EVALUATION OF SHELF LIFE OF LIVE AND GUTTED FISH TREATED WITH A SHALLOT EXTRACT

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ABSTRACT

This study was carried out to evaluate the quality of whole gutted fresh rainbow trout via two methods: the immersion of whole gutted rainbow trout in a shallot extract and the immersion of live fish in a shallot extract before being gutted and vacuum packed and stored at 4 ± 1C. The control and the treated fish samples were periodically analyzed for microbiological (total viable count, psychrotrophic count, *Enterobacteriaceae*), chemical (peroxide value, thiobarbituric acid) and sensory characteristics. The results indicated that immersion of fish in shallot extract increased shelf life of fish stored at refrigeration temperatures.

PRACTICAL APPLICATIONS

Shallot extract is a natural antioxidant. It can improve the quality of food products during refrigerated storage because of the presence of sulfur-containing compounds and polyphenolic derivatives in its composition. Shallot extract promotes health by preventing lipid oxidation, and has been shown to possess antibacterial characteristics. In the food industry, it can be used as alternatives to the synthetic antioxidants because the use of these types of antioxidants is controlled because of concern for their carcinogenic potential.

INTRODUCTION

Fish is a valuable source of protein for human consumption. The significance of long-chain polyunsaturated fatty acids (PUFAs) of the n-3 family has gained attention because of their preventive effect against human cardiovascular diseases (Sallam 2007). However, given that the shelf life of refrigerated fish is relatively short and that there is a growing tendency of consumers to eat fresh rather than processed or frozen foods, research on the application of new preservation methods and technologies that extend the shelf life of fresh fish is required. Plant extracts are regarded as natural preservatives and their use in foods meets the current demands of consumers for mildly processed or natural products (Atrea *et al*. 2009). Many spices and herbs, and their extracts, possess antimicrobial activity, although questions still remain regarding their safe use in food products. Plant extracts and their constituents have a wide spectrum of antimicrobial actions. The composition and structure, as well as the functional groups of the extracts, play an important role in determining their antimicrobial activity. Usually, compounds with phenolic groups are the most effective (Ojagh *et al*. 2010b). Among these, extracts of cinnamon, thyme, shallot, clove, turmeric, rosemary and vanillin have been found to be the most effective against microorganisms. Because of the effect of the direct addition of plant extracts to food, the sensory characteristics of added food may have supplementary applications in food packaging (Seydim and Sarikus 2006; Ojagh *et al*. 2010a). In fact, the hurdle method (defined by Leistner [2000] as an intelligent combination of hurdles) can be cause to increase shelf life storage. Many studies have evaluated the antimicrobial and antioxidant activities of several plant extracts, including rosemary (Cadun *et al*. 2008), oregano (Atrea *et al*. 2009), thyme (Kykkidou *et al*. 2009), cinnamon (Ojagh *et al*. 2010a) and creosote bush (Mendez *et al*. 2011). In these studies, fish fillets were exposed to plant extracts and the results demonstrated both antimicrobial and

antioxidant activities of the plant extracts against foodborne pathogens (including *Enterobacter aerogenes*, *Escherichia coli*, *Salmonella typhi* and *Staphylococcus aureus*), plus extension of the shelf life of the fillets. Regard to the live fish gill and skin rapidly absorb materials in water (Garcia Romeu and Maetz 1964), this study compared the effect of a shallot extract on the shelf life extension of rainbow trout (*Oncorhynchus mykiss*) via two methods: the immersion of whole gutted and live rainbow trout in a shallot extract before being gutted and vacuum packed and stored at 4 ± 1C. Certain microbiological, chemical and sensory parameters were evaluated for all treatments.

