The effect of clove oil on frozen storage stability and quality of rainbow trout (Oncorhynchus mykiss)

Gökkuşağı Alabalığı (Oncorhynchus mykiss)’nin kalitesi ve dondurulmuş depolama stabilitesi üzerine karanfil yağının etkisi

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INTRODUCTION

Frozen and chilled storage are widely used protection methods for fish and fish product, owing to the microbiological growth and some biochemical processes can be minimized with these methods. But, the quality of fish muscle will also deteriorate throughout frozen storage as the fishes have high protein and unsaturated fatty acid contents (Simeonidou et al. 1997; Tironi et al. 2010; Dusukcan et al. 2014). That’s why, some measures must be taken to extend the shelf life of fish in cold storage.

Clove oil is a plant widely cultivated in the world. Its antimicrobial potential was established when its essential oil extracts killed many Gram positive and Gram negative organisms including some fungi. The antimicrobial activity of clove is attributable to eugenol, oleic acids and lipids found in its essential oils (Nzeako et al. 2006). Clove oil has been listed as a “Generally Regarded As Safe” substance by the United States Food and Drug Administration when administered at levels not exceeding 1500 ppm in all food categories. Nontraditional preservation techniques are being developed to satisfy consumer demand with regard to nutritional and sensory aspects of foods. This increasing demand has opened new dimensions for the use of natural preservatives derived from plants and animals. In biopreservation, storage life is extended, and/or safety of food products is enhanced by using natural compounds (Nzeako et al. 2006; Yildiz Oguzhan, 2015). Besides, plant and spices extracts possessing an antioxidant activity preserve the cells from the harm caused by the free radicals and they have an major role in the antioxidant defense (Tuna Keleştemur, 2011; Tuna Keleştemur and Özdemir, 2013).

The impact of essential oils on the shelf-life of fish and fish products has been studied (Harraz et al., 2003; Mahmoud et al., 2006; Goulas and Kontominas, 2007), but there are very limited study (Solanki et al., 2016) on fish, treated with clove oil.
Therefore, the objective of the present study was to determine the effect of vacuum pack and in combination with the addition of clove oil, as a natural preservative, on the shelf-life extension of rainbow trout (Oncorhynchus mykiss) fillets by evaluating microbiological and sensory quality.

**MATERIALS AND METHODS**

**Preparation of fish samples and storage conditions**

Clove oil was acquired from Kalsec® (Kalsec®, Inc, Kalamazoo). Fresh rainbow trout (O. mykiss) samples (varying from 250 to 280 g weight and total rainbow trout 150) were obtained from Keban Dam Lake. Fish samples were placed in ice box and transferred within 1 h to the fish-processing laboratory of the Faculty of Fisheries of Firat University. Fishes were immediately weighed, gutted, headed and filleted. Then fillets were washed with water and were divided to three lots.

**First group:** Control (Control group, no clove oil)
**Second group:** 0.5 Co (0.5 % v/w Clove oil added)
**Third group:** 1 Co (1% v/w Clove oil added)

Clove oil was added onto the surface (two sides) of each fillet using a micropipette followed by mild uniform distribution (directly with gloved fingers) of the oil for each sample. The clove oil ratio was determined by preliminary studies. All samples were vacuum-packaged and stored in -18 °C and analysed once a month during 6 month to determine the microbiological and sensory attributes.

**Microbiological analysis**

Rainbow trout (10 g) obtained from each fillet were transferred aseptically to a Stomacher bag containing 90 ml of sterile 0.1% peptone water (Buffer Peptone Water, LAB M) and homogenised for 1 min using a laboratory blender (Stomacher 400, Lab. Blender, London, UK) at high speed. Tenfold successive dilutions were made with 0.1% peptone water from these homogenates as required. Petri dishes for determination of total anaerobe on brewer anaerobe agar and then incubated at 30 °C for 3 days. Psychrophile was determined on Plate Count Agar (PCA, Merck 1.05463) after incubation at 7°C for 10 days. Lactic acid bacteria were enumerated on de Man Rogosa Sharpe agar (MRS, Oxoid, CM361) incubated at 30°C for 5 days. Yeast and mold bacteria were enumerated on Potato Dextrose Agar (PDA, Merck 1.10130) incubated at 22 °C for 5 days enumerated. Microbiological counts were expressed as log colony-forming units (cfu) per gram of sample (Harrigan 1996; ICMSF, 1986).

