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THE EFFECT OF THE COMPOSITION OF FATTY ACIDS OF BALTIC FISHES AND FROZEN STORAGE PROCESS ON THE ANTIOXIDANT ACTIVITY OF AQUEOUS EXTRACTS OF ROSEMARY AND SAGE, AS WELL AS BHA AND ENDOX

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Background. Natural antioxidants become increasingly important in fish processing and particularly in the preservation phase of fish raw materials. Some of them can protect lipids, containing essential unsaturated fatty acids. The aim of the present study was to determine antioxidant properties of aqueous extracts of rosemary and sage, compared with the activity of BHA (butylated hydroxyanisole) and Endox in lipids of fresh Baltic fishes (herring, sprat, flounder) and those subjected to frozen storage at –25°C for 6 months.

Material and methods. Antioxidant activity of the additives was determined based on β-carotene changes in fish lipids after 10, 20, 40, and 60 min of heating at 50°C.

Results. The plant-origin extracts and the synthetic antioxidants used, inhibited most extensively β-carotene changes in the lipids of non-frozen herring. They were the least effective in non-frozen sprat lipids. The frozen storage lowered the antioxidant action of the additives in relation to β-carotene in lipids of herring and flounder, whereas in sprat lipids the antioxidants used, continued their protective action for β-carotene, during heating. Elongation of the heating time from 10 to 60 min increased the β-carotene losses and their pace followed the logarithmic scale. The activity of the antioxidants in fish lipids decreased along with the increase of polyunsaturated fatty acids ($C_{20:5}$, $C_{22:5}$, and $C_{22:6}$) contents.

Conclusions. The antioxidant properties of plant-origin additives and synthetic antioxidants decreased along with the increased content on unsaturated fatty acids of fish lipids. Six-month-long frozen storage of the fish raw materials resulted in a decrease of the protective properties of the additives used, in relation to the lipids of Baltic herring and flounder. Endox exhibited the best protective properties for lipids of flounder and sprat, while aqueous extract of rosemary was the most effective in relation to sprat lipids.

Key words: fish, herring, sprat, flounder, β-carotene content, antioxidant activity, rosemary, sage, BHA, Endox

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INTRODUCTION

A substantial number of papers, in recent years, have focused on antioxidants, particularly on those of natural origin. Each year this group expands by several new chemical compounds detected in various plants which show lipid-protective properties. What is important, some of those compounds can protect lipids containing essential unsaturated fatty acids (Richheimer et al. 1996, Choi et al. 1996, Winata and Lorenz 1996, Wu and Yen 1998).

Although it is commonly known, that lipid oxidation is the main cause of qualitative changes of the muscle tissue of fishes, there are many aspects of this process, which are not fully understand, because lipid oxidation takes place already in living organisms (Ho et al. 1995), despite of the presence of antioxidants (Sies 1993). As the life processes in a fish terminate, certain changes accelerating lipid oxidation take place in the muscle (Hultin 1992). Xanthine dehydrogenase transforms into xanthine oxidase (Hille and Nishino 1995), iron undergoes oxidation in the proteins of hem (Everse and Hsia 1997), and the cell membranes disintegrate (Hultin 1992, 1995). All this promotes a quick development of oxidative processes in fish raw materials, particularly when they are filleted or grind, even when stored at lower temperatures. Therefore, usage of antioxidants in fish raw materials seems to be a necessity.

Johnsen and Dupree (1991), Gatlin et al. (1992), Brannan and Erickson (1996) demonstrated that α-tocopherol added to the muscle tissue of a catfish effectively decreased the pace of lipid oxidation during its storage at –6°C. α-tocopherol in muscle tissue of a mackerel was increasingly effective along with the time of its chilling storage at 0 to 4°C (Petillo et al. 1998).

Stodolnik (1996), Stodolnik and Samson (1996), and Stodolnik and Szczepanik (1998) demonstrated that 0.01% BHA added to minced muscle tissue of Baltic herring substantially lowered the pace of lipid oxidation, but it did not stop this process completely under conditions of frozen storage (–25°C). They also revealed that antioxidant activity of BHA (butylated hydroxyanisole) calculated based on primary and secondary oxidation products was diversified and it ranged from 34.0 to 57.0% in the muscle tissue of herring caught in different seasons. The other study (Stodolnik 1995) found out that the addition of the seeds of dwarf Japanese quince and wild rose to minced muscle tissue of Baltic herring effectively protected the lipids from oxidation during its storage at –25°C and the antioxidant activity of those additives was estimated to some 40.0%. Ramanathan and Das (1993), studied the effect of 10-% juice of garlic, ginger, onion, turmeric, dried cloves, cinnamon, caraway, and black pepper (to name just a few) on the lipids of salted and cooked narrow-barred Spanish mackerel, *Scomberomorus commerson*. They found out that higher antioxidant activities were associated with dried additives, compared to their aqueous extracts, possibly because of higher content of lipoprotective compounds in the former and also because of different chemical composition of the aqueous extracts and the dried spices.

A rosemary addition to sardine oil lowered lipid oxidation under elevated temperatures (Han et al. 1990). According to Richheimer et al. (1996) the antioxidant properties of rosemary are caused by its components, namely abietic diterpene and coffee-, rosemary- and carnosic acids. Sage also contains compounds of high antioxidant properties (Berry 1990, Ho et al. 1995) of which chinones are particularly active (Weng and Gordon 1992). The above compounds were identified in extracts, using organic solvents. Considering the importance of those plants as a source of natural antioxidants the present authors decided to analyse the effectiveness of aqueous extracts of rosemary and sage in lowering the lipid oxidation of Baltic fishes in comparison to such properties of BHA and Endox.

