PAPER

EFFECT OF AGEING TIME IN VACUUM PACKAGE ON VEAL LONGISSIMUS DORSI AND BICEPS FEMORIS PHYSICAL AND SENSORY TRAITS

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ABSTRACT

Study evaluated the effects of vacuum ageing (2, 4, 6, 8, 10, 12, 16 days) on veal loin (*longissimus dorsi;* LD) and silverside (*biceps femoris;* BF) physical and sensory characteristics. Ageing did not affect cooking loss, increased LD pH and L*, a* and b* in both muscles. Shear force (SF) decreased until day 6 in LD and day 10 in BF. Aroma, flavor and taste were not affected, while texture traits were improved. SF was negative correlated with tenderness and juiciness and positive correlated with BF fibrousness and stringy sensation. Ageing improved texture properties without altering other sensory traits.

- Keywords: meat quality, postmortem ageing, sensory panel, tenderness, veal calves -

INTRODUCTION

Tenderness is one of the main factors affecting consumer's preference (REICKS *et al.*, 2011). Since in EU veal calves are slaughtered at no more than 8 months of age (EU Regulation 1234/2007) consumers expect a tender meat from them. Therefore, ensuring a tender product is a critical aspect for veal producers and retailers, because tenderness is closely related to consumer's satisfaction and they are also willing to pay more for tender meat (DRANSFIELD *et al.*, 1998; FEUZ *et al.*, 2004; ALFNES *et al.*, 2005). Post mortem ageing improves meat tenderness due to the proteolysis of myofibrillar, structural and connective proteins starting from the onset of post-mortem phase (KEMP *et al.*, 2010; NISHIMURA, 2010; OUALI *et al.*, 2013). Nowadays meat cuts are extensively stored vacuum packaged, a practice that does not significantly affect veal aroma, color, appearance, flavor and texture traits when compared to traditional bone-in carcass ageing (NGAPO and GARIÉPY, 2006). In light of the different degrees of tenderness and tenderization rates among muscles (RHEE *et al.*, 2004), this technique can allow to maximize tenderization through the different duration of postmortem ageing, based on specific muscle or commercial cut characteristics. Since veal is not commonly aged in commercial practice, it is necessary to evaluate the effects of long term chilling storage not only on meat tenderness, but also on physical and sensory properties that can affect it. For example, color is an important aspect for veal quality, so preserving veal appearance is essential. However, in some studies prolonged ageing has led to development of off-flavor in beef (SPANIER, 1997). The aim of this study was to evaluate the effect of postmortem ageing time in vacuum package at refrigeration temperature on physical and sensory parameters of veal loin, *(m. longissimus dorsi; LD*) and silverside (*m. biceps femoris; BF*), frozen after ageing and then thawed before quality evaluation, in order to simulate a typical consumer habit (JEREMIAH, 1996). The first cut was selected due to its economic significance, while the second one because its recognition as a less tender hindquarter beef cut when cooked with dry-heat cooking methods (SULLIVAN and CALKINS, 2011), failing thus consumers' expectation for tenderness.

MATERIAL AND METHODS

Two (2) days post mortem, 8 right loin (LD muscle from 6th rib to the 6th lumbar vertebrae) and silverside (BF muscle) whole primal cuts were collected from the carcasses of 8 male milk-fed Holstein veal calves. Calves were similar in age (231±16 d) and sourced from the same farm, being fed the same diet and slaughtered on the same day. Cold carcass weight (163.50±15 kg), conformation (SEUROP conformation score: R), fatness score (European fatness score 1-5: 2) and serum lactate (54.52±1.32 mg/dL) were similar. The serum lactate was determined using blood samples collected during exsanguination by the Central Laboratory of the Veterinary Hospital of the University of Milan using a commercial kit (Sentinel Diagnostics, Milan, Italy). This evaluation was aimed to assess differences in individual animal stress level, which can impair meat tenderness (GRUBER *et al.*, 2010). After collection, each muscle was divided in 8 subsamples and each of them was then vacuum packaged. Subsamples were assigned to one of the seven different postmortem ageing treatments: 2, 4, 6, 8, 10, 12 and 16 days randomized, while the remaining one was used to determine chemical composition. Subsamples distribution between treatments was done ensuring that each portion of the muscle was equally represented in every ageing time, as reported by MANDELL *et al.* (2001). All subamples were kept at 0°C until the end of the established ageing period before being frozen at -20°C, as done by CAMPO *et al.* (2000) and MANDELL *et al.* (2001). Prior to measurement, subsamples were thawed for 24 hours at 4°C and from each subsample a 1.50 cm steak was removed for sensory evaluation, while the remaining part was used for physical and chemical analysis.

