

Measuring the Amount of Water Activity (a_w) in Hot Smoked Rainbow Trout (*Oncorhynchus Mykiss*) in Various Salinity Percentages

Raheleh Esmailnia

Master Degree in Aquatics Breeding and Propagation; Department of Natural Resources; Islamic Azad University
Lahijan Branch, Iran

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ABSTRACT

Since smoked fishes are amongst fishery products of the northern areas of the country, it seems essential to conduct a study on the manner of maintaining the quality of this product throughout its storage period.

In the present study 15 Rainbow Trout were washed, their viscera were removed afterwards, and then they were salted (the wet smoking method) for 3 hours in different salt concentrations including 10%, 15%, and 20% and then they were hot smoked in the Atmos smoking machine. They were then packed under vacuum conditions and kept in the refrigerator at 4°C.

Salinity of 10 percent, which has been usual method of smoking industrial fish in Bandar-e Anzali Research Center, is considered as control treatment, and treatments of 15 and 2 percent are as comparable treatments. Experiments related to qualitative changes and measuring a_w have been performed on fish, respectively at 0, 15, 30, 45 days. Progress in Lipids' pungency measured by several biochemical parameters such as Free Fatty Acids (FFA), Peroxide (Pv) and Thiobarbituric Acid (TBA) as well as total Bacterial Flora (total count).

Moreover, according to performed tests on 45th day of retention, the amount of bacteria did not even reach to allowable amount. However the amount of FFA, Pr, and TBA had increasing trend but through decreasing a_w , by smoking, salting, and packing in vacuum decreasing a_w , we made the gradient of this increase extremely slow. And the most appropriate a_w to Rainbow Trout and Silver Carp has been measured respectively 0.727 and 0.748. In general, results indicated that the smoked treatments and packed ones under vacuum, in all measured parameters of fat rancidity and bacterial rancidity and a_w , have had significant differences in various treatments and maintaining days ($P < 0.05$). And such types of hot smoking, vacuum packaging, salt percentages and maintenance in the fridge at 4°C have caused to retain the product quality during the 45 days of storage.

Rainbow trout, according to the obtained results from chemical and microbial factors and organoleptic test is consumable until day of 45, but from that day as a result of intensive reduction in its taste (organoleptic test) and taking out of the standard range of chemical factors its using is not recommended. As well as, due to bacterial and chemical factors and organoleptic test it can be concluded that rainbow trout is appropriate to this method of smoking.

KEYWORDS: Rainbow Trout (*Oncorhynchus Mykiss*); Hot Smoked; Vacuum Packaging; Qualitative Control; Active Water; Activated; Corruption

INTRODUCTION

The Specie which is used in this study is rainbow trout (*Oncorhynchus Mykiss* that is a valuable commercial species and greatly is used by consumers around the world (Cakli et al., 2006).

According to this fact that nowadays seafood as healthy and protein foods are much consumed, the importance of its maintenance, health principles and the quality preservation is necessary. This is due to high corruptibility of seafood, especially fish, during transportation and maintenance before using and cooking. One of the most important factors issues in fish corruptibility is the existence of different microorganisms, including bacteria, fungi, mold and yeast. These microorganisms can highly grow and reproduce in wet and humid environment that it is prepare based on high levels of free water in fish texture. On the other hand, fish freshness is one of the main features in food quality.

Smoking is a food preservation method, which has been done traditionally since a few centuries ago. Making Smoked fish through traditional, due to production of carcinogenic substances such as benzopyrene and Benzo(a)anthracene, and secondary infection in non-normative maintenance conditions is not secure in aspect of safety and nutrition health, there it has been tried to improve this process by using of modern and industrial methods of smoking (Hot smoking (Razavi Shirazi, 2007).

