temperature. The total microbial count fell below 1 log cfu.g⁻¹ by the end of the drying process. Furthermore, salting, particularly with partial KCl substitution suppressed myofibrillar protein denaturation. Both MD and TD, induced fragmentation of high molecular weight DNA (long amplicons: 918 bp), while short amplicons (173 bp) remained detectable. TD resulted in lower heme-iron content compared to MD. In general, drying decreased L*, a* and b* values of beef, while salting significantly increased L* and b* values, resulting in a lighter and yellower appearance. MD was an energy-efficient technique and appeared to shorten the drying period and improve the meat's structural quality. Using KCl as a partial substitute for NaCl is recommended, as it did not adversely affect product quality.

Keywords

salting, KCl, DNA, SDS-PAGE, heme-iron, a_w, adulteration, color

Abbreviations

a* - redness; ANOVA - analysis of variance; a_w - water activity; b* - yellowness; C* - Chroma; cfu - colony-forming units; ΔE - total color difference; h - hue angle; KCl - potassium chloride; L* - lightness; MD - microwave drying; NaCl - sodium chloride; PCR - polymerase chain reaction; SDS-PAGE - Sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TAMB - total aerobic mesophilic bacteria; TD - tray drying

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Introduction

Drying is one of the oldest food preservation techniques and is still commonly utilized for processing a wide range of products, including meats. Over time, traditional drying methods have evolved into more industrialized and advanced technologies, enabling the development of novel and value-added meat products (Aykin-Dinçer 2023). Dried meats can be processed into various forms, such as diced or minced, which are commonly used in ready-to-eat meals and pet food formulations (Başlar et al. 2014). They also feature prominently in traditional culinary items like kadi, pastırma, and jerky (Soydan et al. 2014). Traditionally, foods that have water activity values between 0.0-0.6 are categorised as dried or low moisture food products (Mishra et al. 2017), most meat snacks are considered part of this category (Bechtel 2001). The low water activity helps maintain microbiological quality over extended storage, therefore these types of products do not need any preservatives or cold chains (Aykin-Dinçer 2023).

Despite its advantages, dried meat production faces two primary challenges. The first is the reliance on conventional tray dryers, which require prolonged operation at high temperatures resulting in substantial energy use, elevated costs, and considerable environmental impact (Aykin-Dinçer 2021). In this technique, warm air circulates around the meats which placed on metal trays, and drying takes 3 h to a couple of days depending on the product size and drying temperature (Al-Haddad et al. 2025; Olum and Candoğan 2024; Rahman et al. 2005). Additionally, prolonged heat exposure during drying induces significant biochemical and physical alterations in meat, particularly affecting its textural and color attributes. These changes are primarily associated with proteins, including denaturation, aggregation, and contraction of connective tissues (Deng et al. 2014). Among the protein components, sarcoplasmic and myofibrillar fractions are particularly susceptible to thermal degradation, which plays a critical role in the deterioration of meat quality (Chabbouh et al. 2011). To address these challenges, recent studies have explored microwave drying as a promising alternative, offering enhanced energy efficiency while maintaining better product quality compared to conventional methods (Aykin-Dinçer et al. 2021;

Kipçak and İsmail 2020; Özbay and Sarıcoşan 2021; Pawlak et al. 2019). Contrary to tray drying, microwave drying generates volumetric heat inside material through electromagnetic energy by directly targeting the water molecules that cause vibration (Wray and Ramaswamy 2015).

Some traditional dried meat products including jerky (South America), cecina (Spain), bresalo (Italy), lap-yuk (China), yukpo (Korea), and dried goose meat (Türkiye) are generally salted before drying. Dry salting is generally applied in during manufacturing of these products as a pretreatment which enhances products' physicochemical and sensory quality (Vidal et al. 2021; Kabil et al. 2025). These products are widely produced and consumed worldwide owing to their unique sensory characteristics, physicochemical quality and easy manufacturing conditions. On the other hand, elevated sodium content in these salted meat products constitutes a major public health problem. (Vidal et al. 2021; Dias et al. 2022). Due to the well-documented association between high sodium intake and public health risks including hypertension and cardiovascular disease (Ruusunen and Puolanne 2005), researchers have increasingly explored the use of alternative salts such as potassium chloride, magnesium chloride, and calcium chlorides, as well as phosphate salts (sodium or calcium phosphates), to reduce sodium levels without compromising product functionality (Carkcioglu et al. 2016; Cittadini et al. 2020; Cruz-Romero et al. 2021). Among the alternative salts, KCl is the most appropriate and common salt, in terms of organoleptic properties of meat products since it has similar chemical properties with NaCl and substitution NaCl with other alternatives leads to undesirable flavours (Stanley et al. 2017; Vidal et al. 2023).

While less frequently addressed, the potential impact of drying and salting on DNA integrity is becoming a matter of growing relevance. Thermal processing, particularly at elevated temperatures, can cause DNA fragmentation, thereby hindering species identification and adulteration detection—issues of particular importance to consumers seeking accurate labeling and ethical sourcing, such as in halal and kosher-certified products (Mortas et al. 2022). DNA-based methods, especially PCR-based assays, are widely used for species identification. However, DNA degradation due to

thermal processing may hinder the amplification of target sequences (Musto 2011). Thus, maintaining DNA integrity is critical for regulatory compliance and consumer trust, particularly for products subjected to high temperatures for extended periods. In this context, the detection of specific gene sequences becomes essential, particularly in dried meat products subjected to prolonged thermal exposure. Nevertheless, limited data are available regarding the influence of drying methods on the stability and integrity of DNA in meats (Aslan et al. 2009; Frezza et al. 2003; Musto 2011; Musto et al. 2014).

