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PHYSICOCHEMICAL CHANGES IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) AFTER FREEZING

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Abstract: *The aim of this study was to compare some physicochemical changes in the dorsal and ventral muscles of rainbow trout (Oncorhynchus mykiss) after freezing. A total of 45 fish were divided into three experimental groups. The first group consisted of fresh fish (n=15) while the second group encompassed frozen fish at – 18 °C for 15 days (n=15). The third group contained double frozen fish which were frozen at – 18 °C for 15 days, thawed, and frozen again under the same conditions (n=15). Water content, water activity, ash content, crude protein, and fat content were determined. There was a significant difference (p<0.05) between the water content of the dorsal muscle of the fresh fish (76.08%) and the double frozen fish (73.22%). Water activity differed substantially (p<0.05) in the dorsal (0.975) and ventral (0.960) muscles of the frozen trout, while it was significantly higher (p<0.05) in the dorsal muscle of the frozen (0.975) than the double frozen fish (0.967). Significant differences (p<0.05) in crude protein content were measured between the ventral muscle of the fresh (19.27%) and frozen fish (20.11%), as well as between the frozen (20.11%) and double frozen fish (19.29%). The fat content was significantly higher (p<0.05) in the ventral muscle (5.02%) than the dorsal muscle (3.83%) of the double frozen fish. It was concluded that freezing and storage at low temperatures affect water content, water activity, crude protein, and fat content of rainbow trout (Oncorhynchus mykiss).*

Key words: *fish, frozen storage, physicochemical characteristics.*

1. Introduction

The physicochemical characteristics of fish meat are a key factor in determining its safety and quality [27]. Due to its lipid composition, fish meat is considered to be a major source of nutrients for humans [11]. The nutritional value of fish meat may be lost after the onset of hydrolysis and oxidation of fats that occur when stored frozen. Rancid odour is particularly pronounced in fish with a high lipid content [13], [15]. Poor quality of meat after freezing is associated with changes in

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colour, texture, and moisture loss [10]. Organoleptic characteristics such as tenderness and juiciness of meat are directly related to protein loss and their functionality in frozen storage [10], [17]. Proteins are denatured after forming bonds with lipid oxidizing compounds. As a result, there is a change in the water retention capacity of meat [13]. Proteins can also undergo oxidation and form protein aggregates [25]. Knowledge of the chemical composition of fish will help consumers make the right choice when selecting a balanced diet [16]. The objective of the present study was to compare some physicochemical changes in the dorsal and ventral muscles of rainbow trout (*Oncorhynchus mykiss*) after freezing once and twice.

2. Materials and Methods

For the experiment, 45 fresh rainbow trout (*Oncorhynchus mykiss*) of approximately equal size were purchased from a local store in Stara Zagora, Bulgaria. The fish were transported in refrigerated bags to the laboratory where the analyses were performed. The study material was taken from the dorsal and abdominal anatomical region of fresh fish and fish frozen once and twice, determining water content, water activity, protein content, fat and ash content. Fifteen of the fish (n=15) were weighted and analyzed immediately after delivery to the laboratory. The remaining thirty fish (n=30) were frozen in conventional freezers at - 18 °C for 15 days. Then they were thawed at 4 °C for 24 hours. Fifteen fish (n=15) were weighted and material for subsequent analyses was taken. The remaining fifteen thawed fish (n=15) were frozen again under the same conditions. After the second freezing period had expired, the fish were thawed at 4 °C for 24 hours, weighted, and material was taken for the analyses.

2.1. Determining Water Content

The test was carried out by means of an analytical weighing method of drying at 104±2 ºC to a constant mass according to BDS 5712-74 [3].

2.2. Determining Water Activity (A_w)

Water activity (A_w) was determined using a Hygrolab device (Rotronic AG, Switzerland).

2.3. Determining Protein Content

Protein content was determined by the Kjeldahl method according to BDS - EN ISO 5983 [5] on a Kjeltec™ 8400 device (Foss, Denmark).

2.4. Determining Fat Content

Fats were extracted according to BDS ISO 6492 [6] and determined on a Soxtec™ 2050 device (Foss, Denmark).

2.5. Determining Ash Content

The method was based on burning a meat sample in a muffle furnace followed by weight determination according to BDS 9373:1980 [4].

2.6. Statistical Analysis

The results were statistically processed using GraphPad InStat 3 (GraphPad Software, San Diego, CA) and presented as average values with standard deviations.

