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Assessment of frozen storage duration effect on quality characteristics of various horse muscles

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Objective: The study aimed at assessing the effects of frozen storage duration on quality characteristics, lipid oxidation and sensory quality of various horse muscles.
Methods: Five representative muscles: *longissimus dorsi* (LD), *gluteus medius* (GM), *semimembranosus* (SM), *biceps femoris* (BF), and *triceps brachii* (TB) at 24 h post-mortem obtained from 28-mo-old Jeju female breed horses (n = 8) were used in the present investigation. The muscles were vacuum-packaged and frozen at –20°C for 120, 240, and 360 days. All the samples were analyzed for thawing and cooking losses, pH, Warner–Bratzler shear forces (WBSF), color traits, total volatile basic nitrogen (TVBN), thiobarbituric acid reactive substances (TBARS) and sensory traits. The muscle samples analyzed on day 0 of frozen storage (fresh, non-frozen) were used for comparison.
Results: Results revealed that thawing and cooking losses significantly ($p<0.05$) increased in all the muscles after 120 days and then remained unchanged up to 360 days of frozen storage. The TBARS and TVBN contents significantly increased as increasing frozen storage time up to 360 days ($p<0.05$). While, significant decreases in WBSF values were observed for all the muscles with increased frozen storage time ($p<0.05$). Frozen storage variously affected the color traits of the muscles for instance; the redness of LD, GM, and BF muscles showed a decreasing tendency during frozen storage while it was not changed in TB and SM muscles. Furthermore, the frozen storage did not produce detrimental effects on sensory quality as it did not cause flavor and juiciness defects whereas it partially improved the tenderness of all the muscles studied.
Conclusion: Based on the results obtained from our work, it is concluded that frozen storage could be applied to increase the long-term shelf life of horsemeat while still retaining its sensory quality.

Keywords: Horsemeat; Muscle; Frozen Storage; Lipid Oxidation; Sensory Quality

INTRODUCTION

Horsemeat has been regarded as a human food for thousands of years in many countries, and recently is considered as "healthier" due to its higher polyunsaturated fatty acid contents and poly-unsaturated fatty acids/saturated fatty acids ratio in comparison to other conventional red meats [1,2]. According to the data published by the Food and Agriculture Organization (FAO) [3], the world horsemeat production kept constant from the years 1965 to 1990 at an average of 5 thousand tonnes per year and then increased by 40% in 2013 to approximately 700,000 tonnes. Asia is one of the largest horsemeat-producing regions while South America and Western Europe are the largest exporting and importing regions, respectively.

Though horse slaughter for human consumption and trade activities appear quite common in many countries, however, little attention has been paid to the factors affecting its quality characteristics. Furthermore, most studies have only focused on evaluating the effects of pre-harvest factors such as breed, sex and feeding diets, [4] while only few studies have been conducted

to determine the effects of post-harvest factors such as; chilled ageing [5] or muscle types [6] on the quality characteristics of horsemeat. In general, these studies have reported that the pre- and post-harvest factors both have considerable impacts on the horsemeat quality and sensory characteristics.

In the meat industry, in order to keep meats fresh and safe for long periods, meat is usually stored under refrigerated or freezing conditions. Of which, freezing is one of the oldest and most common preservation method because it can increase the long-term shelf life of meat while still retaining properties similar to those of fresh meat [7]. Importantly, the application of frozen storage would partly help to stabilize markets and provide retailers with a higher flexibility [8]. A significant number of studies have been conducted to investigate the effects of freezing methods and frozen storage duration on the quality of conventional red meats such as beef and lamb [7,9]. These studies have indicated that the frozen storage of meat for several months has a considerable impact on meat quality such as; increases in moisture loss and protein denaturation and reduction in number of viable microbes.

Short shelf-life is a major concern in the marketing of any meat type therefore it is necessary to find ways to prolong its shelf-life stability. In fact, horse meat is widely consumed and the exporting and importing of this meat type is quite common thoughout the world. However, to the best of our knowledge, no attention has been paid to the effects of storage method and conditions on the quality and sensory characteristics of horsemeat. In order to promote the consumption, processing and trade activities of horsemeat more detailed studies on the factors (e.g., storage methods) affecting the quality of this meat type are required. The present research aimed at investigating the effects of frozen storage durations (0, 120, 240, and 360 days) at –20°C on the quality characteristics of various horse muscles. Our study results could provide additional scientific information that may be helpful for the standardization of sustainable horsemeat processing, storage and production.