MATERIAL AND METHODS

The material for the present study were Baltic herring, *Clupea harengus membras* Linnaeus, 1761, Baltic sprat, *Sprattus sprattus balticus* (Schneider, 1908), and flounder, *Platichthys flesus* (Linnaeus, 1758). collected near the port of Kołobrzeg. The herring were caught in November, sprat—in February, and flounder — in December. All fishes were iced and within 1–2 days after capture delivered to the laboratory. Packages of 3 to 10 fish were wrapped in polyethylene foil, frozen, and stored at –25°C. In the first part of the study—aimed at determination of the effect of qualitative and quantitative composition of the fatty acids on the activity of antioxidants—the fish lipids were analysed before freezing. In the second part of the study, the fishes were sampled after 4, 8, 12, 20, and 24 weeks of frozen storage at –25°C, defrosted in the air at 18–20°C for 2 hours. In both parts of the study the fishes were filleted, skinned, grind in an electric meat grinder with a 3-mm cutting plate, and analysed. The additives used for lipids were BHA (98% purity) manufactured by "Fluka"; Endox (commercial name of a mixture of: BHA, etoxyquin, citric- and phosphoric acids as chelates and surface active compounds) manufactured by "Kemin"; rosemary (*Rosmarinus officinalis* L.) — by "Kamis-przyprawy" Warszawa — used as aqueous extract; sage (*Salvia officinalis* L.)—by "Herbapol–Lublin"—used as aqueous extract; and mixture of aqueous extracts of rosemary and sage (1 : 1).

Preparation mode: dried leaves of rosemary were grind and sage lives were crushed. Subsamples of leaves weighing 1.5 g were soaked in 24 ml of distilled water and mixed using an electric mixer (300 rpm) at room temperature for 1 h. Subsequently the extracts were filtered through a soft filter paper and subsequently studied.

Lipids of the fish muscle tissue were extracted using a chloroform-methanol mixture (2 : 1) (Linko 1967) in the following way: 25 g of muscle tissue was supplemented by 50 ml of chloroform and 25 ml of methyl alcohol and homogenised at 10 000 rpm, for 2 min; after that 25 ml of methyl alcohol and 25 ml of distilled water were added and the mixture was homogenised for another minute. Subsequently the mixture was filtered into a vacuum flask. The obtained product was separated in a separator. The chloroform layer, after its dehydration with anhydrous sodium

sulphate, was transferred qualitatively to a 100-ml graduated flask. The lipid contents in the extracts were determined by weighing after distilling off the solvents, under decreased pressure and drying at 80°C for 1 h. The fatty acid composition was determined with the method of gas chromatography, following methylation of the fatty acids using BF3. The fatty acids were separated on a PU 4550 Philips chromatograph under the following conditions: glass column, 2.1-m long, 4 mm internal diameter, filled with GP3% SP-2310/2% SP-2300 on chromosorb WAW 100/110 mesh (SUPELCO); FID detector, temperature 250°C; feeder temperature 250°C; column temperature (initial 120°C for 2 minutes; increasing 12° per min, final 225°C for 10 minutes), carrier gas (argon) flow 40 $cm^3 \cdot min^{-1}$; injection about 1 µl.

Composition of individual fatty acids was calculated using the following formula:

$$\text{Acid content} = Ax \cdot 100 \cdot \Sigma A^{-1} \ [\%]$$

where:
Ax, peak surface area of individual ester,
ΣA, total area of peaks representing all esters.

Antioxidant activity was estimated based on β-carotene changes during heating at 50°C using the method of Al-Saikhan et al. (1995). Procedure: amounts of 2 mg of β-carotene were dissolved in 20 ml of chloroform. From that, 3 ml were transferred to Erlenmayer flask, 0.04 g of fish were added with 0.4 g of Tween 80, subsequently mixed, chloroform was evaporated under reduced pressure at 40°C. After that 80 ml of distilled water was added to the flask, well mixed and amount of 3 ml of emulsion was sampled to test tubes and 0.12 ml of aqueous extract of rosemary or sage were added. BHA and Endox were added at 0.01% of emulsion mass. After that the samples were heated at 50°C within 10, 20, 40, and 60 min. β-carotene content was determined spectrophotometrically with the wave length of 463 nm. The reagent sample contained the same components with equal amount of distilled water replacing the lipid extract.

Degradation level of β-carotene (SD) was calculated using the formula:

$$SD = A - B \ [\%]$$

where:
A, absorbance before heating, assumed as 100%,
B, absorbance after 10, 20, 40, and 60 minutes of incubation expressed in percents of absorbance before heating.

Antioxidant activity of the additives used was calculated following the formula:

$$AA = (SDK - SDA)\ 100 \cdot SDK^{-1} \ [\%]$$

where:
SDK, degradation level of β-carotene in control sample,
SDA, degradation level of β-carotene in sample with antioxidant.

All presented study results are arithmetic means, based on three parallel readings. To determine the variability of the results, a standard deviation was calculated using Statistica 6.0 software package.

RESULTS

The lipid content of the muscle tissue of fishes sampled for the present study amounted to 8.7% in Baltic herring, 4.9% in Baltic sprat, and 3.6% in flounder. The qualitative composition of the fatty acids in the fish lipids was the same but it varied quantitatively (Table 1). Unsaturated fatty acids constituted 66.86% in the herring lipids, 72.51%—in sprat lipids, and 73.84%—in the lipids of flounder. The contents of $C_{20:5}$, $C_{22:5}$, and $C_{22:6}$ constituted 16.61, 28.48, and 16.49% of all fatty acids, respectively.

