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Investigation of fatty acid composition, thermal and rheological behavior of yak, cow and horse fats

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ABSTRACT

The objective of this paper was to study the fatty acid composition, and thermal and rheological behavior of Kyrgyz yak (Bos grunniens) visceral fats compared to visceral fats of cow (Bos taurus) and horse (Equus caballus). The result of the study revealed that the content of saturated fatty acids (SFA) in yak fat was higher than those of unsaturated fatty acids (UFA) (about 58.3 and 38.0 %, respectively). The UFA content in yak fat was higher compared to cow fat (about 27.1 %) and lower than that of horse fat (about 60.0 %). The melting temperatures determined using DSC were found to be 55.18±0.71, 54.98±3.01, 37.60±1.92°C for yak, cow and horse fat, respectively. These results were close to the solid-liquid transition temperature determined by oscillation rheology (52.20±0.89°C for yak, 53.56±3.53°C for cow and 33.7±1.84°C for horse fat). Rotational rheological measurements at 35°C have shown that yak fat had shear thinning flow behavior with high a viscosity of 226 mPa•s compared to horse fat, which has Newtonian flow behavior with a viscosity of about 36 mPa•s (γ=100s-1). Results on properties obtained in this study will help to understand the contribution of yak fats to the structural properties of new products with these alternative fat sources.

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1. Introduction

Fat is an important source of essential fatty acids since it facilitates the absorption of fat-soluble micronutrients such as vitamins and trace elements. In addition, fat is an important attribute for determining the consumers' acceptance of food products, that is, of their desirable appearance, flavor, aroma, texture, and mouth feel [1]. Fat tissue is the second most important anatomical and morphological component of eviscerated animals. Animal fat is a natural product with a low level of non-nutritional substances. Moreover, animal fats are mostly saturated, stable at elevated temperatures, and can be stored longer compared to plant-based fats. Reduced oxidation in animal fats ensures less susceptibility to toxins and carcinogens [2].

In recent times, the world's scientific literature pays more attention to the content of fatty acids in animal raw materials of various origin. The majority of studies on the fatty acid compositions have been carried out concerning beef cattle, lamb, goat, pig and chicken fats [3-5]. The thermal behavior of animal fats has been also widely investigated to detect adulteration and identification and to study the oxidative stability and enzymatic interesterification [6-9]. Rheological properties, such as elastic modulus of fats, have been found to be a suitable indicator of the hardness of fats [10-11]. Moreover, these properties can provide information regarding spreadability and mouth feel [12]. Rheological methods were also used to understand the microstructure of fat crystal network [13-14] and to determine the melting and crystallization temperatures [15-16].

An increasing consumer demand for meat-based products, such as sausage, has encouraged research on alternative fat sources, including yak fat. Yak *(Bos grunniens)* is a multi-purpose animal. There are approximately 31,000 heads of

yak, and they are an important cattle species in the Tian-Shan mountain of Kyrgyzstan [17]. Yaks are adapted to extreme living conditions and high altitude (2,000 to 5,000 m above sea level). Living in the severe climatic conditions of high mountains, yaks survive by grazing on highland pastures with widely dispersed feed, mostly composed of grass and herbs. Along with milk, meat and wool, slaughtered yak gives up to 10 kg of visceral fat [18]. Due to the higher carotene content (19.1 mg/kg), yak fat is a typical reddish-yellowish color. For comparison, the cow fat has a carotene content of 7.2 mg/kg [19]. The cholesterol level of subcutaneous yak fat is low and varies from 13 to 17 mg/g [19], while this level in lard is 38 - 76 mg/g and in cow fat is 95 - 140 mg/g [20]. The results of the previous research [3, 21] reported that the main fatty acids in yak body and kidney fat were palmitic acid (C16:0), stearic acid (C18:0), and oleic acid (C18:1). The contents of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in yak body fat were 42.7 and 7.95 %, respectively [3]. Consequently, yak fat can be considered as a potentially useful product and utilization of yak fat as an ingredient of new meat-based products could be of high interest. However, little information is available on the fatty acid profiles, rheological and thermal properties of visceral yak fat.

The aim of this study was to analyze the fatty acid composition of Kyrgyz yak fats. First, fatty acid profile of yak fat was measured using gas chromatography (GC) and the results were compared with fatty acid compositions of cow and horse fat. Cow and horse fat were chosen as reference systems due to their common bovine origin (cow fat) and similar nutritional values (horse fat). Secondly, thermal and rheological properties of yak, cow, and horse fats were studied to determine the processing conditions of yak fat in meat-based products. Finally, the correlation of the thermal and rheological properties of animal fat and its fatty acid composition was calculated.

2. Materials and methods

2.1. Materials and Sample Preparation

Fat samples were collected from animals of the autumn slaughtering season in 2012 (female animals, 2.5 - 3.5 years old, of medium nutritional state). The visceral fats of 6 yaks, 5 cows, and 6 horses were used for each fat sample. All animals were kept in the highland pastures of Issyk-Kul region (the northeastern part of Kyrgyzstan) at an average altitude of 3,800 m above sea level. The climate is continental and characterized by an extraordinary change of climatic conditions, i.e. high-temperature differences during the season and intense solar insolation.