In a recent study the effect of microwave drying and pre-salting by NaCl and KCl on drying characteristics, texture, microstructure and water retention capacity of beef was investigated (Olum and Candogan 2024). Therefore, the main objective of the current study was to assess the effect of these technologies on physicochemical and molecular quality of meat. Investigating the effect of potassium chloride as a substitute for sodium chloride is an important aspect of salted and dried meat products in terms of salt reduction. However, a major problem with this kind of application is lack of information about the effect of new applied techniques and ingredients on the molecular structure of meat. Therefore, the primary aim of this paper is to critically analyse the effect of microwave drying and KCl application on DNA and protein degradation which will help to address these new processing factors on effectiveness of reliable molecular adulteration analysis.

Materials and Methods

Materials. Beef (*M. semimembranosus* muscles) was purchased from a local supermarket in Ankara 48 h postmortem and transported to the laboratory for three replicates in different months. The chemicals used in this study, including sodium hydroxide (NaOH), boric acid (H_3BO_3), sulfuric acid (H_2SO_4), copper (II) sulphate ($CuSO_4 \cdot 5H_2O$), potassium sulfate (K_2SO_4), methyl red, brom cresol green, ethyl alcohol, petroleum ether, acetone, hydrochloric acid (HCl) and other chemicals were of analytical grade and were obtained from Merck Chemicals (Darmstadt, Germany). Chemicals for SDS-PAGE analysis were purchased from BioRad (Ankara, Türkiye) Qiagen Blood & Tissue Kit (Cat no: 69504) were used for DNA isolation which

purchased from Oligomer Biyoteknoloji (Ankara/Türkiye). Specific gene sequences were obtained from Heliksbio (Ankara, Türkiye).

The meat was initially cut into small pieces (3 cm (h) x 2 cm (w) x 2 cm (d)) using a cutting template. and stored at $-80^{\circ}C$ until drying was conducted. Samples were packed in low-density polyethylene bags and before drying the beef pieces were kept at $+4^{\circ}C$ overnight which allowed them to be oxygenated during the thawing period. The pH value of raw beef was 5.67 and the moisture, fat, protein, and ash contents of the raw material were 73.16, 1.62, 23.81, and 1.43 g.100 g⁻¹, respectively.

Experimental design. Drying was carried out with the procedure previously reported by Olum and Candogan (2024) and briefly explained as follows. After thawing, the beef pieces were divided into nine groups, three of which were subjected to tray drying (TD), microwave drying (MD), and microwave-assisted TD (TD+MD). For the other six groups, salting was carried out with NaCl or NaCl + KCl salts as pretreatment before drying, and after that, these portions of beef were subjected to TD, MD, or TD+MD. TD was performed with a tray dryer (İNOKSEN A. Ş., Ankara/Türkiye) at $80^{\circ}C$ and 0.5 m.s⁻¹ air velocity, MD was performed with a custom-made microwave dryer (Dizge Analitik Inc., Türkiye) at 350 watts and 2450 MHz, and TD+MD involved initial TD for 90 min following the approximately 40% moisture loss was achieved, MD was applied. Additionally, for the six salted groups, dry salting with NaCl (w.w⁻¹) or a 50% NaCl + 50% KCl mix with a salt to meat ratio of 1:1 (w.w⁻¹) was applied before drying for 120 min which was decided after preliminary trials considering the moisture loss by salting. Drying lasted until equilibrium moisture content was calculated by carrying out the drying process until the differences between the final two consecutive weighings was less than 0.01 g.

Instrumental color parameters. Instrumental color parameter (CIE L^* -lightness, a^* -redness and b^* -yellowness values, chroma, and hue angle) values of raw material and dried beef samples were measured with a Konica Minolta CR-400 chromameter (Osaka, Japan). The chromometer was calibrated before measurements with a standard white plate (calibration values were $Y=92.7$, $x=0.3133$, $y=0.3193$) (Bulut and Candogan 2022).

Color parameters were obtained from the surface of the meat samples through three different readings for each group. The total color difference (ΔE) was estimated using the color of the raw meat for initial color values (Eq. 1). Chroma (C^*) and hue angle (h) are also two terms to characterize the color (Eq. 2 and Eq. 3).

$$\Delta E = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2} \quad (1)$$

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

$$\text{Hue angle } (h) = \tan^{-1}(b/a) \quad (3)$$

where the subscript '0' refers to initial value of L^* , a^* , and b^* color parameters and values without a subscription refer to the final color parameters after drying.

Microbiological analysis and water activity. Microbiological analysis was carried out for fresh and dried beef samples. Ten grams of dry and fresh meat samples were homogenized with 90 mL of sterile saline solution (0.85% NaCl) to obtain the initial (10^{-1}) diluent, and a series of dilutions were then prepared. Microbial counts were determined by the pour plate method. TAMB (total aerobic mesophilic bacteria) counts were enumerated on Plate Count Agar (PCA, Merck 1.05463, Darmstadt, Germany) medium after incubation at 28-30°C for 48 h. Total yeast and mold counts were determined using Yeast Extract Glucose Chloramphenicol Agar (Merck, Darmstadt, Germany) incubated at 25-28°C for 72 h and 120 h, respectively. Coagulase-positive staphylococci were enumerated by incubating in Baird Parker agar (Merck 1.05406, Darmstadt, Germany) medium enriched with egg yolk tellurite (at 37°C, for 24 h). Lactic acid bacteria (LAB) were enumerated on de Man Rogosa Sharpe Agar (Merck 1.10660, Darmstadt, Germany) medium at 28-30°C after 48 h by the double layered plate method. The most probable number method (MPN) was used to determine coliform group bacteria and *E. coli* count. Tubes containing 10 mL of lauryl sulfate broth were inoculated with 1 mL of the samples and incubated for 48 h at 35-37°C (Anonymous 2005). Microbiological analyses were performed in triplicate, and all bacterial counts are expressed as log₁₀ colony-forming units (cfu.g⁻¹ sample). Water activity (a_w) was measured with a water activity

