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Original Scientific Article

FATTY ACID COMPOSITION OF OSTRICH (*STRUTHIO CAMELUS*) ABDOMINAL ADIPOSE TISSUE

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ABSTRACT

Fatty acid composition of foods has a great impact on nutrition and health. Therefore, the determination and knowledge of the fatty acid composition of food is very important for nutrition. Due to the high nutritional characteristics of ostrich meat and its products, the research determining their quality is of topical interest. The aim of the present investigation was the determination of fatty acid composition of ostrich adipose tissue. The content of fatty acids was determined according to AOAC Official Methods of Analysis and determination was performed using a gas chromatograph with a flame-ionization detector (GC-FID). The results are expressed as a percentage of the total content of fatty acids. The method was validated and whereupon the following parameters were determined: linearity, precision, recovery, limit of detection and limit of quantification. The repeatability was within of 0.99 to 2.15%, reproducibility from 2.01 to 4.57%, while recovery ranged from 94.89 to 101.03%. According to these results, this method is accurate and precise and can be used for analysis of fatty acids in foods. It was concluded that the content of saturated fatty acids (SFA) accounted 34.75%, of monounsaturated fatty acids (MUFA) 38.37%, of polyunsaturated fatty acids (PUFA) 26.88%, of total unsaturated fatty acids (UFA) 65.25% and of desirable fatty acids (DFA) (total unsaturated + stearic acid) 70.37% of the analysed samples. The ratio polyunsaturated/saturated fatty acids accounted 0.77. The most present fatty acid is the oleic (C18:1*n*9c) with 28.31%, followed by palmitic (C16:0) with 27.12% and linoleic (C18:2*n*6c) acid with 25.08%. Other fatty acids are contained in significantly lower quantities.

Key words: ostrich, adipose tissue, fatty acids, validation, GC-FID

INTRODUCTION

In recent decades the interest for ostrich farms in the world has been growing. Great interest in breeding ostriches has appeared also in the Republic of Macedonia over the past decade. According to our regulations, ostriches belong to farm breeding game (15). Otherwise, besides the major ostrich products (dietetic meat and highly esteemed skin) the by-products, including fat are also utilized in the industry as well.

Fats (extra-muscular) in the ostrich carcass are deposited in the abdominal cavity, breast and back (18). Their quantity, composition and properties vary depending on the type of animal (2, 4), genotype (8), diet (7, 12) age (9), sex (4) etc.

On a live weight basis, 5.2% of the live animal is fat, while carcass contains 9.2% knife separable fat (6). The content of abdominal fat accounted for 4.3% (11), or 5.5% (13). Ostrich fat is used in the food industry as an ingredient of processed meat (8, 10). It is also sold locally, where it is used in cooking, as a source of lard (8), for production of oil which is used in cosmetics (3, 16) and as a supplement to pet food, mainly dogs and cats (10). Today, the production of ostrich meat and oil is constantly increasing. Ostrich oil is a source of various commercial products including moisturizing

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creams, body lotion, soap and lipbalm (5). Ostrich oil is a high quality oil with high similarity to human skin lipids (19).

The age and the diet of ostriches are in correlation with the fatty acid composition of fat (9). PUFA high content of ostrich adipose tissue could be a source of essential fatty acids in human and animal diets (10). Ostrich fat has a more advantageous composition of fatty acids than porcine (4), beef, sheep and chicken fat (2).

In recent times consumers are becoming increasingly aware of the importance of food and nutrition for their health. Emphasis is placed on the content of cholesterol and fat or fatty acid composition after it was revealed that some aspects of these components may be a risk factor in cardiovascular disease (17). Knowledge of the quality properties of fat, especially of the fatty acid profile, provides a real view about its quality. Fats which contain more unsaturated fatty acids are appreciated.

Given that our knowledge of ostrich fat is still limited, the aim of this study was to examine the content of fatty acids in abdominal ostrich fat.

MATERIAL AND METHODS

The research was performed on abdominal adipose tissue of seven South African Black ostriches (*Struthio camelus* var. *domesticus*), bred in Republic of Macedonia. Ostriches were reared on a farm in Demir Kapija and were fed with 40% alfalfa and 60% mixture of maize, barley, soya bean, sunflower meal, bran, salt, limestone and vitamins. The birds were slaughtered at the age of 13 to 14 months. The content of fatty acids was determined in seven samples which were frozen and stored into polyethylene bags for 21 days at a temperature of 21°C and then slowly thawed.