Physical and chemical analysis

Chemical composition (dry matter, ether extract, crude proteins and ash) was determined, according to AOAC (2000), on designated samples trimmed from the external fat and connective tissue, and homogenized for 30 seconds. On each subsample subjected to different aging time, a fresh cut surface was created removing a slice perpendicular to the fiber axis and, after blooming for 60 minutes in a dark room at 4°C, its color was assessed by a CR-300 Chroma Meter device (Minolta Camera, Co., Osaka, Japan) calibrated on the CIE L*a*b* (CIE, 1976) color space (Calibration Plate 21533131 Y 93.4 x 0.3456 y 0.3321, Minolta Cameras). The Chroma Meter had an 8-mm measuring area, was set in D-65 lighting, and an average of 10 repetitions was recorded as the value for each sample. pH was measured with a portable pH-meter (HI 98150, HANNA Instruments Inc., Woonsocket, RI, USA) on a homogenate prepared by grinding the slice removed to create a fresh cut surface and mixing it with deionized water. Cooking loss was determined, as described by HONIKEL (1988), as the weight lost after cooking in water bath, until core temperature attained 75°C (monitored with a temperature meter Hanna Instruments HI98840) and 24 hours of storage at 4°C. Before being weighed after cooking, samples were blotted dried. The difference between pre- and post-cooking weights was used to calculate the percentage lost during cooking (cooking loss). After cooking loss determination, from 2.5 cm thick cooked samples six cylindrical cores (1.27 cm in diameter), parallel to fiber orientation, were obtained and used for shear force (SF) evaluation with a Warner-Bratzler shear force texture analyzer (model 4466; Instron Corp., Canton, MA). Peak force (kg/cm^2) was recorded for each core and the average of six values per sample was used for the statistical analysis.

Sensory analysis

Steaks of BF and LD samples were cut to 1.5 cm thick and cooked for 60 seconds at the greatest power (200°C) on double-plated grills, before being cut into 1.5 cm cubes. Core temperature was monitored with a thermocouple (Pentronic AB,198 Gunnebobruk, Sweden) and it was not allowed to exceed 68°C. Sensory evaluation was performed by 10 expert and trained judges (UNI EN ISO 13299:2010), confident with meat sensory evaluation, on each sample aged for 2, 4, 8, 10 and 16 days. Three cubes per samples were presented on white plastic plates to each panelist, which during training and sampling, had access to unlimited water and unsalted crackers and each sample was identified by 3-digit codes. Judges were trained in two tasting session with the aim to allow them to find and familiarized with sensory descriptors relative to veal aroma, taste, flavor and texture. At each judge was asked to evaluate the intensity of each attribute by assigning a score between 1 (absence of the sensation) and 9 (extremely intense sensation). Descriptors (Table 1) includes the main beef sensory parameters and some of the defects that could affect vacuum packaged aged meat.

Statistical analysis

Statistical analysis was performed using SAS® 9.3 (SAS Institute Inc., 2012 Cary NC) software. Data from the physical analysis was analyzed by one-way ANOVA, considering post mortem ageing time as the main effect. Data from sensory profile were analyzed by three-way ANOVA considering the effects of judge, replications and ageing time and their interactions. Least square means were compared according to F test, with the level of significance set at P≤0.05. Pearson correlation analysis was also performed to evaluate the relationship between SF and sensory texture characteristics.

Table 2 - Average chemical composition of the muscles sampled for the trial (least square means±SD).

