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Two-stage dry fractionation of sheep tail and ostrich fats and evaluation of physicochemical properties of their fractions

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ABSTRACT

In Iran, there is a large capacity for animal fat production, which is less used in food products. In this study, the fat obtained from sheep tail and ostrich abdominal tissue were investigated as waste from the slaughterhouse. For this purpose, in the first step, fats were extracted by wet rendering method and their chemical compositions were analyzed. In the second step, the fat was separated into two parts, stearin and olein, by dry fractionation. Then, the olein fraction was divided into two parts, soft stearin and superolein, and their fatty acid composition and physicochemical properties were measured. The sheep tail and ostrich tissues contained 10.66% and 5.18% moisture, 4.10% and 4.29% protein, 0.26% and 0.62% ash, and 84.93% and 88.35% fat, respectively, and the extraction yield were obtained 37.42% and 62.56%, respectively. The gas chromatography results showed that oleic acid, palmitic acid and stearic acid were the main fatty acids in sheep tail fat and oleic acid, palmitic acid, palmitoleic acid and linoleic acid were dominant in the fatty acid profile of ostrich fat. After fractionation, an increase in oleic content of olein and superolein in both fats increased iodine value and refractive index followed by decreasing melting point and L*. Peroxide value of sheep tail fat was 1.54 meqO2/ kg and the fractionation process had no significant effect on it, however, the peroxide value of ostrich fat increased significantly after the separation ($p<0.05$), which could be due to the presence of a considerable portion of linoleic in this fat. Therefore, it can be concluded that the fat extracted by wet rendering from sheep tail and ostrich tissues has an acceptable quality for edible use, and their stearin and soft stearin fractions can be used as an alternative to the hydrogenation process to produce semi-solid fat in food products.

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1- Introduction

In the formulation of fat-based food products such as butter, margarine, shortening, spreads, chocolate, ice cream, and sweets, solid fats provide a suitable mouthfeel, spreadability, aroma, and taste, and suitable solid-like and rheological properties. A major part of this fat is produced through the oil hydrogenation process, which has side effects on the consumer's health, including increasing the probability of cardiovascular diseases and chronic diseases such as obesity, type 2 diabetes, and digestive disorders. Fractionation of natural fats is another approach to preparing solid fat and produces different components with different characteristics that can be used in the food industry and various products. One of the available, relatively cheap, and domestic fat sources, which is also slaughterhouse waste, is animal fat, including beef tallow, sheep tail fat, and ostrich fat. In Iran, there is a large capacity to produce animal fat, although a small amount of it is used in the food industry. Therefore, it is essential to investigate the possibility of using these fat sources in the food industry [1-4].

In Iran, sheep tail fat is the main animal fat produced with the slaughter of more than 10 million sheep/year and the production of 50,000 tons of tail tissue, therefore, it is essential to evaluate the characteristics of this fat and its components as an alternative to fully saturated and trans fats [5]. Sheep tail fat, 15-20% of the carcass weight, has the highest nutritional quality in terms of fatty acid composition among the fats stored in the sheep's body [5-7]. Oleic acid (40%) is the most dominant fatty acid, followed by palmitic acid (26%) and stearic acid (13%) in sheep tail fat [5]. This fat is rich in fat-soluble vitamins and conjugated linoleic acid, which are beneficial for health [8, 9]. Heptadecanoic acid (C17:0), also known as margaric acid, is a single-chain saturated fatty acid in ruminant fat, also found in tallow fat. Studies have shown that the consumption of margaric acid is inversely related to the incidence of coronary heart disease, type 2 diabetes and multiple sclerosis (MS). Also, sheep tail fat, as a food lipid rich in margaric acid, can inhibit the recovery of cancer cells by promoting the production of reactive oxygen species [10]. Sheep tail fat may be used in certain ratios to prepare food products to improve flavor. In a study on the effect of using different animal fats and their combination (beef intermuscular fat, beef kidney fat, sheep tail fat, and their combination) on kavurma (a traditional Turkish cooked uncured meat product), the results showed that sheep tail fat increased the proportion of unsaturated fatty acids (57.7%), but negatively affected the sensory characteristics of the product (general appearance, taste and odor, color and

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overall acceptance of the final product). The results recommended that sheep tail fat should not be applied >30% in kavurma production [11].

Ostrich (*Struthio camelus*) is a large bird, without ability to fly, native to grasslands, and the African Sahara and adapted to live in dry areas [12, 13]. The fat of this bird, 15% of the carcass weight, has been used in the pharmaceutical, cosmetic, and food industries [12, 14, 15]. Ostrich fat contains 35% saturated fatty acids, 38% monounsaturated fatty acids, and 27% polyunsaturated fatty acids, and oleic, palmitic, and linoleic acids are the main fatty acids in this fat [14]. The large amount of polyunsaturated fatty acids in ostrich fat can provide essential fatty acids for humans, moreover this fat contains various compounds such as carotenoids, tocopherol, and flavones, which have therapeutic benefits. In addition, several properties have been reported for ostrich fat, such as antibacterial activity, anti-inflammatory activity, skin protection, facilitating essential phospholipids production, maintaining cell membranes, and developing neurons, brain, and nervous systems [16, 17]. Ghanei (2020) investigated the fatty acid composition and oxidative stability of ostrich fat as a new food source compared to olive oil and stated that ostrich fat has a higher oxidative stability after 32 hours of thermal processing at 170 ºC due to its lower unsaturation than olive oil. Also, small amounts of trans fatty acid were produced in ostrich fat (0.22%) compared to olive oil (0.60%) [18].

