# Effect of different packaging methods on shelf life of hot smoked rainbow trout (*Oncorhynchus mykiss*) during storage at 0-2°C

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**Abstract**

This study was carried out to investigate the effects of three different packing methods including modified atmosphere, vacuum and common packaging on chemical, microbial and sensorial quality and shelf life of hot smoked Rainbow trout (*Oncorhynchus mykiss*) during storage at refrigerated temperature. For hot smoking, rainbow trout were gutted and cut in butterfly form and washed. Fish gutted brining with 15% salt for 4 h and they were hot smoked by using Atmoos smoking cooking room. Then hot smoked samples were packed in modified atmosphere (with gas ratio of, $N_2$:50%, $O_2$:5%, $CO_2$:45%), vacuum and common packaging and stored at refrigerated temperature (0-2°C). Chemical values (total volatile basic nitrogen, moisture, peroxide, pH), microbial values (total microbial count, mold and yeast) and sensorial index (aroma, flavor, color and texture) were determined on 0, 10, 20, 30 and 40 days of storage. The results showed that samples did not have a significant difference in moisture and pH values ($p>0.05$), however significant different was found in peroxide, total count, mold and yeast and TVB-N values ($p<0.05$).Also, results of sensory evaluation have been showed that the score of color, flavor, texture and aroma in modified atmosphere packaging were more than other treatments ($p<0.05$). The Shelf life of the hot smoked trout is specified between 20 to 30 days, 30 to 40 days and at least 40 days in common, vacuum and MAP packaging, respectively. So, using the modified atmosphere for hot smoked trout can enhance quality and increase the shelf life of trout in refrigerated storage condition up to 40 days.

**Keywords**: Rainbow trout, Hot smoking, Shelf life, Modified atmosphere, Vacuum packaging

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## Introduction

Rainbow trout (*Oncorhynchus mykiss*) is a species with high commercial value and much appreciated by European consumers (Cakli et al., 2006). Fish are a cheap source of animal protein with little or no religious rejection of it. It must be preserved in some way because it is disposed of quickly after capture (Silva, 2002). Smoking is a centuries old food preservation technique (Ibrahim et al., 2008). The traditional fish smoking is not a sure method, because product is exposure to second pollution in undesirable storage condition. Also, this way produces cancer material such as benzopirin and benzoanterasen. Thus industrial smoking method improves smoked products (Razavi, 2008). Modified Atmosphere Packaging (MAP) is a type of packaging that in it, the bags was filled by a mixture of gases. The composition of gases in it is different with air composition (Cakli et al., 2006). Vacuum Packaging (VP) is exclusion of the air from the package and thus creating a vacuum also is, in effects a certain type of modification of the atmosphere (Hall, 1994). In study of Dondero et al., (2004) changes in the quality of vacuum packed cold smoked salmon (*Salmo salar*) were evaluated through a systematic study of biochemical, microbiological and sensory analyses during storage at different temperatures. Cakli et al. (2006) in Turkey compared the shelf lives of MAP and vacuum packaged traditional hot smoked rainbow trout (*Oncorhynchus mykiss*). This study was done in National Fish Processing Research Center in Bandar Anzali on industrial hot smoked rainbow trout. The aim of the this study was to investigate the effect of three different packing methods on the shelf life and of hot smoked rainbow trout (*Oncorhynchus mykiss*) at refrigerated temperature (0-2 ºC).

## Materials and methods

For preparing samples, alive rainbow trout at marketing size (277±31 g) were purchased from fish farming in Anzali Harbour. Then fish by using chilled sea water tanks (CSW) with 2 to 1 in ratio ice and water, transferred to National Fish Processing Research Center (Anzali Harbour). Fish were washed, gutted and cut in butterfly form. Hot smoked rainbow trout were prepared according to specific way of flesh smoking in Iranian National Center (food and drug of Guilan University of medical sciences, 2010) and with industrial smoking machine – Atmoos (Germany) and with using *Fagus orientalis* wood. The samples packed in polyethylene bags. For VP and MAP packing carried out with Multi VAC machine (A 300/16-Germany). In each package, there was one fish. Fish were packed in 3 treatments and 3 replicates for each treatment. The treatments were Included: treatment 1 (Hot smoked rainbow trout packed in common packaging or C), treatment 2 (Hot smoked rainbow trout packed in vacuum packaging or VP), treatment 3 (Hot smoked rainbow trout packed in modified atmosphere packaging or MAP). Then all 3 treatments were kept in cold room (0-2 ºC) for 40 days. Sampling has done for 5 times. First step of sampling has done after smoking

