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Fish technology

CHANGES OF THE LIPIDS OF THE MUSCLE TISSUE OF HERRING CONTAINING ADDITIVE OF AMARANTH SEEDS (*AMARANTHUS CRUENTUS*) DURING ITS FROZEN STORAGE

ZMIANY LIPIDÓW TKANKI MIĘŚNIOWEJ ŚLEDZI ZAWIERAJĄCEJ DODATEK NASION AMARANTUSA (*AMARANTHUS CRUENTUS*) W CZASIE ZAMRAŻALNICZEGO PRZECHOWYWANIA

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Minced muscle tissue of herring with addition of 0.25 and 0.50% of minced seeds of amaranth was studied for its content of hydroperoxides, secondary products of oxidation, conjugated dienes and trienes, water-binding capacity, and tissue hardness during 3-month frozen storage at -25°C.

INTRODUCTION

The main reason for lowering quality and consumption suitability of moderately fat and fat fish meat, in particular at minced stage, during frozen storage has been oxidation of their lipids. Results of the earlier study (Stodolnik 1995) suggested, that inhibition of the lipid oxidation of minced muscle tissue of Baltic herring, stored under frozen conditions could be possible through usage of additives of seeds of selected plants.

In the recent years, increased attention has been focused on amaranth□a cereal of relatively high nutritive values. A particular nutritive suitability of amaranth seeds stems from its high content of proteins□in this number globulins and albumins (Czubaszek 1996)□containing substantial amounts of essential amino acids (Konopko and Kondrat 1997). Moreover this cereal contains a substantial amount of iron (exceeding five-fold that of wheat), calcium, and vitamins of the group B as well as vitamins A, E, and C (Malewska 1995). The content of lipids and essential unsaturated fatty acids in the seeds of amaranth is higher than in traditionally cultured cereals (Malewska 1995). Lipid fraction of amaranth revealed presence of squalene (Jankuszew and Oleszek 1997), known as a compound of

low stability (Witas et al. 1981; Witas and Wędzińska 1989). The presence of two steroids, chondrilasterol and its 3-0-glukoside was also stated. The above compounds, subjected to toxicological tests showed no symptoms of harmful properties (Jankuszew and Oleszek 1997). Amaranth seeds were characterised by low content of gliadin, glutenine, and pentosans (Czubaszek 1996) and very low content of saponins□known as anti-nutritional substances (Junkuszew and Oleszek 1996).

A well-known usage of amaranth flour is its role as a qualitative modifier of bread products (Konopko and Kondrat 1997). The seeds of amaranth are used to enhance efficiency of carbohydrate fermentation in alcohol-distilling industry (Pysiak and Sobczak 1997). Beneficial properties of amaranth seed, particularly ground (3%) were observed in relation to yoghurt texture, in particular because of thickening properties of starch present in it (Grega et al. 1999). A valuable feature of amaranth is its small grain size (1–3 μm) and high viscosity of its solution (Cierach et al. 1999), which makes it very useful as a medium, not only in the food industry (Malewska 1995).

In the available literature, there are no papers on effect of amaranth seeds□on the stability of lipid compounds of food, in this number food of fish origin□applied to improve the nutritive quality of those products. In view of the above the present study was aimed at determining oxidative changes of lipids of the muscle tissue of herring supplemented with amaranth seeds and kept under frozen condition.

MATERIAL AND METHODS

Material

The study was based on Baltic herring, caught on the beginning of March. The fish were at a pre-spawning stage and at the moment of delivery to the laboratory their rigor mortis had already faded away. The mean length of the fish was 29.75 cm and their mean weight amounted to 200.15 g. The herring were filleted, skinned, and their muscle tissue was ground in a meat grinder with 3-mm cutting plate. The mince, following its mixing, was divided into 350-g portions supplemented with amaranth seeds (0.25 and 0.50%) and BHA (0.01%). The mince without additives constituted control sample. All experimental treatments of the mince were mixed to achieve a uniform dispersion of the additives used. The mince constituting control sample was also mixed.

All experimental treatments of the mince, divided into sub-samples of about 50 g were formed into rectangular prisms, wrapped in polyethylene foil, frozen at –25°C, and stored at such temperature for 12 weeks. Prior the analyses the fish mince samples were thawed in air at 4°C for 18 hours.

