PAPER

EFFECT OF STORAGE METHOD: AEROBIC REFRIGERATED, VACUUM REFRIGERATED AND FROZEN STORAGE, ON THE QUALITY OF CHURRA SUCKLING LAMB MEAT

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ABSTRACT

Suckling lamb meat is typically refrigerated stored for few days before being sold, although the use of vacuum and frozen storage is increasing. The effect of storage: non-stored (NS), 7-day aerobic refrigerated storage (ARS), 21-day vacuum refrigerated storage (VRS) and 3-month frozen storage (FS) on the suckling lamb quality was investigated using 32 legs (8 per method) of Churra-breed lambs. Raw meat pH, colour and water holding capacity (WHC) and cooked meat texture and oxidative stability were evaluated. ARS showed increased discoloration than NS. VRS showed the highest pH increment and the lowest hardness. FS was the lowest in WHC and lightness.

Keywords: meat colour, meat texture, oxidative stability, packaging, suckling lamb

1. INTRODUCTION

In the European Mediterranean region suckling lamb meat is typically produced in sheep farms with local breeds, and characterised by a white to light pink colour, mild flavour and soft texture (GORRAIZ *et al*., 2000; MARTÍNEZ-CEREZO *et al*., 2005a; SANTOS *et al*., 2007; TEIXEIRA *et al*., 2005). The suckling lamb meat produced under selected conditions in Castilla y León region (Spain) was granted by the EU's Protected Geographical Indication (PGI) "*Lechazo de Castilla y León*" (Commission Regulation EEC No. 2107/99). The PGI lambs must belong to one of the authorized breeds – Churra, Castellana or Ojalada – or their crosses, have to be fed on maternal milk and slaughtered under 35 d old. In 2017 the PGI-protected lambs were approximately 300 thousand, being Churra the predominant breed (SÁNCHEZ, 2018).

Suckling lamb meat is usually stored under aerobic refrigerated conditions in retail meat premises during a short period of time before being sold to the consumers (MARM, 2010). The PGI appellation allows aerobic refrigerated storage for a maximum of 8 d, as well as other storage methods in order to extend the meat's shelf-life as long as storage does not negatively affect the meat colour and edible quality. During aerobic refrigerated storage of carcasses or meat joints ageing takes place resulting in more tenderness, although meat becomes unacceptable to the consumer over time due to microbial spoilage (BELLÉS *et al*., 2017). Edible quality changes in suckling lamb joints during aerobic refrigerated storage have been studied (MARTÍNEZ-CEREZO *et al*., 2005a, 2005b; VIEIRA and FERNÁNDEZ, 2014). These studies reported that 4-5-d storage periods resulted in a decrease in meat hardness and fibrosity and that 4 d could be enough time to obtain a high-quality meat.

Retail premises are increasingly offering vacuum packaged suckling lamb joints, or chops obtained from previously vacuum packaged joints. Vacuum packaging provides an anoxic environment between the meat surface and the packaging retarding microbial spoilage. RUBIO *et al*. (2016) studied the shelf-life of vacuum packaged suckling lamb forelegs stored at 4 ºC and found a suitable storage period of up to 16 d. Ageing is not stopped by vacuum storage during the retail period (BELLÉS *et al*., 2017). Freezing prolongs meat shelf-life; however, freezing and thawing can affect juiciness, flavour and colour to a level that depends on the meat characteristics and the freezing, frozen storage and thawing conditions (LEYGONIE *et al*., 2012). No studies have addressed the effect of frozen storage on the quality of suckling lamb meat after thawing; however, there are a number of studies on its effect on the meat of older lambs (3-4 months old), concluding that thawed meat showed small differences in quality as compared to fresh meat, e.g. lower juiciness (BUENO *et al*., 2013; MUELA *et al*., 2016, 2012).

The storage-related changes in suckling lamb meat have been scarcely studied. The aim of the present study is thus to explore the effect of three different storage procedures (7-d aerobic refrigerated storage, 3-week refrigerated storage under vacuum, or 3-month frozen storage) on the colour and water holding capacity of suckling lamb meat, as well as on the texture and oxidative stability of this meat after cooking.

