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HIGH-DENSITY POLYETHYLENE CARCASS WRAPPING DURING THE FIRST 24 HOURS POST-MORTEM AND ITS EFFECTS ON RABBIT MEAT QUALITY

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Abstract: Post-slaughter chilling is essential for food safety in the meat industry. However, carcasses exposed to cold air in refrigerated chambers may undergo adverse effects on quality parameters. Thus, this study aimed to evaluate the efficiency of high-density polyethylene (HDPE) plastic wrapping on rabbit carcasses during post-slaughter chilling and its impact on the rabbit meat quality. Twenty whole carcasses of male Botucatu rabbits, slaughtered at 90 days of age, were used in a completely randomised design with a 2×2 factorial scheme [HDPE carcass wrapping (present and absent) *vs*. cut (loin vs. thigh)]. After 24 h of slow chilling at 4°C, the carcasses were deboned and physicochemical analyses were conducted to assess quality parameters: pH, colour (*L**, *a**, *b**), water-holding capacity (WHC), cooking loss (CL), sarcomere length (SL), shear force (SF), myofibrillar fragmentation index (MFI) and lipid oxidation by thiobarbituric acid reactive substance (TBARS). The wrapping resulted in lower ($P<0.05$) ultimate pH, red intensity, water-holding capacity and shear force, and higher MFI ($P<0.05$). Sarcomere length was shorter in the unwrapped thigh compared to both wrapped thigh and unwrapped loin ($P<0.05$). The thigh exhibited higher redness, yellowness and shear force ($P<0.05$), while the loin showed greater lightness ($P<0.05$). The unwrapped loin also demonstrated a greater MFI than the unwrapped thigh ($P<0.05$). Notably, no significant effect of either wrapping or cut type was observed on TBARS ($P>0.05$). These findings demonstrated that HDPE wrapping maintained the light colour of rabbit meat, which is more appealing to white meat consumers due to its light pink appearance. Additionally, HDPE wrapping enhanced tenderness, a key attribute in consumers' meat selection, as indicated by higher MFI values ($P<0.05$) and lower shear force measurements ($P<0.05$). In conclusion, wrapping carcasses in high-density polyethylene during the first 24 h post-mortem positively influences the rabbit meat quality, particularly in terms of colour and tenderness, with the loin cut showing superior tenderness compared to the thigh.

Key Words: meat colour, myofibrillar fragmentation index, rabbit production, sarcomere length, shear force, tenderness.

INTRODUCTION

The post-slaughter carcass chilling process is employed to maintain the final product quality by reducing factors that compromise meat tenderness and colour, such as the process of shortening, characterised by the shrinkage or shortening of muscle fibres (Savell *et al.*, 2005; Yan *et al.*, 2022). Fibre shortening can be influenced by the cooling method to which carcasses are subjected before rigor mortis sets in. When muscle fibres are exposed to cold, it causes a sudden stiffening of the muscles, making the product less attractive to consumers, as seen in our previous studies (Dutra *et al.*, 2024a,b).

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This process is more intensely related to the proportion of red fibres present in the muscle. The greater the proportion of red fibres, the higher the chance of shrinkage. Additionally, muscles on the outer parts of the carcass are more likely to suffer from cold shortening, as they are directly exposed to low temperatures in the chilling chamber. Lacking any fat cover, rabbit carcasses are more influenced by the cold, particularly in the hind limbs, which have a higher proportion of red fibres and greater oxidative activity compared to the loin, making them more susceptible to muscle fibre shrinkage (Gomide *et al.*, 2006; Dalle Zotte *et al.*, 2016).

Recent studies (Sampaio *et al.*, 2015; Redondo-Solano *et al.*, 2022; Zhao *et al.*, 2024) have shown that wrapping meat products prevents external factors such as oxygen, moisture and light from altering their qualitative characteristics. The most recommended products are plastic wraps of varying densities, made from organic or inorganic molecules with high molecular weight (polymers) (Kim *et al.*, 2014; Zhang *et al.*, 2022), consisting of polyethylene, a low-cost thermoplastic obtained from the ethylene monomer (CH_2=CH_2). Polyethylene is also characterised by high chemical and moisture resistance, low friction coefficient and easy processing (Islabão, 2005; Khanam *et al.*, 2015). However, there are few studies investigating the effect of carcass wrapping during post-mortem chilling on rabbit meat quality, mainly due to its limited demand and high market price (Cullere *et al.*, 2018).

