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EFFECT OF FREEZING AND FROZEN STORAGE ON FATTY ACID PROFILE OF CALVES' MEAT

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Key words: calves, meat, freezing, fatty acids, linseed, rapeseed, fish oil

The aim of the study was to determine the effect of frozen storage of veal on the composition and proportion of fatty acids in meat fat. Thirty bull calves, divided into 6 equal groups aged from 7 to 90 days, were fed concentrates differing in fat sources. After slaughter the meat samples from the thoracic muscle (fresh and after 3-month frozen storage) were analysed for the level of fatty acids. The composition of fatty acids in meat fat was determined by the type of dietary fat. Analysis of frozen meat samples showed a decrease in $C_{16:1}$ (by approx. 15%) and a higher ($p \leq 0.05$) sum of all fatty acids compared to fresh meat. There was a tendency towards increased PUFA ($C_{18:1}$ and $C_{18:2}$ n-6), EPA and DHA in meat fat after frozen storage.

INTRODUCTION

The health-promoting value of meat depends largely on the fat content and fatty acid composition. Due to the high level of saturated fatty acids, ruminant meat is considered to be the main factor behind several diseases of modern civilization such as obesity, atherosclerosis or cancer [Jimenez-Colmenero *et al*., 2001]. Many recent studies have shown that the appropriate feeding of animals can modify the composition of meat fat towards increasing the level of polyunsaturated fatty acids (PUFA) [Scollan *et al*., 2001; Raes *et al.*, 2003; Cooper *et al.*, 2004]. Feeding oilseeds rich in linoleic acid or fish oil rich in long-chain fatty acids EPA and DHA increases the level of n-3 PUFA in meat and improves the n-6/n-3 acid ratio [Strzetelski *et al*., 2003; Rule *et al*., 1994; Zymon *et al*., 2005]. The composition of fatty acids in meat fat affects not only the palatability and dietetic value, but also the storageability of meat. Freezing is the most natural method of preparing meat for long storage, enabling its full nutritive value to be preserved. During freezing and frozen storage, meat is subject to certain physicochemical qualitative changes, the type and extent of which depend largely on the method and rate of freezing [Petrowić *et al*., 1993]. Most of these changes, including changes in consistency, changes in colour or meat weight loss are associated with the formation of ice crystals. Ice formation results in protein denaturation, leading to lower water binding capacity [Boles & Swan, 1996]. During frozen storage, lipids are subject to some changes, mainly autooxidative and hydrolytic changes. The rate and extent of fat autooxidation depends, among others, on the degree of fatty acid saturation, oxygen exposure, and storage time and temperature [Tomás & Aòón, 1990]. Previous studies were primarily concerned with the effect of the freezing process on the physicochemical and organoleptic properties of meat, mainly buffalo meat and pork, less frequently beef [Berry & Leddy, 1989; Ngapo *et al*., 1999; Kandeepan & Biswas, 2005; Sen & Sharma, 2004]. Studies concerning the effect of freezing on fat composition and quality were conducted mainly with fish [Fernandez-Reiriz *et al*., 1995; Ortiz & Bello, 1992]. The aim of the study was to determine the effect of frozen storage of veal on the composition and proportion of fatty acids in meat fat.

MATERIALS AND METHODS

A total of 30 Polish Holstein-Friesian bull calves were randomly assigned based on the analogue principle to 6 groups (5 animals per group). Bulls were kept on perforated wooden floor in cages equipped with drinkers and feed troughs. Animals were fed individually according to IZ-INRA standards [2001]. Milk replacer, in which dried whey and soy protein concentrate were the main sources of protein, was diluted by dissolving 167 g of the powder in 1 L of water and fed from 7 to 56 days of age. Concentrates were fed throughout the experiment (7 to 90 days). The diets contained (%): ground barley (40.5-55.5), ground wheat (13-30.5), wheat bran (6-13), soybean meal (12.5-15.5), mineral-vitamin preparation (3), and different sources of fat in the form of linseed cv. Omega (10) or Linola (10), rapeseed cv. Spencer (10) or Contact (10), or fish oil (4). At the end of the study, 90-day-old bulls were slaughtered and after 24-h cooling of carcasses meat samples were taken from the thoracic muscle for analysis. Some samples were cold stored at 2-4°C for the next 24 h until analysis, and the other samples were sealed in plastic bags, frozen and stored at -18°C for 3 months. The meat samples were analysed before and after frozen storage for the level of higher fatty acids. Extraction of the total lipids was done us-

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TABLE 1. Fatty acid proportion in vegetable oils and fish oil (g/100 g fatty acids).

