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Alternations in Quality Parameters of Rainbow Trout (*Oncorhynchus Mykiss*) Compared to Albino Golden Rainbow Trout Stored at 0 to 4°C

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Running title**: Quality Parameters of Rainbow and Albino Golden Rainbow Trout During Cold Storage**

Abstract
The purpose of this study was to compare quality parameters of rainbow trout and its hybrid albino golden rainbow trout during cold storage at 0 to 4°C. Control sample was rainbow trout, and the examinations were made on period of three days at 1st, 3rd, 6th and 9th day. Physicochemical parameters were examined. pH value during storage ranged from 6.79 in first day to 7.18 at last 9th day. pH value was in normal limits and didn't show significant differences between two groups. Percent of fat in fish show statistically significant differences and it was higher at albino rainbow trout samples. Free fatty acids (FFA) during cold storage increased about three times from the first to the last day, peroxide value (POV) increased from 0.65 in first day of examinations to 2.33 μeqO2 kg^{-1} at the end. Thiobarbituric acid reactive substances (TBARS) value was ranged from 0.12 to 0.79 MDA mg/kg at the last day. Free α-amino nitrogen (Fan) and total volatile base nitrogen (TVB-N) raised with 0.5 and 3 times respectively. The conclusion was made that albino golden rainbow trout has bigger percent of fat in the meat because of its way of life and biological factors, but important proves during cold storage were that significant differences weren't indicated in lipid oxidation and protein denaturation.

Practical applications
Examination of physicochemical parameters expresses the quality of meat. In this study the emphasis is at higher lipid content in albino rainbow trout muscles. Exclusive gold color of the skin, red tender meat, and the fact that during cold storage shelf life of normal and albino rainbow trout is equal, tells that when it comes to healthy food the albino rainbow trout is mega bargain for consumers.

Key words: cold storage, rainbow trout, albino, quality parameters, o*ncorcynchus mykiss, walbaum*

Introduction

The rainbow trout *(Oncorcynchus mykiss, Walbaum)* whose name refers to the many rainbow-coloured spots on its skin is the leading freshwater cultured species in Europe. Native to the Pacific coastal area in the United States it was introduced into Europe at the end of the 19th century. Its hardiness and fast growth quickly proved particularly well adaptive to aquaculture. Rainbow trout is farmed today in nearly all of the European countries, especially in the coastal countries with a temperate climate. It can occupy many different habitats, moving from freshwater to saltwater and back, or staying permanently in lakes. The optimum water temperature for breeding is below 21°C. Growth and maturation are influenced by water temperature and food. Under normal conditions, trout usually matures at 2.5 - 3 years (Paaver et al. 2003). They are carnivorous and need a diet rich in protein (Hardy 1996). In the right environment, a trout can grow to 350 g in 10 to 12 months and to 3 kg in two years (Austreng 1987).

Golden rainbow trout is an albino form of rainbow trout. Albinism happens due to the missing oxidation activity of the enzyme tyrosinese (Thorgaard et al., 1995). Albinism is a recessive character and albino fish constitutes very small part of the population. The colour disadvantages of albinos greatly decrease their chance to survive in the nature. However, in the farms, they have a much better chance to survive and some promising capabilities for demand on the market. Their number also can be increased through selective breeding (Dobosz et al., 2000). The golden yellow pattern of the fish and its tender meat are attractive for the customers. On the other hand, the specific biology of golden trout contributes to a different proximate composition and sensory properties of the meat compared to the normal trout.

Lipid oxidation is a major cause of quality deterioration in muscle-based foods, where flavour, colour, texture and nutritional value can be negatively affected. The presence of haem pigments and trace amounts of metallic ions make the fish, especially dark flesh fatty fish, prone to lipid oxidation.

