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Combined Effect of Vacuum Packaging and Sodium Acetate Dip Treatment on Shelf Life Extension of Rainbow Trout (*Oncorhynchus mykiss*) during Refrigerated Storage

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ABSTRACT

The present study evaluated the effect of packaging (air, vacuum) with or without addition of sodium acetate (2% W/V) on shelf-life extension of rainbow trout (*Oncorhynchus mykiss*) under refrigeration, for a period of 18 days. Four different treatments were tested: CAP: control air pack; SAAP: sodium acetate treated aerobic packaging; VP: vacuum packaging in the absence of sodium acetate; and SAVP, sodium acetate under VP. Trimethylamine nitrogen (TMA-N), free fatty acids (FFA) and peroxide values (PV) of SAVP samples were lower compared to the other treatments during the entire refrigerated storage period. In the present study, the thiobarbituric acid (TBA) level showed fluctuations during storage indicating that TBA values may not reveal the actual rate of lipid oxidation. Total viable counts for fresh rainbow trout stored aerobically exceeded 7 log CFU g^{-1} after 10-11 days, while treatments SAAP and VP reached the same value on days 12 and 16, respectively. In contrast, SAVP samples did not reach this value throughout the 18-day. Psychrotrophic counts of SAVP samples were significantly ($P< 0.05$) lower compared to the control samples during storage period. As regards sensory evaluation, shelf-life of trout was longest for SAVP (15-16 days), followed by VP (12-13 days), SAAP (9-10 days), and CAP samples (6-7 days). The results indicated that the combined effect of vacuum packaging and sodium acetate on fish samples preserved their good quality characteristics and extended the shelf-life of the treated samples during refrigerated storage, as supported by the results of microbiological, chemical, and sensory evaluation analyses.

Keywords: Packaging, Quality characteristics, Rainbow trout, Sodium acetate.

INTRODUCTION

Fish is an important source of high-quality proteins for humans (Tidwell and Allan, 2001). However, it is highly susceptible to both microbiological and chemical deterioration, due to its high water activity, neutral pH, relatively large quantities of free amino acids, and presence of autolytic enzymes (Jeyasekaran *et al.,* 2006). Traditional methods are used to extend the shelf-life of fish and fishery products involving rapid chilling and ice storage (Himelbloom *et al.,* 1994). Moreover, in order to increase shelf-life of fresh fish, low levels of salt and/or natural preservatives (antimicrobials and antioxidants) have been also used. The preservative effect of salting is mainly due to the decrease in water activity (a_w) and, thus, prevention of growth of many spoilage organisms along with the formation of a more membranous surface, which further inhibits the growth of microorganisms (Leroi and Joffraud, 2000). Thus, to this aim, sodium

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salts such as acetic, lactic, and citric, have been used alone or in combination with other preservation methods such as MAP, vacuum packaging, irradiation, etc. (Rajesh *et al.,* 2002; Sallam, 2007; Goulas and Kontominas, 2007; Manju *et al.,* 2007; Frangos *et al.,* 2010; Mohan *et al.,* 2010). Sodium acetate is found to be effective in preventing microbial growth and improving shelf-life under different storage conditions (Kim *et al.,* 1995). Sodium acetate is an approved (USFDA) flavouring and pH control agent. Zhuang *et al.* (1996) observed that 2% sodium acetate is effective in controlling the growth of natural flora on catfish fillets. Rainbow trout *(Oncorhynchus mykiss*) is a species with high commercial value and much appreciated by Iranian consumers. It is sold as either whole fresh fish or in fillet form. Additionally, vacuum packaged rainbow trout are being consumed. Thus, the objective of the present work was to determine the effect of vacuum packaging individually or in combination with sodium acetate, as a chemical preservative, on the shelf-life extension of fresh rainbow trout (*O. mykiss*) stored under refrigeration (2±1°C) by evaluating certain chemical (pH, TMA, FFA, PV, TBA), microbiological (Total Viable Count, Psychrotrophic bacteria) and sensory parameters.

MATERIALS AND METHODS

Fish Samples

Seventy-five fresh aqua-cultured rainbow trout (1 year-old with average weight and length 300 g and 270 mm, respectively) were purchased from a local aquaculture farm located on River Haraz in North Iran. Fish samples were brought to the laboratory within a 30-minute in iced condition.