Table 1

Percentage composition of fatty acids of lipids of muscle tissue of non-frozen fishes sampled for the study

Fatty acids	Lipids of		
	Herring	Sprat	Flounder
$C_{14:0}$	9.19 ± 0.13	4.88 ± 0.07	4.09 ± 0.08
$C_{14:1}$	0.70 ± 0.02	0.86 ± 0.06	0.55 ± 0.06
$C_{16:0}$	20.00 ± 0.11	18.15 ± 0.15	18.19 ± 0.12
$C_{16:1}$	8.92 ± 0.07	6.17 ± 0.08	17.46 ± 0.11
$C_{17:0}$	1.91 ± 0.03	2.10 ± 0.04	2.10 ± 0.04
$C_{18:0}$	2.04 ± 0.06	2.38 ± 0.06	1.79 ± 0.02
$C_{18:1}$	17.90 ± 0.11	22.24 ± 0.11	23.43 ± 0.20
$C_{18:2}$	3.72 ± 0.09	3.38 ± 0.05	1.66 ± 0.06
$C_{18:3}\ \gamma$	0.44 ± 0.02	0.39 ± 0.03	0.43 ± 0.02
$C_{18:3}\ \alpha$	0.20 ± 0.02	2.63 ± 0.06	1.21 ± 0.04
$C_{18:4}$	3.04 ± 0.10	4.03 ± 0.06	1.02 ± 0.03
$C_{20:1}$	13.59 ± 0.12	1.63 ± 0.02	8.39 ± 0.09
$C_{20:2}$	0.64 ± 0.04	1.13 ± 0.03	0.82 ± 0.07
$C_{20:3}$	0.37 ± 0.03	0.46 ± 0.02	0.50 ± 0.03
$C_{20:4}$	0.73 ± 0.05	1.11 ± 0.04	1.90 ± 0.05
$C_{20:5} + C_{22:1}$	3.19 ± 0.12	10.27 ± 0.08	7.98 ±0.04
$C_{22:5}$	1.72 ± 0.09	2.20 ± 0.06	1.28 ± 0.03
$C_{22:6}$	11.70 ± 0.21	16.01 ± 0.09	7.21 ± 0.05

Lipid analysis of fishes not subjected to freezing, demonstrated that the content of β-carotene changed the most in control samples (without antioxidant additives) (Table 2). In the majority of lipids, the heating up, prolonged from 10 to 60 min, inflicted increased losses of β-carotene. This relationship was not proportional, however (Fig. 1). In fish lipids, without antioxidant additives, the content of β-carotene, determined after incubation (all temperature regimes), was as follows:

lipids of Baltic herring > lipids of Baltic sprat > lipids of flounder.

The use of BHA distinctly inhibited oxidative changes of β-carotene in all analysed fish lipids, though it was the most effective in lipids of flounder (Table 2). In general, Endox showed a stronger antioxidant activity than BHA. Endox activity in relation to fish lipids was as follows: herring > sprat > flounder. Addition of aqueous extract of rosemary distinctly stabilised fish lipids. The average content of β-carotene in fish lipids of the species studied, supplemented with rosemary extract, was as follows: herring > flounder > sprat. A similar pattern was observed after supplementation of aqueous extract of sage, which—in general—had stronger antioxidative potential than rosemary extract. The mixture of aqueous extracts of rosemary and sage inhibited oxidative changes in the lipids studied, similarly as sage extract. The former was the most effective in the flounder lipids, less effective in herring lipids, and the least effective in the lipids of sprat.

Among all lipid additives used, the most effective inhibition of β-carotene changes was observed for the mixture of rosemary and sage. With use of the latter additive, β-carotene level declined by 11.0 percentage point. Sage used alone inflicted a 11.5 percentage point decline, while Endox and BHA—a decline of 13.0 and 16.1 percentage points, respectively (Table 2). Lipids of Baltic herring, supplemented by synthetic antioxidants and plant-origin extracts protected β-carotene the most, with its decline amounting to 7.9 percentage points. All additives were less effective in flounder lipids (decline by 12.1 percentage points) and the least—in sprat lipids (22.9 percentage point decline). (Table 2).

Table 2

Effects of antioxidant supplementation on β-carotene content (%) in fish lipids, during heating (arithmetic mean of the values obtained from all heating regimes)

Lipids of	Without supplementation	Additives				
		Antioxidants		Aqueous extracts		
		BHA	Endox	Rosemary	Sage	Rosemary + sage
Herring	65.6 ± 2.6	85.2 ± 0.4	92.7 ± 1.8	98.2 ± 0.0	93.6 ± 0.8	90.6 ± 1.8
Flounder	44.0 ± 0.0	97.3 ± 0.0	80.6 ± 0.3	77.8 ± 0.8	87.5 ± 0.0	96.3 ± 0.0
Sprat	50.4 ± 0.2	69.2 ± 0.0	87.6 ± 0.3	64.3 ± 0.0	84.4 ± 0.3	80.0 ± 1.3

