Antioxidant activity of freeze-dried Siberian sturgeon (*Acipenser baerii*) ovarian fluid

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Abstract. Ovarian fluid is a mass-production nutritionally valuable by-product of aquaculture of sturgeons. Total antioxidant activity of freeze-dried pasteurized and non-pasteurized ovarian fluid of Siberian sturgeon (*Acipenser baerii*) was determined to estimate its potential acceptability as an anti-inflammatory and anti-age agent in nutraceuticals. Total antioxidant activity was determined using the certified coulometric measurement method. The value of total antioxidant activity was about 18.6 ± 1.1% of that of a well-known source of antioxidants – tomato fruits, if calculated by dry weight. There was no statistically significant difference between the values of activity for pasteurized and non-pasteurized ovarian fluid, so it is reasonable to pasteurize the fluid for its preservation. **Keywords:** ovarian fluid, sturgeon, antioxidant activity, nutraceuticals.

1 Introduction

Ovarian fluid is a mass-production nutritionally valuable by-product of aquaculture of sturgeons including Siberian sturgeon (*Acipenser baerii*) [1]. Ovarian fluid contains protein, fat and little coarse connective tissue. The special importance of fat-containing fish tissues in nutrition is determined by the polyunsaturated fatty acids (PUFAs), fat-soluble vitamins (A, D, E), fat-like substances (phosphatides, sterols and steroids) contained in them. It has been shown that PUFAs have significant anti-inflammatory and antioxidant effects. PUFAs are precursors of resolvins, leukotrienes, and prostaglandins involved in the natural attenuation of the inflammatory process [2]. Clinical studies have shown that eicosapentaenoic and docosahexaenoic acids are helpful when administered orally for the prevention of cardiovascular diseases [3] and the regulation of cholesterol and lipoproteins in blood [4].

Antioxidant activity (AOA) of food products implies their ability to chemically neutralize (reduce) the so-called "reactive oxygen species" (ROS) i.e. biological oxidants formed in living cells as a result of oxygen metabolism during various stresses and inflammatory processes. ROS include the superoxide anion radical ($\bullet O^{-}_{2}$), hydrogen peroxide (H_2O_2), hydroxyl radical (•OH). For example, the AOA of fish PUFA is due to the ability to absorb these particles by double bonds of the carbon skeleton. Antioxidants are natural protective compounds and are produced, in particular, by fish to regulate internal redox processes [5].

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It is reasonable to assume that the level of oxidative stress processes and, therefore, the power of the antioxidant defense system will vary significantly in the tissues of fish of various species living in different environmental conditions, which is consistent with published data on the AOA of fish of various species [6]. For example, peptides with antioxidant properties are formed during enzymatic hydrolysis of the muscles of sardine [7], mackerel [8], white-spotted conger eel [9], and round scad [10]. It has also been described that low-molecular compounds contained in the muscle tissue of a number of fish species have buffering and antioxidant effect, for example, free histidine and dipeptides containing it (carnosine, anserine and others) [11], taurine [12, 13].

Environmental conditions also have a direct effect on the equilibrium and kinetics of antioxidant processes. Thus, for example, it has been shown that in the muscles of Antarctic fish (sluggish rockcod and marbled notothen) the AOA is significantly higher than that of habitual edible fish living in warmer waters. This is due to the greater solubility of oxygen in cold waters (correspondingly, the higher the equilibrium concentration of ROS formed during its metabolism), as well as kinetically slower processes of ROS reduction by antioxidants, which makes fish synthesize more PUFAs and other antioxidants to compensate for these effects [14].

The purpose of this work was to determine the total antioxidant activity of freeze-dried ovarian fluid of Siberian sturgeon. According to these data, it was supposed to evaluate acceptability of the material being studied as raw material for antioxidant therapeutic and prophylactic food supplies. Furthermore, since pasteurization is a cheap and widespread technique for preservation, it was necessary to assess its possible affect on the AOA of ovarian fluid.

2 Materials and Methods

Five female Siberian stureon specimens were collected at a fish farm located in Energetik, Neftekamsk city district, Republic of Bashkortostan, Russia. Fish were anesthetized and dissected, and the ovarian fluid (about 100 ml) samples were taken from ovaries. The samples were transported into the laboratory in liquid nitrogen and kept there at -80 °C till further procedures.

Next, the samples were defrosted and separated from solid tissue fragments by centrifugation at 6000 G for 15 min [15]. The supernatants were freeze-dried to 5.0 ± 0.2 % residual moisture according to the previously described procedure [16]. The residual moisture was controlled by its measuring using the Karl Fischer volumetric titration method with METTLER TOLEDO DL31 automatic titrator.

