



Physicochemical property, fatty acid composition, and antioxidant activity of ostrich oils using different rendering methods

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ABSTRACT

Ostrich Oil is well known for its nutritional, cosmetic, and pharmaceutical uses due to the fact that it has a high content of essential fatty acids. However, the quality of ostrich oil is important in terms of the effectiveness of products containing this oil. This study therefore aimed to prepare ostrich oils from abdominal adipose tissues using two different methods, being the classical method and a developed rendering method. The results revealed that both types of oils had physicochemical properties, heavy metal contents, and fatty acid compositions within the allowable limits set by the Codex Standard for Named Animal Fats CODEX STAN 211–1999. However, the ostrich oil obtained from the classical method had a stronger yellow color than that from the developed method. Furthermore, the total polyunsaturated fatty acids, iron content, and antioxidant activity of the ostrich oil obtained from the developed method were higher than those from the classical method. The developed rendering method can therefore produce high quality ostrich oils and can be easily adapted to processing other ratite oils.

1. Introduction

The flightless ostrich (*Struthio camelus*), the world's largest bird, is adapted to life in hot countries including Thailand and Australia (Lev, 2003). Ostrich oil obtained from adipose tissues of ostrich, is mainly composed of triglycerides and essential fatty acids (EFA) especially alpha-linolenic acid (ALA) and linoleic acid (LA) (Gavanji, Larki, & Taraghian, 2013). The high amount of polyunsaturated fatty acids (PUFA) in ostrich oil could be a great source of essential fatty acids in human food (Horbańczuk et al., 2004). The capability of the ostrich oil to defend membranes from oxidative stress is related to its fatty acid composition (Bennett, Code, Godin, & Cheng, 2008). The results from the DPPH assay showed that ostrich oil ($IC_{50} = 16.6 \text{ mg mL}^{-1}$) exhibited stronger free radical scavenging activity than that of emu oil ($IC_{50} = 24.5 \text{ mg mL}^{-1}$) (Bennett et al., 2008). However, it has been mentioned that antioxidant activities of ostrich oil and emu oil might be due to their fatty acids, vitamins and amino acids. The major constituents of natural unsaturated fatty acids and antioxidants in ostrich oil means that it can be considered as a healthy food and also acts as a good moisturizer (Belichovska, Hajrulai-Musliu, Uzunov, Belichovska, & Arapcheska, 2015). It also consists of various compounds such as carotenoids, tocopherol, and flavones which show therapeutic benefits (Gavanji, Moshirian, Larki, & Taraghian, 2013). Furthermore, there are

several properties of ostrich oil such as antibacterial activity, anti-inflammatory activity, and skin protection. Ostrich oil is therefore currently used in food supplements and cosmetic preparations.

The important factors determining the quality of ostrich oils are physicochemical properties, fatty acid profiles, and antioxidant activities that can vary between classical and developed rendering methods, so a need exists to investigate such variations with regard to different temperature and process. For classical processing method, ostrich adipose tissues were rendered at high temperature without adherent tissue cleaning and drying steps while the adipose tissues in the developed processing method were cleaned of adherent tissues and dried prior to low temperature rendering. Furthermore, the ostrich oil from the classical processing method was kept in polyethylene terephthalate bottles without inert gas while the oil from the developed processing method was stored in amber glass bottles filled with nitrogen gas. Up till now, a characterization and comparison of the quality attributes of the ostrich oils obtained by classical and developed methods has not yet been scrutinized. Nevertheless, the possibility of scientific knowledge regarding ostrich oil components and its antioxidant activities is still limited. The hypothesis is that the low temperature and suitable process may increase the quality of ostrich oils. Taking into account the above mentioned considerations, the aim of this research was to study the effects of temperature and process on the quality of ostrich oils

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including saponification value, iodine value, acid value, peroxide value, fatty acid composition, and antioxidant activity. The main topic behind carrying out this study was to transfer information to the ostrich oil manufacturers about the quality of ostrich oils thus helping them select appropriate temperature and process for rendering ostrich fats. The oils were prepared from ostrich fats using classical and developed processing methods, and then were determined some physicochemical properties, heavy metal contents, fatty acid compositions, and antioxidant activities.

2. Materials and methods

2.1. Ostrich oils

Black-necked ostriches (*S. camelus*) were obtained from Siam Ostrich Farm, an organic farm in Tambon Bo Suphan, Amphoe Song Phi Nong, Suphan Buri Province, Thailand. The ostriches were fed with 20% chicken pellet feed and 80% mixture of Napier grass (*Pennisetum purpureum*), stalk and leaves of baby corn (*Zea mays* L.), and some vitamins including vitamin A (2,000,000 IU), vitamin B1 (220 mg), vitamin B2 (450 mg), vitamin B12 (4.5 mg), vitamin D3 (320,000 IU), vitamin E (2000 mg), and vitamin K3 (330 mg) per kg of food. The ostriches were decapitated at 12 months of age in a commercial abattoir, Suphan Buri Province, Thailand, with the halal slaughter method adapted for ostriches. Abdominal fat tissues were dissected and kept in polypropylene bags at a temperature of -18 °C until the rendering was performed.

