



## EFFECTS OF *CITRUS AURANTIUM* EXTRACT AND PACKAGING UNDER VACUUM ON CHEMICAL, MICROBIOLOGICAL AND SENSORY CHARACTERISTICS OF RAINBOW TROUT DURING STORAGE AT REFRIGERATOR TEMPERATURE

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### ABSTRACT

The intent of vacuum packing is usually to remove oxygen from the container to extend the shelf life of foods. The aim of this study was to determine the effects of *Citrus aurantium* extract and packaging under vacuum on shelf life of Rainbow trout during storage at refrigerator temperature. Fish fillets were immersed in traditional marinades and stored at -18 °C for 56 days. Some chemical and microbial characteristics like total volatile basic nitrogen (TVN), thiobarbituric acid (TBA), water holding capacity (WHC), pH, mesophilic, and psychrophilic bacterial count were performed with 7 days interval. The results were divided into four categories including the first control sample (without extract and packing), the second treatment (packed in the vacuum without extract), the third treatment (immersed in a solution of 1.5% extract for 30 min) and the fourth treatment (immersed in a solution of 1.5% extract for 30 min and packed in vacuum) were stored in a refrigerator (4±1°C). Sensory and some chemical and microbial characteristics like total volatile basic nitrogen (TVB-N), thiobarbituric acid (TBA), and free fatty acids (FFA) were measured at days zero, 5, 10, 15, and 20. It seems that the *Citrus aurantium* extract and packaging under vacuum reduce the oxidation process and increases the shelf life of rainbow trout and can be an appropriate alternative for artificial preservatives.

## 1. Introduction

The rainbow trout (*Oncorhynchus mykiss*) is one of the most widely introduced fishes on a global basis. This fish has the ability to culture in most parts of Iran and has been one of the most desirable farmed fish in the last two decades (Salazar and el., 2016). Rainbow trout is very closely related to salmon which having the highest content of polyunsaturated fatty acids such as eicosapentaenoic acid and docosahexaenoic acid compared to other fish and seafood (LaPatra et al., 2015). Fatty fish

such as rainbow trout has limited shelf life and quality deterioration of this species is mainly caused by rapid growth of microorganisms and lipid oxidation (Fontagné-Dicharry et al., 2014). So, the off-odor and off-taste of the products affect the consumer acceptability (Secci et al., 2016). Today, the addition of synthetic preservatives, antioxidants, colorants to extend shelf life has been revised by authorities due to certain health problems. Use

of natural preservative has been the subject of many investigations (Raeisi et al., 2016).

These days consumers are more willing to consume foods that are free of chemicals and artificial antioxidant. The use of natural antioxidants is recommended to replace synthetic antioxidants. *Citrus aurantium* is as a natural antioxidant that is one of the largest species among plants; it consists of 40 species which are distributed in all continents (Parhiz et al., 2015). Citrus is one of the most important fruits, which is consumed mostly fresh and has been used as an herbal medicine or additive or food supplement. Citrus is believed to possess bioactivities such as antioxidant, anti-inflammatory, antimicrobial, and is suggested to be responsible for the prevention of cancer and degenerative diseases (Abirami et al., 2014). Those bioactivities of citrus are due to the presence of bioactive compounds such as phenolics, flavonoids, essential oil, and vitamins (Park et al., 2015). It is a hybrid between *Citrus maxima* (pomelo) and *Citrus reticulata* (mandarin). Many varieties of bitter orange are used for their essential oil, and are found in perfume, used as a flavoring or as a solvent. The Seville orange variety is used in the production of marmalade. Bitter orange is also employed in herbal medicine as a stimulant and appetite suppressant, due to its active ingredient, synephrine (Marzouk, 2013). Bitter orange supplements have been linked to a number of serious side effects and deaths, and consumer groups advocate that people avoid using the fruit medically (Al-Juhaimi, 2014).