and the other sampling has done with 10 days interval (0, 10, 20, 30 and 40 days). Moisture value was determined with drying method in oven at 105 ºC for 8 h (Parvaneh, 1998). Lee method was used to determine the peroxide value (Pearson, 1997). The pH value determined by using of digital pH-meter (Switzerland Az86p3). Fish muscle (2 g) was homogenized thoroughly with 10 ml of distilled water and the homogenate was used for pH determination. Kjeldahl method was used for determining amount of TVB-N according to Iranian National Standard No.5558 (Parvaneh, 1998). For total microbial count, treatments were prepared according to Iranian National Standard No.8923-1 and mold and yeast counting have done according to Iranian National Standard No.10899-1. Sensory assessments included the evaluation of three parameters (aroma, flavor, color and texture) were conducted by nine trained test panel. According to the method of Watts et al. (1989) and Iranian National Standard No.7431 (1992), 5 categories of acceptability were ranked: excellent quality (5), very good quality (4), good quality (3), acceptable (2) and unacceptable (1). Samples of each treatment were taken at regular intervals and the scores given to the judgments were analyzed. Statistical analysis values were presented as "means ± SD". To statistically analyze data, One-way ANOVA was applied to determine the differences between two groups of chemical and microbial data. The mean value compared using Duncan test at reliability level of 5%. Also, K-Related sample t-test (Friedman test) was used to determine the differences between sensorial. Data analysis was done in SPSS software (release 15.0).

## Results

Table 1 shows the biochemical composition of fresh and hot smoked (*Oncorhynchus mykiss*).

As indicated in Table 2, moisture content of raw rainbow trout was decreased after smoking and during storage. Results showed that there was not significant statistic difference between moisture value for different treatments during 0, 10, 20, 30 and 40 days ($P>0.05$). Also, investigation on the effect of time on the moisture content was showed that there was significant difference in level 5% between mean of moisture in different times in all treatment ($P\leq0.05$) (Table 2).

Changes of pH value of samples are showed in Table 2. pH value decreased to $20^{th}$ day of storage and then increased in all treatment. Results showed that there wasn't significant statistic difference between pH value for different treatments during 0, 10, 20, 30 and 40 days ($P>0.05$). Also, investigation of effect of time on pH value was showed that there was not significant difference between mean of pH value in different times in all treatments ($P>0.05$) (Table 2).

Total volatile bases nitrogen value of all samples is presented in Table 2. The TVB-N value in all treatment increased. There was significant statistic difference between TVB-N value for different treatments during 0, 10, 20, 30 and 40 days ($P<0.05$). Investigation on the effect of time on the TVB-N value was showed, there was significant difference between

mean of TVB-N value in different times in three packaging (P<0.05) (Table 2). Results showed that there was significant statistic difference between peroxide value (PV) for different treatments during phases 2, 3 and 4 of sampling (P<0.05). Also, investigation on the effect of time on the PV value shows, there was significant difference between mean of PV value in different times in three packaging (P<0.05) (Table 2). Changes of peroxide value of samples is shown (Table 2). Results of microbial analyses showed that there was significant difference between total count in all three treatment after 30$^{th}$ day of storage period (P<0.05). But, there was no significant difference in mold and yeast count in different treatments during 0, 10, 20, 30 and 40 days (P<0.05). The level of total count and mold and yeast count in all treatments was found to increase significantly different (P<0.05) with storage period (Table 2).

**Table 1: Biochemical composition of fresh and hot smoked rainbow trout (*Oncorhynchus mykiss*)**

| Hot smoked | fresh | component* |
|---|---|---|
| 60.3± 1.3 | 74.6 ±0.8 | erutsioM (%) |
| 23. 9± 0.21 | 19.1 ± 0.37 | Protein (%) |
| 7.8 ±0.11 | 4.8 ± 0.11 | fat (%) |
| 4.10± 0.29 | 0.98 ± 0.19 | Ash (%) |