Fatty acid	Linseed Omega	Linseed Linola	Rapeseed Spencer	Rapeseed Contact	Fish oil
$C_{14:0}$	0.00	0.13	0.00	0.00	6.92
$C_{16:0}$	8.69	7.52	4.75	4.28	22.51
$C_{18:0}$	3.83	3.18	1.72	1.57	2.33
$C_{18:1}$	18.20	13.00	56.87	67.78	25.23
$C_{18:2}$ n-6	26.76	72.90	30.14	13.61	5.18
$C_{18:3}$ n-3	41.87	2.84	3.71	9.63	3.41
$C_{20:5}$ n-3 EPA	0.00	0.04	0.00	0.03	7.87
$C_{22:6}$ n-3 DHA	0.00	0.06	0.00	0.00	12.91
SFA	12.99	11.04	7.49	6.93	32.39
PUFA n-6	26.76	72.90	30.14	13.61	5.28
PUFA n-3	41.87	2.98	3.71	9.66	25.06

SFA – Saturated Fatty Acids; PUFA – Polyunsaturated Fatty Acids

ing chloroform/methanol (2/1; v/v) according to the method of Folsch *et al*. [1957]. The lipids were transmethylated using NaOH/MeOH followed by BF_3/MeOH. The fatty acid methyl esters were analysed by gas chromatography using a Varian 3400 gas chromatograph (column SUPELCOWAX 10, 30 m; 0.53 mm ID; 1.0 μm; temp. program. 50-280°C; injector 200(C; detector 260(C; helium as the carrier gas – 6 mL/min). The results were analysed statistically by two-way analysis of variance using the SAS package [2000].

RESULTS

The fat sources used in the experiment differed in the profile of fatty acids (Table 1).

Linseed cv. Omega was characterized by the highest proportion of linolenic acid, whereas the fat of linseed cv. Linola had the highest concentration of linoleic acid. Rapeseed cv. Spencer and Contact contained the highest proportion of oleic acid, which accounted for over half of all the acids. Fish oil was characterized by the highest proportion of n-3 PUFA, in particular EPA and DHA.

The composition of fatty acids in veal fat varied according to experimental group (Table 2). In all the groups, there was a tendency towards a lower level of saturated fatty acids (SFA) in relation to the control group, although the differences were not significant ($p>0.05$). A significant decrease ($p\leq0.05$) in the level of palmitic acid $C_{16:0}$, in relation to the control group, was found when feeding linseed cv. Omega, and in the level of stearic acid $C_{18:0}$ when calves received fish oil. Linseed cv. Omega, characterized by a 48% proportion of linolenic acid $C_{18:3}$ n-3, increased approx. 4-fold ($p\leq0.05$) the level of this acid in meat fat in relation to the other groups. Feeding fish oil caused a several-fold increase ($p\leq0.05$) in the level of long-chain n-3 fatty acids in meat fat, including eicosapentaenoic (EPA), docosapentaenoic (DPA) and docosahexaenoic acids (DHA). Feeding calves with linseed cv. Omega, rapeseed cv. Contact, and especially fish oil caused a marked decrease ($p\leq0.05$) in the n-6/n-3 ratio in relation to the control group.

After three months of frozen storage of meat, there was a significant decrease ($p\leq0.05$) in the level of $C_{16:1}$, and an

TABLE 2. Composition of fatty acids in veal fat (% of total fatty acids; n=60).

