

EFFECT OF FROZEN STORAGE ON FAT SOLUBLE VITAMINS CONTENT IN FISH FILLETS

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ABSTRACT

Fat-soluble vitamins content (all-trans-retinol, alpha-tocopherol and cholecalciferol) in edible tissue of Bluefish (*Pomatomus saltatrix*), a typical Black sea pelagic fish, and in Rainbow trout (*Oncorhynchus mykiss*), a typical farmed freshwater fish, were determined and compared on raw state and after frozen storage.

The sample preparation procedure includes saponification and consequent extraction of fat-soluble vitamins with n-hexane. The extract was dried under nitrogen flow and redissolved in methanol. HPLC analysis of methanolic samples was performed on ODS2 Hypersil (250x4,6, 5um) column with a mobile phase of methanol:water = 97:3. The quantification of fat-soluble vitamins was by the method of standard addition. Retinol and cholecalciferol were monitored by UV detection and alpha-tocopherol was detected by fluorescence.

The retinol and cholecalciferol contents in fresh edible tissue of Black sea Bluefish ($38.5 \pm 2.4 \mu\text{g} \cdot 100\text{g}^{-1}\text{ww}$ and $11.2 \pm 1.2 \mu\text{g} \cdot 100\text{g}^{-1}\text{ww}$, respectively) were close to values in the freshwater fish Rainbow trout ($58.9 \pm 2.6 \mu\text{g} \cdot 100\text{g}^{-1}\text{ww}$ and $14.9 \pm 1.1 \mu\text{g} \cdot 100\text{g}^{-1}\text{ww}$, respectively). Alpha-tocopherol content was several fold higher in Rainbow trout ($1648.9 \pm 68.8 \mu\text{g} \cdot 100\text{g}^{-1}\text{ww}$) than in Black sea Bluefish ($427.1 \pm 37.1 \mu\text{g} \cdot 100\text{g}^{-1}\text{ww}$).

Long period of storage affected mostly retinol and alpha-tocopherol contents in two fish species. While cholecalciferol content remained almost unchanged.

Key words: Retinol, Alpha-Tocopherol, Cholecalciferol, Frozen storage, Fish fillet

INTRODUCTION

Fish tissue fats are rich source of fat soluble vitamins and both saturated and unsaturated fatty acids. Fat soluble vitamins are essential nutrients controlling a diversity of biologically important processes in human body. All-trans-retinol (vitamin A) is a fat-soluble unsaturated isoprenoid necessary for growth, differentiation and maintenance of epithelial tissues, and also for reproduction. It takes place in photore-

ception and regulates gene expression and cell division, bone growth, teeth development etc (10). Cholecalciferol (vitamin D₃) plays crucial role for the development, growth, and maintenance of a healthy skeleton from birth until death. Its major function is to maintain calcium homeostasis (6). Alpha-tocopherol (vitamin E) is an important antioxidant which protects against lipid peroxidation (which could contribute to cell membrane weakness), and essential fatty acids and vitamins A from oxidation (2).

Moreover the fat soluble vitamins are considered to be especially susceptible to oxidation. Freezing is one of the easiest and most preferred method of preserving foods. Frozen foods retain their original flavor, color and more of their nutrients. But their final quality is highly dependent on primary condition of the food and duration of freezing. Also the

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storage condition and continuance may influence the amounts of nutrients as fat soluble vitamins (1, 5).

The distinctive flavour of Rainbow trout (*Oncorhynchus mykiss*) and Bluefish (*Pomatomus saltatrix*) characterize them as favourite dishes in the restaurant. The bluefish is Black sea fish. The skin coloration is a grey-blue-green on the upper sides and lightens to white on the lower sides. They are extremely aggressive fishes. Bluefish species are cannibalistic, they are preyed upon at all stages of their life cycle. Edible tissue of bluefish allows preparation in a variety of culinary options (7).

The Rainbow trout is one of the most widely farmed fishes in our country. This fish inhabits cold and clear freshwater ponds. Rainbow trout is a predator with a varied diet - eats almost anything. Trout is the preferred fish for breeding and consumption because of its rapid growth and rich and diverse composition of the meat (7).

The aim of the present study was to evaluate the effect of long-term freezing storage on fat soluble vitamins content in Rainbow trout and Bluefish fillets.

MATERIAL AND METHODS

Samples collection and preparation

Samples of rainbow trout (caught from fish farm, Plovdiv region) and bluefish were purchased from Varna fish market during autumn 2010. All fishes were spitted into two groups – immediately analysed and immediately frozen (fillets with skin) at -20 °C and stored in a home fridge for 1, 3 and 6 months. Six specimens of each fish were used as a material for vitamin analysis. Prior to analysis the head, tail, fins, and viscera of the fish were removed. The edible tissue was filleted with the skin and the samples were homogenized using kitchen homogenizer at 800 rpm for 3 min and analysed.

