Influence of frozen storage on the fatty acid composition of ostrich meat enriched with linseed and rapeseed

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Abstract

The aim of the study was to evaluate the effect of the duration (24 hours, 60 days and 120 days) of frozen storage (−20 ºC) on the fatty acid composition of meat from ostriches supplemented with linseed and rapeseed. The study was carried out on muscles of 40 ostriches raised on five dietary groups: control with no supplementation (C), with 4% linseed (L4); 8% linseed (L8); and 5% rapeseed (R5); or 10% rapeseed (R10) in the diet. As the frozen storage period increased, the fatty acid profile of the ostrich meat in all the "enriched" groups changed, especially treatments L4 and L8. There was a decrease in the polyunsaturated fatty acid content (especially from 61 to 120 days of storage) including linolenic, arachidonic and docosahexaenoic acids. However, storage did not influence the fatty acid profile of ostrich meat up to 60 days. These results suggest that freezing is an acceptable method for preserving ostrich meat (up to 60 days), causing only a small decrease in the fatty acids of ostrich meat enriched with n-3 fatty acids. However, further research on prolonged frozen storage is recommended.

Keywords: Exotic meat; frozen, lipids; n-3; n-6; poultry, diet, *Struthio camelus*
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Introduction

In recent years, consumers have paid particular attention to the quality and health effects of foods. Nowadays they are becoming increasingly aware that food with a high n-3 polyunsaturated fatty acids (PUFA) content and well-balanced polyunsaturated fatty acids/saturated fatty acids (PUFA/SFA) are important for human health for the role that they play in protection against cardiovascular and inflammatory diseases (Dalle Zotte *et al*., 2013). According to the World Health Organization (WHO) recommendations, n-6/n-3 and PUFA/SFA ratios should be below 4 and above 0.4, respectively (WHO/FAO, 2003). Increased interest in enhancing the nutritional quality of meat foods has stimulated research on manipulating their fatty acid composition through nutritional strategies (Hoffman *et al*., 2005; Diaz *et al*., 2011; Polawska *et al*., 2013), including dietary supplementation with n-3 fatty acid-enriched sources such as fish, linseed and rapeseed oils. Ostrich meat is gaining in popularity among modern consumers (Horbanczuk *et al*., 1998, 2008; Sales & Horbanczuk 1998; Cooper & Horbanczuk 2004). When enriched with oil seeds, it has been shown to have beneficial PUFA/SFA (0.4 - 0.7) and n-6/n-3 ratios (2 - 4) (Poławska *et al*., 2013). In addition, the level of inclusion of oil seeds in the diet influences the level of fatty acid deposition (Hoffman *et al*., 2005; Poławska *et al*., 2012; 2013) and it is therefore worthwhile to evaluate a range of oil seed inclusion levels.

Ostrich meat is typically sold as entire muscles (Sales & Horbańczuk, 1998; Hoffman, 2008). Although a large number of these muscles are sold as "fillets" or "steaks", they differ in fibre types as well as chemical composition (Hoffman *et al*., 2005; Girolami *et al*., 2003]). It is therefore important that more than one muscle should be examined when evaluating the effects on ostrich meat of extrinsic factors such as diet on fresh meat (Poławska *et al*., 2013; Dalle Zotte *et al*., 2014), of additives in processed products (Hoffman *et al*., 2014) and of storage (Leygonie *et al*., 2012a).

Supplementation of poultry diet with n-3 enriched sources may increase its susceptibility to oxidation. Thus, the benefit of this composition requires storage conditions that ensure limited lipid oxidation during storage (Filgueras *et al*., 2011). Freezing is a good method of preservation for meat for relatively long periods (Leygonie *et al*., 2012a). However, frozen meat storage can affect the structural and chemical

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properties of muscle foods and influence meat quality (Farouk & Swan, 1998; Leygonie *et al*., 2012b). Some authors have shown that the fatty acid composition of meat could be modified during storage by oxidative and hydrolytic processes (Alvarez *et al*., 2009; Diaz *et al*., 2011), especially that of meat enriched with PUFAs. However, in the current literature there is a shortage of information about the effect of frozen storage time on the fatty acid profiles of ostrich meat. Thus, the aim of this study was to determine the changes in fatty acid profile with special reference to n-3 of ostrich meat supplemented with linseed and rapeseed as related to the frozen storage duration.

