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Comparative investigation of physico-chemical and sensory properties of glazed and non-glazed frozen rainbow trout (*Oncorhynchus mykiss*) thawed with different methods by principal component analysis

M. Naseri[1*], E. Abedi[2], S. Vafa[1], L. Torri[3]

[1]Department of Natural Resources and Environment Engineering, Shiraz University, Shiraz, I. R. Iran
[2]Department of Food Science and Technology, Fasa University, Fasa, I. R. Iran
[3]University of Gastronomic Sciences, Bra, Italy

* Corresponding Author: mnaseri@shirazu.ac.ir
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ABSTRACT- Quality of glazed (G) and non-glazed air-blast frozen rainbow trout *(Oncorhynchus mykiss)* was evaluated after applying different modern (M: microwave oven; U: ultrasonic; HVEFT: high voltage electrical field; O: ohmic heating) and conventional (S: steam cooking; W: water; R: refrigeration) thawing methods. Glazed frozen samples thawed by modern and conventional methods showed an increase in protein and pH contents (except for M/MG). The glazing process negatively affected the TVB-N (total volatile basic nitrogen) and % FFA (free fatty acid) levels and these compound increased after glazing in all thawed samples except for SG and S from conventional and M, MG, U and UG from modern thawing methods, respectively. The TOTOX value of HVEF, HVEFG and OG was not significantly changed after thawing process compared with the fresh fish sample. Glazing could reduce the drip loss and increase the WHC (water holding capacity) in different thawing methods except for MG. MG and HVEFG/RG/WG had the lowest and highest hardness, respectively. The *L** values of HVEF/HVEFG and U were higher than those of fresh and other thawing methods.

INTRODUCTION

Fish is one of the most important sources of animal protein and has been widely accepted as a good source of essential nutrients for human consumption (Arannilewa et al., 2005). More than 70 different fish species are cited for aquaculture in the European Union, but rainbow trout, Atlantic salmon, gilthead sea bream, European Sea Bass, and common carp make up 90% of all fish production in the region (Bostock et al., 2016). However, in countries such as Italy, France, Germany, Denmark, Spain, the United States of America (USA), Iran and the United Kingdom, inland production of rainbow trout has been increasingly practiced to supply domestic demands (Kalbassi et al., 2013). Rainbow trout (*Oncorhynchus mykiss*) is the only cold-water fish species intensively cultivated in Iran, accounting for around 40% of the country's aquaculture production (Kalbassi et al., 2013) and one of the most popular seafood of Iranian families (Zolfaghari et al., 2011). Rainbow trout is known as a medium-fat fish (5–10% fat by weight) and recognized as a good source of polyunsaturated fatty acids (PUFAs), especially ω-3 fatty acids, for human nutrition (Chávez-Mendoza et al., 2014). Factors, such as proximate composition, the weakness of the connective tissue, pH values and the high moisture content of fish make it susceptible to spoilage and highly perishable, due to autolysis, lipid oxidation and microbiological growth (Genç et al., 2015). Accordingly, finding reliable methods of maintaining the quality of seafood is of paramount importance, especially for modern life. Various modern methods of chilling and freezing of fish products are common to extend the shelf-life (Boziaris, 2014; Ersoy et al., 2008). It was reported that the freezing process controlled by heat and mass transfer phenomena (Alizadeh et al., 2007), and its effectiveness were mainly dependent upon internal dehydration or immobilization of water and lowering of temperature (Ersoy et al., 2008). Blast freezing was among the most reputable treatments used in the fish industry. It was shown that this method required a stream of cold air to be circulated at high speed over the fish, usually in a small room or through a tunnel (Boziaris, 2014).

Besides the freezing process, it was reported that the quality of frozen food was also, typically, closely associated with the thawing method (Chevalier et al., 2000; Archer et al., 2008; Genç et al., 2015). Hence,

selecting an appropriate thawing method is of considerable importance to maintain the quality and characteristic properties of the product. It was well-accepted that thawing usually occured more slowly than freezing (Chevalier et al., 2000). Therefore, to preserve the sensory characteristics of food and prevent microbial activities, rapid thawing is desired. Moreover, in the course of thawing-heating of seafood, excessive drip loss and dehydration should be avoided (Genç et al., 2015). Currently, various thawing methods including water (immersion or spray), forced air or air blast, still or ambient air and electric methods (vacuum, microwave and radio frequency) were introduced. It was shown that each of them had different merits and demerits, and there was no one system suiting all purposes (Boziaris, 2014).

Up to now, effect of glazing under ultrasonic, ohmic and high voltage electric field has not been investigated. In the present study, the comparison effect of modern and conventional thawing methods and also glazing/non-glazing on the physico-chemical properties and sensory quality of frozen rainbow trout were determined. The various criteria regarding selecting a suitable thawing method were also discussed.

MATERIALS AND METHODS

Materials

Chemical compounds were purchased from Merck Chemical Co. (Darmstadt, Germany).

Methods

Fish Samples

A total of 30 (15×2=30) rainbow trout (300±2 g body weight) were obtained from a fish farm in Sepidan, Fars, Iran. At harvesting, the fish were packed into polystyrene boxes, covered with ice and immediately transported to Liossa Packaging and Processing Complex at the industrial zone of Sepidan, Fars, Iran. The fish samples were divided into 15 groups. The fish of the first sample group were gutted, washed with tap water, blotted dry, weighed, packed into polyethylene bags and immediately frozen by using an air-blast freezing tunnel at -45±1 °C for 4 h. Then, they were placed in food-grade high-density polyethylene plastic boxes and stored at -18±1 °C for 10 days. The fish of the second sample group were gutted, weighed (W_1), glazed by dipping in container continuously supplied with fresh cold potable water (1.0±0.1 °C for 40 s), drained for 1 min and re-weighed (W_2). Glazing uptake was calculated using Eq. (1), where W_1 and W_2 are the sample weight before and after glazing, respectively (Soares et al., 2015).

$$\text{Glazing uptake (\%)} = \frac{(W_2 - W_1) \times 100}{(w2)} \quad (1)$$

An average glazing uptake of 7.5±0.5% was obtained. Finally, samples were packed into polyethylene bags and immediately stored under constant freezing conditions. Finally, they were frozen by using an air-blast freezing tunnel at -45±1 °C for 4 h. Then, they were placed in food-grade high-density polyethylene plastic boxes and stored at -18±1 °C for 10 days. The size of each frozen fish sample was 5 ×5 ×5 cm^3. The frozen rainbow trout were transported to the Seafood Processing Laboratory of the Department of Fisheries, Shiraz University, Iran.

Thawing Time and Rate

Thawing methods were performed to increase the temperature from -18 to +4 °C. The total thawing time was run from a constant initial temperature (T_i) of -18 °C up to a final temperature (T_f) of 4 °C, at the thermal center of the thawed sample. In order to accurate determination of freezing and thawing temperatures, a type K sensor with a sensitivity of 0.5 °C was placed in the geometrical center of the fish pieces and their temperature data were digitally recorded by mentioned thermocouples. The thawing rate was measured using Eq. (2), where t_p is the thawing time:

$$\text{Thawing rate (°C/min)} = (T_f - T_i)/t_p \quad (2)$$

Thawing Methods

Conventional Thawing Methods

Steam (S), water (W) and refrigerator (R) thawing were carried out in a steam cooker (85±1 °C) (FS-12000P, Parskhazar, Iran), water bath (22±1 °C) and refrigerator (6±1 °C) (RH15D-350, Emerson, Iran), respectively (Fig. 1).

Modern Thawing Methods

Ultrasonic (U) thawing was done by using an ultrasonic bath equipped with a temperature control system (Pacisa SA, Spain). The ultrasound was operated at a working frequency of 25 KHz and 50% amplitude (200 W), with input power up to 400 W (Abedi et al., 2015) (Fig. 2a). The ultrasonic bath consisted of a rectangular tank of internal dimensions 300 ×150 × 150 mm^3, having four transducers at the base of the bath.

High voltage electrical field (HVEF) thawing was undertaken according to Abedi et al., (2015), by using a continuous flow bench scale system (International Power Silicon Company, Iran) (Fig. 2b). In this system, the HVEF is created by a needle electrode that can enhance the thawing rate via production of a corona wind from the needle electrodes (Mousakhani-Ganjeh et al., 2015). The heat transfer for thawing is obtained by multiple points-to-plate electrodes. Frozen fish cube (5 cm) was placed on a rectangular stainless-steel plate, and the electrical field (12 kV for gap 8 cm, with electrical field strength 150 kV/m) was set up between two electrodes. The sharp points of 4 needles (0.4 mm in diameter), were connected to the positive pole of a high-voltage power unit. The electrical current of 5 mA was applied using monopolar mode (DC). In order to record the temperature during thawing, a temperature sensor was inserted into the geometric center of the fish cubes. Ohmic (O) thawing experiments were conducted using a custom-designed laboratory scale, which consisted of a power supply system (voltage 50 V/cm) and the thawing cell (Teflon with dimensions of 100 × 70 ×50 mm^3) (Fig. 2c).