MATERIALS AND METHODS

Animals and sample preparation

Jeju female breed horses (n = 8) with their live weight of about 280 to 350 kg obtained from a local farm in Jeju province, Korea, were used in the present study. The raising procedures, feed content and feeding regimes applied for the animals were similar to those described in our previous study [5]. The animals were transported from the farm to the abattoir using an animal transporter truck and attempts were made to minimize stress in the animals. Before slaughter, the animals were fasted from feed for ~14 h, but given access to water. The slaughter was carried out at a slaughter plant of the National Institute of Animal Science (Suwon, Korea) under commercial conditions. After stunning with a captive bolt, the horses were then slaughtered, dressed, split, and finally transferred to a chilling room (2°C±2°C) for 24 h. The following day, the carcass sides were transferred to a cutting room where five representative muscles including: *longissimus dorsi* (LD), *gluteus medius* (GM), *semimembranosus* (SM), *biceps femoris* (BF), and *triceps brachii* (TB) muscles were taken from both sides (left and right) of each carcass. Thereafter, each muscle was divided into two equal parts (size of parts ranged from 1.0 to 2.5 kg depending on each muscle type), so 4 parts (2 parts/carcass side×2 sides) per carcass were collected. After the initial weighs were recorded, all the samples were placed in oxygen impermeable polyethylene bags, labelled, vacuum-packaged and randomly assigned to 0 (non-frozen, fresh), 120, 240, and 360 days frozen storage groups. The non-frozen (day 0) samples were immediately used for analyses while the other remaining samples were vacuum-packaged, placed in paper boxes and transferred to the freezing room. The frozen storage was carried out at –20°C. On completion of each freezing period, the samples were thawed at 4°C for 16 h, re-weighed to determine freezing loss and then used for further analyses.

pH measurement

The pH values of muscle samples at each frozen storage period were determined in triplicates using a pH*K 21 (NWK-Technology GmbH, Lengenfeld, Germany) equipped with a stainless steel probe, ensuring that the probe was inserted deep into the tissue. The pH meter was calibrated with pH 4 and 7 standards (NWK Technology, Bayerntrasse, Kaufering, Germany) before use.

Color measurement

The instrumental colors were measured on the newly cut surface of samples at five different locations after 30 min of blooming (the blooming was conducted by directly exposing the samples to the atmosphere at room temperature) using a CR-400 chroma meter (2 degree observer and illuminant D65; Minolta Co., Osaka, Japan). Prior to use, the instrument was calibrated with a provided white plate (Y = 86.3, x = 0.3165, and y = 0.3242), and values were expressed according to the Commission International de l'Eclairage (CIE) system and reported as L*(lightness), a* (redness), and b* (yellowness).

Cooking loss and Warner–Bratzler shear force

The cooking loss and Warner–Bratzler shear force (WBSF) of samples at each frozen storage period were determined following the procedures as described in our previous work [5]. Particularly, each muscle sample (3.0-cm thick) of about 200 to 250 g were placed in plastic bags, put in a pre-heated 72°C water bath (Water bath BS-21, Jeiotech Co., Seoul, Korea) and then heated until their core temperature had reached 70°C. The core temperature of samples was monitored using a copper-constantan thermocouple attached to a thermo recorder (Model TR-71U; T and D Corp., Tokyo, Japan). The cooked samples were immediately cooled in a circulatory water bath for 30 min, thereafter; they

were removed from the polyvinylchloride film bags, absorbed with blotting-paper and re-weighed to determined cooking loss. After cooking loss determination, the cooked samples were used for WBSF analysis. For the WBSF analysis, five strip core samples removed parallel to the muscle fibre direction of each muscle were used. The WBSF values (kilogram force, kgf) of each muscle sample were obtained by completely cutting the strips in an Instron Universal Testing Machine (Model 4465, Instron Corp., High Wycombe, UK) using a crosshead speed of 200 mm/min and a 40-N load cell.

Thiobarbituric acid reactive substances

Thiobarbituric acid reactive substances (TBARS) content was determined to evaluate the lipid oxidation levels in samples, following the procedure of Buege and Aust [10]. The samples (5 g each) added with distilled water (15 mL), saturated butylated hydroxyansole (50 μL) and 20 mL of thiobarbituric acid (0.02 M)/ trichloroacetic acid (15% w/v) (TBA/TCA at 1:1 ratio) were homogenized at 11,000×rpm for 15 s using an Ultra-Turrax T25B. The volume of the sample homogenate was adjusted to 50 mL with the TBA/TCA solution and immediately placed on ice. The tube containing homogenate was immersed in a 90°C water bath for 15 min. After removal from the water bath, the sample tubes were immediately placed on ice to cool for 20 min and centrifuged at 3,000×g for 10 min using an Avanti J-E centrifuge (Beckman Coulter Inc., Brea, CA, USA). About 1.5 mL of supernatant was taken and the absorbance was measured at 531 nm using an UV–visible spectrophotometer (ProteomeLab Du-800, Beckman Coulter, Inc., USA). The TBARS content was calculated on the sample weight basis and expressed as mg malondialdehyde/kg (MDA/kg) of meat. Three repetitions were applied for each sample in each treatment.

Total volatile basic nitrogen

The total volatile basic nitrogen (TVBN) content was determined using the method of Min et al [11] with minor modifications. Briefly, a 10 g sample (each) was homogenized with 50 mL of distilled water and filtered through Whatman filter paper (No.1). The filtrate (1 mL each) was placed into the outer space of the Conway tool, and then 1 mL of 0.01 N H_3BO_3 and 2 to 3 drops of Conway reagent (0.066% methyl red:0.066% bromocresol green, 1:1) were also added to the inner space. Thereafter, 1 mL of 50% K_2CO_3 was added to the outer space of the Conway tool and was sealed immediately. The sealed Conway tool was incubated at 37°C for 2 h in an incubator. At final stage, different volumes of 0.02 N H_2SO_4 were added to the inner space to calculate the TVBN; the content was expressed as mg% TVBN/100 g meat.