Fractionation is one of the important and diverse processes of oil/fat modification to improve the quality of edible fats and oils. In this process, fat/oil is separated into different fractions with different melting points, which can be used in food products such as margarine, shortening, salad oil, frying oil, etc. [19]. No need to use chemicals, low cost, and easy performance of the dry fractionation process have made this method popular in the food industry. Hou et al. (2020) investigated the effect of two-stage dry fractionation at temperatures 30 and 25 ºC on the chemical and physical properties of sheep tail fat with the purpose of its application increment. They stated that the stearin samples showed a predominantly β' crystal structure with a higher solid fat content than the olein samples consequently, the stearin fraction could be used to prepare shortening [8]. In another study, Basuni et al. (2011) reported that blending the olein fraction of ostrich fat (from dry fractionation) with sunflower oil increased oxidative stability as well as improved sunflower oil quality during the frying process [13]. Recently, in a study, ostrich fat stearin produced by the dry fractionation method was used in biscuit production to replace it with margarine or hydrogenated oil in different

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proportions (0, 25, 50, and 75%). The results of the sensory evaluation indicated that the characteristics of the biscuit were improved with the use of ostrich fat stearin [20].

According to the studies, the goal of this paper was the extraction of sheep tail fat and ostrich fat from animal tissues and the production of various fractions from them (with the aim of an increment in the use of this type of fat in the food industry). For this purpose, the dry fractionation process was carried out in two stages, and the physicochemical properties of the produced fractions were determined and compared.

1. Materials and methods

2-1. Materials

Sheep tail was procured from the local market. Ostrich abdominal tissue was obtained from Real Gostar Ariana Co. (Isfahan, Iran). Other chemicals were obtained from Merck (Germany) and Sigma-Aldrich (USA) with analytical grade.

2-2. Preparation of sheep tail fat and ostrich fat and determination of their properties

The wet rendering method was used to extract fat from sheep tail and ostrich abdominal tissues. First, the rest of the meat and other additions were separated from the fat tissues and the samples were chopped into 1×1 cm2. Then, the samples were transferred to -20 ºC. In the next step, a specific weight of the samples was transferred into the shot and distilled water was added to the shot at a 2:1 ratio (Figure 1-a). The shot was heated for 20 min at 121 ºC in an autoclave (Ecotech CLAVE 94, Iran). After autoclaving (Figure 1-b), the mixture was first passed through a 1×1 cm2 porous filter cloth to separate the remaining coarse particles (Figure 1-c). Next, the upper phase (containing oil and suspended particles) was passed through another filter cloth with very fine pores to separate the remaining suspended particles (Figure 1-d) and then centrifuged at 10,000 g for 10 minutes (Sigma 6K15, Germany) was performed to separate the fat from the aqueous phase. The obtained fat was heated under a vacuum (20 mmHg) for 10 minutes (160 and 100 ºC, respectively for tail fat and ostrich fat) to remove the remaining moisture and volatile substances. Finally, the fat was placed in dark-colored plastic containers and transferred to a 5 ºC fridge [21, 22].

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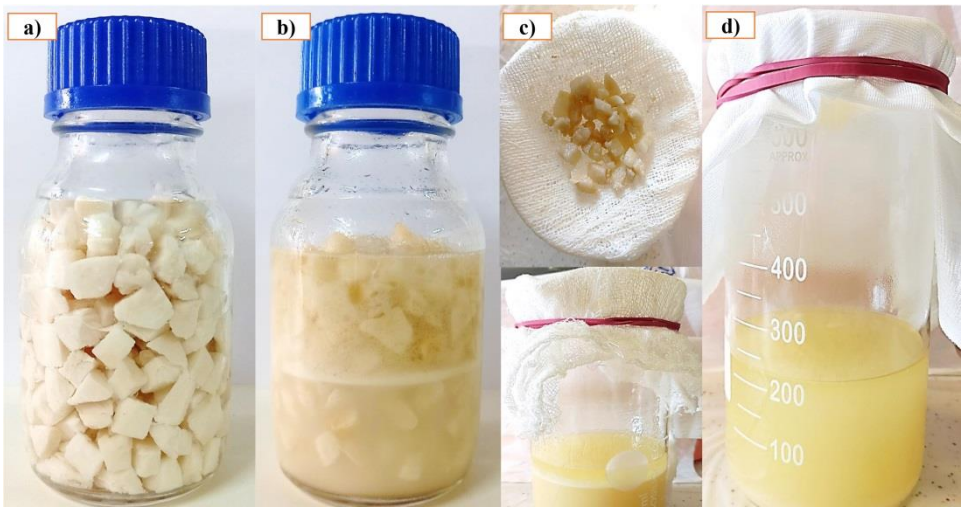

Figure 1- Fat extraction steps: a) Chopped tissue immersed in water before and b) after wet rendering; c and d) fat separation steps.

2-2-1. Fat extraction yield

Fat extraction yield (%) was calculated by the following equation [21]:

Fat extraction yield (%)= (weight of extracted fat /weight of fat in the tissue) ×100

2-2-2. Chemical composition

The moisture content of animal tissue was measured according to AOAC 925/09. The moisture content of fat was determined using a Karl-Fisher apparatus according to AOAC 28/003. The measurement of protein content of animal tissue and fat was done by AOAC 46-13. The fat content of animal tissue was measured according to AOAC 39/960. The ash content in animal tissue and fat was determined according to the AOAC 49-5 Bc [8, 17].

2-3. Fractionation of sheep tail fat and ostrich fat and determination of their characteristics

Crude fat (about 500 g) was heated in a water bath (Memmert wb-22, Germany) at 80 °C for 30 min to melt completely (Figure 2-a). Then, the fat samples were cooled at a 15 ºC/hour rate to 35 and 30 ºC, for sheep tail fat and ostrich fat, respectively (these temperatures were selected according to the melting point of sheep tail fat 38.5 ºC and ostrich fat 35 ºC), and samples were kept for 20 hours at the mentioned temperature until the crystal network was completely formed (Figure 2-b). After that, the olein and stearin fractions were separated from each other using a centrifuge at 10,000 g for 10 minutes (Figure 2-c). The supernatant containing triglycerides with a low melting point was named an olein fraction, and the bottom precipitate containing high melting point triglycerides was separated as a stearin fraction. The obtained olein was again subjected to the dry fractionation process in the second step. For this aim, the olein

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was melted at 80 ºC temperature for 30 min, and cooled to 30 and 25 ºC, for sheep tail fat and ostrich fat, respectively, and kept at this temperature for 20 hours until it crystallized completely. The super olein fraction was separated from soft stearin by centrifugation at 10,000 g for 10 minutes. The fractions olein, stearin, superolein, and soft stearin were stored at 5 ºC for supplementary analysis [8].