Methods

Lipids of the muscle tissue of herring were extracted using a chloroform-methanol mixture (2 : 1) (Linko 1967). Dehydrated chloroform layers of the extracts were used for determination of the following parameters: hydroperoxides, conjugated dienes and trienes of fatty acids, lipid content. Hydroperoxides were determined by an indirect method following their oxidation to malondialdehyde, determination of the latter compound and expression in the form of free malondialdehyde (Schmedes and Hølmer 1989). Conjugated dienes and trienes were determined through absorbance readings under wave lengths of 242 and 278 nm respectively, and results were expressed as absorption coefficients (Paquot 1979). The lipid content was determined by a weighing method after distilling out the solvent and drying the residue at 80°C for 1 hour. The methanol-water layers of the extracts were used for determining malondialdehyde with the aid of TBA (Schmedes and Hølmer 1989). Anti-oxidative activity (AA) of the additives was calculated using the following formula:

$$AA = \frac{K - B}{K} \cdot 100 \ [\%]$$

Where: K, oxidation product content in control sample;

B, content of oxidation products in a sample with additives.

Quantitative analysis of the fatty acids was performed using gas chromatography method, separating methyl esters on a column with GP 3%, SP-2310 (2%), SP-2300 on chromosorb WAW 100/110 masch at detector temperature 120–225°C and argon flow 40 cm^3/min. Individual fatty acid content was determined relating surface areas of all esters peaks, and the result was expressed as a percentage.

Forced exudate was assessed by measuring the area of the spill caused by squeezing 1 g of the mince by a weight of 800 g for 10 minutes, and expressed in cm^2/g.

Hardness of the mince was characterised by the surface area of 1 g of mince squeezed by a weight of 800 g for 10 minutes, and expressed in cm^2/g.

Total protein was determined using the Kjeldahl method.

All analyses were done in triplicate, while the exudate assessment □ in six repetitions.

RESULTS

The muscle tissue of herring used in the present study contained 8.15% of lipids, while the amaranth seeds—7.07% (Tabs. 1, 2). Among the fatty acids of amaranth the dominant item was linoleic acid, followed by oleic acid and palmitic acid, the three constituting jointly 90% of all acids (Tab. 3).

Table 1

Changes of lipid content (%) in the muscle tissue of herring during frozen storage

Sample		Storage time (weeks)						
		0	2	4	6	8	10	12
Control		8.15	6.56	6.48	5.91	6.80	6.18	7.17
Additive:	BHA 0.01%	8.15	4.40	6.44	7.67	6.28	8.58	7.12
	amaranth 0.25%	8.15	6.61	5.24	6.30	6.15	6.51	6.55
	amaranth 0.50%	8.15	6.81	6.75	5.87	6.57	6.75	6.40

Table 2

Chemical composition of amaranth seed

Water %	Total protein %	Lipids %	Dienes	Trienes
			absorption coefficient	
10.02	13.85	7.07	6.02	2.60

Table 3

Fatty acid composition of amaranth seed lipids

Fatty acid	%
C 14 : 0	0.22
C 16 : 0	20.80
C 18 : 0	3.88
C 18 : 1	25.27
C 18 : 2	47.40
C 18 : 3	1.02
C 20 : 0	1.05
C 20 : 1	0.10
C 22 : 0	0.30

In the muscle tissue of fresh herring, before freezing, a presence of peroxides was stated. Their content increased during the frozen storage, mainly within first 6 weeks and this was particularly evident in the mince containing amaranth seeds, in direct proportion to their content. The peroxide content in the latter samples was about 1.5 time (for 0.25% amaranth supplement) and 2 times (for 0.50% amaranth supplement) higher than it was in the mince with BHA supplement or without any additives (Fig. 1). Until the end of the storage period (12 weeks) the above differences between samples were maintained. Inhibiting properties of BHA turned out to be relatively weak in relation to the pace of the peroxide build-up (Fig. 1; Tab. 4).

Table 4

Antioxidative activity (%) of the additives used to the muscle tissue of herring during frozen storage

Additive	Hydroperoxides	Malon-dialdehyde	Dienes	Trienes	Total
BHA 0.01%	22.0	43.4	-3.8	-14.4	11.8
amaranth 0.25%	-28.6	-9.4	1.3	-7.7	-11.1
amaranth 0.50%	-77.0	-7.0	15.6	15.4	-9.7

The studied muscle tissue of herring revealed also the presence of secondary products of oxidation. The content of the latter products dramatically increased up to the second week of storage in the samples with amaranth or without any supplements, maintaining similar level throughout the entire storage period, without any major differences between samples of different content of amaranth seeds (Fig. 2: Tab. 4).