Sensory evaluation

The sensory evaluation for the samples in the present study was performed following the procedure as described in our previous study [5]. Consumer panels consisted of 12 trained members who were staff at the Animal Products Processing Division of National Institute of Animal Science. For the sample preparation, the thawed samples (each) were cut into slices (40 mm×40 mm×4 mm) using a knife, 6 representative slices were used for the sensory evaluation. Cooking was done on open tin-coated grills (surface temperature ranged from 230°C to 240°C) and the samples were cooked for 2 min (turned every 30 s for 3 times). Two sets of grills were used where each the grill was set to cook six slices of samples. The cooked samples were immediately dispensed on individual plates and served to the panelists. Each sample was evaluated by six panelists. The samples were evaluated for tenderness, juiciness and flavor using 7-point scales [12] in which; flavor (1 = undesirable flavor, 7 = desirable flavor); tenderness (1 = very tough, 7 = very tender); juiciness (1 = very dry, 7 = very juicy). After evaluation of each sample, the panelists were asked to rinse their mouth with the distilled drinking water and salt-free crackers.

Statistical analysis

The statistical analysis system (SAS) package (SAS Institute, Cary, NC, USA) [13] was used for analysis of obtained data. The data were analyzed by using the general linear model procedure considering muscle type and frozen storage period as the main effects. The differences between means were compared by using Duncan's multiple range test, and significance was defined at $p<0.05$.

RESULTS AND DISCUSSION

Effect on freezing loss and pH

The thawing loss percent and pH values of horse muscles during frozen storage are presented in Table 1. The thawing loss levels ranged among the muscles from 5.12% to 10.53%, 6.83% to 12.15%, and 6.04% to 12.14% after 120, 240, and 360 days of frozen storage, respectively. It was observed that the thawing loss significantly ($p<0.05$) increased in all the muscles studied after 120 days and did not increase thereafter. Thawing loss is an important quality trait which reflexes the water-holding capacity of frozen/thawed meat. The increased thawing loss in the samples during frozen storage could be due to the formation of ice crystals and the increased size of ice crystals that cause protein denaturation, thus reducing their water-holding capacity [14]. Also, comparing the thawing loss among some muscles showed statistical differences for instance; the loss levels (5.64% to 6.95%) in TB muscle were lower than the level (9.53% to 12.15%) in the GM muscle at all periods examined ($p<0.05$). The variations in the thawing loss levels observed among the muscles could be due to the differences in damaged levels to muscular cells ultrastructure during frozen storage [9]. When compared to the thawing loss levels observed in all muscles in the present study, Muela et al [7] reported lower values (2.68%) for lamb *longissimus lumborum* muscle frozen for 6 months at –18°C and Vieira et al [15]

Table 1. Thawing loss and pH values (mean±standard error) in horse muscles during frozen storage at –20°C

Muscles	Thawing loss (%)				pH			
	0 d (non-frozen)	120 d	240 d	360 d	0 d	120 d	240 d	360 d
LD	0	5.12 ± 1.07^{cB}	6.83 ± 0.96^{bA}	7.06 ± 0.65^{cA}	5.75 ± 0.49^{abB}	5.88 ± 0.03^{aA}	5.85 ± 0.03^{abAB}	5.85 ± 0.05^{bAB}
GM	0	9.53 ± 0.84^{abB}	12.15 ± 0.51^{aA}	11.64 ± 0.68^{abA}	5.67 ± 0.03^{c}	5.65 ± 0.21^{b}	5.68 ± 0.02^{c}	5.66 ± 0.01^{c}
BF	0	7.08 ± 0.53^{bcB}	10.05 ± 1.03^{abA}	9.80 ± 0.70^{bA}	5.71 ± 0.01^{bc}	5.70 ± 0.02^{b}	5.87 ± 0.03^{bc}	5.71 ± 0.03^{c}
TB	0	5.64 ± 0.10^{cB}	6.95 ± 0.38^{bA}	6.04 ± 0.59^{cA}	5.82 ± 0.02^{aB}	5.93 ± 0.02^{aA}	5.92 ± 0.05^{aA}	5.98 ± 0.02^{aA}
SM	0	10.53 ± 0.91^{aB}	11.73 ± 0.90^{abA}	12.14 ± 0.61^{aA}	5.68 ± 0.01^{bc}	5.67 ± 0.02^{b}	5.77 ± 0.05^{bc}	5.68 ± 0.02^{c}

LD, *longissimus dorsi*; GM, *gluteus medius*; BF, *biceps femoris*; TB, *triceps brachii*; SM, *semimembranosus*.
Values with different letters ($^{A-C}$) within each parameter in the same row differ significantly ($p < 0.05$).
Values with different letters ($^{a-c}$) in the same column differ significantly ($p < 0.05$).

also reported lower loss level (3.73%) for beef *longissimus thoracis* muscle frozen for 90 days at –20°C; while Fernandez et al [14] reported similar values (5.33% to 6.40%) for beef LD frozen at –35°C for 45 days. These contrasting results could be related to the differences in inherent characteristics of muscle tissue among the animal species or frozen storage conditions (e.g., temperature and duration etc.) applied among the studies.