Fatty acid	Storage time (in months) n=30		p	Groups* n=10						p	SE	Interaction
	0	3		C	LO	LL	RS	RC	FO			
$C_{14:0}$	1.10	1.15	0.7	1.16	1.21	0.96	1.06	1.13	1.22	0.8	0.06	-
$C_{16:0}$	17.10	17.09	0.9	18.21ab	15.86b	16.61ab	16.47ab	16.86ab	18.58a	0.02	0.27	-
$C_{16:1}$	2.27a	1.93b	0.02	2.06	2.39	1.93	1.81	2.21	2.19	0.2	0.08	-
$C_{18:0}$	12.95	12.86	0.7	13.50ab	12.69b	12.77b	14.08b	13.14ab	11.27c	<0.01	0.17	-
$C_{18:1}$	28.39	28.85	0.7	29.28	29.38	28.50	27.61	30.29	26.64	0.5	0.55	-
$C_{18:2}$n-6	19.41	20.01	0.5	18.91	19.60	22.47	20.58	18.78	17.93	0.1	0.51	-
$C_{18:3}$n-3	1.12	1.00	0.3	0.56b	2.95a	0.59b	0.60b	1.03b	0.64b	<0.01	0.12	-
CLA	0.12	0.11	0.4	0.09	0.09	0.10	0.11	0.13	0.14	0.2	0.01	-
$C_{20:5}$n-3	0.88	1.01	0.5	0.35b	0.63b	0.33b	0.62b	0.59b	3.15a	<0.01	0.16	-
$C_{22:5}$n-3	1.08	1.15	0.4	0.82b	1.19b	0.93b	1.01b	1.11b	1.64a	<0.01	0.05	-
$C_{22:6}$n-3	0.36	0.41	0.4	0.18b	0.28b	0.21b	0.19b	0.37b	1.08a	<0.01	0.05	-
Sum	95.67a	96.14b	0.04	95.91	95.94	96.21	95.87	95.77	95.75	0.9	0.11	-
Others	4.33	3.86	0.4	4.09	4.06	3.79	4.13	4.23	4.25	0.9	0.10	-
SFA	32.67	32.54	0.8	34.70	31.07	31.66	33.02	32.54	32.64	0.08	0.36	-
PUFA n-3	3.81	3.90	0.8	2.00c	5.15b	2.13c	2.66c	3.39c	7.79a	<0.01	0.33	**
PUFA n-6	27.21	27.81	0.7	26.48	26.88	31.04	29.59	25.98	25.11	0.2	0.76	-
n-6/n-3	9.44	9.83	0.5	13.25a	5.41c	14.66a	12.06a	8.58b	3.87c	<0.01	0.60	-

* Concentrates for groups: C – control; LO – with linseed Omega; LL – with linseed Linola; RS – with rapeseed Spencer; RC – with rapeseed Contact; FO – with fish oil; $p>0.05$ – non-significant differences; a, b, c – $p\leq0.05$

increase ($p \leq 0.05$) in the sum of all fatty acids in meat fat compared to fresh meat (Table 2). Frozen meat showed a tendency towards a higher content of PUFA with 18 carbon atoms, including oleic acid $C_{18:1}$, linoleic acid $C_{18:2}$ n-6, and long-chain EPA and DHA.

DISCUSSION

Analysis of the lipid fraction of the thoracic muscle showed that the composition and proportion of fatty acids in meat are determined mostly by the type of fat in calf diets, and less so by frozen storage. From the dietetic point of view, the presence of PUFA, in particular n-3 PUFA, is important. Earlier studies [Strzetelski *et al*., 2003; Zymon *et al*., 2005) showed that feeding calves oilseed and fish oil diets with different proportions of these acids enables the meat to be enriched with PUFA (essential for human health) while reducing the content of undesirable SFA. Feeding system had a significant effect on the amount of n-3 PUFA. In the groups of calves receiving linseed cv. Omega and fish oil, the amount of n-3 PUFA was twice and three times that of animals from the control group.

During frozen storage, there was a significant decrease in the level of $C_{16:1}$ in meat fat. Santos-Filho *et al*. [2005] reported a similar relationship for the composition of fatty acids in goat meat stored at -18°C for 6 months. De Pedro *et al.* [1999] reported a decrease in the level of $C_{16:1}$ in samples of pig subcutaneous fat after 32 months of frozen storage. Contrary to expectations, frozen meat showed no decrease in the level of PUFA, which are particularly susceptible to oxidative processes. On the contrary, there was a tendency towards a higher content of oleic, linoleic, EPA and DHA acids. This may suggest that certain changes (not necessarily oxidative changes) take place during the first three months of frozen meat storage, while the processes of fat oxidation and hydrolysis proceed very slowly and to a small extent, probably as a result of the inactivation of tissue enzymes and microorganisms. The low rate of fat oxidation processes was probably due to the fact that meat samples were tightly sealed in plastic bags and stored without oxygen exposure. According to Santos-Filho *et al*. [2005], storage of meat at -18°C for 6 months leads to oxidative changes and a significant decrease in the level of unsaturated fatty acids in meat fat. The tendency towards increased level of oleic acid was probably due to the synthesis of $C_{18:1}$ from $C_{18:0}$ using Δ-9-desaturase, because the activity of this enzyme decreases very slightly in frozen animal tissues [Klingenberg *et al.*, 1995]. Differences in the sum of all fatty acids resulted from changes in the proportions of individual acids.