* n=3

**Table 2: Changes in Chemical and microbial parameters of hot smoked rainbow trout in different packaging during refrigerated storage under Common packaging (C),Vacuum packaging (VP) and modified atmosphere packaging (MAP)**

| **Factor *** | **treatment** | **Storage time (days)** | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 10 | 20 | 30 | 40 |
| pH | C | 6.23 ± 0.05 $^{a,A}$ | 6.13±0.05 $^{a,A}$ | 6.07± 0.10 $^{a,A}$ | 6.08± 0.03 $^{a,A}$ | 6.16 ± 0.15 $^{a,A}$ |
| | VP | 6.23 ± 0.05 $^{a,A}$ | 6.20± 0.17 $^{a,A}$ | 6.06 ±0.11 $^{a,A}$ | 6.16± 0.11 $^{a,A}$ | 6.26± 0.05 $^{a,A}$ |
| | MAP | 6.23 ± 0.05 $^{a,A}$ | 6.13 ±0.11$^{a,A}$ | 6.06 ±0.11$^{a,A}$ | 6.10± 0.90 $^{a,A}$ | 6.26± 0.57 $^{a,A}$ |
| Moisture (%) | C | 60.30 ± 1.30 $^{a,A}$ | 60.7 ± 1.6 $^{a,A}$ | 56.2 ± 0.3 $^{b,A}$ | 56.20 ± 1.2 $^{b,A}$ | 53.0 0± 0.8 $^{c,A}$ |
| | VP | 60.30 ± 1.3 $^{a,A}$ | 57.9 ±0.3 $^{ab,A}$ | 56.70± 1.4 $^{ab,A}$ | 55.70±1.9 $^{b,A}$ | 54.90± 4.1 $^{b,A}$ |
| | MAP | 60.30 ± 1.3 $^{a,A}$ | 60.0 ±1.9$^{ab,A}$ | 57.20 ±2.01$^{bc,A}$ | 55.90± 1.05 $^{c,A}$ | 55.80 ±1.09 $^{c,A}$ |
| TVB-N (mg N/100g) | C | 26.60 ±2.4 $^{d,A}$ | 29.80 ±0.8 $^{dc,A}$ | 30.30± 0.8 $^{c,A}$ | 36.80± 2.9 $^{b,A}$ | 42.40± 0.8 $^{a,A}$ |
| | VP | 26.60 ±2.4 $^{c,A}$ | 29.30± 0.5 $^{b,A}$ | 30.30± 0.7 $^{b,A}$ | 31.70 ±0.8 $^{ab,B}$ | 34.00 ±1.6 $^{a,B}$ |
| | MAP | 26.60 ±2.4 $^{b,A}$ | 25.60± 0.8 $^{b,B}$ | 26.10±0.8 $^{b,B}$ | 27.5 0±1.6$^{b,C}$ | 30.30 ±0.8 $^{a,C}$ |
| Peroxide (meq g /kg) | C | 0.00 ±0.0 $^{c,A}$ | 1.66 ± 1.1 $^{b,A}$ | 3.86 ± 0.2 $^{a,A}$ | 4.46 ± 0.3 $^{aA}$ | 4.30 ± 0.1 $^{a,A}$ |
| | VP | 0.00 ±0.0 $^{c,A}$ | 0.00 ±0.0 $^{c,A}$ | 0.84 ±0.1 $^{b,C}$ | 2.36 ±0.2 $^{a,B}$ | 2.56 ±0.5$^{a,B}$ |
| | MAP | 0.00 ±0.0 $^{b,A}$ | 0.80 ±1.3 $^{b,A}$ | 2.20 ± 0.6$^{a,B}$ | 2.58 ± 0.2 $^{a,B}$ | 2.93± 0.1 $^{a,B}$ |
| TC (log cfu/g) | C | 2.88 ± 0.7$^{c,A}$ | 5.11 ± 1.3 $^{c,A}$ | 5.30 ± 0.4 $^{b,A}$ | 7.40 ± 0.6 $^{b,A}$ | 8.40 ± 0.4 $^{a,A}$ |
| | VP | 2.88 ± 0.7 $^{d,A}$ | 3.58 ± 0.9 $^{cd,A}$ | 4.40 ± 0.9 $^{c,A}$ | 6.20 ± 0.3 $^{b,A}$ | 7.60 ± 0.6 $^{a,AB}$ |
| | MAP | 2.88 ± 0.7 $^{c,A}$ | 3.70± 0.9 $^{c,A}$ | 3.80± 0.7 $^{c,A}$ | 5.20 ± 0.4 $^{b,A}$ | 6.80 ± 0.6 $^{a,B}$ |
| YMC | C | 2.48±0.6 $^{b,A}$ | 2.60±0.5 $^{b,A}$ | 2.99±0.4 $^{b,A}$ | 4.06±0.7 $^{a,A}$ | 4.26±0.4 $^{a,A}$ |

| | | | | | | |
|---|---|---|---|---|---|---|
| (log cfu/g) | VP | 2.48±0.6 $^{b,A}$ | 2.20±0.5 $^{b,A}$ | 2.37±0.1 $^{b,A}$ | 2.43±0.6 $^{b,A}$ | 4.48±0.6 $^{a,A}$ |
| | MAP | 2.48±0.6 $^{b,A}$ | 2.30±0.4 $^{b,A}$ | 2.86±0.7 $^{b,A}$ | 3.15±0.5 $^{ab.A}$ | 3.74±0.4 $^{a,A}$ |

* Data are expressed as means± SD (n=3)

Different script letters characterize significant differences in each raw (a-d) for different times of storage and in each column (A-C) for different treatments (P<0.05).