Extraction of fat soluble vitamins and HPLC analysis

The sample preparation was performed using the method of Dobрева et al. (3). An aliquot of the homogenized sample (1,000±0.005 g) was weighed into a glass tube with a screw cap and 1% of methanolic L-ascorbic acid and 1M methanolic potassium hydroxide were added. Six parallel samples of fish edible tissue were prepared and

subjected to saponification at 80°C for 20 min. The components of interest were extracted with n-hexane and the extract was evaporated under nitrogen. The dry residue was dissolved in MeOH and injected (20µl) into the liquid chromatography system.

The reversed phase high performance liquid chromatography (HPLC) was used for the vitamin analysis. Three fat soluble vitamins were analysed simultaneously using HPLC system (Thermo Scientific Spectra SYSTEM) equipped with analytical column ODS2 Hypersil™ 250x 4,6mm, 5u. All-trans retinol and cholecalciferol were detected by UV, alpha-tocopherol by fluorescence detection. The mobile phase was 97:3 = MeOH:H₂O, flow rate 1ml/min. The qualitative analysis was performed by comparing the retention times of pure substances: at $\lambda_{\max} = 325\text{nm}$ for retinol; $\lambda_{\max} = 265\text{nm}$ for cholecalciferol and alpha-TP fluorescence at $\lambda_{\text{ex}} = 288\text{nm}$ and $\lambda_{\text{em}} = 332\text{nm}$. The quantitation was done by the method of external calibration comparing the chromatographic peak areas of the corresponding standards (Retinol solution, Fluka; DL-alpha Tocopherol, Supelco; Cholecalciferol, Supelco). The recovery rates were calculated utilizing the external standard method. For the determination of the analytical recoveries, samples of homogenized fish tissue were spiked with a methanolic solution containing known amounts of three fat soluble vitamins. The results were expressed as µg per 100 g wet weight (µg.100g⁻¹ww).

Statistical analysis

The data were analysed using Graph Pad Prism 5 software. The results were presented as means and standard deviations. One-way ANOVA (nonparametric test) statistical analysis was employed for the calculation of differences between diverse periods of frozen storage (significant at p<0.05).

RESULTS AND DISCUSSION

Fat soluble vitamins content in Bluefish and Rainbow trout were analysed in two different states - fresh and frozen. The results obtained for retinol, cholecalciferol and alpha-tocopherol amounts in samples were separated in 4 groups (raw, 1 month, 3 months and 6 months frozen).

Both fish species are of commercial importance for many countries. Surprisingly, the data concerning bluefish's vitamin content in the scientific literature

is very scarce. Rainbow trout and Bluefish are characterized with close amounts for vitamins A (58.9 ± 2.6 and 38.5 ± 2.4 $\mu\text{g} \cdot 100\text{g}^{-1}\text{ww}$, respectively) and vitamin D₃ (14.9 ± 1.1 and 11.2 ± 1.2 $\mu\text{g} \cdot 100\text{g}^{-1}\text{ww}$, respectively). In contrast vitamin E content in raw trout (1648.9 ± 68.8 $\mu\text{g} \cdot 100\text{g}^{-1}\text{ww}$) is about four times higher. In our study significant differences ($p < 0.05$) in retinol and alpha-tocopherol contents between fish species were established.

Figure 1 shows retinol changes in two fish fillets due to the different duration of freezing storage. Our results for retinol content in trout fillets are in the same order with those presented by other authors – Szlinder-Richert et al. (43.1 $\mu\text{g} \cdot 100\text{g}^{-1}\text{ww}$) and Kuhnlein et al. (61.0 $\mu\text{g} \cdot 100\text{g}^{-1}\text{ww}$) (8, 11). Apparent is the tendency to increase retinol losses with increasing storage period. The most significant are differences in vitamin A contents between raw and 6 months stored fillets – 58% for rainbow trout and 40% for bluefish.