Materials and Methods

Forty ostriches (*Struthio camelus* var. *domesticus*) were raised at a commercial farm in Stypułów, western Poland, in 2012/2013. (The farm is under the scientific supervision of the Institute of Genetics and Animal Breeding of the Polish Academy of Sciences.) The birds were raised in a feedlot system with free access to a barn in bad weather. The sides of the pens were made of poles and allowed for interaction between the birds from the various treatments. Ethical clearance was obtained from the local ethical commission (no 27/2009).

Ostriches were raised together in a feedlot system on a commercial ostrich starter diet (215 g crude protein/kg and 11.92 MJ/kg feed until 5 months old. From the age of 5 months (*ca.* 40 kg BW), birds were randomly allocated to five dietary groups: C (control) group, standard diet; L4 group, standard diet supplemented with 4% linseed; L8 group, standard diet supplemented with 8% linseed; R5 group, standard diet supplemented with 5% rapeseed; and R10 group, standard diet supplemented with 10% rapeseed. The standard diet was based on barley, wheat, wheat bran and soybean meal (Poławska *et al*., 2012). All diets contained 150 g crude protein/kg and 10.67 MJ/kg feed. Different dietary fatty acid profiles were achieved by including linseed or rapeseed at various concentrations in the diets. Compared with the control diet, all experimental diets had lower concentrations of SFA (16.1 - 19.9 vs. 21.7% FA_{total}), higher PUFA/SFA (2.6 - 3.6 vs. 2.2) and lower n-6/n-3 (1.2 - 5.6 vs. 6.3) ratios (Poławska *et al*., 2012; 2013).

At 12 months old, when the ostriches' live weight reached 96.3 ± 5.5 kg, they were slaughtered in an EU-approved commercial abattoir for cattle and pigs in Wolbrom (Poland). The ostriches were fasted for 24 h before being electrically stunned. Bleeding and evisceration were performed according to standard slaughtering procedures for ostriches in Poland (Majewska *et al*., 2009). Meat samples were taken from the *gastrocnemius pars interna* (GN) and *iliofibularis* (IF) muscles from the left side of the carcasses, and transported to the laboratory in insulated containers, where they were maintained at −20 ºC until further analyses.

Meat samples were cut into steaks (100 - 120 g each) and vacuum-packed in foil bags (VAC-10DT+GV, Edesa Hostelera S.A., Barcelona). The packs were stored in a freezer at −20 ºC for the duration of the experiment. Samples were analysed at 24 h after slaughter, and after 60 and 120 days of storage.

Fatty acids were extracted from homogenised samples (5 g) of ostrich muscles with the chloroform-methanol procedure of Folch *et al.* (1957). After filtration through filter paper (Filtrak 390), 800 μL filtrate was collected into the vials which were then evaporated under nitrogen in a heating block at 50 ºC. Samples were saponified with 0.5 M KOH in methanol into the heating block (75 ºC). After saponification, samples were esterified with 4% solution of $SOCl_2$ in methanol, then the methyl esters were extracted with heptane and salted out with NaCl to separate the organic layer. Thereafter, 300 μL of esters were transferred to vials and 600 μL of heptane was added.

Fatty acid methyl esters (FAME) were analysed using a GC-7890 Agilent gas chromatograph equipped with a 60 m Hewlett-Packard-88 capillary column (Agilent J&W GC Columns, USA) with 0.25 mm inner diameter and 0.20 μm film thickness. Each sample (1 μL) was injected at a split ratio of 1 : 40. Helium was used as a carrier gas at a flow rate of 50 mL/min. The injector and detector were maintained at 260 ºC. Column oven temperature was programmed to increase from 140 ºC (held for 5 min.) at a rate of 4 ºC/min. to 190 ºC and then to 215 ºC at a rate 0.8 ºC/min.