This study thus aimed to assess the effectiveness of high-density polyethylene (HDPE) wrapping applied to carcasses during the first 24 h of post-mortem chilling on rabbit meat quality.

MATERIAL AND METHODS

Carcass and sample collection

Twenty carcasses of male Botucatu rabbits, slaughtered at 90 d of age with a live weight of 3.5±0.2 kg, were randomly selected. The rabbits were individually reared in flat-deck cages under uniform conditions at the Rabbit Production Unit of the São Paulo State University (FCAV/UNESP), Jaboticabal Campus, SP, Brazil (21°14'S, 48°17'W, 583 m altitude), with approval from the Animal Ethics Committee (CEUA) of the institution (protocol 1631/21). During the fattening phase, the rabbits had *ad libitum* access to water and Coastcross grass hay (*Cynodon dactylon* L. Pers.), and were fed a controlled commercial pelleted diet (14% crude protein, 3% fat, 18% crude fibre, 15% mineral matter, 5% phosphorus, 10% calcium, and 13% moisture), which met their nutritional needs (de Blas and Wiseman, 2010).

The rabbits were slaughtered at a commercial abattoir, following the operational and humane requirements of the Industrial and Sanitary Inspection Regulation for Animal-Origin Products – RIISPOA (Brazil, 2017). All animals underwent a 12-h fasting period before slaughter, followed by rest at the abattoir (Cavani and Petracci, 2004). They were stunned by electronarcosis (110V, 60Hz, 1.40A, with an average of 3 seconds), in accordance with Regulation (EC) No 1099/2009 (Council Regulation, 2009). After stunning, the rabbits were suspended by their hind legs for bleeding, with cuts made to the carotid arteries and jugular veins, followed by skinning and evisceration.

The carcasses were then stored in a chilling chamber at 4°C for 24 h with air circulation speed (0.5 to 3.0 m/s) adjusted for the proper chilling of the carcasses and randomly assigned to two experimental groups. The first group (n=10) consisted of carcasses individually wrapped in sterilised high-density polyethylene (HDPE) plastic, 34 × 49 cm, with an average density of 0.950 g/cm^3 (Zpp Indústria de Embalagens Plásticas Ltda, Santa Rita do Passa Quatro, Sao Paulo State, Brazil). The wrapping covered the carcasses completely, with the lower portion left open to allow for the drainage of exudate released during chilling. The second group (n=10) remained unwrapped, as is standard practice in processing plants.

After 24 h, the carcasses were transported chilled to the Animal Food Analysis Laboratory of the Department of Agricultural and Environmental Biotechnology at São Paulo State University (UNESP), Jaboticabal, SP, where they were deboned. From the caudal portion of the loin and the thigh, the *Longissimus lumborum* (LL) and *Biceps femoris* (BF) muscles were excised for immediate physicochemical analysis.

Analysis of physicochemical meat parameters

pH Determination

Initial pH (pH_i), defined as the pH measured 15 min post-mortem, and ultimate pH (pH_u), defined as the pH measured 24 h post-mortem, were recorded in triplicate using a digital pH meter connected to a digital thermometer and a penetration electrode (Testo 205, Testo Inc., Sparta, NJ, USA) inserted into the BF and LL muscles at the level of the 5th lumbar vertebra. The pH meter was calibrated with two buffer solutions (pH 4.00±0.2 and pH 7.00±0.02, respectively). Temperature compensation was automatically adjusted by the equipment.

Colour

The colour of the left caudal portion of the LL and BF muscles was assessed in triplicate after deboning. Lightness (L^*), redness (a^*), and yellowness (b^*) were determined using the CIELAB method (CIE, 1976) with a Minolta Chromameter (CR-400, Konica Minolta Sensing, Inc., Osaka, Japan) under the following configuration: diffuse lighting/0 viewing angle, D65 illuminant, specular component included and 8 mm aperture size.