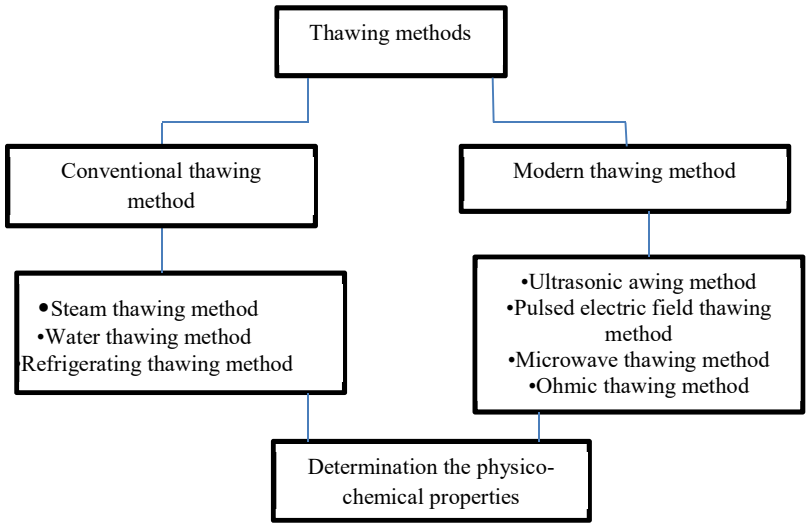

Fig. 1 Diagram of thawing methods.

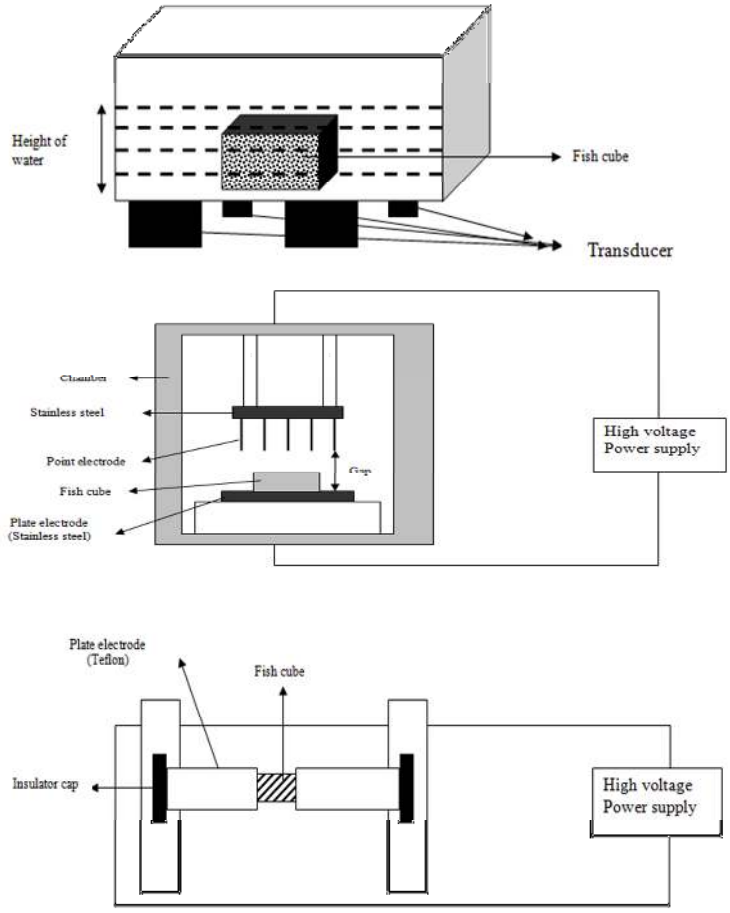

Fig. 2 Representation of ultrasonic bath (a), high voltage electrical field (b) and ohmic thawing (c), as modern thawing methods

The frozen, cut fish sample was sandwiched between two parallel electrodes, by assuring good contact between the electrodes and the sample. Temperature measurements were performed by using thermocouples (Omega Engineering, Inc. USA) from different sections of the sample in the test cell. The process was terminated by recording the sample temperature up to 4 °C. Microwave (M) thawing was performed for 15 min at ca. 90 W, 915 MHz at defrost option to avoid from the cooking

In the microwave process, the depth of penetration (dp) was defined by Eq. (3):

$$dp= \lambda \sqrt{\varepsilon'}/2\pi \varepsilon'' \quad (3)$$

where λ is the wavelength; ε' is the dielectric constant, and ε" is the loss factor or dissipation constant.

Physical and Chemical Analyses

The proximate composition (total fat, protein and ash) of both fresh and thawed samples was determined according to the Association of Official Analytical Chemists (AOAC, 2000). Total volatile basic nitrogen (TVB-N, mg N/100 g) was determined as described by Woyewoda et al. (1986). Free fatty acid (FFA) content and peroxide value (PV) of the samples were determined according to Abedi et al. (2016), and the results expressed as a percentage of oleic acid and meq active oxygen kg^{-1} lipids, respectively. The amount of secondary lipid oxidation products, indicated by the anisidine value (AV), was based on the reaction between α- and β-unsaturated aldehydes (primarily, 2-alkenals) and *p*-anisidine reagent (Abedi et al., 2016). The pH of the homogenized sample mixed with distilled water (pH 7.2±0.1) was determined using a digital pH meter (Fan Azma Gostar, Iran) (Woyewoda et al., 1986).

Water Holding Capacity (WHC) and Drip Loss Measurement

The WHC was assessed as described by Azadian et al. (2012). Drip loss measurements were performed using centrifugation (Soares et al., 2015) and calculated using Eq. (4):

$$\text{Drip loss (\%)} = (W_3 - W_4)/W_3 \quad (4)$$

W_3: weight of frozen samples without glazing; W_4: weight of thawed samples.

Texture Profile Analysis (TPA)

The TPA of the fish was established by using a texture analyzer (Model LFRA 4500, Brookfield Engineering Labs, Inc., USA) equipped with a TA41 probe. For the TPA test, the fish was formed as disks with 5 ×5 cm^2 surface area and 3 cm thickness. Each sample was compressed twice at 8 mm/s to 30% of their original height, with a relaxation time of 10 s between the two compressions. The fish characteristics were analyzed for the following factors: hardness, the force required to attain a deformation of the product's surface (Genç et al., 2015); cohesiveness, the strength of internal bonds making up the product; springiness, the force in which the sample returns to its original size after compression; and chewiness (N*mm), obtaining by multiplying the values for hardness (N), cohesiveness and springiness (mm) (Christensen, 2012).

Color Measurements

Color measurements were carried out on fresh and thawed samples, according to the Hunter *L**, *a**, *b** scale, using a digital colorimeter (MAH2000, Iranian Teb Barez, Shiraz, Iran) (Abedi et al., 2015).

Sensorial Analysis

The fishes were cooked in a steam cooker (FS-12000p, Parskhazar, Iran) at 110±1 °C for 30 min. Sensory evaluation of raw and cooked fishes was conducted by a panel of 30 trained subjects (15 males, and 15 females; aged 20–45 years; mean age, 36 years), who declared to consume fish weekly/monthly. The participants had received an invitation to participate in the study and volunteered based on their interest and availability. Written informed consent was obtained from each subject after the experiment was described to them. All participants declared to have no allergies or intolerances to fish. All tests were conducted in individual booths, and social interaction was not permitted. The experimenter verbally introduced the subjects to the data collection procedure. The fish samples were served under blind conditions, in a transparent plastic cup (25 mL) hermetically sealed with a plastic lid and coded with a random three-digit number. Samples were served in a completely randomized and balanced order among the subjects, following a complete block design, and evaluated at 25 ± 1 °C. All participants served cracker to rinse the lipid sense in the mouth. Additionally, for the raw samples a neutralizer (coffee) was used between the samples to discriminate the odor of the samples. The participants rinsed their mouth with still water before beginning the test and between samples. Subjects were instructed to observe, smell and taste the samples, and to rate their liking for color, odor, taste and flavor, texture and overall acceptability. The panel ranked raw and cooked fish was conducted by a procedure described by Abedi et al. (2016). The consumers took 30±2 min to complete their evaluation in each session.

Statistical Analysis

All experiments were conducted in triplicate. Results were expressed as mean ± standard deviation values. The mean comparison was performed using the Multiple Range Duncan's test ($p < 0.05$). Data were analyzed using analysis of variance (ANOVA) procedure by the means of the SPSS 22 statistical software (SPSS, Inc., New Jersey, USA) except for principal component analysis (PCA) (XLStat software version 19.6, Addinsoft, Paris, France).

