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Proximate Chemical Analysis, Fatty Acid Profile and Microstructural Characteristics of Dromedary Camel Fats (Hump, Renal and Mesentery)

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ABSTRACT

The quality, safety, and suitability of animal fat for processing of a specific meat product is a critical issue. Increasing the human awareness about the health aspects associated with increased intake of animal fat, makes camel fat a suitable raw material for meat processing due to its excellent nutritional contribution. Therefore, the target of this study is examination of the sensory, physicochemical, fat oxidation, fatty acid profile, and other quality parameters of camel fat to evaluate the feasibility for processing of different meat products. To achieve this goal, 30 fat samples each from the hump, renal, and mesentery of Arabian male camels were investigated. The results showed that both the renal and mesenteric fat had honey color and medium-soft texture, while the hump had greyish-white color and hard texture. The sensory panel scores were significantly different between the hump and other fats. Hump fat had significantly ($P<0.05$) higher moisture, protein, and collagen content, while higher fat content was recorded in mesenteric fat. The fatty acid analysis showed that hump had high SFA and very low PUFA in comparison with both renal and mesenteric fat. Camel fat had high oxidation stability, and the mean values were very low in comparison with the levels of quality and acceptability. The ultrastructural analysis showed that hump fat had high elastin fibers which increase its hardness. The results indicated that both renal and mesenteric fat were more suitable for the production of various meat products than the hump.

Key words: Camel fat, chemical analysis, lipid oxidation criteria, hump, ultrastructure.

INTRODUCTION

Animal fat plays an important role in human nutrition (Insufficient fat intake may cause many physiological disorders (Fleming, 2002), while high-fat content is associated with obesity, type 2 diabetes, cancer, and coronary heart disease (McAfee *et al.*, 2010). Fortunately, the emerging properties of camel fat due to low cholesterol and high-unsaturated fatty acids content (Abu-Tarboush and Dawood, 1993) provide the foundation for the expansion as a healthy fat in the human diet.

Animal fat is added during meat processing to achieve several physical and eating quality characteristics, e.g., emulsion stabilization, improvement of the Water-Holding Capacity and cooking loss (Baer and Dilger, 2014). However, increasing consumer awareness about the health risks of animal fat forced meat producers to find healthy alternatives (Grasso *et al.*, 2014). Production of low-fat meat products is one of these solutions, but the decline in fat content adversely affects the flavor and palatability (Muguerza *et al.*, 2001). Therefore, meat processors presented several alternatives to overcome the detrimental impact of fat reduction. Direct addition of vegetable oils is a simple solution, however, it deteriorates the physical quality of meat products (Youssef and Barbut, 2011), therefore, the nutritional contribution and quality attributes of camel fat can provide a promising alternative.

Camel produces a substantial amount of fat under extreme improper management systems (Muzzachi et al., 2015). Fat represents 12-18% of the camel carcass and stored in the hump, renal, and mesentery (Kurtu, 2004), which provides an appropriate raw material to compensate the low-fat content of camel meat, especially in high-fat products. Many consumers prejudiced against the characteristic odor of camel fat despite its acceptable nutritional value, therefore, its incorporation in processed products e.g., dry sausages (Sbihi *et al.*, 2013), emulsion sausage (Mohamed *et al*., 2015), and ground type products (Heydari *et al.*, 2015) may be more acceptable

Data of the chemical structure of camel fats are uncommon, where most of the investigations focused on the overall fatty acid analysis (Kadim *et al*., 2002). Information, e.g., moisture, protein, lipid content, fat oxidation, collagen content and histological structure are

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essential to asset the practical use of camel fat in processing different products. Therefore, this study was designed to investigate the sensory, chemical composition, fat oxidation criteria, fatty acids profile, and microstructural features of the hump, renal, and mesenteric fat.

MATERIALS AND METHODS

Raw material

Ninety fat samples were collected from 5 years old intact Arabian male camels slaughtered at the Municipality slaughterhouse, Cairo (Egypt). One Kg was obtained from the middle portion of the hump, renal, and mesenteric fats after slaughter and carcass preparation. Samples were wrapped in oxygen-permeable films, transported to the laboratory and stored at -20ºC for 24h. Each fat sample was examined three times for sensory, proximate chemical, fat oxidation criteria, collagen content & solubility, instrumental color, fatty acid profile, cholesterol, and ultrastructural analysis.

