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ANIMAL SCIENCE

Effect of ageing on meat quality of the one humped camel (*Camelus dromedarius*)

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Abstract

A total of seven she-camels (3-4 years old) were slaughtered following the normal abattoir procedures in Khartoum state, Sudan. Longissimus thoracis (LT) muscle samples were aged for 1, 3, 5, and 7 days at 1-3° C. Chemical composition, muscle pH, drip loss (DL), water holding capacity (WHC), color, myosin heavy chain isoforms (MyHC) as well as lipid peroxidation (MDA) and vitamin E content were determined at the indicated times during ageing. Ageing time of LT muscle influenced significantly ($P<0.001$) chemical composition except ash. Dry matter and drip losses significantly increased while moisture and protein contents decreased during ageing, however, no differences were found in muscle pH, color, fat peroxidation and WHC although fat peroxidation tended to increase from 5 days of ageing onwards. Electrophoresis of MyHC isoforms indicated the presence of two muscle fiber types only: type I (64.1%) and type IIa (35.9 %), respectively. In conclusion, shelf life of camel meat could be extended in the presence of high levels of vitamin E which helps to maintain lipid stability.

Key words: Ageing, Desert she-camel, Longissimus thoracis, Lipid peroxidation

Introduction

Ageing of meat is a highly complex phenomenon which depends on physicochemical parameters, extent and rate of acidification, changes in osmotic pressure, glycolytic and proteolytic enzymes (Ouali, 1991). After slaughtering, there is a decrease in temperature and pH (Marsh et al., 1988) and an increase in osmotic pressure (Bonnet et al., 1992) and expressible juiciness (Offer and Knight, 1989). Consequently, there is a weakening of the myofibrillar structure and an improvement in tenderness of the cooked beef (Ho et al., 1996; Wegner et al., 2000). Several studies showed that ageing improves the tenderness of most muscles (Campo et al., 2000). The post-mortem ageing of meat is a very important period having a significant effect on its microstructure and quality traits, especially texture, tenderness and water-holding capacity (Zamora et al., 1996).

Camel meat is the least studied type of meat and is wrongly believed to be of lower nutritive value and quality than other types of red meat, inspite of their ability to produce good quality meat at comparatively low cost under extremely harsh environments. In addition, there is lack of research in the improvement of camel meat characteristics (Skidmore, 2005). Investigation of muscle fiber characteristics is of practical importance to meat scientists, breeders, and the meat industry to provide a better understanding of the involvement of muscle fibers with regard to the determination of muscle growth and final meat quality traits (Wegner et al., 2000). Lipid peroxidation is one of the major causes of quality deterioration in raw and cooked meat products during refrigerated or frozen storage. Peroxidation occurs when lack of balance between production of reactive oxygen species (ROS) and the level of antioxidants take places (Favier, 1997). It takes place in polyunsaturated fatty acids (PUFA), the most susceptible being FA of the n-3 family (n-3 PUFA) initiated by (ROS), which are essential for life and growth in animal cells (Kama–Eldin and Yanishlieva, 2002). Lipid peroxidation in meat, with production of malondialdehyde (MDA) and other oxidized products are toxic for consumers

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and harmful to human health (Sasaki et al., 2001; Alderton et al., 2003). The presence of antioxidants prevents peroxidation from happening (Burton and Inglold, 1989). Among antioxidants, vitamin E (as α tocopherol acetate) was reported controlling efficiently lipid oxidation and thus extends shelf life of meat (Arnold et al., 1993; Faustman and Wang, 2000; Kerry et al., 2000). Thus, lipid stability is one of the important factors for maintaining meat quality during storage. The main objective of the present study is to investigate the effect of post-mortem ageing on chemical composition and quality characteristics of *longissimus thoracis* (LT) muscle of one humped camels (*Camelus dromedarius*).

Materials and Methods

Collection of samples

A total of seven she-camels (3-4 years old) fattened by local camel herders in Khartoum state, Sudan were slaughtered following the normal abattoir procedures. Samples of *Longissimus thoracis* (LT) muscle between the 5^{th} to 10^{th} rib were obtained from the right side of the carcasses after 60 minutes post slaughter, placed in plastic bags and transported to meat science laboratory, Faculty of Animal production, University of Khartoum in an insulated box filled with ice. In the laboratory, any visible fat was trimmed and each muscle was then divided into 4 parts, aged for 1, 3, 5, and 7 days at 1-3°C. Samples were then vacuumed, packed and stored at –18°C awaiting the analysis.