MATERIALS AND METHODS

Preparation and Treatment of Fish Samples

Fresh rainbow trout (*O. myciss*) were purchased and brought within an hour to the laboratory in sealed foam polystyrene boxes containing flaked ice. The trout samples were divided into three groups. The first group was gutted and washed with potable water and vacuum packed (control sample [I]). Following gutting and washing, the second group was immersed in shallot extract solution (1.5% v/v) for 30 min before being vacuum packed (II). In the third group, live fish were floated in a shallot extract solution (1.5% v/v) for 30 min (III) before being gutted, washed and vacuum packed. All samples were labeled and stored at 4 ± 1C for 20 days. Sampling was carried out at predetermined time intervals, namely: 0, 5, 10, 15 and 20 days. On each sampling day, three packages were randomly selected from each batch for chemical, microbiological and sensorial analyses to determine the overall quality of the fish.

Chemical Analyses

Determination of pH. The pH value was recorded using a pH meter (713ph Meter, Metrohm, Herisau, Switzerland). Trout muscle (10 g) was thoroughly homogenized with 90 mL of distilled water and the homogenate was used for pH determination (Manju *et al.* 2006).

Assessment of Lipid Oxidation. The peroxide value (PV) was calculated according to the method of Egan *et al.* (1997), and the results were expressed in meq oxygen/lipid. The thiobarbituric acid value (TBA) was determined by a selective third-order derivative spectrophotometric method (Atrea *et al.* 2009). The TBA content was expressed as mg of malondialdehyde (MDA)/kg fish muscle.

Bacteriological Analysis. Total viable counts (TVC) were determined by the pour plate method using plate count agar (Merck, Darmstadt, Germany). The plates were incubated at 37C for 48 h and expressed as log cfu/g of the sample. All plates for TVC and differential counts were prepared in duplicate (Kilinc and Cakli 2005). Psychrotrophic counts (PTCs) were determined using a similar method to that for TVC except that plates were incubated for 7 days at 10C (Ojagh *et al.* 2010a). *Enterobacteriaceae* counts (EBC) were performed using the pour plating method on violet red bile glucose agar (Merck). The plates were overlaid with a virgin layer of the same growth medium before incubation at 37C for 24 h (Sallam 2007).

Sensory Evaluation. The sensory quality of raw fish (texture, color, odor and overall) was evaluated on days 0, 5, 10, 15 and 20 by five trained panelists (Ozogul *et al.* 2006). The fish samples were ranked according to the following four categories: highest quality (E), good quality (A), fair quality (B) and unacceptable quality (C).

Statistical Analysis. On each sampling occasion, three independent samples from each processing condition were subjected to microbiological and chemical quality tests. The data were subjected to analysis of variance by using the Statistical Package for the Social Sciences (SPSS) 16 software for Windows (SPSS Inc., Chicago, IL; 1989–1999). For significantly different treatments, Duncan's multiple range test was used to group the parameter means and the significance was determined at $P < 0.05$.

RESULTS AND DISCUSSION

Chemical Analysis

pH. The changes in pH of the fish samples during vacuum-packed storage at 4 ± 1C are shown in Fig. 1. The initial pH of the fish samples was pH 6.68. The pH of the control samples decreased initially and then increased again. The initial decrease in pH may have been due to the dissolution of CO_2 in the fish samples. Similar observations were made by Mexis *et al.* (2009) and Manju *et al.* (2006). The increase in pH may have been due to the production of volatile basic components, including ammonia and trimethyl amine, by fish spoilage bacteria (Fan *et al.* 2008). In the case of samples from groups II and III, the pH decreased during storage. It can be concluded that the lower pH level of groups II and III, caused by the shallot extract, increased the degree of microbial inhibition and contributed toward extending the preservation of the fish samples by inhibiting the activity of endogenous proteases (Fan *et al.* 2008). Significant differences ($P < 0.05$) were observed in the pH level between the controls and the treated samples (groups II and III) at day 20 of the storage period. No differences ($P > 0.05$) were observed throughout the storage period