Analysis of samples

The fatty acids composition was determined according to AOAC Official Method 996.06 (2005). 30 g abdominal adipose tissue was minced and homogenized, then 0.1 g from the homogenized sample was dissolved in 3.0 ml chloroform and 3.0 ml diethyl ether. The mixture was transferred into a 10 ml glass vial and then evaporated to dryness in 40°C water bath under nitrogen stream. The conversion into fatty acid methyl esters (FAMEs) was achieved by adding 2.0 ml 7% BF_3 reagent and 1.0 ml toluene. The vial was heated in oven at 100 °C for 45 min. Every 10 min the vial was shook gently. After heating, the vial was cooled down to room temperature (20-25°C) and 5.0 ml distilled water, 1.0 ml hexane and 1.0 g sodium sulfate anhydrous were added. The sample was shaken on vortex for 1 min. When the layers were separated, the top layer was transferred into another vial containing 1.0 g sodium sulfate anhydrous. Determinations of FAMEs were carried out on a GC-FID 5890 (Agulent-USA).

Preparation of standards

The individual fatty acid methyl ester standards (FAMEs): myristic acid (C14:0), myristoleic acid (C14:1), pentadecanoic acid (C15:0), palmitic acid (C16:0), palmitoleic acid (C16:1), heptadecanoic acid (C17:1), stearic acid (C18:0), oleic acid (C18:1*n*9c), linoleic acid (C18:2*n*6c), conjugated linoleic acid (CLA) were purchased from Sigma (Sigma-Aldrich, Germany). Individual FAMEs standards were used for preparation of stock standard mixture (50 mg/ml) from which six working standards (0.5 – 30.0 mg/ml) were prepared by diluting with n-hexane. Furthermore, from these six working standards calibration curves were produced. For construction of the calibration curve, the aforementioned working standards were analyzed in triplicate. Identification and contents of the fatty acids were carried out by comparing sample FAME peak retention times and peak area with those obtained for FAME mix standard.

Gas chromatograph (GC) analyses

Analyses of the FAMEs were performed on a GC-FID 5890. The analysis was carried out using a column HP88 (J&W 112 -8867; 250°C; 60m x 250mm x 0.2 mm, Agilent, USA). In Table 1 the column temperature parameters are given.

Table 1. Column temperature parameters

	Rate °C/min	Temperature °C	Hold Time min	Run Time min
Initial	/	70	1	1
Ramp 1	5	100	2	9
Ramp 2	10	175	2	18.5
Ramp 3	3	220	5	38.5

Table 2. Linearity of the method

Fatty acids	Retention time (min)	Coefficient of correlation (r^2)
Myristic	20.000	0.99991
Myristoleic	20.851	0.99990
Pentadecanoic	22.016	0.99879
Palmitic	22.759	0.99994
Palmitoleic	23.500	0.99877
Margaroleic	24.479	0.99886
Stearic	25.794	0.99992
Oleic	26.575	0.99983
Linoleic	27.796	0.99998
Conjugated linoleic	29.330	0.99994

Injector and detector temperatures were kept at 250ºC and 300ºC, respectively. Helium was used as a carrier gas at a flow rate of 1.4 mL/min with split ratio 200:1 and nitrogen was used as a make up gas at a flow rate of 23 mL/min. 1 µL volume of each sample was injected two times into GC-FID for separation and identification of the FAMEs.

Method validation

A guideline for validation of chromatographic methods was used for validation of the method (20). Within the validation procedure linearity, precision and recovery, limit of detection (LOD) and limit of quantification (LOQ) were investigated.

About the data obtained from the examination of the fatty acid profile, arithmetic mean ($\overline{x}$), standard deviation (SD) and coefficient of variation (CV) were calculated.

RESULTS

Linearity

The linearity of the method was estimated by performing of 3 replicates of FAME mix standard solution in a range from 0.5 to 30.0 mg/ml at six concentration levels. Table 2 indicates the retention time and coefficient of correlation (r^2) for fatty acids.

Limit of detection and limit of quantification

The results for LOD and LOQ were calculated from the mean noise value (analysed in six blanks) multiplied by 3 and 10 respectively. In Table 3 values for LOD and LOQ are presented.