Water-holding capacity (WHC)

Two grams of sample, collected from the cranial left section of the deboned cuts, were weighed on an analytical balance. The samples were placed between two sheets of filter paper and subjected to a pressure of 10 kg for five minutes. After this period, the weights were removed and the samples were reweighed. Results were expressed as a percentage using the formula: (Final Weight×100) / Initial Weight (Hamm, 1960).

Lipid oxidation

Lipid oxidation was assessed using the thiobarbituric acid reactive substances (TBARS) test, as described by Vyncke (1970). Extraction was performed with trichloroacetic acid on 5 g of ground sample from the caudal right portion of the cuts. Following the colour reaction after heating with thiobarbituric acid, readings were taken using a spectrophotometer with a wavelength of 532 nm. Results were expressed as mg of malondialdehyde (MDA) per kg of sample.

Cooking loss (CL)

Cooking loss was evaluated using samples from the caudal and medial sections of the LL and BF muscles, respectively, following Honikel's methodology (1987). Each sample, of similar dimensions and weight, was individually wrapped and cooked in one single batch in a water bath at 85°C for 40 min. The samples were allowed to cool to room temperature and then reweighed to calculate the cooking loss percentage, using the following formula:

$$CL\ (\%) = (\text{Initial weight} - \text{Final weight}) \times 100 / \text{Initial weight}.$$

Shear force (SF)

Subsamples of 1 cm^2 were taken from the samples used in the cooking loss analysis. These were positioned with the fibres perpendicular to the Warner-Bratzler device attached to the Texture Analyser TA-XT2i. The force required to shear the samples was measured in Newtons, according to the method used by Lyon *et al.* (1998).

Sarcomere length

Sarcomere length was determined using the phase contrast microscopy method. Approximately 0.5 g of sample from the cranial right portion of the LL and BF muscles was homogenised in a Turrax (Marconi MA102, Marconi Equipamentos Para Laboratórios Ltda., Piracicaba, São Paulo, Brazil) with 30 mL of a mixed solution of 0.08 mol/L potassium chloride and 0.08 mol/L potassium iodide, at a speed greater than 15 000 rpm for 60 s. With myofibrils in suspension, microscopic readings were performed using an optical microscope (Novel BM2100, Nanjing Jlangnan Novel Optics Ltd., China) with 1000× magnification (100× objective and 10× ocular) (Cross *et al.*, 1981). Twelve observations per slide were recorded, and sarcomere length was defined as the average length of the myofibrils and the number of sarcomeres recorded.

Myofibrillar Fragmentation Index (MFI)

The MFI was determined following the methods proposed by Culler *et al.* (1978) and Gornall *et al.* (1949). Subsamples of 3 g from the cranial right portion of each muscle were homogenised for 1.5 min with 30 mL of extraction solution and centrifuged, with sequential dispersions and centrifugations. Protein concentration was determined using the biuret method (Gornall *et al.*, 1949), with readings taken using a spectrophotometer at 540 nm. MFI was calculated as MFI=absorbance×200.

Statistical analysis

The experimental design used was a completely randomised design with a 2×2 factorial arrangement. Two independent variables, wrapping and muscle, were set as fixed effects. The main effects and their interactions were tested. Individual carcasses were considered experimental units for the meat quality traits. All data were analysed using two-way ANOVA, and mean comparisons were performed with Tukey's test, with a significance level set at 5%. Data were analysed using the "General Linear Models" procedure of the Statistical Analysis System (SAS).

RESULTS AND DISCUSSION

No significant difference was observed for the initial pH (pH_i) and ultimate pH (pH_u) when comparing the thigh and loin (Table 1). However, the use of the wrapping led to significantly lower ($P<0.05$) pH_u values in the meat. Immediately after slaughter, the muscle pH of rabbits is close to neutrality and stabilises within a few hours (ultimate pH)

Table 1: Estimated means for pH_i, pH_u, lightness (L^*), red intensity (a^*), yellow intensity (b^*), water-holding capacity (WHC), lipid oxidation (TBARS) and cooking loss (CL). Interaction between cut type and wrapping regarding sarcomere length (SL), shear force (SF) and myofibrillar fragmentation index (MFI).