Individual fatty acids were identified by comparing retention times with those of a standard FAME mixture (Supelco 37 Component FAME Mix, 47885-U–10 mg/mL in methylene chloride, analytical standard, Sigma-Aldrich Co.) and expressed as a percentage of total fatty acids.

An analysis of variance using Statistica (version 9, StatSoft Inc., USA) was conducted with diet and storage time as factors. Significant differences (P <0.05) between the means were determined by Tukey's test.

Results and Discussion

Means for the fatty acid composition in the two muscles as related to duration of frozen storage (up to 120 days) and the types of diet are shown in Tables 1a,b and 2a,b.

Table 1a Saturated and monounsaturated fatty acid composition (% of fatty acids total) of ostrich *gastrocnemius pars interna* muscle enriched with two levels of rapeseed and linseed as related to frozen storage time

Fatty acids[1]		Diet[3] (D)							Interaction
	Storage time (S)	C	R5	R10	L4	L8	Mean	SEM	D x S
SFA	24 h	35.43	36.17	35.79	37.17	38.58	36.63		
	60 d	36.40	37.23	36.75	39.88	39.78	38.01		
	120 d	36.65	38.40	37.52	40.89	40.24	38.74		
	Mean	36.16	37.26	36.68	38.57	39.53		0.57	NS
16:0	24 h	16.76	15.93	14.29	13.84	13.54	14.87		
	60 d	16.91	16.06	17.12	16.15	16.90	16.63		
	120 d	17.58	16.97	17.91	16.09	16.92	17.09		
	Mean	16.99	16.15	16.89	15.36	15.08		0.12	NS
18:0	24 h	11.04	11.18	11.19	11.42	11.83	11.33		
	60 d	10.74	10.30	10.37	11.26	11.20	10.77		
	120 d	11.09	10.65	11.69	12.04	11.51	11.40		
	Mean	10.86	10.89	10.93	11.67	11.53		0.10	NS
MUFA	24 h	37.28	36.82	37.05	31.76	31.27	34.84		
	60 d	36.75	35.17	35.79	30.49	30.04	33.65		
	120 d	36.48	35.18	35.04	30.39	30.36	33.49		
	Mean	36.84[a]	35.72[a]	35.95 [a]	30.67[b]	30.55[b]		0.38	NS
16:1	24 h	4.45	4.62	4.78	4.73	4.42	4.60		
	60 d	5.89	5.75	5.70	5.04	5.20	5.52		
	120 d	5.90	5.57	5.95	5.27	5.54	5.65		
	Mean	5.41[a]	5.08[ab]	5.15[ab]	4.99[b]	5.20[ab]		0.07	NS
18:1n-9	24 h	24.76	25.17	25.18	22.99	21.72	23.96		
	60 d	25.15	25.46	25.94	22.80	22.36	24.34		
	120 d	25.32	25.60	24.08	22.52	22.12	23.93		
	Mean	25.01[a]	25.38[a]	25.10[a]	22.76[b]	22.01[b]		0.17	NS

[1] SFA: sum of saturated fatty acids; MFA: sum of monounsaturated fatty acid.
[2] C: control group; L4: diet with 4% of linseed supplementation; L8: diet with 8% of linseed supplementation; R5: diet with 5% of rapeseed supplementation; R10: diet with 10% of rapeseed supplementation.
[A-C] means within rows/columns with different letters differ at $P < 0.001$.
[a,b] means within rows/columns with different letters differ at $P < 0.05$.
[v-z] values within rows and columns (significant interaction) with different letters differ at $P < 0.05$.