Meat quality evaluation

Quality characteristics of *longissimus thoracis* muscles included: pH, water holding capacity (WHC), drip loss (DL) and meat color coordinates (*l*, a*, b**). Muscle pH was measured 60 min post slaughter, 1, 3, 5 and 7 days postmortem using a portable pH meter (Hanna waterproof pH meter, Model H I 9025, Italy) with temperature adjusting probe inserted at same depth each time into the muscle. Water holding capacity (WHC) was expressed as percentage of the expelled water using filter paper method as the total wetted area less than meat film area (cm^2) relatively to the sample weight (g). Drip loss was calculated from the difference in muscle weight before and after ageing. Meat color co-ordinates *l** (lightness), *a** (red-green) and *b** (yellow-blue) were measured using a Minolta CR100 chromameter (Minolta Co., Ltd., Japan).

Chemical analysis

The chemical composition of the LT muscle (dry matter, protein, intramuscular fat and ash) was determined according to the standard methods of AOAC (1990). Crude protein was determined using a Foss Tecator Kjeltec 2300 Nitrogen/Protein Analyzer. Intramuscular fat was determined by Soxhlet extraction of the wet sample, using petroleum ether. Ash content was determined by ashing samples in a muffle furnace at 500°C for 24 h. The results were expressed on dry matter bases.

Electrophoresis of myofibrillar proteins

Muscle fibre types were determined by quantifying the different myosin heavy chain (MyHC) isoforms using Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Talmadge and Roy (1993). Bovine albumin was used as a reference sample. Each hole of gel was loaded with 4-5 µg of myofibrillar protein and electrophoresis was performed at a constant voltage of 70 volts for 30 hours at 4°C. The relative proportions of slow isoforms (% MyHC I) and fast isoforms (% MyHC IIa) were determined after staining of gels in a solution of Coomassie Blue R250 and quantifying using Image Quant TL v2003.

Malondialdehyde (MDA) determination

As a marker of lipid oxidation intensity, MDA was extracted from LT muscle homogenized powder (1g) by the procedure of Botsoglou et al. (1994). Briefly, 5 ml of 0.8% butyl hydroxytoluene in hexane and 8 ml of 5% thiobarbituric acid in H_2O were added to camel meat sample. After crushing and adding 2 ml of hexane, the mixture was centrifuged for 10 min at 10,900 g to recover the aqueous phase. Then 0.7 ml of 5% trichloroacetic acid and 1.5 ml of 0.8% thiobarbituric acid were added to the previously filtered aqueous phase. The sample was heated in a water bath at 70°C for 30 min, cooled in an ice bath and, after addition of 1 ml n-butanol, centrifuged for 10 min at 680g to recover the complex MDA-TBA_2 present in the upper phase.

Meat MDA content was determined by HPLC as described by Agarwal and Chase (2002). An aliquot (180 µl) of this solution was injected into an HPLC system (Perkin–Elmer Instruments, Shelton, Connecticut) equipped with an RP C18 column (ODB 5 lm, 4.6 250 mm, Interchim, Montluçon, France). The mobile phase (flow rate: 0.6 ml/min) was composed of potassium phosphate (0.05 M, pH 6.8) in methanol. MDA was detected by fluorescence (excitation at 515 calibration curve).

Determination of α tocopherol acetate content

α -tocopherol acetate was assayed by High Performance Liquid Chromatography (HPLC) and Ultraviolet (UV) absorption as described by Scislowski et al. (2005). Before the extraction step, α -tocotrienol acetate (22.5 µg/ml) was added as internal standard to muscle powder samples to determine extraction efficiency. Powder of camel LT muscle (1 g) was treated by heating at 80 C° in 11% KOH in ethanol for 20 min and α- tocopherol was extracted twice with 5 ml of hexane. After elimination of hexane by evaporation, α-tocopherol and α-tocotrienol acetate were dissolved in 160 µl of tetrahydrofurane and 240 µl of methanol/dichloromethane (65/35, vol/ vol). Finally, α-tocopherol and α-tocotrienol concentrations were determined by UV spectrophotometer (Kontron, model 430) at 292 nm using Kroma System 2000 software (Kontron Instruments, Zurich, Switzerland).

Statistical analysis

Data were subjected to statistical analysis to test the effect of ageing time (days) on camel meat characteristics by ANOVA using general linear model procedure of SAS (2001). Least squares means were calculated and significance was declared at *(P<0.05*). Principal Component Analysis (PCA) was used to interpret the relationship between chemical analysis (dry matter, moisture, protein, fat and ash), muscle pH, color co-ordinates (*a*, l** and *b**), WHC, drip loss, vitamin E and lipid peroxidation (total: 15 variables). They were carried out taking into account the most explicit variables for each analysis to work out inter-relationships between all the parameters. The p variables observed on the 7 (n) camels were represented by projection in the main plan constituted by the two first factor axes. These factor axes were calculated from the eigenvectors of the p variables and the correlation matrix of the data (Jobson, 1992).