Preparation of Fish Samples and Packaging

The fish samples were scaled, gutted, and washed in potable water. The whole scaled and gutted fish were then divided into four lots and packaged as follows: Lot I (CAP), the samples were packed without vacuum (air pack); Lot Π (SAAP), the samples were treated in sodium acetate (2% w/v) for 30 minutes and then packed without vacuum (air pack); Lot III (VP), the samples were vacuum packed; Lot IV (SAVP), the sodium acetate treated samples were vacuum-packed. All samples were packaged in Low-density Polyethylene/Polyamide/Low-density Polyethylene (LDPE/PA/LDPE) barrier pouches (2 samples per pouch), 75 µm in thickness, having an oxygen permeability of 52.2 ml m^{-2} day^{-1} atm^{-1}, and a water vapor permeability of 2.4 g m^{-2} day^{-1} at 0% relative humidity (RH), at 25°C. Pouches containing the trout samples (control and treated) were heat-sealed using a BOSS N48 vacuum sealer (Boss GmbH, Germany) and stored under refrigeration (2±1°C) for a period of 18 days. Sampling was carried out at predetermined time intervals, namely: 0, 3, 6, 9, 12, 15, and 18 days. At each sampling day, 3 samples were randomly chosen for chemical, microbiological, and sensory analysis. Sampling was done in triplicate and the mean values were taken.

Chemical Analysis

The anterior-dorsal halves of each fish were homogenized. Appropriate quantities of homogenized fish were used for determination of the following chemical parameters. Proximate analysis was determined using the method of AOAC (1998). The pH value was recorded using a digital pH meter (Multiline P4 Wtw). Fish muscle (10 g) was homogenized thoroughly with 20 ml of distilled water and the homogenate was used for pH determination. Trimethylamine nitrogen (TMAN) was determined using the method of AOAC (1990). FFA analysis, expressed as % of oleic acid, and PV, expressed as meq of peroxide oxygen kg^{-1} fat, were determined according to the Egan *et al.* (1981) method. Thiobarbituric acid (TBA) (mg malondialdehyde kg^{-1} fish flesh) was

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determined using the method of Namulema *et al*. (1999).

Microbiological Analysis

Twenty-five grams of fish samples were aseptically removed from anterior dorsal region and homogenized for 60 seconds in a stomacher (lab blender 400; Seward Medical, London, UK) containing 225 ml of 0.85% physiological saline solution. For microbial enumeration, 0.1 ml samples of serial dilutions (1:10, diluents, 0.85% normal saline) of fish homogenates were spread on plates of agar materials. Total viable counts (TVC) were enumerated on Plate Count Agar (PCA, Oxoid, CM325), and incubated at 37°C for 48 hours. Psychrotrophic count (PTC) was determined in a similar method to that for (TVC), except that plates were incubated at 7°C for 10 days (Cousin *et al.,* 1992).

Sensory Evaluation

Sensory analysis was conducted on rainbow trout according to the European Community (EC) grading scheme by a taste panel consisting of five people from the laboratory staff, trained in grading fish (Howgate *et al.,* 1992). The appearance of the skin, eyes, gills, surface slime, and the odor of each fish was assessed into four quality grades. In this EC grading scheme, excellent quality (perfect condition), high quality (slight loss of excellent characteristics), good quality (some deterioration, but fit for sale) and unfit for sale were assigned by E, A, B and C grades, respectively. The total grade of each fish was estimated from the grades attributed by each panelist and the final grade of each sample was estimated from the fish examined during the days of evaluation.

Statistical Analysis

Statistical analysis was determined based on triplicate analysis for each sample at each specific storage time. Data were subjected to analysis of variance (ANOVA). The least significant difference (LSD) procedure was used to test for differences between means ($P< 0.05$). In the case of sensory evaluation, a nonparametric ANOVA of Kruskal-Wallis was used.

