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Fish technology

**LIPOXYGENASE ACTIVITY OF SELECTED TISSUES AND ORGANS
OF BALTIC HERRING**

**AKTYWNOŚĆ LIPOOKSYGENAZY WYBRANYCH TKANEK
I NARZĄDÓW ŚLEDZI BAŁTYCKICH**

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Activity of lipoxygenase of the muscle tissue, skin, gills, fins, and gonads of Baltic herring (*Clupea harengus* L.) was analysed. Effect of gonad maturity (the fishing season) on the activity of this enzyme present in the muscle tissue was demonstrated. The substrate specificity of the enzyme was also determined.

INTRODUCTION

Freezing storage of fishes is associated with oxidation processes of their lipids, which may be either chemical and/or enzymatic. Among the enzymes catalysing oxidation reactions of lipids an important role is played by lipoxygenases. Those enzymes demonstrate variable activity dependant mostly on the fish species and on the kind of tissues and organs. Therefore their role in the lipid changes of fish raw materials during frozen storage can be also dependant on the advancement and rigorousness of their mechanical processing.

The molecular weight of fish lipoxygenases ranges from 65 to 114 kDa (Borhan et al. 1984; Ushio et al. 1989; German and Creveling 1990; Boyington et al. 1993) and it may be associated with diverse sensitivity of those enzymes to chemical and physical factors. They show high stereospecificity, substrate specificity, and position specificity (Kuhn et al. 1986; Mohri et al. 1992; Hultin 1994). In view of the above, the activity of lipoxygenases, in the biological cycle of a given fish species, can change also because of the differences in the qualitative and quantitative composition of the substrate. Harris and Tall (1994), analysing substrate specificity of lipoxygenase of the muscle tissue of mackerel, demonstrated a high preference of the enzyme towards linoleic acid (18:2), docosahexaenoic acid (22:6), arachidonic acid (20:4), and 1,3-diacyl-glycerol of linolenic acid. Substantially lower activity

of lipoxygenase was stated in the presence of triacyl-glycerols and linolenic acid (18:3) and docosapentaenoic acid (20:5). Neither of the two families of polyunsaturated acids, i.e. n-3 and n-6 appeared to be particularly preferred because similar substrate activity was observed for both α -linolenic acid (n-3) and γ -linolenic acid (n-6). A minimal effectiveness of the enzyme in the presence of triacyl-glycerol of linolenic acid, 1,2-diacyl-*sn*-glycero-3-phosphocholine (lecithin), and oleic acid was observed.

NADH (nicotinamide adenine dinucleotide), NADPH (nicotinamide adenine dinucleotide phosphate), and ATP (adenosine triphosphate) play crucial role in processes of enzymatic oxidation of fish lipids (Hochstein and Emster 1963; Slabyj and Hultin 1984; Hultin 1988, 1992; Decker and Hultin 1990). Fish lipoxygenases were more effective in the presence of NADH and NADPH than they were without them, but NADH exhibited a higher catalytic effect than NADPH (McDonald et al. 1979; Hultin et al. 1982; McDonald and Hultin 1987; Decker and Hultin 1992; Huang et al. 1998). Depending on the concentration, NADH can catalyse or inhibit processes of enzymatic oxidation of fish lipids. In the concentration exceeding 10.0 μM of NADH a decrease of lipoxygenase activity of the muscle tissue of Atlantic herring and a catfish was observed (Slabyj and Hultin 1982; Eun et al. 1992). On the other hand, the enzyme extracted from the muscles of cod and a flatfish was inhibited by NADP in concentrations above 0.1 mM (McDonald et al. 1979; McDonald and Hultin 1987).

The action of NADH is associated with presence and the concentration of iron ions (Fe^{+2}) and ATP or ADP (Slabyj and Hultin 1982; Hultin et al. 1982). ATP and products of its decomposition applied in the concentration of 0.1 mM catalysed the enzymatic system of catfish in variable levels (Eun et al. 1992). ATP turned out to be the most effective was, while AMP was the least effective.

Catalytic activity of fish lipoxygenase is primarily affected by the presence of iron ions (Hultin et al. 1982, 1988; Decker and Hultin 1990, 1992; Eun et al. 1991, 1993). The study of Hultin (1977) demonstrated that iron ions are present in the muscle tissue of fishes in sufficiently high concentrations to catalyse lipid oxidation. Aleško-Ožerskij et al. (1986) determined that the iron content in the muscle tissue of clupeid fishes was 1.34 mg/100 g. The muscle tissue of cod contained between 3.1 and 9.8 $\mu\text{g/g}$ of tissue (Falandysz and Ośmiałowski 1990), while that of flatfishes 0.03 $\mu\text{g/g}$ of the tissue (Decker et al. 1989).