* **Corresponding Author:** Raheleh Esmailnia, Master Degree in Aquatics Breeding and Propagation; Department of Natural Resources; Islamic Azad University Lahijan Branch, Email:ahmadpoor_h@yahoo.com

On the basis of this fact that making this product in Iran is traditionally, making smoked fish through industrial method is related to exact estimate of aw , and other relevant factors. In this study, according to measurement of aw by aw meter device which is called *Lab swift aw*, the measurement of biochemical indices such as free fatty acids (FFA) (Anons, 1988) and peroxide (PV) (Anons, 1988) and tiobarbiotic acid (TBA) (Egan, H.1997), as well as and total bacterial flora (total count) (Ibrahim sallam, K, 2007), and also measuring salt absorption has been performed according to the "Parvane" reference, 1991.

The quality of smoked fish during retain period of 45 days in the fridge at 4°C has been evaluated and its impact on durability period has been investigated; and to enhance its durability, in addition to reduction by hot smoking (Mirza khani, 2011) and cooking within Atmoos and saltation, respectively in the percentage of 10 (as a control), 15, 20, some other activities such as packaging under vacuum and keeping in the fridge at 4°C have been carried out to prevent oxidative corruption. In order to achieve a relative balance between maintaining humidity and freshness, as well as elimination and inactivation of microorganisms, an indicator which is called water activity (aw), can be used (Gram and Huss, 2000).

Therefore, the optimal combination of maintaining freshness on the one hand, and controlling microorganism through reducing humidity within fish texture on the other hand are considered as a challenge for nutritionists. In order to achieve a relative balance between maintaining humidity and freshness, as well as elimination and inactivation of microorganisms, an indicator which is called water activity (aw), can be used (Gram and Huss, 2000).

Aw is a measure to evaluate how water can be involved in a chemical or physical reaction. Microorganisms, without existence of this water cannot continue their life and growth. The amount of water required for all germs is not the same, and necessary water for the continuation of life is called water activity. By definition, the water activity is the ratio of vapor pressure in nutrients, at a certain temperature, to vapor pressure of pure water at the same temperature, which is demonstrated as following equation (Robinson, 1984).

$$aw = \frac{P}{P_0}$$

aw = water activity

P = vapor pressure in nutrients

P_0 = vapor pressure of pure water at the same temperature and conditions

aw in nutrition science belongs to applying in product development, quality control and food safety. As well as it has become a very important scale to evaluate and control of food safety and quality (Roos, 1993).

Currently, over 50 percent of foods disappear in third world countries due to lack of proper packaging and maintenance. While in industrial countries the rate of this type of waste is less than 5 percent, and the food in the hands of countries has always been as a weapon against third world developing countries. Therefore, proper packaging of food is considered as one of applied methods to prevent food waste (Jahanbazi Goojani, 2003).

Vacuum packaging also is a type of modified atmosphere packaging system, in which the air is removed from the package and the package is sealed (Cakliet al., 2006). Therefore, a combination of smoking methods, vacuum packaging, and storing in the refrigerator 4°C can increase durability of seafood (Dhananjaya and Stroud, 1994).

Therefore, with regard to the existing limitations, this study intends to determine the proper aw for smoked rainbow trout which can be used as a sample for estimating the (aw) of other fishery products and other food products as well. Moreover we can increase the durability of rainbow trout through the process of salting, smoking, packing under vacuum conditions, and also keeping them in the refrigerator at 4°C.

Method of Research:

Materials (instruments): rainbow trout with an average weight of 650 grams, metalized bags for vacuum packaging

Spices: salt NaCl- onion- lemon juice- tomato paste- chloroform- methanol- distilled water- Glacial acetic acid- potassium iodide- thiosulfate (0.01 m), neutralized ethanol, phenolphthalein 0.5 cc, ethanolic potassium hydroxide (0.1 m), phenolphthalein (0.01 m), and physiologic serum

Equipment

A Balance with accuracy of 0.0001g (MEHLER model), spectrophotometer device (HACH 2000 /DR Model), vacuum packaging device (500/400 DZQ), laboratory glassware, industrial smoke machine Atemouse, aw meter device which is called Lab swift- aw

Sampling

Initially rainbow trout with an average weight of 650 g have been considered. These fish were bought from a fish farm in Khomam. Then, they was transferred to Aquaculture Research Center of Iran (in Bandar-e Anzali) inside polystyrene with complete ice cover.