RESULTS AND DISCUSSION

Thawing Time and Rate

Significant differences ($p \leq 0.05$) in thawing time were observed among the compared thawing methods (Table 1). It was noticed that the thawing times recorded for modern methods (ranging from 5 to 8 min) were typically lower than those found for conventional thawing methods (ranging from 15 to 610 min). The lowest value was for M (5 ± 0.5) and MG (6 ± 0.5) and the highest values were recorded for R (610 ± 1). Comparing the thawing times reported for the glazed frozen and non-glazed frozen samples processed with the same thawing method, significantly higher values for glazed samples were observed only when the

conventional thawing methods were considered. On the contrary, no significant differences in thawing time were evident between the glazed or non-glazed samples processed by the same modern techniques. Conversely, significant differences in thawing time were noticed between the glazed or non-glazed samples processed by the same conventional approaches. The order of thawing time was as fallow: RG > R > WG > W > SG > S and O/OG> U/UG> HVEF/HVEFG> M/MG for conventional and modern thawing methods, respectively. The interval temperature considered for evaluating the thawing process was constant (from -18 to 4 °C; Δ22 °C) for all samples and therefore, the thawing rate (°C/min) reduced with increasing thawing time. Comparing the thawing rate reported for the glazed frozen and non-glazed frozen samples processed by the same thawing method, significantly higher values for non-glazed samples were observed based on the modern thawing methods, except for HVEFG and OG thawing methods. Not significant differences (p> 0.05) in thawing rate were noticed between the glazed or non-glazed samples processed by the same conventional approaches. The highest thawing rate (°C/min) belonged to M (4.40 ± 0.2) and HVEFG (4.40 ± 0.2).

In the microwave process, the depth of penetration (dp, m) was defined by Eq. (5):

$$dp = \lambda_0 \sqrt{\varepsilon'}/2\pi\,\varepsilon'' \quad (5)$$

where λ(m) is the wavelength; ε' is the dielectric constant, and ε'' is the loss factor or dissipation constant. Microwaves have less depth of penetration in foods with more moisture and salt contents. When glazing is conducted with water, most of the microwave energy is absorbed in this layer and overheating on the product surface occurs. On the other hand, conversely, in the current study, the choice of frequency (915 MHz) was based on Eq. (5). The low frequency is more suitable for thawing, as it enables the microwave energy to act on the product to a depth of up to 20 cm, whereas a maximum penetration depth of only 10 cm was achieved at 2450 MHz (Regier et al., 2016).

Corona wind was defined as ‘‘the movement of uncharged air particles due to collisions with ionized air particles moving in an electrostatic field to produce an appreciable wind’’ (Goodenough et al., 2007). It was reported that corona wind was the main mechanism for heat transfer by HVEF, due to turbulence and vortices generated in the liquid portion of fish during thawing (Mousakhani-Ganjeh et al., 2105). This approach decreased the thawing time with an improvement in the thawing rate compared with the airflow alone. The voltage (12 kV) and gap (8 cm) were chosen for thawing of the fish cube, based on the results of Mousakhani-Ganjeh et al. (2015) and Rahbari et al. (2018). They showed that these settings provided a high electrical field Reynolds number for enhancement of the heat transfer coefficient and a low thawing loss.

Ultrasonic process is an alternative technique for thawing process. However, disadvantages of ultrasonic technology, such as poor penetration, scattering and attenuation of energy, are due to density differences between the frozen and thawed regions, localized heating and the high-power requirement. Whole barriers improved by the relaxation mechanism. It was indicated that frozen foods were capable of absorbing more acoustic energy when applying a frequency in the range of relaxation frequency of ice crystals in the food, which occured more rapidly with increased thawing rate (Kissam et al., 1982). In the current work, a frequency of 25 KHz with an input power of 200 W of the ultrasonic bath activated the relaxation mechanism for the thawing process. Additionally, cavitation occured without surface over-heating, due to the high absorption of the ultrasound near the surface, which eliminated poor ultrasonic penetration and ultrasonic attenuation with frequency (Miles et al., 1999).

Ohmic heating (direct resistance heating) was done via placing liquid and solid foods between electrodes that are heated simultaneously by passing an electric current through them. The electrical conductivity of food, electrical field strength and frequency were key factors affecting ohmic thawing efficacy. An increase in salt concentration or moisture content increased the electrical conductivity of the food, resulting in a reduction of the thawing time (Table 1) (Seyhun et al., 2013; Varghese et al., 2014; Boonchoo et al., 2015). In the glazing process, ice induced a high-conductivity medium, which increased the efficiency of ohmic heating.

Proximate Composition

The proximate composition of fresh fish and its changes due to the freezing-thawing processes were determined (Table 1). In general, it was reported that the proximate composition of fish varied with species, season and its reproductive cycle (Woyewoda et al., 1986). After the freezing-thawing process, the lipid content of conventional (S, W and R) and modern (M, U, HVEF and O) thawing samples were significantly increased (p<0.05) in comparison to the fresh sample. Typically, the conventional samples were richer in fat than modern samples. Glazing decreased the fat content of frozen samples after the thawing process, especially for conventional methods. However, glazing had a negative effect on microwave thawing. Fat content of MG (34.8 ± 0.3) was more than that of M (32.6 ± 0.4). These changes might also be related to accumulation of energy on surface layer and overheating on the product surface which induce to the weakening of the lipid–protein bonds that in turn facilitate the fat extraction. Moreover, protein denaturation could accelerate the bond breakage. Also, oily fish containing surplus fat as peripheral triacylglycerols in muscles, which might aggravate fat loss when the temperature was applied to the fish (García-Arias et al., 2003). García-Arias et al. (2003) reported that highest fat content and drip-loss phenomenon belonged to R fillets compared with thawing by M. The protein content of conventionally thawed samples (C, W and R) was significantly (p<0.05) lower than that in fishes processed by modern thawing methods (M, HVEF, O and U) and in the fresh sample. The protein content of the frozen samples decreased after the thawing processes. Modern thawing produced smaller losses in protein, which might be due to the lower leaching and the shorter time (Table 1) of

actuation of the modern system (García-Arias et al., 2003). Under the same thawing condition, glazed frozen samples thawed by modern and conventional methods showed an increasing in protein content. Glazing had negative effect on protein content of M thawing. In a work conducted by García-Arias et al. (2003), after frozen storage, followed by thawing (by conventional and modern methods), fillets showed significant decreases in several amino acids including cyst(e)ine, particularly, and also arginine, lysine, histidine, phenylalanine, tyrosine, leucine, isoleucine, methionine, valine, alanine, glycine, proline, serine and threonine. Ozone formation during the application of HVEF is an important factor affecting protein denaturation. It was reported that ozone oxidized aromatic monomeric and cysteine units of the proteins and was capable of converting the thiols group of cysteine into disulfide crosslinks (Mousakhani-Ganjeh et al., 2015). According to the result of Arzeni et al. (2012), total free sulfhydryl groups decreased after ultrasonic process which might be due to several reasons including generating hydroxyl radicals and other free radicals (Abedi et al., 2015; Tiwari et al., 2015) and rapid formation and then collapse of gas bubbles, which resulted in localised pressure and temperature (hydrodynamic shear forces) over short periods of times (a few microseconds) which induced to aggregate state of protein due to protein denaturation (O'Sullivan et al., 2016). The protein denaturation of frozen fish mince wash water under ohmic heating was reported by Huang et al. (1997).
The ash contents of conventional thawing (S-SG, W-WG and R-RG) methods were significantly more than modern (M-MG, U-UG, HVEF-HVEFG and O-OG) thawing methods (Table 1).

Physico-Chemical Properties

pH Measurement

Table 2 shows several quality indices of pH measured in samples after the freezing-thawing processes were involved. It was reported that pH of fresh fish muscles was close to neutrality (Genç et al., 2015). In the current research, the initial pH of the fresh sample was 6.61 ± 0.02. Significant changes were observed in the pH value after the freezing-thawing processes; both modern and conventional thawing processes decreased the pH. In the current work, the pH levels of the samples thawed by modern techniques were significantly (p<0.05) higher than those observed in samples thawed by conventional methods. Lower pH values which have seen after freezing-thawing processes might be due to several reasons. One could be the production of lactic acid, depending on species, age, catching season, gender and feeding status (Tokur et al., 2006). Another was the FFA content increase that observed in this study (Table 2) in post-thawing samples. Thirdly, the release of hydrogen ions might occur, due to a possible denaturation of buffer proteins after formation of exudate compounds (Rodriguez-Turienzo et al., 2011; Leygonie et al., 2012; Soares et al., 2015). Fourth, there could be an enhancement in the concentration of solutes due to a loss of water from meat (Leygonie et al., 2012; Ali et al., 2015). Another explanation for the reduction in pH was reported to be the release of hydrogen atoms due to deamination of proteins promoted by microbial or enzymatic reaction (Leygonie et al., 2012). Finally, possible fat oxidation in fish species with high fat content can lead to a reduction in pH value, if the proposed thawing method is not conducted properly (Genç et al., 2015). In the current study, S samples, followed by W samples had the lowest pH levels. The pH of glazed samples (except for M/MG) were higher than those in non-glazed samples. The more alkaline pH of the glazed fish might be attributed to the relatively lower fat (Table 2) and FFA contents (Table 2). Genç et al. (2015) reported that the pH value of meagre (*Argyrosomus regius*) fillets increased after the glazing-thawing process in the thawing methods tested (air, R, W and M). However, Ersoy et al. (2008) and Javadian et al. (2013) found a decrease in pH value of thawed samples using refrigeration, water and air at ambient temperature and microwave oven.