Ostrich oils were prepared by dry rendering processes including classical and developed methods. For developed method, ostrich fat samples were thawed at room temperature, cleaned of adherent tissues, washed thoroughly with distilled water, made them waterless by using tissue papers to absorb the residual surface moisture on fat tissues, cut into very small pieces, and then chopped into a smooth paste. The paste was extracted by rendering method at temperature below 50 °C until the samples had completely melted. When the clear oil was cooled down to room temperature, it was kept refrigerated at 4 °C in amber glass bottles filled with nitrogen gas to prevent oxidation and photo-degradation.

In case of the classical method, the ostrich fat samples were thawed and cut into small pieces without cleaning, washing, drying, and chopping before being rendered at a temperature between 90 and 100 °C. After cooling to room temperature, the oil was filtered through fine muslin and stored at 4 °C in polyethylene terephthalate bottles without inert gas until the analyses were performed.

2.2. Physicochemical properties

The effects of rendering temperature and process (such as cleaning of adherent tissues, washing, and drying) on the physicochemical properties of ostrich oils from different methods were studied. The oil samples obtained by classical and developed methods were analysed for their saponification values, iodine values, acid values, peroxide values, refractive indices, heavy metal contents, and color intensities.

This is a list of reagents used in physicochemical properties. Potassium hydroxide, potassium iodide, sodium thiosulfate, and Wijs solution were bought from Sigma-Aldrich Chemical Corporation (St. Louis, MO, USA). All other analytical reagents were obtained from Merck Chemical Company (Darmstadt, Germany). The titrations were performed by using an autotitrator Titrino plus 848 (Metrohm, Herisau, Switzerland).

2.2.1. Saponification value (SV)

The saponification value is expressed by the weight (mg) of potassium hydroxide needed to saponify 1 g of oil. The oil was saponified by refluxing with a known excess of 0.5 meq L⁻¹ ethanolic potassium hydroxide, and then the excess potassium hydroxide was titrated with

0.5 meq L⁻¹ standard hydrochloric acid solution until the equivalence point was reached. The equivalence point was determined by the maximum inflection point on a titration curve.

2.2.2. Iodine value (IV)

The iodine value is expressed as g of iodine (I₂) per 100 g of oil. The Wijs method was used to determine this value. The oil sample (0.3 g) was placed into a glass-stoppered iodine flask, was dissolved in 10 mL of carbon tetrachloride. Exactly 20.0 mL of Wijs solution was added, mixed the solution, and put the flask in the dark for 30 min. After keeping, 15 mL of 10% w/v potassium iodide solution and 100 mL of freshly prepared distilled water were added. The iodine was titrated with 0.1 meq L⁻¹ standard sodium thiosulfate solution until the equivalence point was occurred.

2.2.3. Acid value (AV)

The acid value is described as mg of potassium hydroxide desired to neutralize the free acids present in 1 g of oil. This value was determined by direct potentiometric titration of the oil sample (10.0 g) in a mixture of 125 mL of isopropyl alcohol: toluene (1:1 v/v), against 0.1 meq L⁻¹ standard potassium hydroxide solution to the equivalence point.

2.2.4. Peroxide value (PV)

The peroxide value is defined as the milliequivalents of peroxide oxygen per 1 kg of oil. Exactly 1.0 g of potassium iodide and 30 mL of a mixed solvent (glacial acetic acid: chloroform, 3:2 v/v) were poured into 5.0 g of the oil sample and the mixture was swirled thoroughly. The solution was diluted with 30 mL of distilled water to prevent free iodine loss. The amount of liberated iodine was determined by potentiometric titration with 0.1 meq L⁻¹ standard sodium thiosulfate solution in order to achieve the equivalence point.

2.2.5. Refractive index (RI)

Refractive indices of the oil samples were determined using the digital refractometer NAR-1T Liquid (Atago Co. Ltd., Tokyo, Japan) at 30 ± 0.1 °C. A prism was opened out by means of a screw head and a small quantity of oil was applied on the prism. The prism was closed up carefully by tightening the screw cap. The refractometer was then left standing for 3 min before recording in order to equilibrate the temperature of the oil sample with that of the instrument. The instrument was adjusted using the compensator dial and the refractive index was determined.

2.2.6. Heavy metal contents

Ultrapure water ASTM type I, 18.3 MΩ x cm resistivity, produced by a GenPure equipment (TKA Wasseraufbereitungssysteme GmbH, Germany), nitric acid obtained from Merck, Darmstadt, Germany, and ICP multi-element standard solution XIII bought from Agilent Technologies, Santa Clara, USA were used to analyzed heavy metals.

The concentrations (mg/kg) of copper (Cu), iron (Fe), arsenic (As), and lead (Pb) were determined according to our previous report with some modifications (Limmatvapirat et al., 2015). Approximately 0.3 g of each oil sample was digested by adding 7.0 mL of 60% v/v nitric acid solution for 45 min using a microwave digester Model ETHOS ONE (Milestone Corporation, Sorisole, Italy). The heavy metal concentrations were determined by 7500ce Inductively Coupled Plasma - Mass Spectrometer (ICP-MS) (Agilent Technologies, Santa Clara, USA) after the digestate was diluted with ultrapure water.