Dispersion analysis was performed to compare the significance of the differences between the different experimental groups. Statistical significance was determined at $p<0.05$.

3. Results and Discussion

The average initial weight of the fresh rainbow trout (0.414 g ± 0.112) was equal to that of the frozen trout (0.414 g ± 0.070), while the weight of the twice frozen trout was insignificantly lower (0.394 g ± 0.061) ($p>0.05$). The values of water activity and its changes in the muscles of the rainbow trout (*Oncorhynchus mykiss*) after freezing are presented in Tables 1 and 2. Water activity showed significant differences ($p<0.05$) between the abdominal (0.960) and dorsal (0.975) muscles of the trout that was frozen once, but not in the fresh and twice frozen trout (Table 1). The dorsal muscles of the fish frozen once (0.975) and twice (0.967) showed significant differences ($p<0.01$) in water activity, while no such differences were found between the fresh and once-frozen fish, as well as between the fresh and twice-frozen fish (Table 2). Powrie [20] concluded that the available water of frozen foods decreases due to ice crystal formation, and the water activity of the non-frozen liquid phase decreases because of increased concentration of hydrophilic solutes. Otherwise, the water activity of frozen foods depends on temperature [2]. According to Leygonie *et al.* [14], decreased thawing time below 50 min results in low amount of exudate and melting of ice in the extracellular space causing increased water activity.

Table 1

Comparing physicochemical indicators of abdominal and dorsal muscles of rainbow trout (Oncorhynchus mykiss)

Indicator	State of fish	Abdominal muscles x̄±SD	Dorsal muscles x̄±SD	Significance (p)
Water activity	fresh	0.967±0.007	0.969±0.003	ISD
	frozen once	0.960±0.014	0.975±0.002	*
	frozen twice	0.967±0.006	0.967±0.006	ISD
Water content [%]	fresh	73.91%±3.94	76.08%±2.04	ISD
	frozen once	73.69%±2.82	74.99%±1.35	ISD
	frozen twice	73.37%±1.58	73.22%±1.73	ISD
Ash [%]	fresh	1.23%±0.52	1.30%±0.18	ISD
	frozen once	1.61%±0.14	1.54%±0.42	ISD
	frozen twice	1.33%±0.15	1.29%±0.09	ISD
Crude protein [%]	fresh	19.27%± 0.31	19.82%±0.25	ISD
	frozen once	20.11%±0.25	20.33%±0.24	ISD
	frozen twice	19.29%±0.40	19.98%±0.41	ISD
Fat [%]	fresh	3.80%±0.43	3.25%±0.63	ISD
	frozen once	4.52%±0.69	3.25%±0.45	ISD
	frozen twice	5.02%±0.44	3.83%±0.16	*

*p<0.05; differently marked values show significant differences

ISD-insignificant difference, p>0.05

Water content also showed no significant differences (p>0.05) between the abdominal and dorsal muscles of the fresh trout, the once and twice frozen trout. The dorsal muscles of the fresh (76.08%) and twice-frozen fish (73.22%) showed significant differences (p<0.05) in the water content, while none were found between the fresh and once-frozen fish and between the once- and twice-frozen fish. No significant differences were observed in the values for the water content of the abdominal muscles after freezing (Tables 1 and 2). According to Siddique *et al*. [22], moisture content is not the same for individual fish and is influenced by the type and size of the fish, the season of harvesting and processing. The authors find an inverse relationship between moisture and lipid content. Reduced moisture content leads to an increase in lipids. Özyurt *et al*. [18] support this claim. Zhelyazkov *et al*. [26] studied the chemical composition of mackerel and found that low water content is due to losses in thawing. This is probably the reason for the lowest water content in double frozen trout in the study.

Ash content showed no significant differences (p>0.05) between the abdominal and dorsal muscles of the fresh, once-, and twice-frozen trout. No significant differences were found in the ash values of the abdominal and dorsal muscles after freezing (Tables 1 and 2). According to Sharaf [21], the reduction of ash content is a result of the process of thawing and loss of moisture. Gandotra *et al*. [9] report that as the storage time in frozen state increases, the ash content in the muscles decreases. According to Karki *et al*. [12], fish food and size could influence ash content. Arannilewa *et al*. [1] did not find significant changes in the ash content of tilapia during 60 days of frozen storage. The ash content of rainbow trout in the present study also remained the same during frozen storage.