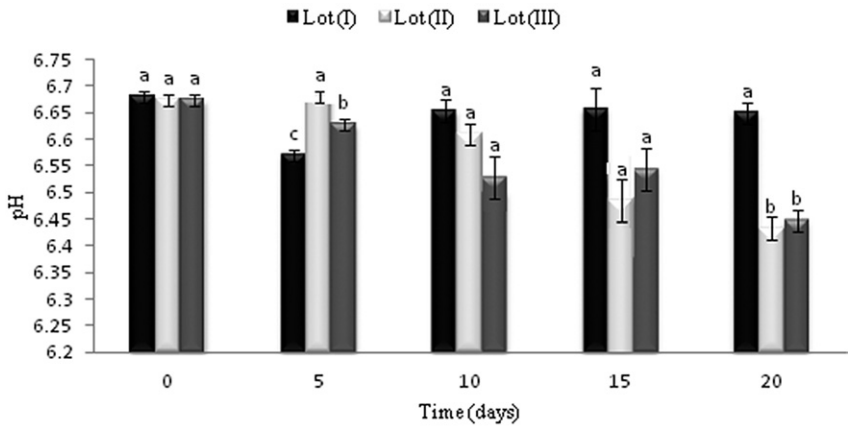

FIG. 1. CHANGES IN PH OF FISH SAMPLES DURING REFRIGERATED STORAGE
Lot (I): control sample, Lot (II): immersed whole gutted fish in shallot extract, Lot (III): immersed live fish in shallot extract. Bars represent the error bar. Same small letters on the bar indicate no significant difference between the different treatment and the control group on the particular sampling day ($P < 0.05$).

between the two treated samples. These results are in agreement with those of Mexis *et al*. (2009), who reported that oregano essential oil was effective in controlling pH of rainbow trout fillets stored at 4 ± 1C.

Lipid Oxidation. Lipid peroxidation is the oxidative deterioration of PUFAs in fish tissue, creating off odors and off flavors and thereby shortening the shelf life of food. The PV and the TBA are both well-established methods for determining oxidation of lipids in products (Barakat *et al*. 2006).

The effect of the shallot extract on the changes in the PV of the fish lipids is shown in Fig. 2. The initial PV (meq peroxide/kg fish muscle) in the raw trout was 0.86. The PV values of the controls and treated samples (groups II and III) increased significantly ($P < 0.05$) with the time spent in storage. In comparison with the initial PV value in the control samples, a considerable increase was observed on day 10 (6.70 meq/kg), followed by a decrease to 4.67 meq/kg at the end of the storage period. The secondary reactions of the carbonyl compounds and volatilization may have been the cause of this decrease (Vidya and Srikar 1996). Significant differences ($P < 0.05$) were observed in the PV between group I and groups II and III at days 10 and 20 of the storage period. Also, a significant difference ($P < 0.05$) was observed between groups I and III at day 15. No difference ($P > 0.05$) was observed throughout the storage period between groups II and III. The results of the present study indicate that the shallot extract is effective at delaying lipid peroxidation in trout meat stored under refrigeration (4 ± 1C). These results are in agreement with those of Mexis *et al*. (2009), who reported that oregano essential oil was effective at delaying the production of primary lipid oxidation products in rainbow trout fillets stored at 4 ± 1C.

The effect of the shallot extract on the changes in the TBA of fish lipids is shown in Fig. 3. The initial TBA (mg MDA per kg of fish sample) in the raw trout was 0.33. The TBA values of the controls and immersed samples (group II) increased to a maximum during storage by day 15, followed by a slight decrease in this value. The decrease may have represented the breakdown of MDA because of tertiary