2. MATERIAL AND METHODS

2.1. Meat samples and storage

Thirty-two legs from Churra PGI "*Lechazo de Castilla y León*" lambs were sampled. According to the PGI specifications, the lambs were fed on ewe`s milk, slaughtered between 20 and 35 d of age, and their carcasses weighed between 4.5 to 7.0 kg. Legs were purchased from the local market on 8 different days, up to 6 legs per day, during a 1-

month period. All the sampled legs had to come from a different lamb and the post-mortem time at purchasing had to be one day. Twenty-four legs had to come from males and eight from females in order to resemble the distribution of gender in the market. After sampling all legs were carried to the lab at 0-6 ºC, wrapped with a cling film (polyvinyl chloride, PVC; oxygen permeability of 580 ml m^{-2} h^{-1}), and stored at 3 ºC until the following day.
Afterwards, the covering film, the tail, and the epiploic fat adhered on the leg surface were removed and the legs were weighed. The legs, weighing 785 g±184 g (standard deviation) were randomly assigned to one of the following storage groups, (i) Non-stored (NS), with no further storage; (ii) aerobic refrigerated storage (ARS) at 3 ºC for 7 d with the legs wrapped with PVC cling film; (iii) vacuum-packaged refrigerated storage (VRS) at 3 ºC for 21 d using a 150-μm film (polyamide/polyethylene 30/120; oxygen permeability of 1.25 ml m^{-2} h^{-1}); and (iv) frozen storage (FS), freezing the legs wrapped with PVC cling film in a freezing tunnel (-25 ºC for 60 min) and then stored at -18 ºC for 90 d followed by a 12-h (3 ºC) thawing period. The proportion of male-female samples in the assignment was balanced to adjust for the potential confounding effect of sex (8 legs per group with 2 out of them being from female lambs), which could be expected to be of little or no significant (MIGUÉLEZ *et al*., 2008). The ARS, VRS and FS groups would be representative, respectively, for whole lamb joints stored in a cool room, packaged under vacuum and frozen, for extending shelf-life. The NS group would serve as a control to compare the quality changes due to storage method.

2.2. Sample preparation and meat analysis

2.2.1 Raw meat

After the storage, the covering film was removed from the legs, the legs were weighed, and pH was measured in the *semimembranosus* muscle using a 52-32 puncture pH electrode (Crison Instruments, Barcelona, Spain). Colour was measured at three different points on the external surface of *gracilis* muscle where it showed the thinnest epimysium and lack of visible fat, using a spectrophotometer CM-700d (Konica Minolta Sensing Inc., Osaka, Japan) operating in triplicated with a D65 illuminant, SCI mode, 11 mm aperture for illumination and 8 mm for measurement, and 10° visual angle. The results were expressed according to the CIE *L*a*b** system and the ratio of reflectance at 630 nm and at 580 nm (R_{630}/R_{580}) was calculated as discoloration index in cut meat surfaces exposed to oxygen due to storage, i.e. the lower the ratio, the higher the proportion of metmyoglobin relative to oxymyoglobin plus deoxymyoglobin (AMSA, 1995).
The top-round-cap muscle group – *semimembranosus*, *adductor* and *gracilis* muscles – was then separated from the NS legs on the following day of purchasing or from the rest of the legs after the storage. This muscle group was sliced transversely into two equal-sized parts and 1 h after slicing the water holding capacity (WHC) as assessed in duplicate determining the percentage of expressible juice released by a 300 mg (290-310 mg) meat sample placed on filter paper after a 5 min compression under a 1-kg weight (GRAU and HAMM, 1957), and the colour of the cut surface following the above-mentioned procedure were determined. Chroma (the squared root of $a^{*2}+b^{*2}$) was calculated to better describe the vividness of colour in the meat surface (YOUNG *et al*., 1999).

2.2.2 Cooked meat

The legs, without the top-round-caps, were roasted (180 ºC) in a forced-air oven rotating them every 15 min until the leg core temperature reached 80 ºC, and then tempered at