Table 1b Polyunsaturated fatty acid composition (% of fatty acids total) of ostrich *gastrocnemius pars interna* muscle enriched with two levels of rapeseed and linseed as related to frozen storage time

Fatty acids[1]	Diet[2] (D)							Interaction	
	Storage time (S)	C	R5	R10	L4	L8	Mean	SEM	D x S
PUFA	24 h	27.29^{xy}	27.01^{xy}	27.16^{xy}	31.07^{v}	30.15^{vx}	28.54		
	60 d	26.33^{y}	25.79^{z}	27.15^{xy}	30.70^{vx}	30.13^{vx}	28.02		
	120 d	26.26^{y}	25.77^{z}	26.46^{y}	28.02^{x}	28.40^{x}	26.98		
	Mean	26.63	26.19	26.92	29.93	29.78		0.27	0.03
18:2n-6	24 h	16.24	15.92	16.13	16.81	15.96	16.21		
	60 d	17.18	15.51	17.46	17.60	17.47	17.04		
	120 d	16.46	16.15	17.14	17.27	17.07	16.82		
	Mean	16.56	15.99	16.89	17.10	16.89	16.69	0.15	NS
18:3n-3	24 h	1.71	1.76	1.84	5.77	4.29	3.07		
	60 d	1.68	1.80	1.79	5.33	4.18	2.96		
	120 d	1.61	1.51	1.64	4.35	4.09	2.64		
	Mean	1.67^{B}	1.70^{B}	1.76^{B}	5.15^{A}	4.19^{A}		0.03	NS
20:4n-6	24 h	8.78	8.59	8.54	7.04	8.38	8.27		
	60 d	7.40	7.73	7.31	6.65	7.38	7.29		
	120 d	7.31	7.39	7.15	5.48	6.39	6.74		
	Mean	7.83^{a}	7.89^{a}	7.66^{ab}	6.39^{b}	7.38^{ab}		0.41	NS
20:5n-3	24 h	0.18	0.27	0.31	0.49	0.45	0.34		
	60 d	0.17	0.33	0.30	0.48	0.43	0.34		
	120 d	0.19	0.37	0.31	0.48	0.41	0.35		
	Mean	0.18^{C}	0.34^{B}	0.30^{B}	0.48^{A}	0.43^{A}		0.01	NS
22:6n-3	24 h	0.38	0.47	0.34	0.96	1.07	0.64^{a}		
	60 d	0.29	0.42	0.29	0.74	0.77	0.50^{ab}		
	120 d	0.25	0.35	0.22	0.64	0.63	0.42^{b}		
	Mean	0.31^{B}	0.41^{B}	0.28^{B}	0.78^{A}	0.82^{A}		0.01	NS
n-6/n-3	24 h	11.02^{v}	9.80^{x}	9.91^{x}	3.30^{z}	4.19^{yz}	7.64		
	60 d	11.49^{v}	9.11^{x}	10.41^{vx}	3.70^{z}	4.62^{y}	6.40		
	120 d	11.60^{v}	10.56^{vx}	11.19^{v}	4.16^{yz}	4.57^{y}	6.91		
	Mean	11.37	9.82	10.50	3.72	4.46		0.15	0.04
PUFA/SFA	24 h	0.77	0.75	0.76	0.84	0.78	0.78^{a}		
	60 d	0.72	0.69	0.74	0.77	0.76	0.74^{ab}		
	120 d	0.72	0.67	0.71	0.69	0.71	0.70^{b}		
	Mean	0.74^{AB}	0.70^{B}	0.73^{AB}	0.76^{A}	0.75^{A}		0.02	NS

[1] PUFA: sum of polyunsaturated fatty acids; n-6/n-3: ratio of n-6 to n-3 fatty acids; PUFA/SFA: ratio of polyunsaturated to saturated fatty acids.
[2] C: control group; L4 diet with 4% of linseed supplementation; L8: diet with 8% of linseed supplementation; R5: diet with 5% of rapeseed supplementation; R10: diet with 10% of rapeseed supplementation.
[A-C] means within rows/columns with different letters differ at P <0.001.
[a,b] means within rows/columns with different letters differ at P <0.05.
[v-z] values within rows and columns (significant interaction) with different letters differ at P <0.05.