Preparation of samples was done in accordance with the general guidelines on sampling CAC/GL 50-2004 (Codex Alimentarius, 2007) and ISO 3100-1-91, 1991 [22-23]. Primary fat samples of 200 g each were taken from three different parts of each animal visceral fat. A composite sample was obtained by mixing the primary samples. The composite fat sample was minced and placed in steel cups and then kept at 110°C for 30 to 60 minutes in a heating chamber of Model FD 56 (Binder, Tuttlingen, Germany) until a clear melt appeared. The molten samples were filtered using 5.0 µm filters (Sartorius, CA Membran) and divided into three aliquots by types of analyses performed: for fatty acid analysis, rheological analysis, and DSC analysis. Each analysis was done at least in three times in order to avoid inaccuracy.

2.2. Measurement of Fatty Acid Profile

The sample preparation procedure was performed according to application note described by David et al. (2002) [24-25]. To do so, 100 mg of each melted fat sample was transferred into a test tube; 100 µL of 2 M KOH in methanol was added to each sample and the content of tubes was vortexed for 2 - 3 minutes. Upon cooling, 5 mL of hexane was added to the sample and the content of the test tube was thoroughly mixed; afterward, it was centrifuged. The clear supernatant was removed from the test tube and injected to a gas chromatography (Agilent 7890 A, USA), which was equipped with flame ionization detector (FID) and DB-Wax column (30 m × 0.25 mm × 0.25 µm, Agilent Technologies, USA). Nitrogen was used as a carrier gas with a flow rate of 6.4941 mL/min. The temperature of the detector was at 275 °C. An initial column temperature of 80°C was held for 3 min, then increased at 7°C/min to a final temperature of 240 °C, where it was held for 10 min. Identification of peaks was based on the comparison of their retention times with those of the standard fatty acids (Carl Roth GmbH, Karlsruhe, Germany). Fatty acids were identified by comparison of retention times to those of a standard FAME mixture (Carl Roth, Karlsruhe, Germany). The fatty acid contents were calculated based on a percentage of the peak area.

2.3. Differential Scanning Calorimetry (DSC)

DSC measurements were carried out by using a Simultaneous DSC/TG Thermal Analyzer STA409C (Netzsch Gerätebau GmbH, Selb, Germany). Indium (melting temperature 156.6°C, ΔH = 28.45 J/g) was used for calibration of the instrument and an empty pan was used as a reference. At 80°C 15 min melted fat samples of 10 - 30 mg were weighed in aluminum pans and sealed with a pierced lid and cooled until - 20°C and kept overnight at this temperature in order to ensure solid crystallization. DSC analyses of the samples were done in a temperature range from - 10 to 80°C at a rate of 5°C/min. All data recordings were carried out using nitrogen as a purge gas (75 mL/min) to reach the desired temperatures and to prevent samples from oxidation. All samples were analyzed at least three times. The liquid fraction LF at T_i was determined from equation 1 [26]:

$$LF = \frac{\int_{T_0}^{T_i} AdT}{\int_{T_0}^{T_f} AdT} \cdot 100\% \quad (1)$$

where T_0 is the temperature where the melting process begins (T_{onset}) and T_f is the temperature of the end of the melting process (T_{offset}). The low melting (LM) and high melting (HM) peaks in the DSC curve were established according to their melting temperatures, i.e - 10 to 23°C for the low melting peak and 23 to 80°C for the high melting peak.

2.4. Rheological Measurements

Steady and dynamic rheological experiments were carried out using a rheometer MCR 302 (Anton Paar, Graz, Austria) equipped with concentric cylinder geometry CC27. All measurements were conducted after equilibration of temperature at 70, 60, 50, 40, and 35°C. Samples were placed into the measurement chamber in the molten state at 80 °C with the equilibration time 15 min, 0.1°C.
The individual flow curves were recorded by decreasing the shear rate from 100 to 5 s^{-1} (20 data point in total) within 60 s. High shear stress was chosen as an initial value to ensure a homogenous sample distribution and movement. The power law equation (Eq. 2) was used for samples with non-Newtonian flow behavior (35°C), while the Newtonian law (Eq. 3) was applied to samples with linear flow behavior in the temperature range from 40 to 70°C:

$$\tau = K \cdot \dot{\gamma}^n \quad (2)$$

$$\tau = \eta \cdot \dot{\gamma} \quad (3)$$

where τ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), K is the consistency coefficient (Pas^n), and n is the flow behavior index. The non-Newtonian case was treated as a shear-thinning fluid ($n < 1$) [27]. In the case of the Newtonian fluid ($n = 1$) Eq. 2 can be simplified to Eq. 3, in which η is the viscosity. In addition, the activation energy E_a was calculated at maximum shear stress ($\dot{\gamma}_{max} = 100\ s^{-1}$) according to the Arrhenius-type relationship (Eq. 4, 5) at the temperature range 40 - 70°C:

$$\eta(T) = A\exp\left(-\frac{E_a}{R \cdot T}\right) \quad (4)$$

$$\ln\eta = \ln A + \left(\frac{E_a}{R}\right) \cdot \left(\frac{1}{T}\right) \quad (5)$$

where A is the pre-exponential factor, R is the ideal gas constant (8.31 J/mol·K), T is the absolute temperature (K), and E_a is the activation energy (J/mol) [28].