2.2.7. Color intensity

The yellow color intensities of ostrich oils were determined using a Hitachi U-2900 UV-Vis Spectrometer (Hitachi High-Technologies Corporation, Tokyo, Japan). The oil was placed in a cuvette and recorded its absorbance at 540 nm (Palanisamy et al., 2015). Each sample was analysed in triplicates.

2.3. Fatty acid composition

Fatty acids including lauric acid (Lot No. N-12A-S8-T), myristic acid (Lot No. N-14A-M), palmitic acid (Lot No. N-16A), stearic acid (Lot No. N-18A), oleic acid (Lot No. U-46A-MA14-W), LA (Lot No. U-59A-M), ALA (Lot No. U-62A-M), and methyl heptadecanoate (C17:0) (Lot No. N-17M-A8-X), were obtained from Nu-Chek Prep, Inc., Minnesota, USA and the purity was at least 99%. Chloroform, methanol, sulfuric acid, ethyl acetate, and hexane were of analytical reagent grade from Merck KGaA, Darmstadt, Germany.

2.3.1. GC chromatographic instruments

The fatty acid composition was analyzed using a Model 6890N Gas Chromatography - Flame Ionization Detector (GC-FID) (Agilent Technologies, Santa Clara, California, USA) and an automated liquid sampler. A capillary fused silica column HP-INNOWax 19091N-113 (30 m × 0.32 mm × 0.25 µm film of polyethylene glycol) from Agilent Technologies, Santa Clara, California, USA was operated under programmed conditions. In order to study the column temperature programming, a slope of 10 °C along with a starting temperature of 180 °C and a final temperature of 240 °C at a flow rate of 1.5 mL min⁻¹ gave optimal separation. Peak identification and quantities of fatty acids were achieved by direct comparison of peak areas and retention times of fatty acid methyl esters (FAMES) in oil samples with those obtained from FAME mix standards.

2.3.2. Internal standard solution

Methyl heptadecanoate used as an internal standard was soluble in chloroform to achieve a stock solution at a concentration of approximately 5000 µg mL⁻¹.

2.3.3. Standard stock solutions

Seven standards of fatty acids selected based on their different chain length and double bond positions were individually weighed in 20-mL volumetric flasks and then dissolved with methanol: sulfuric acid: chloroform (17: 3: 20 v/v/v) to obtain stock solutions with an approximate concentration range of 250–4500 µg mL⁻¹.

2.3.4. Fatty acid mixture solution

Mixed-component solutions of fatty acids were arranged by placing each of the individual fatty acid stock solutions into a 5-mL volumetric flask and diluting them to volume with methanol: sulfuric acid: chloroform (17:3:20 v/v/v) to obtain fatty acid mixture solutions with an approximate concentration range of 48–800 µg mL⁻¹.

2.3.5. Synthesis of fatty acid methyl esters (FAMES)

FAMES were produced using one-step extraction and direct transesterification process between fatty acids and methanol under high temperature conditions as previously described (Indarti, Majid, Hashim, & Chong, 2005) after suitable modifications. The conversion into FAMES was achieved by heating 3.0 mL of individual fatty acid mixture solutions in a heat block (AccuBlock™ Digital Dry Bath, Labnet International, Inc., New Jersey, USA) at 90 °C for 30 min. After the solution was cooled to room temperature (25–30 °C), 1000 µl of ultrapure water and 500 µl of internal standard solution were added, and the mixture agitated with a vortex mixer for 1 min. The upper aqueous layer was discarded and 1 g of anhydrous sodium sulfate was added to the lower organic layer. The clear solution of FAMES was immediately analyzed using a GC-FID Model 6890N Network GC System. The linearity and range of standard fatty acids were prepared via serial dilution, covering the ranges of each component assay level. For generating of calibration curves, the standard solutions were performed in triplicate.

2.3.6. Analysis of samples

Each oil sample (50 mg) was accurately weighed into a 5-mL volumetric flask, then a mixture of methanol: sulfuric acid: chloroform

(17:3:20 v/v/v) was added to adjust the volume. After mixing, the 3.0 mL solution was transferred into a glass GC vial and fatty acids in this solution were converted quantitatively into the corresponding methyl esters using the acid-catalyzed esterification method and one-step extraction as described above. The extracted FAMES containing the internal standard were immediately analyzed in triplicate by GC-FID.

2.4. Antioxidant assay

6-Hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (trolox), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and ferrous chloride (FeCl₂) were obtained from Sigma-Aldrich Chemical Corporation (St. Louis, MO, USA). Tween 20 and linoleic acid were bought from Nu-Chek Prep, Inc., Minnesota, USA and P.C. Drug Center, Bangkok, Thailand, respectively. Ammonium thiocyanate (NH₄SCN) and di-sodium hydrogen orthophosphate (Na₂HPO₄) were purchased from Ajax Finechem, New South Wales, Australia. Hydrochloric acid and potassium dihydrogen phosphate (KH₂PO₄) were obtained from QR&CTM, Kuala Lumpur, Malaysia. Ethanol and ethyl acetate were obtained from Merck KGaA, Darmstadt, Germany. The absorbance of all samples was read using a Hitachi U-2900 UV-Vis Spectrometer (Hitachi High-Technologies Corporation, Tokyo, Japan).