Table 2a Saturated and monounsaturated fatty acid composition (% of fatty acid total) of ostrich *iliofibularis* muscle enriched with two levels of rapeseed and linseed as related to frozen storage time

Fatty acids[1]		Diet[2] (D)							Interaction
	Storage time (S)	C	R5	R10	L4	L8	Mean	SEM	D x S
SFA	24 h	38.25	38.07	37.86	37.05	36.68	37.58		
	60 d	39.36	38.54	37.84	38.85	37.30	38.37		
	120 d	40.18	39.26	38.91	39.99	38.42	39.35		
	Mean	39.26	38.62	38.20	38.63	37.13		0.59	NS
16:0	24 h	21.15	20.83	19.09	15.93	16.04	18.61^{b}		
	60 d	22.82	21.28	19.86	16.63	16.94	19.51^{a}		
	120 d	23.31	21.77	19.79	16.86	16.92	19.73^{a}		
	Mean	22.43	21.29	19.58	16.47	16.63		0.16	NS
18:0	24 h	9.64	9.51	10.02	10.44	9.80	9.88		
	60 d	9.85	9.46	9.90	10.56	10.31	10.02		
	120 d	9.99	9.30	9.68	10.64	10.42	10.01		
	Mean	9.83	9.42	9.87	10.55	10.18		0.13	NS
MUFA	24 h	34.80	35.30	34.11	31.64	31.99	33.57		
	60 d	32.70	34.15	33.20	30.53	30.22	32.16		
	120 d	32.89	33.58	32.28	30.76	30.06	31.91		
	Mean	33.45^{a}	34.34^{a}	33.20^{a}	30.98^{b}	30.76^{b}		0.42	NS
16:1	24 h	6.54	6.16	5.87	4.78	4.83	5.64^{b}		
	60 d	6.70	6.31	5.92	5.17	5.23	5.87^{ab}		
	120 d	6.99	6.61	6.10	5.42	5.37	6.10^{a}		
	Mean	6.74^{a}	6.36^{a}	5.96^{a}	5.12^{b}	5.14^{b}		0.10	NS
18:1n-9	24 h	27.13	28.01	27.07	24.86	26.17	26.65		
	60 d	26.88	27.99	27.15	25.21	25.85	26.61		
	120 d	26.77	27.84	27.18	25.61	25.56	26.59		
	Mean	26.93^{a}	27.95^{a}	27.13^{a}	25.23^{b}	25.86^{b}		0.22	NS
	Mean	0.67^{B}	0.68^{B}	0.73^{AB}	0.79^{A}	0.81^{A}		0.04	NS

[1] SFA: sum of saturated fatty acids; MUFA: sum of monounsaturated fatty acids.
[2] C: control group; L4: diet with 4% of linseed supplementation; L8: diet with 8% of linseed supplementation; R5: diet with 5% of rapeseed supplementation; R10: diet with 10% of rapeseed supplementation.
$^{A-B}$ means within rows/columns with different letters differ at P <0.001.
^{a,b} means within rows/columns with different letters differ at P <0.05.
$^{v-z}$ values within rows and columns (significant interaction) with different letters differ at P <0.05.

Table 2b Polyunsaturated fatty acid composition (% of fatty acid total) of ostrich *iliofibularis* muscle enriched with two levels of rapeseed and linseed as related to frozen storage time