Regarding pH, we observed that the LD and TB muscles significantly ($p<0.05$) increased in pH values approximately by 0.13 and 0.11 units after 120 days of frozen storage, respectively and did not increase thereafter. Whereas, the other remaining muscles (GM, BF, and SM) showed no pH changes up to 360 days of frozen storage. The ultimate pH values of non-frozen (day 0) or frozen muscles in this study were almost similar to values reported for the fresh horse muscles in literature [1,5,6]. This study for the first time, we assessed the effect of frozen storage duration on horsemeat pH values, and these obtained results suggest that there were differences in biochemical changes (e.g., glycolytic rate/or others) among the horse muscles before freezing or during freezing/thawing process. For other red meat species, the effect of freezing and thawing process on pH has also been investigated for instance; Muela et al [7] reported that the frozen storage had no effect on lamb meat pH whereas, an increase in pH values was observed in beef samples after 30 days frozen storage at –20°C [16].

Effect on cooking loss and WBSF

Results on cooking loss and WBSF are presented in Table 2. Cooking loss is an important quality trait reflecting the water holding capacity of muscle tissue, in which the greater the loss level the lower the water holding capacity. The outcome of our analysis showed that all the muscles significantly ($p<0.05$) increased in cooking loss after 120 days of frozen storage and did not increase thereafter. The cooking loss levels ranged among the muscles from 25.99% to 28.83% and from 29.74% to 31.62% at 0 (non-frozen) and 120 days frozen storage periods, respectively, with the averaged increase approximately by 4.47% after 120 days frozen storage. Similar to our finding, previous studies on other red meat species have also reported that freezing and thawing reduced water-holding capacity which resulted in increased cooking loss of muscle tissues [9,15]. This phenomenon could be attributed to the denaturation or ultrastructure changes of muscle proteins caused by freezing and thawing process [9]. The cooking loss levels in all non-frozen muscles (day 0) in the present work were similar to the levels (26.55% to 27.76%) reported for the various horse muscles at 24 h postmortem in literature [5,6], but higher than the values (18.28% to 20.99%) reported for 7 days-chilling aged beef LD cooked under the same conditions [17]. Also, lower cooking loss levels (12% to 14%) have been reported for lamb LD muscle after 6 months of frozen storage [7]. These contrasting results could be related to the physicochemical characteristics difference of muscle tissues among the meat species studied.

WBSF value is usually considered as an important indicator which predicts the tenderness ratings of meat in which the lower instrumental WBSF values obtained from meat are highly related to tenderness and consumer satisfaction [18]. Our results depict

Table 2. Cooking loss and Warner–Bratzler shear force (WBSF) values (mean±standard error) of horse muscles during frozen storage at –20°C

Muscles	Cooking loss (%)				Warner–Bratzler shear force (kgf)			
	0 d (non-frozen)	120 d	240 d	360 d	0 d	120 d	240 d	360 d
LD	26.16 ± 0.95^{bB}	32.11 ± 1.45^{aA}	32.34 ± 1.59^{aA}	29.74 ± 1.16^{aAB}	5.39 ± 0.04^{aA}	2.68 ± 0.20^{bB}	2.74 ± 0.12^{aB}	2.79 ± 0.23^{aB}
GM	26.69 ± 0.76^{abB}	32.51 ± 0.75^{aA}	30.48 ± 1.08^{aA}	30.53 ± 0.89^{aA}	5.09 ± 0.58^{abA}	3.60 ± 0.21^{aB}	3.00 ± 0.23^{aB}	3.46 ± 0.29^{aB}
BF	25.99 ± 0.62^{bB}	32.46 ± 1.12^{aA}	31.53 ± 1.17^{aA}	30.95 ± 0.81^{aA}	3.62 ± 0.10^{bA}	2.84 ± 0.27^{abB}	3.23 ± 0.25^{aAB}	3.01 ± 0.23^{aAB}
TB	28.79 ± 0.75^{aB}	33.07 ± 1.15^{aA}	33.48 ± 1.38^{aA}	31.62 ± 1.28^{aA}	3.81 ± 0.25^{abA}	3.02 ± 0.33^{abB}	3.03 ± 0.34^{aB}	2.86 ± 0.45^{aB}
SM	28.83 ± 0.72^{aB}	33.52 ± 0.71^{aA}	31.84 ± 0.99^{aAB}	31.03 ± 0.53^{aAB}	4.57 ± 0.21^{abA}	3.03 ± 0.30^{abB}	3.01 ± 0.21^{aB}	2.74 ± 0.12^{aB}

LD, *longissimus dorsi*; GM, *gluteus medius*; BF, *biceps femoris*; TB, *triceps brachii*; SM, *semimembranosus*.
Values with different letters ($^{A-C}$) within each parameter in the same row differ significantly ($p < 0.05$).
Values with different letters ($^{a-c}$) in the same column differ significantly ($p < 0.05$).

