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FROM MUSCLE TO DELIGHT: EXPLORING SALTING METHODS' IMPACT ON MUSCULAR NUCLEI DYNAMICS IN ARTISAN PASTRAMI

DE LA MATERIE PRIMĂ LA DELICIU: EXPLORAREA IMPACTULUI METODELOR DE SĂRARE ASUPRA DINAMICII DIMENSIUNII NUCLEILOR DIN ȚESUTUL MUSCULAR ÎN PASTRAMA ARTIZANALĂ

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ABSTRACT | REZUMAT

Histometry (morphometry), a quantitative histological method, offers a precise evaluation of the composition of meat. Recent research highlights its applicability for detecting unauthorized tissues. On the other hand, the investigation of meat products, specifically the evolution of the muscular nucleus area resulting from various salting techniques and prolonged ageing periods, remains relatively uncharted territory, particularly in the context of assessing their freshness. This preliminary study aims to examine the histometric measurements of nucleus area in Romanian artisan pastrami, which has been subjected to diverse salting methods for a defined period. It seeks to provide valuable insights into the potential correlation between salting techniques and the quality attributes of Romanian artisan pastrami, with a particular focus on assessing its freshness. The pastrami samples under investigation were prepared following a traditional artisan recipe and utilized 3 different types of salt: iodized salt (IS), natural unionised salt (NUS), and natural salted mineral water (NSMW). A control sample, consisting of unprocessed meat, was included for comparison. Subsequently, the histological specimens were obtained at three time points: T0 (24 hours post-production), T1 (30 days), and T2 (60 days). The area of the nuclei decreased with time, being higher for the NSMW treatment at all time points of the research. In conclusion, our study reveals that the applied salting methods significantly influenced the size of muscular nuclei during pastrami refrigerated storage, with NSMW resulting in the largest nuclei. However, this phenomenon can be attributed to nuclear hyperhydration further research is needed to establish a correlation between these results and the freshness of the meat products.

Keywords: artisan meat products, muscular nuclei morphometry, salting methods'

Histometria (morfometria), metodă histologică cantitativă, este aplicată în diferite domenii, inclusiv pentru a oferi o evaluare precisă a compoziției produselor din carne. Cercetările recente evidențiază aplicabilitatea sa și în detectarea țesuturilor neautorizate. Pe de altă parte, cercetarea produselor și a preparatelor din carne, în special a evoluției suprafeței nucleului muscular rezultată în urma diferitelor tehnici de sărare și a perioadelor prelungite de maturare, rămâne un subiect relativ necunoscut, în special în contextul evaluării prospețimii acestora. Acest studiu preliminar își propune să analizeze valorile histometrice ale suprafeței nucleului din pastrama artizanală de porc, care a fost supusă unor metode diverse de sărare pentru o perioadă definită. Acesta urmărește să ofere informații cu privire la potențiala corelație dintre tehnicile de sărare și atributele de calitate ale pastramei artizanale, cu un accent deosebit pe evaluarea prospețimii acesteia. Probele cercetate au fost preparate după o rețetă artizanală tradițională, fiind utilizate trei tipuri diferite de sare: sare iodată (IS), sare naturală neiodată (NUS) și apă minerală sărată naturală (NSMW). Pentru comparație, a fost inclus în studiu și un eșantion martor – carne proaspătă neprocesată. Ulterior, au fost obținute probe histologice în trei momente de timp: T0 (24 de ore după producție), T1 (30 zile) și T2 (60 zile). Suprafața nucleilor a scăzut în timp, cu excepția nucleilor din lotul NSMW. Prezentul studiu relevă faptul că metodele de sărare aplicate au influențat semnificativ dimensiunea nucleilor musculari în timpul depozitării la temperatura de refrigerare a pastramei artizanale, în situația NSMW rezultând cele mai mari suprafețe nucleare. Acest fenomen poate fi atribuit hiperhidratării nucleare, fiind necesare cercetări suplimentare pentru a stabili o corelație între aceste rezultate și prospețimea produselor din carne.