Trait	*Longissimus dorsi*	*Biceps femoris*
Moisture, g kg^{-1}	751.72±5.12	754.91±3.32
Ash, g kg^{-1}	12.34±1.04	11.72±0.65
Crude Protein, g kg^{-1}	211.50±3.22	212.24±3.96
Ether extract, g kg^{-1}	24.52±3.24	21.20±2.47

RESULTS AND CONCLUSIONS

Physical and chemical characteristics

The average chemical composition of LD and BF muscles is summarized in Table 2. Results are consistent with data reported for lean veal meat in some national food composition databases (Denmark: National Food Institute, 2009; USA: United States Department of Agriculture, 2011). Although a significant effect (P=0.05) of ageing time on LD pH was found (Table 3), it increase only from 2 to 8 days of ageing, while no significant differences were evident since the day 4 of ageing. Regarding BF, its pH was not affected by ageing time. This last data is consistent with other studies that found no differences in veal pH during 7 (REVILLA *et al.*, 2006) or 14 days of ageing (OLIETE *et al.*, 2006), and the review by

Table 1 - Descriptors, definitions and standards for sensory analysis.

Attribute		Definition
Aroma	Veal	Aroma associated with cooked veal loin
	Metallic	Aroma associated with blood or rare meat
	Off flavor	Aroma associated with meat at the end of shelf life
Taste	Salty	Salty taste
	Sweet	Sweet taste
Flavor	Veal	Flavor associated with cooked veal loin
	Metallic	Flavor associated with blood or rare meat
	Off flavor	Flavor associated with meat at the end of shelf life
Texture	Tender	The force needed to masticate the meat ready for swallowing (chewing 5 times)
	Fibrous	Presence of fibers during swallowing
	Juicy	The degree of juice released while chewing the meat
	Stingy	Production of a large quantity of saliva for swallowing

NGAPO and GARYÉPI (2006), that suggests post-mortem ageing did not increase veal pH. However, although the reported slight differences in LD pH values across post mortem times, it fell within the normal range (5.40-5.70) for both muscles. Cooking loss (Table 3) was not affected by ageing time in either muscle. Findings are in agreement with other works (KLONT *et al*. 2000; MANDELL *et al*. 2001), even if is difficult to make a comparison with the previous study due to the different cooking methods and endpoint temperatures to between studies. However, for both muscles, results of the present study are intermediate between the cooking loss values reported by the previous authors (19.1-38.2%). Regarding color parameters (Table 3), ageing increased lightness (L*), redness (a*) and yellowness (b*) in both muscles (P≤0.01). This concurs with MANDELL *et al*. (2001), which suggested color parameters tended to increase only during the first week of ageing, before becoming stable. INSAUSTI *et al*. (1999) also found L* to increase during vacuum storage in *longissimus dorsi* of young cattle. Lightness increasing can be attributed, as reported by KLONT *et al*. (2000), to the increasing of meat light scatter properties due to post mortem protein denaturation and degradation. OLIETE *et al*. (2006) found an increasing of a* and b* measured 1 hour after blooming in vacuum packaged veal and young cattle *longissimus dorsi*. These studies attributed the increasing in redness to the faster blooming of aged meat. Indeed, the more meat is aged the faster it blooms because of the reduced activity of enzyme that compete for oxygen with Mb. The rising of yellowness was, instead, attributed the increasing of metmyoglobin formation during storage time. A lightness increasing can exert a positive effect on veal appearance, while an improvement of redness can represent a negative factor. Indeed, in several studies on veal carcasses, a decreasing in lightness and an increasing in redness moving from lightest to darkest veal was reported, while b* was not related to color score (DENOYELLE and BERNY, 1999; HULSEGGE *et al*. 2001; LAGODA *et al*. 2002; VANDONI and SGOIFO ROSSI, 2009). The magnitude of L*, a* and b* increasing, higher than those found in the study of MANDELL *et al*. (2001), could be promoted by the combination of freezing and thawing and blooming time, this latter not applied by MANDELL *et al*. (2001), that can have exacerbated the impact of ageing on

Table 3 - Effect of ageing time on veal LD and BF physical traits (least square means±SEM).