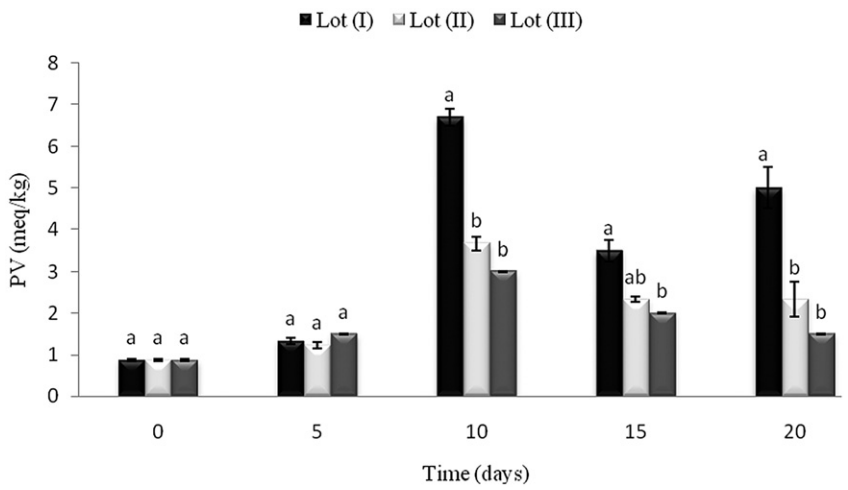

FIG. 2. CHANGES IN PEROXIDE VALUE (PV) VALUE OF FISH SAMPLES DURING REFRIGERATED STORAGE
Lot (I): control sample; Lot (II): immersed whole gutted fish in shallot extract; Lot (III): immersed live fish in shallot extract. Bars represent the error bar. Same small letters on the bar indicate no significant difference between the different treatment and the control group on the particular sampling day ($P < 0.05$).

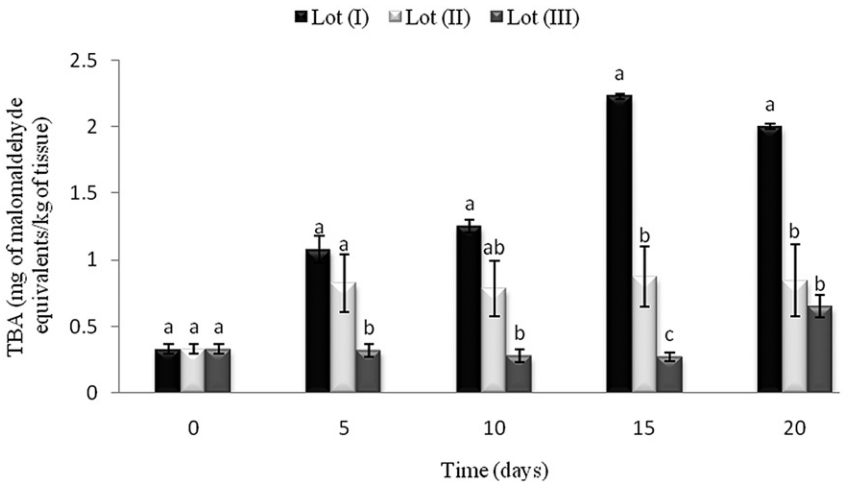

FIG. 3. CHANGES IN THIOBARBITURIC ACID VALUE (TBA) VALUE OF FISH SAMPLES DURING REFRIGERATED STORAGE
Lot (I): control sample; Lot (II): immersed whole gutted fish in shallot extract; Lot (III): immersed live fish in shallot extract. Bars represent the error bar. Same small letters on the bar indicate no significant difference between the different treatment and the control group on the particular sampling day ($P < 0.05$).

degradation (Ozden *et al*. 2007; Pezeshk *et al*. 2011). The TBA values of the samples from the live fish that were immersed in the shallot extract (group III) increased to a maximum during storage up to day 20. By day 10 of storage and beyond, significantly lower differences ($P < 0.05$) were found in the TBA value of the treated samples (groups II and III) compared with the controls. Similar results were obtained in trout fillets and sea bream (Goulas and Kontominas 2006; Mexis *et al*. 2009). In this study, group III showed a lower TBA value ($P < 0.05$) in comparison with group II (Fig 3). In the case of most plant extracts, the antioxidant activity of their phenolic compounds is related to their ability to break down free radicals, donate hydrogen atoms or electrons, or chelate metal cations (Ojagh *et al*. 2010a).