Cuvinte cheie: produse artizanale din carne, morfometria nucleilor musculari, tipuri de sărare

The specialized analytical approach of histological examination in meat product analysis serves as a powerful tool for the direct differentiation and identification of individual constituents derived from both animal and plant origins (1, 8, 9, 11). It imparts crucial insights into the spatial distribution, dimensions, and abundance of these components. Traditionally, this assessment tends to favour a qualitative methodology, involving the identification of particular tissues and an assessment of their appropriateness for the desired final product (2). Nevertheless, situations arise, particularly when dealing with raw materials like mechani-

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cally separated meat and plant additives in meat products, where it becomes imperative not only to identify these components but also to gauge their proportions quantitatively (14, 15, 16). The process of identifying individual constituents hinges on their morphological attributes and their responsiveness to various staining techniques. On the other hand, the quantitative assessment revolves around quantifying the number and dimensions of analyzed components relative to the sample's overall area (7).

The prospect of streamlining and objectifying histometry has been substantiated through specialized computer programmes designed for image analysis (7, 9). Image analysis assumes a pivotal role in furnishing quantitative descriptions and precise specifications of information derived from macroscopic or microscopic scans, thereby enabling meticulous data processing, detailed sample comparisons, and a plethora of result presentation methods (3). The process involves input through digital cameras, but standard photographs can also be transformed into digital format for analysis. Notably, the ability to compare presently scanned objects from microscopic slides with those previously acquired enhances data evaluation through statistical means. In this regard, Palka et al. (1999), used histometry (morphometry) to determine if the alterations in the diameter and length of sarcomeres during heating can exert an impact on the textural characteristics of beef (12). Similar parameters have been determined in relation to a meat's capacity to bind water under varying pH conditions (13). Image analysis has also proved invaluable in the assessment of bone fragments within final meat products and the evaluation of component size and quantity in mechanically separated meat, particularly in relation to the separator and raw materials employed (5, 9).

Romanian artisan pork pastrami is a culinary gem cherished for its rich flavours and traditional craftsmanship. This delectable product represents a blend of artistry and gastronomy, with meticulous preparation methods handed down through generations. To preserve and enhance its exceptional quality, it is imperative to delve into a comprehensive investigation of this meat product. By analysing the nuclear changes and potential correlations with salting techniques, this study seeks to provide valuable insights into the quality attributes of Romanian artisan pastrami. The findings of this research can contribute to the optimization of salting methods and enhance the freshness and overall quality of artisan meat products.

MATERIALS AND METHODS

The experimental batches were obtained by utilizing a recipe supplied by a manufacturer of artisanal meat products in Prahova County, as depicted in Fig. 1. The pork leg meat (*Biceps femoris*) used in this study was sourced from commercial hybrids. The iodized salt used for the IS batch was obtained from EUROSALT, which had a particle size of 2 mm and a minimum purity of 99%. The natural non-iodized salt used for the NUS batch was obtained from the Târgu Ocna salt mine. This salt had a moisture level of approximately 0.2% and a particle size of 2 mm, with water-insoluble compounds not exceeding 1.2%. The NSMW batch involved the use of natural saline mineral water from Matița village, located in Prahova County. The control (C) group consisted of fresh, unprocessed meat.

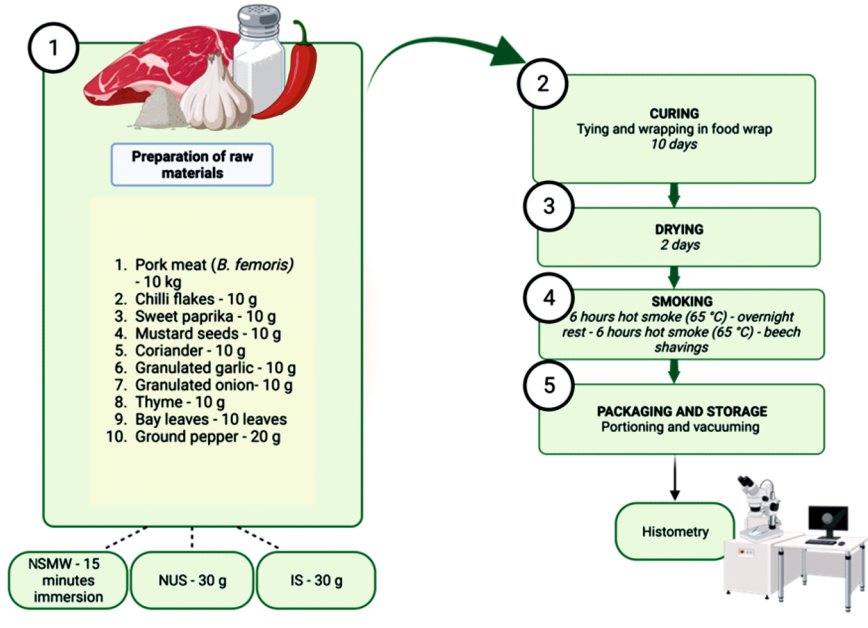

Fig. 1. Sample preparation procedure (made via www.Biorender.com)