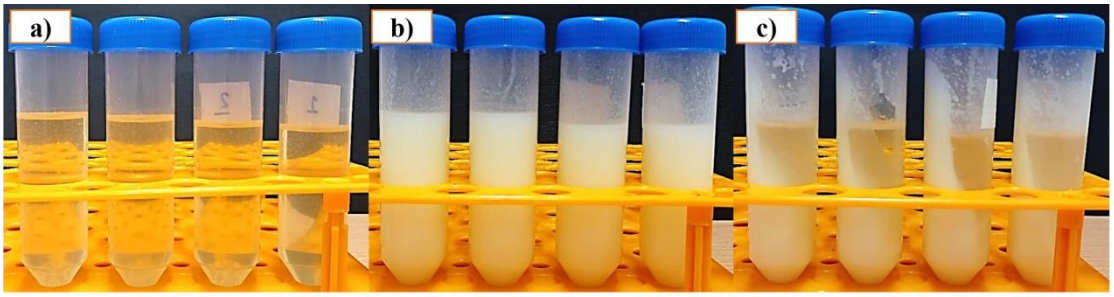

Figure 2- Fat fractionation steps: a) samples heated at 80 ºC; b) kept at a specific temperature (35 and 30 ºC for sheep tail and ostrich fats, respectively) and c) fractionated sample.

2-3-1. Fractionation yield

The fractionation yield (%) was calculated according to the bellow equation [8]:

Fractionation yield (%)= (weight of olein/soft olein or stearin/soft stearin/weight of fat before processing) ×100

2-3-2. Fatty acid composition

The fatty acids in the fats were first methylated and injected into a gas chromatography machine (Agilent 7890 A, USA) for identification. The HP-88 column was 100 meters long, 250 micrometers in diameter, and the thickness of the stationary phase was 0.2 micrometers, nitrogen gas was used as the carrier gas with a 1.1 ml/min flow rate. The temperature programming, defined for the column, was staying at 150 ºC for 1 min, then it reached 190 ºC at 5 ºC/min rate and was kept at this temperature for 2 min. After that, the temperature increased at 5 ºC/min rate to 240 ºC and was kept at this temperature for 8 min. The injection volume was 1 µL in split mode on a 1 to 30 scale. The detector was a flame ionization detector (FID) with a temperature of 250 ºC and 150 ºC injection temperature. Based on the retention time, the fatty acid was identified and the area under the peak showed the amount of the desired fatty acid [21].

2-3-3. Acid value and free fatty acid (%)

The acid value of fats was determined according to AOAC 28/032 [22, 23].

2-3-4. Peroxide value

Peroxide value was measured according to AOCS Cd 53-8 [16].

2-3-5. Iodine value

Determination of iodine value was done according to AOCS Cd 1-25 [16].

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2-3-6. Refractive index

The refractive index was determined according to AOCS Cc 7-25 [16].

2-3-7. Melting point

The melting point was measured by the dropping method according to AOCS Cc 18-80 [8].

2-3-8. Color

The color of the samples was measured with a colorimeter (Nippon ZE6000, Japan) based on the L*, a*, and b* color system. In this system, the L* parameter showed the degree of brightness (whiteness/blackness), the a* parameter showed the tendency to be red/green, and the b* parameter showed the degree of yellowness/blueness. If the indices a* and b* were negative, it indicated the predominance of green and blue colors, respectively. The important point is that L*, a*, and b* factors should be determined based on the average daylight, which is shown by D65 and is standard [21].

2. Results and discussion

3-1. Extraction yield

The yield of fat extraction from sheep tail tissue and ostrich tissue using the wet rendering method is illustrated in Figure 3-a. Heating led to the denaturation of proteins, reduction of fat viscosity as well as the increase of extraction yield. In a study on the extraction of fat from a type of tuna (skipjack tuna eyeballs), it was stated that wet rendering could weaken the bond between the oil and the protein matrix in the tissue followed by facilitating the fat release from the tissue. The wet rendering approach has a potential application for fat extraction without the use of toxic substances and is known as a green process [21]. Therefore, the wet rendering method was used in this study. As seen, the yield of fat extraction from sheep tail tissue and ostrich tissue was 44.1% and 70.8%, respectively, indicating the fat was not completely extracted by the wet rendering method. Also, the fat extraction yield from ostrich tissue was higher than sheep tail tissue, which might be because the ostrich matrix is weaker than the sheep tail matrix, and therefore, more fat was extracted from the ostrich tissue network. Elhamirad et al. (2011) reported the yield of fat extraction from sheep tail tissue using dry rendering under vacuum (80 ºC for 2 hours) as 64.66% [7]. Hou et al. (2020) also obtained 66.7% fat extraction yield by dry rendering method (120 ºC for 4 hours) [8]. In a study on the preparation of ostrich fat from abdominal adipose tissue, Ponphaiboon et al. (2018) reported 72.72% and 66.7% extraction yields using the classical dry rendering and the developed one (preparation of a soft paste and extraction at <50 ºC), respectively. They stated that the presence of moisture, heat, oxygen, and light affected the yield

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of fat extraction and the production of unpleasant odors during the separation of fatty tissue from non-fatty tissue, so in the developed method, the extraction yield decreased by reducing the temperature process. Moreover, due to the use of lower temperature, clean fat tissue, and light-protected packaging, the intensity of yellow color and unpleasant odor in the resulting fat was reduced [16]. Also, Ashkezari et al. (2022) reported the yield of fat extraction from the abdominal, front chest, and heart tissues of the ostrich as 62.45, 40.35 and 38.58%, respectively [15]. In general, the difference in the type of method used and the duration of heating can be the reason for the difference in extraction yield. An increment in the duration of heating could remove the fat from the tissue more easily and increase the extraction yield by further weakening the protein network. However, more extraction time could increase the possibility of fat hydrolysis due to the presence of water and high temperature in the wet rendering environment [23].

