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Fish technology

INHIBITION OF LIPID OXIDATION IN THE MINCED MUSCLE TI SSUE OF THE BALTIC HERRING THROUGH USAGE OF HAWTHORN AND ELDER FLOWERS IN THE CONDITIONS OF FROZEN STORAGE

INHIBI TOWANIE UTLENIANIA LIPIDÓW ZMIELONEJ TKANKI MIĘŚNIOWEJ ŚLEDZI BAŁTYCKICH PRZEZ ZASTOSOWANIE KWIATÓW GŁOGU I BZU CZARNEGO W CZASIE ZAMRAŻALNICZEGO PRZECHOWYWANIA

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The oxidation rate of the lipids of minced muscle tissue of the Baltic herring was analyzed. The tissue had been stored for 3 months at the temperature of –25°C, with addition of plant raw materials: hawthorn flower (0.5%), elder flower (0.5%), and the mixture of those flowers (0.25% + 0.25%) supplementing buthylated hydroxyanisole (BHA)—a synthetic phenolic antioxidant.

INTRODUCTION

Lipids of the muscle tissue of herring are a good source of unsaturated fatty acids, so their protection against oxidation is necessary if they are to play nutritive and therapeutic role. Minced muscle tissue is particularly susceptible to oxidation due to: increased content of oxygen (Hwang and Regenstein 1988), dispersion of microsomal enzymes (Slabyj and Hultin 1984) and metal pro-oxidants (Hultin et al. 1990).

In the earlier works on oxidation of lipids of mince muscle tissue of the Baltic herring (Stodolnik 1994, 1995, 1996) it was determined, that this process is significantly inhibited by the addition of such plant-origin substances as: seeds and fruits of wild rose, seeds of dwarf quince and pepper, rowan, and mixture of the rowan and hawthorn berries. It was concluded further, that they constitute a source of natural antioxidants.

Among plant-origin raw materials, obviously containing compounds of proven anti-oxidative properties, the leading role play flowers of hawthorn and elder, being used for treating heart- and circulatory-system diseases.

It has been known from the literature (Ożarowski and Jaroniewski 1989), that the basic active compounds occurring in the flowers of hawthorn are flavonoids constituting 0.1–1.0%. Those flavonoids comprise, among others: vitexin, isovitexin, apigenin, rutin, quercetin, cempherol, and glycozyde bindings. Among the polyphenols, the most important are: 3-biozyde leucoanthocyanides and bi- and oligomeric leucoanthocyanides derivatives of flavone, binded with catechin and epicatechin. In addition to that, there occur phenolic acids, such as caffeic and chlorogenic. Flowers of the elder contain also such flavonoids as: rutin, quercetin, isoquercetin, astrogalin and polyphenolic acids such as caffeic and chlorogenic (Ożarowski and Jaroniewski 1989).

Hayase and Kato (1984) concluded from their studies that chlorogenic acid inhibited in over 80% production of hydroperoxides from linolenic acid. Subsequent studies (Hemeda and Klein 1990) proved that chlorogenic acid has greater potential for inhibiting peroxidase than quercetin and rutin. Further studies on antioxidants (Rodriguez de Sotillo et al. 1994) also proved, that chlorogenic and caffeic acids are good inhibitors of lipid oxidation. Particularly good is caffeic acid which antioxidative activity is higher than that of BHA.

Rutin, quercetin, and particularly isoquercetin, which are present in the flowers of hawthorn and elder, are good natural antioxidants of lipids (Hemeda and Klein 1990; Haraguchi et al. 1990).

The relevant bibliography lacks information on publications analyzing antioxidative properties of the hawthorn and elder or compounds extracted from them. In view of the above the present authors decided to study in what degree flowers of the hawthorn and elder can influence the process of oxidative changes of the lipids of the herring muscle tissue, known to oxidize easily also in the conditions of frozen storage. The study was to be compared with the concurrent results obtained using synthetic phenolic antioxidant BHA. To avoid possible alternation of chemical and therapeutic properties of the active compounds in the course of extraction and fractionation the present authors decided to use flowers of hawthorn and elder in their natural form.