	Ageing time							
	2 d	**4 d**	**6 d**	**8 d**	**10 d**	**12 d**	**16 d**	**p**
				pH				
LD	5.54±0.04 a	5.62±0.04 ab	5.63±0.04 ab	5.70±0.04 b	5.68±0.04 b	5.71±0.04 b	5.70±0.04 b	0.05
BF	5.60±0.04	5.59±0.04	5.63±0.04	5.63±0.04	5.68±0.04	5.68±0.04	5.67±0.04	NS
				cooking loss, %				
LD	25.41±0.53	26.16±0.53	25.72±0.53	25.96±0.53	25.98±0.53	25.79±0.53	25.66±0.53	NS
BF	28.81±0.11	28.55±0.11	29.87±0.11	28.81±0.11	29.85±0.11	27.62±0.11	28.77±0.11	NS
				L*				
LD	48.20±0.68 a	50.32±0.47 b	51.00±0.54 bc	52.91±0.93 cd	52.80±0.90 cd	52.49±0.86 cd	53.08±0.90 d	≤0.01
BF	48.51±0.53 a	50.46±0.43 b	50.71±0.52 b	52.74±0.75 c	53.04±0.55 c	53.12±0.56 c	53.88±0.55 c	≤0.01
				a*				
LD	9.71±0.47a	10.27±0.34 a	12.82±0.40b	12.49±0.68 b	12.52±0.66 b	12.57±0.63 b	12.53±0.66 b	≤0.01
BF	10.85±0.25 a	11.27±0.20 a	13.70±0.25b	14.86±0.34 b	14.87±0.27 b	14.11±0.25 b	14.03±0.26 b	≤0.01
				b*				
LD	9.86±0.25 a	10.52±0.17 b	11.97±0.20c	12.22±0.34 c	12.41±0.33 c	12.73±0.31 c	12.87±0.33 c	≤0.01
BF	10.58±0.25 a	11.23±0.20 b	12.46±0.25c	13.62±0.34 d	13.65±0.27 d	13.39±0.25 d	13.22±0.26 d	≤0.01
				SF, kg				
LD	2.89±0.15 a	2.59±0.11 ab	2.42±0.16 bc	2.21±0.15 c	2.12±0.18 c	2.09±0.21 c	2.05±0.17 c	≤0.01
BF	2.89±0.13 a	2.73±0.09 ab	2.65±0.13 ab	2.45±0.13 bc	2.22±0.11 cd	2.19±0.11 cd	1.96±0.11 d	≤0.01

a,b,c,d in the same row indicates significant differences between the different ageing times.

meat color stability. Indeed, freezing and thawing promote myoglobin denaturation, increase susceptibility to oxidation and reduce the activity of metmyoglobin reducing enzymes. These effects, coupled with the loss of NADH (cofactor of these enzymes) in the exudate, reduce meat color and oxidative stability as reviewed by LEYGONIE *et al*. (2012).

Post mortem ageing of LD reduced SF (P≤0.01), but there were no further improvements in tenderness after 8 days of ageing (Table 3). These findings are consistent with MANDEL *et al*. (2001), who reported decreases in SF for LD and semimembranosus muscles comparing veal aged for 2 days with veal aged for at least 7 days, but the same study found no differences in SF for ageing periods beyond 7 days. Furthermore, REVILLA *et al*. (2006) also found a reduction in SF loin during 7 days of ageing. Eight days of ageing was needed to significantly decrease SF values for 2 days aged BF; there was no further improvement in SF values until BF was aged for 16 days. This slower tenderization rate of BF compared to LD agrees with data relative to this muscle collected from lean beef carcasses graded as USDA quality grade Select (GRUBER *et al*., 2006).