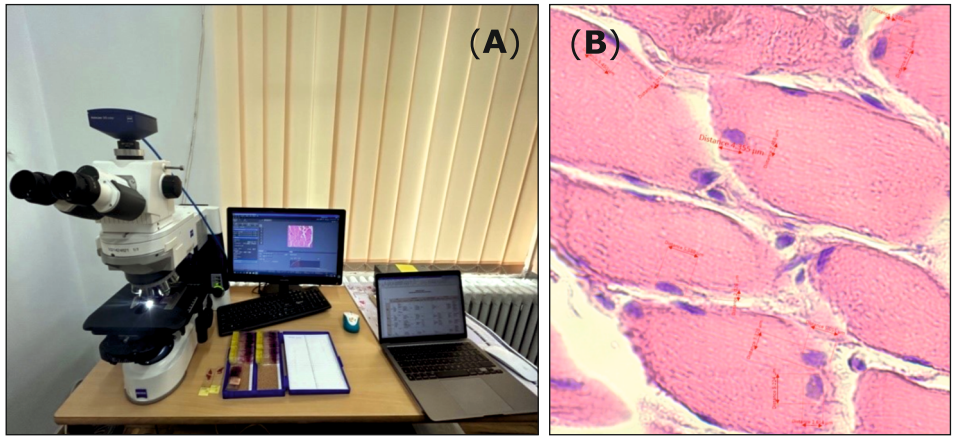

Fig. 2. (A) Zeiss ZEN 3.4 system,
(B) Nuclear measurements using the Zeiss ZEN 3.4 system

The meat samples were divided into approximately uniformly measured portions, averaging around 30 cm in length and 10 cm in diameter, with an average weight of around 1500 g. After being seasoned, the obtained batches (IS, NUS, and NSMW) underwent a curing process while being covered in foil. The curing process took place in a controlled environment at a temperature of 4°C and a relative humidity of 60% for a 10-day period. The drying stage was conducted at 4°C and 60% humidity for two days. The meat was subjected to smoking in an artisanal smoking chamber, using beech shavings as the smoking agent. This stage ran for a total of 6 hours, during which the temperature was consistently maintained at 65°C. The smoking procedure was carried out in two phases.

After obtaining the experimental batches, the final products were individually weighed and sealed securely using a GORENJE VS120W device (Fig. 1). The storage conditions were maintained at a refrigeration temperature of 2±0.5°C for the entire research period (60 days). Histometric measurements were made at 30-day intervals, delineating the assessment timeline as T0 (24 hours post-processing), T1 (30 days post-processing), and T2 (60 days post-processing). In this regard, the histological specimens were obtained according to ISO 21118:2019.

Histometry

Employing the ZEISS ZEN 3.4 software (Fig. 2), the muscular nucleus area (computed as the area of the nucleus =π x semimajor axis x semiminor axis) was assessed within samples of Romanian artisan pastrami. A total of ten measurements were meticulously acquired from serial histological sections.

Statistical data analysis

In the context of statistical analysis for this experiment, the normal distribution of the collected data was assessed using the Shapiro-Wilk test. Subsequently, based on the outcome of the normality test, distinct statistical methods were employed. For datasets that exhibited a normal distribution, the analysis of variance (ANOVA) was applied, and when it was significant, it was followed by the Tukey-Kramer post-hoc test. This approach allowed for the comparison of means between multiple groups while maintaining control over Type I error rates. In cases where the data did not conform to a normal distribution, the Kruskal-Wallis test, a non-parametric alternative to ANOVA, was utilized. Subsequently, the Conover post-hoc analysis was performed to discern significant differences between groups. These statistical methods were selected to ensure robust and accurate analyses, accommodating both normally and non-normally distributed datasets, thus facilitating a comprehensive evaluation of the experimental results. The statistical analysis was performed using MedCalcTM v22.007.