The viscoelasticity and phase transition temperatures of the fats were studied in an oscillatory temperature sweep experiment at constant strain γ of 10^{-3} % (Linear viscoelastic range) and the constant angular frequency of 1 Hz. The temperature profile of the cooling-heating-cycle was set from 60 to 0°C followed by 0°C to 60°C. The sample temperature was decreased and increased within the experiments by cooling and heating rates of 0.5 °C/min to ensure sufficient sample equilibration. The measured elastic G' and loss G'' moduli provide detailed information on the fat elasticity (stored energy in the form of deformation) and fat viscosity (energy dissipation as heat by internal friction), as well as on the melting and crystallization behavior of the fats. The crystallization and melting temperatures from oscillatory measurements were determined by the loss factor $tan\ \delta = G''/G'$. The loss factor relates the viscous properties was denoted by G'' versus the elastic properties denoted by G'. Thus, values above 1 thus indicate more viscous flow behavior while any value below 1 is related to elastic network response [26]. The RHEOPLUS V 3.61 software (Anton Paar, Ostfildern, Germany) was used for the rheological data evaluation. Steady and dynamic rheological measurements were carried in triplicate.

2.5. Statistical Analysis

Analysis of variance (ANOVA) was used to test the differences between the fatty acid contents, thermal and rheological data (Group 1) and investigated animal fats (Group 2). Duncan test was applied to compare the difference between the means (SPSS software version 16 (SPSS Inc., Chicago, IL), with the 95% confidence interval.

3. Results and discussion

3.1. Fatty Acid Composition

The identified saturated fatty acids (capric, lauric, tridecylic, myristic, pentadecylic, palmitic, margaric, stearic, nonadecylic, arachidic, heneicosylic and behenic acids) and unsaturated fatty acids (myristoleic, ginkgolic, palmitoleic, heptadecenoic, oleic, linoleic, paullinic and conjugated linoleic acid) of yak, cow, and horse fat are listed in Table 1. A significant difference ($P < 0.05$) between the fatty acids content and animal types are obvious (Table 1). In general, the mean difference of the content of palmitic (C16:0, from 19.36 to 26.29 %), stearic (C18:0, from 3.61 to 34.33 %), and oleic acids (C18:1, from 22.34 to 52.2 %) were found to be significant in all fats ($P < 0.05$). The amount of these acids accounts for three-quarters of the total amount of fatty acids. When compared to lard fat, which is widely used in the meat product industry, the content of palmitic acid in yak fat was lower, 19.36 %, whereas lard contains 22.68 % [29].

Table 1. *Fatty acid composition (% of total fatty acids) of visceral yak, cow and horse fats*

	Fatty acids	Yak fat	Cow fat	Horse fat
1	Capric acid (10:0)	$0.08^{l,m,B} \pm 0.00$	$0.09^{j,k,A} \pm 0.02$	ND
2	Lauric acid (12:0)	$0.05^{m,o,C} \pm 0.00$	$0.14^{h,i,j,A} \pm 0.00$	$0.10^{e,B} \pm 0.01$
3	Tridecylic acid (13:0)	$0.17^{k,C} \pm 0.00$	$0.31^{g,h,B} \pm 0.01$	$0.36^{e,A} \pm 0.04$
4	Myristic acid (14:0)	$2.12^{f,C} \pm 0.01$	$3.66^{d,A} \pm 0.04$	$2.50^{d,B} \pm 0.06$
5	Pentadecylic acid (15:0)	$0.30^{j,B} \pm 0.00$	$0.44^{g,A} \pm 0.01$	$0.24^{e,C} \pm 0.02$
6	Palmitic acid (16:0)	$19.36^{c,B} \pm 0.06$	$26.29^{b,A} \pm 0.26$	$20.05^{b,B} \pm 1.61$
7	Margaric acid (17:0)	$1.15^{g,B} \pm 0.00$	$1.13^{f,B} \pm 0.01$	$2.58^{d,A} \pm 1.44$
8	Stearic acid (18:0)	$34.33^{a,A} \pm 0.13$	$33.54^{a,B} \pm 0.31$	$3.61^{d,C} \pm 0.28$
9	Nonadecylic acid (19:0)	$0.17^{k,B} \pm 0.00$	$0.12^{i,j,k,B} \pm 0.00$	$2.38^{d,A} \pm 0.03$
10	Arachidic acid (20:0)	$0.40^{i,A} \pm 0.00$	$0.12^{i,j,k,B} \pm 0.00$	$0.10^{e,C} \pm 0.02$
11	Heneicosylic acid (21:0)	$0.13^{k,l,A} \pm 0.00$	$0.07^{j,k,B} \pm 0.02$	$0.08^{e,B} \pm 0.00$
12	Behenic acid (22:0)	$0.08^{l,m,B} \pm 0.00$	$0.10^{j,k,B} \pm 0.01$	$0.42^{e,A} \pm 0.03$
13	Myristoleic acid (14:1)	$0.13^{k,l,C} \pm 0.01$	$0.21^{h,i,j,B} \pm 0.01$	$0.35^{e,A} \pm 0.11$
14	Ginkgolic acid (15:1)	$0.19^{k,B} \pm 0.00$	$0.18^{h,i,j,B} \pm 0.00$	$0.24^{e,A} \pm 0.02$
15	Palmitoleic acid (16:1)	$2.33^{e,B} \pm 0.01$	$2.60^{e,A} \pm 0.02$	$0.82^{e,C} \pm 0.04$
16	Heptadecenoic acid (17:1)	ND	ND	$0.07^{e,A} \pm 0.10$
17	Oleic acid (18:1)	$32.06^{b,B} \pm 0.12$	$22.34^{c,C} \pm 0.20$	$52.20^{a,A} \pm 4.01$
18	Linoleic acid (18:2)	$2.52^{d,B} \pm 0.20$	$1.19^{f,C} \pm 0.49$	$5.10^{c,A} \pm 0.89$
19	Conjugated linoleic acid	$0.54^{h,B} \pm 0.04$	$0.29^{g,h,i,C} \pm 0.22$	$1.17^{e,A} \pm 0.15$
20	Paullinic acid (20:1)	$0.27^{j,B} \pm 0.00$	$0.29^{g,h,i,A} \pm 0.00$	$0.09^{e,C} \pm 0.00$
	Unidentified fatty acid	3.63	6.90	7.56
	∑ (SFA) %	58.34	66.01	32.40
	∑ (UFA) %	38.03	27.09	60.04
	MUFA	34.97	25.62	53.77
	PUFA	3.06	1.47	6.27
	Σ (SFA + UFA) %	96.37	93.11	92.44
	SFA / UFA	1.5	2.4	0.5
	UFA / SFA	0.7	0.4	1.9
	UFA : SFA	1 : 1.5	1 : 2.4	1 : 0.5