Table 3. Limit of detection and limit of quantification

Fatty acids	LOD (µg/ml)	LOQ (µg/ml)
Myristic	0.04	0.17
Myristoleic	0.05	0.14
Pentadecanoic	0.10	0.24
Palmitic	0.05	0.21
Palmitoleic	0.09	0.24
Margaroleic	0.03	0.10
Stearic	0.07	0.24
Oleic	0.09	0.31
Linoleic	0.06	0.28
Conjugated linoleic	0.04	0.23

Table 4. Repeatability, reproducibility and accuracy of the method

Fatty acids	Repeatability (n=6) RSD, %			Reproducibility (n=9) RSD, %			Recovery %
	Sample			Sample			
	1	2	3	1	2	3	
C14:0	1.94	1.71	1.35	3.15	3.28	2.94	95.7
C14:1	1.15	1.43	1.37	2.99	3.14	3.56	97.1
C15:0	1.23	1.10	1.22	2.01	2.15	2.07	94.9
C16:0	1.44	1.36	1.35	2.97	3.01	3.44	98.1
C16:1	0.99	1.14	1.41	3.46	3.42	3.01	97.5
C17:1	1.71	1.46	1.52	3.47	3.36	3.45	94.5
C18:0	1.12	1.41	0.99	2.96	2.84	3.01	101
C18:1*n*9c	1.47	1.52	1.31	3.14	3.36	3.27	97.5
C18:2*n*6c	1.22	1.37	1.15	3.71	4.13	4.01	98.1
CLA	1.77	2.01	2.15	4.43	4.57	4.07	96.1

Precision and accuracy of the method

The precision of the method was evaluated through repeatability and reproducibility and the results are expressed as the relative standard deviation (RSD, %) (Table 4). Repeatability of the method was established by six fold analyses of three different samples in one day, while the reproducibility was established by three fold analyses of three different samples in three consecutive days. The recovery (%) of the method was established by spiking a sample with a standard working solution at one concentration level (10.0 mg/ml), and assaying it in triplicate (Table 4). Accuracy of the method was verified through the recovery.

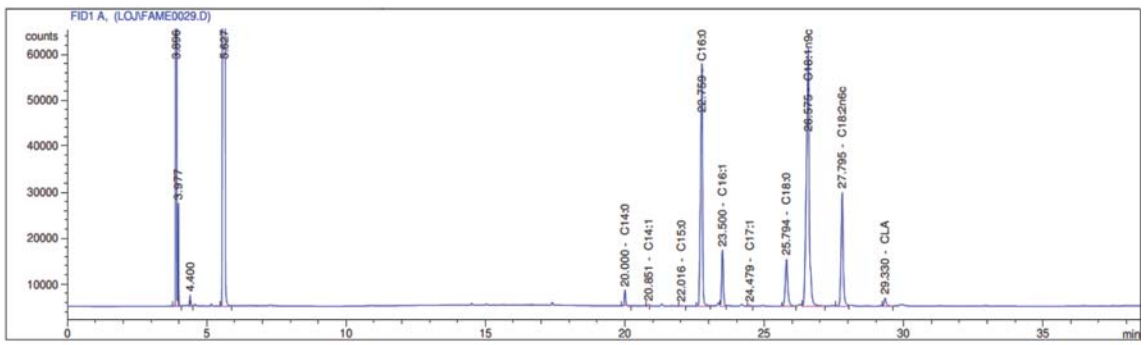

Figure 1. Chromatogram from fatty acid composition in abdominal adipose tissues of ostrich

The fatty acid profile of ostrich fat

The fatty acid composition of the abdominal ostrich fat is presented in Table 5. The results of the examination showed that oleic (28.31%) acid was

Table 5. Mean ($\overline{X}$), standard deviation (SD) and coefficient of variation (CV) for fatty acid composition (% of total fatty acids present) in abdominal ostrich fat (n=7)

Fatty acids		$\overline{X}$	SD	CV
Designation	Trivial name			
C14:0	Myristic	2.16	0.15	6.94
C14:1	Myristoleic	0.20	0.03	15.0
C15:0	Pentadecanoic	0.35	0.10	28.6
C16:0	Palmitic	27.1	1.10	4.06
C16:1	Palmitoleic	9.73	1.50	15.4
C17:1	Margaroleic	0.13	0.02	15.4
C18:0	Stearic	5.12	1.12	21.9
C18:1*n*9c	Oleic	28.3	2.01	7.10
C18:2*n*6c	Linoleic	25.1	1.07	4.27
CLA	Conjugated linoleic	1.80	0.20	11.1

presented with the highest percentage, followed by palmitic (27.12%) and linoleic (25.08%) acid. Palmitoleic (9.73%), stearic (5.12%) and myristic (2.16%) acid participated with a significantly lower percentage. Other fatty acids were found in insignificant quantities and will not be discussed.

In the total content of fatty acids (Table 6), MUFA were contained in the greatest amount (38.37%), followed by SFA (34.75%) and least present were PUFA (26.88%). Total UFA participated with 65.25% and DFA with 70.37%. The ratio of polyunsaturated to saturated fatty acids amounted 0.77.