Processing

After opening the abdominal area and removing all the viscera and washing the trout carefully we will prepare them to be placed in the salt water. Three different containers are considered in such a manner that the first container is labeled treatment (1) the second container is labeled treatment (2), and the third container is labeled treatment (3) (FRW, 2004).

Each container will contain 5 fish from each sample. The Percentage of used salt in three containers respectively was 10, 15 and 20 percent. The reason of choosing these percentages of salt was that Anzali research center, usually has added 10 percent of salt, and because different saltiness were significant to researchers, to make a significant difference, second and third treatments respectively were assumed 15 and 20 percent. Since In traditional method of smoking fish is immersed in saturated salt water and without specifying the exact percentage of salt, choosing 20 percent saltiness is reasonable. Spices which are used as essence of fish before smoking, are turmeric, black pepper, cardamom powder, coriander, nutmeg and cumin, and also, materials such as onion, lemon juice and tomato paste that were separately formulated for each treatment. We mixed ingredients well together. And Fish will carefully remain in this essences and flavorings about 3 hours, so that they absorb salt and flavorings. We write the number of treatments on each container, and put them in refrigerated up to 3 hours. After 3 hours, we will remove fish from essences, then wash them, and stay to remove their water out, and finally hung them (Mirza khani, 2011).

Hot Smoking Method:

The fish were smoked using Iran's National Fish Processing Research center's popular method (Mirzakhani, 2011). The fish were assigned specific codes and then placed into the Atemouse in order to prevent confusion regarding the treatments. Atemouse is a device to hot smoking different types of. Temperature degree and time will set up According to the type of fish. Rainbow trout needs initial temperature of 60°C, to cook which is set up on 1 hour. After initial cooking, straw of alder, which are soaked from the night before will be put in exhaust part of device. This section consists of 3 parts. The first part, which straws will be there, then after rotation and warming straw they will be in lower part that is the part producing smoke, and after creating a lot of smoke, this smoke will be exited through a pipe from this part and will enter to device. In this case, the device temperature will be set on 60°C, for an hour, and in this way fish will be smoked in addition to cooking. After one hour, the device temperature will be set again on 70°C for one hour; and the next step of cooking along with smoking device temperature will be 80°C, for two hours. So, in general the time duration of cooking these fish is about 5 hours in temperature 60°C to 80°C (Mirza khani, 2011).

Vacuum Packaging Method:

We will wait for a while for the fish to cool down after smoking them and then we will begin the packaging stages. Now, we put samples which are inside the metalized bags in vacuum packager and then packing them; half of fish will be send to Rasht Science and Technology Park to measure aw, and the other half will be transmit to Qazvin Pirayeh Zist Laboratory to perform chemical and T.C. experiments. Fish were kept for 45 days in the fridge at 4°C. Conducted chemical tests to investigate the process of oxidation and corruption of fish including the measurement of free fatty acids (FFA) (Anon, 1988), measurement of peroxide (PV) (Anon, 1988), measurement of Thiobarbituric acid (TBA) (Egan et al. 1997) and measurement of bacterial flora, as Total count (Ibrahim sallam, 2007). Aw well as measuring aw has been done in Rasht Science and Technology Center. Experiments were conducted with two replications on days of 0, 15, 30 and 45. Measuring the salt absorption has been performed according to the Parvaneh reference (Parvaneh, 1991).