The fatty acids were reported as the mean ± standard deviation of three independent measurements.
ND not detected, *SFA* total saturated fatty acids, *UFA* total unsaturated fatty acids, *MUFA* total monounsaturated fatty acids, *PUFA* total polyunsaturated fatty acids.
[a-o] Differences within the fatty acids content are statistically significant ($p < 0.05$)
[A-C] Differences within the investigated fats are statistically significant ($p < 0.05$).

The content of oleic acid (18:1) in horse fat was significantly higher ($P < 0.05$), 52.20 %, whereas in cow and yak fats it was significantly lower ($P < 0.05$) 22.34 and 32.06 %, respectively. These results are in compliance with reports in the literature relating to distinct fatty acid compositions of horse fat [30-31]. The oleic acid content of yak fat was similar as in lard 29.94% [30] and 38.24% [29].

Linoleic acid (18:2) belongs to one of two families of essential fatty acids and its amount in horse fat was 5.10 %, while in yak and cow fats the content of linoleic acid was as low as 2.52 and 1.19 %, respectively. Similarly, the percentage of conjugated linoleic acid in horse fat (1.17 %) was also higher than yak and cow fat (0.54 % and 0.29 %, respectively). Cow fat contains the highest amount of SFA (66.01 %) and the lowest amount of UFA (27.09 %), which is similar to previous results [6]. Yak fat contains high amounts of SFA (58.34 %) and lower amounts of UFA (38.03 %). These findings go in line with the result obtained by Liu et al. [31], where it was shown that yak hepatic lipids have a higher content in SFA (58.11 %) than in UFA (41.89 %). A relatively high content of UFA (60.04 %) was determined in horse fat, which is close to the results obtained by Tonial et al. (2009) [32]. The content of MUFA (53.77 %) and PUFA (6.27 %) in horse fat were higher than those of yak and cow fats. Calculation of the UFA/SFA ratio (Table 1) of yak fat is 0.7, which is higher than in cow fat (0.4). Finally, the highest UFA/SFA ratio was observed for horse fat (1.9). The data in Table 1 were analyzed using ANOVA test on SPSS software version 16 (SPSS Inc., Chicago, IL). Significant differences between fatty acid content and investigated fats were compared with Duncan's test at a significance level of $P \leq 0.05$.

3.2. Thermal Behavior

Differential scanning calorimetry (DSC) was used to characterize the fat thermal behavior by monitoring associated changes in enthalpy upon heating. Fig. 1 shows the melting thermograms from - 10 to 80°C for the three animal fats studied (cow, yak, and horse). The fat melting profile of animal fats exhibited two endothermic DSC peaks. The minor peak for