MATERIAL AND METHODS

Material

Minced muscle tissue of the Baltic herring (*Clupea harengus* L.) of "S" size was used in the present study. The fish were caught on the beginning of March 1996 on the fishing grounds of "Barka" fisheries enterprise in Kołobrzeg. The herring represented the "spring" spawning stock, fourth and fifth stage of gonad maturity of the Maier's scale, and the post-mortem stage when they were examined. The fish were skinned and filleted. The dorsal muscle were separated and minced in a grinding machine with 2.5-mm strainer. The minced meat obtained was mixed with plant additives and divided into 350-g samples. The plant

additives, previously dried at 40°C and ground, were added in the following amounts: hawthorn flower (*Crataegus oxyacantha* L.)—0.5%, elder flower (*Sambucus niora* L.)—0.5%, mixture of hawthorn flower (0.25%) and elder flower (0.25%), BHA (0.01%). Minced meat without additives constituted the control sample. The dosage of plant-origin additives was based on the earlier studies (Stodolnik 1994).

Individual meat-sample alternatives were divided into about 55-g, 1-cm-thick bars, and packed in polyethylene foil sealed with adhesive tape. Such prepared samples were frozen using air blast freezing at the temperature of –25°C and stored at the same temperature for a period of 3 months.

The minced meat was chemically analyzed after 0, 2, 4, 6, 9, and 12 weeks of frozen storage, following defrosting in the air of the temperature 4°C.

Methods

The lipids were extracted using chloroform-methanol mixture (2:1) (Linko 1967). Separated layers of extracts: chloroform and methanol-water were transferred separately quantitatively to 100-cm^3 measuring flasks. The extracts obtained served for the following assays:

1) Content of lipids—using weight method after distilling out chloroform and drying the residue at the temperature of 80°C.
2) Content of hydroperoxides in chloroform extracts of lipids—using indirect method through their oxidation with $FeCl_3$ to malonaldehyde and its further reaction with 2-thiobarbituric acid measured through absorbance readings at the wave length of 532 nm (Schmedes and Hølmer 1989). The concentration of malonaldehyde was read from a standard curve prepared for 1,1-3,3-tetraethoxypropane (TEP by Merck) and the results were expressed in milligrams of malonaldehyde contained in 1 kg of the muscle tissue.
3) Content of malonaldehyde in methanol-water extracts—using 2-thiobarbituric acid according to the method of Schmedes and Hølmer (1989). The absorbance of the solutions was read from the standard curve prepared for 1,1-3,3-tetraethoxypropane. The final results were expressed in milligrams of malonaldehyde contained in 1 kg of the muscle tissue.
4) Content of dienes and trienes—using spectrophotometric readings of absorbance of diluted chloroform extracts of lipids in the ratio of 1:10 to 1:40; in the full range of ultraviolet spectrum, giving intensive band at the wave length of 242 nm for dienes and 278 nm for trienes. The results were expressed as absorption coefficient (Paquot 1982).
5) Content of carotenoids—using spectrophotometric reading of absorbance of chloroform extracts of lipids at the wave length of 463 nm and using the standard curve prepared for β-carotene.

Solubility of the proteins in 0.5% solution of NaCl—using the method described in the earlier work (Stodolnik 1994).

Antioxidative activity (AA) of the additives used was calculated using the formula of Al-Saikhan et al. (1995):

$$AA = \frac{\text{Increment rate of oxidation products in the control sample} - \text{Increment rate of oxidation products in the sample with additive}}{\text{Increment rate of oxidation products in the control sample}} \times 100\ [\%]$$

RESULTS AND DISCUSSION

The muscle tissue of herring sampled for the present study contained 4.3% of lipids (Tab. 1). It showed presence of hydroperoxides in amount equal to 6.6 mg of malonaldehyde in 1 kg of tissue (Fig. 1). During the storage of the muscle tissue of herring at the temperature of –25°C the increment of the amount of hydroperoxides took place and the additives used, inhibited in different rate the dynamics of their buildup. The hawthorn flowers were the ones which most effectively prevented the oxidation of the fatty acids to hydroperoxides, which was observed throughout the entire period of frozen storage of the minced muscle of the fish.