meter (Aqualab 4TE, Addium WA/USA) with a measurement range of 0.05-1.00 at 24°C (Akpınar et al. 2019). Each group was analysed for three replications (n=3).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Alteration of meat myofibrillar protein fractions with various drying processes were examined with sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the procedures reported by Toldra, Miralles and Flores (1992) for protein extraction and Laemmli (1970) for SDS-PAGE analysis. 10-15 g meat samples homogenized in 40 mL K-phosphate buffer and centrifuged at 1200 g for 20 min. and filtered to obtain sarcoplasmic proteins. Pellet was homogenised with 40 mL 8M urea (containing 1% β merkaptotanol) and centrifuged at 1200 g for 20 min and filtered to obtain myofibrillar proteins. Bovine serum albumin standard was used to determine the protein concentrations. 4x Laemmli sample buffer (Biorad) was used to adjust protein content to 30 μ g and samples were heated at 95°C for 10 min. Samples were loaded onto gels consisting of 4% stacking gel and 12% resolving gel which are obtained from Biorad as ready-to-use gel solutions. After separation gels were washed with a destaining solution containing Commassie Brilliant Blue R-250 (1 g.L⁻¹). Gel images were acquired with a charge-coupled device (CCD) camera, and the image records were focused using FUJI FILM Science Lab 97 Image Gauge, Version 3.0, software (Fuji Film, Tokyo, Japan).

DNA isolation and DNA quality. The Qiagen Blood and Tissue Kit (Qiagen Blood & Tissue Kit, Cat. no: 69504) was used, and the suggested protocol was applied for DNA isolation. 25 mg sample, 180 μ L extraction buffer (Buffer ATL) and 20 μ L proteinase K was mixed and incubated at 56°C at 3 h at thermal block. Following that 200 μ L buffer and 200 μ L 96% ethyl alcohol was added to the sample and centrifuged with DNeasy spin columns (8000 rpm, 1 min). Afterwards, 500 μ L buffer AV1 was added and centrifuged and 500 μ L buffer AV2 was added and centrifuged at 14 000 rpm for 3 min. Then 200 μ L buffer AE was added and centrifuged at 8000 rpm for 1 min. The integrity of isolated DNA was determined via 0.01 g.mL⁻¹ (w.v⁻¹) agarose gel electrophoresis. Gels were stained with (0.5–1 μ g.mL⁻¹) ethidium bromide and

photographed under UV light. A 1 kb DNA ladder was used as a marker. The DNA yield was measured using a UV-visible spectrophotometer (NanoDrop, ND 100, Thermo Fisher Scientific, MA, USA), and the A_{260}/A_{280} and A_{260}/A_{230} absorption ratios were used as a purity index.

Primer design and PCR amplification. The specific gene sequences of extracted mitochondrial (mtDNA) and nuclear (nDNA) DNA from beef *semimembranosus* muscle were chosen from previous studies (Musto et al. 2014) to define DNA quality. Specific primer sequences belong to Beef *semimembranosus* mitochondrial gene for cytochrome b (F-5'- TCG ACC TTC CAG CCC CAT CGA A -3' and R- 5'- ACA GGC ATT GGC TGA GCA GTC G -3' for long amplicons and FF: 5'- CGA CCT TCC AGC CCC ATC GA -3' and RR: 5'- AGC CGT AGT TCA CGT CTC GGC -3' for short amplicons) targeting 918-bp and 173-bp site were obtained from Heliksbio (Ankara, Türkiye). The PCR reaction was conducted with a Tetrad 2 Thermal cycler (BioRad, Ankara, Türkiye). The concentrations of compounds in the PCR mix 50 μ L of reaction mixture containing 38.8 μ L sterile distilled water, 5 μ L*5 PCR tampon, 1 μ L dNTP, 1 μ L Forward primer (10 pmol), 1 μ L reverse primer (10 pmol), 0.2 μ L (Taq DNA polymerase (5u.mL⁻¹), 3 μ L of extracted DNA, 4 μ L MgCl₂ (25mM). For blind control DNA was replaced with the distilled water. PCR cycling was completed with an initial denaturation at 94°C for 4 min. followed by 35 cycles of denaturation at 94°C for 30 sec, at 63°C for 45 sec, and extension at 72°C for 90 sec. Then the final extension was conducted at 72°C for 10 min. Gene regions obtained with the amplification were resolved by electrophoresis on 0.015 g.mL⁻¹ (w.v⁻¹) agarose gel stained with ethidium bromide (0.5-1 g.mL⁻¹) and visualized under UV light. Appropriately sized bands were identified by comparison with a 100bp DNA helix (Musto et al. 2014).

Heme-iron content. Total heme-pigment analysis was performed according to the method described by Hornsey (1956). Briefly, 10 g of sample was put into a 50 mL centrifuge tube and then homogenized for 30 sec, with 20 mL acid-acetone mixture (40 mL acetone, 9 mL water, and 1 mL concentrated HCl) using a homogenizer (Micra D-9, MICCRA GmbH, Germany). Another 20 mL of acid-acetone solution was added, and the mixture was sealed with

parafilm and left in the dark for 1 h before being centrifuged at 2200g for 10 min. The supernatant was then filtered, and the absorbance of the filtrate was measured against reagent blank (acid-acetone mixture) at 640 nm. The total heme pigment concentration was calculated as μ g hematin.g⁻¹meat by multiplying the absorbance by the factor 6800 and dividing by the sample weight. Heme-iron content was calculated by multiplying the total heme-pigment concentration in meat (μ g hematin.g⁻¹ meat) by the factor of 0.0882 μ g iron. μ g⁻¹ hematin (Clark et al. 1997).