RESULTS AND DISCUSSIONS

According to the Shapiro-Wilk test, the data at time T0 followed a normal distribution (P=0.6185) (Table 1).

Table 1

Shapiro-Wilk test results for the normal distribution of the data

Data and Factor Code	Shapiro-Wilk test for the Normal distribution
T0 - Treatment	W=0.9781 accept Normality (P=0.6185)
T1 - Treatment	W=0.8676 reject Normality (P=0.0002)
T2 - Treatment	W=0.9562 accept Normality (P=0.1243)
NSMW - Time	W=0.9126 reject Normality (P=0.0046)
IS - Time	W=0.9636 accept Normality (P=0.2226)
NUS - Time	W=0.9603 accept Normality (P=0.1720)

Table 2

Descriptive statistics for the T0 moment

Factor	n	Minimum	25th percentile	Median	75th percentile	Maximum
C	10	9.9104	10.582	13.670	18.149	23.129
IS	10	8.5870	10.165	11.506	14.493	16.175
NUS	10	6.8770	8.438	11.872	15.605	20.959
NSMW	10	13.1610	16.781	22.586	27.804	31.659

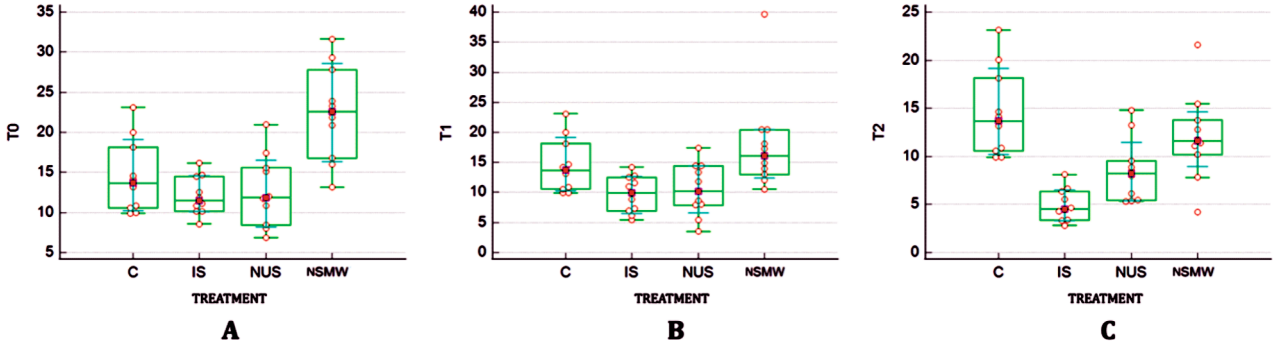

Fig. 3. Multiple comparison graph for the studied treatments at T0 (A), T1 (B) and T2 (C)

Additionally, significant differences were observed between the treatment groups, as indicated by the ANOVA analysis (P<0.001).

Moreover, according to the Tukey-Kramer test, the NSMW treatment significantly differed from all other treatment groups. Descriptive statistics for batch C showed that data ranged from a minimum of 9.9104 µm to a maximum of 23.129 µm (Table 2).

The interquartile range (IQR), representing the middle 50% of the data, was between 10.582 µm and 18.149 µm, with a median value of 13.670 µm. For the IS treatment, data ranged from 8.5870 µm to 16.175 µm, with an IQR of 10.165 µm to 14.493 µm and a median of 11.506 µm. The NUS treatment data ranged from 6.8770 µm to 20.959 µm, with an IQR of 8.438 µm to 15.605 µm and a median of 11.872 µm. Lastly, the NSMW treatment data spanned from 13.1610 µm to 31.659 µm, with an IQR of 16.781 µm to 27.804 µm and a median of 22.586 µm. Based on the observed means, the ranking from highest to lowest nucleus area was: NSMW (22.4621 ± 6.0218 µm) > C (14.4525 ± 4.6014 µm) > NUS (12.6982 ± 4.5140 µm) > IS (12.0746 ± 2.3940 µm) (Fig. 3 - A).