TVB-N Measurement

The TVB-N index was applied to evaluate food spoilage. Microbial degradation of protein and non-protein nitrogen compounds lead to the production of volatile nitrogen (Mousakhani-Ganjeh et al., 2015). This index is produced mainly as a result of microbial activity and could increase during storage (Mousakhani-Ganjeh et al., 2015). However, it is an insufficient indicator of quality during the initial stages of the seafood decay. In addition, TVB-N values differ from species to species (Genç et al., 2015). The TVB-N value of fresh fish should not exceed 15 mg/100 g (Etienne, 2005). However, the acceptable threshold of TVB-N reported in the literature was in the range of 30–35 mg N/100 g (Genç et al., 2015). In the current investigation, the TVB-N value of the fresh fish was 6.34 ± 0.19 mg N/100 g, which was well below those reported by Genç et al. (2015), Javadian et al. (2013) and Tokur et al. (2006). According to Table 2, the TVB-N of samples thawed by conventional and modern methods were higher than that in the fresh sample. Significant differences were found among the TVB-N values of samples thawed by both modern (M, U, HVEF and O) and conventional (S, W and R) techniques. As mentioned above (section thawing time and rate), there was a significant difference in the thawing times between thawing procedures (Table 1). In conventional thawing, the efficient heat transfer mechanism was at first convection between the sample and thawing medium, and then conduction within the sample (Bozkurt and İçier, 2012). However, in M, U, HVEF and O, radiation between the sample and thawing medium occurred initially, and then conduction/convection within the sample was performed. In thawed treatments, the thermal conductivity of the frozen part was more than the unfrozen part (Bozkurt and İçier, 2012) and thawed surface layer on the defrosted sample acted as an insulator and causeed longer treatment times, especially in conventional thawing methods. It was reported that increasing in thawing time in conventional thawing methods compared with modern thawing methods

resulted in an increase in the microbial activity and enzymatic reaction (Bozkurt and İçier, 2012). Among modern technologies for thawing, HVEF (7.52 ± 0.07) and HVEFG (7.65 ± 0.08) with no significant difference (p>0.05) had lower TVB-N values. Some reasons to justify the ability of corona discharge to reduce the microbial load are production and release of negative ions of the air (NIA), air ions of N_2^+, O_2^+, N^+, O^+, and O^- and ozone. Ozone is produced by several steps. First, excitation of oxygen electrons, then splitting of oxygen molecules and, finally, the reaction of oxygen atoms with other oxygen molecules to create ozone (Mousakhani-Ganjeh et al., 2015). Ozone and NIA can inactive pathogenic and spoilage effects of microbes, and decrease the TVB-N production by microorganisms. Contradictory results have been documented for the TVB-N value (Mol et al., 2004; Boonsumrej et al., 2007; Ersoy et al., 2008), mainly due to species, catching season and region, gender and age of the fishes. The glazing process negatively affected the TVB-N levels, which increased after glazing, in all thawed samples (except for SG and S). That result might be justified by the defrosting of the glazed sample, which could accelerate microbial growth and increase the TVB-N content. There were no significant differences (p<0.05) in TVB-N levels between HVEF-HVEFG and U-UG thawing methods. The results were in concurrence with the findings of Ersoy et al. (2008). The highest TVB-N values belonged to SG and S fishes.

FFA measurement

FFAs are more prone to oxidation than triglycerides, and thus their presence in oils increases the possibility of lipid degradation (Cozzolino et al., 2005). Our results showed that the FFA content of HVEF and HVEFG samples were significantly lower than that in fish thawed by conventional (W, S and R) and other modern (U, M and O) methods (p<0.05, Table 2). The results might attribute to different thawing times (Table 1) and fat contents (Table 2) of various thawing methods. The higher FFA amount observed in W, S and R samples might be due to the suitable conditions (high time and % fat content) for enzymatic reactions and unfolding of the globin moiety (in myoglobin) during the conventional thawing processes, compared with modern methods. The glazing process could increase the %FFA contents during M (0.69 ± 0.03) and MG (1.02 ± 0.04), U (0.72 ± 0.05) and UG (0.83 ± 0.02). MG had the highest FFA content when compared with the other modern thawing procedures evaluated. The relative loss factor of ice enhances with temperature (according to Eq. 3). Thus, the penetration depth of wave is higher at lower temperature (freezing temperature), and heat rapidly induces the melting of ice. Also, thawing does not occur uniformly in large blocks having an asymmetrical and angular shape. Consequently, some portions of the food may cook while other sections remain frozen, triggering activation of oxidation enzymes. In UG process, glazing might cause to increase the FFA content due to poor penetration, scattering and attenuation of energy between the frozen and thawed regions and localized heating. On the contrary, Javadian et al. (2013) reported a significant increase of the FFA content in ambient air thawed samples. They indicated that this increase was related to the faster lipid hydrolysis at ambient air room temperature than to low temperature.

PV, AV and TOTOX Measurements

Our results showed that the PV significantly (p<0.05) changed over the freezing-thawing process. Glazing process could enhance the PVs in MG, UG and SG. When comparing the different thawing methods, the PVs of MG (2.84 ± 0.12) and SG (2.75 ± 0.07) were significantly (p<0.05) higher than those measured in fresh and other defrosted samples. This result was probably due to the high energy liberated by the microwave, which might activate lipid oxidation and result in increased peroxide formation (Boonsumrej et al., 2007). Alternatively, due to the unfolding of the globin moiety (in myoglobin) during heating, freezing, frozen storage and thawing (Leygonie et al., 2012), iron could oxidize the lipid and cause an increase in FFA, TVB-N and PV (Rodríguez et al., 2012). The portion of unfrozen water is also important in the creation of oxidation, as chemical reactions can occur during frozen storage and initiate primary lipid oxidation (peroxidation) in the frozen sample. Consequently, radical compounds and secondary lipid oxidation compounds may form upon thawing. It was reported that lipid oxidation accelerated during the frozen–thawing process and caused changes in odor, flavor, color and healthfulness of the product (Rodríguez et al., 2012).

The p-anisidine method was used to measure the alpha-beta unsaturated aldehydes of fats and oils (Boziaris, 2014). These secondary products could cause rancid, putrid, fatty, pungent and other off-flavors. Frozen storage is not necessarily sufficient to prohibit the occurrence of oxidation. Freezing and thawing of muscle tissue enhance secondary products of lipid oxidation and their accumulation, primarily at the cellular membrane level. These phenomena might result from damage to cell membranes by ice crystals and following release of pro-oxidants, including heme iron. As shown in Table 2, the highest AV was found in S (p<0.05). However, according to the results of Boonsumrej et al. (2007), the AV of shrimps was higher in those thawed by microwave and ultrasound wave in comparison to shrimps thawed at refrigerator temperature. In contrast, Javadian et al. (2013) reported a significantly high secondary lipid oxidation in air- and refrigerator-thawed samples, respectively. There were no significant differences (p>0.05) in the AV between the glazing and non-glazing fishes, thawed by U, HVEF, O, W and R. Accordingly, it was concluded that glazing treatment had no significant effect (p>0.05) on the reduction of AV. The total oxidation value (TOTOX), calculated by the formula 2PV+AV, demonstrates overall oxidation state. This index has been used to determine the presence of lipid oxidation products, such as hydroperoxides, aldehydes and ketones (Deepika et al., 2014). According to the results of this study, the TOTOX value of HVEF, HVEFG and OG was not significantly (p<0.05) changed after the freezing-thawing process compared with the fresh fish sample. However, this value was higher in SG and MG than that in other treatments (p<0.05, Table 2).