Statistical analysis. Statistical analysis was performed using SAS system 9 (SAS Inc., NC, USA) (SAS 2002). Data from three replications for each group (n=3) were analyzed with analysis of variance (ANOVA) to compare differences between groups, with significance set at $p < 0.05$. Post-hoc comparisons were conducted using the least significant difference (LSD) procedure for mean separation ($p < 0.05$). Tukey test was conducted as a comparison method. The statistical analysis focused on evaluating the effects of drying and salting treatments on microbial counts, DNA concentration, heme-iron content, color, pH, water activity and chemical composition.

Results and Discussion

Instrumental color parameters. Color is one of the most important sensory attributes that is influenced by the processing conditions. During heat application the color change of meat is basically due to the Maillard reaction, myoglobin oxidation and protein denaturation (Shi et al. 2021). The CIE instrumental color parameters (L^* , a^* , and b^*) of dried meats obtained from the nine different drying methods are given in Table 1. The structural changes in proteins affect the instrumental color values by altering the light-emitting features of the meat surface (Renerre and Labas 1987). The L^* , a^* , and b^* values of fresh meat were 43.56, 23.96, and 12.61, respectively. MD and TD methods decreased the L^* , a^* , and b^* values. The decrease in L^* value was associated with the moisture decrease in previous studies (Bak et al. 2012; Guizani et al. 2008; Sanabria et al. 2004; Seong et al. 2015). Our results are consistent with the data reported by Bodruk et al. (2023), where L^* parameters of dried beef snacks were between 24.57-46.66 with different predrying conditions. Also decrease in L^* ,

a^* , and b^* values in dried beef were attributed to the protein denaturation with heat exposure in this study. Moreover, in the present study the a^* value decreased after drying in the MD and TD groups, reaching values between 8.34 and 9.21 ($p < 0.05$), possibly due to denatured myoglobin hemochrome formation with elevated temperatures during drying. Fan et al. (2024), examined the color changes of meat with hot air and microwave drying, and found lower L^* , a^* , and b^* values in hot air dried meat compared to MD which is attributed to lower heat exposure in MD caused less protein denaturation and better color resistance. In contrast to earlier findings, our results indicate that there are no significant differences in color parameters among drying methods (TD, MD and TD+MD) due to the excessive moisture loss in all groups. Bak et al. (2012) examined color stability of ham with different drying process levels and reported that the decrease in moisture content and a^* value was almost linear. Elevated temperatures cause heat-induced denaturation of myoglobin; thus, changing the red color and resulting in drab-brown color of meats (Suman et al. 2016; Su et al. 2024). Several studies associated the decrease in a^* value with metmyoglobin formation during long drying periods (von Gersdorff et al. 2021) and heating (Guizani et al. 2008; Sanabria et al. 2004). The moisture content of meat products decreases, and oxidation and denaturation of proteins increase during drying. Therefore, heme in myoglobin is gradually exposed and this results in myoglobin oxidation, oxymyoglobin decreases, and metmyoglobin increases. This causes a reduction in L^* , a^* and b^* values (Shi et al. 2021). The initial b^* value was 12.6, and it decreased to 7.16 with MD and TD ($p > 0.05$).

A clear effect of salting was observed with a significant increase in L^* and b^* values ($p < 0.05$). This is due to the excessive salt deposition on the meat surface, which results in a yellower and lighter appearance of the meat. It was well documented that color coordinates change with the diffusion of salt into meat and salting of meat products has a significant effect on color (Pérez-Álvarez and Fernández-López 2008). Similar findings were reported by Sanabria et al. (2004) for dry-cured ham, where the b^* value increased with salting ($p < 0.05$). This increase in yellowness is attributed

to the migration and subsequent accumulation of dissolved salt on the surface. There was no significant change in the a^* values ($p > 0.05$) caused by salt pretreatments with NaCl or NaCl and KCl. The acceptable meat color was associated with the high a^* values of these types of over dried products (Bodruk et al. 2023). Therefore, novel drying methods must be developed with a focus on improving a^* values.

Chroma or saturation is a qualitative expression of colorfulness. As chroma value increases, the color intensity that humans can perceive also increases (Pathare et al. 2013). The hue angle (h) is a parameter that expresses the qualitative aspects of colors, which determines how different a particular color is from a gray color with equivalent L^* values. Hue angle is generally used to define the color properties of vegetables and meats. Total color difference (ΔE), another color parameter, is the expression of the vectorial distance between the first and last coordinates of the color and is an indicator of color gradient (Pathare et al. 2013). The ΔE (total color difference), C^* , and h values for the nine groups of dried meats are given in Table 1. The ΔE of samples decreased with salting. The C^* value exhibited a general increase in dried meats subjected to salting, with alterations similar to the b^* value. While there were no significant differences among C^* values in the salted groups ($p > 0.05$), these values had higher magnitude when compared to unsalted meats. This trend is consistent with a study conducted by Maskan (2001), which indicated that the change in chroma values is parallel with the alteration in the b^* value since chroma is an expression of the stability of the yellow color. Similarly, the hue angle (h) generally increased in the salted groups. The h values were 41° , 43° , and 46° in the TD, MD, and TD+MD groups, respectively, indicating closer to red. The h values were around 65° in salted-dried meats, expressing a shift towards yellow. This observation aligns with the study conducted by Parolari et al. (2016), which found the h value was around 60° during the ripening period of dry-cured hams without adding nitrite.