Sensory investigations

Nine professional panelists received three initial training sessions to be familiar with the sensory parameters of camel fats. The panelists were asked to describe each fat sample and the score of different parameters using 8-points rating scale. Small 1 x 1 cm cubes were incised from the central portion of each sample and dried by a paper towel. Three cores from each sample were coded, presented in a white plate, and evaluated under natural light. The color categories include white, greyish-white, honey, pale-yellow, and yellow scales. The odor was described as slight, moderate, strong, intense, and pungent. The hardness of fat was estimated by hand feel and assigned as hard, medium-hard, medium, medium-soft, and soft. Hard fat has a high resistance to finger push, while the soft fat shows low initial resistance to the finger pressure, and the medium hardness denotes intermediate criteria. All tests were carried out under controlled conditions following the AMSA Guidelines (2016).

Proximate chemical analysis

Each fat sample was minced three times using Fama Fabbrica meat mincer (Italy). The samples were mixed thoroughly after each mincing time. Three replicates from each sample were used to determine the proximate chemical composition using the procedure of AOAC (2013). For the determination of moisture contents, 10g sample was dried to obtain a constant weight (100ºC/ 16-18h). The protein content was determined using the micro distillation Kjeldahl method. Six extraction cycles with petroleum ether (boiling point 60-80 °C) in a Soxhlet apparatus were used for the determination of total lipids, while ash content was determined by the muffle furnace ignition at 500 ºC for 5h.

Collagen content and solubility

Two gram fat sample was hydrolyzed in a sealed tube with 50 ml 6N HCL at 105 ºC for 18h and filtered through a sintered glass funnel. Five ml filtrate was neutralized with NaOH solution to pH 6.9–7.0 and diluted again with distilled water to 10 ml. Hydroxyproline content was determined according to Neuman and Logan (1950), total collagen was calculated using 7.25 conversion factor (Goll et al., 1963). Five gram sample was homogenized with 50 ml distilled water for 2 min, centrifuged at 1500g for 30 min., and hydrolyzed for 18hr. Soluble collagen (mg%) was calculated from the soluble hydroxyproline using 7.14 conversion factor (Mahendrakar et al., 1989).

Cholesterol content

For saponification of cholesterol ester, two gram fat sample was mixed in a tightly capped tube with 0.6 ml potassium hydroxide (33%) and 9.4 mL absolute ethanol (95%), heated at 60 ºC for 20 min in a water bath with frequent shaking. The aliquot was cooled, and the cholesterol was extracted three times with 10 ml hexane, dissolved in dimethylformamide and derivatized by hexamethyldisilazane and trimethylchlorsilane for 30 min (AOAC, 2013). The cholesterol content (mg/100g) was determined using a commercial enzymatic kit (MAK043, Sigma Aldrich, St. Louis, USA) and a Unico (1200Series, USA) spectrophotometer. The absorbance of the sample was read at 500 nm against a blank (5-cholestane, Sigma).

Measurement of pH value

A triplicate five gram from each sample was homogenized with 20 ml distilled water for 15s. Three readings were taken for each homogenate using a digital pH meter with a probe-type combined electrode (Senso Direct 330). The average pH value was calculated from the readings of each replicate (Kandeepan *et al.*, 2009).

Thiobarbituric Acid Reactive Substances

Five gram sample was homogenized with 15 ml double distilled water for one min, filtrated and one ml filtrate was mixed with one ml each of trichloroacetic acid (15%), 2-TBA and 50 µl 7.2% butylated hydroxyanisole. The mixture was heated for 15 min in a boiling water bath, cooled, and finally centrifuged for 15 min. A Unico 1200 spectrophotometer (USA) was calibrated using a blank prepared from double distilled water at 531 nm. The absorbance of the sample was multiplied by 7.8 conversion factor to obtain TBARS content (mg malonaldehyde/kg) (Du and Ahn, 2002).

Peroxide value

Fat samples were melted in a hot air oven at 70ºC, poured into a clean beaker and filtered. Five g of the melted fat was shaken thoroughly with 30 ml glacial acetic acid chloroform mixture (60:40 v/v) to solubilize the fat. After that, half ml of saturated potassium iodide was added, mixed for 1 min and kept in a dark place. After 10 min, 30 ml H_2O_2 and 0.5 ml 1% starch solution were added then slowly titrated with 0.1 M sodium thiosulphate until the development of a yellow color. The peroxide value (milliequivalent peroxide/ kg fat) was obtained from the used sodium thiosulphate time its molarity (AOAC, 2013).