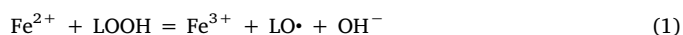
2.4.1. Assay of DPPH radical-scavenging activity

DPPH assay is an accurate and convenient experiment carried out to determine the antioxidant capacity of a sample pertains to its ability to neutralize DPPH radicals by donating hydrogen atoms or electrons (Kurechi, Kikugawa, & Kato, 1980). A deep violet colored solution of the stable DPPH radicals shows a strong absorption band at 517 nm. The absorbance decreases due to the reduction of the DPPH radicals (violet color) to the diamagnetic molecule 2,2-diphenyl-1-picrylhydrazine (yellow color) in the presence of antioxidants.

The DPPH radical scavenging activities of oil samples were evaluated by the previous method (Bennett et al., 2008). The oil samples were separately dissolved in ethyl acetate to obtain various test concentrations. A 0.1 mmol L⁻¹ DPPH in ethyl acetate was freshly prepared. An aliquot of 1.0 mL of the sample was added to 3.0 mL of 0.1 mmol L⁻¹ DPPH and put at ambient temperature for 90 min in the dark. Absorbance at 517 nm was immediately determined against a blank. Trolox was chosen as a standard antioxidant. The 50 percent inhibitory concentration (IC₅₀) was determined using linear regression.

2.4.2. Assay of lipid oxidation

The principle of lipid peroxidation method involves the formation of lipid hydroperoxides (LOOH) at high temperature which activate the oxidation of ferrous ions to ferric ions (Eq. (1)) and is consequently determined the quantity of peroxy radicals (LO•) which is achieved in the linoleic acid emulsion, owing to the lipid oxidation reaction (Kitts, Yuan, Wijewickreme, & Thompson, 1999).



Subsequently, the ferric ion reacts with ammonium thiocyanate (NH₄SCN) to give the ferric thiocyanate complex ion (FeSCN²⁺) that has a maximum absorbance at 500 nm. High absorbance is associated with high peroxidation of linoleic acid emulsion.

Lipid peroxidation - ammonium thiocyanate method was used to determine the antioxidant activities of ostrich oil samples according to the reported method (Sen Gupta & Ghosh, 2013) with some minor modifications. A 0.2 mol L⁻¹ linoleic acid emulsion was prepared by accurately weighing 0.28 g of linoleic acid into a 50-mL volumetric flask and adjusted the final volume with 0.56% (w/v) Tween 20 in 0.2 mol L⁻¹ phosphate buffer solution (pH 7.0). The oil sample (400 mg) was mixed with 5.0 mL of linoleic acid emulsion and 4.6 mL of phosphate buffer solution and then incubated in a heat block (AccuBlock™ Digital Dry Bath, Labnet International, Inc., New Jersey, USA) at

Table 1
Percentage yield and physicochemical properties of ostrich oils.

Parameters	Ostrich oil obtained from a classical method	Ostrich oil obtained from a developed method	Permissible limits of edible animal oils*
Yield (%)	72.5 ^a	66.7 ^b	–
Saponification value (mg KOH/g oil)	191.35 ± 0.96 ^a	195.82 ± 3.85 ^b	190–200
Iodine value (Wijs; g I ₂ /100 g oil)	31.35 ± 0.46 ^a	30.90 ± 1.00 ^b	36–47
Acid value (mg KOH/g oil)	0.18 ± 0.01 ^a	0.10 ± 0.00 ^b	2.0
Peroxide value (mEq O ₂ /kg oil)	0.72 ± 0.27 ^a	2.53 ± 0.12 ^b	10
Refractive index (30 °C)	1.4560 ± 0.0000 ^a	1.4560 ± 0.0000 ^a	1.448–1.460
Heavy metal concentration (mg kg ⁻¹)			
As	0.002 ± 0.000 ^a	0.002 ± 0.001 ^a	0.1
Cu	ND	ND	0.4
Fe	0.461 ± 0.084 ^a	0.803 ± 0.148 ^b	1.5
Pb	ND	ND	0.1

*Codex Standard for Named Animal Fats CODEX-STAN 211–1999, FAO/WHO.

The data are the means of three independent experiments ± standard deviations (n = 3).

^a, ^bValues in the same row with different superscript letters differ significantly (P < 0.05).

ND: not determined.

40 °C in the darkness. Aliquots of 300 µl were withdrawn at 1, 2, 3, 4, 5, 6, and 7 d of the incubation time and tested for lipid peroxidation by sequentially adding 4.3 mL of 75% (v/v) ethanol, 0.2 mL of 30% (w/v) ammonium thiocyanate solution, and 0.2 mL of 0.2 mol L⁻¹ ferrous chloride solution. After mixing, the solution was left at room temperature for 3 min, and then the absorbance of the reaction solution was measured at 500 nm against the corresponding blank. The same solution, without any additives was taken as control sample. Trolox was used as a standard antioxidant.