Fatty acids[1]	Diet[2] (D)							Interaction	
	Storage time (S)	C	R5	R10	L4	L8	Mean	SEM	D x S
PUFA	24 h	26.95	26.63	28.03	31.31	31.33	28.85^{a}		
	60 d	25.93	26.31	27.96	30.62	30.49	28.26^{ab}		
	120 d	25.63	25.86	27.11	29.75	29.62	27.59^{b}		
	Mean	26.17^{b}	26.27^{b}	27.70^{ab}	30.56^{a}	30.48^{a}		0.31	NS
18:2n-6	24 h	17.25	17.16	18.92	18.09	17.90	17.86		
	60 d	17.71	17.59	18.30	18.11	17.69	17.88		
	120 d	17.11	17.75	18.46	18.05	18.20	17.91		
	Mean	17.36	17.50	18.56	18.08	17.93		0.12	NS
18:3n-3	24 h	1.86	1.96	2.07	5.40	5.31	3.32		
	60 d	1.79	1.85	2.00	5.21	5.20	3.21		
	120 d	1.66	1.65	1.89	4.59	4.64	2.89		
	Mean	1.77^{b}	1.82^{b}	1.99^{b}	5.07^{a}	5.05^{a}		0.05	NS
20:4n-6	24 h	5.77	5.60	5.99	5.21	5.65	5.64^{a}		
	60 d	5.49	5.08	5.66	4.81	5.07	5.22^{b}		
	120 d	5.33	5.06	5.58	4.21	4.66	4.97^{b}		
	Mean	5.53^{ab}	5.25^{ab}	5.74^{a}	4.74^{b}	5.13^{ab}		0.30	NS
20:5n-3	24 h	0.49	0.43	0.50	1.30	1.20	0.78^{a}		
	60 d	0.47	0.42	0.50	1.10	1.09	0.72^{ab}		
	120 d	0.45	0.41	0.49	0.95	0.93	0.65^{b}		
	Mean	0.47^{B}	0.42^{B}	0.50^{B}	1.12^{A}	1.07^{A}		0.02	NS
22:6n-3	24 h	0.58	0.48	0.55	1.31	1.27	0.84^{a}		
	60 d	0.47	0.36	0.47	1.20	1.14	0.73^{ab}		
	120 d	0.45	0.29	0.39	0.97	0.98	0.62^{b}		
	Mean	0.50^{B}	0.38^{B}	0.47^{B}	1.16^{A}	1.13^{A}		0.01	NS
n-6/n-3	24 h	7.86^{x}	7.93^{x}	7.98^{x}	2.91^{y}	3.03^{y}	5.94		
	60 d	8.17^{vx}	8.24^{vx}	7.86^{x}	3.01^{y}	3.01^{y}	6.06		
	120 d	8.77^{v}	9.71^{v}	8.68^{v}	3.42^{y}	3.49^{y}	6.81		
	Mean	8.26	8.63	8.17	3.11	3.18		0.17	0.04
PUFA/SFA	24 h	0.70	0.70	0.74	0.85	0.85	0.77		
	60 d	0.66	0.68	0.74	0.79	0.82	0.74		
	120 d	0.64	0.66	0.70	0.74	0.77	0.70		
	Mean	0.67^{B}	0.68^{B}	0.73^{AB}	0.79^{A}	0.81^{A}		0.04	NS

[1] PUFA: sum of polyunsaturated fatty acids; n-6/n-3: ratio of n-6 to n-3 fatty acids;
PUFA/SFA: ratio of polyunsaturated to saturated fatty acids.
[2] C: control group; L4: diet with 4% of linseed supplementation; L8: diet with 8% of linseed supplementation;
R5: diet with 5% of rapeseed supplementation; R10: diet with 10% of rapeseed supplementation.
$^{A-B}$ means within rows/columns with different letters differ at $P < 0.001$;
^{a,b} means within rows/columns with different letters differ at $P < 0.05$;
$^{v-z}$ values within rows and columns (significant interaction) with different letters differ at $P < 0.05$.

Conclusions

A decrease was noted in the PUFA concentration in ostrich meat as related to frozen storage duration. The results suggest that for up to 60 days freezing (−20 ºC) is an acceptable method of preservation for

ostrich meat enriched with n-3 fatty acids. However, the changes in PUFA profile in the second period of storage (61 - 120 days) indicate that further research should be conducted to evaluate a prolonged frozen storage for 180 days or longer. It would also be worthwhile to evaluate the effect of prolonged frozen storage of ostrich muscle on other quality parameters such as drip loss, colour stability and sensory attributes. However, it is always a challenge to conduct such studies, especially when one considers sensory analyses, although the use of TBARs as a measurement of lipid oxidation may be more feasible.

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