Bacteriological Analysis

The present study focused on monitoring the following: TVCs, psychrotrophic bacteria and *Enterobacteriaceae*. Variations in the value of TVC during refrigerated storage are presented in Fig. 4. The initial TVC of the whole gutted rainbow trout was 2.14 (log cfu/g). A TVC value of 7 log cfu/g (ICMSF 1986) is considered the upper acceptable limit for freshwater and marine species. By day 20 of storage, the TVC values in the trout tissues from groups II and III were still below 6 log cfu/g, whereas that of the controls had reached a count of 7.44 by 20 days. By day 5 of storage and beyond, significantly lower differences ($P < 0.05$) were found between the TVC values of the treated samples (groups II and III) compared with the controls. In the present study, the TVC values for the treated samples (groups II and III) were much lower than the limit mentioned earlier, throughout the 20-day storage period. The significant reduction in TVC observed in the treated samples of trout can be attributed to the antibacterial effect of plant extracts on aerobic spoilage bacteria. No difference ($P > 0.05$) was observed throughout the storage period between groups II and III. Reports concerning the biological

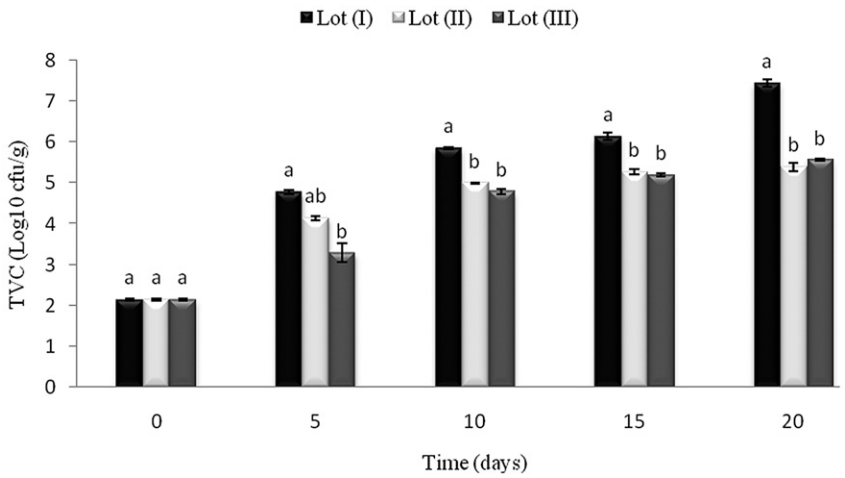

FIG. 4. CHANGES IN TOTAL VIABLE COUNTS (TVCs) VALUE OF FISH SAMPLES DURING REFRIGERATED STORAGE
Lot (I): control sample; Lot (II): immersed whole gutted fish in shallot extract; Lot (III): immersed live fish in shallot extract. Bars represent the error bar. Same small letters on the bar indicate no significant difference between the different treatment and the control group on the particular sampling day ($P < 0.05$).