Table 1

Changes in extraction of lipids (g/100 g) from the muscle tissue of herring during frozen storage

Kind of sample	Storage time (weeks)					
	0	2	4	6	9	12
Control	4.3	3.9	4.2	4.5	4.4	4.6
Additive used:						
Elder flower 0.5%	4.3	4.7	4.3	4.5	4.4	4.4
Hawthorn flower 0.5%	4.3	3.7	4.4	4.5	4.1	4.6
Elder flower 0.25% + hawthorn flower 0.25%	4.3	4.7	4.6	5.3	4.3	4.4
BHA 0.01%	4.3	3.4	4.5	4.0	3.0	4.2

Table 2

Antioxidative activity [AA (%)] of the additives used for the muscle tissue of herring during frozen storage

Kind of additive	Hydroperoxides	Malonaldehyde	Dienes	Trienes	Total
Elder flower 0.5%	21.8	16.4	0.6	–2.3	9.1
Hawthorn flower 0.5%	41.3	27.6	–10.1	–6.6	13.0
Elder flower 0.25% + hawthorn flower 0.25%	22.9	22.5	10.4	–6.6	12.3
BHA 0.01%	38.7	30.0	3.7	–16.2	14.1

The flowers of elder inhibited the formation of hydroperoxides as long as up to 6th week of storage. The mixture of the elder flowers with hawthorn flowers and BHA were slowing down oxidation of the lipids past 5th and 6th week of storage, respectively.

Antioxidative activity of the additives used, calculated based on the content of the primary products of oxidation, revealed that the hawthorn flowers most extensively

inhibited the oxidation of the herring lipids (41.3%). The second in the raw was BHA (38.7%) followed closely by the mixture of elder and hawthorn flowers (22.9%), and the elder flowers (21.8%) used as a single additive (Tab. 2).

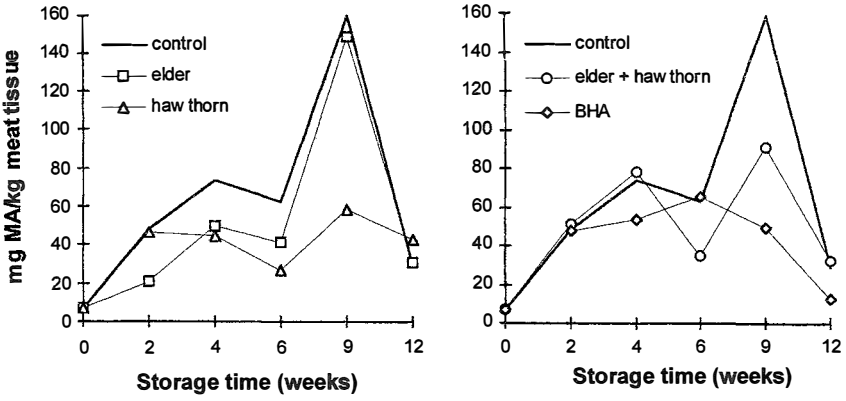

Fig. 1. Changes in hydroperoxides content in the muscle tissue of herring during frozen storage (chloroform layer of the extract)

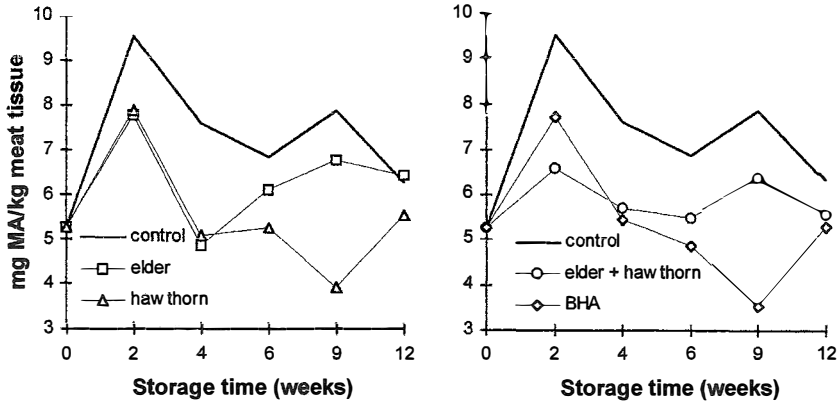

Fig. 2. Changes in malonaldehyde content in the muscle tissue of herring during frozen storage (methanol layer of the extract)