CONCLUSION

The composition and proportions of fatty acids in meat fat are determined by the feeding system and less so by frozen storage conditions. Changes that occurred in the composition of fatty acids in frozen meat stored for 3 months are inconsistent with the mechanism of fat autooxidation. The increase in PUFA level may suggest that during frozen storage, processes other than oxidative may occur in meat fat and be responsible for the observed changes in the fatty acid profile. Frozen meat samples can be stored for three months without significant changes to the fatty acid profile, provided that appropriate packing that limits the exposure to atmospheric oxygen is used. Freezing as a method of meat preservation causes only a small decrease in nutritive value, and natural health-promoting substances such as unsaturated fatty acids, are largely retained.

REFERENCES

1. Berry, B.W., Leddy, K.F., Effect of freezing rate, frozen storage temperature and storage time on tenderness values of beef patties. J. Food Sci., 1989, 54, 291–296.
2. Boles, J.A., Swan, J.E., Effect of post-slaughter processing and freezing on the functionality of hot-boned meat from young bull. Meat Sci., 1996, 44, 11–18.
3. Cooper S.L., Sinclair L.A., Wilkinson R.G., Hallett K.G., Enser M., Wood J.D., Manipulation of the n-3 polyunsaturated fatty acid content of muscle and adipose tissue in lambs. J. Anim. Sci., 2004, 82, 1461-1470.
4. De Pedro E., Murillo M., Salas J., Peña F., Effect of storage time on fatty acid composition of subcutaneous fat. Unpublished work, supported by CEE (Project n.800-ct90-0013), 1999.
5. Fernandez-Reiriz M.J., Pastoriza L., Sampedro G., Herrera J.J., Changes in lipids of whole and minced rayfish (*Raja clavata*) muscle during frozen storage. Z. Lebensm. Unters. Forsch., 1995, 200, 420-424.
6. Folch J., Lees M., Stanley G.H.S., A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 1957, 226, 497-509.
7. IZ-INRA (2001). Standards for Cattle, Sheep and Goats Nutrition (in Polish). Research Institute of Animal Production, Kraków (Poland).
8. Jimenez-Colmenero F., Carball J., Cofrades S., Healthier meat and meat products: their role as functional foods. Meat Sci., 2001, 59, 5-13.
9. Kandeepan G., Biswas S., Effect of low temperature preservation on microbial and sensory quality of buffalo meat. Livestock Research for Rural Development 2005, 17, 11.
10. Klingenberg I.L., Knabe D.A., Smith S.B., Lipid metabolism in pig fed beef tallow or high-oleic acid sunflower oil. Compen. Biochem. Phys., 1995, 110B, 183-192.
11. Ngapo, T.M., Babare, I.H., Reynolds, J., Mawson, R.F., Freezing rate and frozen storage effects on the ultrastructure of samples of pork. Meat Sci., 1999, 53, 159–168.
12. Ortiz H., Bello R., Composition and stability of fatty acids from deboned cachama and sardine meat during freezer storage. Arch. Latinoam. Nutr., 1992, 42 (4), 460-464.
13. Petrović L., Grujić R., Petrović M., Definition of the optimum freezing rate-2. Investigation of the physico-chemical properties of beef *m. longissimus dorsi* frozen at different freezing rates. Meat Sci., 1993, 33, 319–331.
14. Raes K., De Smet S., Balcaen A., Claeys E., Demeyer D., Effect of diets rich in n-3 polyunsatured fatty acids on muscle lipids and fatty acids in Belgian Blue double-muscled young bulls. Reprod. Nutr. Dev., 2003, 43(4), 331-45.
15. Rule D.C., Busboom J.R., Kercher C.J., Effect of dietary canola on fatty acid composition of bovine adipose tissue, muscle, kidney, and liver. J. Anim. Sci., 1994, 72, 2735-2744.
16. Santos-Filho J.M., Morais S., Rondina D., Beserra F., Neiva J.N., Magalhaes E.F., Effect of cashew nut supplemented diet, castra-