Table 1. Proximate composition analyses (g/100 g dry weight) of fresh and freeze-thawed rainbow trout samples

	Sample	Thawing time (min)	Thawing rate (°C/min)	Fat (%)	Protein (%)	Ash (%)
Modern thawing	Fresh	-	-	31.3 ± 0.3^{h}	48.6 ± 0.2^{a}	7.3 ± 0.2^{a}
	MG	6 ± 0.5^{ij}	3.70 ± 0.1^{b}	34.8 ± 0.3^{c}	46.2 ± 0.3^{d}	6.0 ± 0.0^{f}
	M	5 ± 0.5^{jk}	4.40 ± 0.2^{a}	32.6 ± 0.4^{gh}	47.1 ± 0.2^{bc}	6.2 ± 0.0^{ef}
	UG	7 ± 0.5^{hi}	3.14 ± 0.1^{c}	33.2 ± 0.2^{h}	46.9 ± 0.1^{c}	6.2 ± 0.0^{ef}
	U	6 ± 0.5^{ij}	3.70 ± 0.1^{b}	33.0 ± 0.2^{efg}	46.7 ± 0.2^{c}	6.2 ± 0.0^{e}
	HVEFG	5 ± 0.5^{jk}	4.40 ± 0.2^{a}	31.0 ± 0.2^{h}	47.5 ± 0.2^{b}	6.2 ± 0.0^{ef}
	HVEF	6 ± 0.5^{ij}	3.70 ± 0.2^{b}	32.8 ± 0.2^{fg}	46.7 ± 0.1^{c}	6.2 ± 0.0^{ef}
	OG	7 ± 0.5^{hi}	3.14 ± 0.0^{c}	33.4 ± 0.2^{e}	46.7 ± 0.1^{c}	6.2 ± 0.0^{e}
	O	8 ± 0.5^{gh}	2.75 ± 0.1^{d}	33.9 ± 0.1^{d}	46.0 ± 0.2^{d}	6.2 ± 0.0^{ef}
Conventional thawing	SG	16 ± 0.5^{e}	1.37 ± 0.1^{f}	37.3 ± 0.3^{b}	42.3 ± 0.1^{g}	6.8 ± 0.0^{b}
	S	15 ± 0.5^{f}	1.46 ± 0.1^{f}	38.2 ± 0.2^{a}	40.5 ± 0.3^{i}	6.8 ± 0.0^{b}
	WG	124 ± 1^{c}	0.18 ± 0.0^{g}	34.9 ± 0.3^{c}	44.4 ± 0.2^{f}	6.4 ± 0.0^{d}
	W	121 ± 1^{d}	0.18 ± 0.0^{g}	36.9 ± 0.3^{b}	41.2 ± 0.4^{h}	6.4 ± 0.0^{d}
	RG	610 ± 1^{a}	0.04 ± 0.0^{h}	35.2 ± 0.3^{c}	45.0 ± 0.3^{e}	6.6 ± 0.0^{c}
	R	605 ± 1^{b}	0.03 ± 0.0^{h}	37.8 ± 0.4^{a}	42.6 ± 0.3^{g}	6.6 ± 0.0^{c}

G: glazed frozen fish; HVEF: high voltage electrical field-thawed frozen fish; M: microwave-thawed frozen fish; O: ohmic-thawed frozen fish; R: refrigerated-thawed frozen fish; S: steam-thawed frozen fish; U: ultrasonic-thawed frozen fish; W: water-thawed frozen fish. Values are shown as mean ± standard deviation (n=3). Means with different letters in the same column are significantly different ($p < 0.05$).

Table 2. Physico-chemical changes of fat, drip loss and WHC indices of fresh and freeze-thawed rainbow trout samples

	Sample	pH	TVB-N (mg N/100 g)	FFAs (g oleic acid/100 g oil)	PV (meq O_2/kg)	AV	TOTOX value	Drip loss	WHC
Modern thawing	Fresh	6.61 ± 0.02^{a}	6.34 ± 0.19^{j}	0.50 ± 0.10^{i}	1.40 ± 0.14^{hi}	0.24 ± 0.01^{de}	3.04 ± 0.14^{hi}	13.4 ± 0.3^{k}	81.8 ± 0.2^{a}
	MG	6.28 ± 0.02^{g}	8.76 ± 0.20^{c}	1.02 ± 0.04^{d}	2.84 ± 0.12^{a}	0.41 ± 0.02^{c}	6.06 ± 0.12^{a}	21.2 ± 0.1^{b}	69.3 ± 0.2^{k}
	M	6.45 ± 0.02^{d}	8.07 ± 0.08^{de}	0.69 ± 0.03^{gh}	2.36 ± 0.10^{b}	0.17 ± 0.03^{gh}	4.89 ± 0.11^{b}	13.0 ± 0.2^{l}	73.2 ± 0.4^{h}
	UG	6.34 ± 0.03^{ef}	8.26 ± 0.10^{d}	0.83 ± 0.02^{e}	1.70 ± 0.06^{ef}	0.21 ± 0.03^{efg}	3.61 ± 0.07^{e}	15.2 ± 0.2^{i}	77.0 ± 0.3^{d}
	U	6.38 ± 0.02^{e}	8.13 ± 0.08^{d}	0.72 ± 0.05^{fg}	1.56 ± 0.09^{fgh}	0.23 ± 0.02^{de}	3.35 ± 0.10^{f}	16.6 ± 0.2^{h}	76.4 ± 0.4^{f}
	HVEFG	6.58 ± 0.01^{b}	7.65 ± 0.08^{g}	0.34 ± 0.05^{j}	1.44 ± 0.09^{hi}	0.18 ± 0.04^{fgh}	3.06 ± 0.10^{hi}	13.7 ± 0.2^{k}	79.0 ± 0.3^{b}
	HVEF	6.53 ± 0.02^{bc}	7.52 ± 0.07^{g}	0.35 ± 0.03^{j}	1.49 ± 0.10^{ghi}	0.17 ± 0.02^{gh}	3.15 ± 0.10^{gh}	13.1 ± 0.1^{l}	78.2 ± 0.4^{c}
	OG	6.51 ± 0.02^{c}	8.21 ± 0.10^{d}	0.47 ± 0.03^{i}	1.42 ± 0.10^{hi}	0.26 ± 0.03^{d}	3.10 ± 0.10^{hi}	17.3 ± 0.1^{g}	74.8 ± 0.2^{g}
	O	6.46 ± 0.03^{d}	7.89 ± 0.08^{ef}	0.51 ± 0.02^{i}	1.61 ± 0.07^{fg}	0.24 ± 0.02^{de}	3.46 ± 0.07^{f}	19.4 ± 0.1^{d}	70.8 ± 0.4^{j}
Conventional thawing	SG	6.11 ± 0.03^{i}	9.25 ± 0.15^{b}	1.38 ± 0.04^{c}	2.75 ± 0.07^{a}	0.68 ± 0.04^{a}	6.18 ± 0.08^{a}	19.0 ± 0.2^{e}	67.8 ± 0.2^{l}
	S	5.93 ± 0.02^{k}	10.02 ± 0.18^{a}	1.43 ± 0.02^{bc}	1.88 ± 0.11^{cd}	0.45 ± 0.01^{b}	4.21 ± 0.11^{c}	21.8 ± 0.1^{a}	62.2 ± 0.2^{m}
	WG	6.16 ± 0.03^{h}	6.93 ± 0.15^{h}	1.48 ± 0.05^{b}	1.82 ± 0.11^{de}	0.25 ± 0.01^{de}	3.89 ± 0.11^{d}	18.0 ± 0.2^{f}	76.0 ± 0.2^{f}
	W	6.04 ± 0.02^{j}	6.61 ± 0.14^{i}	1.65 ± 0.03^{a}	2.02 ± 0.08^{c}	0.22 ± 0.02^{def}	4.26 ± 0.08^{c}	20.0 ± 0.1^{c}	72.2 ± 0.2^{i}
	RG	6.31 ± 0.05^{fg}	7.71 ± 0.13^{fg}	0.62 ± 0.03^{h}	1.23 ± 0.05^{j}	0.17 ± 0.01^{gh}	2.63 ± 0.05^{k}	14.6 ± 0.1^{j}	77.4 ± 0.3^{d}
	R	6.19 ± 0.04^{h}	7.07 ± 0.10^{h}	0.78 ± 0.01^{ef}	1.35 ± 0.02^{ij}	0.15 ± 0.01^{h}	2.85 ± 0.02^{j}	15.2 ± 0.2^{i}	76.9 ± 0.2^{e}

G: glazed frozen fish; HVEF: high voltage electrical field-thawed frozen fish; M: microwave-thawed frozen fish; O: ohmic-thawed frozen fish; R: refrigerated-thawed frozen fish; S: steam-thawed frozen fish; U: ultrasonic-thawed frozen fish; W: water-thawed frozen fish. AV = anisidine value; FFAs = free fatty acids; PV = peroxide value; TOTOX = total oxidation; VB-N = total volatile base nitrogen. Values are shown as mean ± standard deviation (n=3). Means with different letters in the same column are significantly different ($p < 0.05$).