Materials and methods

Materials

The rainbow trout was taken from the fish market store Plovdiv. Twenty four fish were bought 12 hours post mortem cooled in flake ice. The fish were brought to the laboratory of the Department of Meat and Fish Technology of the University of Food Technologies - Plovdiv, where they were divided into two groups. 12 fish of rainbow trout *(Oncorcynchus mykiss, Walbaum)* were control group and 12 fish of albino golden rainbow trout were experimental samples. Examination of the parameters was made on the first day. After that, the two groups of fish were packed in polyethylene zipper bags that were tagged and then stored in a refrigerator at 0 - 4°C. The fish samples were examined at 1st, 3rd, 6th and 9th day of the storage, and the five repetition were made independently for each parameter. The fish were on the age of 13 months and with almost identical mass that varied from 380-400 g. For the examined parameters concerning the pH value, % fat in meat, lipid oxidation and protein denaturation we used blended fish meat which contains all the muscles excluded the internal organs, skeletal system, bones, head and the fins.

Methods

Preparation of samples for chemical analysis. Average laboratory sample was prepared by homogenization of the fish meat samples. From the average laboratory sample, the quantity required has calibrated, as described in the appropriate method for research.

pH value of the samples was determined from a meat-water mixture (Korkeala et al. 1986). 10 g of the homogenized sample was put into 90 ml of distilled water and the pH value was measured with pH meter Microsyst MS 2004 (Microsyst, Plovdiv), equipped with temperature and combined pH electrode type Sensorex Combination Recorder S450 CD (Sensorex pH Electrode Station, Garden Grove, CA, USA).

% fat in 100g fish meat was made with the method of Bligh and Dyer 1959. In flask 100g of meat and 300 ml chloroform were putted, wrapped with aluminium foil and left in refrigerator for one night. Next day the content of flask was filtered and then putted on vacuum rotary evaporator and the % of lipid in 100 g meat was measured.

Free fatty acids

The acid value was measured using free fatty acids as an indication of hydrolytic rancidity of fish oil (Gheisari, 2011). FFA in the oil extracted from a fish sample were determined by titration with a solution of potassium hydroxide (KOH) with 0,1N concentration and with phenolphthalein as a colour indicator. The acid value (or free fatty acid content) was determined by AOAC method 940.28 (AOAC,

2012). The oil sample (0.2 g) was dissolved in 10 cm^3 ethanol and titrated with 0.1M NaOH solution using phenolphthalein indicator until pink colour disappeared. The acid value and the percentage fatty acid were calculated from the expression bellow:

$$\text{Acid Value} = \frac{56 \text{ molarities of NaOH} \times \text{titer value}}{\text{Weight of fish oil}}$$

Free Fatty Acid = 0.503 × Acid Value, % Oleic acid

Peroxide value

Peroxide value is one of the most widely used tests for oxidative rancidity. Peroxide value is a measure of the concentration of peroxides and hydroperoxides formed in the initial stages of lipid oxidation. Peroxide value was determined spectrophotometrically based on the oxidation of Fe (II) to Fe (III) in the presence of hydroperoxides and the formation of a coloured complex between obtained Fe (III) and thiocyanate (SCN) as described by (Schmedes and Hølmer 1989) and perfected with few modifications. The absorbance was measured by twin ray spectrophotometer UV-VIS Camspec, model M550 (Camspec Ltd, Sawston, Cambridge, United Kingdom).

TBARS

TBARS was determined using the method of (Botsoglou et al. 1994), following recommendations of (Jebelli Javan et al. 2012). A 2 g sample was transferred into a 25 mL centrofugated tube, and volumes of 5% aqueous TCA (8 cm^3), and 0.8 mL BHT in hexane (5 cm^3) were successively added. The content of the tube was Ulta-Turraxed for 30 s at high speed and centrofugated for 3 min at 3000 g, and the top hexane layer was discarded. The bottom aqueous layer was made to a 10 mL volume with a 5% TCA, and 2.5 cm^3 aliquot was pipetted into a screw-capted tube to which a volume (1.5 cm^3) of 0.8% aqueous TCA was added. Following incubation for 30 min at 70°C, the tube was cooled under tap water, and reaction mixture was submitted to third-derivative spectrophotometry against blank reaction mixture. Aliquots of standard solutions were pipetted into screw-capped tubes and diluted to 2.5 cm^3 volume with 5% TCA. A 1.5 cm^3 0.8 % TCA was added in each tube and the reaction was carried out as prescribed. Calibration curves were constructing by plotting values of peak height at 521.5 nm were measured using twin ray spectrophotometer UV-VIS Camspec, model M550 (Camspec Ltd, Sawston, Cambridge, United Kingdom), as they are printed on the instrumental chart in arbitrary units, versus known concentration of MDA in the final reaction mixtures. MDA in samples was calculated using formula:

$$\text{MDA} = \frac{16 \cdot C \cdot V}{W}, \mu g/kg, \text{ where}$$

C is the MDA concentration (ng/cm^3) in the sample extracts according to the calibration curve,
V is a dilution factor of sample extract (cm^3) if any, and
W is the weight (kg) of the sample.