Results and Discussion

Chemical composition

The overall mean of moisture, dry matter, crude protein, intramuscular fat and ash content were: 74.9, 25.1, 19.9, 4.8 and 1.2 respectively in *longissimus thoracis* (LT) muscle (Table 1), which within the range reported by Dawood and Alkanhal (1995), Al-Owaimer (2000), Al-Ani (2004), Kadim and Mahgoub (2006) and Kadim et al. (2008) for moisture (70-77)%, intramuscular fat (0.5-9.8)%, ash content (1-1.3)% but slightly lower than the range of crude protein (20-23)%. Significant differences were observed in chemical composition during ageing time in moisture, dry matter, crude protein and intramuscular fat, however no differences were found in ash content. Moisture content was found to decrease significantly during ageing which is associated with significant increase in dry matter. Crude protein was significantly lower in day 7 compared to days 1, 3 and 5 which could be attributed to escape of proteins in the purge or drip during ageing. This was confirmed previously by Savage et al. (1990) who indicated significant amount of protein lost from postmortem muscle; most of them are water-soluble, sarcoplasmic proteins. Intramuscular fat showed some inconsistent variations between time points which may be explained by sampling variations.

Table 1. Chemical composition of *longissimus thoracis* muscle (LT) from desert she-camels during ageing.

Parameters	Ageing time				SEM	Effect of cold storage *(P- value)*
	1	3	5	7		
Moisture (%)	76.0^{a}	75.1^{b}	74.6^{bc}	73.7^{c}	0.22	0.0004
Dry matter (%)	23.9^{c}	25.0^{b}	25.4^{ab}	26.3^{a}	0.22	0.0004
Crude protein (%)	20.6^{a}	20.2^{ab}	19.5^{ab}	19.1^{b}	0.21	0.0463
Intramuscular fat (%)	4.23^{ac}	6.8^{a}	2.86^{c}	5.61^{ab}	0.50	0.02
Ash (%)	1.12	1.15	1.16	1.18	0.02	0.37

a,b,c means with different superscripts within a row are significantly different at (P<0.05).

Table 2. Quality characteristics of aged *longissimus thoracis* (LT) muscle from desert female camels.

Parameters	Ageing time (Days)				SEM	Effect of cold storage *(P- value)*
	1	3	5	7		
Muscle pH	5.72	5.59	5.58	5.58	0.024	0.08
Muscle color:						
l*	27.50	27.59	28.13	29.26	0.59	0.73
a*	12.98	14.01	13.77	13.18	0.29	0.57
b*	14.08	15.34	14.76	15.55	0.30	0.33
Drip loss	1.58^{c}	3.37^{b}	4.24^{ab}	5.51^{a}	0.40	0.0001
WHC	1.36	1.28	1.22	0.95	0.10	0.51
MyHC isoforms (%):						
Ia	67.32	63.40	63.57	62.08	1.70	0.75
IIa	32.68	36.60	36.92	37.92	170	0.75
MDA (µg/ g muscle)	0.08	0.08	0.14	0.24	0.03	0.14
Vitamin E (µg/ g muscle)	16.39	24.45	12.80	18.35	2.01	0.39

a,b,c means with different superscripts within a row are significantly different at (P<0.05).

Meat quality of camel meat

The overall mean of muscle pH during ageing was 5.63 (Table 2) which were higher than the values reported by Soltanizaheh et al. (2008) who indicated that pH of 5.5, and lower than Kadim, Mahgoub et al. (2009) who reported pH of 5.79 in camel LT muscle. These differences could be attributed to the glycogen storage in the muscle at the time of slaughter. The pH declined rapidly to 5.72 in day one and then in slow rate until the ultimate point. This goes in accordance with the fact that camels are animals with a high gluconeogenesis capacity due to the presence of hump. The amount of enzymes in its glycolytic pathway causes slower glycogen degradation and pH decline (Immonen and Puolanne, 2000; Soltanizaheh et al., 2008). No significant difference was found in pH values during ageing of muscles which fits with the results of Kadim et al. (2009) who found no significant effect of ageing on ultimate pH (Table 2).