RESULTS AND DISCUSSION

Proximate Analysis

Proximate composition of fresh rainbow trout showed 72.33% moisture, 2.02% crude fat, 19.25% crude protein, and 1.14% ash. The proximate composition of the rainbow trout reported in different studies (González-Fandos *et al.,* 2005; USDA, 1987) showed some degree of differences, especially for the lipid content. Such variations in the chemical composition of fish is greatly related to the nutrition, catching season (spawning cycles), sexual variation, fish size, living area, as well as the other environmental conditions (Pacheco-Aguilar *et al.,* 2000). The compositional variation, due to the reasons mentioned above, may possibly lead to changes in the sensory attributes, including taste, odor, texture, color, and surface appearance, which control the acceptability of fish as food (Flick and Martin, 1992; González-Fandos *et al.,* 2005). Also, the abovementioned composition may affect the microbial growth (González-Fandos *et al*., 2005).

pH

The changes in pH during chilled storage are shown in Figure 1. The initial pH of rainbow trout was 6.73, which is in agreement with that of Chytiri *et al.* (2004) and Mexis *et al.* (2009). In the case of CAP and SAAP samples, pH increased slowly during two weeks and, then, decreased. The significantly ($P< 0.05$) higher pH values recorded for CAP and SAAP may be attributed to the rapid spoilage of the

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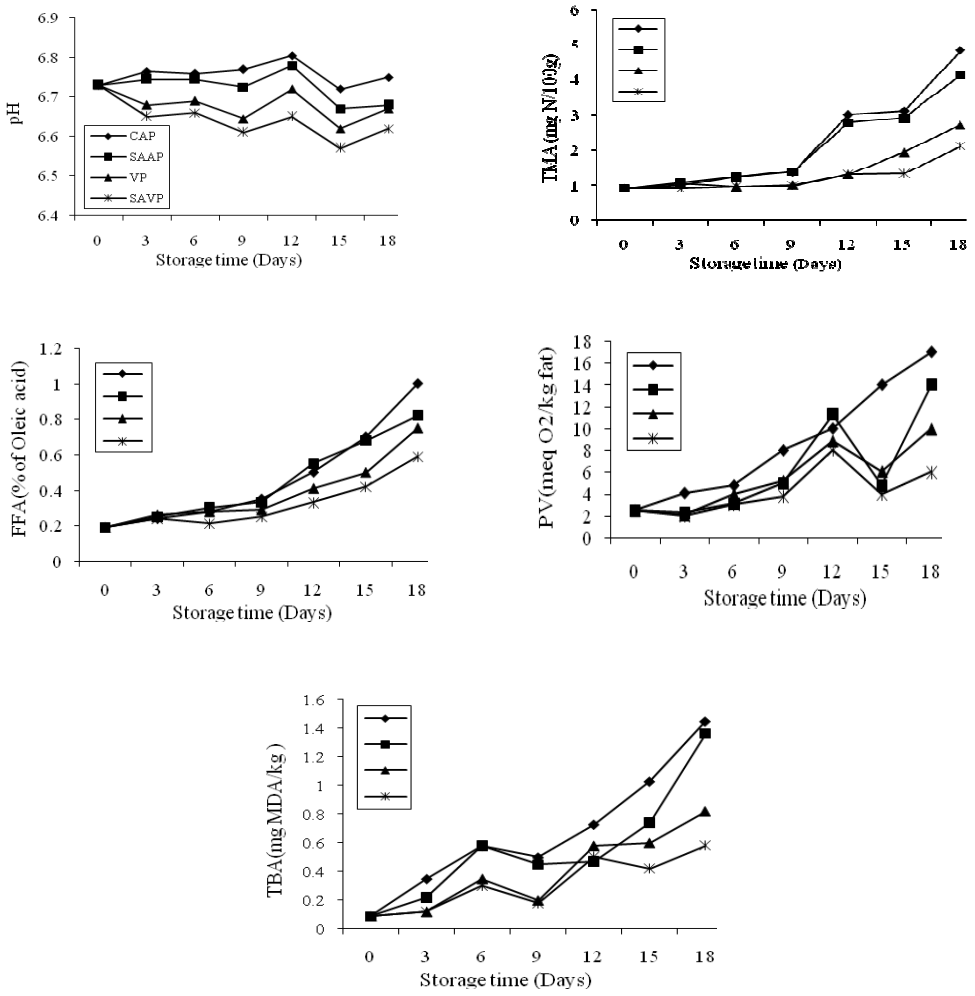

Figure 1. Chemical changes in rainbow trout packed under air and vacuum (sodium acetate treated and untreated) during storage at 2±1ºC. Each point is the mean of three samples. (FFA) free fatty acids, (TMA) Trimethylamine, (PV) peroxide values.

product and the formation of alkaline compounds of autolysis and bacterial metabolites in the rainbow trout fillet (Atrea *et al.,* 2009). Such behavior has also been noted for control air package and sodium acetate vacuum package pearl spot (*Etroplus suratensis*) by Manju *et al.* (2007). A slight drop in pH (6.57) was observed in samples treated with sodium acetate stored under vacuum packaging (SAVP).