Sensory analysis

The F values of ageing time for aroma, taste, flavor and texture parameters of LD and BF sensory profile are reported in Table 4 and Table 5, respectively. Results indicated that postmortem ageing time affected (P ≤0.01) sensory texture of both LD and BF. In particular, postmortem ageing improved LD sensory tenderness (P≤0.01). Tenderness was higher at day 4 days in comparison with day 2, and at day 16 in comparison to day 4 (Tab. 4). Increasing ageing from 2 to 4 days also improved juiciness (P≤0.01) and reduced stringy sensation (P≤0.01). Improvements in eating quality associated with ageing were also perceived for fibrousness, with significant reduction (P≤0.01), starting from the 8^{th} days post mortem. These results are consistent with Mandell *et al*. (2001), which found an increase in perceived LD tenderness comparing samples aged 2 days with the average of the values recorded for samples aged 7 and 14 days, while no significant difference was detected increasing ageing period from 7 to 14 days. BF sensory analysis (Table 5), showed that perceived tenderness significantly increased with ageing (P≤0.01) from days 2 and 4 to day 8, and also between 8 to 16 days post mortem. Juiciness was improved (P≤0.01) from 2 to 8 days of ageing, but no further. Fibrousness was also reduced (P≤0.01) from 2 to 8 days and from 8 to 16 days of ageing and stringy sensation decreased (P≤0.01) from 2 to 4 days and from 4 to 10 days of ageing, but no further. The improvement of perceived tenderness and juiciness, as well as reductions in fibrousness and stringy rankings, are common when sensory panels evaluate the effects of postmortem ageing on beef palatability attributes (JEREMIAH and GIBSON, 2003; MILLER *et al*. 1997 and

Table 4 - Effect of ageing time on LD sensory profile (least square means).

Descriptors	F value	2 d	4 d	8 d	10 d	16 d	SEM	p
				Aroma				
Veal	1.47	6.98	6.77	6.62	6.92	6.65	0.20	N.S.
Metallic	1.32	4.18	4.29	4.59	4.17	4.87	0.32	N.S.
Off flavor	3.02	2.33	2.15	2.52	2.37	2.87	0.22	N.S.
				Taste				
Sweet	1.14	4.67	5.27	5.07	5.20	5.38	0.32	N.S.
Salty	1.47	3.81	4.32	3.83	3.68	4.22	0.30	N.S.
				Flavor				
Veal	0.89	6.62	6.82	6.50	6.82	6.95	0.24	N.S.
Metallic	1.56	4.12	4.61	4.34	3.92	4.77	0.32	N.S.
Off flavor	1.64	2.97	2.21	2.96	2.88	3.08	0.31	N.S.
				Texture				
Tender	16.21	4.92 a	6.35 b	6.77 bc	6.88 bc	6.94 c	0.23	≤0.01
Juicy	10.82	4.75 a	5.79 b	5.95 b	6.34 b	6.33 b	0.26	≤0.01
Fibrous	4.94	5.05 a	3.95 b	3.87 b	3.70 b	3.57 b	0.30	≤0.01
Stringy	3.89	4.68 a	3.96 ab	3.62 b	3.32 b	3.34 b	0.29	≤0.01

a,b,c in the same row indicates significant differences between the different ageing times.

Table 5 - Effect of ageing time on BF sensory profile (least square means).

Descriptors	F value	2 d	4 d	8 d	10 d	16 d	SEM	p
				Aroma				
Veal	1.00	6.69	6.60	6.80	7.04	6.93	0.18	N.S.
Metallic	1.50	4.15	4.43	4.31	3.81	3.71	0.29	N.S.
Off flavor	0.19	2.31	2.40	2.42	2.35	2.26	0.19	N.S.
				Taste				
Sweet	0.74	5.00	5.12	5.34	5.41	5.31	0.27	N.S.
Salty	2.27	3.73	3.84	4.33	3.88	4.33	0.28	N.S.
				Flavor				
Veal	1.47	6.38	6.48	6.67	6.94	6.52	0.20	N.S.
Metallic	2.47	4.04	4.78	4.44	4.25	4.36	0.29	N.S.
Off flavor	1.54	3.04	3.00	2.58	3.27	2.29	0.22	N.S.
				Texture				
Tender	15.43	3.80 a	4.52 a	5.65 b	6.38 bc	6.64 c	0.31	≤0.01
Juicy	5.83	4.27 a	4.57 ab	5.13 b	5.80 b	5.80 b	0.29	≤0.01
Fibrous	9.88	6.58 a	5.34 b	4.78 bc	4.15 cd	3.76 d	0.33	≤0.01
Stringy	21.37	6.64 a	5.81 ab	5.00 bc	4.41 cd	3.39 d	0.30	≤0.01

a,b,c,d in the same row indicates significant differences between the different ageing times.