Muscle color development could be influenced by many factors (Table 2) including: myoglobin concentration, ultimate pH, muscle fiber type and cooling rate (Faustman and Cassens, 1990). The overall mean values of color coordinates *(l*, a* and b*)* during ageing were 28.1, 13.5 and 14.9, respectively indicating that meat from camel was moderately brighter and less red. These findings were in agreement with Kadim et al. (2009) who reported no significant effect in muscle color during ageing time.

Drip loss is a result of shrinkage of the myofibrils due to postmortem drop of pH and attachment between thin and thick filaments at the onset of rigor mortis and denaturation of myosin. Ageing affected drip loss significantly ($P<0.001$), however, no differences were observed between day 3 and 5 on one hand, day 5 and 7 on the other hand (Table 2). This goes in line with the fact that drip loss could increase linearly with a decrease in the length of the sarcomeres in muscle cells (Honikel et al., 1986).

Water holding capacity (WHC) is an important meat quality characteristic due to its influence on the nutritional value, appearance, palatability and processing properties of meat (Offer and Knight, 1988a; Lawrie, 1979). Many factors were reported previously affecting WHC (Hamm, 1986; Offer and Knight, 1988a,b; Honikel, 2004; Puolanne and Halonenm, 2010). In the present results, the overall mean of WHC was 1.2; however it decreased from 1.36 in day one to 0.95 in day 7 with no significant difference among ageing days. This indicated that an improvement in WHC which agreed with Lawrie (1979) who stated that WHC improved with ageing.

Myosin heavy chain isoforms

Ageing is the process that causes an increase in tenderness over time and involves specific degradation of structural proteins (Hwang et al., 2003). Electrophoresis of myosin heavy chain isoforms (SDS-PAGE) indicated the presence of two different muscle fiber types (slow MyHC I and fast MyHC IIa) in camel (LT) muscle, (Table 2 and Figure 1) as previously described (Abdelhadi et al., 2012). The fast isoforms (MyHC IIx and MyHC IIb), described in bovine muscle (Picard and Cassar-Malek, 2009) have not been revealed in all the studied samples.

The mean percentages of type I and type IIa were 64.1 and 35.9 %, respectively. This generally goes in line with the results of Kadim et al. (2009)

and Rose et al. (1992) who observed a higher proportion of type I compared to type IIa in (LT) muscle of breeding and racing camels. Tenderness is related to the high percentage of type I (with a high oxidative activity) which is able to use mainly fatty acids as energy-yielding nutrients. This agreed with Calkins et al. (1981), Dransfield et al. (2003) and Jurie et al. (2007) who reported that the oxidative capacity of a muscle was related to marbling and/or tenderness. No significant effect of ageing on the proportion of MyHC fiber types in she-camel (LT) muscle.

Lipid peroxidation and Vitamin E

Several factors can affect lipid oxidation in meat during ageing and packing including light, oxygen concentration, temperature, presence of anti- and pro-oxidants and abundance of unsaturated fatty acids (Jakobsen and Bertelsen, 2000). The overall mean of Malondialdehde (MDA) level in LT muscle was 0.14 µg/ g muscle. Low MDA values revealed during ageing of LT muscle in day 1 and 3 (0.08 µg/ g), respectively, however, these values increased to 0.14 and 0.24 µg/ g in day 5 and 7. No differences in MDA levels among ageing days which goes in line with the findings of Durand et al. (2006) from Charolais cull cows aged for 5 days and Durand et al. (2010) who reported MDA level was not modified by ageing time from 1 to 12 days whatever the ageing method (entire carcass *vs.* under vacuum).

Vitamin E (α-tocopherol) is an important lipid-soluble antioxidant that protects cell membranes from oxidation by reacting with lipid radicals produced in the lipid peroxidation chain reaction. This would remove the free radical intermediates and prevent the oxidation reaction from continuing. The antioxidant function is considered to be the most important function of this vitamin (Bell, 1987; Herrera and Barbas, 2001; Traber and Atkinson, 2007). No significant effect of ageing on vitamin E during ageing days was found. The overall mean of vitamin E in the present study was three fold higher (17.8 µg/ g) than that reported in bovine rumsteack (5 µg/ g) by Durand et al. (2007 and 2010) in LT muscle of cows fed with plant extracts combined with vitamin E. Thus, camel meat could be a good source of vitamin E to fulfill the recommended daily requirements (15 mg/ day) by the Food and Nutrition Board (2000). MDA values were low in day 1 and 3 (0.08 µg/ g) and high in day 5 and 7 (0.14 and 0.24 µg/ g) which could be related to vitamin E levels in day 1 and 3 (16.4 and 24.5 µg/ g) as well as in the later (12.8 and 18.4 µg/ g) and to the low vitamin E values in day 5 and 7. These findings goes in line with Fausman and Wang (2000) and Kerry et al. (2000) since vitamin E is considered to control lipid oxidation in meat from many animal species (Table 2). To our knowledge no previous reports were found describing the effect of ageing of camel LT muscle on lipid peroxidation and vitamin E content in camel meat.