Trimethylamine Nitrogen (TMA)

TMA is produced by the decomposition of TMAO due to bacterial spoilage and enzymatic activity (Frangos *et al.,* 2010). Low initial TMA content (0.9 mg N 100 g^{-1}) (Figure 1) indicates that the trout was of good quality, in agreement with the initial TMAN values (day 0) reported for rainbow trout stored under VP (Cakli *et al.,* 2006; Frangos *et al.,* 2010). The highest concentrations of TMA (4.82) were observed for samples packaged in the presence of air followed by SAAP samples (4.12), then VP (2.70) and, lastly, by samples treated with sodium acetate, under vacuum packaging (2.10). Lower TMA value of SAVP samples might be due to the antimicrobial effects of sodium acetate in combination with vacuum packaging in this

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sample over the growth of bacteria. Similar observations were reported earlier by Manju *et al.* (2007) and Rajesh *et al.* (2002). A wide range of TMA values have been reported by various authors as acceptability limits for various fish species: i.e. 1 mg N 100 g^{-1} for sea bream (Kyrana *et al.,* 1997); 5 mg N 100 g^{-1} for sea bass (Masniyom *et al.,* 2002); 5-10 mg N 100 g^{-1} for sardines (Özogul *et al.,* 2004); 12 mg N 100 g^{-1} for hake (Ruíz-Capillas and Moral, 2001); 10-15 mg N 100 g^{-1} as a general limit for fish (Connell, 1990). The great variation in the proposed limit values may be attributed to the fact that TMA values are influenced by fish species, season, initial bacterial count as well as storage conditions (Connell, 1990). Given the great variation in TMA acceptability limits for various fish species reported, and TMA values shown in Fig. 1, as well as data based on sensory (Eye) scores (Table1) and microbiological (TVC) data (Figure. 2), a more realistic TMA limit of acceptability for fresh rainbow trout, of approximately 2.0 mg N/100 g may be proposed, On the basis of this limit, CAP, SAAP, VP and SAVP rainbow trout exceeded this value on days 9, 10, 15 and 17 days, respectively, which could be used to mark the shelf-life of rainbow trout.

Free Fatty Aacid (FFA)

Production of FFA is measured to study the progress of lipid hydrolysis and has been used to establish the degree of deterioration of food products. FFA is a triacylglyceride product formed by either chemical or enzyme mediated hydrolysis (Barthet *et al.,* 2008). In this study, FFA increased from the initial value of 0.19 (expressed as % of oleic acid) to the final value of 1, 0.82, 0.75, 0.59 in CAP, SAAP, VP and SAVP on day 18 of storage period, respectively (Figure 1). Possibly, lipid hydrolysis occurred to a great extent at the end of the storage period. Since the release of FFA content increased with time, as found in this study, it is reported that there is a relationship between FFA

Table 1. Sensory acceptability of CAP [a], SAAP [b], VP [c], and SAVP [d] samples of rainbow trout according to European Community (EC) grading scheme.

Days of storage	Initial	CAP [a]					SAAP [b]					VP [c]					SAVP [d]				
	0	3	6	9	12	15	18	3	6	9	12	15	18	3	6	9	12	15	18	3	6	9	12	15	18
Skin	E	E	A	B	B	C	C	E	A	A	B	B	C	E	A	A	B	B	C	E	E	A	A	A	B
Eye	E	A	B	B	C	C	C	A	A	B	C	C	C	A	A	B	B	C	C	E	A	A	B	B	C
Odour	E	A	B	C	C	C	C	E	A	A	B	B	C	E	A	A	A	B	C	E	E	A	A	A	B
Gill	E	A	B	C	C	C	C	A	A	B	C	C	C	E	A	A	B	B	C	E	A	A	A	B	B
Surface slime	E	A	B	B	C	C	C	A	A	B	C	C	C	A	A	B	B	C	C	A	A	B	B	B	C

[a] Control air pack, SAAP; [b] Under aerobic packaging treated with sodium acetate; [c] Under vacuum packaging in the absence of sodium acetate, [d] Treated samples with sodium acetate, under VP.