that frozen storage resulted in significant decreases in shear force values for all the muscles studied ($p<0.05$). On day 0 of frozen storage (non-frozen) the WBSF values varied among the muscles in the following order: LD≥GM≥SM≥TB≥BF, being their mean values of 5.396, 5.09, 4.57, 3.81, and 3.62 kgf, respectively. However, after 120 days frozen storage, the order was changed as following: GM≥SM≥TB≥BF≥LD, being their mean values of 3.60, 3.03, 3.02, 2.84, and 2.68 kgf, respectively. Thus, the LD muscle reduced approximately a half shear force value after 120 days frozen storage. Similar to our finding on horsemeat, studies on beef LD muscle have also found that the frozen storage and thawing process resulted in shear force reduction and tenderness improvement [19]. The decreases in shear force values observed on all muscles in the present study could be related to: i) either the breakdown of the muscle fibres during proteolysis, although the proteolysis is paused due to suppression of calpain activity during frozen storage, however, the activity will be restored and re-activated at a faster rate during thawing [20]; ii) the loss of structural integrity caused by large ice crystals that disrupts the physical structure and break myofibrils [9].

According to the tenderness classification proposed by Belew et al [21], all the horse muscles within this investigation could be considered as 'tender' (3.2<WBSF<3.9 kg) after 120 to 360 days frozen storage. The shear force values of frozen samples (120 to 360 days) in the present work were also lower than values reported for horsemeat cuts determined after 24 h postmortem and similar to values for the 30 days-chilling aged samples in our previous work [5]. Furthermore, when compared to the shear force values (5.24 to 5.95 kgf) reported for the same muscles of 6 months old foals [1], all the frozen muscle samples in the present work had much lower values.

Effect on color traits

Color is well recognized as an important quality trait which significantly affects the salability and attractiveness of meat to consumers [22]. The color traits (L*, a*, and b*) measured on day 0 (non-frozen) and during frozen storage periods (120, 240, and 360 days) for the 5 muscles studied are shown in Table 3. With regard to the L* (lightness), results depict that there was a significant ($p<0.05$) difference in values among the muscles as measured on day 0, in the following order: LD>BF≥GM≥SM≥TB, being their mean values of 36.36, 34.47, 34.44, 32.72, and 32.54. The results suggest that the LD muscle was lighter and TB muscle was darker in comparison to the other remaining muscles, which agrees with findings of Tateo et al [1]. Whereas, no statistical differences in L*-values occurred among the muscles after 120 or 240 and 360 days of frozen storage ($p>0.05$). In general, the L*-values in all muscles measured at non-frozen or frozen stages were almost equal to the values reported for various horse muscles chilling aged for 2 to 30 days in our previous studies [5]. The frozen storage showed a significant effect on L* values, as it caused the increases in L* values for all muscles (except LD muscle) after 120 days. In contrast to our results, studies on lamb and beef meats have reported that freezing and thawing process tend to reduce lightness values [15,23].

Regarding a* (redness), values significantly differed among the muscles studied on day 0 (non-frozen) or frozen storage periods. For instance, the LD muscle had significantly higher a* values than GM and SM muscles at 0 and 120 days of frozen storage ($p<0.05$), while after 360 days the TB showed the reddest color in comparison to other muscles. These results support other findings indicating redness differences among horse muscles [1,5,6]. The reason responsible for the redness differences could be related to the different thawing loss levels which resulted in different loss of sarcoplasmic proteins (e.g., myoglobin) among the muscles studied. For the frozen storage effect, we observed that three muscles including; LD, GM, and BF had significantly ($p<0.05$) reduced a* values after 240 days, whereas the two muscles that did not change in their a* values throughout the frozen storage were TB and SM. These results suggest that the LD, GM, and BF muscles seemed to have lesser color stability in comparison to the TB and SM muscles. The redness reduction observed on some muscles could be related to the denaturation of myoglobin molecules during frozen storage and thawing, which results in the loss of optimum color presentation [9]. Similar results have reported for beef and lamb meat, there was decreased redness values with an increasing duration of frozen storage [7,15]. Though the a* values tended to decrease in some muscles after 240 days of frozen storage as mentioned above, however, when compared to values of various horse muscles chilling aged under vacuum condition at 4°C for 30 days [5], all the frozen muscle samples in the present study presented similar values.