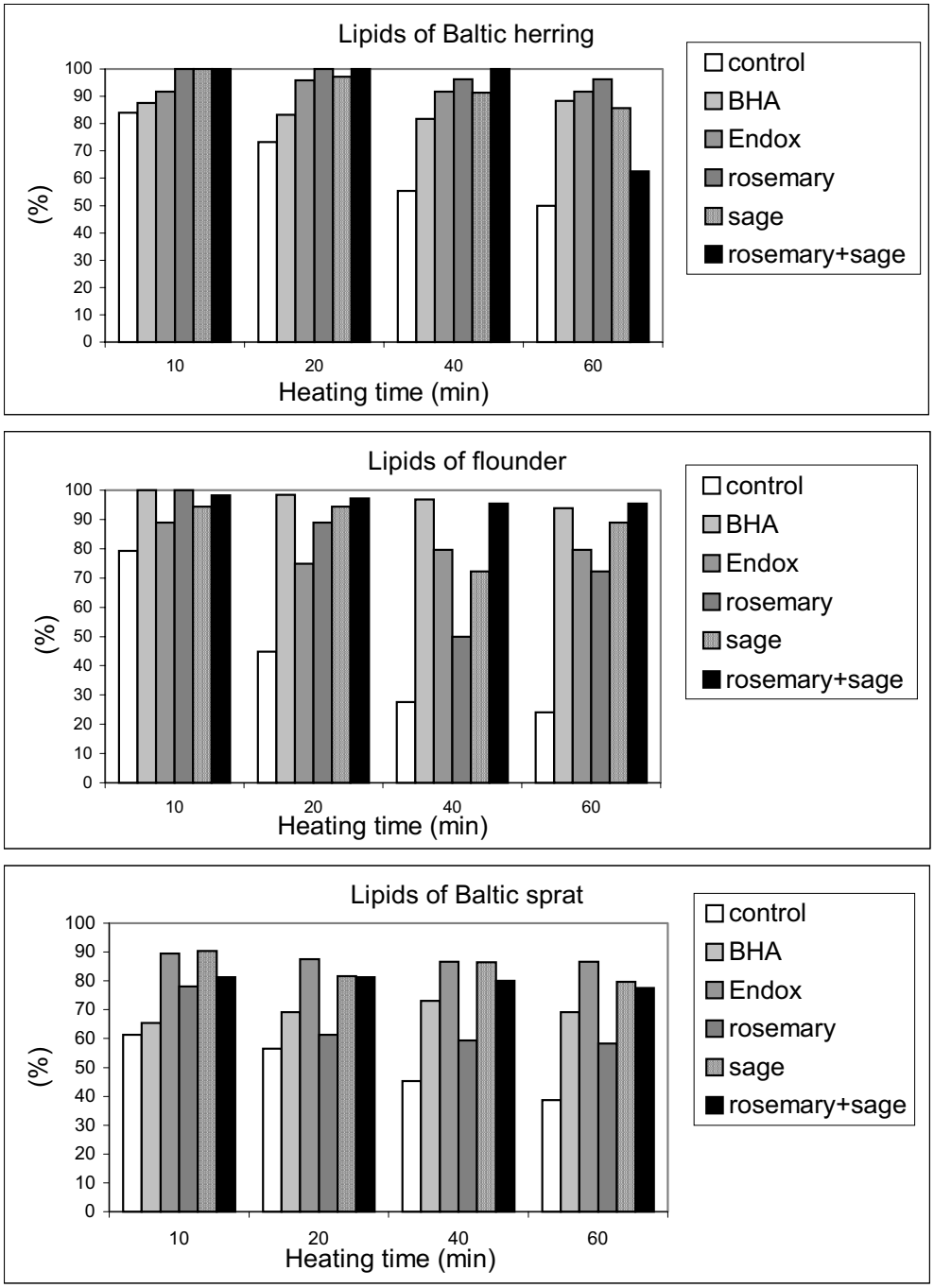

Fig. 1. Effect of heating time on changes in β-carotene content in lipids of the muscle tissue of non-frozen fish

The degradation level of β-carotene in lipids of herring without supplements was two-fold higher than that observed in this fish protected by antioxidants. The former value increased proportionally with time up to the second month of frozen storage, to decline in subsequent months, proportionally with time (Table 3). In the lipids of herring, supplemented with Endox and with extract mixture of rosemary and sage, the level of β-carotene degradation started to increase after three months (Fig. 2). β-carotene level did not change distinctly through the entire period of frozen storage for herring lipids treated with BHA and extracts of rosemary and sage.

Table 3

Effect of duration of heating on β-carotene degradation level (%) in lipids of herring analysed during 6-month frozen storage (arithmetic mean of the values obtained from analysed time-periods of storage)

Sample studied	Duration of heating [min]				$\bar{x}$
	10	20	40	60	
Control	22.5 ± 0.9	33.6 ± 0.6	52.3 ± 1.0	61.0 ± 0.3	42.3
With:					
BHA	19.5 ± 1.6	18.8 ± 1.6	18.2 ± 0.7	20.3 ± 1.0	19.2
Endox	29.8 ± 1.7	26.1 ± 1.7	27.9 ± 0.8	28.7 ± 0.2	28.1
Rosemary	7.9 ± 0.4	15.2 ± 1.9	18.4 ± 0.6	17.3 ± 0.8	14.7
Sage	1.5 ± 1.2	15.1 ± 1.5	14.8 ± 0.8	17.6 ± 0.7	15.0
Rosemary+sage	19.5 ± 0.8	19.9 ± 0.9	23.4 ± 0.6	30.3 ± 1.8	23.3

The level of β-carotene degradation showed a growing tendency in lipids of flounder without antioxidants. In the same fish, supplemented with the additive studied, no explicit tendencies were observed (Fig. 3). Endox and aqueous extracts of sage demonstrated the strongest protective properties in relation to β-carotene in the lipids of flounder sampled in sequential months of frozen storage (Table 4). Slow changes of β-carotene degradation level were observed in lipids of sprat, with and without the additives, monitored throughout 6 months of frozen storage (Fig. 4). The highest degradation level of β-carotene was observed, however, in the lipids of this fish without additives, and it was two- or three-fold higher than in the remaining samples (Table 5). The strongest protective properties, as determined within the entire frozen storage of sprat, were exhibited by synthetic antioxidants. In lipids, however, with BHA the β-carotene breakdown decreased with time of frozen storage. Plant-origin additives to lipids of Baltic herring were less efficient than the synthetic antioxidants in inhibition of β-carotene degradation. The levels of the latter compound decreased in the last months of frozen storage (Fig. 4).