Prior to AOA determination, the samples were rehydrated by dissolution of 1 g of a sample in 100 mL of distilled water. AOA was determined using a certified coulometer Expert-006-Antioxidanty (Econix-Expert Ltd, Moscow, Russia) at a constant current intensity of 50 mA: 20 mL of a background solution (0.2 M KBr in 0.1 M H_2SO_4) was poured into the conductometric cell, the electrodes were put in and the generator was switched on; when indicational current was achieved, 2 mL of sample solution was added; the titration endpoint was recorded when the preliminary value of the potential was achieved. AOA value (in C/g) was calculated as product of current and titration time per 1 g of the sample. This method is described in details in [17, 18].

The AOA values were presented as means ± standard deviation (SD). Significance of the difference between groups of samples (pasteurized and non-pasteurized) was evaluated using Mann–Whitney *U* test; the level of significance considered was $p < 0.05$. Data analysis was performed using IBM SPSS Statistics 26 software.

3 Results and Discussion

The values of AOA of the samples are presented on Fig. 1.

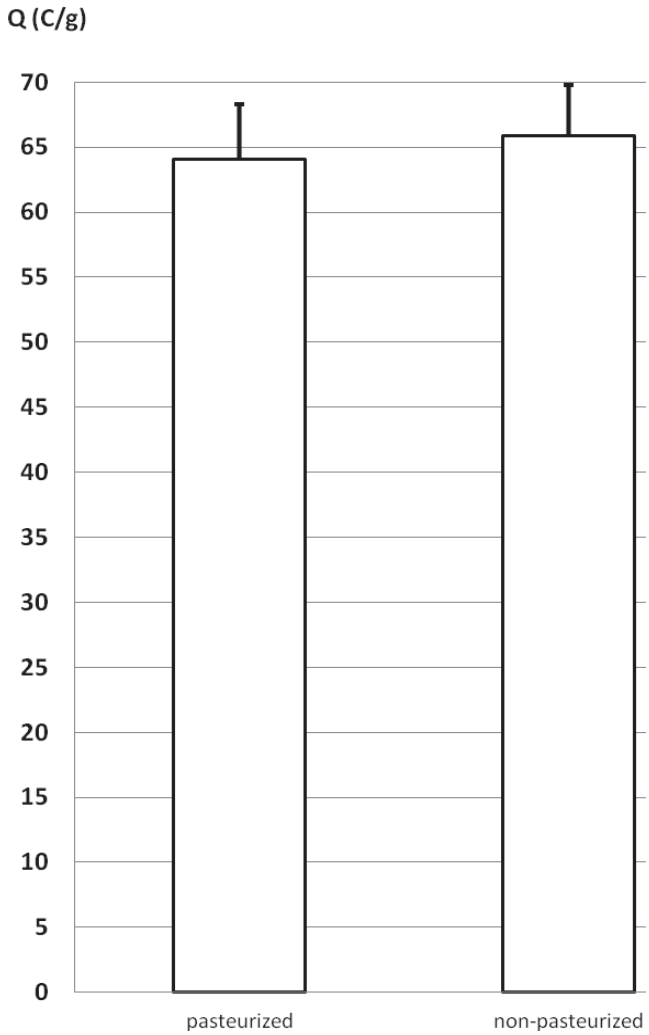

Fig. 1. Total antioxidant activity of freeze-dried pasteurized and non-pasteurized Siberian sturgeon ovarian fluid at constant 50 mA current intensity (means ± SD).

The AOA of Siberian sturgeon ovarian fluid turned out to be quite significant. This indicates a high bioactive potential of this product, which is also consistent with the previously obtained data on modeling the oxidative effect on cell culture, in which, when ovarian fluid lyophilisate was added to the medium, a decrease in the marker of stress and aging (β-galactosidase activity) without proliferation growth was observed, which indicates an improvement in the state of cells [19]. The antioxidant activity of sturgeon ovarian fluid was about 18.6 ± 1.1% of the value previously obtained by a similar method for tomato fruits, if calculated by dry weight [18]. Tomatoes are a major component of the so-called "Mediterranean diet", and their regular consumption is correlated with a reduction in mortality from cardiovascular diseases [20].

The Mann–Whitney *U* test has shown that there was no statistically significant difference between AOA of pasteurized and non-pasteurized ovarian fluid. Thus, pasteurization does not significantly affect the AOA of the ovarian fluid, so it is reasonable to increase its shelf life and partially disinfect it by pasteurization.

4 Conclusion

The freeze-dried ovarian fluid, an aquaculture by-product, may be used (both pasteurized and non-pasteurized) as an anti-inflammatory and anti-age agent in nutraceuticals. Such approach would increase profitability and decrease waste production in sturgeon aquaculture.

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