Results

pH value of the fish Fig. 1, was growing over time from 6.79 at the beginning to 7.18 at the last 9th day. These values are in the upper limits for rainbow trout and showed that 9 days is limit of fresh fish stored at 0 - 4°C. **% fat in 100g** fish meat showed statistically significant differences between control and experimental samples Fig. 2. Golden rainbow trout have significant higher percents of fat and they reach to 4.51g fats in 100g fish meat. Values of examined parameters that refers to lipid oxidation are showed in Tab. 1. **FFA** of the examined samples was going up to 0.31 % oleic acid during storage at 0 to 4°C for 9 days. **Peroxide value** of two samples was taking an upward trend from 0.048 μeq O_2 kg^{-1} to 0.110 μeq O_2 kg^{-1} on 9th day of refrigeration. **Tiobarbituric acid reactive substances** (TBARS) increased to 0.79 MDA mg/kg for nine days of cold storage.

Discussion

The results obtained allow us to conclude that under the conditions of this experiment, both groups control and experimental didn't show significant differences in quality parameters during cold storage at 0 to 4°C for nine days. Significant difference were noticed in %fat/100g meat. This correlation between the examined parameters shows that the significantly higher percent of fat in meat of albino trout didn't affect on storage time in cold storage conditions at 0 to 4°C for nine days. The *post mortem* pH limit of acceptability is

usually 6.8~7.0. In this study, on 6th day the fish was at the ultimate limit and on the ninth day crossed the limit to 7.18. However, no significant difference in pH ($p^* > 0.05$) was observed between two control and experimental samples chilled fish. Similar results for pH trends were also reported by (Gandotra et al. 2012) were storied the *Mystus seenghala* at 4 ± 1°C.

The acid value test was used to measure the amounts of free fatty acids and shows lipolysis. In our case the % of oleic acid in lipids at control and experimental samples during cold storage didn't show statistically significant difference The primary lipids' oxidation products in fish oil are mainly due the formation of hydroperoxides, which are measured by the peroxide value test (Hosseini et al. 2010, Ojagh et al. 2010). In this study peroxide value of control and experimental samples didn't show significant difference during storage at 0 to 4°C for nine days. Our results are similar of the data reported by (Viji et al. 2015) for stored in ice sutchi catfish (*Pangasianodon hypophthalmus*). TBARS is a value which shows the degree of accumulation of one of the secondary products of lipid oxidation, namely malondialdehyde (MDA). Fresh golden rainbow trout showed TBARS than 0.12 mg MDA/kg was determined. The limit of acceptability for TBARS is 2 mg MDA/kg, and then the fish usually develop a very unpleasant rancid smell and taste.

Conclusion

The albino rainbow trout which is a result of the albinism in rainbow trout is known as yellow golden rainbow trout. The golden yellow pattern of the fish and its tender meat are attractive for the consumers. The specific biology of golden trout contributes to a different proximate composition of the meat compared to the normal trout. In our study the percentage of lipids in 100 g of meat showed significant difference while comparing the rainbow trout to albino golden rainbow trout, whereas lipid oxidation examined parameters didn't show significant changes. Finally, the conclusion of our study is that the albino golden yellow trout had higher percent of fat, golden yellow colour, tender meat, attractive appearance, which makes it appealing to the consumers.