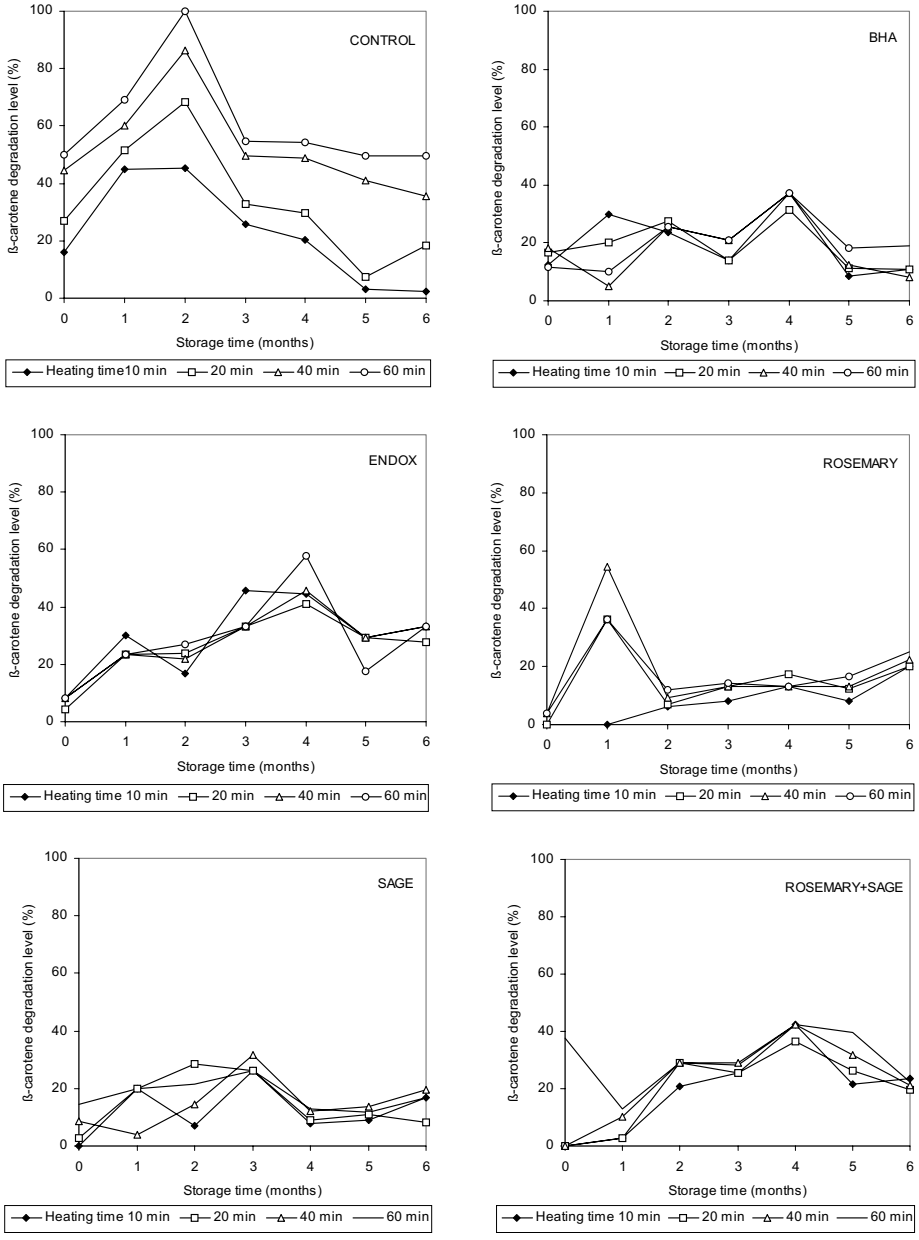

Fig. 2. Effect of frozen storage time of herring on the degradation level of β-carotene in the lipids during heating at 50°C (arithmetic mean based on the values recorded at individual time-periods of heating)