	pH_i	pH_u	Colour			WHC (%)	TBARS (mg MDA kg^{-1})	CL (%)
			L*	a*	b*			
Cut (C)								
Thigh	6.44	5.98	45.86^{A}	16.00^{B}	7.18^{B}	63.60	1.24	28.55
Loin	6.52	5.90	53.13^{B}	6.32^{A}	0.44^{A}	62.50	0.89	29.71
Wrapping with HDPE (W)								
Wrapped	6.48	5.85^{A}	50.61	10.39^{A}	3.29	61.17^{A}	1.21	27.65
Unwrapped	6.57	6.03^{B}	48.51	11.65^{B}	4.13	64.93^{B}	0.92	30.62
CV (%)								
	2.08	1.95	8.98	50.56	10.26	3.9	25	7.33
P-value								
P (C)	0.445	0.784	<0.001	<0.001	<0.001	0.556	0.898	0.444
P (W)	0.235	0.001	0.174	<0.001	0.19	<0.001	0.234	0.379
P Int. (CxW)	0.555	0.753	0.731	0.435	0.451	0.547	0.334	0.321

Cut (C)	Wrapping with HDPE (W)		CV	*P*-value		
	Wrapped	Unwrapped	(%)	*P* (C)	*P* (W)	*P* (CxW)
SL (µm)						
Thigh	1.77^{b}	1.68^{Aa}	3.11	<0.001	0.456	<0.001
Loin	1.79	1.80^{B}				
SF (N)						
Thigh	51.53^{Ba}	70.71^{Bb}	25.24	<0.001	0.738	0.001
Loin	37.38^{Aa}	54.50^{Ab}				
MFI						
Thigh	104.35^{b}	84.26^{Aa}	11.24	0.001	<0.001	<0.001
Loin	108.25^{b}	92.83^{Ba}				

Means followed by different uppercase letters in columns and lowercase letters in rows differ significantly according to Tukey's test ($P<0.05$); pH_i: initial pH (15 min post-mortem); pH_u: ultimate pH (24 h post-mortem); TBARS: thiobarbituric acid reactive substances; MDA: Malondialdehyde; HDPE: high-density polyethylene; C: cut; W: wrapping with HDPE; CV: coefficient of variation.

(Ouhayoun and Dalle Zotte, 2010), which is highly relevant, as the pH at 24 h post-mortem is one of the best indicators of meat quality. The pH values recorded for the cuts, whether wrapped or not, were similar to those found by other authors (Daszkiewicz and Gugołek, 2020; Składanowska-Baryza and Stanisz, 2019). However, it is important to note that lower pH values have greater bacteriostatic function, more effectively inhibiting the growth of proteolytic microorganisms during storage (Hulot and Ouhayoun, 1999; Dalle Zotte, 2002), which may indicate that HDPE-wrapped carcasses have higher microbiological quality than unwrapped ones, suggesting the need for further research on the topic.

The wrapping had an effect on colour, with a higher ($P<0.001$) intensity of red (a^*) observed in the unwrapped cuts compared to the wrapped ones (Table 1). One of the most relevant colour coordinates for the rabbit meat industry is the intensity of red (a^*). Low positive values of a^* indicate a lower tendency towards redness, resulting in a pinkish-red meat, which is more attractive to the modern consumer who seeks to incorporate white meats into their diet. This demonstrates the importance of protecting carcasses with HDPE to maintain a desirable colour of rabbit meat.

The intensity of red in unwrapped carcasses was more pronounced due to the continuous exposure of the cuts to cold air during chilling. These values depend on the myoglobin content in the muscles and the surrounding oxygen pressure, which contribute to colour changes in meat during chilled storage (Kozioł *et al.*, 2017). In post-mortem muscle, active mitochondria consume oxygen, reducing metmyoglobin expression and giving the exposed surface a more reddish hue (Ramanathan and Mancini, 2018). Packaging used to cover fresh meat is generally minimally permeable to moisture, preventing surface desiccation of the meat (Faustman and Cassens, 1990). Similar results were observed by Rea *et al.* (1972), who reported that covered carcasses or cuts retained a fresher appearance and higher consumer acceptability due to the protective effect of the plastic, which minimised the negative impacts of chilling.