Sensorial scores of samples is shown in Table 3. There was siginificant diffrerence between aroma, flavor, color and texture scores in different treatments just at 30$^{th}$ day of storage (P≤0.05). A significant decrease was found in aroma, color and texture scores in all treatments (P≤0.05). Also this decrease for flavor score was found in all treatments except in modified atmosphere packaging (P≤0.05) (Table 3).

**Table 3: Changes in sensory attributes of hot smoked rainbow trout in different packaging during refrigerated storage under Common packaging (C), Vacuum packaging (VP) and modified atmosphere packaging (MAP)**

| Factor * | treatment | Storage time (days) | | | | |
|---|---|---|---|---|---|---|
| | | 0 | 10 | 20 | 30 | 40 |
| **Aroma** | C | 4.43± 0.8 $^{a,A}$ | 3.38± 1.0 $^{b,A}$ | 3.67± 0.8 $^{b,A}$ | 1.67± 0.9 $^{c,B}$ | ND |
| | VP | 4.43± 0.8 $^{a,A}$ | 3.67± 0.8 $^{ab,A}$ | 4.00 ± 0.9$^{ab,A}$ | 2.81± 1.4 $^{b,A}$ | ND |
| | MAP | 4.43± 0.8 $^{a,A}$ | 3.38± 1.0 $^{b,A}$ | 3.81± 0.8 $^{ab,A}$ | 3.62± 1.2 $^{ab,A}$ | 3.37±1.0$^{b}$ |
| **Color** | C | 4.62± 0.5 $^{a,A}$ | 3.48± 0.9 $^{b,A}$ | 3.86± 0.9 $^{b,A}$ | 1.62± 1.0 $^{c.C}$ | ND |
| | VP | 4.62± 0.5 $^{a,A}$ | 3.76± 1.0 $^{b,A}$ | 3.38± 0.9 $^{b,A}$ | 2.33± 1.1 $^{c,B}$ | ND |
| | MAP | 4.62± 0.5 $^{a,A}$ | 3.33± 1.0 $^{b,A}$ | 4.05± 0.9 $^{ab,A}$ | 3.95± 0.8 $^{b,A}$ | 3.31± 1.0 $^{b}$ |
| **Flavor** | C | 4.24± 0.7 $^{a,A}$ | 3.71± 1.0 $^{ab,A}$ | 3.57± 1.1 $^{b,A}$ | 1.76± 1.1 $^{c,C}$ | ND |
| | VP | 4.24± 0.7 $^{a,A}$ | 3.76± 0.8 $^{a,A}$ | 3.90± 0.7 $^{a,A}$ | 2.81± 1.4 $^{b,B}$ | ND |
| | MAP | 4.24± 0.7 $^{a,A}$ | 3.95± 0.5 $^{a,A}$ | 4.00± 0.9 $^{a,A}$ | 4.43± 0.5 $^{a,A}$ | 3.93± 0.5 $^{a}$ |
| **Texture** | C | 4.43± 0.7$^{a,A}$ | 3.57± 1.0 $^{b,A}$ | 3.33± 0.9 $^{b,A}$ | 1.86± 1.3 $^{c,B}$ | ND |
| | VP | 4.43± 0.7 $^{a,A}$ | 3.62± 1.0 $^{bc,A}$ | 4.00± 0.8 $^{B,A}$ | 2.09± 1.5 $^{c,B}$ | ND |
| | MAP | 4.43± 0.7 $^{a,A}$ | 3.62± 1.0 $^{b,A}$ | 4.05± 0.9 $^{ab,A}$ | 4.43± 0.5 $^{a,A}$ | 3.60± 1.0 $^{b}$ |

* Data are expressed as means± SD (n=3)

ND: not detected

Different script letters characterize significant differences in each raw (a-d) for different times of storage and in each column (A-C) for different treatments (P<0.05).