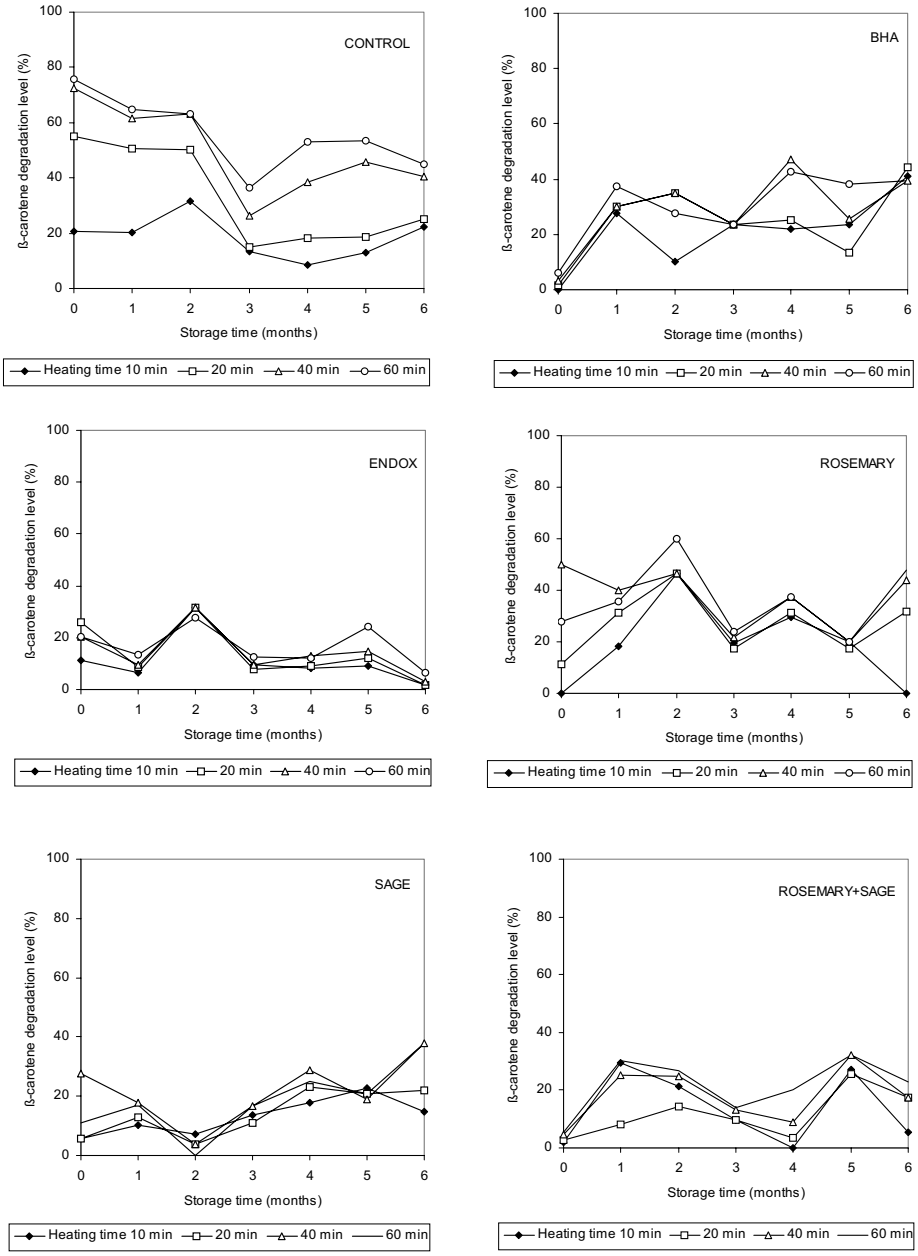

Fig. 3. Effect of frozen storage time of flounder on the degradation level of β-carotene in the lipids during heating at 50°C (arithmetic mean based on the values recorded at individual time-periods of heating)