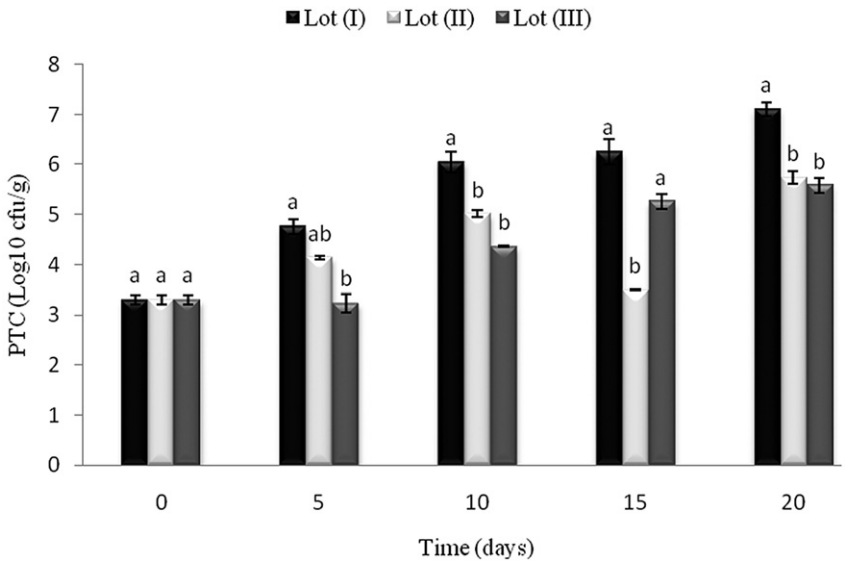

FIG. 5. CHANGES IN PSYCHROTROPHIC COUNTS (PTCs) VALUE OF FISH SAMPLES DURING REFRIGERATED STORAGE
Lot (I): control sample; Lot (II): immersed whole gutted fish in shallot extract; Lot (III): immersed live fish in shallot extract. Bars represent the error bar. Same small letters on the bar indicate no significant difference between the different treatment and the control group on the particular sampling day ($P < 0.05$).

effects of shallots are scarce compared with other *Allium* species such as onion and garlic. Shallots were recently reported to show antioxidant, antibacterial, hypocholesterolemic and free radical-scavenging abilities (Adeniyi and Anyiam 2002; Wongmekiat *et al.* 2008). This result is in agreement with that of Mexis *et al.* (2009), who reported that the natural antibacterial was effective in retarding the growth of TVC in rainbow trout fillets during refrigerated storage. The shelf life of fresh fish is largely reduced by the growth and biochemical activities of gram-negative, PTC strains of *Pseudomonas*, *Achromabacter*, *Flavobacterium* and *Moraxella* species in the presence of atmospheric O_2 (Ozden *et al.* 2007). In our study, the initial PTC (day 0) of trout meat was 3.3 log cfu/g (Fig. 5). An increase in the PTC of trout meat during storage was observed. By day 5 of storage and beyond, significantly lower differences ($P < 0.05$) were noticed between the PTC of treated samples (groups II and III) compared with the controls. No difference ($P > 0.05$) was observed throughout the storage period between groups II and III, expect for day 15 when group II was significantly lower than group III ($P < 0.05$). Kykkidou *et al.* (2009) reported similar results for swordfish.

Enterobacteriaceae, a hygiene indicator, were also found to be members of the microbial populations associated with the spoilage of fresh whole gutted rainbow trout during refrigerated storage. This finding is in agreement with the results reported for different fish species, including salmon (Sallam 2007), Mediterranean octopus (Atrea *et al.* 2009), and rainbow trout (Mexis *et al.* 2009), in which *Enterobacteriaceae* were part of the microflora found at the end of the shelf life of products under refrigerated storage. The changes in the value of the EBC during storage are presented in Fig. 6. The initial EBC of rainbow trout was 1.78 log cfu/g. The EBC of both the controls and the treated samples significantly increased ($P < 0.05$); by the end of the storage period (day 20). However, much lower counts of 3.54 and 3.81 log cfu/g were found in groups II and III, respectively (Fig. 6). Although *Enterobacteriaceae* can grow at low temperatures, their propagation was slow during refrigerated storage, possibly because their growth rate is lower than the other Gram-negative psychrotrophic spoilers (Sallam 2007), Nonetheless, the spoilage potential of *Enterobacteriaceae* must be taken into consideration, especially in the case of polluted water or a delay in chilling after the fish are caught (Ozden *et al.* 2007).