## Discussion

Mean of moisture in hot smoked rainbow trout was 60.33% at the day 0. Water amount was decreased significantly during storage in all treatments (P<0.05). In spite of, there was no significant different between moisture treatments in different times (P>0.05), generally water reduction in air packing is more than VP and in VP is more than MAP. This result is the same as obtained results by Dharmaveer et al. (2007). Lack of any difference on pH value between different packing has been previously reported by Pantazi et al. (2008). The decrease of pH value until the 20$^{th}$ day can be related to different reasons such as absorption of $CO_2$ in fish muscle,

decomposing of $CO_2$ to carbonic acid (in proportion of $CO_2$ density in MAP) (Lannelongue et al., 1982), production of acid by lactic acid bacteria (Stohr et al., 2001) and presence of fermentative carbohydrates (Matos et al., 2005). The increase of pH can be the result of volatile bases compound such as ammoniac and tri-methylamine or fish bacterial spoilage (Hyytia et al., 1999).

The significant increase of TVB-N in all packs may originate from a combination of microbial activity and autolytic deamination of amino acids produced of decomposition of proteins and separation of amine because of bacterial spoilage (Muratore and Licciardello, 2005). In this study, there was a considerable different in the amount of TVB-N in 3 types of packing during sampling time ($P<0.05$). Similar results were obtained by Cakli et al. (2006). Acceptable limit of TVB-N for smoked marine product based on EU is 35 mg N per 100 g flesh (EEC, 1995). According to this index, hot smoked rainbow trout can be consumed lower than 30 days under common packing, maximum 40 days under vacuum packing and more than 40 days under modified atmosphere packing.

An increasing in the amount of peroxide was observed in all treatments. Then peroxide value decreased just in common packaging. In spite of the increase of peroxide in all treatments, the amount of peroxide is lower than critical limit of peroxide (5 mili equivalent per 1 kg of fat) (Parvaneh, 1998) in $40^{th}$ day of storage. The mean amount of peroxide in the sample packed in vacuum was lower than other types ($P<0.05$). It can be related to presence of minimum oxygen which is necessary for oxidation in vacuum packing. Fagan et al. (2004) observed the similar result in mackerel fillets in MAP and in presence of air. Vermieren et al. (1999) believed the reason of oxidation in VP packing is that oxygen isn't omitted completely and may enter through packing materials.

In all three packages, microbial computation significantly raised by increasing preservation period ($P<0.05$). Significant difference was observed in total count between various treatments only in days 30, 40 of storage time ($P<0.05$). VP and MAP treatments have preventive effects on total count. At the absence of O2 and at presence of $CO_2$ a bacteria-static effect is applied to aerobic flora growth (Sivertsrik et al., 2002). Acceptable maximum level of total count for smoked fish is 7 log cfu/g (ICMSF, 1986).

Until $10^{th}$ day of storage the average of mold and yeast was decreased at first, and until the end of storage period was increased. This increasing procedure in all 3 packages was significant ($P<0.05$). There was no significant difference in yeast and mold in all sampling phases. Generally the presence of aerobic microflora such as fungi (particularly yeasts) in VP packaging can be because of incomplete air removing from package (Vermieren et al., 1999) or cause of other factors such as food ability in oxygen grasping (Smith et al., 1986), storage period and temperature (Matos et al., 2005). The acceptable level of yeast in smoked products was determined 4 (Log cfu/g) (Leroi et al., 2001).

Generally significant decrease in aroma, texture and color in every three treatments was observed ($P<0.05$). About

flavor significant decrease just observed in VP and common packaging ($P<0.05$). Such significant decrease was observed in cold smoked salmon packed in vacuum (Dondero et al., 2004). The quality decrease in texture factor of smoked samples can be due to the tightening effect of smoke on protein of muscle (Goulaas and Kontominas, 2005). Some of researchers impute it to formaldehydes because it can react with amino group and denature protein (Horner, 1997). Also it can be because of autolysin enzyme (Truelstrup et al., 1996). Changes in aroma, color and flavor can be because of microorganism growth and operation (Ozogul et al., 2004). Sensory evaluation result indicates that after 30 days, there was significant difference between 4 factors in 3 treatments ($P>0.05$). In 40$^{th}$ day only MAP samples were examinable. Hot smoked rainbow trout in common packaging became undesirable earlier than VP and in VP became undesirable earlier than MAP. Similar results were reported by Pantazi et al. (2008). In regard of chemical, microbial and sensorial scores hot smoked rainbow trout in common packaging less than 30 days, in VP packaging between 30 to 40 days and in modified atmosphere packaging more than 40 days in refrigerator temperature can be consumable.

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