20 ºC for one h. The *biceps femoris* and *semitendinosus* muscles were separated, individually vacuum packaged and frozen at -25 ºC for up to 15 d until further instrumental texture and oxidative stability analyses, which were carried out after a 12-h 3 ºC thawing period.
The texture profile analysis (TPA), as predictor for sensory texture (RUIZ DE HUIDOBRO *et al*., 2005), was determined in three 1.0-cm thick cubes cut from the long head of the *biceps femoris* muscles after being tempered at 21 °C for 1 h using a TA-XT2i analyser. The operating conditions used were a 25-kg load cell, 0.5 mm/s test speed, 10 mm/s pre- and post-test speeds, compression percentage of the initial height of 80%, perpendicularly to the muscle fibre, and a 10-s elapse between the 2 compression cycles. Oxidative stability was determined in duplicate using the thiobarbituric acid reactive substances (TBARS) test (NAM and AHN, 2003). For this purpose, the *semitendinosus* muscle was transversely cut in two similar parts. One was used for immediate analysis and the other was wrapped with PVC cling film, stored for two days at 3 ºC under darkness and then analyzed. Results were expressed as the increment in TBARS during storage, i.e the difference between TBARS before and after the two days storage.

2.3. Statistical analysis

The experimental data were analyzed using a univariate analysis of variance (ANOVA) with storage method as fixed factor – to assess weight losses the NS group was excluded of the model. When the fixed factor showed significance ($P < 0.05$) the ANOVA was followed by the least square difference (LSD) test. The SPSS Statistics software (version 23; IBM, Somers, NY, USA) was used.

3. RESULTS AND DISCUSSION

3.1. Raw meat

The mean colour values (± standard deviation) of the *gracillis* muscle for the 32 legs before storage were as follows: L^*, 44.0±2.4; a^*, 6.6±1.4; b^*, 11.6±2.3, and the mean pH was 5.74±0.08. No differences were found among the legs assigned to the different storage methods ($P > 0.05$).
The highest weight losses due to storage were found in the VRS (Table 1). The ARS weight losses were comparable to those found in legs submitted to frozen storage and thawing (FS) suggesting the suckling lamb meat to have a good ability to reabsorb water during thawing. In contrast, FS lamb showed the highest expressible juice percentage (Table 1), indicating the lowest WHC. Decreases in meat WHC due to frozen storage have been widely recognized and related to disruption of the muscle fibre structure and denaturation of muscle proteins (LEYGONIE *et al*., 2012). Freezing meat at slow freezing rates, i.e. using air temperatures between -20 and -33 ºC (as done in the present study) results in significant water diffusion from the muscle fibres into the intercellular spaces, fibre separation and myofibril damage (GRUJIĆ *et al*., 1993).
The pH of NS, ARS and FS meat did not differ significantly among them; however, a higher pH was found in the VRS meat (Table 1). The pH increment during refrigerated storage has been attributed to a switch from a glycolytic to an amino acid-degrading microbial metabolism (NYCHAS *et al*., 2008). In contrast to the present results, CALLEJAS-CARDENAS *et al*. (2014) found no significant change in lamb pH after a similar vacuum storage period. The discrepancy among studies could be attributed to the differences between the lamb ages at slaughter, i.e. 3-months vs 3-weeks in the former and present study, respectively, and explained by a faster microbial growth and/or lower muscle

glycogen content in the meat from the younger lambs. In spite of that the pH of thawed meat tends to be lower than prior to freezing (LEYGONIE *et al*., 2012), as seen in this study and in previous studies on light lamb (MUELA *et al*., 2010), frozen storage showed no effect on pH.

Table 1. Effect of storage method on leg weight loss, expressible juice of *adductor* muscle, pH and colour characteristics[a] of *gracilis* muscle surface and *adductor* muscle cut surface (after 1 h of slicing) in suckling lamb legs.

	NS (n=8)	ARS (n=8)	VRS (n=8)	FS (n=8)	SEM	*P*-level
Weight loss (%)	-	0.85[b]	1.29[a]	0.88[b]	0.136	*
Expressible juice (%)	19.9[b]	17.6[b]	21.4[b]	26.7[a]	1.462	**
pH	5.70[b]	5.75[b]	5.96[a]	5.79[b]	0.017	***
Colour of muscle surface						
*L**	42.88[a]	43.11[a]	45.23[a]	39.98[b]	0.990	**
*a**	7.34	8.02	6.76	6.22	0.630	n.s.
*b**	11.60	10.70	8.76	8.80	1.004	n.s.
R_{630}/R_{580}	2.52[a]	2.05[b]	2.43[a]	1.99[b]	0.111	**
Colour of muscle cut surface						
*L**	40.28[a]	37.54[a]	39.06[a]	33.32[b]	1.264	**
*a**	7.62	9.37	9.20	8.01	0.724	n.s.
*b**	15.12	17.42	16.05	16.63	0.611	#
Chroma	16.09	19.81	18.53	18.59	0.785	#