Table 3. Mean values (mean±standard error) for color characteristics of horse muscles during frozen storage at –20°C

Muscle	CIE L* (Lightness)				CIE a* (Redness)				CIE b* (Yellowness)			
	0 d (non-frozen)	120 d	240 d	360 d	0 d	120 d	240 d	360 d	0 d	120 d	240 d	360 d
LD	36.36 ± 0.41^{a}	37.32 ± 0.28^{a}	37.00 ± 0.99^{a}	35.16 ± 1.42^{a}	22.16 ± 0.52^{aA}	22.15 ± 0.38^{aA}	18.23 ± 0.38^{aB}	18.04 ± 0.21^{cbB}	10.62 ± 0.35^{a}	10.78 ± 0.47^{a}	9.12 ± 0.42^{a}	8.95 ± 0.30^{a}
GM	34.44 ± 0.57^{bC}	41.45 ± 0.75^{aA}	38.43 ± 0.87^{aB}	37.46 ± 0.72^{aB}	19.22 ± 0.56^{bcA}	19.02 ± 0.49^{cA}	17.78 ± 0.84^{aB}	17.18 ± 0.43^{cB}	8.56 ± 0.40^{b}	10.57 ± 0.21^{a}	9.69 ± 0.41^{a}	9.24 ± 0.18^{a}
BF	34.47 ± 0.78^{bC}	40.02 ± 0.75^{aA}	37.21 ± 1.03^{aB}	35.03 ± 0.92^{aCB}	20.15 ± 0.43^{abA}	20.71 ± 0.31^{bA}	18.43 ± 0.87^{aB}	18.37 ± 0.17^{bB}	8.91 ± 0.35^{b}	10.71 ± 0.34^{a}	9.20 ± 0.37^{a}	8.95 ± 0.24^{a}
TB	32.54 ± 0.28^{cB}	39.47 ± 0.29^{abA}	37.30 ± 1.43^{aA}	34.98 ± 0.41^{aAB}	20.56 ± 0.41^{ab}	21.16 ± 0.56^{ab}	19.09 ± 0.74^{a}	20.01 ± 0.51^{a}	8.69 ± 0.27^{b}	9.60 ± 0.46^{a}	10.05 ± 0.65^{a}	8.71 ± 0.28^{a}
SM	32.72 ± 0.59^{cC}	39.46 ± 0.95^{abA}	37.48 ± 1.23^{aAB}	35.15 ± 0.77^{aBC}	18.44 ± 0.44^{c}	19.36 ± 0.46^{c}	18.39 ± 0.83^{a}	18.41 ± 0.44^{b}	8.24 ± 0.38^{b}	10.78 ± 0.39^{a}	9.60 ± 0.62^{a}	9.41 ± 0.46^{a}

CIE, Commission International de l'Eclairage; LD, *longissimus dorsi*; GM, *gluteus medius*; BF, *biceps femoris*; TB, *triceps brachii*; SM, *semimembranosus*.
Values with different letters ($^{A-C}$) within each parameter in the same row differ significantly ($p<0.05$).
Values with different letters ($^{a-c}$) in the same column differ significantly ($p<0.05$).

Unlike the L* and a*, the b* (yellowness) values were not significantly affected by the frozen storage duration ($p>0.05$). Contrary results have reported on beef in which the frozen storage caused a decrease in b* values [15] while, an increase in b* value caused by frozen storage was noted for lamb meat [7]. These contrasting results could be related to the differences in frozen storage condition or meat species.

Effect on total volatile basic nitrogen and thiobarbituric acid reactive substances

Table 4 shows the TVBN and TBARS contents of horse muscles measured during frozen storage. The TVBN content is considered as the important index of meat's freshness [11]. Our results depict that the TVBN contents varied among the muscles throughout the frozen storage but were not statistically different ($p>0.05$). Regarding the frozen storage effect, we observed that the TVBN content did not increase up to 240 days of frozen storage, but significantly increased in all muscles after 360 days in comparison to the non-frozen samples. It was reported that the level of TVBN content usually increases during spoilage either by bacterial or enzymatic degradation of proteins [24,25]. In the present study, however, the spoilage bacteria (e.g., aerobic and lactic acid bacteria) were only found in few muscles at very low number (below 1.0 to 2.0 $\log_{10}$cfu/g meat) and no *Escherichia coli* were detected in all muscles at all periods examined (data not shown). The results indicating the TVBN content increases in the samples with increased frozen storage time, therefore, could be related to the increased protein degradation by muscle enzymic activity. In this regard, muscle proteins will be decomposed into ammonia, hydrogen sulfide and ethyl mercaptan etc. which together make up TVBN [25]. Also, increased proteolysis could be the factor responsible for the thawing loss increase (Table 1), shear force and redness reductions (Tables 2, 3, respectively) in muscles with increased frozen storage time as described above. Previous studies have also reported that chilling storage at 4°C significantly increased the TVBN contents in pork muscles [25]. Though the results showed an increase in TVBN content in all muscles with increased frozen storage time, however, the levels of TVBN observed in the muscles frozen for 240 days in present work were much lower than levels (4.47 to 35.02 mg%) reported for pork muscles chilling aged at 2 to 30 days at 4°C [24,25].