The content of conjugated dienes in the lipids of the muscle tissue of herring during the frozen storage was reduced by amaranth supplement, especially in the dose of 0.50%, despite the high content of dienes in the lipids of this cereal additive (Tabs. 2, 4). BHA did not show such properties (Tabs. 4, 5).

Table 5

Changes of the absorption coefficient value for conjugated fatty acids (dienes) of herring muscle tissue lipids during frozen storage

Sample		Storage time (weeks)						
		0	2	4	6	8	10	12
Control		12.33	6.37	14.20	5.40	2.53	3.42	2.29
Additive:	BHA 0.01%	12.33	7.71	14.62	3.90	3.00	3.90	2.83
	amaranth 0.25%	12.33	8.46	15.54	2.92	1.50	2.16	3.00
	amaranth 0.50%	12.33	6.65	11.53	2.85	1.57	2.08	2.28

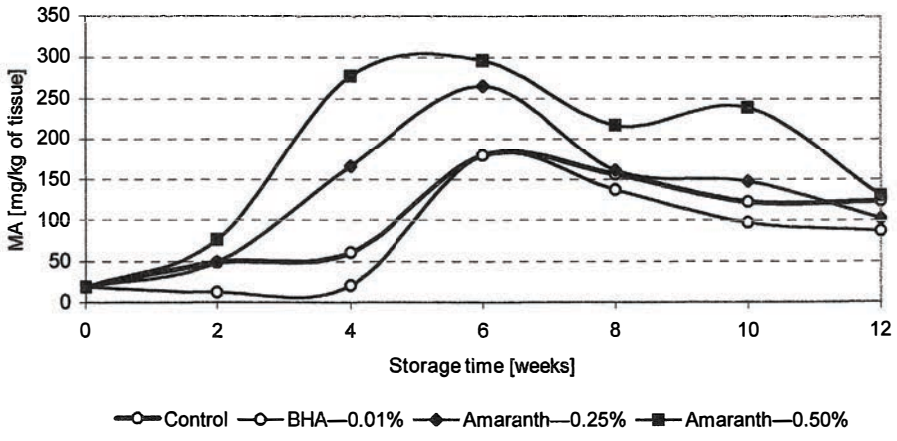

Fig. 1. Changes of hydroperoxides content (expressed in the form of free malondialdehyde) in the muscle tissue of herring during frozen storage (chloroform layer of the extracts)

Supplement of 0.50% of amaranth seeds to the muscle tissue of herring in a similar way inhibited formation of both dienes and trienes, while in the dose of 0.25% this additive similarly as BHA catalysed build-up of trienes during the frozen storage (Tabs. 4, 6).

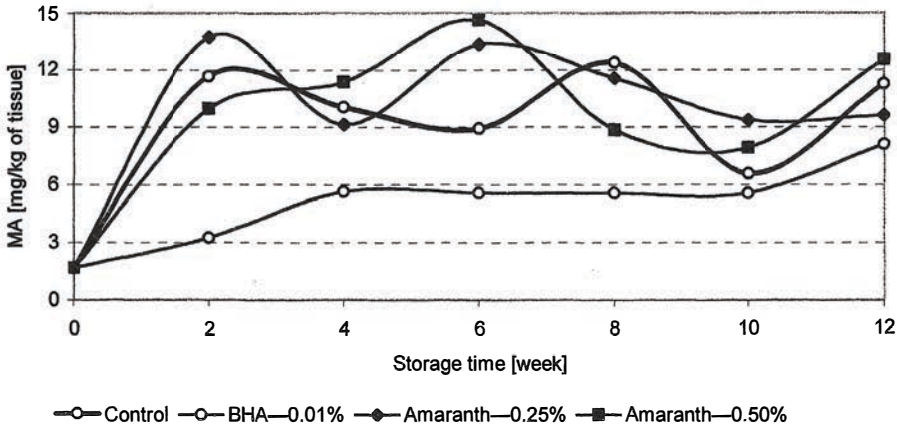

Fig. 2. Changes of malondialdehyde (MA) content in the muscle tissue of herring during frozen storage (methanol-water layer of the extracts)

Table 6

Changes of the absorption coefficient value for conjugated fatty acids (trienes) of herring muscle tissue lipids during frozen storage

Sample		Storage time (weeks)						
		0	2	4	6	8	10	12
Control		1.91	1.06	1.42	1.10	0.60	0.66	0.54
Additive:	BHA 0.01%	1.91	1.65	1.69	0.93	0.78	0.62	0.72
	amaranth 0.25%	1.91	1.24	1.72	0.95	0.53	0.62	0.88
	amaranth 0.50%	1.91	0.99	1.09	0.55	0.49	0.52	0.62

The lipids of amaranth seeds were characterised by a high content of trienes (about 30%) in overall amount of conjugated acids (Tab 2). The content of latter acids in the conditions of the muscle tissue of the herring decreased more than that of the muscle tissue of these fish alone or in the tissue supplemented with BHA during frozen storage (Tab. 6).