[a]: *L**, lightness; *a**, redness: *b**, yellowness; R_{630}/R_{580}, wavelength reflectance ratio (630 nm and 580 nm wavelengths).
NS: Non-stored, 2-day post-mortem refrigerated legs. ARS: refrigerated legs (2-day post-mortem) wrapped with air-permeable cling film and stored for 7 days at 3 ºC. VRS: refrigerated legs (2-day post-mortem) stored under vacuum for 21 days at 3 ºC. FS: refrigerated legs (2-day post-mortem) wrapped with air-permeable cling film, frozen and stored for 3 months at -18 ºC and then thawed at 3 ºC overnight.
SEM: Standard error of the mean.
P-level: n.s.: not significant; *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.
[ab]: Means in the same row showing different superscripts are significantly different ($P < 0.05$; least significant difference test).

Colour in the muscle leg surface was affected by storage (Table 1). FS resulted in a significant lower *L** value as compared to NS, ARS and VRS. Accordingly, other studies found frozen storage to decrease *L** in comparison with not-frozen meat (MOORE and YOUNG, 1991; MUELA *et al*., 2010). This difference should be attributed to changes affecting light scattering on the meat surface and could negatively affect the colour preference by lamb consumers, which has been strongly related to *L** (CALLEJAS-CÁRDENAS *et al*., 2014; HOPKINS, 1996). It might specially affect suckling lamb meat, characterized by a bright white to light pink colour (ERASMUS *et al*., 2017). As another adverse effect on leg appearance, freezing tended to produce a more pronounced red-blood colour into the superficial large blood vessels visible on the leg internal face (Fig. 1). The differences found for R_{630}/R_{580} ratio suggest that meat discoloration occurred during those storage methods allowing the higher exposure of meat to oxygen, i.e. ARS and FS, since the R_{630}/R_{580} ratio is inversely related to metmyoglobin formation (AMSA, 1995) during aerobic storage (MANCINI and HUNT, 2005). Discoloration due to oxygen exposure is

considered to be a regular phenomenon (MCKENNA *et al*., 2005; MOORE, 1990). A decrease in that ratio was previously observed during a 10-d aerobic refrigerated storage period in suckling lamb chops (MATEO *et al*., 2018). MUELA *et al*. (2016) also reported discoloration in the meat of 3-month age lambs after one month of frozen (-18 ºC) storage as detected by a trained sensory panel.