Effect of Thawing on Drip Loss and WHC

The effect of the various thawing methods on drip loss and WHC of trout are depicted in Table 2. Drip loss has been shown to be indicative of deteriorative changes in frozen foods (Akter et al., 2012). In the present study, the type of thawing method significantly ($p<0.05$) affected the drip loss of the samples. Water holding capacity (WHC) is a suitable determinant of fish quality subjected to the freezing-thawing process. This characteristic directly affected product appearance, production profitability and sensory quality, such as juiciness (Woyewoda et al., 1986; Leygonie et al., 2012). The order of WHC of modern and conventional thawing methods were as HVEFG> HVEF> UG/RG> R> U/WG> OG> M> W> O> MG> S> SG. Glazing could increase the WHC of thawed fishes. Our results showed that the WHC of HVEFG (79.0 ± 0.3) and S (62.2 ± 0.2) were significantly ($p<0.05$) higher and lower than those of other treatments, respectively, ($p<0.05$, Table 2). Javadian et al. (2013) reported a decrease in WHC after thawing of kutum (*Rutilus kutum*). The pH, ionic strength and oxidation directly affected the efficiency of myofibrillar proteins and muscle protein cells to bind and entrap water (Ali et al., 2015). The WHC is a selected functional property of proteins, and thus it was reported that the oxidative changes, denaturation and aggregation of proteins (especially myosin) during the freezing-thawing process decreased their ability to hold water (Zhu et al., 2004; Mørkøre and Lilleholt, 2007; Genç et al., 2015). In general, freezing and subsequent thawing treatments cause the denaturation of fish muscle proteins. Various stress

factors, such as the production of FFAs (Table 2), oxidizing lipids (Table 2) and an increase in intracellular ionic strength, due to the migration of water to the extracellular spaces, as well as heme pigments and oxidative enzymes were assumed to be important causes of denaturation (Leygonie et al., 2012). According to Table 2, HVEF/HVEFG (against with SG/S) with low FFAs, PV and AV proned to high WHC of proteins. Proteins of cold-water fish, such as trout, tend to be more susceptible to freeze denaturation than those of warm-water fish (Genç et al., 2015). The failure of protein solubility has been attributed to the formation of stable covalent linkages. Thus, frozen storage of proteins causes the formation of hydrogen and disulfide bonds (Regier et al., 2016). Several treatments such as ultrasonic process (Arzeni et al., 2012) and HVEF (Mousakhani-Ganjeh et al., 2015) can form S–S bonds through oxidation of SH groups, and markedly affect the gel network structure and mechanical strength. Although, intermolecular disulfide cross-links are not necessary for gelation, but can provide an important role for stable gels. On the other hand, ultrasonic modify the protein structure via intermolecular hydrophobic interactions specially after glazing process and prone to the formation of gel network (Arzeni et al., 2012; Mousakhani-Ganjeh et al., 2015).

Glazing could reduce the drip loss and increase the WHC in modern and conventional thawing methods. However, glazing had negative impact on M thawing and drip loss significantly ($p< 0.05$) increased after thawing of MG frozen fish. García-Arias et al. (2009) noted the total sulfhydryl content decreased due to losses of sulfur amino acids in defrosted samples. According to Ali et al. (2015) and García-Arias et al. (2009), the solubility decreased in thawed samples would be related to the loss of cyst(e)ine throughout the formation of insoluble compounds that ultimately reduces the WHC and increases drip loss (Leygonie et al., 2012). Changes in the stability of fish muscle proteins during frozen storage have been shown to depend on species and their habitat temperatures (Davies et al., 1994). Boonsumrej et al. (2007) and Xia et al. (2012) reported high drip loss in microwave-thawed tiger shrimp (*Penaeus monodon*) and porcine longissimus muscle, which was consistent with our results. Zhu et al. (2004) have also found a significant reduction of drip loss, by applying high pressure thawing to Atlantic salmon snap-frozen by liquid nitrogen. They reported that pressurization rate and pressure holding time could affect drip loss, as well.

Textural Characteristics

Oxidation of myofibrillar proteins during frozen storage and thawing may have a significant influence on meat texture. The impact of different thawing methods on the texture of rainbow trout is shown in Table 3. Significant differences ($p<0.05$) in hardness, cohesiveness, springiness, and chewiness were found among samples processed using different thawing methods. Furthermore, the hardness of defrosted samples reduced in comparison to the hardness of the fresh fish. Hardness and chewiness of S were significantly ($p<0.05$) much lower than those of other thawed samples. Tenderness of meat was shown to be increased with freezing and thawing processes (Leygonie et al., 2012). Several factors might contribute to the enhancement of tenderness [e.g., frozen storage duration, the degree of muscle protein aging before freezing, enzyme-induced deterioration of the muscle fibers during aging (proteolysis) and loss of structural integrity due to ice crystal production] (Leygonie et al., 2012). Contradictorily, Genç et al. (2015) noticed a significant increase in hardness of water-thawed fillets. They argued that the increase in hardness has been a function of fillet thickness so that thinner fillets showed higher hardness and vice versa. Glazing treatment increased the hardness, except for MG (821 ± 54). Treatment with glazing could reduce drip loss during thawing (Table 2) and cause a reduction in the damaged protein content (Table 2). Thus, an increased quantity of muscle fibers per surface area seemed to increase the toughness. Textural properties (hardness, cohesiveness, springiness, chewiness) of S were significantly ($p<0.05$) lower than those of the fresh sample and other thawing methods (Table 3). There were not significant differences ($p<0.05$) among RG (1122 ± 29), WG (1052 ± 79), HVEFG (1072 ± 53) thawing methods. MG and HVEFG/RG/WG had the lowest and highest hardness, respectively, among the samples thawed by modern methods. Although microwave heating could produce fast thawing, it induced a pronounced protein denaturation and destabilization. Thus, it introduced to be an undesirable method to defrost frozen muscle proteins (Genç et al., 2015). There was no significant difference ($p<0.05$) among thawed sample with fresh fish (except S and SG). The springiness of conventional thawing samples (W/WG and R/RG) were not significant ($P> 0.05$) with fresh sample but were significantly ($P< 0.05$) more than those in modern and S/SG thawed samples. Chewiness of RG (559 ± 30) was significantly ($p<0.05$) more than other thawing method.

Color Measurement

The color values L* (lightness), a* (redness–greenness) and b* (yellowness–blueness) observed for the fish samples are shown in Table 3. During freezing-thawing processes, color changes could occur due to lipid oxidation and pigment degradation (Ali et al., 2015; Xia et al., 2012). One reason explaining the color changes, is denaturation of the globin moiety of the myoglobin molecule, occurring at some phase during freezing, frozen storage and thawing. It was reported that denaturation resulted in an increased susceptibility of myoglobin to autoxidation and metmyoglobin production that caused a subsequent loss of the optimum thawed frozen color (Leygonie et al., 2012). A second explanation could be the metmyoglobin reducing activity (MRA). MRA is the ability of a fish to reduce the ferric (Fe^{3+}) state myoglobin to its ferrous (Fe^{2+}) state. The metmyoglobin (brown) was reduced to myoglobin (red). However, MRA activity was decreased during freezing and thawing, in which conversion of metmyoglobin (brown) to myoglobin or oxymyoglobin was stopped.

Table 3. Texture and color parameters of fresh and freeze-thawed rainbow trout samples