Different cuts showed statistical significance for their colour parameters, with greater lightness (L^*) and higher intensities of red (a^*) and yellow (b^*) in the thigh and loin. These differences are attributed to intrinsic factors and the individual characteristics of the muscle fibres in each evaluated cut, reinforcing the importance of considering the specific properties of each muscle when assessing rabbit meat quality. In rabbit meat, higher a^* and b^* values are usually associated with a lower L^* (darker colour), which is indirectly related to a higher myoglobin content in the hind leg muscles compared to the loin muscles (Kozioł *et al.*, 2015). Tůmová *et al.* (2014) corroborated these findings by evaluating the fibre characteristics of Czech rabbits of three different sizes, finding that over 90% of the fibres in the LL were white, while less than 10% were red, oxidative and slow-twitch. Similarly, Dalle Zotte and Ouhayoun (1998) observed that 98% of the fibres in the LL were white, glycolytic and fast-twitch, while only 2% were red, oxidative and slow-twitch.

The colour findings have significant implications for the meat industry, as visual appearance is critical for consumer acceptance and is often used as an indicator of freshness (Joo *et al.*, 2013). This is particularly relevant in the rabbit meat market, where rabbit meat is predominantly sold as whole carcasses or in cuts (Abdullatif *et al.*, 2023; Siddiqui *et al.*, 2023).

Regarding water-holding capacity (WHC) and cooking loss (CL), no significant differences were observed between cuts, but the wrapping had a significant effect ($P<0.05$) on water-holding capacity. The unwrapped group showed 6.15% higher water retention compared to the wrapped group. Analysis of water-holding capacity in conjunction with pH revealed that the pH_u for the wrapped group remained close to the isoelectric point of meat proteins (5.5 to 5.8). This condition results in the gradual denaturation of glycolytic enzymes, leading to greater water release, as these denatured proteins are unable to bind water (Rübensam, 2000).

However, the lower pH_u values in the wrapped samples correlated with increased tenderness as well, suggesting that the water release does not necessarily compromise the overall meat quality. The higher tenderness in the wrapped group may be attributed to the optimised enzymatic activity favoured by the low pH of the muscles at the end of the 24-h post-mortem period. Calpains, calcium-dependent enzymes, play a crucial role in this tenderisation process by proteolytically degrading structural proteins, which weakens myofibrils and enhances meat texture. This process occurs after pH stabilisation, as calpains facilitate the dissociation of actomyosin, contributing to improved tenderness, marking the resolution phase of rigor mortis (Aberle *et al.*, 2001).

Conversely, pH_u values above the isoelectric point (>5.8), observed in the unwrapped group, result in the disappearance of positive charges in the meat, leaving only excess negative charges. This results in the repulsion of myofibrillar filaments, increasing the spacing between them and allowing for greater water retention but potentially compromising tenderness (Offer, 1991; Ramos and Gomide, 2017). No effect of wrapping or cut type was observed on the lipid stability of rabbit meat.

The results related to meat tenderness showed a significant interaction between cut type and wrapping for sarcomere length (SL), shear force (SF), and MFI (Table 1), with the loin intrinsically more tender than the thigh (P<0.05). Additionally, the wrapping of carcasses during the first 24 h of chilling significantly improved the tenderness of both cuts, proving to be an accessible and effective solution for enhancing the final quality of the products.

When exposed to low temperatures before rigor mortis sets in, muscles may undergo a reduction in SL, especially those with red fibres, which can increase SF and compromise meat tenderness (Heinemann *et al.*, 2002). This pattern was observed in the present study, particularly with the *Biceps femoris*, a locomotor muscle with high oxidative metabolism and more red fibres compared to the *LL*, thus more susceptible to shrinkage (Gomide *et al.*, 2006; Dalle Zotte *et al.*, 2016).

The use of HDPE wrapping seems to have mitigated the effect of cold shortening on meat tenderness, as the wrapped thigh showed a greater SL compared to the unwrapped thigh (1.77 *vs.* 1.68 µm), as seen in Figure 1. Additionally, both cuts in the unwrapped group exhibited higher SF values (P<0.05): 70.71 N and 51.53 N (unwrapped and wrapped thigh) and 54.50 N and 37.38 N (unwrapped and wrapped loin). In one of the few similar studies on rabbit carcasses covered individually with loosely fitting polythene bags, Jolley *et al.* (1983) also found that unheated burger mix and raw fine comminute made from uncovered carcasses had higher compression resistance compared to those processed from covered carcasses during rapid chilling; they also did not observe an effect of carcass covering on the sarcomere length of the longissimus, corroborating the findings of this study.