At T1 moment, the data did not follow a normal distribution, with a Shapiro-Wilk test result of P=0.0002 (Table 1)and significant differences between the treatment groups were observed according to the Kruskal-Wallis test (P=0.006791). Additionally, based on the post-hoc Conover analysis, the NSMW treatment significantly differed from the IS and NUS treatments, and the IS treatment also significantly differed from the C treatment. Descriptive statistics for Factor C showed a wide range of nucleus area values, from 9.9104 to 23.129 µm (Table 3).

The median nucleus area was 13.670 µm, suggesting that the central tendency was in the lower to middle portion of the range, with some outliers on the higher end. The IQR was relatively wide, spanning from 10.582 to 18.149 µm, indicating significant variability. Factor IS exhibited a narrower range, with a minimum of 5.4400 and a maximum of 14.234 µm. The median nucleus area was 9.934 µm, and the IQR (6.928 to 12.512 µm) was relatively narrow, suggesting less variability. There were fewer outliers compared to Factor C. Factor NUS displayed the narrowest range, with a minimum of 3.5160 µm and a maximum of 17.420 µm. The nucleus' median area was 10.215 µm, and the IQR (7.876 µm to 14.425 µm) indicated moderate variabi-

Table 3

Descriptive statistics for the T1 moment

Factor	n	Minimum	25th percentile	Median	75th percentile	Maximum
C	10	9.9104	10.582	13.670	18.149	23.129
IS	10	5.4400	6.928	9.934	12.512	14.234
NUS	10	3.5160	7.876	10.215	14.425	17.420
NSMW	10	10.5630	12.995	16.100	20.415	39.635

Table 4

Descriptive statistics for the T2 moment

Factor	n	Minimum	25th percentile	Median	75th percentile	Maximum
C	10	9.9104	10.582	13.670	18.149	23.129
IS	10	2.8400	3.376	4.538	6.356	8.105
NUS	10	5.3520	5.441	8.230	9.537	14.820
NSMW	10	4.2080	10.191	11.608	13.785	21.602

Table 5

Descriptive statistics for the NSMW treatment

Factor	n	Minimum	25th percentile	Median	75th percentile	Maximum
C	10	9.9104	10.582	13.670	18.149	23.129
T0	10	13.1610	16.781	22.586	27.804	31.659
T1	10	10.5630	12.995	16.100	20.415	39.635
T2	10	4.2080	10.191	11.608	13.785	21.602

lity, with a few outliers on the higher end. Factor NSMW showed a wide range, from 10.5630 µm to 39.635 µm, with a median nucleus area of 16.100 µm. The IQR (12.995 µm to 20.415 µm) suggested moderate variability, and there were outliers on the higher end, notably exceeding the other factors. Based on the observed means, the ranking from highest to lowest nucleus area at T1 was: NSMW (18.0316 µm ± 8.3124 µm) > C (14.4525 µm ± 4.6014 µm) > NUS (10.5145 µm ± 4.4615 µm) > IS (9.6797 µm ± 3.1321 µm) (Fig. 3 B). According to the Shapiro-Wilk test, at time T2, the data followed a normal distribution (P=0.1243) (Table 1). Significant differences were observed between the treatment groups, as indicated by the ANOVA analysis (P<0.001). Additionally, based on the Tukey-Kramer test, the C treatment significantly differed from the IS and NUS treatments, and the IS treatment also differed from the NSMW treatment.

According to the descriptive statistics shown in Table 4, the C factor exhibits a substantial range in its measurements, spanning from 9.910 µm to 23.130 µm.

The data is moderately positively skewed, as evidenced by the median (13.670 µm) being less than the mean, and the 75th percentile (18.150 µm) being greater than the median. For the IS factor, there is less variability in the measurements, with values ranging from 2.84 µm to 8.11 µm. The data appears to be positively skewed, with the median (4.540 µm) being less than the mean, and the 75th percentile (6.360 µm) being greater than the median.

The NUS factor also demonstrates variability, with measurements ranging from approximately 5.350 µm to 14.820 µm. The data seems to be positively skewed, as the median (8.230 µm) is less than the mean, and the 75th percentile (9.540 µm) is greater than the median. Lastly, the NSMW factor displays a range of measurements from 4.210 µm to 21.60 µm. The data may have a positive skew, as the median (11.610 µm) is less than the mean, and the 75th percentile (13.790 µm) is greater than the median. Based on the observed means, the ranking from highest to lowest nucleus area at T2 was C (14.4525 µm ±4.6014 µm) > NSMW (12.0151 µm ± 4.6052 µm)>NUS (8.5191 µm±3.3052 µm) > IS (4.9634 µm±1.6854 µm) (Fig. 3 C).