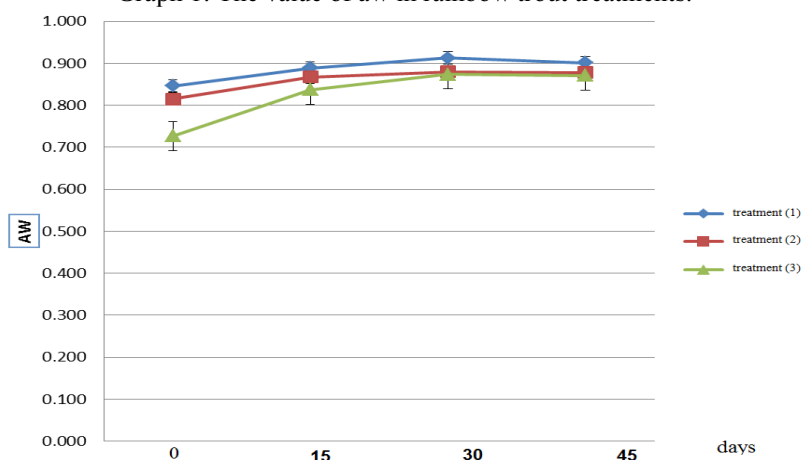
Statistical Analysis

Collected data and figures have been analyzed randomly and in factorial by using in Excel and SPSS software; and means were compared through Duncan test at level of 5% ($P = 0.05$).

RESULTS

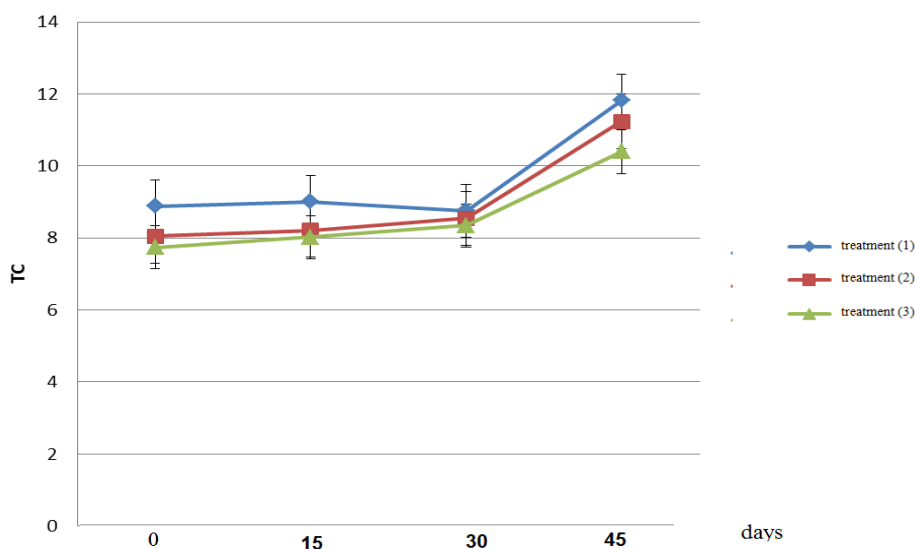
Appropriate aw for rainbow trout is as a criterion to the health of fish, because the activity of different types of microorganisms occurs in their certain aw areas (J. Olley et al, 1984)

Graph 1: The value of aw in rainbow trout treatments:



According to this diagram and Analysis of variance (ANOVA) among hot-smoked rainbow trout treatments of 1, 2 and 3, there is significant difference in the value of aw ($P < 0.05$) and there is a significant difference between phases of 0, 15, 30, 45 ($P < 0.05$). The most appropriate aw is appeared in treatment of 3 (20% NaCl).

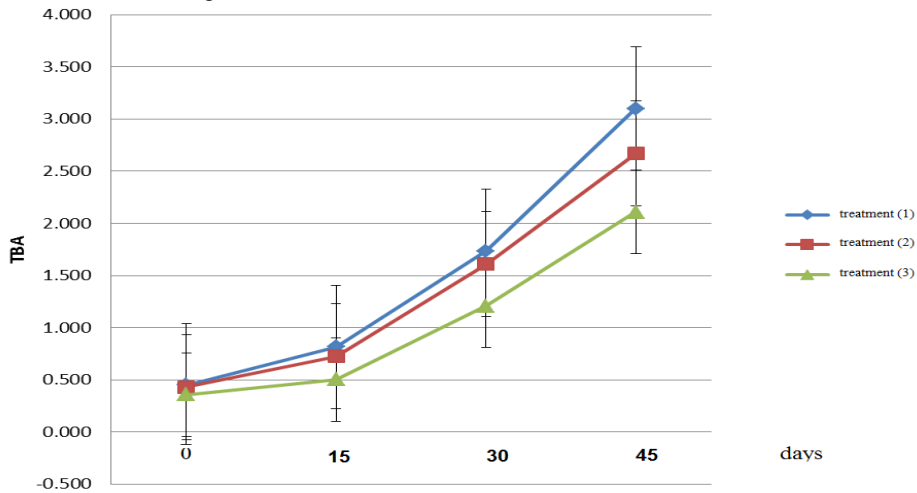
Graph 2: The value of T.C. in treatments of rainbow trout:



Microorganisms are the main causes of food corruption. The changes in total microbial load of hot-smoked rainbow trout have been shown in above diagram. Evaluated microbial load in hot-smoked rainbow trout has been measured on day 45, equal to $\log \text{cfu/g } 10/4 \pm 0.0042$. The limit of growing total microbial load which is permitted to use has been reported $16.11 \log \text{cfu/g}$ (Rezai et al., 2004).

On the basis of this standard, smoked rainbow trout can be consumed until day 45 regardless microbial load. According to ANOVA and Duncan test, there is a significant difference between treatments over passing time ($P < 0.05$). Rainbow trout treatment 3, has the lowest T.C among other treatments, in the phase of 0 and 45.

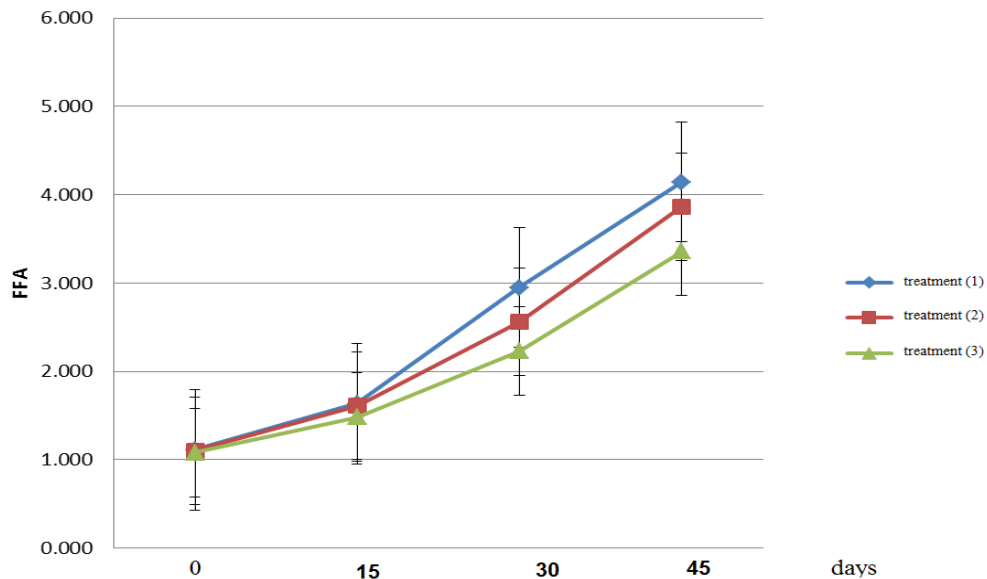
Graph 3: the value of TBA in rainbow trout treatments



TBA is one of indices which is used to estimate the rate of fat oxidation in fish meat (Couliara et al., 2004). According to the diagram and Table 3, TBA in different treatments has had increasing trend, but this trend due to the reduction of aw in smoking and addition of NaCl, or salting down, has been decreased; vacuum packaging also has been effective in reduction of this increasing trend. The amount of 3-4 gram Malone aldehyde in fish meat/ kg, indicating lowering its quality (Karkam, H., and Boran, 1996). This situation just can be observed in treatment 1 of rainbow trout.

According to ANOVA and Duncan test, there is a significant difference between treatments of rainbow trout ($P < 0.05$) and also there is a significant difference in aspect of storage time ($P < 0.05$). Due to comparing rainbow trout treatments, treatment 3 in phase 45 has the lowest TBA compare to other treatments of rainbow trout in this phase.