Analysis of the water-binding capacity by the muscle tissue of herring containing amaranth seeds demonstrated that the values obtained were similar to those of the mince without any supplement (Tab. 7). The texture changes of the mince containing amaranth seeds, as well as without them, were similar (Tab. 8).

The present study indicated that the muscle tissue of herring with the supplement of amaranth seeds in the doses of 0.25 and 0.50% retains the same physical properties as the muscle tissue without such supplement during frozen storage, despite high protein and low water content (Tab. 2) and high starch content (45–65% of dry weight) (Witkowska et al. 1999). According to Cierach et al. (1999) a much higher, amounting to 3–6%, supplement

of amaranth seeds added to a meat-fat mince improves water detention and rheological properties of meat products.

Table 7

Changes in forced exudate value (cm^2/g) of herring muscle tissue during frozen storage ($\bar{x}$ ±SD)

Sample	Storage time (weeks)				
	0	2	4	8	12
Control	8.25 ±0.28	8.36 ±0.40	9.55 ±0.43	10.16 ±0.26	11.30 ±0.45
Additive: amaranth 0.25%	8.16 ±0.37	9.66 ±0.28	—	10.00 ±0.29	10.36 ±1.46
amaranth 0.50%	8.45 ±0.41	10.10 ±0.52	10.38 ±0.56	9.96 ±0.55	10.68 ±0.41

Table 8

Changes in hardness value (cm^2/g) of herring muscle tissue during frozen storage ($\bar{x}$ ±SD)

Sample	Storage time (weeks)				
	0	2	4	8	12
Control	4,86 ±0.67	5,00 ±0.10	4,86 ±0.15	4,90 ±0.66	4,73 ±0.15
Additive: amaranth 0.25%	5,63 ±0.65	4,26 ±0.21	—	5,03 ±0.57	4,13 ±0,83
amaranth 0.50%	5,20 ±0.10	4,60 ±0.17	4,66 ±0.49	4,73 ±0.42	4,60 ±0.36

The presently observed increased content of lipid oxidation products in the muscle tissue of herring, supplemented with amaranth seeds may be a result of a high content of easy oxidising linoleic acid and almost twice lower content of stable, oleic acid in the seeds (Tab. 3). At the same time linoleic acid present in the lipids of amaranth seeds did not constitute a source of its diene compounds, because their content was lower than in control sample and in the sample with BHA during frozen storage.

Linoleic acid constitutes a small fraction of lipids in the muscle tissue of herring, ranging from detectable traces to 4.34% (Stodolnik and Podeszewski 1972; Szczygielski 1989). Therefore enrichment of this acid content, is justified for nutritional reasons. Explanation of the changes of conjugated dienes of fatty acids in the presently conducted study is not easy, because in technological processes, the mechanism of transformation of linoleic and linolenic acids into dienes is poorly known. It chiefly occurs under presence of lactic acid bacteria in the production processes of fermented products (Jiang et al. 1998) and also through free-radical oxidation during thermal processing (Bartnikowska et al. 1999). Intensification of fatty acid oxidation and also transformation of dienes to oleic and stearic acids can be a cause of decrease of content of conjugated dienes in food subjected to technological processes (Lin et al. 1995).

The presently observed stabilisation of content of primary and secondary products of oxidation, occurring from week 6 on, of frozen storage of analysed experimental treatments of mince and decrease of the content of conjugated dienes and trienes of fatty acids may suggest inhibition of the oxidation process of the lipids of the muscle tissue of herring during its frozen storage. It may result from decreasing activity of lipoxygenase, present in the muscle tissue of herring under influence of also lipid oxidation products during frozen storage. As demonstrated by Stodolnik and Samson (1998) the activity of lipoxygenase of the muscle tissue of the Baltic herring gradually decreases during storage of such raw material at -25°C.

The results of the present study indicate that amaranth seeds can be used in the fisheries industry for manufacturing products based on minced meat. The seeds can enrich the products with essential linoleic acid, although they must be accompanied by an antioxidant, preferably a natural one.