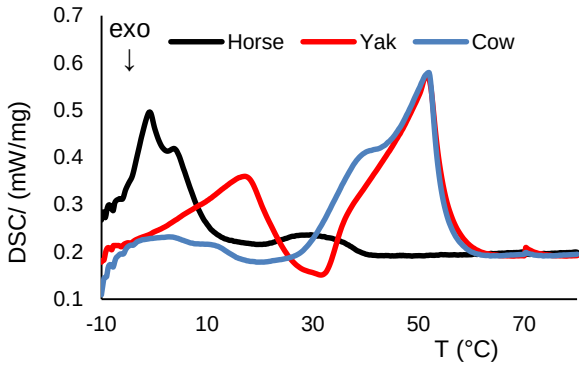


Figure 1. *DSC melting curve of cow, yak and horse visceral fats in a temperature range of -10 to 80°C*

yak fat was found in the low-temperature region (15.98 ± 0.62°C), while the major peak was observed in the high-temperature region (51.31 ± 0.46°C). On the contrary, for horse fat, the major endotherm peak was observed at 5.17 ± 1.13°C and a minor peak at 28.70 ± 1.19°C, which is attributed to its large amount of unsaturated fatty acids. Thus, the wide range of melting temperatures of the animal fats can be explained by various melting temperature of individual fatty acids. As previously reported [20, 33], the low-temperature endotherm peak was assigned to olein fraction and the high-temperature endotherm peak to stearin fraction. For example, the melting point of stearic acid is 69.6°C, whereas that of oleic acid is 13.4°C. The melting points of polyunsaturated fatty acids of the C_{18} series are even lower (- 5°C) [34]. Integral area (∫A) and enthalpy (H) of high melting (HM) and low melting (LM) peaks of yak, cow and horse visceral fats calculated from DSC curves (Eq. 4) are given in Table 2. The $\int A_{HM} / \int A_{LM}$ –ratio (1.01, 2.27 and 0.48 for yak, cow and horse fat, respectively) in Table 2 and SFA/UFA-ratio (1.5, 2.4 and 0.5 for yak, cow and horse fat, respectively) in Table 1 are almost similar. Therefore, the obtained low melting (LM) peak can be attributed to UFA and high melting (HM) peak to SFA. The specific heat parameter of the experimental fats was also determined by DSC. The peak area of both low and high-temperature regions was used to determine the melting enthalpies ΔH of the samples. The melting enthalpies ΔH in the high-temperature region were observed for yak fat = 67.93 J/g, cow fat = 71.86 J/g. These values are significantly higher than the melting enthalpy determined for horse fat = 3.93 J/g. The lard had the lower melting enthalpy of 44.36 J/g [9], which is comparable to yak and cow fats, but considerably higher than the melting enthalpy of horse fat.

Table 2. *Integral area (∫A) and enthalpy (H) of high melting (HM) and low melting (LM) peaks of yak, cow and horse visceral fats calculated from DSC curves*

DSC Parameters	Yak fat	Cow fat	Horse fat
$\int A_{HM}$ (W^2/g^2)	$10.67^{c,B} \pm 0.34$	$13.62^{c,A} \pm 0.55$	$4.63^{c,C} \pm 0.42$
$\int A_{LM}$ (W^2/g^2)	$10.6^{c,A} \pm 0.29$	$5.97^{d,C} \pm 0.46$	$9.73^{a,B} \pm 0.16$
H_{HM} (J/g)	$67.93^{a,B} \pm 0.34$	$71.86^{a,A} \pm 0.21$	$3.93^{d,C} \pm 0.62$
H_{LM} (J/g)	$63.3^{b,A} \pm 0.36$	$30.54^{b,B} \pm 0.73$	$7.06^{b,C} \pm 0.79$
$\int A_{HM} / \int A_{LM}$	1.01	2.27	0.48
$\int A_{LM} / \int A_{HM}$	0.99	0.44	2.10

DSC parameters were reported as the mean ± standard deviation of three independent measurements
[a-d] Differences within the thermal properties are statistically significant ($p < 0.05$)
[A-C] Differences within the investigated fats are statistically significant ($p < 0.05$).

3.3. Viscosity

Shear rate dependence of apparent viscosity of yak fat shows the strong shear thinning behavior at 35°C, while horse fat showed a Newtonian behavior. No flow-curve data were obtained from cow fat samples at 35°C (Fig. 2). The flow behavior index (*n*) and consistency index (*K*) values of yak fat were obtained by fitting the shear rate versus apparent viscosity data to a power law model (Eq. 2). The values of the flow behavior index, *n*, of yak fat were

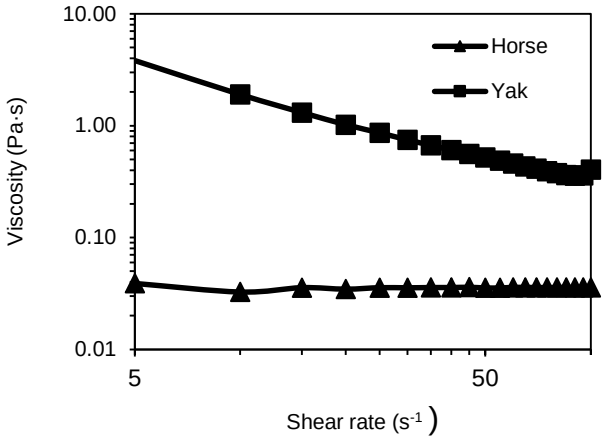


Figure 2. *Viscosity as a function of shear rate for yak, cow (not measurable) and horse visceral fats at 35°C.*