Vacuum packing is a method of packaging that removes air from the package prior to sealing. This method involves (manually or automatically) placing items in a plastic film package, removing air from inside, and sealing the package. Shrink film is sometimes used to have a tight fit to the contents. The intent of vacuum packing is usually to remove oxygen from the container to extend the shelf life of foods and, with flexible package forms, to reduce the volume of the contents and package. Vacuum packing reduces atmospheric oxygen,

limiting the growth of aerobic bacteria or fungi, and preventing the evaporation of volatile components. It is also commonly used to store dry foods over a long period of time, such as cereals, nuts, cured meats, cheese, smoked fish, coffee, and potato chips (crisps). On a shorter term basis, vacuum packing can also be used to store fresh foods, such as vegetables, meats, and liquids, because it inhibits bacterial growth. Vacuum packing greatly reduces the bulk of non-food items. For example, clothing and bedding can be stored in bags evacuated with a domestic vacuum cleaner or a dedicated vacuum sealer. This technique is sometimes used to compact household waste, for example where a charge is made for each full bag collected. In an oxygen-depleted environment, anaerobic bacteria can proliferate, potentially causing food-safety issues. Vacuum packing is often used in combination with other packaging and food processing techniques (Mohan et al., 2016). This study was to determine the effects of *Citrus aurantium* extract and packaging under vacuum on shelf life of rainbow trout during storage at refrigerator temperature.

## 2. Materials and methods

### 2.1. Sample preparation

Rainbow trout weighing  $25 \pm 250$  were purchased from a trout farm located in "Noor" city in north of Iran. It transferred to Tehran university laboratory, then washed with drinkable water, and also abdominal drain and scaling were done and it cut off into 3-27 pieces. For the first day, 3 pieces were used as sample of first day and the rest of them were divided into 4 sections. Section one was placed in vacuum packing as control or blank sample. Another part of sample (section 2) was placed in *Citrus aurantium* which was extracted by GC Mass Chromatography at 4° C for 20 days. Section 3 was stored under vacuum section and the last sample was stored in vacuum packing with the *Citrus aurantium* which was extracted by GC Mass Chromatography. Chemical tests (TBA, TVB, FFA), Sensory evaluation (taste, odor, taste, color, appearance and texture),

microbial tests (Coliforms, psychrophilic, mold, yeast and total count of aerobic mesophilic bacteria) were carried out on the 4 samples of rainbow trout for 3 times within 20 days (zero, five, tenth, fifteenth and twentieth).

## 2.2. Determination of Thiobarbituric Acid (TBA) and Free Fatty Acid (FFA)

The TBA value (mg malonaldehyde (MDA) kg<sup>-1</sup> of fish flesh) was determined colorimetrically by the Porkony and Dieffenbacher method. Free fatty acid (%) was determined by the Kirk and Sawyer method (Binsi et al., 2016).

## 2.3. Determination of Total Volatile Base Nitrogen (TVB-N)

The amount of TVB-N (mg 100 g<sup>-1</sup> fish flesh) was determined by the direct water distillation method according to Goudlas and Kontoinas (Binsi et al., 2016).

## 2.4. Microbiological analysis

Psychrotrophic (TAPC) counts were performed using standard microbiological methods (Wu et al., 2014). Enumeration of Coliform was determined by pour technique according to AOAC, 1 mL of diluents was mixed with 10 - 12 mL of VRBA (Violet Red Bile Salts Agar)/Mac-Conkey agar, then after solidification overlaid with VRBA With MUG (5 - 10 mL) and incubated at 37°C for 24 hours, all the colonies having 0.5 mm in diameter were presumed as coliforms (Wu et al., 2014).

## 2.5. Sensory evaluation

The samples in each sampling period by panelist trained sensory parameters in accordance with the grading of the Council of Europe (EC) was graded. Sample of fish from each of the randomly in the same containers for evaluating the sensory properties Such as appearance, texture, color and odor part of the same fish that to evaluate the taste were Steamed. In panelist were put at the disposal of grading scheme EC, the excellent quality (E),

good quality (A), medium quality (B) bad quality (C) respectively are given scores 4,3,2,1 and finally 3 as it was considered acceptable for human consumption (Yang et al., 2014).

## 2.6. Statistical procedure

The results were analyzed by repeated measure ANOVA test using SPSS Inc. software (v. 16.0, Chicago, IL).