Acid value

The titration method with potassium hydroxide described by The American Oil Chemists' Society (AOCS, 2009) was used to estimate the acid value. Ten gram fat sample was mixed with 50 ml alcohol/ether mixture (v/v), heated slowly to 75-80°C in a water bath with frequent shaking for 10 min to dissolve the sample and finally

cooled to room temperature. Three drops phenolphthalein indicator was added to the mixture and titrated against 0.1 N KOH to faint pink color. The acid value was calculated from the volume of KOH used in the titration x normality (0.1) x molecular weight (56) divided the sample weight.

Instrumental color evaluation

The instrumental color values of three cubes (2 cm) from each fat sample were measured shortly after carcass preparation using CIE system (Hunt et al., 1991) using Konica Minolta Cromameter (CR 410, Japan) previously adjusted with a white plate and light trap. Standard black and white ceramic tiles were used to adjust the colorimeter. The average CIE values of L* (lightness), a* (redness), and b* (yellowness) were obtained from three readings at three different locations on the surface of each sample.

Fatty acid profile

The total lipid fractions from each fat sample were extracted in duplicate using chloroform-methanol, hexane mixture (Fernandes et al., 2009). The extracted lipids were saponification at 60ºC for 30 min with NaOH (0.5 N) in methanol, then methylated with a mixture of boron trifluoride-methanol (IUPAC, 1987). The resultant fatty acid methyl esters were separated and analyzed by an automated gas-liquid chromatograph equipped with a 1.8 m×3.2mm stainless steel column packed in GP 10% sp 2330 on 100/200 Chromosorb WAW. A standard was also chromatographed with each sample to confirm the sample identity. The free fatty acids percentages were calculated from the obtained peaks (Slover and Lanza, 1979).

Light microscopy

Three 1x1 cm frozen blocks from fat samples were fixed in 10% formol saline for 24h. Fixed samples were overnight washed under running tap water, dehydrated in serial dilutions of alcohol. After dehydration, all specimens were cleared in xylene and embedded for 24hr at 56ºC in paraffin. A 4 microns thin slices were prepared by slide microtome, collected on glass slides, deparaffinized, and stained by Verhöeff-van Gieson Stain (Verhöeff, 1908).

Scanning Electron Microscopy

The topographical details of camel fats were analyzed using Scanning Electron Microscopy according to the method of (Ketnawa and Rawdkuen, 2011). Two cubes (2 x 2 x 3 mm) from each deep-frozen fat sample were fixed in phosphate-buffered glutaraldehyde (2.5%) at 4°C for 4 hours. The fixed samples were washed three times with 0.1M phosphate buffer saline for 15 min each. The samples were post-fixed using 1% osmium tetroxide for 2h and dehydrated in upgrading concentration of ethanol (50-100) for 30 min each. The dehydrated samples were critical point dried, coated with gold, examined under QUANTA FEG 250 SEM (Hillsboro, Oregon, USA) SEM.

Statistical analysis

All data of sensory evaluation, chemical composition, fat oxidation criteria, instrumental color (L*, a*, and b*) and fatty acid profile were analyzed using SPSS 23.0 for windows (SPSS Inc., Chicago, IL, USA). All values were recorded as mean ± SE and the one-way ANOVA test was used to compare between means. The least-square difference test was used to detect the significance between means, which was considered at $P<0.05$.

RESULTS AND DISCUSSION

The distribution of the camel fats (Table 1) showed that most of the hump samples had a dull greyish color and hard texture, however, the majority of renal and mesenteric fat showed honey color and medium-soft texture. The grey color of hump fat may be due to its high connective tissue content (Table 3 and Fig. 1&2) a matter which verified the observation of Wood (1984). The high saturated fat and connective tissue contents of hump can explain its hard texture. The sensory panel scores of camel fat revealed the presence of significant differences in color, texture, and consistency, however, the odor scores were not different among the three types of fat (Table 2). Unfortunately, data were not available for comparing the obtained sensory criteria with camel fat or even with other fats. Carcass fat plays a core role in determining the quality characteristics of meat products (Adeyemi and Sazili, 2014). The sensory quality and nutritional value of fat are the most important criteria that determine the acceptability and processing ability of animal fat (Olivares *et al.*, 2010). The color, flavor, and texture are the chief sensory attributes of animal fat (Lida *et al.*, 2015), where good quality fat has a firm texture, white color, and an acceptable flavor.