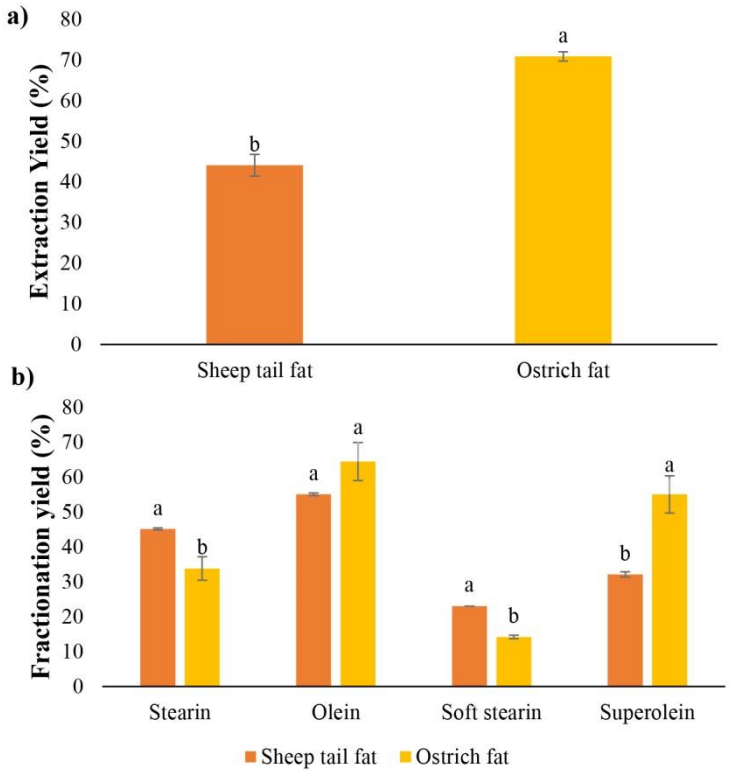

Figure 3- a) Extraction yield of sheep tail and ostrich fats; b) Fractionation yield of sheep tail and ostrich fats. In each parameter small different letters demonstrate significant differences among the treatments ($p < 0.05$).

3-2. Chemical content of animal tissues and their fat

Table 1 shows the chemical content of sheep tail tissue and ostrich tissue and their fats. Sheep tail tissue had 93.84%, 10.66%, 4.10%, and 0.26% fat, water, protein, and ash contents, respectively. On the other hand, the fat, water, protein,

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and ash contents in ostrich tissue were determined as 88.35, 5.18, 4.29, and 0.62% respectively. Hou et al. (2020) also obtained the content of fat, moisture, protein, and ash in the sheep tail tissue as 84.1, 13.4, 3.5, and 0.2%, respectively [8]. Also, Al-Baidhani and Al-Mousavi (2019) reported the chemical content of ostrich tissue including moisture, protein, fat, and ash as 5.56, 5.16, 87.88, and 1.03%, respectively. This difference in the chemical content of the tissue could be caused by the effect of various factors such as the breed, age, and type of diet of the animal [17]. After the wet rendering process, the fat content of sheep tail fat increased to 99.66%, although the protein content decreased to 0.33% and the moisture and ash content were not seen. Similarly, the content of moisture, protein, and ash in extracted ostrich fat was also insignificant. Amani et al. (2011) investigated the physicochemical properties of ostrich, beef, buffalo, sheep, and chicken fat, and reported the moisture content in ostrich and sheep fat as 0.01% and 0.03%, respectively [13]. Therefore, according to the small amounts of protein, moisture, and ash and the increase in fat content in sheep tail and ostrich fats, it seems that the wet rendering method has been successful in separating impurities from fat; Although the extraction yield could be increased by the increment in process duration.

Table 1- Proximate analysis of animal tissues and fats.

Sample	Sheep tail		Ostrich	
	Tissue	Fat	Tissue	Fat
Moisture (%)	10.66±0.57	Trace	5.18±0.02	Trace
Protein (%)	4.10±0.16[a]	0.33±0.00[b]	4.29±0.00	Trace
Ash (%)	0.26±0.02	Trace	0.62±0.00	Trace
Fat (%)	84.93±1.07[b]	99.66±0.00[a]	88.35±0.87[b]	100.00±0.00[a]

Different letters demonstrate significant differences between tissue and fat in each animal source ($p < 0.05$).

3-3. Fractionation yield

Figure 3-b shows the effect of fat type on fractionation yield. As observed, the fractionation yield of stearin in sheep tail fat (45.1%) was significantly higher than ostrich fat (33.8%). The olein content fractionated from sheep tail fat (0.55%) was lower than that of ostrich fat (64.4%) ($p<0.05$). Similarly, the fractionation yield of the soft stearin from the super olein was more in sheep tail fat than in ostrich fat. The content of super olein in sheep tail fat and ostrich fat was determined as 32.1% and 54.9%, respectively. The difference in the fatty acid composition of the two types of fat,

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which led to the difference in the saturation level and the melting point of the fat, might be a reason for the higher fractionation yield of the stearin from the olein, followed by the soft stearin from super olein in sheep tail fat. Hou et al. (2020) reported that the dry fractionation yield of stearin and soft stearin from sheep tail fat at 30 and 20 ºC were 45.9 and 35.0%, respectively [8]. In another study on separation of the liquid phase of sheep tail fat by solvent fractionation method, the yield of the solid fraction (stearin) was 26.35% [7]. In the dry fractionation method, the presence of coarse crystals can help to increase the fractionation yield. These crystals can trap some of the liquid phase of olein, which correlated to an increase in the solid fraction content and the produce of soft stearin. On the other hand, the presence of solvent in the solvent fractionation method can separate the solid crystals more efficiently and purer from the olein fraction, and therefore, the fractionation yield of stearin is lower, but the final product can be more saturated with a higher melting point.