## 3. Results and discussions

### 3.1. Chemical analysis

Amount of (TBA), (TVB) (FFA) had increasing trend over time in all Treatments But this increase in control samples was higher than others samples and the lowest increase in samples simultaneous application of packaging under vacuum and *Citrus aurantium* extract was observed and this increase in the sample *Citrus aurantium* extract was lower than samples under vacuum packed Due to the low free fatty acids in treatments containing *Citrus aurantium* extract may live on Antibacterial activity may be *Citrus aurantium* extract and thus reduce microbial activity as well as the enzymes secreted in fat fillet attributed (Table 3). Also The difference increases the amount of TBA treatments during the period Can be Attributed to lipid oxidation and peroxide converted into substances such as aldehyde (Table 2). TBA low in treatments containing *Citrus aurantium* and packed under vacuum can be attributed to antioxidant properties *Citrus aurantium* and prevent fat oxidation by packaging under vacuum (Al-Juhaimi, 2014). These results are according to the previous researches performed by kang et al, 2006 which is showing samples contained *Citrus aurantium* extract were significantly protective lipid oxidation. Increase in TVB during storage can relate associated with activity spoilage bacteria TVB consisting of trimethylamine, ammonia and other nitrogenous compounds escape seafood is associated with corruption and by bacterial spoilage, autolytic enzymes, D-amino acids and nucleotides generated are produced (Wu et al., 2014). Low levels of TVB in

treatments containing *Citrus aurantium* and application of vacuum packaging and *Citrus aurantium* extract compared to the control can be due to antibacterial properties and decreased activity of phenolic compounds *Citrus aurantium* and a population of bacteria for amine oxidation and removal of non-protein nitrogen compounds due to less oxygen for vacuum packaging or both (Yang et al., 2014). Through inhibition of proteolytic bacteria that cause spoilage, inhibit the activity of these bacteria and break down protein and thus prevent their release nitrogen compounds. In general, the results of this study are consistent with previous studies showed that the use of different types of antioxidants, including vitamin C, alone or in combination with packaging mullet fillets stored oxidation process frozen delay (Table 1).

### 3.2. Microbiological analysis

Microbial test results showed that the number of psychrophilic microorganisms, mesophilic aerobic microbes, total count, coliform] had increased over time in all treatments but this increase was higher than other treatments in control samples and in

samples containing *Citrus aurantium* extract and packaged under vacuum. The lowest increase was observed. As well as increasing the number of microbes in the samples with *Citrus aurantium* extract have been lower than control sample and vacuum packed samples. Generally, the reduction number of microbes that can be attributed to antimicrobial phenolic compounds in the extract. Also microbial counts in the samples packaged under vacuum can be attributed to the lack of sufficient oxygen for bacteria. The results were consistent with previous research results, as studies have shown that extracts of lemon, cloves, cinnamon and rosemary, especially cinnamon has an antibacterial activity against Gram positive bacteria and Gram-negative bacteria (Diao et al., 2014), *Citrus* fruits is a rich source of flavonoid glycosides, coumarins and glycosides (Shahnan et al., 2007) and the citrus flavonoids have a large spectrum of biological activity including antibacterial, antioxidant and antifungal activities. Manthey and Grohmann (2001) reported that polyphenol compounds such as *p*-coumaric, ferulic, and sinapic acids and narirutin were present in citrus extract.

**Table 1.** Effect of *Citrus aurantium* extract and packaged under vacuum and simultaneous application of both on The TVB during storage at refrigeration temperatures for 20 days (mean±SD)

Twentieth day	Fifteenth day	Tenth day	Fifth day	Zero	Treatment
30/79±0/39 <sup>a</sup>	24/21±0/27 <sup>d</sup>	16/83±0/16 <sup>g</sup>	10/9±0/08 <sup>n</sup>	7/75±0/06 <sup>m</sup>	Control sample (T1)
27/57±0/09 <sup>f</sup>	22/52±0/20 <sup>e</sup>	16/52±0/06 <sup>d</sup>	10/40±0/04 <sup>c</sup>	7/31±0/07 <sup>b</sup>	Vacuum packaging rainbow trout (T)
26/54±0/05 <sup>t</sup>	22/32±0/08 <sup>f</sup>	16/04±0/03 <sup>s</sup>	10/17±0/07	7/15±0/04 <sup>i</sup>	<i>Citrus aurantium</i> extract 1.5% (T3)
24/62±0/12 <sup>d</sup>	21/18±0/44 <sup>g</sup>	14/50±0/02 <sup>k</sup>	10/38±0/03 <sup>i</sup>	6/22±0/10 <sup>n</sup>	<i>Citrus aurantium</i> extract 1.5% percent and vacuum packaging (T4)

Means in each column followed by different letters are significantly different (p<0.05).