The data of proximate chemical analysis (Table 3) revealed the presence of some differences between the fat from the three anatomical locations. The most evident difference was in moisture content. The hump had a significantly higher ($P<0.05$) moisture and lower ether-extractable lipids. However, the differences between the renal and mesenteric fats were slight. The hump fat had significantly higher collagen content and significantly ($P<0.05$) lower collagen solubility. The hump fat had significantly higher cholesterol contents (Table 3). In general, camel carcass with exception of the hump contains lower cholesterol level than other red meat animals (Abdel-Raheem *et al.*, 2019).

The fat oxidation criteria (TBARS, acid value, and peroxide number) indicated that camel fats exhibit good lipid stability. Moreover, hump fat was more stable than either renal or mesenteric fats. Mean fat oxidation values for camel fats followed a similar pattern and were correlated with fat saturation (Tables 3 and 4). The obtained results showed that an increase in total lipid contents (Table 3) and UFA (Table 4) were comparable to one another and the increase in fat oxidation criteria. The increase in the level of UFA in mesenteric fat was correlated with an increase in the susceptibility to fat oxidation. The obtained results were in agreement with Li *et al.* (2017). The results also indicated that all fat oxidation criteria were very low in comparison with limits of acceptability of fats.

Color evolution (Table 3) revealed the presence of significant differences in instrumental color parameters between different types of fat. Hump fat had a significantly lower L* value probably due to the greyish white color and the high connective tissue content (Table 2 & 3). Wood (1984) explained that the grey color of young fat was due to the higher connective tissue content, which lowers the lightness value. The results also indicated that both renal

Table 1: Number of camel fat samples from different anatomical locations in the different sensory classes

Color	Hump	Renal	Mesenteric
White	1	7	1
Greyish-white	25	3	1
Honey	4	20	28
Pale-yellow	0	0	0
Yellow	0	0	0
Odor			
Slight	0	0	0
Moderate	0	0	0
Strong	20	22	26
Intense	10	8	4
Pugnent	0	0	0
Hardness			
Hard	22	0	0
Medium-hard	8	0	0
Medium	0	5	3
Medium-soft	0	24	26
Soft	0	1	1

Table 2: Sensory attributes of dromedary camel fat from different anatomical locations

	Color	Odor	Hardness
Hump	6.00 ± 0.30^{a}	5.00 ± 0.20^{a}	5.01 ± 0.18^{a}
Renal	7.30 ± 0.17^{b}	5.30 ± 0.17^{a}	6.11 ± 0.14^{b}
Mesentery	7.73 ± 0.13^{ab}	5.19 ± 0.10^{a}	7.90 ± 0.22^{c}

$^{a–c}$ Values with different superscripts are significantly ($P<0.05$) different.

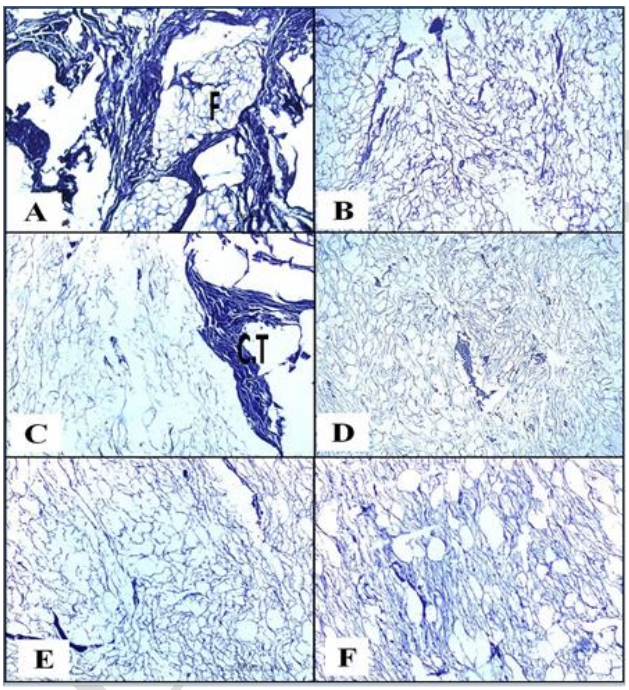

Fig 1: Light micrographs of raw camel fats (A-B hump fat; C-D renal fat and E-F mesenteric fat) stained with Verhöeff-van Gieson Stain and magnification power X40. The symbol F is pointed to fat while C.T for connective tissue.

and mesenteric fats had high yellowness value. Such data were correlated with both sensory evaluation and fatty acid profile. The highly unsaturated animal fat is relatively yellow in color because it contains a yellow carotenoid pigment which is associated with too much polyunsaturated fatty acids (Maw *et al.*, 2003).