3-4. Fatty acid composition

To identify the fatty acid composition of sheep tail fat, ostrich fat, and their fractions (stearin, soft stearin, olein, and super olein), methylated fat samples were injected into the gas chromatography system and the results are presented in Table 2. As shown, oleic acid (41.8%), palmitic acid (26.8%), and stearic acid (11.9%) are the major fatty acids in sheep tail fat. Also, myristic acid (6.4%), palmitoleic acid (3.8%), margaric acid (2.6%), linolenic acid (2.2%), and linoleic acid (1.6%) were other fatty acids in sheep tail fat. In a study on sheep tail fat, oleic acid (44%), palmitic acid (20%), and stearic acid (9%) were introduced as the main fatty acids in sheep tail fat [8]. Doosti et al. (2020) also reported that about 47% of the fatty acids of sheep tail fat were saturated (palmitic, stearic, margaric, and myristic acids) and the rest were unsaturated fatty acids (about 53%), most of which was oleic (38%) [2]. After the first stage of dry fractionation, the unsaturation level (%) and especially the amount of oleic acid in the olein fraction increased to 54.8 and 46.9%, respectively. In addition, the stearic acid content in the stearin fraction (14.0%) increased compared to crude fat, which was caused by the crystallization of fatty acids with a melting point higher than 35 ºC (the temperature of the first stage of fractionation). After fractionation of olein by decreasing the temperature of fractionation (from 35 to 30 ºC), the unsaturated fatty acids content increased and the unsaturation degree in super olein fraction reached 55.4%. Also, stearic acid and palmitic acid in the soft stearin fraction were reported as 13 and 25%. Similarly, Hou et al. (2020) stated that after the two-stage dry fractionation process, the amount of saturated fatty

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acids in stearin (42.45%) and soft stearin (43.71%) was higher compared to crude fat (38.58%), while unsaturated fatty acids content and specifically oleic acid content of stearin and soft stearin decreased to 40.65% and 39.70%, respectively, compared to crude fat (44.93) [8]. Simanpoor et al. (2012) also observed that during the fractionation process of fat, the palmitic acid and stearic acid contents of the stearin fraction increased and the amount of oleic acid improved in the olein fraction [24].

On the other hand, the chromatography results showed the presence of oleic acid, palmitic acid, palmitoleic acid, linoleic acid, stearic acid, and linolenic acid in ostrich fat 39.1, 30.9, 11.6, 10.6, 5.3 and 1.5%, respectively and the total unsaturation content was determined as 62.8%. In a study on the fatty acids composition and physicochemical properties of ostrich fat extracted by the supercritical fluid method, oleic acid (40.7%) was the main unsaturated fatty acid, followed by linoleic acid (7.38%) and palmitoleic acid (7.13%). Belichovska et al. (2015) reported that oleic acid (28.31%), palmitic acid (27.12%), linoleic acid (25.08%), palmitoleic acid (9.73%), stearic acid (12.1%) 5) and myristic acid (2.16%) were the main fatty acids in ostrich fat. Also, the total unsaturation content and desirable fatty acids (total unsaturation + stearic acid) in this fat were 62.25 and 70.37%, respectively [14]. Amani et al. (2011) stated that oleic acid was the major unsaturated fatty acid (46.75%) and palmitic acid was the predominant saturated fatty acid (28.50%) in ostrich oil [13]. During the first stage of the fractionation process, the content of oleic acid and palmitic acid in the stearin fraction decreased and increased to 35.9% and 35.0%, respectively. In general, the unsaturation degree in stearin and olein fractions was reported as 57.9 and 63.4%, respectively. Crystallization of saturated fatty acids with a melting point higher than 30 ºC (temperature of the first stage) and their separation from the liquid fraction led to a decrease in the unsaturation level of the stearin fraction compared to olein fraction. In the second stage of dry fractionation, the unsaturation degree of superolein reached 65.8%, the major part of which was oleic acid (41.1%). On the other hand, the palmitic acid of the superolein fraction decreased to 28.2%; Its content in the soft stearin fraction was reported as 31.3%. A reduction in the fractionation temperature from 30 to 25 ºC decreased the content of long-chain saturated fatty acids in the olein fraction. Similarly, it was observed that after dry fractionation of crude ostrich fat, the content of oleic acid and palmitic acid increased in the olein and stearin fractions, respectively [13]. In another research on the dry fractionation of ostrich fat and the use of its stearin

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fraction to produce biscuits, Busani et al. (2017) confirmed these results and reported that the content of oleic acid increased from 46.7% in crude fat to 57.4% in the olein fraction. On the other hand, the content of palmitic acid in the stearin fraction (55.8%) was dramatically increased in comparison with crude fat (28.5%) [20].

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Table 2- Fatty acid composition of unfractionated, stearin, olein, soft stearin, and super olein of sheep tail fat and ostrich fat samples.