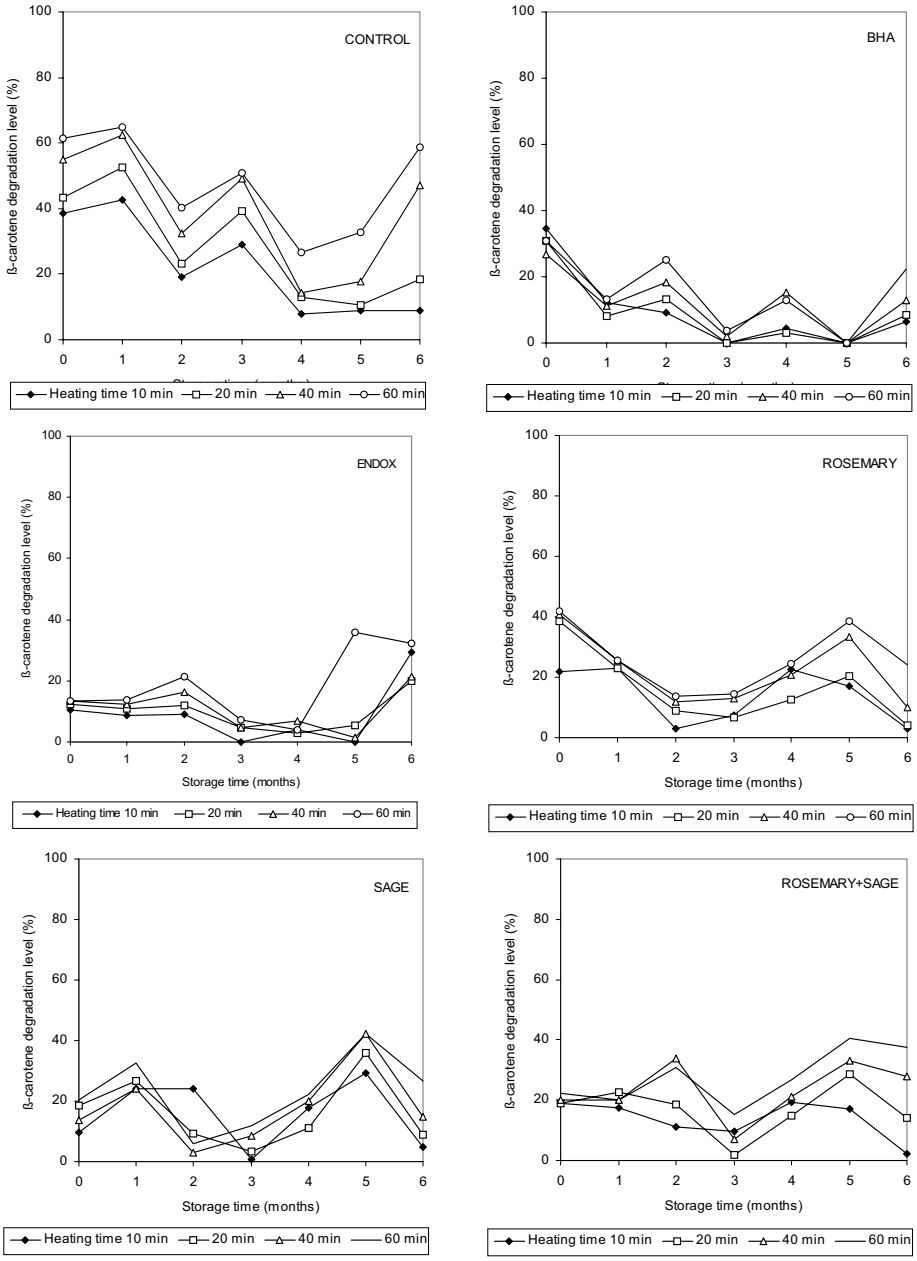

Fig. 4. Effect of frozen storage time of sprat on the degradation level of β-carotene in the lipids during heating at 50°C (arithmetic mean based on the values recorded at individual time-periods of heating)

Table 4

Effect of duration of heating on β-carotene degradation level (%) in lipids of flounder analysed during 6-month frozen storage (arithmetic mean of the values obtained from analysed time-periods of storage)

Sample studied	Duration of heating (min)				$\bar{x}$
	10	20	40	60	
Control	20.1 ± 1.3	33.2 ± 0.9	49.7 ± 0.5	55.9 ± 0.7	39.7
With:					
BHA	21.1 ± 0.5	24.7 ± 0.7	29.1 ± 0.6	30.7 ± 0.6	26.4
Endox	11.2 ± 0.3	13.9 ± 0.3	14.6 ± 0.5	16.7 ± 0.4	14.1
Rosemary	19.1 ± 0.2	26.7 ± 0.3	37.0 ± 0.7	36.0 ± 0.8	29.7
Sage	13.2 ± 0.5	14.2 ± 0.3	21.6 ± 0.1	18.4 ± 0.4	16.8
Rosemary+sage	13.5 ± 0.2	11.6 ± 0.3	18.1 ± 0.4	21.6 ± 0.0	16.2

Table 5

Effect of duration of heating on β-carotene degradation level (%) in lipids of sprat analysed during 6-month frozen storage (arithmetic mean of the values obtained from analysed time-periods of storage)

Sample studied	Duration of heating (min)				$\bar{x}$
	10	20	40	60	
Control	22.1 ± 0.4	28.6 ± 0.4	39.8 ± 0.0	47.9 ± 0.4	34.6
With:					
BHA	9.5 ± 0.1	15.9 ± 0.1	12.3 ± 1.8	15.4 ± 0.6	13.3
Endox	8.8 ± 0.4	9.7 ± 0.2	10.9 ± 0.3	18.3 ± 0.5	11.9
Rosemary	13.9 ± 02	16.3 ± 0.3	22.1 ± 0.4	26.1 ± 2.8	19.6
Sage	15.8 ± 0.6	16.2 ± 0.3	18.0 ± 0.6	23.2 ± 0.6	18.3
Rosemary+sage	13.7 ± 0.6	17.0 ± 1.7	23.3 ± 0.8	27.6 ± 0.8	20.4

Taking into account the entire period of frozen storage of all fishes studied, the changes in β-carotene levels in lipids were the slowest when accompanied by aqueous extract of sage and Endox. The other antioxidants studied, inhibited changes in β-carotene in lower extent (Table 6). The highest effectiveness of the analysed additives was observed for lipids of sprat, while the lowest—for the lipids of herring and flounder. Six-month storage at –25°C weakened the antioxidative properties of the plant extracts and the antioxidants in relation to β-carotene of herring- and flounder lipids. The above substances remained still effective, however, towards β-carotene in sprat lipids during heating.

The most effective antioxidant, taking into account the impact of frozen storage of two fish studied on the changes of β-carotene level, was Endox, while for one fish species—rosemary. The most characteristic were the lipids of sprat, where β-carotene was protected the most by the synthetic antioxidants (Table 7).

Table 6

Effect of antioxidant supplementation on β-carotene content (%) in fish lipids analysed during 6-month frozen storage (arithmetic mean of the values obtained from analysed time-periods of heating and storage)

Lipids of	Without supplementation	Additives				
		Antioxidant		Aqueous extracts		
		BHA	Endox	Rosemary	Sage	Rosemary + sage
Herring	57.7	80.8	71.9	85.3	85.0	76.7
Flounder	60.3	73.6	85.9	70.3	83.2	83.8
Sprat	65.4	86.7	88.1	80.4	81.7	79.6

Table 7

The most effective antioxidants for lipids of individual fish, analysed during the entire frozen storage period, based on the mean values of β-carotene degradation during heating

Lipids of	Two most effective antioxidants or additives	
	1	2
Herring	rosemary	sage
Flounder	Endox	rosemary + sage
Sprat	Endox	BHA

DISCUSSION

The diversified effect of fish lipids analysed before the frozen storage—as demonstrated in the present study—on the degradation level of β-carotene during heating is associated with the composition of their fatty acids. The highest stability of β-carotene in herring lipids and the highest activity of antioxidative additives coincided with the lowest content of unsaturated fatty acids, constituting 66.9% compared to their content in lipids of sprat and flounder. The lowest values of β-carotene stability and the lowest effectiveness of the additives, observed in sprat lipids was probably associated with a higher content of unsaturated fatty acids. The latter was by 8.5 percentage points higher in sprat than it was in herring. Moreover, the unsaturated fatty acids sprat contained more (39.3%) polyunsaturated acids ($C_{20:5}$, $C_{22:5}$, and $C_{22:6}$) than in herring lipids (24.8%).