Sensory Evaluation

Table 1 shows the results of the sensory analysis of rainbow trout samples under chilled storage. The control trout samples maintained a high (E) and good quality (A) during the first 10 days of storage. The quality of the controls decreased on day 10 (A), and they were no longer acceptable by day 20 (C). The limits of acceptability for texture, odor and color were reached after 10 days for the control samples and after approximately 15 days for groups II and III. The sensory evaluation results appeared to be correlated with the microbial and chemical analyses. Because of high rates of microbial growth and lipid oxidation, the control samples of whole gutted trout showed spoilage, with an off odor and a slimy, discolored appearance after 15 days of storage. A good correlation between microbial, chemical and sensory data was previously documented by several studies (Nerantzaki *et al.* 2005; Mexis *et al.* 2009; Ojagh *et al.* 2010a). The antioxidant and antimicrobial activities of herbal extracts have been shown to delay the effects of

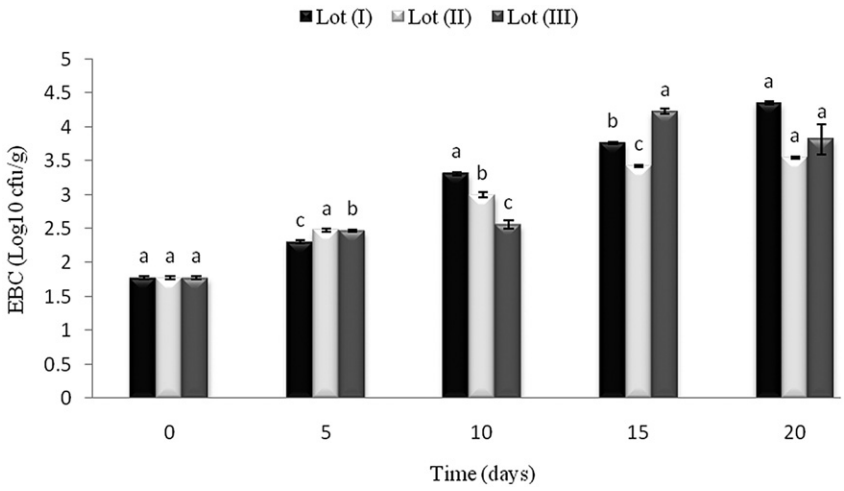

FIG. 6. CHANGES IN *ENTEROBACTERIACEAE* COUNTS (EBCs) VALUE OF FISH SAMPLES DURING REFRIGERATED STORAGE
Lot (I): control sample; Lot (II): immersed whole gutted fish in shallot extract; Lot (III): immersed live fish in shallot extract. Bars represent the error bar. Same small letters on the bar indicate no significant difference between the different treatment and the control group on the particular sampling day ($P < 0.05$).

oxidation, prolonging the shelf life of products while maintaining the quality. Shallot extract treatments yielded beneficial effects on texture, odor, color and the overall acceptability of rainbow trout during storage.

CONCLUSION

In this study, the immersion of both live and gutted fish in a shallot extract caused the delay of bacterial and oxidative decay under refrigerated storage. The immersion of live fish in the shallot extract had the same effect as the immersion of gutted fish in the shallot extract on the delay of bacterial and oxidative decay under refrigerated storage, by extending its shelf life for five more days. The future repetition of similar studies on others fish by different natural antioxidant and antibacterial could produce more conclusive and practical results.

TABLE 1. CHANGES IN ATTRIBUTES SCORES OF FISH SAMPLES REFRIGERATED STORAGE

Sensory attributes	Treatment	Storage period (days)				
		0	5	10	15	20
Texture	Lot I	E	A	A	B	C
	Lot II	E	E	E	A	B
	Lot III	E	E	E	A	B
Odor	Lot I	E	A	A	B	C
	Lot II	E	E	E	A	B
	Lot III	E	E	E	A	B
Color	Lot I	E	A	A	B	C
	Lot II	E	E	A	A	B
	Lot III	E	E	A	A	B
Overall	Lot I	E	A	A	B	C
	Lot II	E	E	A	A	B
	Lot III	E	E	A	A	B

Lot (I): control sample, Lot (II): immersed whole gutted fish in shallot extract, Lot (III): immersed live fish in shallot extract.
E, highest quality; A, good quality; B, fair quality and C, unacceptable.

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