Fatty acid (%)	Sheep tail fat					Ostrich fat				
	Unfractionated	Stearin	Olein	Soft stearin	Superolein	Unfractionated	Stearin	Olein	Soft stearin	Superolein
Lauric acid	0.492±0.06^{a}	0.278±0.01^{b}	0.292±0.03^{b}	0.365±0.02^{b}	0.319±0.04^{b}	0±0.00^{c}	0±0.00^{c}	0±0.00^{c}	0±0.00^{c}	0±0.00^{c}
Myrestic acid	6.402±0.32^{a}	5.219±0.51^{bc}	4.381±0.06^{c}	5.774±0.60^{ab}	5.215±0.03^{bc}	0.817±0.01^{d}	0.839±0.02^{d}	0.805±0.04^{d}	0.829±0.07^{d}	0.756±0.05^{d}
Palmitic acid	26.854±0.80^{cd}	26.115±0.60^{de}	25.254±0.24^{def}	25.126±0.90^{ef}	24.15±0.50^{f}	30.86±0.20^{b}	35.041±0.25^{a}	30.147±0.33^{b}	31.308±0.56^{b}	28.189±0.10^{c}
Palmitoleic acid	3.859±0.00^{b}	3.497±0.42^{b}	3.941±0.01^{b}	3.192±0.16^{b}	4.053±0.51^{b}	11.582±0.32^{a}	11.084±0.25^{a}	11.638±0.74^{a}	11.264±0.5^{a}	11.783±0.15^{a}
Margaric acid	2.571±0.01^{b}	2.674±0.05^{a}	2.511±0.03^{b}	2.333±0.03^{c}	2.346±0.06^{c}	0±0.00^{d}	0±0.00^{d}	0±0.00^{d}	0±0.00^{d}	0±0.00^{d}
Stearic acid	11.941±0.80^{bc}	13.924±0.60^{a}	12.362±0.08^{bc}	12.777±0.90^{ab}	11.059±0.50^{c}	5.306±0.20^{d}	5.948±0.25^{d}	5.406±0.16^{d}	5.289±0.04^{d}	4.947±0.10^{d}
Oleic acid	41.799±0.90^{bc}	43.275±0.1^{b}	46.865±0.77^{a}	43.278±0.80^{b}	46.956±0.50^{a}	39.08±0.09^{d}	35.934±0.20^{e}	40.397±0.48^{cd}	38.873±0.50^{d}	41.16±0.30^{c}
Linoleic acid	1.637±0.03^{f}	1.161±0.06^{g}	1.573±0.00^{f}	2.339±0.09^{e}	1.585±0.05^{f}	10.652±0.02^{b}	9.589±0.02^{d}	10.083±0.23^{c}	10.422±0.05^{b}	11.312±0.01^{a}
Linolenic acid	2.222±0.04^{b}	2.061±0.01^{c}	1.86±0.02^{d}	1.925±0.07^{d}	2.376±0.08^{a}	1.517±0.02^{e}	1.358±0.03^{g}	1.322±0.00^{g}	1.472±0.06^{ef}	1.598±0.00^{e}

Different letters demonstrate significant differences between sheep tail and ostrich fats and their fractions in each row ($p < 0.05$).

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3-5. Physicochemical characteristics

To evaluate the quality of fat samples before and after fractionation, the acid value was measured as an indicator of the content of free fatty acids in the fat samples (Table 3). According to the data, the acid values were within the permissible range determined by the Codex standard (<2 mg potassium hydroxide/kg fat) for animal fats [16]. Also, the acid values of raw and fractionated sheep tail fat samples (0.69-0.72 mg potassium hydroxide/kg fat) were significantly higher than raw ostrich fat samples and its fractions (0.28-0.29 mg potassium hydroxide/kg fat) ($p<0.05$). It presented that sheep tail fat samples have higher free fatty acid content than ostrich fat. Nevertheless, no significant difference was observed in the acid value of raw and fractionated parts in one type of fat, which confirmed that the dry fractionation process was not effective on the free fatty acid content in this study. Similarly, Busani et al. (2017) reported the free fatty acid content of crude fat, olein and stearin separated by dry fractionation method from ostrich abdominal tissue as 0.10, 0.09, and 0.08%, respectively [20]. In another study on the separation of the liquid phase of sheep tail fat by solvent fractionation with acetone, Elhamirad et al. (2011) measured the acid value of crude fat and olein 0.6 and 0.56 mg potassium hydroxide/kg fat, respectively [7].

The iodine value, correlating to the number of double bonds and the degree of unsaturation, was investigated (Table 3). As expected, the iodine value of ostrich fat was higher than sheep tail fat, which was consistent with the data obtained from gas chromatography of the samples. The fractionation process was also effective on iodine value by changing the composition of fatty acids in the fractions separated from raw fat. The stearin and super olein samples, with the decrement and increment in the oleic acid (%) in their structures, had the lowest and highest iodine value among the sheep tail fat samples, respectively (47.94 and 53.19 gr I2/100 gr fat, respectively). On the other hand, with the increase of saturation from 37 to 42% in stearin fraction compared to the raw sample of ostrich fat, the iodine value reduced from 67.03 to 61.59 gr I2/100 gr fat. In addition, the highest iodine was reported in the super olein sample of ostrich fat with the highest total unsaturated level (65.8%), which was in agreement with the gas chromatography data. Similarly, Hou et al. (2020) stated that the iodine value of the stearin and soft stearin fractions (53 gr I2/100 gr fat) was lower than that of the olein and super olein fractions (58 gr I2/100 gr fat) [8]. Also, Simanpoor et al. (2012) observed that the iodine value of liquid fraction increased from 47.78 to 51.55 gr I2/100 gr fat with the increase in the unsaturation degree resulting from the third step of three-stage solvent

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fractionation of sheep tail fat by acetone [24]. In another paper on the application of ostrich fat in food, the iodine value of raw fat, stearin, and olein fractionated from ostrich tissue were measured as 79, 49, and 58 gr I2/100 gr fat by dry fractionation method, respectively, and it was stated that the higher iodine value confirmed more unsaturation degree of the olein fraction [13].

The refractive index of crude and fractionated fats at 50 ºC is also shown in Table 3. The refractive index in raw ostrich fat (1.4649) was significantly higher than crude sheep tail fat (1.4637), which was related to the higher content of unsaturated fatty acids and unsaturation in ostrich fat (62.8%) compared to sheep tail fat (50.2%). During the fractionation process and by increasing the number of double bonds, the refractive index in the olein and super olein fractions of ostrich fat (1.4646 and 1.4650, respectively) and sheep tail fat (1.4642 and 1.4647, respectively) enhanced; However, the refractive index in the stearin fraction decreased and the lowest refractive index was observed in the stearin of sheep tail fat (1.4630) with the lowest iodine value (47.94 gr I2/100 gr fat). Therefore, the fractionation process and the type of oil with a change in fatty acid composition could affect the refractive index of fat. Elhamirad et al. (2011) also reported that the refractive index of the olein fraction of sheep tail fat (1.459) was higher than its crude fat (1.454) (at 25 ºC) [7].