3. Results and discussion

3.1. Ostrich oils

Rendering is the most conventional method for preparing animal oils. This method serves to both expel moisture and sterilize at the same time. From Table 1, the yield of ostrich oil obtained from the developed rendering method (66.7%) was lower (P < 0.05) than that from the classical rendering method (72.5%). However, the yellow color intensity of the ostrich oil obtained from the classical method was higher than that from the developed method. This developed method was the rendering using cleaned and waterless fat tissues, lower temperature, nitrogen steam flushing, and light protected packages, while the classical method was a process using wet fat tissues, higher temperature, without nitrogen gas flushing, and transparent packages. The results suggest that the percentage yield and bad odor are affected by cleaning the fat tissues from nonfat tissues, moisture, heat, oxygen, and light.

3.2. Physicochemical properties of oils

3.2.1. Saponification value, iodine value, acid value, and peroxide value

As shown in Table 1, all values of physicochemical properties were within the permissible limits set by the Codex Standard for Named Animal Fats CODEX STAN 211–1999, FAO/WHO (Sbihi, Nehdi, & Al-Resayes, 2013). A comparison between oil samples obtained by classical and developed methods, the results showed the significant differences (P < 0.05) in physicochemical properties except the peroxide values. The peroxide value of the ostrich oil obtained from a classical method (0.72 ± 0.27 mEq O₂/kg oil) was 3.5 times lower than that from a developed method (2.53 ± 0.12 mEq O₂/kg oil). Lipid oxidation leads to the production of unstable hydroperoxides, which further break down into secondary oxidation products including alcohols, aldehydes, ketones, acids, and hydrocarbons which are causes of rancidity. In general, high temperature of rendering can induce lipid oxidation. During the progress of oxidation, peroxide values will reach the maximum value and then decrease to zero (Juita, Dlugogorski, Kennedy, & Mackie, 2013). The oil sample obtained from a classical

method had low peroxide value but high yellow color intensity (3.22 ± 0.01) that represented the complete autooxidation. However, the presence of peroxides indicated ongoing oxidation process. The high yellow color intensity of the oil derived from a classical method was associated with the presence of peroxides that reduced the quality of the oil (Palanisamy et al., 2015). Therefore, in case of the oil obtained by a classical method, the peroxide value was decreased with increasing yellow color intensity. Nevertheless, the higher peroxide value indicated the partial lipid oxidation occurred in the oil derived from a developed method.

Saponification values, iodine values, and acid values of ostrich oil samples met the required international standards as shown in Table 1. However, the acid value of the oil sample obtained from a classical method (0.18 ± 0.01 mg KOH/g oil) was higher (P < 0.05) than that from a developed method (0.10 ± 0.00 mg KOH/g oil). It suggested that the oil sample obtained from the classical method had greater free fatty acid content than that from the developed method. Free fatty acids can accelerate the oxidation of oils, and consequently the acid value has a major effect on the stability and flavor quality of oils. The extraction using high temperature in a classical rendering method affected the production of free fatty acids in the oil. Thus, the higher extraction temperature would produce ostrich oils with greater acid values. At the beginning of the study on the effect of temperature, moisture, and adherent tissue cleaning, the hypothesis was based on the fact that the ostrich oil obtained from the developed method exhibited better quality than that from the classical method because iodine value and acid value of ostrich oils obtained from the developed method were significantly lower than those from the classical method (Table 1).

3.2.2. Refractive index

Refractive index of oils increases with the increase in degree of unsaturation and chain length of fatty acids. The different types of oils exhibited different refractive indices while the values of the same types were close. This suggests that refractive index can be adopted in the identification of oils and plays a crucial role in the quality control of oils. The refractive indices of the ostrich oil samples obtained by classical and developed methods were quite similar with respect to the allowable limit (Table 1) indicating the purity and authenticity of both oil samples (Sharma, Giriprasad, & Goswami, 2013).

3.2.3. Heavy metal concentrations

Normally, the quality of edible oils with respect to their toxicity can be decided by the measurement of heavy metals. Cu and Fe are known to increase the oxidation of oils during processing and storage while As and Pb are important owing to their toxicity. In case of edible oils, the concentration of Fe was usually higher than other heavy metals (Zhu, Fan, Wang, Qu, & Yao, 2011). As shown in Table 1, the contents of Fe

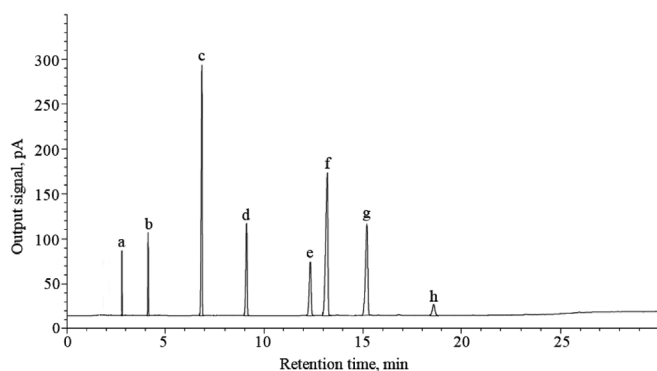


Fig. 1. GC chromatogram of standard FAMES prepared using a one step extraction-transesterification method. (a, methyl laurate; b, methyl myristate; c, methyl palmitate; d, methyl heptadecanoate; e, methyl stearate; f, methyl oleate; g, methyl linoleate; h, methyl linolenate).

and As in both ostrich oil samples were found within the allowable limits while those of Pb and Cu were not detected. The concentration of Fe in the ostrich oil derived from the developed method ($0.803 \pm 0.148 \text{ mg kg}^{-1}$) was about 1.74 times higher than that from the classical method ($0.461 \pm 0.084 \text{ mg kg}^{-1}$) but still lower than the permissible limits of edible animal oils (1.5 mg kg^{-1}) according to Codex Standard for Named Animal Fats CODEX-STAN 211–1999. The results showed that both ostrich oil samples should pose no risk to human health owing to low levels of As and Pb and showed their stability against oxidation due to the low contents of Cu and Fe.