Decker and Hultin (1990) observed a directly proportional relation between the concentration of iron ions and the number of compounds reacting with TBA (2-thiobarbituric acid) produced in the processes of lipid oxidation catalysed by the enzymatic system of the muscle tissue of mackerel. Ferric chloride (FeCl_3) along with its concentration increase (up to 0.06 mM) caused increase of lipoxygenase of white and red muscles of Atlantic herring (Slabyj and Hultin 1982). According to Hultin et al. (1982) the Fe^{+2} form exhibited

two times higher effectiveness in catalysing lipid oxidation, than the Fe^{+3} form under the same concentrations (up to 0.15 mM). The documented importance of iron ions in activation of fish lipoxygenase proves the importance—of bleeding and rinsing the fish before storage—in lowering the content of iron ions catalysing peroxidative activity of enzymes.

The studies completed so far, analysed lipoxygenase activity in a number of fish species. No information, however, is available on the presence and activity of lipoxygenase of the Baltic herring. This species constitutes an important share of the catches and it is a basic raw material for the production of salted products, marinates, and others, based on the fresh and frozen fish. Taking into account that the quality of Baltic herring during storage is limited, in wide extent, by oxidative changes of lipids—it is very likely that such changes take place also under influence of enzymes. In view of the above the present study was initiated aimed at identification of enzymatic oxidation of lipids in selected tissues and organs of Baltic herring, determination of the substrate specificity, and the effect of fishing season on activity of lipoxygenase of the muscle tissue of this fish species.

MATERIAL AND METHODS

Material

The present study was based on Baltic herring caught on Baltic fishing grounds off Kołobrzeg from February 1998 to February 1999. The herring collected represented the spring-spawning stock at 4th (February 1998, February 1999), 5th (April 1998) and 6th (November 1998) stages of gonad maturity in the Maier's scale. The herring were sampled for the experiment after fading of their rigor mortis. The following tissues were sampled from herring: muscle tissue, eggs, milt, skin, fins, and gills. They were grind in a meat grinder using a 2.5 mm plate and prepared for the enzyme extraction.

The substrate specificity of lipoxygenase and the entire muscle tissue of herring (white and red jointly, natively formed) was determined in the respect of the following lipids: oleic-, linoleic-, α -linolenic-, and docosenoic acids; triacyl-glycerol of oleic acid (Fluca AG), 1,2-diacyl-*sn*-glycero-3-phosphocholine (lecithin from eggs—Merck), squalene (Janssen Chimica), and also lipids extracted from the muscle tissue of the analysed fish.

Methods

Lipids from the dissected and grind tissues and organs of herring were extracted with a chloroform-methanol mixture (2 : 1) (Linko 1967). The lipid content was determined by a weight method following the distilling of the solvents and drying the remaining matter at 80°C within 1 h, expressing the units in g/100 g.

Lipoxygenase was extracted with phosphate buffer (10.0 mM, pH 7.0) according to modified method of Harris and Tall (1994).

The activity of lipoxygenase was determined through incubation at 25°C in the following arrangement: 2.0 cm³ of enzyme extract from 0.4 cm³ of hydro-lipid emulsion (25.0 mg of lipids in 1.0 cm³ of emulsion), 2.6 cm³ of phosphate buffer (10.0 mM, pH 7.0), and FeCl₃ (0.015 mM). The determination proceeded according to modified methods of Harris and Tall (1994) and Slabyj and Hultin (1984). The enzyme activity was determined based on the malondialdehyde (AM) content after 24-h incubation of the mixture. Malondialdehyde was determined using 2-thiobarbituric acid (Merck) following the method of Schmedes and Hølmer (1989). The malondialdehyde content was determined from a standardisation curve prepared for 1,1-3,3 tetraethoxypropane and the results were expressed in nmol AM/mg of protein.

The protein content in extracts of the enzyme was determined spectrophotometrically at the wavelength of 280 nm. The protein concentration was read from a standardisation curve, prepared for bovine albumin and the results were expressed in mg of protein/cm³.

All analyses were done in triplicate. The results were treated statistically using standard deviation.