0.23 at 35°C. The consistency index, *K*, was 10.93 $Pa \cdot s^n$ for yak fat. The smaller *n* values the greater the departure from Newtonian behavior. As shown by DSC analysis, horse fat

melted already at 28.6°C, therefore it has the lowest viscosity of 35.7 ± 0.6 mPa·s compared to yak fat at 35°C. There is a negative correlation between UFA/SFA ratio and average melting temperature of the individual fat, i.e. the melting points decrease with an increase in unsaturation of fatty acids [2]. Therefore, at 35°C yak fat exhibits shear-thinning behavior with a high effective viscosity of 226.8 ±1.4 mPa·s than horse fat due to the low ratio of UFA/SFA. The viscosity of the widely used lard fat at 35°C was reported to be 42.8 mPa·s [15]. Consequently, industrial application, e.g. replacing lard fat in sausages by the addition of yak fat becomes possible when mixed with horse fat, and, thus obtaining the melting and crystallization behavior of lard. As mentioned above, by the DSC analysis, increasing the temperature from 35 to 70°C caused the progressive phase transition from solid to the liquid-like state for cow and yak fats. Thus, at T ≥ 40°C all fat samples exhibited distinct Newtonian flow behavior for share rates obtain approximately greater 10 s^{-1}(Fig. 3). Comparison of the viscosity values at 70 °C of fats showed that yak fat has almost the same viscosity (15 ± 0.2 mPa·s) as cow (15 ± 0.5 mPa·s) and horse fats (12 ± 0.3 mPa·s).

An Arrhenius-type relationship (Eq.4) was employed to estimate the activation energy for fat samples. A least squares linear regression was used to estimate the activation energy from the slope of Eq. 5. The activation energy requirement for the cow fat $E_{a,\,cow}$ = 30.46 ± 0.5 kJ/mol with R^2 = 0.996 is higher and for horse fat $E_{a,\,horse}$ = 25.5 ± 0.6 kJ/mol with R^2 = 0.999 is lower, which indicate that the viscosity of cow fat was more sensitive to temperature changes compared to horse fat. The activation energy of yak fat was $E_{a,\,yak}$ = 29.5±0.7 kJ/mol with R^2 = 0.995. Similar values of activation energy (26.5 kJ/mol) were reported of lard at 35 - 90°C [15], 26.3 kJ/mol of coconut fat containing 90% of saturated fatty acids at 30-90°C [16], 36.5 kJ/mol of rendered cow fat at 10 - 62 °C [35] and 20.1 kJ/mol at 58 - 94°C [36].

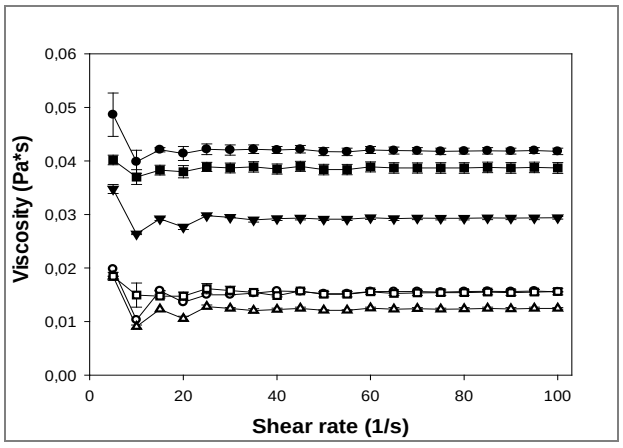


Figure 3. *Viscosity as a function of shear rate for yak (○, ●), cow (□, ■) and horse visceral fats (Δ, ▼) at 40 and 70 °C, respectively.*

3.4. Viscoelastic Behavior

Figure 4 shows elastic G' and loss G'' moduli obtained in temperature sweep experiments throughout the entire cooling (Fig. 4A) and heating processes (Fig. 4B), as well as the behavior of the loss factor *tan* δ (Fig. 4C). Fully melted fats showed a weak viscoelastic liquid structure with the loss modulus (G″) higher than the elastic modulus (G′) at a temperature range from 60 to 50°C. The shift in G' and G'' of cow and yak fat during heating and cooling were almost similar behavior (Fig. 4B) due to their similar fatty acid compositions. The significant difference (P≤0.05) between yak and horse fat is more obvious (Fig. 4A), which is explained by the different fatty acid composition. Уак fat samples continued to develop their microstructure during cooling at 37.43 ±0.94°C, for horse fat samples this temperature is at 10.34 ±2.94°C.

A standard procedure for determining the liquid-solid (l-s) or solid-liquid (s-l) transition temperatures is done by calculating loss factor *tan* δ = G''/G', which provides a measurement of crystallization $T_{l\text{-}s}$ and melting $T_{s\text{-}l}$ temperatures. With decreasing loss factor *tan* δ the sample progressively solidifies and the viscoelastic behavior changes from viscous to elastic, i.e. when loss factor *tan* δ > 1 sample obtains liquid and viscous properties, and when *tan* δ < 1 it becomes solid and elastic [28].