The TBARS method is usually used to measure the secondary products of lipid oxidation in meat. Our results depict that the TBARS values in non-frozen muscles (day 0) were lower than those in the frozen samples, the content increased from 120 days of frozen storage, but statistical differences with respect to non-frozen samples only appeared after 360 days under frozen storage ($p<0.05$). Looking at individual muscles, the increasing rate of TBARS contents varied among the muscles studied in the following order: TB>SM>GM>BF>LD, being their mean values of 0.39, 0.38, 0.37, 0.33, and 0.26 mg MDA/kg. These results suggest that the highest lipid oxidation level seemed to occur in the TB muscle, whereas the lowest level occurred in the LD muscle. Similar observations have been reported for other red meat types such beef and lamb meat, in which higher TBARS contents were found in frozen meat samples than those in fresh meat samples [7,15]. It is well recognized that horsemeat generally contains a higher content of unsaturated fatty acids (UFAs) which are not stable and easy to oxidized [26]. However, the TBARS levels in all muscles frozen after 360 days at –20°C in the present study were much lower than levels (1.94 mg MDA/kg) reported for beef LD muscle frozen for 90 days at the same temperature [15]. These suggest that the freezing or frozen storage seemed to have minor effect on oxidation/degradation of UFAs present in intramuscular fat content, and the increased lipid oxidation during the frozen storage could be due to the cellular structures damage, especially membrane lipids [27]. The TBARS content has been used as a predictor of the rancid perception [28]. High level of these secondary products often causes rancid and off-flavors. Wood et al [29] found that the TBARS values above 0.5 mg MDA/kg indicates a level of lipid oxidation products which impart a rancid flavor and odor that can be detected by consumers. Regarding this, the TBARS values in all frozen samples were below the critical limit of 0.5 mg MDA/kg meat in the present study. Thus, it can be concluded from our results that frozen storage of horse muscles for 360 days did not result in excessive oxidation of lipids.

Effect on sensory characteristics

Results on sensory evaluation of horse muscles at different frozen storage periods (0, 120, 240, and 360 days) are presented in

Table 4. Total volatile basic nitrogen (TVBN) and thiobarbituric acid reactive substances (TBARS) (mean±standard error) of horse muscles during frozen storage at –20°C

Muscle	TVBN (mg%)				TBARS (mg MAD/kg fresh meat)			
	0 d (non-frozen)	120 d	240 d	360 d	0 d	120 d	240 d	360 d
LD	0.12 ± 0.00^{bB}	0.17 ± 0.01^{aB}	0.18 ± 0.01^{aB}	32.10 ± 0.27^{aA}	0.08 ± 0.00^{abB}	0.24 ± 0.06^{aB}	0.26 ± 0.04^{aB}	0.34 ± 0.04^{aA}
GM	0.16 ± 0.00^{aC}	0.18 ± 0.01^{aBC}	0.21 ± 0.02^{aB}	30.94 ± 0.09^{aA}	0.09 ± 0.01^{abB}	0.24 ± 0.04^{aB}	0.27 ± 0.08^{aAB}	0.46 ± 0.22^{aA}
BF	0.16 ± 0.00^{aB}	0.19 ± 0.02^{aB}	0.19 ± 0.01^{aB}	31.34 ± 0.15^{aA}	0.10 ± 0.01^{aB}	0.22 ± 0.03^{aB}	0.24 ± 0.06^{aB}	0.43 ± 0.23^{aA}
TB	0.16 ± 0.00^{aB}	0.17 ± 0.01^{aB}	0.19 ± 0.01^{aB}	31.05 ± 0.11^{aA}	0.07 ± 0.01^{bB}	0.22 ± 0.07^{aAB}	0.26 ± 0.06^{aAB}	0.45 ± 0.12^{aA}
SM	0.17 ± 0.01^{aB}	0.19 ± 0.01^{aB}	0.20 ± 0.01^{aB}	31.20 ± 0.11^{aA}	0.09 ± 0.01^{abB}	0.23 ± 0.03^{aAB}	0.31 ± 0.08^{aAB}	0.47 ± 0.16^{aA}

LD, *longissimus dorsi*; GM, *gluteus medius*; BF, *biceps femoris*; TB, *triceps brachii*; SM, *semimembranosus*.
Values with different letters ($^{A-C}$) within each parameter in the same row differ significantly ($p<0.05$).
Values with different letters ($^{a-c}$) in the same column differ significantly ($p<0.05$).

Table 5. Mean values (7 point-scale, mean±standard error) for sensory characteristics of horse muscles evaluated during frozen storage at –20°C