3.3. Fatty acid composition

Methyl heptadecanoate used as internal standard was eluted from the column at a retention time of 9.1 min as shown in Figs. 1–3. In GC chromatograms, fatty acid components in the ostrich oil samples were well separated and the analysis took 19 min per sample to avoid baseline drift as shown in Figs. 2 and 3. The contents of individual fatty acids in the sample mixtures were subsequently analyzed by GC-FID and calculated from the respective linear regression equations. The correlation coefficient (r) values of the regression equation for each fatty acid standard ranged from 0.9997 to 0.9999. The results demonstrate that seven FAMES had good linearity.

Both ostrich oil samples exhibited similar chromatographic profiles as shown in Figs. 2 and 3. The percentage of fatty acids, total saturated fatty acids (total SFA), total polyunsaturated fatty acids (total PUFA), and the ratios of PUFA/SFA in the ostrich oil samples are summarized in Table 2. The results showed that the three dominant fatty acids in both oil samples were oleic acid, LA, and palmitic acid, followed by

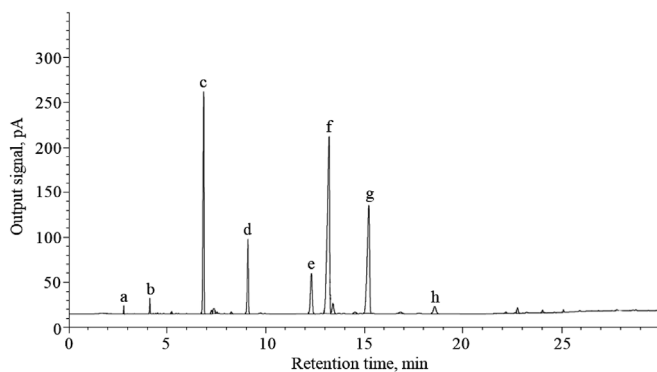


Fig. 2. GC chromatogram of FAMES in the ostrich oil sample from the classical rendering method prepared using a one step extraction-transesterification method. (a, methyl laurate; b, methyl myristate; c, methyl palmitate; d, methyl heptadecanoate; e, methyl stearate; f, methyl oleate; g, methyl linoleate; h, methyl linolenate).

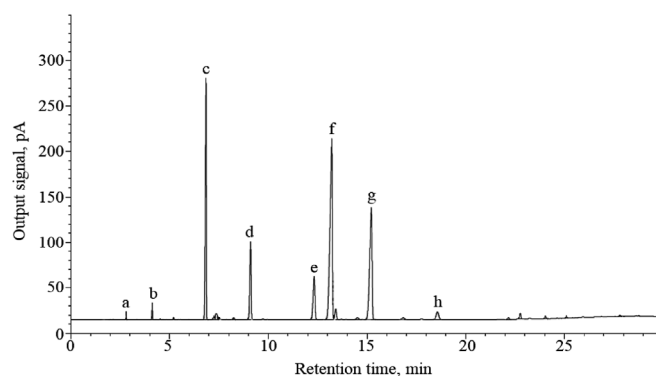


Fig. 3. GC chromatogram of FAMES in the ostrich oil sample from the developed rendering method prepared using a one step extraction-transesterification method. (a, methyl laurate; b, methyl myristate; c, methyl palmitate; d, methyl heptadecanoate; e, methyl stearate; f, methyl oleate; g, methyl linoleate; h, methyl linolenate).

Table 2

Fatty acid composition (percentage of fatty acids) of ostrich oils.

Fatty acids	Percentage of fatty acids	
	classical method	developed method
Lauric acid ($C_{12:0}$)	0.32 ± 0.04^a	0.23 ± 0.01^b
Myristic acid ($C_{14:0}$)	0.82 ± 0.04^a	0.93 ± 0.01^b
Palmitic acid ($C_{16:0}$)	23.33 ± 0.26^a	28.42 ± 0.05^b
Stearic acid ($C_{18:0}$)	8.55 ± 1.14^a	5.07 ± 0.05^b
Total SFA	33.01 ± 1.48^a	34.66 ± 0.11^b
Oleic acid ($C_{18:1n9c}$)	39.95 ± 0.77^a	34.60 ± 0.01^b
Linoleic acid ($C_{18:2n6cc}$)	25.11 ± 0.51^a	27.73 ± 0.01^b
Alpha-linolenic acid ($C_{18:3n3ccc}$)	1.93 ± 0.05^a	3.02 ± 0.00^b
Total PUFA	27.04 ± 0.56^a	30.74 ± 0.02^b
PUFA/SFA	0.82: 1	0.89: 1

*SFA: saturated fatty acid; PUFA: polyunsaturated fatty acid.