The color changes in dried meat products are mainly associated with the biochemical process such as Maillard reaction, lipid oxidation and protein denaturation (Aykın-Dinçer 2023).

Table 1. CIE L^* , a^* , b^* , ΔE , C^* , and h color parameters of raw material and dried beef samples

Groups	L^*	a^*	b^*	ΔE	C^*	h
Raw material	43.56±1.62 ^{ab}	23.96±1.32 ^a	12.61±0.96 ^{bc}	-	-	-
TD	21.46±2.76 ^c	8.81±3.07 ^b	7.16±2.60 ^c	28.44±3.14 ^a	11.32±4.16 ^c	41.23±2.34 ^b
MD	23.62±2.24 ^c	8.34±0.44 ^b	7.16±0.84 ^c	20.38±2.08 ^{cd}	11.79±0.91 ^c	43.32±1.80 ^b
TD+MD	23.11±2.44 ^c	9.21±2.18 ^b	9.82±2.24 ^c	21.93±3.26 ^{bc}	15.04±3.64 ^{bc}	46.25±2.06 ^b
NaCl+TD	53.52±6.52 ^{ab}	8.16±1.36 ^b	19.90±2.59 ^{ab}	24.81±2.78 ^b	22.14±2.26 ^a	68.55±3.49 ^a
NaCl+MD	41.75±6.37 ^b	13.40±6.63 ^b	18.09±4.70 ^{ab}	19.43±2.45 ^{cd}	20.79±5.01 ^{ab}	62.58±2.28 ^a
NaCl+TD+MD	42.95±6.43 ^{ab}	10.77±1.50 ^b	21.46±1.18 ^a	18.20±1.28 ^{cd}	20.64±3.50 ^{ab}	61.84±3.75 ^a
NaCl/KCl+TD	55.38±10.31 ^a	8.82±3.96 ^b	20.75±1.41 ^a	20.81±5.44 ^{cd}	24.49±3.49 ^a	64.88±5.66 ^a
NaCl/KCl+MD	48.72±6.38 ^{ab}	10.36±3.43 ^b	21.81±2.46 ^a	20.11±4.24 ^{cd}	24.05±3.36 ^a	66.37±5.05 ^a
NaCl/KCl+TD+MD	45.34±6.61 ^{ab}	10.98±1.07 ^b	22.09±1.95 ^a	16.93±3.20 ^d	25.10±1.08 ^a	62.68±4.42 ^a
n	27	27	27	27	27	27
P value	<.0001	0.033	<.0001	0.0016	<.0001	<.0001
η^2	0.31	0.31	0.31	0.31	0.31	0.31

a-d: The difference between groups carrying different letters in the same column is significant.

*Mean ± standard deviation

Quantitative research generally associated these reactions with the decrease in L^* , a^* and b^* values (Gao et al. 2024; Ma et al. 2021; Mediani et al. 2022). Furthermore, in dry-cured meat products, both salt and moisture content significantly influence surface color by affecting protein solubility and altering light reflectance properties. However, the present study involves a substantially higher degree of dehydration compared to traditional dry-cured products, resulting in a markedly different surface structure. The presence of excessive surface salt crystals, due to the high dry-salting ratio, interferes with light reflectance and hinders the accurate assessment of color parameters. Consequently, evaluating the specific impact of different salt types on color becomes challenging. These structural and compositional differences limit the comparability of our findings with existing literature on conventionally cured meat products.

Heme-iron content. Meats undergo physical and chemical changes, especially nutritional losses, by the effect of elevated temperatures applied in drying and cooking. Maintaining the heme-iron content as much as possible with the applied technological processes is one of the vital expectations regarding the nutritional quality of processed meat.

Fig. 1 demonstrates the significant decrease in heme-iron content of raw meat (17.25 $\mu\text{g iron.g}^{-1}$ meat, data not shown) with drying ($p<0.05$). TD resulted in lower heme-iron content compared to MD ($p<0.05$) which points out more degradation in MD ($p<0.05$). This result may be explained by the fact that increased drying period in TD resulted in further degradation in heme-iron content compared to MD (Cross et al. 2012; Goran et al. 2016). Shi et al. (2021) reported that with identical drying temperatures, heme-iron content decreased with increasing drying time during hot air drying of beef jerky. They also indicated that increasing drying

temperature leads to heme-iron reduction. These results were associated with metmyoglobin formation during drying and were consistent with previous studies by Jansuittivechakul et al. (1985) and Zdanowska-Sasiadek et al. (2018). Similar results were obtained in a previous study by Liu et al. (2022) comparing different drying methods using conventional oven drying and convection drying that utilized radiation in the form of electromagnetic waves. Heme-iron content was higher with radiation and convection drying which utilized high temperature (120°C), than at the lower drying temperature (85°C) utilized by conventional tray dryer.

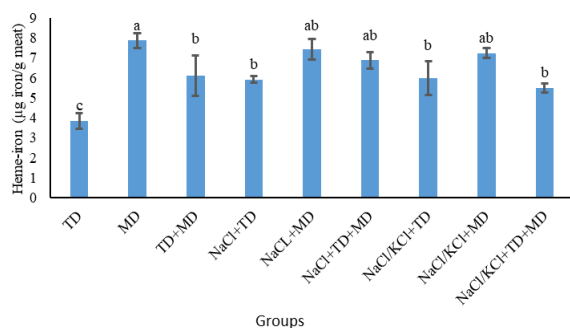


Figure 1. Changes in heme-iron content of dried meats with different drying methods (^{a-c}: The difference between groups carrying different letters in the same column is significant; *error bars: $\pm$ standard error)

The decrease observed with TD compared to MD could be attributed to myoglobin denaturation speed. The temperature suddenly increases with MD, which might have caused rapid myoglobin denaturation, so iron might be turned into an insoluble form and remain in the structure. A similar explanation was reported previously as slow heating increased non-heme iron content compared to fast heating (Buchowski et al. 1988; Purchas et al. 2003). Buchowski et al. (1988) suggested that by providing high levels of protein denaturation with high-temperature applications, the conversion of iron in myoglobin and hemoglobin to a fixed form can be achieved, and its removal as leakage by cooking can be prevented. This explanation is consistent with the results of the study conducted by Purchas et al. (2004), where increasing processing temperature resulted in an increase in denaturation level of myoglobin that caused a decrease in soluble heme iron content. Considering these approaches

together, rapid heating and high cooking temperature in MD resulted in the preservation of heme-iron in the meat itself. Coagulating heme proteins with rapid heating and high-temperature applications can achieve higher heme-iron ratios in meat.