CONCLUSIONS

1. Supplement of amaranth seeds (0.25 and 0.50%) added to minced muscle tissue of herring accelerates oxidation of lipids to hydroperoxides and secondary products of oxidation during frozen storage.
2. Supplement of amaranth seeds to minced muscle tissue of herring, especially in the dose of 0.50% inhibited production of dienes and trienes of fatty acids during frozen storage.
3. Muscle tissue of herring containing 0.25 and 0.50% of amaranth seeds had similar water-binding capacity and hardness as the tissue without supplements, during frozen storage.
4. Enrichment of minced muscle tissue of herring with grind amaranth seeds is justified, because of their high content of linoleic acid. It must be accompanied, however, by an antioxidant, preferably a natural one.

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REFERENCES

Bartnikowska E., M.W. Obiedziński, S. Grześkiewicz, 1999: Sprzężone dieny kwasu linolowego – niedawno wykryte związki o działaniu antykancerogennym występujące w mleku i jego przetworach [Conjugated dienes of linoleic acid□recently discovered compounds of anticancerogenous properties, present in milk and diary products]. Przegl. Mlecz., 3: 86–91. (In Polish).

Cierach M., H. Ostoja, H. Konopko, 1999: Wpływ dodatku mączki z ekspandowanych nasion amarantusa na jakość farszów mięsnych drobno rozdrobnionych [Effect of the meal of expanded seeds of amaranth on quality of finely grind minced meat]. Sympozjum „Jakość zdrowotna żywności i żywienia", Białystok, 16–17 września, 1999: 119–120. (In Polish).

Czubaszek A., 1996: Ocena jakości czterech próbek amarantusa [Quality assessment of four samples of amaranth]. 26 Sesja Naukowa Komitetu Technologii i Chemii Żywności PAN „Postępy przechowalnictwa i ocena jakości żywności", Szczecin, 27–28 czerwca 1996: 10. (In Polish).

Grega T., M. Sady, M. Wszołek, H. Gambuś, 1999: Ocena jakości jogurtu z dodatkiem ziarna amarantusa (*Amaranthus cruentus*) [Quality assessment of yogurt supplemented with amaranth seeds (*Amaranthus cruentus*)]. 30 Sesja Naukowa Komitetu Technologii i Chemii Żywności PAN „Nauka o żywności na progu XXI wieku", Kraków 14–15 września 1999: 252. (In Polish).

Jiang J., L. Bjoerck, R. Fonden, 1998: Production of conjugated linoleic acid by dairy starter cultures. A. Appl. Microbiol., **85**: 91–102.

Jankuszew M., W. Oleszek, 1997: Saponiny nasion szarłatu (*Amaranthus cruentus*); wyodrębnianie, struktura i aktywność biologiczna [Saponins of amaranth seeds (*Amaranthus cruentus*); separation, structure, and biological activity]. 28 Sesja Naukowa Komitetu Technologii i Chemii Żywności PAN „Postępy w technologii i chemii żywności", Gdańsk, 1997: 26. (In Polish).

Konopko H., Z. Kondrat, 1997: Badania wybranych właściwości fizycznych nasion amarantusa [Study on selected physical properties of amaranth seeds]. 28 Sesja Naukowa Komitetu Technologii i Chemii Żywności PAN „Postępy w technologii i chemii żywności", Gdańsk, 1997: 260–261. (In Polish).

Lin H., T.D. Boylston, M.J. Chang, L.O. Luedecke, T.D. Schultz, 1995: Survey of the conjugated linoleic acid contents of dairy products. J. Dairy Sci., **78**: 2358–2365.

Linko R.R., 1967: Fatty acids and other components of Baltic herring flesh lipids. Ann. Univ. Turku, Ser. A, **101**: 7–121.

Małewska M., 1995: Rośliny przyszłości – źródło cennych surowców pokarmowych [Plants of the future□a source of valuable food products]. Wiad. Zielarskie, 6: 10–11. (In Polish).

Paquot C., 1979: Standard methods for the analysis of oils, fats and derivatives. Pergamon Press, Oxford: 71–74.

Pysiak B., E. Sobczak, 1997: Możliwości wykorzystania żyta z dodatkiem amarantusa w gorzelnictwie rolniczym [Utilization possibilities for rye supplemented with amaranth in distilling industry in rural areas]. 28 Sesja Naukowa Komitetu Technologii i Chemii Żywności PAN „Postępy w technologii i chemii żywności", Gdańsk, 1997: 221–222. (In Polish).