Graph 4: free fatty acids in the rainbow trout treatments



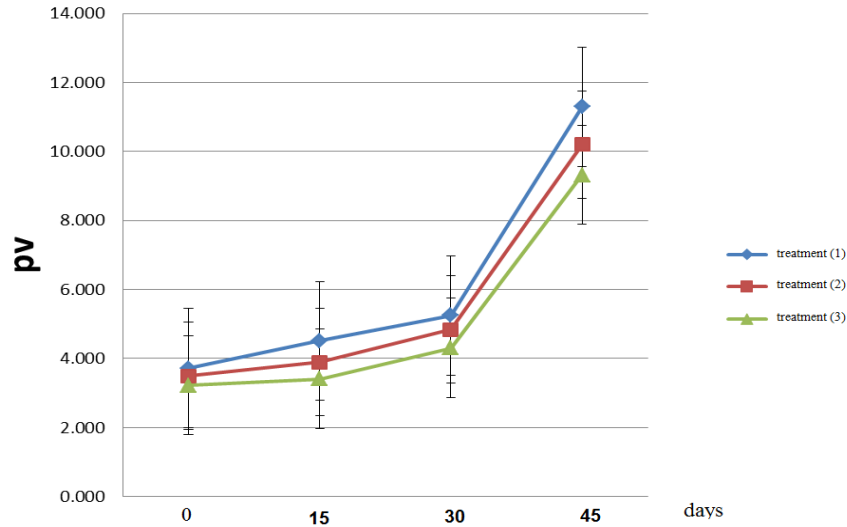
According to the chart, the amount of free fatty acids has increased, in all treatments of hot smoked rainbow trout through vacuum packaging method. The Increase of FFA in samples cause unpleasant taste and smell, because it reacts with proteins and result in protein denaturation and tissue changes (Lodas et al., 2004). Similar results has been obtained in studying the effect of vacuum packaging on durability time of *Pampus Argenteus*, during 19 days of storage at temperature of 4°C (Perez et al., 2004).

Reduction of aw due to heating by Atemouse and addition of NaCl and significant impact of vacuum packaging result in fat hydrolysis reduction. As well as, Fagan et al., in a research on the impact of packaging in

modified atmosphere on the Mackerel and Salmon qualitative parameters have found similar conclusions. This result suggests that the reduction of O₂ in packaging has significant impact on reduction of fat hydrolysis and increase of storage time. According to ANOVA and Duncan, the difference between data and the storage time has been significant ($P < 0.05$).

According to ANOVA and Duncan test, there is a significant difference between treatments of rainbow trout in aspect of FFA data ($P < 0.05$). The treatment (3) rainbow trout has the lowest FFA levels in phase 45 in comparison with the other rainbow trout treatments in this phase.

Graph 5: The PV treatments rainbow trout:



In different treatments of Rainbow trout PV has had increasing trend and significant difference ($P < 0.05$). The increase of PV more than 5 mill equivalents of O₂ in 1000 g fat indicate reduction of fish quality (Karkam, H., and Boran, 1996); and permitted amount of peroxide in fish fillets for human consumption is 10 mill equivalents of O₂ in 1000 g fat, which in this study, treatment of 2 and 1 at temperature of 4°C the amount of peroxide will be more than permitted value.