**Table 2.** Effect of *Citrus aurantium* extract and packaged under vacuum and simultaneous application of both on The TBA during storage at refrigeration temperatures for 20 days (mean  $\pm$  SD)

Twentieth day	Fifteenth day	Tenth day	Fifth day	Zero	Treatment
0/76 $\pm$ 0/01 <sup>a</sup>	08 <sup>m</sup> 0 $\pm$ 0/19	0/29 $\pm$ 0/01 <sup>g</sup>	0/26 $\pm$ 0/01 <sup>n</sup>	08 <sup>m</sup> 0 $\pm$ 0/19	Control sample (T1)
0/65 $\pm$ 0/02 <sup>f</sup>	0/11 $\pm$ 0/02 <sup>b</sup>	0/24 $\pm$ 0/01 <sup>d</sup>	0/21 $\pm$ 0/01 <sup>c</sup>	0/11 $\pm$ 0/02 <sup>b</sup>	Vacuum packaging rainbow trout (T <sub>2</sub> )
0/51 $\pm$ 0/02 <sup>t</sup>	0/09 $\pm$ 0/01 <sup>i</sup>	0/22 $\pm$ 0/05 <sup>s</sup>	0/14 $\pm$ 0/03 <sup>k</sup>	0/09 $\pm$ 0/01 <sup>i</sup>	<i>Citrus aurantium</i> extract 1.5% (T3)

**Table 3.** Effect of *Citrus aurantium* extract and packaged under vacuum and simultaneous application of both on The FFA during storage at refrigeration temperatures for 20 days (mean $\pm$ SD)

Twentieth day	Fifteenth day	Tenth day	Fifth day	Zero	Treatment
2/6 $\pm$ 0/01 <sup>a</sup>	1/8 $\pm$ 0/01 <sup>d</sup>	1/02 $\pm$ 0/01 <sup>g</sup>	0/6 $\pm$ 0/005 <sup>n</sup>	0/4 $\pm$ 0/01 <sup>m</sup>	Control sample (T1)
2/2 $\pm$ 0/01 <sup>f</sup>	1/6 $\pm$ 0/01 <sup>e</sup>	0/9 $\pm$ 0/005 <sup>d</sup>	0/4 $\pm$ 0/005 <sup>c</sup>	0/4 $\pm$ 0/01 <sup>b</sup>	Vacuum packaging rainbow trout (T <sub>2</sub> )
2/003 $\pm$ 0.005 <sup>t</sup>	1/1 $\pm$ 0/01 <sup>f</sup>	0/7 $\pm$ 0/01 <sup>s</sup>	0/4 $\pm$ 0.01 <sup>k</sup>	0/2 $\pm$ 0/01 <sup>j</sup>	<i>Citrus aurantium</i> extract 1.5% (T3)
1/8 $\pm$ 0/01 <sup>d</sup>	1/03 $\pm$ 0/01 <sup>g</sup>	0/5 $\pm$ 0/01 <sup>k</sup>	0/2 $\pm$ 00/01 <sup>i</sup>	0/1 $\pm$ 0/01 <sup>n</sup>	<i>Citrus aurantium</i> extract 1.5% and vacuum packaging (T4)

**Table 4.** Effect of *Citrus aurantium* extract and packaged under vacuum and simultaneous application of both on the total count of aerobic mesophilic bacteria during storage at refrigeration temperatures for 20 days (mean  $\pm$  SD)