Principal component analysis (PCA)

During ageing there was a decrease in moisture, protein, ash and an increase in drip loss with no effect on fiber type I and IIb, lipid peroxidation and vitamin E. The Principal component analysis (PCA) explained about 36.1% of the total variance which can be condensed in two new principal components: PC1 and PC2 which explained 21.4 and 14.7% of the variance, respectively (Figure 2a). As shown in Fig. 2b, PC1 discriminates the ageing groups of muscles according to time (days). PC2 discriminates muscle samples aged for 1 and 7 days from those aged for 3 and 5 days. However, PC2 discriminates samples mainly on their fiber type proportions. High correlation coefficient was found between intramuscular fat and ash ($r= 0.90$, $P<0.05$), moisture and fat ($r=0.67$, $P<0.05$) and moisture with ash ($r=0.58$, $P<0.05$). However, negative correlation coefficient was found between drip loss and muscle protein; intramuscular fat and ash contents, respectively (-0.48, -0.73 and -0.74). Lipid peroxidation was negatively correlated with muscle fibers type I ($r= -0.40$) which could be attributed to the oxidative activity of type I muscle fibers (use fatty acids to obtain energy) (Jurie et al., 2002; Oury et al., 2007). Also dry matter showed negative correlation with intramuscular fat and ash (-0.67 and -0.58), as well as muscle pH and muscle fibers type IIa ($r= -0.42$). The proportions of types I and IIa muscle fibers as well as moisture and dry matter content were negatively correlated ($r= -1.0$, $P<0.05$). It could be concluded that ageing had decreased moisture, improved WHC and increased drip loss, dry matter, lipid peroxidation with no effect of ageing on fiber type and vitamin E.

Conclusion

Chemical composition and drip loss in LT muscle of camels were significantly affected by ageing. Inspite of the insignificant differences, lipid peroxidation tended to increase in days 5 to 7 which could indicate high risk of ageing meat from this type that contained high levels of intramuscular fat longer than 7 days. High levels of vitamin E in camel meat compared to bovine would help to increase shelf life of camel meat products.

Figure 1a **Figure 1b**

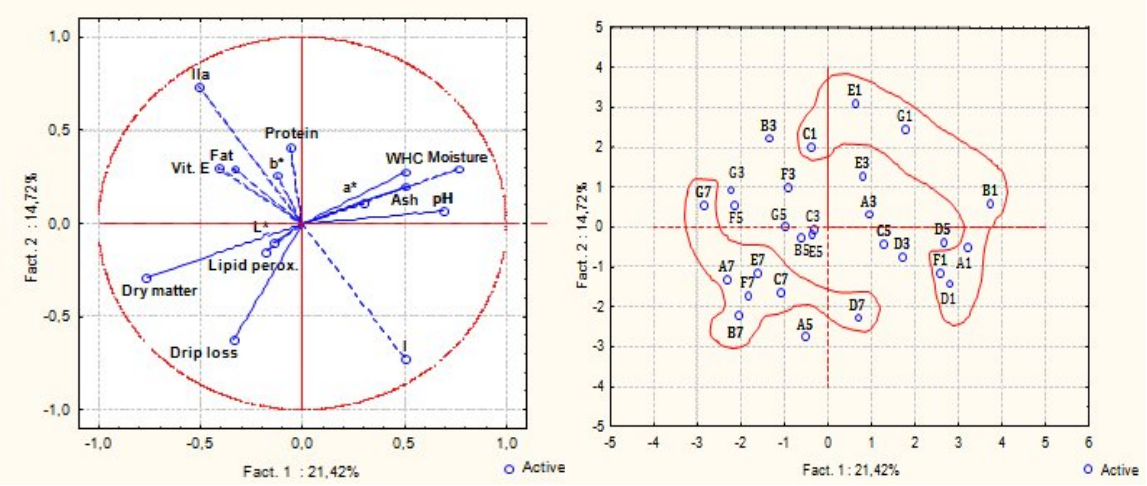

Figure 1. Plot for the variables (1a) and for the animals (1b) in the multivariate space following a principal component analysis (PCA). *In Figure 2a: WHC= Water holding capacity, I = MyHC I, IIa = MyHC II. In Figure 1b: A, B, C, D, E, F and G= Distribution of animals according to ageing group. 1, 2, 3…7=Days of ageing.*

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