RESULTS AND DISCUSSION

Table 1

Lipoxygenase activity (nmolAM/mg of protein) of selected tissues and organs of Baltic herring

Item	Enzyme activity
Fins (all)	95.50 ±0.00
Muscle tissue (light and dark muscles in natural arrangement)	70.00 ±0.05
Roe	67.32 ±0.00
Skin	57.12 ±0.00
Milt	53.92 ±0.00
Gills	33.69 ±0.00

Lipoxygenase extracted from the analysed tissues, morphological structures, and the organs of Baltic herring caught in April 1998 (first batch) exhibited variable activity in the processes of oxidation of lipids separated from the muscle tissue of those fish (Tab. 1). The highest activity was recorded for the lipoxygenase of fins, which exceeded activity of this enzyme from the muscle tissue by 36.4 percentage points. The enzyme from gills shown the lowest activity, which was 48% of that stated for

the muscle tissue (Tab. 1, Fig. 1). The activity of the enzyme from the hard roe was similar to that of the muscle tissue. An interesting feature was higher activity of the enzyme of the roe than that of the milt. The activity of this enzyme from the herring skin was almost by 20 percentage points lower than that of the muscle tissue (Tab. 1, Fig. 1). The other authors (Hsieh et al. 1992 a, b) studying the activity of lipoxygenase of shad, trout, catfish, salmon, perch, and *Plecoglossus altivelis* observed a different relation. The activity of the enzyme

Fig. 1. Lipoxygenase activity (%) of individual tissues and herring organs in relation to the activity of the muscle tissue

Table 2

Lipoxygenase activity (nmol AM/mg of protein) of the muscle tissue of herring in relation to selected substrates

Substrate	Enzyme activity
α -linolenic acid	102.42 $\pm$ 0.00
Lipids of muscle tissue of Baltic herring	47.21 $\pm$ 0.00
Linoleic acid	18.79 $\pm$ 0.00
Squalene	15.74 $\pm$ 0.05
Oleic acid	14.92 $\pm$ 0.06
Docosenoic acid	13.75 $\pm$ 0.00
Triacyl-glycerol of oleic acid	12.92 $\pm$ 0.04
1,2-diacyl- <i>sn</i> -glycero-3-phosphocholine (lecithin)	11.74 $\pm$ 0.00

Table 3

Effect of the fishing season of the Baltic herring on lipoxygenase activity (nmol AM/mg of protein) of the muscle tissue

Fishing season	Gonad maturity (Maier's scale)	Lipoxygenase activity
Feb 1998	IV	124.80 $\pm$ 0.00
Feb 1999	IV	102.20 $\pm$ 0.00
Apr 1998		
Batch 1	V	70.00 $\pm$ 0.05
Batch 2	V	57.20 $\pm$ 0.03
Nov 1998	VI	89.00 $\pm$ 0.04
Average		88.64 $\pm$ 26.58

from the gills was generally higher than that of the skin. No activity of enzymes oxidising lipids was observed in the muscle tissue of the fish species listed above.

The study of Wang et al. (1991) proved that the enzymatic activity of the Atlantic herring skin was lower than that of the white- and red muscles. It was a similar tendency as that formulated in the present study on Baltic herring. It seems that the catalysing effectiveness of oxidative transformations by enzymatic system is determined, most of all, by genetic features and selective dividing, differentiating individual body parts of a given species in the respect of chemical composition and enzymatic activity

The analysis of the substrate specificity of lipoxygenase of the muscle tissue of herring was carried out on the fish caught in February 1999 (Tab. 3), after 3 weeks of frozen storage at -25°C . The present study revealed that the most preferred substrate for lipoxygenase was α -linolenic acid. The enzyme shown the activity which was five times lower in the presence of linoleic acid and almost seven times lower activity while exposed to oleic acid (Tab. 2). The enzyme slightly more actively oxidised oleic acid in the

free form, than it did in the estrified form. The lipoxygenase exhibited the lowest specificity towards 1,2-diacyl-*sn*-glycero-3-phosphocholine and it was only 11.4 percentage points of the activity stated for linolenic acid (Tab. 2). It was interesting that the enzyme oxidised also squalene at a similar rate as it oxidised oleic acid.

Based on the obtained results (Tab. 2) it can be concluded, that lipoxygenase of the muscle tissue of herring prefers fatty acids of more than 2 double bonds. Because the content of the fatty acids of 3 and more double bonds in the analysed lipids of the muscle tissue was much lower than the content of mono-unsaturated acids (Tab. 4), it was probably the reason of substantially lower activity of this enzyme in the presence of lipids extracted from the muscle tissue of herring, than in the presence of α -linolenic acid.