Table 6. Total fatty acids (%) and ratio between them in ostrich fat (n=7)

Fatty acids	$\overline{X}$	SD	CV
Saturated (SFA)	34.8	2.47	7.11
Monounsaturated (MUFA)	38.4	3.56	9.28
Polyunsaturated (PUFA)	26.9	1.27	4.72
Unsaturated (UFA)	65.3	4.83	7.40
Desirable (DFA)*	70.4	5.95	8.45
PUFA/SFA	0.77	0.02	2.60

* DFA - desirable fatty acids (total unsaturated + stearic acid)

DISCUSSION

Determination of fatty acids is usually carried out by gas chromatography, but in special cases it may be necessary to process separations with high pressure liquid chromatography (HPLC). The highest value of HPLC is for volatile fatty acids (short chain fatty acids), for preparative scale separations or for studying isotopically labeled fatty acids. A rapid and simple method for volatile fatty acids by HPLC analysis with ultraviolet detection has been reported (22). In this study we analyzed biological samples which contain long chain fatty acids in the range from C14 to C24, by using (GC-FID) method as more suitable.

The results of the present study show that in abdominal ostrich fat oleic, palmitic and linoleic acid were presented with highest concentration, followed by palmitoleic, stearic and myristic. A similar sequence of quantitative presence of fatty acids in abdominal ostrich fat was found by other authors as well (4, 8, 16). Such an order was found also in breast ostrich fat (12, 19).

From SFA the mostly present was palmitic (Table 5). Sales and Franken (16) found a similar value (28.44%). Depending on the diet, the content of palmitic acid ranged from 32.50% to 33,47% (7), and depending on the genotype from 30.3% to 30.6% (8). Frontczak et al. (4) obtained a lower value (24.98%). Content of stearic acid was nearest to the values (5.34%) (4) and 4.8 to 5.6% (8), and a slightly higher content (6.26%) was found by Sales and Franken (1996) (16) and Hoffman et al. (7) (6.93 to 9.71%).

The abundant MUFA were oleic (28.31%) and palmitoleic (9.73%). These values were higher than those (19.38 to 22.77% and 5.39 to 9.07%) reported by Hoffman et al. (7), while Hoffman et al. (8), Frontczak et al. (4) and Sales and Franken (16) obtained higher values of oleic acid (30.1 to 33.7%, 42.76% and 36.94%, respectively). Close values for palmitoleic acid (9.2 to 10.5%) were found by Hoffman et al. (8), while Frontczak et al. (4) and Sales and Franken (16) determined lower values (5.89%, 8.44%, respectively).

The concentration of linoleic acid (25.08%), determined in present study was higher than the values reported in the literature (4, 7, 8, 16).

In terms of the total fatty acids content (Table 6), Hoffman et al. (8) reported higher values for MUFA (42.4 to 43.9%) and SFA (37.9 to 40.7%) and lower for PUFA (16.9 to 18.8%).

Frontczak et al. (4) found a higher value for MUFA (51.54%), and lower for SFA (31.26%) and PUFA (17.14%). Hoffman et al. (7) suggested that in the abdominal fat pads SFA dominated (46.71 to 48.92%), followed by MUFA (28.23 to 29.84%) and PUFA (22.28 to 23.52%). The UFA value of 65.25% was slightly lower than that of Frontczak et al. (4) (68.66%).

To assess the nutritional quality of ostrich fat, PUFA/SFA ratio was determined, as well as the content of DFA. Stearic acid, one of the dominant saturated fatty acids has health promotional benefit, i.e. reduces blood cholesterol (14). The value of DFA (70.37%) in the present study, was somewhat lower than the published by Frontczak et al. (4) (74.02%)

and higher than those of Hoffman et al. (8) (64.1 to 67.7%). PUFA/SFA ratio of 0.77 is in compliance with the recommended value (> 0.4) of WHO (21).

The content of the certain fatty acids in the abdominal fat of the African Black ostrich reared in Republic of Macedonia is within the frame of the data published by other authors, but in the present study a slightly higher percentage of PUFA was determined than in the results of other authors. The high content of the unsaturated fatty acids indicates that abdominal ostrich fat has high nutritional value.

CONCLUSION

In abdominal ostrich adipose tissue monounsaturated fatty acids dominate (38.37%). The content of saturated (34.75%) and polyunsaturated (26.88%) is lower. Desirable fatty acids are present in high percentage (70.37%). The oleic (28.31%), palmitic (27.12%) and linoleic (25.08%) are the dominant fatty acids. The ratio of polyunsaturated to saturated fatty acids is in compliance with those recommended by the World Health Organization (> 0.4).

In general, ostrich fat is characterized by a high content of unsaturated fatty acids, unlike other animal fat, so it can be considered as a healthy food and used in different ways in the human diet.

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