Crude protein content did not show significant differences (p>0.05) between the abdominal and dorsal muscles of the fresh trout and the trout frozen once and twice. The abdominal muscles of the fresh fish (19.27%) and the fish frozen once (20.11%), as well as of the fish frozen once (20.11%) and twice (19.29%) showed significant differences (p<0.05) in crude protein content, while no such differences were established between the fresh fish and the fish frozen twice. No significant differences were found in the crude protein values of the dorsal muscles after freezing (Tables 1 and 2). The results obtained from this study of fresh rainbow trout (*Oncorhynchus mykiss*) are close to those reported by Zhelyazkov and Stratev [28], Emir Çoban [7], and Taşkaya *et al*. [23], with crude protein values from 18.24% to 21.67% for fresh rainbow trout. Mazumder *et al*. [16] determined the protein content of *Amblypharyngodon mola* (18.46%), *Gudusia chapra* (15.23%), *Puntius chola* (14.08%), *Chanda nama* (18.26%), *Pseudeutropius atherinoides* (15.84%), and *Ailia coila* (16.99 %) fish after freezing and storage at -18 °C. In the present study, protein content showed fluctuation with the highest values observed in trout frozen once. Pawar and Magar [19] compared protein content with moisture content, reporting a relationship between these chemical indicators. The relationship is that the increased protein content in frozen storage is due to the reduced amount of water content. According to Arannilewa *et al*. [1], a decrease in protein content is due to the denaturation of proteins during freezing. Emire and Gebremariam [8] support the claim that denaturation of proteins leads to an alteration in their amount. This process produces a polypeptide chain after the proteins have been damaged by the slow freezing method applied.

Table 2

Comparing the physicochemical indicators of fresh rainbow trout (Oncorhynchus mykiss) and trout frozen once and twice

Indicator	Muscles	Fresh trout x̄±SD	Trout frozen once x̄±SD	Trout frozen twice x̄±SD	Significance (p)
Water activity	Abdominal muscles	0.967±0.007	0.960±0.014	0.967±0.006	ISD
	Dorsal muscles	0.969±0.003^{ab}	0.975±0.002^{ac}	0.967±0.006^{bd}	**
Water content (%)	Abdominal muscles	73.91%±3.94	73.69%±2.82	73.37%±1.58	ISD
	Dorsal muscles	76.08%±2.04^{a}	74.99%±1.35^{ab}	73.22%±1.73^{b}	*
Ash (%)	Abdominal muscles	1.23%±0.52	1.61%±0.14	1.33%±0.15	ISD
	Dorsal muscles	1.30%±0.18	1.54%±0.42	1.29%±0.09	ISD
Crude protein (%)	Abdominal muscles	19.27%±0.31^{a}	20.11%±0.25^{b}	19.29%±0.40^{a}	*
	Dorsal muscles	19.82%±0.25	20.33%±0.24	19.98%±0.41	ISD
Fat (%)	Abdominal muscles	3.80%±0.43	4.52%±0.69	5.02%±0.44	ISD
	Dorsal muscles	3.25%±0.63	3.25%±0.45	3.83%±0.16	ISD

*p<0.05; differently marked values show significant differences

**p<0.01; differently marked values show significant differences

ISD – insignificant difference, p>0.05

Fat content showed significant differences (p<0.05) between the abdominal (5.02%) and dorsal (3.83%) muscles of the trout frozen twice, but not in the fresh and once frozen trout (Table 1). Freezing did not affect the fat values of the abdominal and dorsal muscles of the trout (Table 2). Emir Çoban [7] determined 4.82% fat in raw rainbow trout, while Zhelyazkov and Stratev [28] found 6.56% fat in fresh rainbow trout (*Oncorhynchus mykiss*). Different fat values in male and female rainbow trout (*Oncorhynchus mykiss*) have been published by Videv *et al.* [24], which are 4.86% in male and 4.46% in female fish. According to Siddique *et al.* [22], it can be assumed that for the different fresh fish values obtained, seasonal conditions, age, and sex of the fish are accountable. Taşkaya *et* *al.* [23] determined 4.11% fat in frozen and thawed fish. After 60 days of frozen fish storage, Arannilewa *et al.* [1] determined 7.2% fat. According to the authors, fat oxidation is responsible for changes in frozen storage.

4. Conclusion

Freezing and frozen storage affect water activity, water content, crude protein, and fat content of rainbow trout (*Oncorhynchus mykiss*). Changes in the chemical composition are observed in freezing once and twice. Freezing once over a period of 15 days affects the physicochemical indicators to a lower extent than freezing twice.

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