Twentieth day	Fifteenth day	Tenth day	Fifth day	Zero	Treatment
8/25 $\pm$ 0/31 <sup>a</sup>	7/35 $\pm$ 0/23 <sup>d</sup>	5/54 $\pm$ 0/28 <sup>g</sup>	4/25 $\pm$ 0/20 <sup>n</sup>	3/65 $\pm$ 0/2 <sup>m</sup>	Control sample (T1)
7/57 $\pm$ 0/27 <sup>f</sup>	5/56 $\pm$ 0/25 <sup>e</sup>	4/60 $\pm$ 0/26 <sup>d</sup>	3/75 $\pm$ 0/41 <sup>c</sup>	3/24 $\pm$ 0/27 <sup>b</sup>	Vacuum packaging rainbow trout (T <sub>2</sub> )
5/57 $\pm$ 0/24 <sup>t</sup>	4/25 $\pm$ 0/13 <sup>f</sup>	3/38 $\pm$ 0/15 <sup>s</sup>	2/65 $\pm$ 0/25 <sup>k</sup>	2/07 $\pm$ 0/14 <sup>i</sup>	<i>Citrus aurantium</i> extract 1.5% (T3)
2/02 $\pm$ 0/12 <sup>n</sup>	2/28 $\pm$ 0/17 <sup>i</sup>	3/07 $\pm$ 0/17 <sup>k</sup>	4/11 $\pm$ 0/22 <sup>g</sup>	5/26 $\pm$ 0/28 <sup>d</sup>	<i>Citrus aurantium</i> extract 1.5 % and vacuum packaging (T4)

Means in each column followed by different letters are significantly different (p<0.05).

**Table 5.** Effect of *Citrus aurantium* extract and packaged under vacuum and simultaneous application of both on the *Coliforms* during storage at refrigeration temperatures for 20 days (mean  $\pm$  SD)

Twentieth day	Fifteenth day	Tenth day	Fifth day	Zero	Treatment
8/433 $\pm$ 1/32 <sup>a</sup>	8/29 $\pm$ 0/38 <sup>d</sup>	6/57 $\pm$ 0/25 <sup>g</sup>	5/35 $\pm$ 0/46 <sup>n</sup>	3/85 $\pm$ 0/13 <sup>m</sup>	Control sample (T1)
6/79 $\pm$ 0/61 <sup>f</sup>	5/59 $\pm$ 0/38 <sup>e</sup>	4/59 $\pm$ 1/38 <sup>d</sup>	3/53 $\pm$ 0/49 <sup>c</sup>	3/23 $\pm$ 0/20 <sup>b</sup>	Vacuum packaging rainbow trout (T <sub>2</sub> )
6/68 $\pm$ 0/37 <sup>t</sup>	4/46 $\pm$ 0/45 <sup>f</sup>	4/20 $\pm$ 0/18 <sup>s</sup>	3/33 $\pm$ 0/41 <sup>k</sup>	2/88 $\pm$ 0/16 <sup>i</sup>	<i>Citrus aurantium</i> extract 1.5% (T3)
4/19 $\pm$ 0/27 <sup>d</sup>	2/10 $\pm$ 1/82 <sup>g</sup>	3/84 $\pm$ 0/15 <sup>k</sup>	2/12 $\pm$ 0/15 <sup>i</sup>	0/92 $\pm$ 0/20 <sup>n</sup>	<i>Citrus aurantium</i> extract 1.5 % and vacuum packaging (T4)

**Table 6.** Effect of *Citrus aurantium* extract and packaged under vacuum and simultaneous application of both on the psychrophilic microorganisms during storage at refrigeration temperatures for 20 days (mean  $\pm$  SD)

Twentieth day	Fifteenth day	Tenth day	Fifth day	Zero	Treatment
8/66 $\pm$ 0/39 <sup>a</sup>	7/68 $\pm$ 0/32 <sup>d</sup>	6/11 $\pm$ 0/26 <sup>g</sup>	4/73 $\pm$ 0/24 <sup>n</sup>	4/02 $\pm$ 0/20 <sup>m</sup>	Control sample (T1)
7/01 $\pm$ 0/26 <sup>f</sup>	5/87 $\pm$ 0/34 <sup>e</sup>	5/01 $\pm$ 0/17 <sup>d</sup>	4/56 $\pm$ 0/21 <sup>c</sup>	4/01 $\pm$ 15 <sup>b</sup>	Vacuum packaging rainbow trout (T <sub>2</sub> )
5/95 $\pm$ 0/28 <sup>t</sup>	4/70 $\pm$ 0/22 <sup>f</sup>	3/65 $\pm$ 0/25 <sup>s</sup>	3/20 $\pm$ 0/25 <sup>k</sup>	2/65 $\pm$ 0/16 <sup>i</sup>	<i>Citrus aurantium</i> extract 1.5% (T3)
5/70 $\pm$ 0/23 <sup>d</sup>	4/61 $\pm$ 0/17 <sup>g</sup>	3/57 $\pm$ 0/22 <sup>k</sup>	2/84 $\pm$ 0/15 <sup>i</sup>	2/26 $\pm$ 0/19 <sup>n</sup>	<i>Citrus aurantium</i> extract 1.5% and vacuum packaging (T4)