The analysis of the content of the secondary oxidation products demonstrated that the mixture of the hawthorn and elder flowers inhibited their buildup at the similar rate as it was recorded for the hydroperoxides. The remaining plant-origin additives and BHA less actively inhibited the production of aldehydes than they did for the primary oxidation products

(Fig. 2). Antioxidative activity, of the additives used, calculated based on the content of the secondary oxidation products, was lower than the one calculated based on the hydroperoxides content. Furthermore, the potential of the flowers added to inhibit lipid oxidation was lower than that of BHA (Tab. 2).

The changes of the fatty acids to diene associations during the frozen storage of the herring muscle tissue were inhibited at low rate and it occurred only under the influence of the elder and hawthorn flowers and BHA. None of the additives, however, did inhibit the transformation of the fatty acids' bonds into triene structures (Figs. 3, 4; Tab. 2).

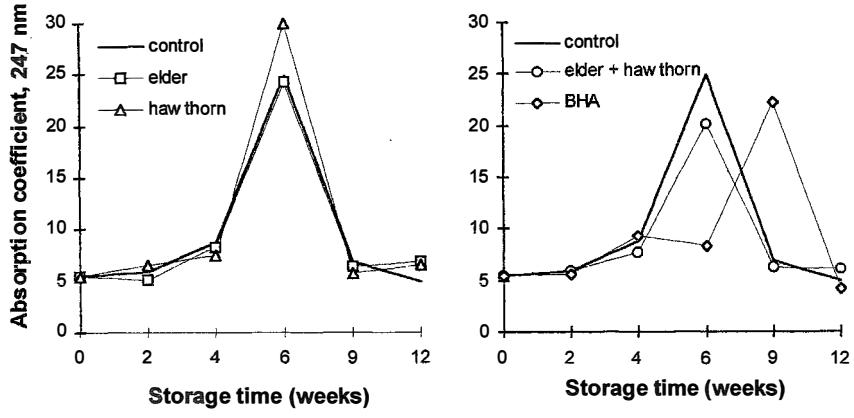

Fig. 3. Content of dienes in the lipids of muscle tissue of herring during frozen storage

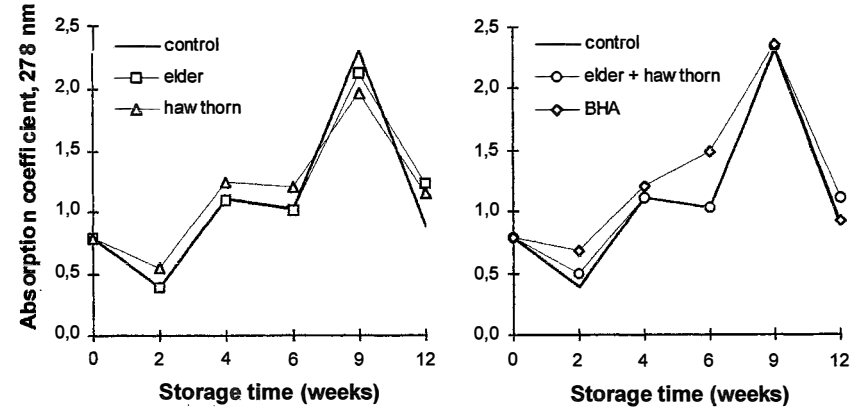

Fig. 4. Content of trienes in the lipids of muscle tissue of herring during frozen storage

The overall potential for lipid oxidation inhibition of the herring muscle tissue, stored for 3 months at the temperature of –25°C, calculated based on all evaluation factors used, was—in the case of the hawthorn flowers and its mixture with the elder flowers—slightly lower than it was for BHA. The elder flowers, however, and in the quantities two times higher, than in the mixture, inhibited the process of lipid oxidation of the muscle tissue of herring at the lowest rate. They exhibited more than twice lower activity than the remaining additives (Tab. 2).