Schmedes A., G. Hølmer, 1989: A new thiobarbituric acid (TBA) method for determining free malonodialdehyde (MDA) and hydroperoxides selectively as a measure of lipid peroxidation. J. Am. Oil Chem. Soc., **66**, 6: 813–817.

Stodolnik L., 1995: Activity of selected plant raw materials in inhibition of lipids changes of Baltic herring minced meat tissue during frozen storage. Acta Ichth. Piscat., **25**, 1: 79–87.

Stodolnik L., Z. Podeszewski, 1972: Lipidy przemysłowych ryb bałtyckich. Cz. II. Kwasy tłuszczowe frakcji lipidowych [Lipids of commercially-exploited Baltic fishes. Part 2. Fatty acids of lipid fractions Lipids of commercially-exploited Baltic fishes. Part 2. Fatty acids of lipid fractions]. Bromat. Chem. Toksykol., 3: 295–300. (In Polish).

Stodolnik L., E. Samson, 1998: Wpływ zamrażalniczego przechowywania na aktywność lipooksydazy tkanki mięśniowej śledzia bałtyckiego [Effect of frozen storage on activity of lipoxygenase of the muscle tissue of Baltic herring]. Manuscript. Department of Refrigeration. Agricultural University of Szczecin. (In Polish).

Szczygielski M., 1998: Zmiany kwasów tłuszczowych w lipidach tkanki mięśniowej śledzia bałtyckiego (*Clupea harengus membras* L.) [Changes of fatty acids in the lipids of the muscle tissue of the Baltic herring (*Clupea harengus membras* L.) in a year cycle]. PhD thesis. Manuscript. Agricultural University of Szczecin. (In Polish).

Witas T., P. Śledziewski, J. Wędzińska, 1981: Zmiany procesów oksydacji skwalenu [Changes in processes of squalene oxidation]. Med. Wet., **37**, 1: 46–48. (In Polish).

Witas T., J. Wędzińska, 1989: Aktywność β-karotenu i przeciwutleniaczy w procesach utleniania skwalenu [Activity of β-carotene and antioxidants in the processes of squalene oxidation]. Zesz. Nauk. WSM w Szczecinie, 35: 269–284. (In Polish).

Witkowska A., M. Konopko, M. Borowska, K. Hukałowicz, R. Markiewicz, 1999: Zawartość błonnika pokarmowego w nasionach szarłatu przed i po ekspandowaniu [Alimentary cellulose content in amaranth seeds before and after expanding]. Sympozjum „Jakość zdrowotna żywności i żywienia", Białystok, 16–17 września, 1999: 48–49. (In Polish).

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ZMIANY LIPIDÓW TKANKI MIĘŚNIOWEJ ŚLEDZI ZAWIERAJACEJ DODATEK NASION AMARANTUSA (*AMARANTHUS CRUENTUS*) W CZASIE ZAMRAŻALNICZEGO PRZECHOWYWANIA

STRESZCZENIE

Analizowano zawartość nadtlenków, wtórnych produktów utleniania, skoniugowanych dienów i trienów kwasów tłuszczowych, zdolność utrzymywania wody oraz twardość zmielonej tkanki mięśniowej śledzi bałtyckich z dodatkiem 0,25 i 0,50% nasion amarantusa w czasie trzymiesięcznego przechowywania w temperaturze -25°C.

Stwierdzono, że dodatek zmielonych nasion amarantusa w obu zastosowanych ilościach przyśpiesza utlenianie lipidów do hydronadtlenków i wtórnych produktów utleniania podczas przechowywania zamrażalniczego, szczególnie przy ich zawartości 0,50%.

Nasiona amarantusa, przede wszystkim w ilości 0,50%, zawarte w tkance mięśniowej śledzi inhibitowały powstawanie dienów i trienów podczas przechowywania zamrażalniczego. Tkanka mięśniowa śledzi z dodatkiem 0,25 i 0,50% nasion amarantusa charakteryzowała się takimi samymi właściwościami utrzymywania soków tkankowych oraz teksturą jak farsz bez takich dodatków w czasie zamrażalniczego przechowywania.

Przeprowadzone badania wskazują, że nasiona amarantusa mogą być zastosowane do wyrobu przetworów rybnych na bazie mięsa rozdrobnionego w celu ich wzbogacenia w niezbędny kwas linolowy, jednakże łącznie z antyoksydantem, najlepiej pochodzenia naturalnego.

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