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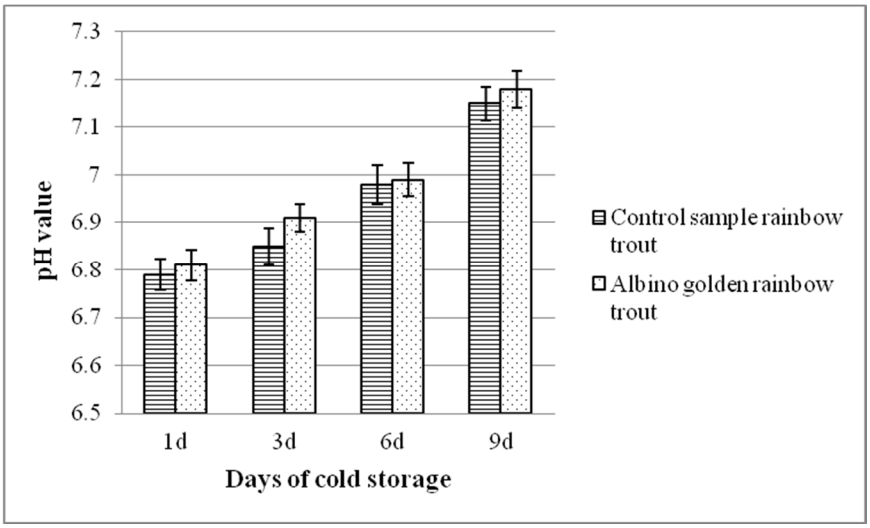

Figure 1. pH value in rainbow trout (o*ncorcynchus mykiss,Walbaum)* and albino golden rainbow trout during storage at 0 to 4°C for 9 days

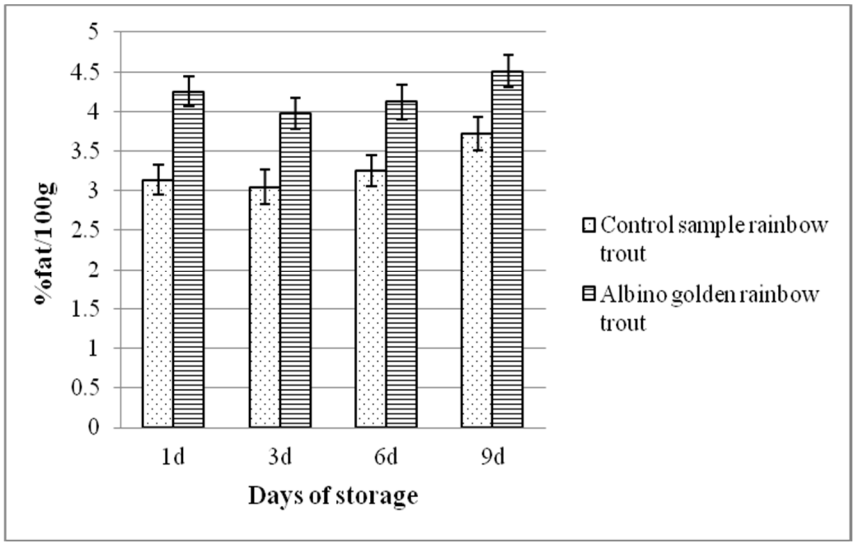

Figure 2. % fat/100g meat in rainbow trout (o*ncorcynchus mykiss,Walbaum)* and albino golden rainbow trout during storage at 0 to 4°C for 9 days

Table 1. Values of examined parameters that refers to lipid oxidation in meat of rainbow trout (*oncorcynchus mykiss,Walbaum)* and albino golden rainbow trout during storage at 0 to 4°C for 9 days

Examined parameter	**Control sample - Rainbow trout (*Oncorhynchus mykiss*) stored at 0 to 4°C**				**Albino golden rainbow trout (*Oncorhynchus mykiss*) stored at 0 to 4°C**				**(p<0.05) ***
Days of storage	1d	3d	6d	9d	1d	3d	6d	9d	
Free fatty acid, % Oleic acid	0.04±0.01	0.05±0.01	0.09±0.02	0.17±0.05	0.05±0.01	0.06±0.01	0.11±0.2	0.21±0.07	0.387
Peroxide value, $\mu eqO_2 kg^{-1}$	0.65±0.04	0.95±0.06	1.36±0.07	2.33±0.06	0.70±0.05	1.11±0.06	1.65±0.08	2.55±0.08	0.462
TBARS, MDA mg/kg	0.12±0.02	0.37±0.04	0.56±0.06	0.73±0.04	0.13±0.03	0.42±0.04	0.61±0.07	0.79±0.05	0.414