Table 4

Qualitative and quantitative composition of the fatty acids (%) in the lipids extracted from the muscle tissue of Baltic herring collected during different fishing seasons

Degree of unsaturation*	Acid	Fishing season			
		Feb '98	Apr '98	Nov '98	Feb '99
C 14:0	Myristic acid	5.12	7.22	5.86	6.18
C 14:1	Myristoleic acid	0.59	0.51	0.47	0.51
C 16:0	Palmitic acid	18.25	12.37	15.84	15.63
C 16:1	Palmitoleic acid	6.70	3.80	6.87	6.84
C 17:0	Margaric acid	1.47	1.19	1.32	1.40
C 18:0	Stearic acid	1.85	1.42	1.87	1.58
C 18:1	Oleic acid	21.84	12.21	17.83	13.74
C 18:2	Linoleic acid	2.98	2.00	2.30	2.68
C 18:3	α-Linolenic acid	2.15	1.02	1.30	1.53
C 18:3	γ-Linolenic acid	0.32	0.33	0.32	0.33
C 18:4	Octadecatetraenoic acid	2.04	1.22	1.93	2.05
C 20:1	Eicosenoic acid	3.40	15.00	9.19	9.39
C 20:2	Eicosadinoic acid	0.57	0.46	0.45	0.50
C 20:3	Eicosatrienoic acid	0.22	0.42	0.27	0.27
C 20:4	Eicosatetraenoic acid	0.90	0.52	0.74	0.53
C 20:5	Eicosapentaenoic acid	0.15	0.00	0.00	0.00
C 22:1	Docosenoic acid	12.75	30.19	21.95	24.58
C 22:5	Docosapentaenoic acid	1.51	1.53	1.32	1.64
C 22:6	Docosahexaenoic acid	17.18	8.60	10.17	10.60

*number of carbon atoms : number of double bonds

Linolenic acid constituted a preferred substrate also for the enzyme extracted from the skin of sardine (Mohr et al. 1990, 1992) and the gills of rainbow trout (Stone and Kinsella 1989). On the other hand, the activity of the enzymatic system of the skin of smelt, and ayu (*Plecoglossus altivelis*) in the presence of linolenic acid was minimal (Zhang et al. 1992a, b). Lipoxygenase of the muscle tissue of Baltic herring analysed in the present study

showed lower effectiveness in oxidative processes of the natural substrate (lipids extracted from the muscles), than in the presence of linolenic acid alone. Harris and Tall (1994) observed a similar relation in the case of the enzyme of the muscle tissue of mackerel.

The present work demonstrated low substrate specificity of lipoxygenase of the muscle tissue of Baltic herring in relation to the remaining analysed compounds, in this number also linoleic acid. The latter was no particularly desirable substrate also for the enzymatic system of the skin of smelt and *Plecoglossus altivelis* and the gills of rainbow trout (Hsieh et al. 1988; Zhang et al. 1992a, b). On the other hand a high activity of the enzyme from the skin of sardine, gills of menhaden and the muscle tissue of mackerel in the presence of this acid was observed (Mohri et al. 1990, 1992; Harris and Tall 1994; Grün and Barbeau 1995; Grün et al. 1996). In general, linoleic acid has been considered as a preferred substrate of lipoxygenases of plant-origin raw materials (Hatanaga 1989).

The present study revealed a low activity of the enzyme extracted from the muscles of Baltic herring in the presence of oleic acid. This regularity was visible also in the case of lipoxygenase of the muscle tissue of mackerel (Harris and Tall 1994).

Lipoxygenases have also certain ability to catalyse oxidation of neutral lipid substrates, such as for example esters of fatty acids. Mohr et al. (1992) observed high activity of the enzyme extracted from the skin of sardine during oxidation of esters of linoleic acid. It was comparable with the activity of this enzyme in the presence of free fatty acids. This feature, however, is typical for plant-origin lipoxygenases (Christopher et al. 1970; Satoh et al. 1976). Lipoxygenase of the muscle tissue of Baltic herring, as demonstrated by the present study, more actively oxidised free oleic acid, than it did in estrified form.