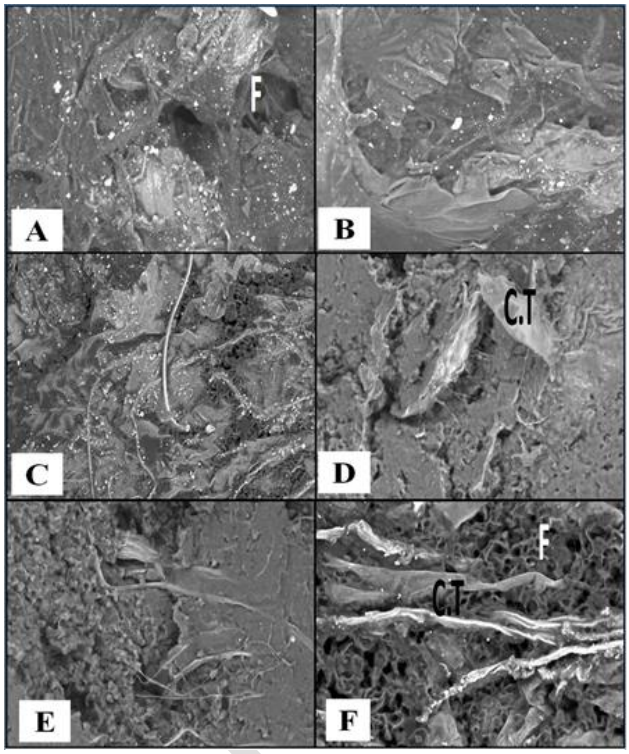

Fig. 2: Scanning electron micrographs of raw camel fats (A-B hump fat; C-D renal fat and E-F mesenteric fat). Magnification power X250 in A, C and E while X500 in B, D and F. The symbol F is pointed to fat while C.T for connective tissue.

Histological examination is used to display the microscopic structure as a tool to investigate the texture of camel fat. The light microscopic assay may require the use of a specific staining technique to enhance the visibility of connective tissue elements, fat cell orientation, and architecture of the fibrous tissues. The light microscopic micrographs of hump fat stained with Verhöeff-van Gieson stain demonstrated a strong matrix formed of dense elastic connective tissue holds numerous fat globules of nearly uniform size in-between, moreover, other fat cells were surrounded by a loose connective tissue (Fig. 1 a&b). In general, the fat cells were less abundant and less evident in both renal and mesenteric fat (Fig. 1 c&d - e&f), where some fat droplets form large ones. The differences in the amount of elastic connective tissue were the predominated feature between the different types of fat. The hump showed the highest content, while mesenteric fat had few fibers. The scanning electron micrographs showed that the architecture of hump was characterized by the presence of fat cells embedded in a less dense non-fibrous matrix and connective tissue fibers of various types and degrees of cross-linking. In some areas, a compact connective tissue bundle completely obscures the structure beneath (Fig. 2 a&b). The compact connective tissue bundles were not evident in both renal (Fig. 2c) and mesenteric fats (Fig. 2e), however, lower amounts of fibrous connective fibers were found. Uniformly distributed fat globules as monitored by the presence of multiple pores in a less dense matrix were more clear in mesenteric fat, however, the fat globules were lower in number (Fig. 2 e-f).

Fat is an indispensable ingredient in the meat industry. The quality characteristics of meat products e.g., tenderness, mouthfeel, flavor and shelf life rely upon the physical,

Table 3: Proximate chemical composition (g/100 g) and physicochemical criteria of dromedary camel fat from different anatomical locations