tion and time of storage on fatty acid composition and cholesterol content of goat meat. Small Ruminant Research 2005, 57, 51-56.
17. Scollan, N.G., Choi N.J., Kurt E., Fisher A.V., Enser M., Wood J.D., Manipulating the fatty acid composition of muscle and adipose tissue in beef cattle. Br. J. Nutr., 2001, 85, 115–124.
18. Sen, A.R., Sharma, N., Effect of freezing and thawing on the histology and ultrastructure of buffalo muscle. Asian-Austral. J. Anim. Sci., 2004, 17, 1291–1295.
19. Strzetelski J., Borowiec F., Niwińska B., Zymon M., Effect of two linseed oily cultivars and soluble dried maize on fatty acid composition of calves meat. Ann. Anim. Sci., 2003, Suppl.2, 65-69.
20. Tomás M.C., Aòón M.C., Study on the influence of freezing rate on lipid oxidation in fish (salmon) and chicken breast muscles. Intern. J. Food Sci. Technol., 1990, 25, 718–721.
21. Zymon M., Strzetelski J.A., Kowalczyk J., The effect of fish oil in calf diets on the fatty-acid content of *Musculus thoracis* intramuscular fat. J. Anim. Feed Sci, 2005, 14, Suppl.1, 319-322.

WPŁYW MROŻENIA I ZAMRAŻALNICZEGO PRZECHOWYWANIA NA PROFIL KWASÓW TŁUSZCZOWYCH MIĘSA CIELĄT

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Celem pracy było określenie wpływu przechowywania mięsa cielęcego w warunkach zamrażalniczych na skład i proporcje kwasów tłuszczowych tłuszczu mięsa. 30 byczków podzielono na 6 grup po 5 sztuk i żywiono indywidualnie od 7. dnia życia preparatem mlekozastępczym (do 56. dnia) i mieszanką treściwą (do 90. dnia), w skład której wchodziły śruty zbożowe (67,5-71%), otręby pszenne (6-13%), poekstrakcyjna śruta sojowa (12,5-15,5%), premiks mineralno-witaminowy (3%) oraz 10% nasion lnu odmian *Omega* lub *Linola*, 10% nasion rzepaku odmian *Spencer* lub *Contact* lub 4% oleju rybnego. W grupie kontrolnej mieszanka nie zawierała dodatkowego tłuszczu. W 90. dniu życia byczki ubito i pobrano do analiz próbki mięsa z *Musculus thoracis*. Połowę próbek z każdej tuszy zamknięto w woreczkach foliowych, zamrożono i przechowywano w temperaturze -18°C przez okres 3 miesięcy. W próbkach mięsa (przed i po okresie mrożenia) oznaczono zawartość kwasów tłuszczowych metodą chromatografii gazowej. Wyniki opracowano statystycznie przy użyciu dwuczynnikowej analizy wariancji. Skład kwasów tłuszczowych tłuszczu mięsa był uwarunkowany głównie rodzajem tłuszczu w diecie, a tylko w niewielkim stopniu zamrażalniczym przechowywaniem. Nasiona lnu *Omega* (48% $C_{18:3}$ n-3) zwiększyły ($p\leq0,05$) udział kwasu linolenowego w tłuszczu mięsa w stosunku do innych grup, zaś olej rybny spowodował wzrost ($p\leq0,05$) zawartości długołańcuchowych kwasów EPA, DPA i DHA w tłuszczu mięsa. Analiza mrożonych próbek mięsa wykazała spadek zawartości kwasu $C_{16:1}$, a także wyższą zawartość sumy wszystkich kwasów tłuszczowych w porównaniu do mięsa świeżego($p\leq0,05$). Odnotowano także tendencję do wzrostu poziomu PUFA, w tym $C_{18:1}$, $C_{18:2}$ n-6, a także EPA i DHA w tłuszczu mięsa po okresie przechowywania zamrażalniczego.