The present study revealed variable activity of lipoxygenase of the muscle tissue of Baltic herring caught in different seasons (Tab. 3). It is evident from the obtained results that lipoxygenase of the muscle tissue of Baltic herring fished in February of two consecutive years varied in their activity by 19 percentage points. The muscle tissue of herring at 4th stage of gonad maturity peroxidative enzymes have higher activity than they had at 5th and 6th stage. Reasons for such activity of lipoxygenase can be attributed to more active feeding of the fish at 4th stage of gonad maturity, than the fish caught just before their spawning period. The herring collected for the study in April had twice demonstrated differences in activity of the enzyme, which indicates discrepancies in the values determined. They can be also attributed to individual differences between different specimens of fish.

Seasonal variability can be also noted in the qualitative and quantitative composition of the fatty acids and lipids of the muscle tissue of herring (Tab. 4). It was possibly important for lipoxygenase, because the lipids extracted from the muscle tissue of fish constituted the substrate of the enzyme.

Lipids of the muscle tissue of herring caught in February 1998, compared with the lipids of the fish in the remaining fishing seasons contained more polyunsaturated fatty acids of long carbon chain C18 (18:2, 18:3, 18:4) from 6.59 to 7.49%; C20 (20:2, 20:4, 20:5—which was not stated in the lipids from the remaining fishing seasons) from 1.30 to 1.84%; C22 (22:5, 22:6) from 12.24 to 18.69% (Tab. 4). Lipids of the muscle tissue of herring caught in February 1998 and 1999 were characterised by the highest content of linolenic acid (α and γ jointly), which was 2.47 and 1.86% respectively (Tab. 4). As demonstrated by the present study the lipoxigenase of the muscle tissue of Baltic herring is highly specific towards linolenic acid (Tab. 2) which can be partly attributed to higher activity of this enzyme of the analysed fishing season.

The lowest enzymatic activity was stated in the muscle tissue of herring in the April catches of 1998 (Tab. 3). The lipids of the muscle tissue of those fish, compared to those of the remaining fishing seasons contained the lowest number of polyunsaturated fatty acids of the carbon chain length of C18 (18:2, 18:3, 18:4) 4.56%; of C20 (20:4) 1.40%; and of C22 (22:6) 10.13% (Tab. 4).

Lipoxigenase activity in the muscle tissue of herring caught in November 1998 was on a level approximating the mean values of the analysed fishing periods.

No information on the effect of the fishing period and the gonad's maturity on the activity of the enzymatic system oxidising lipids had been found in the available literature. It can be concluded, based on the present study, that the importance of the lipoxigenase of the muscle tissue of the Baltic herring diminishes as the spawning season approaches.

CONCLUSIONS

1. Lipoxigenase activity of the analysed tissues and organs of Baltic herring was variable. The respective organs were arranged a decreasing order in the respect of this enzyme's activity: fins, muscle tissue, roe, skin, milt, gills.
2. Substrate activity of the lipoxigenase of the muscle tissue of herring was the highest in the presence of α -linolenic acid, while the lowest—in the presence of lecithin. The respective compounds were arranged in a decreasing order in the respect of the activity: α -linolenic acid, squalene, oleic acid, docosenoic acid, triacyl-glycerol of oleic acid, lecithin.
3. Activity of lipoxigenase was the highest in the muscle tissue of herring caught in February (4th stage of gonad maturity) while the lowest—in April (5th stage of gonad maturity in the Maier's scale)

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AKTYWNOŚĆ LIPOKSYGENAZY WYBRANYCH TKANEK I NARZĄDÓW ŚLEDZI BAŁTYCKICH

STRESZCZENIE

Analizowano aktywność lipoksygenazy tkanki mięśniowej, skóry, skrzel, płetw oraz gonad śledzi bałtyckich (*Clupea harengus* L.). Określono wpływ okresu połowu ryb na aktywność tego enzymu zawartego w tkance mięśniowej, a także oznaczono jego specyficzność substratową.

Aktywność lipoksygenazy analizowanych tkanek i narządów śledzi układała się następująco w kierunku zmniejszających się wartości: płetwy, tkanka mięśniowa, ikra, skóra, mlecz, skrzel. Specyficzność substratowa lipoksygenazy tkanki mięśniowej śledzi była największa w środowisku kwasu α -linolenowego i obniżała się w następującej kolejności: lipidy tkanki mięśniowej śledzi, kwas linolowy, skwalen, kwas oleinowy, kwas dekozanowy (C22:1), triacyloglicerol kwasu oleinowego, lecytyna. Aktywność lipoksygenazy była największa w tkance mięśniowej śledzi złowionych w lutym (IV stadium dojrzałości gonad), a najmniejsza w kwietniu (V stadium dojrzałości gonad według skali Maiera).

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