	Hump	Renal	Mesentery
Chemical composition (g/100 g)			
Moisture	23.01±1.03[a]	15.07±1.38[b]	15.62±1.13[b]
Protein	3.85±0.05[a]	3.07±0.07[b]	2.73±0.05[c]
Fat	72.77±2.06[a]	81.34±1.20[b]	81.16±1.9[b]
Ash	0.15±0.02[a]	0.13±0.09[b]	0.13±0.07[ab]
Collagen content (g %)	0.51±.013[a]	0.40±.019[b]	0.34±.038[c]
Collagen solubility (g %)	0.033±0.003[a]	0.05±0.005[b]	0.043±0.004[b]
Cholesterol mg/100g	81.5±0.11[a]	53.45±0.13[b]	50.46±.09[c]
pH	6.98±0.03[a]	6.11±0.04[b]	6.79±0.03[c]
Fat oxidation criteria			
TBARS (mg/kg)	0.11±0.09[ab]	0.13±0.04[a]	0.15±0.05[b]
Acid value	0.80±0.09[a]	1.01±0.10[b]	1.23±0.11[c]
Peroxide (mequiv O_2/kg)	2.45±0.02[a]	2.66±0.03[a]	2.91±0.05[b]
Instrumental color			
Lightness	68.83±1.84[a]	75.61±1.11[b]	70.18±1.90[c]
Redness	10.08±0.45[a]	5.77±0.08[b]	1.633±0.07[c]
Yellowness	12.7±0.30[a]	14.53±1.40[b]	16.47±1.35[c]

[a–c] Values with different superscripts are significantly (P<0.05) different.

Table 4: fatty acid profile of dromedary camel fat from different anatomical locations

	Hump	Renal	Mesentery
C8:0	ND	ND	0.09±0.002
C10:0	0.10±0.01[a]	ND	0.04±0.001[a]
C12:0	0.44±0.02[a]	0.37±0.02[a]	0.10±0.001[b]
C14:0	6.30±0.33[a]	6.78±0.98[a]	0.38±0.002[b]
C14:1	1.13±0.24[a]	1.90±0.11[a]	4.94±0.76[b]
C15:0	0.27±0.01[a]	2.85±0.45[b]	1.77±0.23[b]
C16:0	33.87±2.50[a]	31.88±2.79[a]	27.23±2.77[b]
C16:1	0.31±0.02[a]	0.47±0.02[a]	ND
C18:0	10.0±1.10[a]	1.94±0.32[b]	ND
C18:1	41.88±3.45[a]	53.10±4.57[b]	62.76±5.78[c]
C18:2	0.10±0.02[a]	0.23±0.01[a]	0.40±0.002[a]
C18:3	0.03±0.01[a]	0.07±0.01[a]	0.10±0.001[a]
C20:0	4.90±0.92[a]	0.02±0.003[b]	0.15±0.001[b]
C22:0	0.01±0.001[a]	0.02±0.004[a]	0.33±0.02[a]
Unknown	0.66±0.02[a]	0.37±0.07[a]	0.55±0.03[a]
Total Saturated fatty acids	55.89±3.34[a]	43.86±4.52[b]	30.09±2.69[c]
Monounsaturated fatty acids	43.32±3.69[a]	55.47±4.98[b]	67.70±4.98[c]
Polyunsaturated fatty acids	0.13±0.04[a]	0.30±0.04[b]	0.50±0.01[c]
Total Unsaturated fatty acids	43.45±4.38[a]	55.77±3.33[b]	68.86±4.45[c]

[a–c] Values with different superscripts are significantly (P<0.05) different.

oxidative, and thermal properties of animal fats (Mallika *et al.*, 2019). The obtained data showed that the proportion of the individual fatty acids, collagen content, and histological findings determined the hardness of the fat and its suitability for combination in meat products. Consequently, both renal and mesenteric fats are ideal, while hump fat is unsuitable for the production of emulsion type products, as the general role governing the suitability of fat for emulsification stipulated that higher UFA and soft texture improve the emulsifying capacity of fat (Santhi *et al.*, 2017). In this respect, the high melting points of both palmitic and stearic acids in hump fat, and high connective tissue content (Table 4 and Fig. 1) contribute to its hardness and bad emulsifying capacity. Moreover, the high connective tissue content has a determinate impact on meat emulsion stability due to its difficult dissolving ability and thermal instability (Kandeepan *et al.*, 2009). On the other hand, the low connective tissue content and the high-level oleic acids (0-15°C melting point) were responsible for the softness of renal and mesenteric fats. Concerning the use of animal fat in ground-type meat products soft fat is less suitable because it results in oily appearance, lack of cohesiveness, and rapid susceptibility to oxidative rancidity (Maw *et al.*, 2003), however, hard fat exhibited a granular appearance on cutting.

Conclusions

From the obtained data, it can be concluded that camel fats differ significantly in suitability for processing different meat products. Both renal and mesenteric fats are more suitable for the production of emulsion-type products with special care for the possibility of lipid oxidation, while the hump fat is more acceptable for the production of ground-type products.

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