	Sample	Hardness (N)	Cohesivenes s	Springiness (mm)	Chewiness (N*mm)	*L**	*a**	*b**
Modern thawing	Fresh	1298 ± 62^{a}	0.58 ± 0.08^{a}	0.95 ± 0.01^{a}	715 ± 62^{a}	49.0 ± 0.2^{c}	13.1 ± 0.1^{a}	29.3 ± 0.2^{a}
	MG	821 ± 54^{g}	0.53 ± 0.03^{a}	0.77 ± 0.09^{bc}	373 ± 54^{efg}	35.3 ± 0.2^{l}	7.1 ± 0.1^{h}	21.4 ± 0.2^{f}
	M	974 ± 32^{def}	0.51 ± 0.03^{a}	0.74 ± 0.07^{bc}	418 ± 32^{de}	38.4 ± 0.1^{k}	7.5 ± 0.2^{h}	22.4 ± 0.1^{e}
	UG	982 ± 62^{def}	0.54 ± 0.02^{a}	0.59 ± 0.04^{de}	313 ± 62^{fg}	48.2 ± 0.1^{d}	10.4 ± 0.2^{e}	24.4 ± 0.2^{e}
	U	966 ± 30^{def}	0.57 ± 0.08^{a}	0.76 ± 0.09^{bc}	419 ± 30^{de}	48.7 ± 0.1^{c}	10.6 ± 0.1^{e}	24.2 ± 0.4^{cd}
	HVEFG	1072 ± 53^{bc}	0.55 ± 0.08^{a}	0.54 ± 0.09^{e}	319 ± 53^{fg}	51.2 ± 0.1^{a}	11.4 ± 0.2^{cd}	27.6 ± 0.2^{b}
	HVEF	1006 ± 42^{cde}	0.56 ± 0.10^{a}	0.70 ± 0.06^{cd}	395 ± 42^{def}	50.4 ± 0.2^{b}	11.2 ± 0.2^{d}	27.4 ± 0.3^{b}
	OG	955 ± 29^{def}	0.51 ± 0.08^{a}	0.62 ± 0.10^{de}	305 ± 29^{g}	41.3 ± 0.3^{h}	9.1 ± 0.2^{f}	23.8 ± 0.3^{d}
	O	917 ± 20^{f}	0.53 ± 0.03^{a}	0.61 ± 0.07^{de}	296 ± 20^{g}	40.4 ± 0.2^{i}	8.8 ± 0.2^{f}	22.7 ± 0.2^{e}
Conventional thawing	SG	728 ± 44^{h}	0.39 ± 0.04^{b}	0.55 ± 0.07^{e}	156 ± 44^{h}	42.2 ± 0.2^{g}	7.4 ± 0.2^{gh}	20.4 ± 0.2^{g}
	S	368 ± 24^{i}	0.35 ± 0.03^{b}	0.56 ± 0.04^{e}	72 ± 24^{i}	39.0 ± 0.1^{j}	7.35 ± 0.1^{gh}	20.5 ± 0.4^{g}
	WG	1052 ± 79^{bcd}	0.53 ± 0.02^{a}	0.92 ± 0.08^{a}	513 ± 79^{bc}	47.6 ± 0.1^{e}	11.5 ± 0.1^{c}	27.7 ± 0.2^{b}
	W	925 ± 22^{ef}	0.52 ± 0.04^{a}	0.91 ± 0.03^{a}	438 ± 22^{cde}	45.7 ± 0.1^{f}	11.4 ± 0.1^{cd}	27.6 ± 0.3^{b}
	RG	1122 ± 29^{b}	0.56 ± 0.05^{a}	0.89 ± 0.06^{a}	559 ± 30^{b}	48.8 ± 0.1^{c}	12.0 ± 0.2^{b}	28.0 ± 0.3^{b}
	R	995 ± 63^{cdef}	0.57 ± 0.02^{a}	0.84 ± 0.05^{ab}	476 ± 63^{cd}	48.2 ± 0.2^{d}	11.1 ± 0.2^{d}	27.7 ± 0.1^{b}

G: glazed frozen fish; HVEF: high voltage electrical field-thawed frozen fish; M: microwave-thawed frozen fish; O: ohmic-thawed frozen fish; R: refrigerated-thawed frozen fish; S: steam-thawed frozen fish; U: ultrasonic-thawed frozen fish; W: water-thawed frozen fish. Values are shown as mean ± standard deviation (n=3). Means with different letters in the same column are significantly different ($p < 0.05$).

In addition to thawing, lipid oxidation and activity of β-hydroxyacyl coenzyme A-dehydrogenase during freezing and thawing processes could be accelerated the destruction of MRA (Leygonie et al., 2012). Color changes could also be associated with metmyoglobin formation, which has been shown to be initiated when pro-oxidants (i.e., free radicals) produced by lipid oxidation during frozen storage (Leygonie et al., 2012; Ali et al., 2015). According to the results, color changes of samples subjected to the freezing-thawing process were considerable. There were significant differences (P> 0.05) in the L*, a* and b* values among various thawing methods. The pattern of color changes in S was similar to that observed in M. The fillets thawed using the microwave oven with glazing were darker (i.e., had lower L* values) compared to the others. The L* values of HVEF/HVEFG and U were higher than those in fresh and other thawing methods. Increasing in lightness could be related, partly, to the ozone and free radical production during the HVEF (Mousakhani-Ganjeh et al., 2015) and ultrasonic processes, respectively (Abedi et al., 2015; Tiwari, 2015). The lowest a* values were recorded for MG, M, SG and S fillets while the highest values were measured in samples thawed in the RG. It seems that overheating near the surface was a problem associated with the M processing, due to runaway heating and oxidation, thereby it reduced the values of a^* and b^* (Seyhun et al., 2013). The color pattern resulting from conventional heating (W/WG and R/RG) was similar to that obtained from modern thawing methods (HVEF and HVEFG).

Sensory Measurement

Sensory results of the assessments of raw and cooked samples are shown in Table 4. The scores in eyes for raw trout samples including HVEF, HVEFG, OG, UG, W, WG, R and RG; in texture for raw trout samples such as HVEF, HVEFG, OG, UG, W, WG, R and RG and in general appearance for raw trout samples including HVEF, HVEFG, U, UG, O, OG, W, WG, R and RG presented no significant difference (P> 0.05) with those of fresh fish. And also. A similar tends were shown for cooked samples in taste, odor, color and texture. The lowest score for the eyes was found in S, SG, M and MG. The results of raw and cooked samples also showed that S and SG had lower texture quality in comparison to the other groups. Furthermore, the taste of S and SG scored lower than the taste of other thawing methods. According to raw and cooked samples, the texture of samples of conventional and modern techniques (except for MG) was improved when the glazing process was used.

Effect of Thawing Methods on Overall Fish Properties

Fig. 3 shows the PCA biplot of the properties analyzed in all fresh and freeze-thawed rainbow trout samples. PC1 and PC2 explained 74.9% of the total variance of the dataset, contributing 52.6 and 22.3%, respectively. Samples were discriminated along PC1 based on the impact of the thawing method. The fresh fish on the right-hand side of the graph was described by high levels of thawing time, pH, proteins, color (L, a, b) and texture (springiness, chewiness, hardness, cohesiveness) parameters while it was opposed to S and SG samples. S and SG samples described by high driploss and content of fat, FFA and oxidation products. Along PC2, samples were positioned as a function of the thawing methods. Fishes treated with conventional processes (R, RG, W, WG, S, SG) are correlated to the negative values of PC2 while samples processed with modern thawing method are correlated to positive values of PC2 and high values of thawing rate, protein content and pH.

Table 4. Sensory acceptability of raw and cooked rainbow trout samples.