The data are the means of three independent experiments $\pm$ standard deviations ($n = 3$).

^a, ^bValues in the same row with different superscript letters differ significantly ($P < 0.05$).

stearic acid, ALA, myristic acid, and lauric acid. The major fatty acid profiles of both oil samples were partially consistent with the previous report (Gavanji, Larki, et al., 2013). Moreover, the ratio of PUFA/SFA is widely used to appraise the nutritional value of dietary fat for human consumption. According to the nutritional recommendations, the PUFA/SFA ratio in the human diet should be greater than 0.45 (Abdelhadi et al., 2017). As shown in Table 2, the PUFA/SFA ratios of ostrich oil samples (> 0.8) were in compliance with the nutritional recommendation (> 0.45). Last but not least, the ostrich oil contains high amounts of essential fatty acids, dissimilar other natural oils, and it can therefore be considered a food supplement to promote health. Furthermore, the results revealed that the total PUFA of the ostrich oil obtained from the developed method was higher than that from the classical method (Table 2). It is interesting to note that high-PUFA oils seemed to have potent antioxidant activity. Therefore, the quality of ostrich oils obtained from the developed method should be better than that from the classical method.

3.4. Antioxidant assay

3.4.1. DPPH radical-scavenging activity

A dose-dependent capacity for radical scavenging activity of DPPH radicals was examined for ostrich oil samples. The concentrations of ostrich oils and trolox which produced 50% neutralization (IC_{50}) for scavenging activities of the DPPH radicals were calculated and are listed in Table 3. The antioxidant capacity can be arranged in the order, trolox $>$ ostrich oil obtained from the developed method $>$ ostrich oil obtained from the classical method. The IC_{50} value of the ostrich oil

Table 3
Comparison of antioxidant activities of ostrich oils.

Samples	Scavenging of DPPH, IC ₅₀ (mg mL ⁻¹)	Percentage inhibition of linoleic acid oxidation, 2.4 mg mL ⁻¹ , Day 5
Ostrich oil from classical method	185.27 ± 0.69 ^a	30.56 ± 3.43 ^a
Ostrich oil from developed method	39.92 ± 1.51 ^b	41.12 ± 1.83 ^b
Trolox	0.004 ± 0.000	58.20 ± 5.18

The data are the means of three independent experiments ± standard deviations (n = 3).

^a, ^bValues in the same column with different superscript letters differ significantly (P < 0.05).

derived from the developed method was 4.6 times lower than that from the classical method because antioxidants, especially phenolic compounds were damaged by high temperature from the classical method (Bennett et al., 2008). The results revealed that the DPPH radical-scavenging activity of the ostrich oil derived from the developed method was significantly stronger than that from the classical method (Table 3).

3.4.2. Lipid oxidation in a linoleic acid emulsion

The ostrich oil exhibited lipophilic antioxidant activity by diffusing in the linoleic acid emulsion and functioning as a highly efficient radical scavenger. Beyond 5 days the inhibition of lipid peroxidation reduced from maximal level of inhibition. Consequently, the antioxidant activity of the ostrich oil samples obtained from the classical and developed methods showed maximum potency for up to 5 days, beyond which the effectiveness decreased steadily and most radicals in the reaction mixture were observed, as indicated by the higher absorbance values. From Table 3, the percentage inhibition of the ostrich oil obtained from the developed method was higher (P < 0.05) than that from the classical method while trolox exhibited inhibition of 58.20% against lipid peroxidation.

The percentage inhibition of linoleic acid oxidation of the oil obtained from the developed method was significantly higher than that from the classical method as shown in Table 3. The results indicate that the ostrich oil obtained from the developed method had a stronger antioxidant capacity than that from the classical method. This suggests that the degradation of antioxidants in ostrich oils obtained from the developed method could be reduced considerably due to the low temperature rendering. Some antioxidants, especially phospholipids and tocopherol in ostrich oils were degraded by high temperature of a classical method (Gavanji, Larki, et al., 2013). For this experiment, the percentage inhibition of linoleic acid oxidation of the ostrich oil derived from the developed method was significantly higher than that from the classical method (Table 3) reflecting higher antioxidant activity of the oil from the developed method.

In this study, two antioxidant assays including DPPH radical-scavenging activity and lipid oxidation in a linoleic acid emulsion were used to assess the determination of potential radical scavenging activities of ostrich oils. In both antioxidant assays, ostrich oils derived from the developed method exhibited significantly higher antioxidant activity than those from the classical method (Table 3). Consequently, these results indicate that ostrich oils derived from the developed method had significantly better quality of antioxidants than those from the classical method.