Very similar activity of the antioxidants of the hawthorn flowers and BHA determined in the course of the present study (13.0% and 14.1% respectively), suggests, most probably, the influence of predominately phenolic compounds contained in the former additive, on the inhibition of the lipid oxidation. In the course of in vitro studies, a high activity of procyanidynes was demonstrated (Masquelier 1987), the compounds also present in the flowers of hawthorn (Ożarowski and Jaroniewski 1989) and considered to be "scavengers" of the peroxide radicals. In the model conditions used, activity of procyanidynes isolated from grapes was 10 times higher than that of ascorbic acid and about 2 times higher than that of catechins, anthocyanins, and rutin.

Good antioxidative properties of the hawthorn flowers may result also from the water solubility of the compounds contained in them. Such affinity to water would give its compounds better dispersion in the minced muscle tissue of the herring, compared to BHA, which is not soluble in water. Low content of lipids in the analyzed tissue, amounting to 4.3% probably did not allow for their sufficient contact with the synthetic antioxidant.

It was revealed in the earlier study (Stodolnik 1996), that the activity of BHA in inhibition of the lipids of the muscle tissues of herring which amounted to 6.4% was higher than it was presently determined and reached 20.2%. The above can support the presumption about the impact of lipid content on the dispersal of BHA, and on the associated potential for inhibition of the oxidative changes of the fatty acids. In the other studies (Jóźwiak 1996) based on the herring muscle tissue and the waste material resulting from its processing—higher antioxidative activity of BHA was stated. The above-mentioned tissue exhibited higher lipid content and was also stored in frozen conditions.

The present study demonstrated positive impact of BHA on the stability of carotenoids contained in the muscle tissue of herring during frozen storage (Tab. 3). It was revealed in the course of the other study (Jóźwiak 1996) that Endox, containing BHA protected carotenoids, which are a constituent of the skin and fins of herring during their 6-month-long storage period at the temperature of –25°C.

Table 3

Carotenoid content (mg/100 g) in the muscle tissue of herring during frozen storage

Kind of sample	Storage time (weeks)					
	0	2	4	6	9	12
Control	0.245	0.158	0.198	0.200	0.150	0.115
Additive used:						
Elder flower 0.5%	0.245	0.356	0.394	0.316	0.356	0.257
Hawthorn flower 0.5%	0.245	0.178	0.218	0.277	0.210	0.180
Elder flower 0.25% + hawthorn flower 0.25%	0.245	0.316	0.332	0.318	0.285	0.198
BHA 0.01%	0.245	0.277	0.277	0.265	0.245	0.166

The plant-origin additives enriched the fish raw material in carotenoids. It was particularly true for the elder flowers, which also influenced change in its color during the frozen storage at the temperature of –25°C (Tab. 3).

The more effective antioxidative activity of the hawthorn flowers than the elder flowers might have been caused by the higher content of the active substances or by their synergetic activity, as it was the case for their mixture with the elder flower. The antioxidative activity of the plant-origin additives used is certainly determined by their qualitative and quantitative composition. In the studies of Stodolnik (1996) on the natural antioxidants it was stated, that dried hawthorn fruits, added in amount of 0.5% to the muscle tissue of herring did not show ability for inhibiting oxidation of the lipids to hydroperoxides, nor to secondary oxidation products: dienes and trienes in the conditions o frozen storage. On the other hand, the fruits of hawthorn used in the mixture with the rowan berries in the amount 0.25% each, acted as antioxidants.

The presently conducted study indicates similar effectiveness of BHA and the plan-origin raw materials in the oxidation inhibition of the herring muscle tissue. The above supports the idea of substituting synthetic antioxidants with the naturally occurring ones. In particular, because flavonoids and polyphenols can act in multilateral ways on the inhibition of lipid changes namely: as compounds blocking free radicals (Pratt and Watts 1964; Torel et al. 1986), as substances making complexes with metal catalyst of oxidation reactions (Bartnikowska 1995), preventing reactions caused by single active oxygen atom (Baumann et al. 1980), inhibiting the activity of oxidative enzymes e.g. lipoxygenases (King and Klein 1987; Lyckander and Malterud 1992).