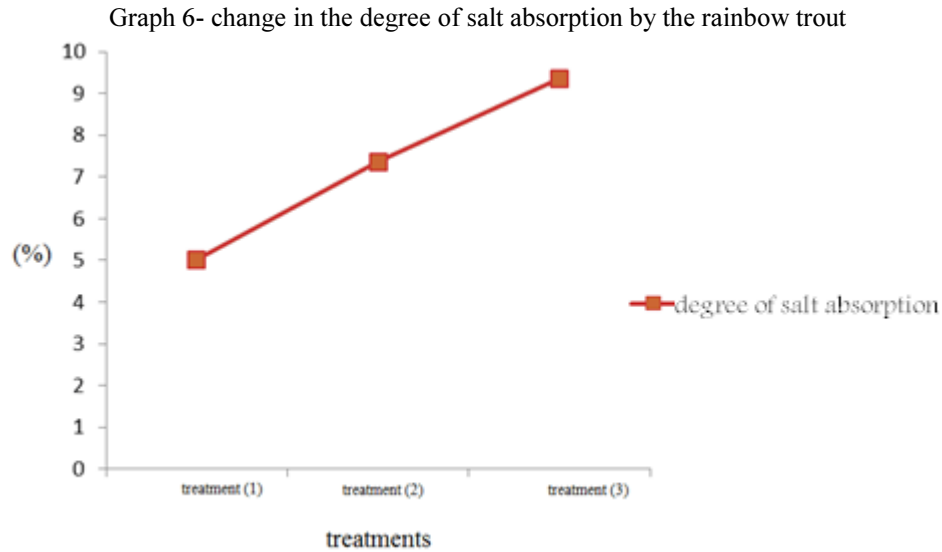
(Figure 5) making smoked along with heating and adding NaCl, especially in treatment 3 of rainbow trout, as well as, vacuum packaging affect increasing trend of peroxide in the present study and result in decrease of changes, which is consistent with the results of Fagan's, et al. (2004) study, on the impact of packaging on the quality of mackerel and salmon fish, as well as the study of Anelich et al. on African catfish. According to ANOVA and Duncan test data between treatments has been significant during time ($P < 0.05$). According to ANOVA and Duncan test, there is significant difference between PV and treatments over time ($P < 0.05$). Comparing the number of PV in different treatments shows that only in treatments 3, in the Phase 45, the PV is not higher than permitted value (Karkam, H., and Boran, 1996).

Salt Absorption in Rainbow Trout:

The increase of salt concentration in the essence which treatments are in it will increase salt absorption treatments. According to ANOVA and Duncan test, salt absorption in the tissue of treatments has significant difference ($P < 0.05$).

Treatments	Salt concentration in the essence	salt absorption in rainbow trout
1	%10	5.02%
2	%15	7.36%
3	%20	9.36%

Table 1 - Changes in the amount of salt absorption of rainbow trout (Parvaneh, 1991)

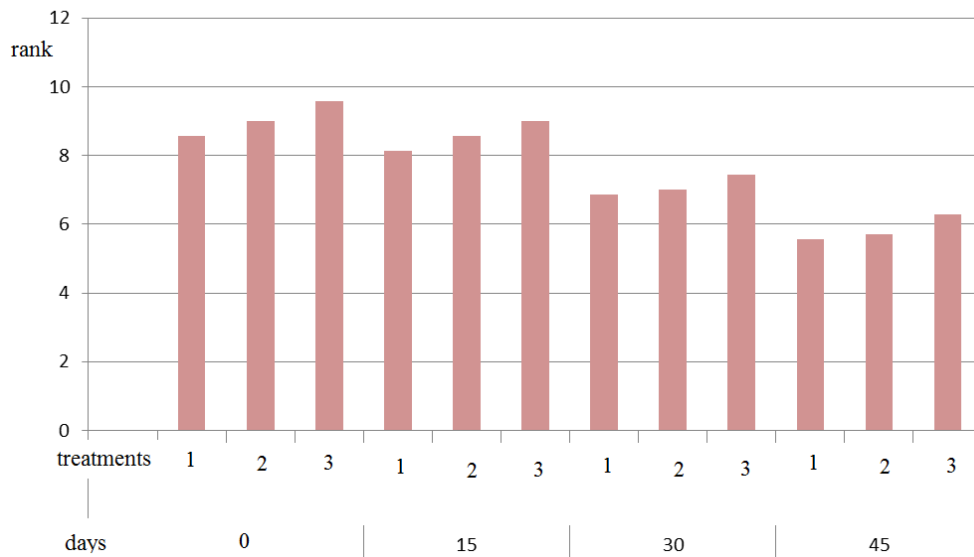


Organoleptic Test

In order to sensory evaluation of hot smoked rainbow trout, during maintenance period the method of Gallas and ketaminas in 2007 was used. Therefore, a group of 7-member panel was applied. All evaluators were members of laboratory, who before starting the test, trained sensory evaluation in 3 session. Sensory evaluation were performed on taste, color, smell, and texture. Fish samples were stored at temperature of 4°C. Sensory evaluation were carried out under equal light and temperature conditions, which these conditions were stable during all experiment period. To rating and scoring a scale of 0 to 10, where 10 was the highest and 0 the lowest score, was used.