A small level of β-carotene degradation occurred in the flounder lipids. Those lipids apparently contained the highest percentage of unsaturated fatty acids, but the earlier mentioned polyunsaturated fatty acids constituted the lowest share compared to the fatty acids composition of herring and sprat. Those acids ($C_{20:5}$, $C_{22:5}$, and $C_{22:6}$) in fish lipids are the most sensitive to oxidation (Takahashi et al. 1985).

Kołakowska et al. (2000) demonstrated that flounder lipids are two times less susceptible to oxidation in the photooxidation test UV than the lipids of Baltic herring. The above-mentioned authors found that the content of polyunsaturated fatty acids in spat lipids was over two-fold higher than that of flounder, which is consistent with the present findings.

The antioxidants used demonstrated weaker protective properties in relation to fish lipids following their frozen storage at –25°C, although the differences between lipids of individual fish species were not so pronounced, as they were in fresh fish. β-carotene degradation levels after such storage were fairly similar in lipids of herring and flounder. Sprat lipids during frozen storage tended to be more stable than the lipids of the two remaining species, as evidenced by β-carotene content values observed during heating. It can be concluded that the influence of fish lipids, and particularly their oxidation products on changes of β-carotene during heating depends not only on the content and composition of the fatty acids, but also on natural presence of anti- and prooxidants, predetermined genetically. Fish muscle tissue contains only one important oxidation inhibitor representing the group of tocopherols—α-tocopherol. According to published records, the muscle tissue of righteye flounders (Pleuronectidae) contains 0.4 mg·100 g^{-1} of α-tocopherol and that of lefteye flounders (Bothidae)—104 mg·100 g^{-1} of this compound (Wheaton and Lawson 1985). The muscles of Atlantic herring contain from 1.7 to 2.1 mg·100 g^{-1} of this inhibitor (Engelhardt et al. 1975). α-tocopherol has a potential to stabilise lipids, including carotenoids during storage (Wasson et al. 1991). During frozen storage, however, α-tocopherol disintegrates (Engelhardt et al. 1975), which may affect the dynamics of lipid oxidation and the content of oxidation products during storage. This may affect the activity of antioxidants added to lipids, during their heating. According to Witas and Wędzińska (1989) β-carotene may also exert antioxidative action on lipid components such as squalene within temperatures ranging from –5 to 5°C. On the other hand β-carotene may promote oxidation at 37°C even in the presence of antioxidants. During heating, lipids may undergo non-enzymatic browning reactions in the presence of phospholipids. Such reactions occur within lipid phase and they produce melanoids of antioxidative properties (Gogolewski et al. 1988). Creation of such compounds may be aided by a generally low level in fishes of α-tocopherol—well known as a phospholipid samples (Bandarra et al. 1999). Food storage may be also affected by naturally occurring Maillard reactions, observed also in non-lipid components and producing a group of compounds of antioxidative properties (Smith and Alfawaz 1995). For the reasons mentioned above, the presently observed lowering of antioxidant activity of the used plant extracts and synthetic antioxidants to the lipids of frozen fishes was not correlated to the time of their storage at –25°C and the time of their heating at 50°C.

The above suggests that frozen storage and heating of fish raw materials is associated with complex processes of lipid transformation. The present study revealed

that despite quantitative differences in the composition of the fatty acids of lipids in the fish studied, the employed extracts of rosemary and sage demonstrated antioxidant activity comparable to those of BHA and Endox. Based on this conclusion, aqueous extracts of those plants may be recommended for use, to actively inhibit oxidation processes of unsaturated fatty acids of Baltic sprat, herring, and flounder, intended for frozen storage and processing.

CONCLUSSIONS

The analysed fish lipids, without antioxidants, caused the most extensive changes of β-carotene during heating. β-carotene was the most stable, however, in lipids of Baltic herring and the least stable—in lipids of flounder.

In the majority of lipids analysed, the prolongation of heating from 10 to 60 minutes resulted in increased losses of β-carotene and a logarithmic dynamics of those changes.

The most effective inhibitors of β-carotene decomposition were the mixture of rosemary and sage extracts, sage extracts, followed by BHA and Endox. The least effective was rosemary extract used alone.

The used synthetic antioxidants and plant extracts were the most effective in stabilising β-carotene in the lipids of Baltic herring and they were least effective in lipids of Baltic sprat.

The activity of antioxidants in fish lipids was adversely correlated with the increased content of polyunsaturated fatty acids: $C_{20:5}$, $C_{22:5}$, and $C_{22:6}$.

Six-month-long fish storage at –25°C reduced antioxidant activity of the studied antioxidants and plant-origin extracts during heating in relation to β-carotene in lipids of Baltic herring and flounder. In the lipids of sprat, however, the substances used continued their effective protection of β-carotene during heating.

The most effective antioxidative protection of β-carotene in lipids of frozen sprat and flounder subjected to heating was exerted by Endox and in Baltic herring—by rosemary.

The antioxidants best protecting β-carotene in the lipids of all fishes studied (arranged in decreasing order of their activity) were: sage extract > Endox > BHA > mixture of rosemary and sage extracts > rosemary.

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