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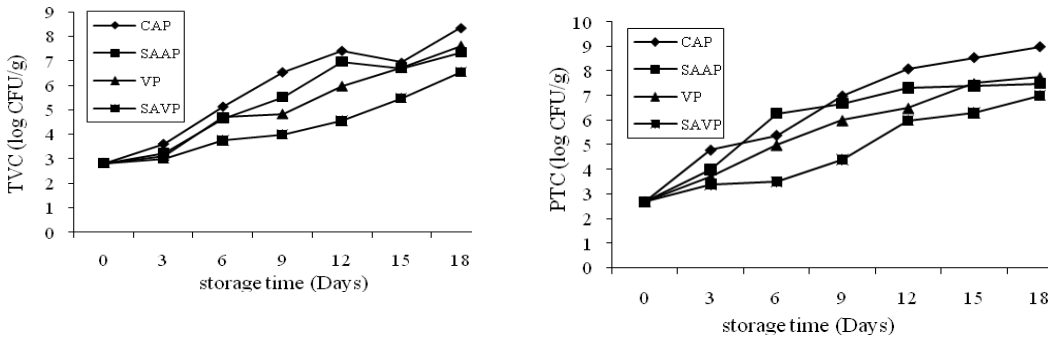

Figure 2. Microbiological changes in rainbow trout packed under air and vacuum (sodium acetate treated and untreated) during storage at 2±1ºC. Each point is the mean of three samples. (TVC) Total viable counts, (PTC) Psychrotrophic count.

release and loss of freshness (Özogul *et al.,* 2005; Rodríguez *et al.,* 2006). During chilling storage, FFA formation has been reported to be produced during a first stage (before the end of the lag phase is attained) as a result of endogenous enzyme (namely, lipases and phospholipases) activity (Whittle *et al.,* 1990). Later on (after the end of the lag phase), microbial activity should be important, so that FFA formation should mostly be produced as a result of bacterial enzyme activity.

Peroxide Value (PV)

Rainbow trout is rich in monounsaturated (50%) and polyunsaturated (26%) fatty acids (Kotakowska *et al.,* 2006) and, therefore, very sensitive to lipid oxidation, which limits its shelf-life. In the present study, primary (PV) and secondary (TBA) oxidation products were determined as indicators of the degree of lipid oxidation. Changes in *PV* are shown in Figure 1. The initial *PV* value was 2.5 meq O_2 kg^{-1} fish fat. However, the initial *PV* values were found to be 1.12 to 1.23 for sliced salmon (Sallam, 2007), 1.17 for farmed rainbow trout (Rezaei and Hosseini, 2008) and 5.60 for wild turbot (Özogul *et al.,* 2006). No significant difference ($P> 0.05$) was observed between CAP, SAAP, *VP* samples in this study, but this index exhibited a marked increase ($P< 0.05$) during storage period. According to the literature, a *PV* value of 10-20 meq O_2 kg^{-1} oil is considered as the upper limit for foodstuffs (Huss, 1995; Özogul *et al.,* 2005; Özogul *et al.,* 2006). The *PV* values of all samples remained within the proposed acceptable level throughout the entire storage period.

Thiobarbituric Acid (TBA)

TBA value is an index of lipid oxidation measuring malondialdehyde (MDA) content (Goulas and Kontominas, 2007). *TBA* value in CAP, SAAP, VP, SAVP samples increased from an initial value of 0.09 (mg malondialdehyde kg^{-1} of fish) to a final value of 1.45, 1.36, 0.82, 0.58, respectively, after 18 days of storage period (Figure 1). There was a trend towards an increase in *TBA* value up to a certain point (day 6) during the storage period, followed by either a decrease in their value (day 9) and, then, increased during the entire storage period. SAVP samples exhibited still lower *TBA* values than the other treatments. This is in agreement with Manju *et al.* (2007) who observed a reduction in *TBA* values of sodium acetate-treated Pearlspot (*Etroplus suratensis*) during chill storage to control samples. Moreover, Shalini *et al.* (2000) also observed lower *TBA* values in sodium acetate-treated vacuum-packed *Lethrinus lentjan* fillets during