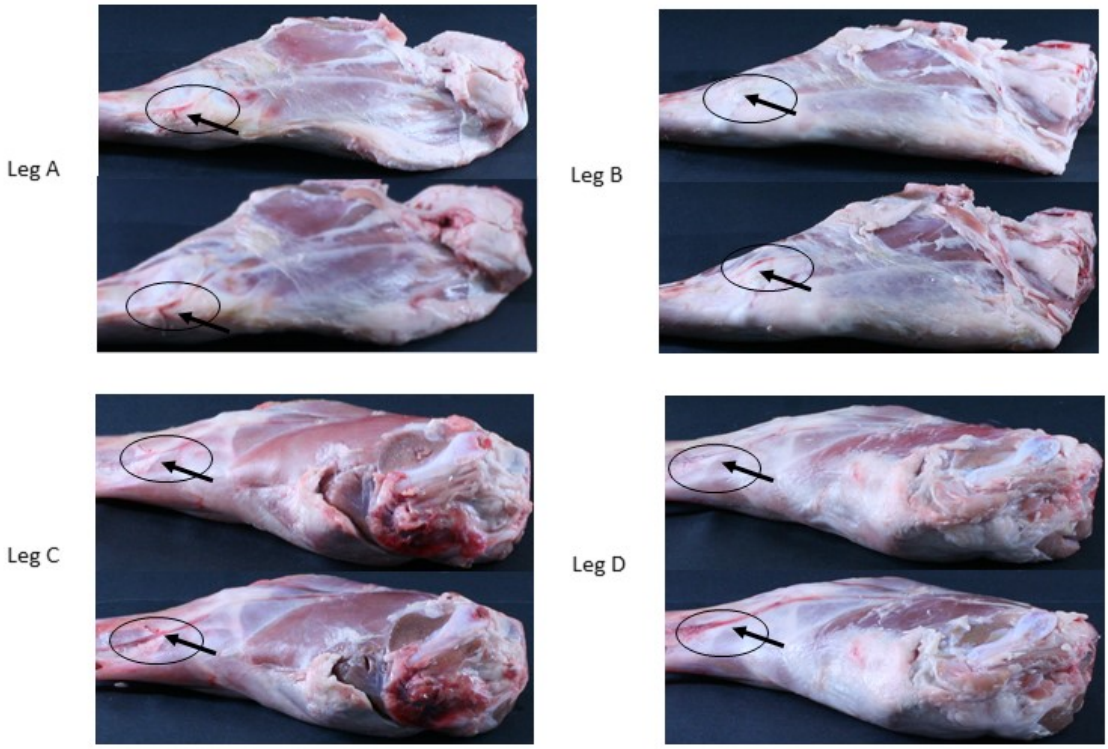

Figure 1. Pairs of photographs from four of the legs used in this study (A to D) just before being freezing (top photo of the pair) and just after thawing (bottom); an arrow and ellipse have been drawn in each photo to locate and highlight the main difference in leg appearance due to frozen storage.

The effect of the storage on the colour of cut meat after the 1-hour blooming period was significant for L^* and near-to-significant for b^* and chroma (Table 1). L^* value was the lowest in the FS meat confirming the results obtained for the colour in the whole leg muscle surface and suggesting a lower consumer colour acceptance. Furthermore, a near-to-significant differences suggest b^* higher chrome values in ARS as compared to NS meat, which should not be considered an advantage for ARS meat because suckling lamb meat is not valuated by a more vivid colour (more chroma) but by a white pale pink colour (ERASMUS *et al*., 2017). Other studies have reported an increase in b^* due to ageing as the most relevant change in the colour of sliced ruminant meat (BOAKYE and MITTAL, 1996; VIEIRA and FERNÁNDEZ, 2014). The latter of them, studying specifically suckling lamb, reported a significant increase in the b^* of cut meat surface due to a 5-d dry ageing period. However, none of those studies explained this effect.

3.2. Cooked meat

Table 2 shows the effect of storage on the TPA of cooked lamb. Hardness was significantly affected ($P < 0.05$) by storage, masticability was near-to-significantly affected ($P < 0.1$) and

no effect was detected on elasticity and cohesiveness. Significant differences in hardness were found between NS exhibiting the highest values and VRS lamb with the lowest.
There is a general agreement that refrigerated aerobic storage tends to decrease the instrumental hardness of lamb (MARTÍNEZ-CEREZO *et al*., 2005b; STARKEY *et al*., 2015), with this decrement depending on storage length. The effect of refrigerated storage on suckling lamb hardness has been however scarcely studied. VIEIRA and FERNÁNDEZ (2014) reported a decrease in instrumental and sensory hardness in cooked suckling lamb due to a 5-d storage period of carcasses when carcasses were chilled under a conventional regimen (2 ºC for 24 h); however, hardness did not decrease with storage when carcasses were slowly chilled (12 ºC for 7 h and then 2 ºC for 22 h).

Table 2. Effect of storage method on the texture profile analysis (TPA) in suckling lamb cooked meat and the lipid oxidation calculated as increment in thiobarbituric-acid reactive substances during a two-day aerobic refrigerated storage period.

	NS (n=8)	ARS (n=8)	VRS (n=8)	FS (n=8)	SEM	*P*-level
TPA						
Hardness (N)	18.90^{a}	17.06^{ab}	15.35^{b}	16.45^{ab}	8.814	*
Elasticity	0.45	0.43	0.43	0.43	0.013	n.s.
Cohesiveness	0.45	0.44	0.43	0.44	0.009	n.s.
Masticability (N)	3.87	3.24	2.88	3.10	0.264	#
Lipid oxidation due to storage (Δ mg malonaldehyde/kg of meat)	4.15	3.98	5.48	3.60	0.522	#

NS: Non-stored, 2-day post-mortem refrigerated legs. ARS: refrigerated legs (2-day post-mortem) wrapped with air-permeable cling film and stored for 7 days at 3 ºC. VRS: refrigerated legs (2-day post-mortem) stored under vacuum for 21 days at 3 ºC. FS: refrigerated legs (2-day post-mortem) wrapped with air-permeable cling film, frozen and stored for 3 months at -18 ºC and then thawed at 3 ºC overnight.
SEM: Standard error of the mean.
P-level: n.s.: not significant; # $P < 0.1$; *: $P < 0.05$.
^{ab}: Means in the same row showing different superscripts are significantly different ($P < 0.05$; least significant difference test).