Usage of antioxidants of natural origin is also justified because BHA adversely affects the texture of muscle tissue of herring. In the present study this aspect was revealed as diminished extractivity of the lipids and proteins during frozen storage (Tabs. 1, 4) and has been a confirmation of the earlier studies (Stodolnik 1996).

Presently conducted study on lipid oxidation of the muscle tissue of herring containing additives of the hawthorn and elder flowers may suggest, that the biologically active compounds contained by the additives, may also affect the inhibition of lipoxidases of the fish

Table 4

Protein solubility (g/100 g) in the muscle tissue of herring during frozen storage

Kind of sample	Storage time (weeks)					
	0	2	4	6	9	12
Control	7.0	6.9	7.0	7.7	7.8	7.1
Additive used:						
Elder flower 0.5%	7.5	7.1	7.2	7.4	8.3	7.5
Hawthorn flower 0.5%	7.4	6.9	7.3	7.8	8.0	7.1
Elder flower 0.25% + hawthorn flower 0.25%	7.6	7.0	7.0	6.9	8.2	6.7
BHA 0.01%	7.7	7.2	6.7	6.6	7.3	6.8

raw material. This follows the argumentation of Eun et al. (1992) that not only synthetic phenolic antioxidants, but also natural ones, such as β-carotene of rosemary, slow down accordingly the activity of lipoxidases. Confirmation, however, of such statement in relation to the hawthorn and elder flowers may occur only in the way of analytic research procedure.

CONCLUSIONS

1. The plant-origin additives and BHA used for the muscle tissue of herring stored at the temperature of –25°C exhibited variable influence on inhibition of the buildup of the studied products of lipid oxidation.
2. Transformations of lipids of the herring muscle tissue to hydroperoxides in the course of its frozen storage were most extensively inhibited by the hawthorn flower, then by BHA which was closely followed by the mixture of the hawthorn and elder flowers. Least effective was the elder flower applied as a sole additive.
3. Formation of secondary products of the oxidation in the muscle tissue of herring during its frozen storage was the most extensively inhibited by BHA, only slightly worse—by the hawthorn flower. It was followed by the mixture of the flowers. The least effective was the elder flower.
4. None of the additives used inhibited formation of trienes during the frozen storage. On the other hand, the mixture of the hawthorn and elder flowers was the only additive to inhibit changes in fatty acid bonds into diene associations.
5. Antioxidative activity of the analyzed plant-origin raw materials and BHA added to the muscle tissue of herring stored for 3 months at the temperature of –25°C calculated based on all assessed factors of the lipid transformations was as follows: BHA>hatwhorn flower>mixture of hawthorn flowers and elder flowers>elder flower.

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INHIBITOWANIE UTLENIANIA LIPIDÓW ZMIELONEJ TKANKI MIĘŚNIOWEJ ŚLEDZI BAŁTYCKICH PRZEZ ZASTOSOWANIE KWIATÓW GŁOGU I BZU CZARNEGO W CZASIE ZAMRAŻALNICZEGO PRZECHOWYWANIA

STRESZCZENIE

Analizowano szybkość utleniania lipidów zmielonej tkanki mięśniowej śledzi bałtyckich, przechowywanej w temperaturze –25°C, zawierającej dodatek surowców roślinnych: kwiat głogu (0,5%), kwiat bzu czarnego (0,5%) i mieszanina tych kwiatów (0,25% + 0,25%) w odniesieniu do fenolowego syntetycznego przeciwutleniacza BHA. Określano zawartość hydronadtlenków, wtórnych produktów utleniania, dienów i trienów kwasów tłuszczowych oraz karotenoidów. Oznaczano także wpływ zastosowanych dodatków na rozpuszczalność białek tkanki mięśniowej śledzi w 5% roztworze NaCl.

W wyniku przeprowadzonych badań stwierdzono, że aktywność antyoksydacyjna analizowanych surowców roślinnych i BHA w stosunku do lipidów tkanki mięśniowej śledzi przechowywanej w temp. –25°C przez 3 miesiące układała się następująco: BHA 14,1%, kwiat głogu 13,0%, mieszanina kwiatów głogu i bzu czarnego 12,3%, kwiat bzu czarnego 9,1%.

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