CAMPO *et al*. 1999). In the latest study a multivariate approach (Principal Component Analysis) was used to differentiate aged from unaged meat. This indicated that aged meat was characterized by tenderness and juiciness sensation, while unaged meat was characterized by fibrousness and residue (similar to stringy sensation) ones. In the present study post mortem ageing did not affect aroma, flavor and taste for both muscle. This disagrees with MANDELL *et al*. (2001), where meat flavor was improved by ageing veal more than 7 days. There was a low incidence for the panel detecting undesirable palatability attributes such as metallic aroma and flavor and off flavor. This concurs with JEREMIAH and GIBSON (2003), that found low levels of off flavor and metallic aroma/flavor attributes in beef, regardless of post mortem ageing time. Furthermore, the same authors, did not find differences in off or metallic aroma and salt and metallic flavor prolonging ageing time until 28 days. The lack of effect of ageing time on negative sensory descriptors is an important outcome, as some past studies have reported increases in undesirable flavor and aroma defects for beef after prolonged ageing (SPANIER *et al*. 1997 and MONSÓN *et al*. 2005).

Correlation between SF and texture sensory traits

Based on Pearson correlation coefficients to examine the relationship of sensory texture characteristics and SF for both muscles, there was a negative relationship between SF and sensory tenderness (r=-0.67; P≤0.01 and r=-0.83; P≤0.001 for LD and BF respectively) and juiciness (r=-0.53; P≤0.05 and r=-0.72; P≤0.01 for LD and BF respectively). The negative correlation between SF and sensory tenderness is in agreement to the findings of SHACKELFORD *et al*. (1999) in beef and MONTEIRO *et al*. (2013) in veal. Positive correlations were found between SF and fibrousness (r= 0.78; P≤0.01) as well as SF and stringy sensation (r= 0.78; P≤0.01) for BF. These findings agrees with the positive correlation between fibrousness and SF found by Caine *et al*. (2003) and the negative correlation between SF and juiciness found by MONTEIRO *et al*. (2013). The lack of relationship between SF with stringy and fibrousness rankings for LD muscle in respect to BF muscle could be explained by the lower collagen content of LD relative to BF (RHEE *et al*. 2004). Indeed, fibrousness and stringy sensation were lower in LD muscle and the lower detectability could have been at the basis of the lack of significant interaction.

Our results indicate that postmortem ageing under vacuum conditions improved the instrumental and sensory tenderness rankings for veal *m. longissimus dorsi* and *m. biceps femoris*, without any negative effects on the main meat sensory traits such as aroma, flavor, taste and juiciness measured after frozen storage and thawing. Ageing, coupled with freezing and thawing, have, however, reduced oxidative stability in both muscles, without affecting other veal technological properties as cooking loss and pH. There were different postmortem tenderization trends for each muscle evaluated in the study. The improvements in LD tenderness and related sensory traits occurred mainly during the first week of postmortem ageing, while in BF postmortem ageing effects were also evident until the tenth day. At these experimental conditions, a minimum period of 4 days for LD muscle and 8 days for BF muscle was necessary to obtain a perceivable tenderizing effect. A prolonged ageing, for at least one week for veal LD and two weeks for veal BF can be applied for frozen veal, mainly destined for ho.re.ca market, in which product appearance is a secondary trait, while tenderness is the primary goal. Vacuum ageing could be also apply for fresh veal market, considering indeed its potentially lower impact than that emerged in this study on oxidative stability, as veal will not undergone to freezing and thawing process before being prepared for retail exposition.

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