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refrigerated storage. Higher *TBA* value in CAP, SAAP samples are probably due to exposure of the fish lipid to atmospheric oxygen. The absence of O_2 in vacuum samples lead to decrease in lipid oxidation and, therefore, decrease of *TBA* value. Lower *TBA* value in SAVP samples are probably due to antibacterial effects of sodium acetate on bacterial enzymatic reaction related with oxidation. In the present study, the *TBA* level showed fluctuations during storage indicating that *TBA* values may not reveal the actual rate of lipid oxidation since malondialdehyde can interact with other components of the fish body and produce secondary metabolites that include carbohydrates, furfural, alkenals, alkadienals, and other aldehydes and ketones (Botsoglou *et al.,* 1994). According to Connell (1990), TBA values of 1-2 mg MDA kg^{-1} of fish flesh are usually regarded as the limit beyond which fish will normally develop an objectionable odor/taste. In all the samples, *TBA* values were within the limit throughout the storage period.

Total Viable Count (TVC)

The initial (day 0) *TVC* (Figure 2) of trout was 2.8 log CFU g^{-1}, which is a relatively low bacterial load, in agreement with the results of Chytiri *et al.* (2004) for whole ungutted rainbow trout and Frangos *et al.* (2010) for rainbow trout fillets. CAP (control air pack), SAAP (under aerobic packaging treated with sodium acetate), and VP (under vacuum packaging in the absence of sodium acetate) exceeded the value of 7 log CFU g^{-1} for *TVC*, considered as the upper acceptability limit for fresh marine species (ICMSF, 1986) on days 10-11, 12, and 16 of storage, respectively, while SAVP (sodium acetate treated samples under VP) samples did not reach this value throughout the 18-day. In other studies, Manju *et al.* (2007) found that surface treatment with sodium acetate (2%) was equally effective in inhibiting microbial growth and extending storage life of Pearlspot (*Etroplus suratensis*) to 15 days compared to 10 days for the control vacuum pack samples and 8 days for air-stored samples. Moreover, sodium acetate treatment has been reported to cause a significant reduction in microbial population and extend the shelf-life of various refrigerated fish such as channel cat fish (*Ictalurus punctatus*) fillets (Zhuang *et al.,* 1996), cod (*Gadus morhua*) fillets (Boskou and Debevere, 2000) and slice salmon (Sallam, 2007).

Psychrotrophic Count (PTC)

The Gram-negative psychrotrophic bacteria are the major group of microorganisms responsible for spoilage of aerobically stored fresh fish at chilled temperatures (Gram and Huss, 1996). In this study, the initial *PTC* of rainbow trout samples was 2.7 log CFU g^{-1} and continuously rose and reached about 7 log CFU g^{-1} on days 9, 10-11, 13-14, and 18 of storage period in CAP, SAAP, VP, SAVP samples, respectively (Figure 2). Psychrotrophic counts of cold stored fish fillets was about 1 log CFU g^{-1} higher than the total plate counts, indicating that fish bacterial flora is composed mainly of psychrotrophic bacteria (Duan *et al.,* 2010). Gimenez *et al.* (2002) reported an aerobic Psychrotrophic count (7 log CFU g^{-1}) for farmed filleted rainbow trout stored under vacuum and modified atmosphere packages after 10 and 14-17 days, respectively. Furthermore, Arashisar *et al.* (2004) found that the Psychrotrophic count of rainbow trout stored in vacuum and air package was 7 log CFU g^{-1} after 6 and 5 days, respectively. There were significant ($P< 0.05$) differences in PTC between CAP and (SAVP, VP), while no significant difference were found between CAP and SAAP. Therefore, combination of sodium acetate and vacuum packaging significantly delayed the microbial growth and, consequently, extended the shelf-life of trout samples.