Considering pre-salted dried meats, the heme-iron content of TD was well-preserved with salt. While the heme-iron content was $3.85 \mu\text{g}\cdot\text{g}^{-1}$ in TD, it was higher as 5.91 and $5.97 \mu\text{g}\cdot\text{g}^{-1}$ with NaCl and NaCl/KCl pre-salting processes, respectively. No significant difference was observed between NaCl and KCl salts in terms of preserving the heme-iron content ($p>0.05$) with all drying methods. The shorter heating period in salted groups may be the reason for higher heme-iron content. Few studies have simultaneously addressed alternative drying technologies and salt substitution, making this research a valuable addition to the current body of knowledge. Kristensen and Purslow (2001) reported similar findings regarding the protective effects of salting on heme-iron content in thermally processed meats, showing that KCl exerts comparable protective effects to sodium chloride NaCl. This phenomenon has been attributed to the increased ionic strength imparted by salt addition. Consistent with this, Cheng et al. (2007) observed no significant differences in heme iron content when NaCl was replaced with KCl in pork patties. However, the existing literature predominantly focuses on conventional meat products such as jerky, pastrami, fermented sausages, and salami, which typically retain a moisture content between 30% and 50%. In contrast, the present study investigated beef samples subjected to extensive drying until equilibrium moisture content is reached. This substantial difference in moisture levels limits the comparability of the current findings with previous studies.

Microbiological analysis and water activity. Drying is recognized as the most common preservation technique that prevents microbiological development and extends shelf life by reducing water activity in food structures (Argyropoulos et al. 2011). The water activity of the final products ranged from 0.30 to 0.35, and no significant differences were observed among all drying methods applied ($p>0.05$). Similar trends in a_w values were observed by Bodruk et al. (2023) in puffed dried beef snacks which were found between

0.16-0.38. Several perishable food products are generally dried to a_w values below 0.5, which is adequate to prevent microbial growth at ambient temperature during storage (Bonazzi and Dumoulin 2011). Leistner (1985) have analysed 42 intermediate-moisture meat products that have a_w values between 0.20-0.78, in terms of microbial quality and found that meets with a_w values below 0.69 were microbiologically stable at 25°C for 3 months.

Initially, the total aerobic mesophilic bacteria count of fresh meat was 2.03 log cfu.g⁻¹sample, and the lactic acid bacteria count was 2.19 log cfu.g⁻¹ sample. Coagulase-positive staphylococci, coliform group bacteria, *E. coli*, sulfite-reducing anaerobic bacteria, yeast, and mold were not detected in fresh meat. In dried meat, the total aerobic mesophilic bacteria count decreased below 1 log cfu.g⁻¹sample at the end of the drying process. This reduction can be attributed to the high heat treatment and low water activity of the final products (a_w around 0.25) (Akpınar et al. 2019).

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The results obtained from the SDS-PAGE gel image of myofibrillar proteins belonging to meats dried with different techniques are shown in Fig. 2. In general, the drying techniques affected protein profiles. Raw beef myofibrillar proteins mainly consist of 250, 150, 100, 50, 37, 20, and 10 kDa molecular weight bands. All drying procedures led to degradation in the myosin-heavy chain (250 kDa). The band around 150 kDa molecular weight, which characterizes degraded myosin, is present only in raw beef (Deng et al. 2014). In Fig. 2, a prominent trend of weakening protein bands within the range of 100 kDa-150 kDa was observed, thought to correspond to peptides formed through the degradation of myosin. These results are similar to those reported by Chabbouh et al. (2011). Moreover, it is worth emphasizing that, in general, TD exhibited more intense bands than MD.

Analysis of the salted groups revealed that the band densities for the groups treated with both NaCl and NaCl/KCl combination were more pronounced than those in the unsalted groups. This observation was more pronounced in the TD group. While band formation in the 20-10 kDa range was noticed in the groups dried after salting, no protein bands were

found in the groups dried with TD and MD likely due to the preserving effect of the salting process on low molecular weight peptides and free amino acids in this range. Similarly, Candogan et al. (2009) stated that for fermented sausages, the drying process after curing caused slight bands in the SDS-PAGE image of low molecular weight peptides and free amino acids. Greiff (2015) reported that extractability of salt soluble proteins increased with increasing salt concentration and partial replacement of Na⁺ with K⁺ would not affect the protein solubility.

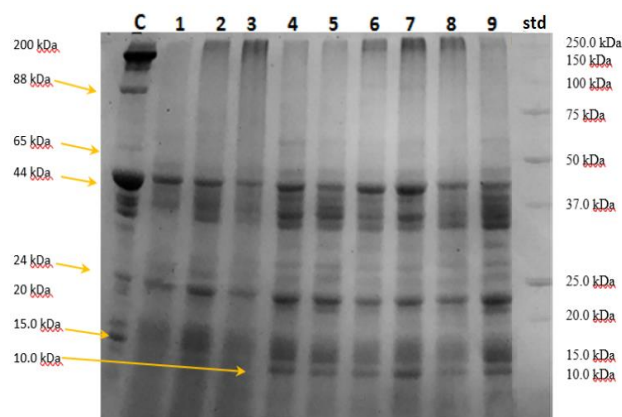


Figure 2. SDS-PAGE images of raw material and dried beef samples.