Muscles	Flavor				Tenderness				Juiciness			
	0 d (non-frozen)	120 d	240 d	360 d	0 d	120 d	240 d	360 d	0 d	120 d	240 d	360 d
LD	4.29±0.15^{a}	4.34±0.16^{a}	4.00±0.10^{a}	4.24±0.18^{ab}	4.28±0.27^{aB}	4.74±0.16^{aA}	4.50±0.23^{aA}	4.77±0.22^{aA}	4.48±0.25^{a}	4.29±0.19^{a}	4.02±0.20^{a}	3.99±0.14^{a}
GM	4.23±0.18^{a}	4.17±0.13^{a}	4.35±0.21^{a}	4.24±0.11^{ab}	3.90±0.21^{abB}	3.81±0.21^{bcB}	4.33±0.33^{aA}	4.76±0.15^{aA}	4.31±0.24^{a}	3.75±0.16^{a}	3.72±0.25^{a}	3.78±0.18^{a}
BF	3.82±0.33^{b}	3.89±0.26^{a}	4.43±0.18^{a}	3.91±0.17^{b}	3.19±0.31^{cB}	3.71±0.45^{bcAB}	4.50±0.35^{aA}	4.37±0.22^{aA}	4.15±0.20^{ab}	4.25±0.23^{a}	4.00±0.28^{a}	3.92±0.23^{a}
TB	3.91±0.17^{b}	3.80±0.12^{a}	4.15±0.16^{a}	4.24±0.11^{ab}	4.07±0.22^{abB}	4.50±0.14^{abAB}	4.46±0.27^{aAB}	4.52±0.13^{aA}	3.92±0.23^{b}	3.76±0.16^{a}	3.72±0.25^{a}	3.75±0.20^{a}
SM	4.04±0.16^{ab}	4.32±0.72^{a}	4.21±0.20^{a}	4.41±0.14^{a}	3.33±0.21^{bcB}	3.40±0.38^{cB}	4.58±0.28^{aA}	4.74±0.21^{aA}	3.77±0.25^{b}	3.93±0.18^{a}	4.13±0.10^{a}	3.81±0.13^{b}

LD, *longissimus dorsi*; GM, *gluteus medius*; BF, *biceps femoris*; TB, *triceps brachii*; SM, *semimembranosus*.
Values with different letters ($^{A-C}$) within each parameter in the same row differ significantly ($p < 0.05$).
Values with different letters ($^{a-c}$) in the same column differ significantly ($p < 0.05$).

Table 5. In comparison among the muscles for flavor on day 0 of frozen storage (fresh, non-frozen), the panelists gave significantly higher scores for the LD and GM muscles than for the other remaining muscles ($p<0.05$). On day 120, 240, and 360 of frozen storage, however, the panelists gave similar flavor scores for all the muscles. The frozen storage also showed no effect on flavor since no statistical differences in scores between the day 0 (fresh, non-frozen) and frozen (120, 240, and 360 days) samples were found ($p>0.05$), suggesting that the flavor characteristic of the horse muscles was maintained unchanged up to 360 days of frozen storage. Similar to our results, previous studies also reported that frozen storage (1 to 10 months) did not affect the flavor characteristic of beef [15]. Muela et al [30] also reported no statistical differences in flavor scores occurred between non-frozen (fresh) and frozen (1, 9, and 15 months) lamb *longissimus lumborum* muscle.

Tenderness, is an important quality criterion determining the overall acceptability of meat. Results showed that significantly ($p<0.05$) higher tenderness scores were given for the LD muscle on day 0 and day 120 of frozen storage in comparison with those of other remaining muscles, but after 240 to 360 days of frozen storage no statistical differences were reported for all the muscles evaluated ($p>0.05$). Similar to the tenderness results observed for the non-frozen muscles (day 0) in the present work, previous workers [5,6] also reported higher scores for the chilling aged or unaged horse LD muscle than other muscles. Frozen storage appeared to improve the tenderness, since all the 360-days frozen muscle samples had significantly higher scores compared with those of the 0-day (fresh) frozen samples ($p<0.05$). These sensory tenderness results are in line with the instrumental shear force results as shown in Table 2. Also, our results are in good agreement with Vieira et al [15], who reported higher tenderness scores for 90-day frozen beef samples than for the non-frozen samples. A recent study on lamb meat also revealed that fresh samples had lower tenderness scores as compared with those frozen for 9 months at –18°C [30].

For juiciness, on day 0 of frozen storage higher scores were given for the LD and GM muscles in comparison with other muscles ($p<0.05$), whereas no statistical differences in scores among the muscles were stated by the panelists after 120, 240, and 360 days of frozen storage. Though there were differences observed in thawing loss (Table 1), no effect of frozen duration was noted by the panelists for juiciness scores ($p>0.05$). Similar results have been observed and reported for beef muscle frozen for 3 months at the same temperature [15], and for lamb *longissimus lumborum* muscle frozen for 21 months at –18°C [30]. From our sensory evaluation results and previous observations, it could be said that frozen storage has little effect on eating quality traits of meat, and the mainly affected trait may be tenderness.

CONCLUSION

This study for the first time assessed the changes in technological and sensory qualities of horse muscles during frozen storage at –20°C for 120, 240, and 360 days. In general, the frozen storage produced both negative (e.g., increased cooking and thawing loss or discoloration phenomena etc.) and positive (e.g., improved the tenderness) effects on horse muscles. Though the frozen storage caused some defects in meat quality, all the values obtained for the quality traits examined in all muscles during frozen storage in this investigation were almost similar to those reported for the same meat type or other fresh and frozen conventional red meat species in literature, and generally are in the acceptable limits. Our findings could be useful information for meat producers and processors in order to standardize the horsemeat storage process. The study results also provide a brief overview on how the frozen storage affected horsemeat quality and thus, further study is needed to clarify the muscle ultrastructure changes or proteolytic activity in horsemeat during frozen storage. Also, more attention should be paid to solutions (e.g., suitable freezing/thawing temperature and rate) to reduce the thawing and cooking loss or discoloration phenomena in some muscles caused by the frozen storage.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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