The melting point data are shown in Table 3. The melting point of crude tail fat (38.5 ºC) was significantly higher than ostrich fat (35 ºC). Also, during the fractionation process, the melting point of stearin fraction in sheep tail fat and ostrich fat increased with the saturation level increment. However, the melting point of the olein fraction decreased with a decrease in the fractionation temperature followed by an increase in the number of double bonds and iodide values of both fats and the super olein samples of sheep tail fat and ostrich fat, with 15 and 20 ºC melting point, respectively, was in a liquid state at room temperature (25 ºC). Similarly, in another research, it was stated that fractionation of sheep tail fat by dry fractionation reduced the melting temperature of olein, so that the melting point of super olein (5.32 ºC) was lower than crude fat (37.30 ºC) [8]. These results were consistent with the data of Simanpoor et al. (2012). They determined the melting point of raw sheep tail fat, the first stage, the second stage, and the third stage of solid fractions as well as the liquid fraction from the third stage as 41.0, 52.5, 48.5, 40.2, and 11.5 ºC, respectively. Busani et al. (2017) also reported the melting point of crude ostrich fat, stearin, and olein as 25.5, 54, and 20 ºC, respectively [20].

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The peroxide value was investigated as a parameter related to chemical deterioration in various types of fat before and after the fractionation process, and the results are shown in Table 3. As illustrated, the peroxide value of the raw and fractionated sheep tail fat samples did not show any significant difference and was in the range of 1.39-1.72 meq oxygen/kg fat. Although raw ostrich fat had a relatively low peroxide value (1.69 meq oxygen/kg fat), its content increased significantly after the fractionation process. The higher level of unsaturation and especially the presence of linoleic acid (~10%) could lead to the sensitivity of ostrich fat to the fractionation process, which was confirmed by the data of gas chromatography and iodine value. Therefore, ostrich fat is more sensitive to processing and storage conditions than sheep tail fat. Nevertheless, Busani et al. (2017) did not observe a significant difference in the peroxide values of ostrich fat, as well as its olein and stearin fractions, and measured their values as 0.90, 0.85 and 0.87 meq oxygen/kg fat, respectively [20]. In another research on sheep tail fat and their fractions using a three-stage solvent fractionation method, the induction time was investigated as a factor of oxidative stability and it was stated that induction time was reduced in the solid and liquid fractions obtained from the third stage of fractionation with increasing unsaturation level [24].

To evaluate the appearance of the sample, L*, a*, and b* parameters were measured using a colorimeter (Table 3). Raw ostrich fat showed a larger L* parameter than sheep tail fat, indicating that ostrich fat was more white than sheep tail fat. On the other hand, the fractionation process led to a change in brightness in different fractions, so that stearin followed by soft stearin had the highest brightness, and the brightness in olein and especially super olein decreased. In addition, the raw fat samples of sheep tail fat and ostrich fat showed no significant difference in a* parameter (greenness-/redness+) (-4.27 and -4.38, respectively). After the fractionation process, a* parameter increased in stearin and soft stearin samples of both types of fat. On the other hand, the greenness of the sheep tail fat super olein increased significantly, although its amount in the olein of sheep tail fat and ostrich fat and super olein of the ostrich fat showed no significant difference with the crude fat samples. There is a possibility that the green pigments were separated along with the olein fraction from the stearin fraction after the dry fractionation process, and therefore, the intensity of the green color in the stearin part decreased. Also, the higher values of b* in ostrich fat samples compared to sheep tail fat confirmed that ostrich fat (8.27) was more yellow than sheep tail fat (1.02). The dry fractionation process increased the yellowness in the fractions compared to raw sheep tail fat, which was the contrary for ostrich fat. The difference in the type and breed of animals and the diet used in their breeding could be the reason for the difference in the color of these two types of fat [25]. Also, more unsaturation of ostrich fat compared to tallow fat and more xanthophyll absorption in birds compared to mammals could lead to a higher intensity of yellowness in ostrich fat compared to sheep tail fat. The presence of a large amount of linoleic acid in ostrich fat and its fractions could lead to more oxidation and the production of polar compounds, increasing the intensity of the yellowness in this fat compared to sheep tail fat [21]. In investigating the color of sheep tail fat and its fractions using the Lovibond device, Simanpoor et al. (2012) observed that the increment in the unsaturation degree enhanced yellow color intensity relating to the presence of xanthophylls in the liquid part from the third stage during the three-stage solvent fractionation process, while the yellowness in the solid parts obtained from the first and second stages was low [24]. In general, it could be said that during the dry fractionation process, the increase in the content of unsaturated fatty acids and the solubility of unsaturated pigments soluble in oil such as xanthophyll in the olein and super olein fractions decreased and increased the whiteness and color intensity, respectively [26].

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Table 3- Physicochemical analysis of unfractionated, stearin, olein, soft stearin, and super olein of sheep tail fat and ostrich fat samples