**Table 7.** Effect of *Citrus aurantium* extract and packaged under vacuum and simultaneous application of both the sensory characteristics

Reception	Flavor	Texture	Odor	Color	Treatment
3 $\pm$ 0/000 <sup>a</sup>	3 $\pm$ 0/000 <sup>a</sup>	1 $\pm$ 0/000 <sup>a</sup>	3 $\pm$ 0/000 <sup>a</sup>	1 $\pm$ 0/000 <sup>a</sup>	Control sample (T1)
2 $\pm$ 0/000 <sup>b</sup>	2 $\pm$ 0/000 <sup>b</sup>	1 $\pm$ 0/000 <sup>a</sup>	2 $\pm$ 0/000 <sup>b</sup>	1 $\pm$ 0/000 <sup>a</sup>	Vacuum packaging rainbow trout (T <sub>2</sub> )
2 $\pm$ 0/000 <sup>b</sup>	2 $\pm$ 0/000 <sup>b</sup>	1 $\pm$ 0/000 <sup>a</sup>	2 $\pm$ 0/000 <sup>b</sup>	1 $\pm$ 0/000 <sup>a</sup>	<i>Citrus aurantium</i> extract 1.5% (T3)
1 $\pm$ 0/000 <sup>c</sup>	1 $\pm$ 0/000 <sup>c</sup>	1 $\pm$ 0/000 <sup>a</sup>	1 $\pm$ 0/000 <sup>c</sup>	1 $\pm$ 0/000 <sup>a</sup>	<i>Citrus aurantium</i> extract 1.5% and vacuum packaging (T4)

Means in each column followed by different letters are significantly different (p<0.05)

Singh et al. (2010) stated that constituents such as gamma-terpinene, terpinolene, alpha-

terpinene, hesperidin, neohesperidin etc. are responsible for the preservative action in

*Citrus aurantium* extract. The result of the present study indicated a positive role of *Citrus aurantium* extract on rainbow trout during storage at refrigerator temperature.

### 3.3. Sensory evaluation

As can be seen in Table 7. The use of *Citrus aurantium* extract and packaged under vacuum in rainbow trout have no effect on color and texture and treatments containing *Citrus aurantium* extract and packaged under vacuum are similar in terms of color and texture with control treatment. The factor of odor and flavor samples containing *Citrus aurantium* extract and vacuum packaging have had odor and flavor better than the control samples and the samples vacuum packed. The overall acceptability comments containing *Citrus aurantium* extract and packed under vacuum gained points better than the control and vacuum packed Sha'banpour et al. (2012) using thyme extract with vacuum packing in a refrigerated temperature on Rainbow trout and their results coincided the sensory properties of this study. Also, the results are consistent with the study of Chouliara et al. (2011) for Shank Fish.

### 4. Conclusions

The effect of *Citrus aurantium* extract and packaged under vacuum and simultaneous application of vacuum packaging and *Citrus aurantium* extract to evaluate the shelf-life and anti-oxidant rainbow trout to examine in the refrigerator for up to 20 days were studied. The results showed that all samples compared to control samples have had higher shelf life and antioxidant properties as well as the simultaneous application of vacuum packaging and orange extract antimicrobial and antioxidant properties have been more and better compared to the control samples and other specimens also sensory evaluation of all the samples have been acceptable But samples containing combined use *Citrus*

*aurantium* extract and packed under vacuum to gained the more points. Therefore orange juice and packaged under vacuum, especially using their combined can be used for preservation of Rainbow trout for human consumption.

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