Abbreviations: C: raw beef, 1: TD, 2: MD, 3: TD+MD, 4: NaCl+TD, 5: NaCl+MD, 6: NaCl+TD+MD, 7: NaCl/KCl+TD, 8: NaCl/KCl+MD, 9: NaCl/KCl+TD+MD. std: bovine serum albumin standard.

However, low-density protein bands, which concentrate mainly in the molecular range of 20 kDa, were observed in the groups with KCl/NaCl salt combination compared to the groups salted with NaCl. Armenteros et al. (2009) reported similar findings in dry-cured meat fillets subjected to salting with NaCl and KCl, indicating that meats salted and dried with KCl had higher myofibrillar band densities between 25 and 37 kDa than meats salted with 100% NaCl. This phenomenon was attributed to the preventive effect of NaCl on proteolytic enzyme activity, which decreased with the use of KCl. Based on the comprehensive findings of SDS PAGE analysis, the MD and TD methods and the use of different salts before drying play key roles in protein degradation.

DNA integrity and PCR analysis. Nanodrop results can be used to support the electrophoresis image of extracted DNA. The most essential prior condition for PCR analysis is achieving DNA extraction with minimum detectable value (Arun et al. 2013; Bauer et al. 2003). While the DNA concentration of raw beef was $17.5 \text{ ng.}\mu\text{L}^{-1}$, DNA concentrations in dried meats were between $23.83\text{--}42.93 \text{ ng.}\mu\text{L}^{-1}$ (Table 2). Even though DNA concentration was expected to be high in raw meat, it was lower than the dried meat.

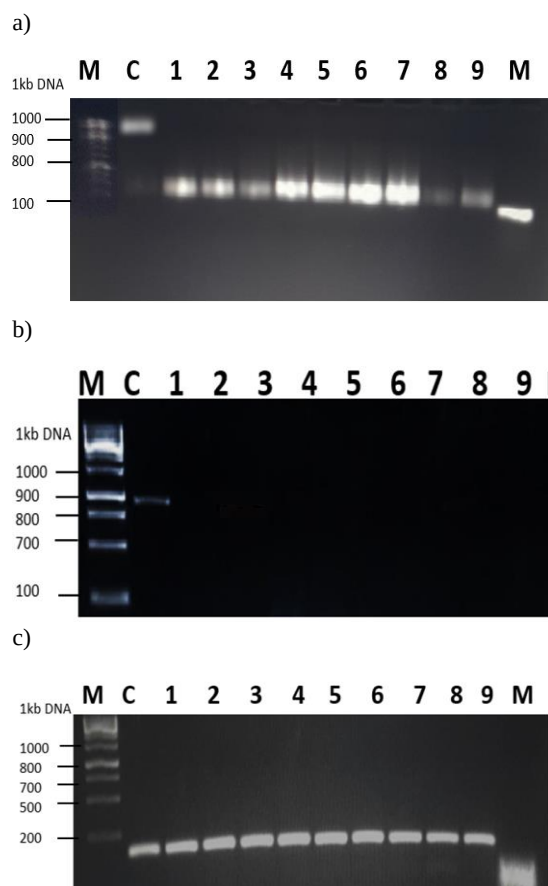


Figure 3. a: Electrophoretic profile of total DNA on $0.01 \text{ g.mL}^{-1}(\text{w.v}^{-1})$ agarose gel by different drying techniques. b and c: Representative agarose gel electrophoresis images of PCR products using two primer sets: (b) results obtained with the primer set belongs to long amplicons (CYTB F and CYTB R), and (c) results obtained with the primer set belongs to short amplicons (CYTB FF and CYTB RR). *M: Marker, C: control, 1:TD, 2: MD, 3: TD+MD, 4: NaCl+TD, 5: NaCl+MD, 6: NaCl+TD+MD, 7: NaCl/KCl+TD, 8: NaCl/KCl+MD, 9: NaCl/KCl+TD+MD.

This result indicates DNA fragmentation in the groups dried at elevated temperatures due to the high absorbance of fragmented DNA under UV light. Increasing DNA degradation resulted in high DNA concentration in dried meats (Musta et al. 2014). When assessing the purity of DNA with a nanodrop spectrophotometer, absorbance at 260, 230 and 280 is generally utilized. Approximately $1.8 \text{ ng.}\mu\text{L}^{-1}$ ratio at 260/280 is considered to indicate pure DNA. Furthermore, the 260/230 index can be used as a secondary benchmark for DNA purity. For pure nucleic acid, the 260/280 ratio is generally lower than the related 260/230 ratio, which is generally measured as $2.0\text{--}2.2 \text{ ng.}\mu\text{L}^{-1}$. If the 260/230 ratio is lower than the expected value, this indicates impurities with absorbance at 230 nm (Bunu et al. 2020; Nagar and Schwessinger 2018). The purity index (260/280 and 260/230 ratio) of extracted DNA from dried meats, obtained with different drying techniques, is given in Table 2. No significant differences were observed between DNA purity indexes among the dried meats ($p>0.05$). When evaluating the 260/280 ratio, it became obvious that only the salt-treated groups had ideal purity indexes, which were around 1.82. Contrarily, the lower purity indexes observed in the unsalted groups, around 1.62, indicated DNA fragmentation. Furthermore, having low values below $2 \text{ ng.}\mu\text{L}^{-1}$ in 260/230 ratios demonstrates evidence for impurities in DNA.