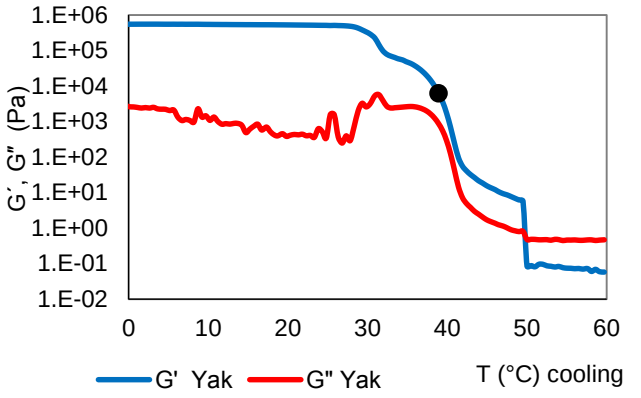
Fig. 4C also shows the changes in loss factors *tan* δ during the process of heating and cooling. As shown in Fig. 4C, the loss factor *tan* δ for horse fat upon heating at a temperature range from 0 to 40°C is higher than that of cow fat. At temperatures higher than 40°C, loss factor *tan* δ of cow fat increases more than that of horse fat. Thus, the liquid-solid transition temperatures at *tan* δ < 1 for yak fat was $T_{l\text{-}s}$, yak = 37.43± 0.94°C, for cow fat $T_{l\text{-}s}$, cow = 38.40±0.77°C, and for horse fat $T_{l\text{-}s}$, horse = 10.34±2.94°C. The solid-liquid transition temperatures at *tan* δ < 1 of yak fat $T_{s\text{-}l}$, yak = 52.2 ±0.89 °C found to be similar to that of cow fat $T_{s\text{-}l}$, cow = 53.56 ±1.91°C. The temperature sweep method applied to lard fat showed the solid-liquid transition temperature was 47°C [15]. Horse fat has solid-liquid transition temperature at $T_{s\text{-}l}$, horse = 33.7±1.84°C (Table 3) and it is comparable with that of the goose fat ($T_{s\text{-}l}$, goose = 38.5°C) [15].

Table 3. *Temperature range of crystallization ($T_{l\text{-}s}$, °C) and melting ($T_{s\text{-}l}$, °C) of yak, cow and horse visceral fats, according to oscillatory temperature sweep and DSC melting curve*

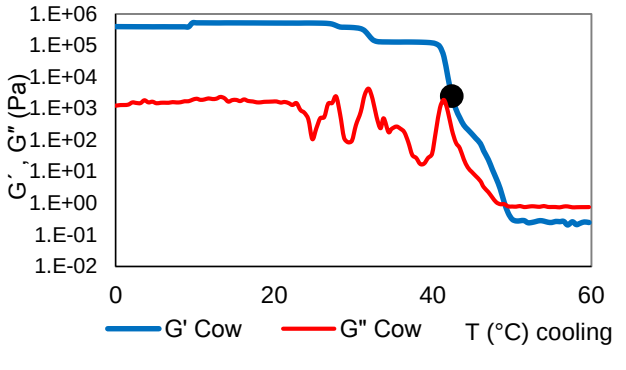
	Oscillatory temperature sweep		DSC (melting curve)			
	$T_{l\text{-}s}$	$T_{s\text{-}l}$	$T_{peak\ 1}$	$T_{peak\ 2}$	$T_{peak\ 3}$	$T_{peak\ end}$
Yak fat	$37.43^{a,B} \pm 0.94$	$52.20^{a,A} \pm 0.89$	$15.98^{b,D} \pm 0.62$	$30.01^{a,C} \pm 2.28$	$51.31^{a,B} \pm 0.46$	$55.18^{a,A} \pm 0.71$
Cow fat	$38.40^{a,B} \pm 0.77$	$53.56^{a,A} \pm 3.53$	$18.55^{a,D} \pm 1.91$	$27.33^{a,C} \pm 4.72$	$50.77^{a,B} \pm 2.86$	$54.98^{a,A} \pm 3.01$
Horse fat	$10.34^{b,B} \pm 2.94$	$33.70^{b,A} \pm 1.84$	$5.17^{c,D} \pm 1.13$	$19.70^{b,C} \pm 1.07$	$28.70^{b,B} \pm 1.19$	$37.60^{b,A} \pm 1.92$

The temperature ranges were reported as the mean ± standard deviation of three independent measurements.
$^{a\text{-}d}$ Differences within the transition temperatures are statistically significant ($P \leq 0.05$)
$^{A\text{-}C}$ Differences within the investigated fats are statistically significant ($P \leq 0.05$).