A Product with a score less than 6 was identified as unacceptable product. This method of scoring completely is consistent with the reference of Gallas and ketaminas in 2007. Analyzing Organoleptic indices along with chemical experiments (as a supplementary method) to determine the extent of corruption and durability of fish and its products is necessary (standard 1995, 3580). Sensory evaluation is considered as a proper method to estimate durability of fish and its products during storage time. (Tang et al, 2001)

Figure 7: The results of rainbow trout organoleptic test



According to figure 6, it can be concluded that by passing time, within 45 days, smell and taste of smoked rainbow trout will be in associated with reduction, so that based on measure 6 in this test, the best taste related to

treatment 3 is on day zero. And treatments of 1 and 2, on day 45, according to reference of Goulas and Ketaminas in 2007 is inedible. Other results of this section are high score of treatment 3 during 45 days of experimentation, compared to other treatments.

CONCLUSION AND DISCUSSION

With regard to the results, treatment 3 with 20% salt concentration and the figure 0.727 can be introduced as the most suitable water activity for the hot smoked rainbow trout. (Due to the low aw of these products and limitation of bacterial activity, necessary aw for Gram-negative bacteria is -0.97, for Gram-positive bacteria: 0.90, for Halophilic bacteria: -0.75, for yeast: -0.88, fungi: 0.80) (J.OLEY, 2004).

the present study, in which using industrial smoking along with heating and cooking, as well as adding NaCl and keeping in the fridge of 4°C significantly lowered increasing trend of aw during maintenance, indicate that keeping the quality of product is due to applying industrial smoking method along with keeping in the fridge of 4°C. Adding salt with concentrations up to 20 percent, as well as, packaging in vacuum have had a considerable impact on this product.

Aw contribute limiting or slowing unpleasant reactions, such as lipid oxidation, degradation of vitamins, enzymatic reactions. In addition it preserve product quality and increase its durability (Wolf Ouckworth, 1984). Aw to measure water without solutes is 100 percent that are available to use by microorganisms. But when certain substances such as salt and sugar are dissolved in water, aw decreases, and water are less accessible to microorganisms, in other words it will be blocked (Neumeiger and Ross and Thomson.1997).

Changes of aw in the samples indicate proteins denaturation, because the capacity of maintenance active water is directly related to protein myofibrils linked (Suvanich et al., 2003). There are similar results on packaging in vacuum and modified atmosphere and sardines' chemical changes (Ozgoles et al., 2003).

Moreover, hot smoking hot along with a high concentration of salt, and vacuum packaging and keeping at fridge of 4°C have positive impacts on lipid oxidative corruption factors and reduce them and the speed of their corruption. So that smoked and packed under vacuum fish has retain its quality largely, at intervals of 30 to 45 days at fridge of 4°C.

Rainbow trout of treatment 3, in aspect of organoleptic test, as well as other chemical and microbial factors can be used up to day of 45, and over that period, due to exceeding the permitted reference of Goulas and Ketaminas (Goulas, 2007 colleagues) cannot be used. In addition, by comparing treatments of rainbow trout during the study period (45 days) in different treatments and phases, it can be conclude that the lowest aw of rainbow trout is belong to treatment 3 in phase zero. Moreover the amount of TC, TBA, FFA, and PV in rainbow trout treatment 3 is the lowest level among the other treatments in the phase 45; and even just PV of rainbow trout treatment 3 in phase 45 pass not the limit. Rainbow trout treatment 3 in aspect of organoleptic test has had the highest scores in different phases. The results demonstrate that rainbow trout, with salinity of 20% , in comparison with other treatments is the most suitable fish to this method of smoking, in terms of keeping quality and preventing corruption, as well as organoleptic test.

The results of this study are consistent with other studies performed in the field of vacuum packaging, (Egan, 1997 et al 2007). Hence, using hot smoked fish in this type of packaging, is recommended due to maintenance of above mentioned fish quality.

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