Fig. 3 illustrates the intensity of extracted DNA from dried beef samples. While the DNA band at 1000 kb DNA only appeared in raw beef, no DNA band was seen at this point for all the groups undergoing elevated temperatures during drying. This indicates fragmentation of long DNA chains due to high heat application. Similarly, Şakalar et al. (2012) conducted agarose gel electrophoresis of DNA extracted from cooked meats and observed a DNA band only for raw material and unclear DNA bands were present in cooked samples. Accordingly, Musta et al. (2014) examined the DNA quality of microwave-cooked meats at different cooking times (2.5, 5, 7.5, and 10 min) and observed advanced DNA degradation with increased cooking times.

Table 2. DNA concentration (ng.µL⁻¹) and purity index (A₂₆₀/280, A₂₃₀/280 ratios) for raw and dried beef samples

Groups	DNA concentration (ng.µL ⁻¹)	A ₂₆₀ /280	A ₂₆₀ /230
Raw beef	17.50±11.34	1.82±0.10	0.65±0.55
TD	25.30±2.26	1.62±0.12	0.23±0.04
MD	28.40±5.77	1.65±0.16	0.26±0.11
TD+MD	27.63±9.54	1.68±0.07	0.27±0.05
NaCl+TD	22.53±8.94	1.82±0.05	0.46±0.18
NaCl+MD	23.83±10.21	1.72±0.21	0.61±0.56
NaCl+TD+MD	25.10±8.35	1.71±0.15	0.34±0.12
NaCl/KCl+TD	37.46±14.81	1.86±0.03	0.53±0.26
NaCl/KCl+MD	35.63±22.25	1.76±0.21	0.66±0.64
NaCl/KCl+TD+MD	42.93±20.16	1.87±0.15	1.23±0.82
n	30	30	30
P value	0.6733	0.6397	0.2799
η ²	0.31	0.31	0.31

*Mean ± standard deviation

Several food processing applications can degrade the DNA and at the end it affects the molecular based detection analysis (Şakalar et al. 2012).

To determine the DNA integrity, specific gene sequences of mitochondrial DNA (mtDNA) specific to bovine semimembranosus muscle were selected based on the study by Musto et al. (2014). Primers were selected to yield large (amplicon length 918 bp) and small (amplicon length 173 bp) fragments. Obtained PCR products were analyzed with 0.015 g.mL⁻¹ (w.v⁻¹) agarose gel and gel images of PCR products are illustrated in Fig. 3b for long amplicons, and Fig. 3c for short amplicons. DNA bands were detected in all samples for CYTB FF and CYTB RR primer sets designed only for short amplicons (173 bp). DNA bands were not observed in PCR images of primer sets designed for long amplicons due to the degradation of DNA long amplicons with long drying times. Studies show that DNA chains are broken due to long heat application

periods in several meat samples (Aslan et al. 2009; López-Andreo et al. 2012; Musto 2011; Musto et al. 2014). Therefore, the size of DNA fragments gradually degrades into smaller fragments in processed meats with high heat applications and processing time (Şakalar et al. 2012). Considering DNA degradation in processed meat samples, there is a greater chance of detecting shorter amplicons. A considerable amount of literature has been published on food adulteration. These studies revealed that DNA long amplicons can be degraded with processing conditions especially in high temperature while short amplicons from the same sequence are still amplified across several stages of food processing (Chen et al. 2025; Fernandes et al. 2021; Lo and Shaw 2018). European legislation also uses methods for detection of DNA sequences of foods that aim to detect short DNA amplicons (Bauer et al. 2003).

Conclusions

This study focused on the effects of pre-salting which constitutes an important step in the production of traditional dry-cured meat products and microwave drying (MD) on the molecular quality of beef cubes dried until equilibrium moisture content. Given the growing demand for low-sodium foods for health reasons, the study also explored the impact of KCl substitution for NaCl in the salting process which is one of the most frequently stated problems with traditionally dry-salted meat products. This research represents the first study to assess the effect of MD and KCl salting on DNA and myofibrillar protein integrity, heme-iron content and color of fully dried meat. The results showed that there were no significant differences in terms of color values between TD and MD. While the salting process significantly changed the color values, the type of salt used had no impact on instrumental color parameters. The current research also showed that heme iron content decreased with all drying techniques and the heme-iron reduction with TD was more pronounced than MD due to the high heat application period. Additionally, pre-salting increased the heme-iron content with TD. Examining the impact of drying techniques on DNA quality and integrity is also important for detecting adulteration. The applied drying techniques degraded the DNA long amplicons but did not affect the short amplicons which is an important finding that proves the DNA short amplicons can still be detectable and be used for adulteration analysis in processed meat. According to the results of SDS-PAGE protein analysis, the salting process protected protein integrity and more intense band densities were detected with the NaCl/KCl combination.

MD is a promising novel technology for reducing drying time and is a good alternative for TD for better preserving the heme-iron content. Although reducing Na content by substitution with KCl did not have any effect with the longer heat treatment duration applied in this study, which continued until equilibrium moisture content, the results of the present study may not be directly applicable to dried meat products such as jerky, pastrami, fermented sausage, etc. However, it provides valuable insight into the biochemical quality of meat with the application of MD and use of KCl. Further studies need to be carried out in order to validate the

biochemical quality features of microwave dried, reduced salt meat products.

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Author Contributions

Conceptualization, E.O. and K.C.; methodology, E.O. and K.C.; investigation, E.O.; writing – original draft preparation, E.O.; writing – review and editing, K.C.; supervision, K.C. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement

The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest

The authors declare no conflicts of interest.

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