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Sensory Evaluation

Freshness grades of raw rainbow trout within different treatment are shown in Table 1. According to the results of the sensory analysis, air packed samples maintained excellent to high quality (grades E and A) up to day 3 of storage. In contrast with these results, such grades were observed at day 6 for SAAP and VP samples, whereas SAVP samples (exception surface slime factor) have an excellent to high quality up to day 9. As determined by sensory analysis data, the observed shelf-life of trout samples was longest for SAVP (15-16 days) followed by VP (12-13 days), SAAP (9-10 days) and CAP samples (6-7 days). Manju *et al.* (2007) reported that addition of sodium acetate at 2% (dip treatment) extended the shelf-life of refrigerated Pearlspot. The use of sodium acetate and vacuum-packaging was found to extend the shelf-life of rainbow trout samples in the present study also. Thus, vacuum-packaging, in conjunction with 2% sodium acetate, can be safely used to extend the shelf-life of rainbow trout samples up to 15 days at 2±1°C. Similar to our results obtained for CAP and SAVP rainbow trout, a shelf-life of 5-6 and 15 days was reported for rainbow trout air storage condition at 2±1°C (Arashisar *et al.,* 2004) and ice-stored Pearlspot (*Etroplus suratensis*) (Manju *et al.,* 2007), respectively.

CONCLUSIONS

Based primarily on sensory and also on microbiological data, the shelf-life of fresh rainbow trout during aerobic storage was approximately 6-7 days. Addition of 2% sodium acetate extended product shelf-life by 9-10 days. Shelf-life for samples stored under vacuum packaging was approximately 12-13 days, whereas the combination of sodium acetate (2% W/V) and VP conditions resulted in shelf-life extension of trout by approximately 15-16 days.

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اثر ترکیبی بسته بندی در خلاء و غوطه وری در استات سدیم بر افزایش زمان ماندگاری قزل آلای رنگین کمان (Oncorhynchus mykiss) طی نگهداری در دمای سرد

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چکیده

مطالعه حاضر، استفاده از بسته بندی (خلاء، هوا) یا به همراه استات سدیم (۲ %وزنی/حجمی) را به منظور افزایش زمان ماندگاری قزل آلای رنگین کمان (Oncorhynchus mykiss)طی ۱۸ روز نگهداری در یخچال مورد بررسی قرار داد. ۴ تیمار مختلف مورد آزمایش قرار گرفتندCAP :، گروه کنترل که در هوا بسته بندی گردیدند SAAP :، نمونه های تیمار شده با استات سدیم که در هوا بسته بندی گردیدندVP :، نمونه های ماهی که بدون تیمار با استات سدیم در خلاء بسته بندی شدند: و SAVP، نمونه هایی که تحت تیمار با استات سدیم و در خلاء بسته بندی شدند. مقادیر تری متیل آمین نیتروژن(TMA) ، اسید های چرب آزاد (FFA) و پراکسید (PV) نمونه های SAVP در مقایسه با سایر تیمارها در سراسر دوره نگهداری در یخچال کمتر بود. در مطالعه حاضر، میزان اسید تیوباربیتوریک (TBA)طی دوره نگهداری نوساناتی را نشان داد که بیانگر این است که میزان TBA نمی تواند نرخ واقعی فساد چربی را بیان کند. مقادیر بار باکتریایی کل (TVC)برای نمونه های تازه قزل آلا که در هوا بسته بندی گردیدند به ۷ $\log CFUg^{-1}$ بعد از ۱۱-۱۰ روز رسید، در حالیکه تیمارهای SAAP و VP به ترتیب در روزهای ۱۲ و ۱۶ به این مقدار رسیدند. حال آنکه نمونه های نمونه های SAVP در طول ۱۸ روز نیز به این مقدار نرسیدند. شمار باکتریهای سرماگرا برای نمونه های SAVP بطور معنی داری ($P<0.05$) در مقایسه با گروه کنترل در طی دوره نگهداری کمتر بود. با توجه به ارزیابی حسی، زمان ماندگاری قزل آلا برای گروه SAVP 16-15 روز، برای تیمار VP 13-12روز، برای نمونه های SAAP 10-9 روز و برای نمونه های CAP 7-6 روز بود. نتایج آزمونهای میکروبی، شیمیایی و حسی نشان داد که اثر ترکیبی بسته بندی در خلاء و استات سدیم موجب حفظ کیفیت و افزایش زمان ماندگاری فیله ماهی قزل آلای رنگین کمان طی دوره نگهداری در دمای سرد می گردد.

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