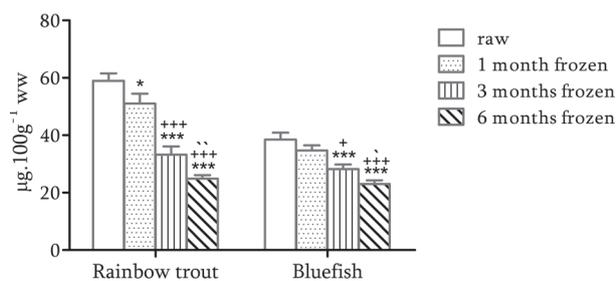


Fig. 1. Vitamin A changes during frozen storage on fish fillets, $\mu\text{g} \cdot 100\text{g}^{-1}\text{ww}$, (mean \pm SD)

*** $p < 0.001$ vs raw; * $p < 0.05$ vs raw; +++ $p < 0.001$ vs 1 months frozen; + $p < 0.05$ vs 1 months frozen; ```` $p < 0.01$ vs 3 months frozen; ` $p < 0.05$ vs 3 months frozen

Similar time-dependent decrease in both fish species depending on continuance of storage was observed for vitamin E content (fig. 2). Szlinder-Richert et al. also presented data for vitamin E in raw rainbow trout – 930.0 $\mu\text{g} \cdot 100\text{g}^{-1}\text{ww}$ (11). The quantity is similar to our result.

The vitamin E quantities in both fish samples decreased most significantly after six months fridge storage. Alpha-tocopherol was reduced to a greater degree, compared with vitamin A – 53% for trout fillets and 67% for bluefish fillets. Differences in the be-

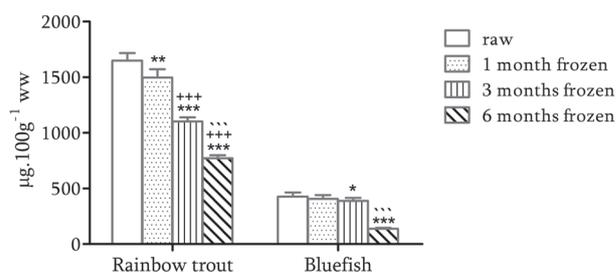


Fig. 2 Vitamin E changes during frozen storage on fish fillets, $\mu\text{g} \cdot 100\text{g}^{-1}\text{ww}$, (mean \pm SD)

*** $p < 0.001$ vs raw; ** $p < 0.01$ vs raw; * $p < 0.05$ vs raw; +++ $p < 0.001$ vs 1 months frozen; ```` $p < 0.001$ vs 3 months frozen

haviour of both vitamins were noticeable only in the changes in the vitamin E content in bluefish tissue – our results showed no significant differences in tocopherol amount at the third month of storage.

The observed changes in the contents of the vitamins A and E are explained by the high content of hydroperoxides in frozen fish tissue (4). They are stable at the low temperature and oxidize the vitamins.

Rainbow trout and Bluefish are amongst the fishes comprising high amounts of cholecalciferol – both fish exhibit very close values. Our data for vitamin D₃ content in raw bluefish tissue (fig. 3) is in accordance with the vitamin D₃ content (7.0 $\mu\text{g} \cdot 100\text{g}^{-1}\text{ww}$) for same fish species given by Lu et al. (9). Also similar to our data for cholecalciferol amounts were presented by Szlinder-Richert et al. (8.0 $\mu\text{g} \cdot 100\text{g}^{-1}\text{ww}$) and Kuhnlein et al. (19.7 $\mu\text{g} \cdot 100\text{g}^{-1}\text{ww}$) (8, 11).

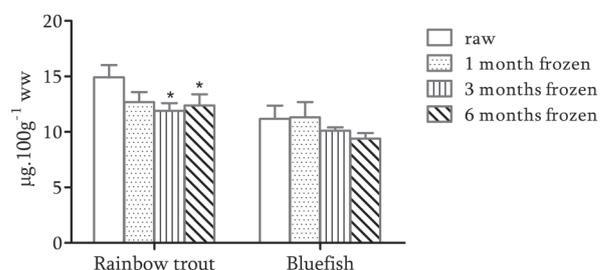


Fig. 3. Vitamin D₃ changes during frozen storage on fish fillets, $\mu\text{g} \cdot 100\text{g}^{-1}\text{ww}$, (mean \pm SD)

* $p < 0.05$ vs raw

No significant changes were detected for cholecalciferol content in bluefish samples for the whole period of storage (fig. 3). On the other hand in rainbow trout a significant ($p < 0.05$) time-dependent decrease (16%) in this vitamin was established.

Aubourg et al. investigated losses of alpha-TP content in edible tissue of horse mackerel (1). The results of their analysis are very similar to our observations. They reported significant losses of alpha-tocopherol in fish fillets, stored at -20 °C, for three months (about 27%) and for 7 months (about 40%) of storage.

CONCLUSIONS

Long-term storage results in a time dependent decrease in fat soluble vitamins content.

The most significant changes were found for retinol and alpha-tocopherol ($p < 0.001$), where the most dramatically decrease was obtained in vitamin E content (67%) in bluefish fillet during six months storage.

In contrast cholecalciferol remained almost unchanged after long-term freezing storage.

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