Regarding the MFI, results showed that wrapped carcasses had higher indices for both thigh and loin (104.35 and 108.25, respectively), compared to the unwrapped group (84.26 and 92.83, respectively). This index is known to predict at least 50% of the variation in meat tenderness (Hopkins *et al.*, 2000). As pointed out by Taylor *et al.* (1995), MFI is a measure of the average size of myofibrils and is directly associated with tenderness. Higher values generally indicate a softer meat texture. Previous studies, such as that by Culler *et al.* (1978), suggest that meat with MFI above 60 can be considered to have satisfactory texture.

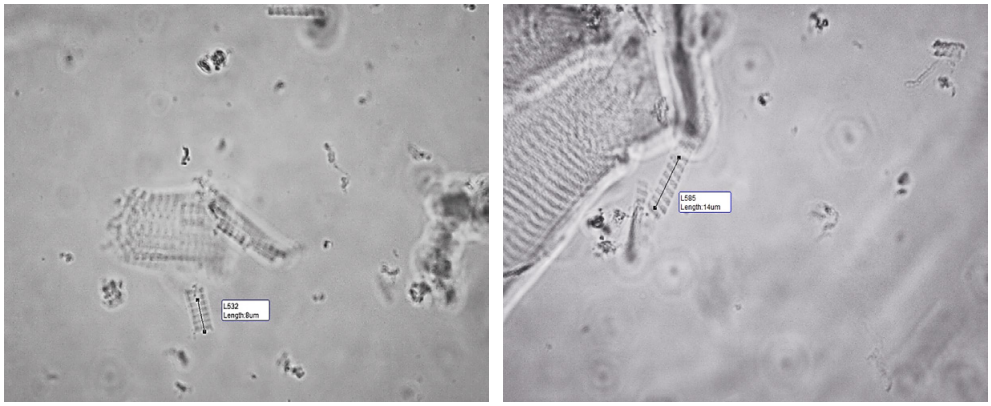

Figure 1: Sarcomere length calculated by dividing the myofibril length by the number of sarcomeres, using an optical microscope at 1000x magnification. L585) Micrograph showing sarcomere length measurement from the *Biceps femoris* muscle of rabbit carcasses wrapped in high-density polyethylene. The sarcomeres, visible as repeating units, appear elongated due to the wrapping process. L532) Micrograph showing a sarcomere length measurement from the *Biceps femoris* muscle of unwrapped rabbit carcasses. The sarcomeres appear shorter in comparison to those in the wrapped carcasses.

Although both wrapped and unwrapped samples had MFI values above 60, the results demonstrated that plastic wrapping favoured the maintenance of rabbit meat tenderness for both cuts, as evidenced by the higher MFI in wrapped samples.

That said, the greater water release in the wrapped samples should not be viewed solely as a detriment. While increased moisture loss can affect texture and juiciness, which were not observed in this study, and may also be associated with nutrient loss, the positive implications of enhanced tenderness and lower pH indicate a complex interaction in which quality parameters can coexist favourably. This balance among colour, pH, WHC and tenderness parameters underscores the need for a multifaceted approach to assessing rabbit meat quality, highlighting the importance of further studies on this topic.

CONCLUSIONS

Wrapping carcasses in high-density polyethylene plastic during the first 24 h post-mortem as an effective way of preserving the quality of rabbit meat. This technique mitigates the adverse effects of slow chilling at 4°C on prime cuts, particularly the thigh, which is particularly susceptible to cold shortening.

As a result, wrapped carcasses yield a more tender meat characterised by longer sarcomere lengths, reduced redness and lower shear force compared to unwrapped carcasses, while maintaining lipid stability. This combination enhances the sensorial appeal for consumers, despite the decrease in water-holding capacity. Additionally, the rabbit loin is proved to be lighter and more tender than the thigh, a distinction that is valuable for both consumers and producers, highlighting opportunities for optimising meat cuts to improve marketability.

Therefore, using high-density polyethylene wrapping is recommended as a practical and cost-effective method of preserving meat quality during the critical initial hours post-mortem in the rabbit industry.

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