Results from previous studies seem to agree with the decrease in instrumental hardness resulting from VRS. MARTÍNEZ-CEREZO *et al*. (2005b) found in suckling lamb meat from three different breeds, one of them Churra, that a 16-d vacuum storage period significantly decreased the TPA-20%-compression hardness as assessed in raw meat and associated this effect to degradation of muscle structure. Furthermore, using the same meat samples, MARTÍNEZ-CEREZO *et al*. (2005a) reported a clear and steady tenderization effect as assessed in cooked meat by a sensory panel. BÓRNEZ *et al*. (2010) also evaluated the effect of a 21-d refrigerated storage of suckling lamb joints under modified atmospheres on the hardness of meat after cooking and observed a decrease in the shear-force. The meat of 1-month age suckling lambs, as that in this study, is considered to be tenderer and easier to swallow as compared with meat from weaned older lambs, i.e. 2-month age lambs (GORRAIZ *et al*., 2000), and tenderness in suckling lamb seems to be highly valued by consumers (SAÑUDO *et al*., 2007; VIEIRA and FERNÁNDEZ, 2014). Notwithstanding, an excess in tenderness might not be desirable. Studies are needed to order to establish a low-value tenderness threshold for consumers.

In partial agreement with our results, no significant effect of a 3-month -18 ºC storage was found on the sensory tenderness of meat from lambs weighing twice as much as those of this study (BUENO *et al*., 2013; MUELA *et al*., 2012). However, frozen meat, in contrast with non-frozen meat, can show similar or lower instrumental hardness and, at the same time, higher toughness as perceived by consumer panels (LEYGONIE *et al*., 2012). This discrepancy between instrumental and sensory results has been attributed to the lower juiciness in thawed meat after cooking together with the positive relationship between sensory juiciness and tenderness. The effect of frozen storage on the sensory tenderness of suckling lamb meat deserves further study.

The increment in TBARS value during the 2-d aerobic storage period in cooked meat was near-to-significantly affected by the previous storage ($P < 0.1$; Table 2). The mean TBARS values just after cooking were 1.6±0.9 mg of malonaldehyde/kg of cooked meat, with no differences between treatments ($P = 0.610$). The value for the TBARS increment in VRS cooked meat was higher than those from the other storage methods. No studies have been found on the oxidative stability of cooked suckling lamb to compare with our results. In light lamb raw meat, neither an up-to-6-month frozen-storage of meat from 3-4-month age lambs (MUELA *et al*., 2010) nor a 18-d vacuum refrigerated storage of suckling lamb (RUBIO *et al*., 2016) resulted in increased TBARS values. A TBARS value of 2 mg of malonaldehyde/kg was suggested as the threshold for raw beef, just before being cooked, over, which rancidity flavour can be perceived by consumers (CAMPO *et al*., 2006). However, the application of this threshold to the present study should be not reliable because the TBARS thresholds for warmer-over-flavour detection appears to depend on different factors such as species, animal age, or whether the meat is raw or cooked (FERNÁNDEZ *et al*., 1997). Studies should be done to determine the TBARS levels in cooked suckling lamb above which oxidized flavours are detectable.

4. CONCLUSIONS

The use of three commonly used methods for increasing the shelf-life of Churra suckling lamb joints could diminish the meat acceptability with regard to non-stored meat. Aerobic storage for 7 d can result in discoloration at the joint muscle surface and increased colour intensity in the surface of fresh cut chops; therefore, it seems to be not advisable to use longer aerobic storage periods to that already established in the PGI's standard (8 d). Vacuum storage (21 d) would result in increased meat pH and softer meat, and frozen storage would give muscle surface discoloration in thawed meat joint and lower water holding capacity. Sensory studies are required for evaluating the association between L^* value and chroma in suckling lamb chops and consumer purchasing intent, and that of tenderness, WHC and TBARS on the meat edible quality.

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