Regarding the evolution of nuclear areas over time for the NSMW treatment, the Shapiro-Wilk test indicated that the data was not normally distributed (P= 0.0046) (Table 1). Additionally, the Kruskal-Wallis test revealed significant differences between the research time points (P=0.002231). Post-hoc Conover analysis further showed significant differences between time point T0 and all other time points, as well as between T1 and T2. As shown in Table 5, the data observed at T0 exhibited a narrower range compared to C, with values ranging from 13.1610 µm to 31.659 µm.

The data displayed positive skewness, with the median (22.586 µm) closer to the 75th percentile (27.804 µm), indicating a distribution skewed towards higher values. For T1, the data ranged from 10.5630 µm to an unusually high maximum of 39.635 µm, suggesting the presence of a significant outlier, as the maximum value greatly exceeded the IQR. The data was positively skewed, with the median (16.100 µm) below the midpoint of the 25th percentile (12.995 µm) and the 75th percentile (20.415 µm). At T2, the values ranged from 4.2080 µm to 21.602 µm, with the data again showing positive skewness. The median (11.608 µm) was positioned between the 25th percentile (10.191 µm) and the 75th percentile (13.785 µm). Additionally, based on observed means, the classification from highest to lowest nucleus area was presented as follows: T0 (22.4621 µm ± 6.0218 µm) > T1 (18.0316 µm) > C (14.4525 µm ± 4.6014 µm) > T2 (12.0151 µm ± 4.6052 µm) (Fig. 4 A).

For the IS treatment, the Shapiro-Wilk test indicated that the data followed a normal distribution (P=

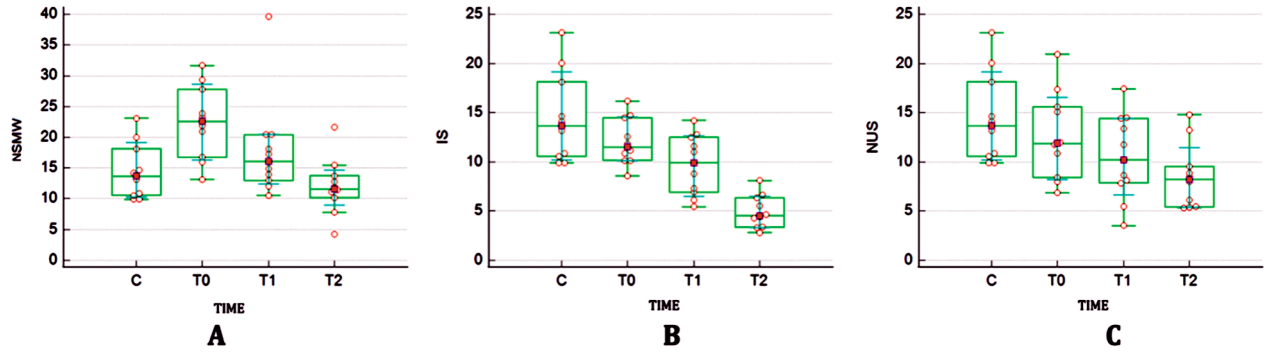

Fig. 4. Multiple comparison graph regarding the evolution in time of the nuclei area for NSMW (A), IS (B), and NUS (C) treatments

0.2226) (Table 1). The ANOVA analysis also indicated significant differences between the research time points (P<0.001).According to the Tukey-Kramer test, significant differences were observed between time point T2 and all other time points, as well as between C and T1. As displayed in Table 6, Factor C had the widest range and the largest IQR, with values ranging from approximately 9.91 µm to 23.13 µm and an IQR of 7.57 µm. Factor T0 also exhibited a positive skew, ranging from about 8.59 µm to 16.18 µm, with an IQR of 4.33 µm. Factor T1 had a range from approximately 5.44 µm to 14.23 µm, with a smaller IQR of 5.21 µm. Finally, Factor T2 had the narrowest range (2.84 µm to 8.10 µm) and the smallest IQR of 2.98 µm. Additionally, the classification based on observed means was as follows: C (14.4525 µm ± 4.6014 µm) > T0 (12.0746 µm ± 2.3940 µm) > T1 (9.6797 µm ± 3.1321 µm) > T2 (4.9634 µm ±1.6854 µm) (Fig. 4 B).