		Raw				Cooked		
	Sample	Eyes	Texture	Overall	Taste	Odor	Color	Texture
Modern thawing	Fresh	4.8 ± 0.7^{a}	4.8 ± 0.6^{a}	4.6 ± 0.4^{a}	4.4 ± 0.6^{a}	4.2 ± 0.7^{ab}	4.8 ± 0.4^{a}	4.7 ± 0.8^{a}
	MG	3.6 ± 0.6^{cde}	2.8 ± 0.5^{ef}	3.1 ± 0.9^{cd}	2.5 ± 0.3^{ef}	1.9 ± 0.6^{e}	1.9 ± 0.6^{e}	3.1 ± 0.5^{de}
	M	3.8 ± 0.9^{bcde}	3.2 ± 0.3^{de}	3.6 ± 0.3^{bc}	3.1 ± 0.8^{cde}	2.7 ± 0.4^{d}	2.4 ± 0.9^{e}	3.3 ± 0.7^{cd}
	UG	4.1 ± 0.6^{abcd}	4.0 ± 0.3^{abc}	4.1 ± 0.3^{ab}	3.6 ± 0.6^{abcd}	3.7 ± 0.6^{abc}	4.3 ± 0.6^{abc}	4.1 ± 0.6^{abcd}
	U	4.0 ± 0.3^{abcde}	3.9 ± 0.3^{bcd}	4.0 ± 0.5^{ab}	3.2 ± 0.6^{bcde}	3.3 ± 0.3^{cd}	4.0 ± 0.3^{abc}	4.0 ± 0.3^{abcd}
	HVEFG	4.6 ± 0.2^{ab}	4.4 ± 0.3^{ab}	4.4 ± 0.3^{ab}	4.2 ± 0.5^{a}	4.2 ± 0.5^{ab}	4.6 ± 0.4^{ab}	4.5 ± 0.8^{ab}
	HVEF	4.3 ± 0.2^{abcd}	4.1 ± 0.3^{abc}	4.1 ± 0.6^{ab}	3.9 ± 0.4^{abc}	3.8 ± 0.5^{abc}	4.5 ± 0.7^{ab}	4.1 ± 0.3^{abcd}
	OG	4.0 ± 0.2^{abcde}	3.5 ± 0.3^{cde}	4.3 ± 0.3^{ab}	3.1 ± 0.2^{cde}	3.2 ± 0.4^{cd}	3.8 ± 0.2^{bc}	3.9 ± 0.6^{abcd}
Conventional thawing	O	3.8 ± 0.9^{bcde}	3.2 ± 0.2^{de}	3.9 ± 0.3^{ab}	2.9 ± 0.4^{de}	3.5 ± 0.5^{bcd}	3.5 ± 0.5^{cd}	3.5 ± 0.5^{bcd}
	SG	3.5 ± 0.6^{de}	2.3 ± 0.5^{fg}	2.7 ± 0.4^{de}	1.7 ± 0.6^{fg}	1.8 ± 0.3^{e}	2.7 ± 0.5^{de}	2.1 ± 0.4^{ef}
	S	3.1 ± 0.3^{e}	1.9 ± 0.4^{g}	2.2 ± 0.7^{e}	1.2 ± 0.3^{g}	1.4 ± 0.3^{e}	2.1 ± 0.4^{e}	1.9 ± 0.5^{f}
	WG	4.6 ± 0.2^{ab}	4.6 ± 0.6^{ab}	4.5 ± 0.4^{a}	4.2 ± 0.5^{a}	4.3 ± 0.5^{ab}	4.2 ± 0.2^{abc}	4.3 ± 0.6^{abc}
	W	4.2 ± 0.2^{abcd}	4.5 ± 0.5^{ab}	4.2 ± 0.3^{ab}	3.9 ± 0.4^{abc}	4.0 ± 0.4^{abc}	4.3 ± 0.6^{abc}	4.0 ± 0.9^{abcd}
	RG	4.6 ± 0.4^{ab}	4.7 ± 0.3^{ab}	4.6 ± 0.3^{a}	4.4 ± 0.5^{a}	4.4 ± 0.2^{a}	4.3 ± 0.4^{abc}	4.3 ± 0.6^{abc}
	R	4.5 ± 0.2^{abc}	4.6 ± 0.6^{ab}	4.3 ± 0.2^{ab}	4.1 ± 0.4^{ab}	4.3 ± 0.3^{ab}	4.1 ± 0.2^{abc}	4.2 ± 0.6^{abcd}
	MG	3.6 ± 0.6^{cde}	2.8 ± 0.5^{ef}	3.1 ± 0.9^{cd}	2.5 ± 0.3^{ef}	1.9 ± 0.6^{e}	1.9 ± 0.6^{e}	3.1 ± 0.5^{de}
	M	3.8 ± 0.9^{bcde}	3.2 ± 0.3^{de}	3.6 ± 0.3^{bc}	3.1 ± 0.8^{cde}	2.7 ± 0.4^{d}	2.4 ± 0.9^{e}	3.3 ± 0.7^{cd}

G: glazed frozen fish; HVEF: high voltage electrical field-thawed frozen fish; M: microwave-thawed frozen fish; O: ohmic-thawed frozen fish; R: refrigerated-thawed frozen fish; S: steam-thawed frozen fish; U: ultrasonic-thawed frozen fish; W: water-thawed frozen fish. Values are shown as mean ± standard deviation (n=3). Means with different letters in the same column are significantly different ($p < 0.05$).

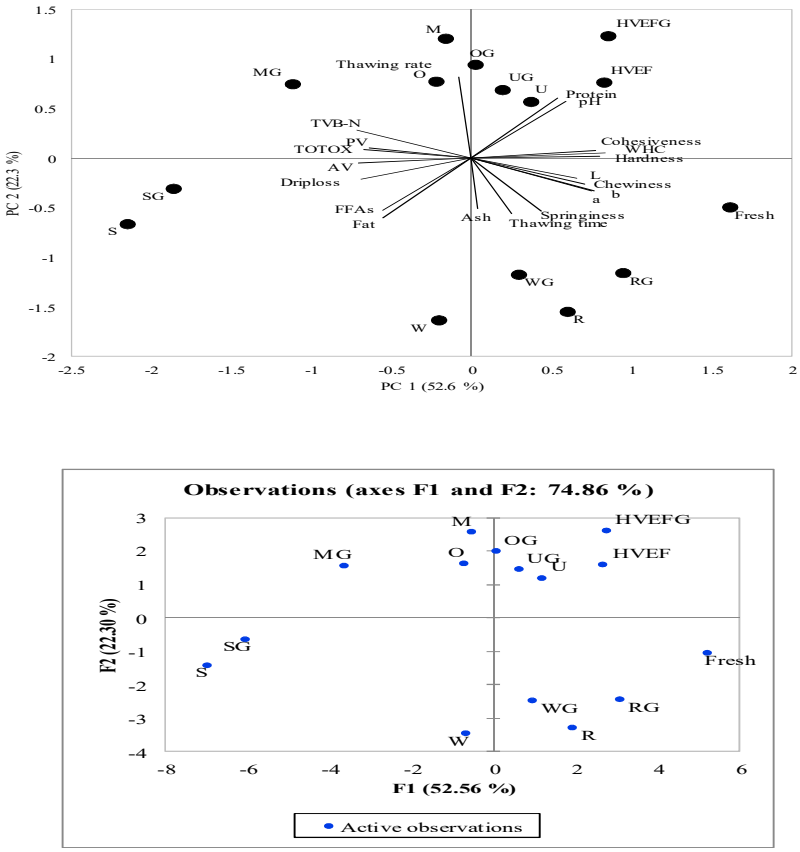

Fig. 3. Score plot (a) and loading plot (b) from the principal component analysis applied on thawing methods

CONCLUSIONS

In most parameters studied, the thawing methods, including modern (M/MG, U/ UG, HVEF/HVEFG, O/OG) and conventional (S/SG, W/WG, R/RG) methods, differed significantly from each other. The glazing process could improve the physico-chemical, color and textural properties of samples thawed by the conventional methods more than the

modern techniques. Although the M/MG samples required the shortest time to defrost, microwave thawing did not seem to be an appropriate method, due to the high PV and TOTOX value and to the relevant color changes. Furthermore, a significant increase of the FFA content was found in thawed glazed samples (MG, UG and OG) as a result of the increased thawing time required. S-SG and HVEF/HVEFG received the lowest and highest sensory scores, respectively. In conclusion, RG and HVEF/HVEFG can be considered the best thawing methods for air-blast frozen rainbow trout.

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Ethical statement

Conflict of Interest: The authors declare that they do not have any conflict of interest.
Ethical Review: "This study does not involve any human or animal testing"

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بررسی مقایسه‌ای خواص فیزیکوشیمیایی و حسی دیفراست با روش‌های مختلف در قزل‌آلای رنگین کمان منجمد با و بدون لعاب، تحت روش آنالیز خوشه‌ای چند متغیره

محمود ناصری[۱*]، الهه عابدی[۲]، سارا وفا[۱]، لوئیسا توری[۳]

[۱]بخش مهندسی منابع طبیعی و محیط زیست، دانشکده کشاورزی، دانشگاه شیراز
[۲] بخش مهندسی علوم و صنایع غذایی، دانشکده کشاورزی، دانشگاه فسا
[۳]دانشگاه علوم گوارش، برا، ایتالیا

*نویسنده مسئول

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واژه‌های کلیدی:
میدان الکتریکی با ولتاژ بالا
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ماهی قزل آلا
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چکیده- کیفیت قزل آلای رنگین کمان (*Oncorhynchus mykiss*) منجمد شده با لعاب و بدون آن، پس از خروج از انجماد به روش های متداول (بخاردهی، آب و یخچالی) و نوین (مایکروویو، میدان الکتریکی با ولتاژ بالا و روش اهمیک) مورد مقایسه قرار گرفت. نمونه های منجمد شده با لعاب، پس از خروج از انجماد به روش متداول و نوین (به جز نمونه M و MG) افزایشی را در میزان pH و پروتئین نشان دادند. فرایند لعاب دهی به صورت منفی بر میزان TVNB (میزان کلی نیتروژن باز فرار) و FFA% (میزان اسید چرب آزاد) اثر گذاشت و میزان این ترکیبات بعد از لعاب دهی در تمامی نمونه ها بعد از خروج از انجماد به استثنای SG (بخاردهی با لعاب) و S (بخاردهی) در روش متداول و M، MG، U و UG از روش مدرن افزایش یافت. میزان TOTOX در نمونه های HVEF HVEFG و OG پس از خروج از انجماد نسبت به نمونه ماهی طبیعی تغییر معناداری نداشت. لعاب دهی، میزان از دست دادن آب را کاهش و میزان ظرفیت نگهداری آب را در تمام نمونه های خروج از انجماد به استثنای MG افزایش داد. نمونه های MG و HVEFG/RG/WG به ترتیب دارای بیشترین و کمترین میزان سفتی بافت بودند. میزان L^* در HVEF/HVEFG و U نسبت به نمونه ماهی تیمار نشده و دیگر تیمارها بزرگتر بود.