4. Conclusions

Saponification values of ostrich oils obtained from the classical and developed methods met the required international standard. Heavy metal contents and refractive indices of both types of ostrich oils were

within the allowable limits indicating the safety and purity of oil samples. The ostrich oil obtained from the developed method exhibited higher quality than that from the classical method because the oil obtained from the developed method exhibited lower iodine value and acid value and had higher antioxidant activity than that from the classical method. In addition, the total PUFA and ratio of PUFA/SFA of the ostrich oil obtained from the developed method were higher than those from the classical method dealing with health-promoting properties. The results revealed that the developed method using cleaned and low moisture content of fat tissues, low temperature rendering, nitrogen steam flushing, and light protected containers could preserve the quality of ostrich oils including physicochemical properties, fatty acid profiles, and antioxidant activities. In light of the foregoing considerations, ostrich oil samples from abdominal adipose tissues prepared from classical and developed processing methods seem to be used in the pharmaceutical, food, and cosmetic industries on account of their physicochemical properties, heavy metal contents, fatty acid compositions, and antioxidant activities.

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References

- Abdelhadi, O. M. A., Babiker, S. A., Bauchart, D., Listrat, A., Rémond, D., Hocquette, J. F., et al. (2017). Effect of gender on quality and nutritive value of dromedary camel (*Camelus dromedarius*) longissimus lumborum muscle. *Journal of the Saudi Society of Agricultural Sciences*, 16(3), 242–249.
- Belichovska, D., Hajrulai-Musliu, Z., Uzunov, R., Belichovska, K., & Arapcheska, M. (2015). Fatty acid composition of ostrich (*Struthio camelus*) abdominal adipose tissue. *Macedonian Veterinary Review*, 38(1), 53–59.
- Bennett, D. C., Code, W. E., Godin, D. V., & Cheng, K. M. (2008). Comparison of the antioxidant properties of emu oil with other avian oils. *Australian Journal of Experimental Agriculture*, 48(10), 1345–1350.
- Gavanji, S., Larki, B., & Taraghian, A. H. (2013a). A review of application of ostrich oil in pharmacy and diseases treatment. *Journal of Novel Applied Sciences*, 2, 650–654.
- Gavanji, S., Moshirian, S. M. H., Larki, B., & Taraghian, A. H. (2013b). Comparison of “nano ostrich oil” and “ostrich oil” on human skin. *International Research Journal of Applied and Basic Sciences*, 5(8), 1016–1020.
- Horbańczuk, J. O., Malecki, I., Cooper, R. G., Jóźwik, A., Klewicz, J., Krzyżewski, J., et al. (2004). Cholesterol content and fatty acid composition of two fat depots from slaughter ostriches (*Struthio camelus*) aged 14 months. *Animal Science Papers And Reports*, 22, 247–251.
- Indarti, E., Majid, M. I. A., Hashim, R., & Chong, A. (2005). Direct FAME synthesis for rapid total lipid analysis from fish oil and cod liver oil. *Journal of Food Composition and Analysis*, 18(2–3), 161–170.
- Juita, D., Dlugogorski, B. Z., Kennedy, E. M., & Mackie, J. C. (2013). Roles of peroxides and unsaturation in spontaneous heating of linseed oil. *Fire Safety Journal*, 61, 108–115.
- Kitts, D. D., Yuan, Y. V., Wijewickreme, A. N., & Thompson, L. U. (1999). Antioxidant activity of the flaxseed lignan secoisolariciresinol diglycoside and its mammalian lignan metabolites enterodiol and enterolactone. *Molecular and Cellular Biochemistry*, 202(1–2), 91–100.
- Kurechi, T., Kikugawa, K., & Kato, T. (1980). Studies on the antioxidants. XIII. Hydrogen donating capability of antioxidants to 2,2-diphenyl-1-picrylhydrazyl. *Chemical and Pharmaceutical Bulletin*, 28(7), 2089–2093.
- Lev, E. (2003). Traditional healing with animals (zootherapy): Medieval to present-day levantine practice. *Journal of Ethnopharmacology*, 85(1), 107–118.
- Limmatvapirat, C., Limmatvapirat, S., Charoentearaboon, J., Wessapan, C., Kumsum, A., Jenwithayamornwech, S., et al. (2015). Comparison of eleven heavy metals in *Moringa oleifera* lam. products. *Indian Journal of Pharmaceutical Sciences*, 77(4), 485–490.
- Palanisamy, U. D., Sivanathan, M., Subramaniam, T., Radhakrishnan, A. K., Haleagrahara, N., Sundralingam, U., et al. (2015). Refining ostrich oil and its stabilization with curcumin. *Journal of Nutritional Health & Food Engineering*, 2(2), 00051.
- Sbihi, H. M., Nehdi, I. A., & Al-Resayes, S. I. (2013). Characterization of Hachi (*Camelus dromedarius*) fat extracted from the hump. *Food Chemistry*, 139(1), 649–654.
- Sen Gupta, S., & Ghosh, M. (2013). In vitro antioxidative evaluation of α - and β -carotene, isolated from crude palm oil. *Journal of Analytical Methods in Chemistry* 351671.
- Sharma, H., Giriprasad, R., & Goswami, M. (2013). Animal fat-processing and its quality control. *Food Processing & Technology*, 4(8), 252.
- Zhu, F., Fan, W., Wang, X., Qu, L., & Yao, S. (2011). Health risk assessment of eight heavy metals in nine varieties of edible vegetable oils consumed in China. *Food and Chemical Toxicology*, 49(12), 3081–3085.