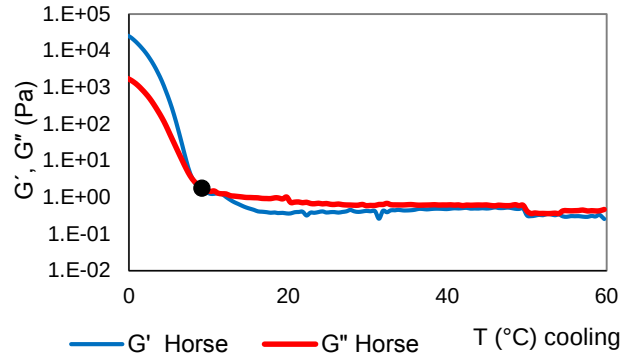
As previously mentioned Table 3 summarizes the melting temperatures obtained from the DSC measurements and the phase transition temperature range during the cooling and heating of cow, yak, and horse fat based on *tan δ*, *G′*, and *G″* data. Comparison of the transition temperatures during heating, given in Table 3, shows the temperatures obtained from the DSC measurements ($T_{peak\ end}$ 55.18±2.5, 54.98±3.01, 37.6±1.92°C for yak, cow and horse fat, respectively) agreed with the transition temperatures obtained from oscillatory tests (for yak 52.2±0.89 °C, cow 53.56±3.53°C and horse fat 33.7±1.84°C), especially in case of $T_{peak\ end}$. This observation is in line with the results reported by López-Martínez et al. (2014), who concluded that the G′ behavior of monoglycerides – oil systems are clearly associated with the thermal transition behavior observed by DSC [37].



(B)

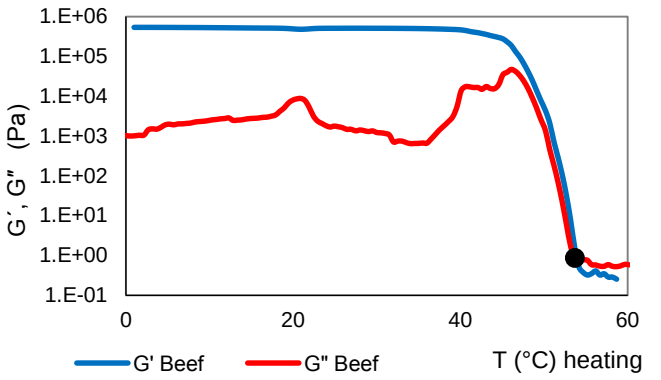


(A)

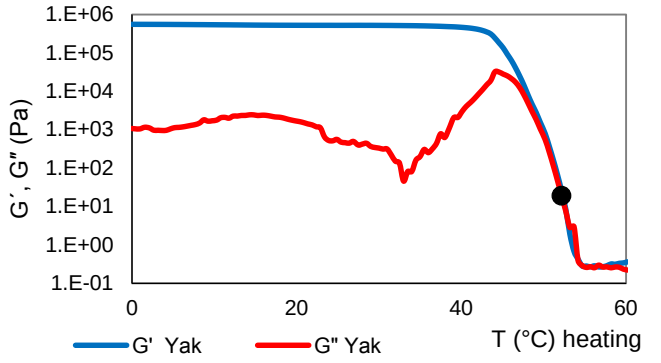


(C)

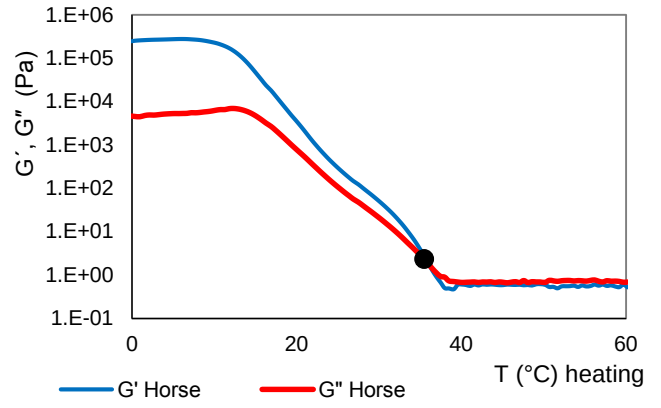
Figure 4A. *Temperature sweep: Changes of moduli G' and G" during the cooling /solidification process (A) cow fat; (B) yak fat; (C) horse fat.*



(A)



(B)



(C)

Figure 4B. *Changes of moduli G' and G" during the heating /melting process (A) cow fat; (B) yak fat; (D) horse fat*

4. Conclusion

The fatty acid composition, rheological and thermal properties of yak visceral fat were investigated and compared with cow and horse fats. The fatty acid content of the fats varied considerably depending on the animal species. The yak fat may be considered to be more valuable than cow fat, due to its higher UFA content. Based on these results we can conclude that yak and horse meat has higher nutritional values compared to a cow meat, due to the richer sources of polyunsaturated fatty acids. The difference in fatty acid composition directly influences the physical properties of animal fats such as the rheological and thermal behavior of the animal fat. Rheological measurements have shown that yak fat had more complex rheological behavior compared to horse fat at 35 °C, but at temperatures above 40 ºC, all fat samples show Newtonian behavior for shear rates greater than approximately 10 s^{-1}. Calculations on the activation energy from 40 to 70 °C resulted in the following order: cow > yak > horse fat. Phase transition temperatures were measured using two different methods, DSC and rheological oscillatory measurements. The melting temperatures, obtained from the oscillatory tests, were close to the transition temperatures obtained from DSC measurements. Furthermore, oscillatory measurements were most suitable for the assessment of the structural changes as a function of temperature. DSC analysis showed that for yak fat: the melting process takes place within a broader temperature range compared to that of cow and horse fat. Thus, industrial application of yak fat is of practical interest. Obtained parameters provide useful information for the development of new products, optimization of industrial processes and control of quality and authenticity of yak fat.

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