In the case of the NUS treatment, the Shapiro-Wilk test indicated that the data were normally distributed (P=0.1720) (Table 1). The ANOVA analysis showed significant differences between the research time points (P=0.021). The Tukey-Kramer test indicated significant differences between time points T2 and C. As shown in Table 7, T0 exhibited a positive skew, ranging from about 6.88 µm to 20.96 µm, with a moderate IQR of 7.17 µm. Factor T1 displayed positive skewness as well, with values ranging from approximately 3.52 µm to 17.42 µm and an IQR of 6.55 µm. Finally, Factor T2 had the narrowest range (5.35 µm to 14.82 µm) and a relatively smaller IQR of 4.11 µm. Additionally, the classification based on observed means was as follows: C (14.4525 µm ± 4 .6014 µm) > T0 (12.6982 µm ± 4 .5140 µm) > T1 (10.5145 µm ± 4.4615 µm) > T2 (8.5191 µm ± 3.3052 µm) (Fig. 4 C).

Regarding the comparison of our findings with similar results in the literature, there were no similar references identified regarding the evolution of the nucleus area. On the other hand, at T0, a noteworthy observation is that the data exhibited a normal distribution across all treatments, signifying a consistent pattern in nucleus area measurements. However, the selection of treatment significantly impacted these measurements throughout the study. Notably, NSMW consistently yielded the highest nucleus area at all time points, suggesting its potential influence on meat quality. This phenomenon could be attributed to the higher salt concentration in the NSMW treatment, which

Table 6

Descriptive statistics for the IS treatment

Factor	n	Minimum	25th percentile	Median	75th percentile	Maximum
C	10	9.9104	10.582	13.670	18.149	23.129
T0	10	8.5870	10.165	11.506	14.493	16.175
T1	10	5.4400	6.928	9.934	12.512	14.234
T2	10	2.8400	3.376	4.538	6.356	8.105

Table 7

Descriptive statistics for the NUS treatment

Factor	n	Minimum	25th percentile	Median	75th percentile	Maximum
C	10	9.9104	10.582	13.670	18.149	23.129
T0	10	6.8770	8.438	11.872	15.605	20.959
T1	10	3.5160	7.876	10.215	14.425	17.420
T2	10	5.3520	5.441	8.230	9.537	14.820

is known to affect the osmotic balance within muscle tissues, potentially resulting in larger nuclear areas (4, 6, 10, 17). It is important to acknowledge that sample variability, encompassing differences in meat quality, cut, or preparation, could also contribute to the observed variations in nuclear areas. Furthermore, it is imperative to recognize that the chosen measurement technique may introduce variability into the results, with discrepancies in sample preparation or microscopy methods potentially affecting the accuracy of nuclear area measurements. Conversely, other treatments exhibited fluctuations in their effects, underscoring the intricate interplay of factors intrinsic to meat processing. The observed temporal changes in nucleus area measurements suggest that ageing, curing, or other processing-related phenomena contribute to structural modifications in the meat over time. These stages likely impact meat quality, emphasizing the necessity of vigilant monitoring to ensure consistent product attributes. The discerned variability within treatment groups underscores that different treatments exert varying effects on nucleus area measurements. This accentuates the critical role of judicious treatment selection in meat processing to attain desired product characteristics. In addition, it is pertinent to acknowledge that a comprehensive evaluation of freshness should encompass sensory attributes, consumer perception, and physico-chemical determinations.

CONCLUSIONS

In conclusion, this study accentuates the significance of both treatment type and time point in shaping the muscle nuclear area of pork pastrami. The consistent trend of larger nucleus areas associated with NSMW treatment, amid fluctuating effects with other treatments, adds depth to our understanding of meat quality evolution. It is imperative to note that while these findings shed light on aspects of meat quality, they cannot provide a direct assessment of freshness. For a comprehensive evaluation of meat product freshness, future investigations should adopt a multidimensional approach that integrates sensory and physico-chemical attributes alongside the parameters studied herein.

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