Properties	Sheep tail fat					Ostrich fat				
	Unfractionated	Stearin	Olein	Soft stearin	Superolein	Unfractionated	Stearin	Olein	Soft stearin	Superolein
Acid value (mg KOH/kg oil)	0.70±0.05^{a}	0.72±0.00^{a}	0.71±0.00^{a}	0.70±0.00^{a}	0.69±0.00^{a}	0.29±0.04^{b}	0.28±0.00^{b}	0.28±0.00^{b}	0.28±0.00^{b}	0.28±0.00^{b}
Iodine value (g I2/100g oil)	48.26±2.71^{cd}	47.94±3.03^{d}	51.64±0.71^{cd}	49.34±0.07^{d}	53.19±0.79^{c}	67.03±2.17^{a}	61.59±1.08^{b}	66.72±0.14^{ab}	66.03±0.61^{ab}	70.36±1.62^{a}
Refractive index (50 ºC)	1.4637±0.000^{cde}	1.4630±0.000^{e}	1.4642±0.000^{abcd}	1.4636±0.000^{de}	1.4647±0.000^{ab}	1.4649±0.000^{a}	1.4636±0.000^{de}	1.4646±0.001^{abc}	1.4638±0.000^{bcde}	1.4650±0.000^{a}
Melting point (ºC)	38.5±0.70^{b}	42.5±2.12^{a}	32.0±1.41^{d}	42.5±0.70^{a}	20.5±0.70^{f}	35.0±0.00^{c}	43.5±0.70^{a}	28.0±1.41^{e}	37.0±0.00^{bc}	15.0±0.00^{g}
Peroxide value (mEq O2/kg oil)	1.55±0.10^{c}	1.69±0.22^{c}	1.72±0.39^{c}	1.48±0.21^{c}	1.39±0.13^{c}	1.69±0.13^{c}	6.96±0.10^{b}	9.08±1.38^{a}	7.59±0.17^{b}	9.68±0.41^{a}
Color										
L*	63.75±0.05^{f}	71.42±0.00^{b}	67.71±0.05^{d}	70.96±0.11^{b}	57.08±0.49^{h}	67.50±0.28^{d}	72.56±0.01^{a}	66.22±0.15^{e}	69.90±0.11^{c}	62.65±0.55^{g}
a*	-4.27±0.014^{bc}	-3.46±0.000^{a}	-4.03±0.113^{b}	-3.36±0.636^{a}	-4.71±0.042^{c}	-4.38±0.007^{bc}	-3.46±0.028^{a}	-4.14±0.162^{b}	-3.50±0.035^{a}	-4.35±0.028^{c}
b*	1.02±0.02^{h}	4.53±0.09^{e}	3.69±0.00^{f}	3.78±0.31^{f}	1.33±0.17^{g}	8.27±0.09^{a}	6.00±0.00^{c}	5.92±0.04^{c}	6.50±0.05^{b}	5.19±0.19^{d}

Different letters demonstrate significant differences between fractions of sheep tail and ostrich fats in each row ($p < 0.05$).

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3. Conclusion

In this research, fat was extracted by wet rendering method from sheep tail tissue and ostrich abdominal tissue as slaughterhouse waste and then it was subjected to the dry fractionation process. According to the chemical composition of sheep tail fat and ostrich fat after the extraction process, it seemed that the wet rendering method succeeded in separating the impurities from the fat and the obtained fats had good quality for edible application. In addition, the evaluation of the fatty acid composition and physicochemical properties of the fractions showed that the stearin and soft stearin fractions of the sheep tail and ostrich fats, with more saturated content and higher melting point, can be used as a substitute for hydrogenated fats in the food products such as margarine, shortening, spreads, chocolate, and ice cream that require solid fat. Also, the super olein component obtained from the sheep tail fat with a high oleic acid content could be used in frying oil production. Therefore, the possibility of using these fractions in food products could be investigated in future studies. Moreover, it is possible to investigate the solvent and detergent fractionation methods to produce a more saturated stearin fraction with a higher melting point.

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چکیده

در کشور ایران ظرفیت زیادی برای تولید چربی حیوانی وجود دارد که کمتر در تولید محصولات غذایی مورد استفاده قرار گرفته است. در این مطالعه، چربی حاصل از بافت دنبه گوسفندی و بافت شکمی شترمرغ به‌عنوان ضایعات حاصل از کشتارگاه مورد بررسی قرار گرفت. به این منظور، در مرحله اول چربی به روش حرارت‌دهی مرطوب استخراج شد و ترکیب شیمیایی آن مورد بررسی قرار گرفت. در مرحله دوم، چربی به روش جزءبه‌جزءسازی خشک به دو بخش استئارین و اولئین تقسیم شد و سپس جزء اولئین مجددا با این روش به دو بخش استئارین نرم و سوپر اولئین جداسازی و ترکیب اسید چرب و خصوصیات فیزیکوشیمیایی آن‌ها اندازه‌گیری شد. بافت دنبه و شترمرغ به ترتیب حاوی ۱۰/۶۶٪ و ۵/۱۸٪ رطوبت، ۴/۱۰٪ و ۴/۲۹٪ پروتئین، ۰/۲۶٪ و ۰/۶۲٪ خاکستر و ۸۴/۹۳٪ و۸۸/۳۵٪ چربی بودند و راندمان استخراج چربی از این بافت‌ها به ترتیب ۴۴/۱٪ و ۷۰/۸٪ بدست آمد. نتایج کروماتوگرافی گازی نشان داد اسیدهای اولئیک، پالمتیک و استئاریک اسیدهای چرب اصلی تشکیل دهنده چربی دنبه و اسیدهای اولئیک، پالمتیک، پالمیتولئیک و لینولئیک اسیدهای چرب غالب در ساختار چربی شترمرغ بودند. پس از فرایند جزءبه‌جزءسازی با افزایش محتوای اولئیک اسید در بخش‌های اولئین و سوپر اولئین در هر دو نوع چربی، مقادیر عدد یدی و ضریب شکست افزایش و خصوصیات نقطه ذوب و شاخص L* کاهش یافت. عدد پراکسید چربی دنبه ۱/۵۴ میلی‌اکی والان اکسیژن در کیلوگرم روغن بود و فرایند جزءبه‌جزءسازی اثر معناداری بر آن نداشت، اگرچه عدد پراکسید چربی شترمرغ پس از فرایند جزءبه‌جزسازی به طور چشمگیری افزایش یافت. به طور کلی، می‌توان نتیجه‌گیری کرد چربی استخراجی به روش حرارت‌دهی مرطوب از دنبه گوسفندی و شترمرغ و اجزای آن‌ها دارای کیفیت قابل قبول برای مصارف خوراکی است و می‌توان از بخش‌های استئارین و استئارین نرم این چربی‌ها به‌عنوان جایگزین فرایند هیدروژناسیون برای تولید چربی نیمه جامد در محصولات غذایی استفاده کرد.