**Sensory analysis**

Ten experienced panelists, staff members of the department of fish processing and technology, who had experience on fish and fish products were chosen to evaluate the quality of fillets. Panelists were asked to evaluate appearance, odor and texture of raw samples. Appearance, odor and texture were scored on a 10-point hedonic scale. A score of zero was used as a rejection attribute point (Simeonidou et al., 1997).

**Statistical analysis**

The study has been composed of three independent repetitions. By using the SAS program, the data was processed. According to General Linear Model (GLM) procedures, averages of the least squares were designated through Fisher’s Least Significant Difference (LSD) test and the statistical significance level was taken as 0.05 hereby (SAS 1999).

**RESULTS AND DISCUSSION**

**Microbiological changes during frozen storage**

Deterioration of fish mainly occurs as a result of bacteriological activity leading to loss of quality and subsequent spoilage. Changes in the microbial flora (total anaerobe, psychrophilic bacteria, lactic acid bacteria, mold-yeast) of rainbow trout fillets during frozen storage are illustrated in Fig 1.

Initial (1st month) total anaerobe counts was 0.95 log cfu/g in all groups (Fig 1). After storage of two months, significant differences (p<0.05) were found between control group and clove oil treated groups. Bacteria populations increased in all groups during storage. However, the lowest bacteria counts were obtained from Group 1Co. At the end of the storage period of 6 months 4.55, 3.14 and 2.06 log10 cfu/g were measured for Control, 0.5 Co and 1 Co group, respectively. These results show that the addition of clove oil had effect on the total anaerobe bacteria of frozen trout. Similar studies show the effectiveness of essential oils such as clove applied to mean samples to prolong their shelf life, inhibiting microorganism growth and measuring the antioxidant activity and therefore increasing the product's shelf life (Burt, 2004)

The psychrophilic bacteria counts of control and other groups were recorded during frozen storage. Initial populations of psychrotrophic bacteria of Control, 0.5 Co and 1 Co samples were 2.14, 1.88 and 1.16 log10 cfu/g, respectively. Psychrophilic bacteria counts of Control, 0.5Co and 1Co groups were 8.11, 7.12 and 7.20 log10 cfu/g at the end of the storage period, respectively. Based on the statistical analysis, clove oil treated groups had significantly lower (p<0.05) counts when compared to control samples during storage. Control and 0.5Co, 1Co samples exceeded the value of 6 log cfu/g for psychrotrophic bacteria count, considered as the upper acceptability limit for fish and fish products, after 4 months and 6 of storage, respectively. Therefore, compared with the control samples, a shelf life extension of 2 months was achieved for clove oil treated samples, as determined by psychrotrophic bacteria counts. Our results for psychrophilic bacteria are in agreement with findings of fresh fish species stored with other essential oils (Erkan, 2007; Ozyilmaz 2007; Attouchi and Sadok, 2011; Emir Çoban and Patır 2013).
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Figure 1. Changes in the microbial flora of rainbow trout fillets during frozen storage. **Control**: without clove oil vacuum packaged. **0.5 Co**: added clove oil 0.5 % v/wt, vacuum packaged. **1 Co**: added clove oil 1 % v/wt, vacuum packaged.

Vacuum-packed food is stored in the absence of oxygen and some microorganisms have restricted growth. Instead, the bacterial population occurs mainly of lactic acid bacteria. In vacuum-packed seafood, lactic acid bacteria are problematic as they produce typically sour odors and flavours. Lactic acid bacteria counts of Control, 0.5Co and 1Co groups (LAB) were determined as 1.50, 1.51 and 1.40 log10 cfu/g, respectively at the beginning of storage. LAB counts in all groups increased during storage (Fig 1). Increases in treated groups were less than the control group. These results may explain the inhibitory effects of essential oils on LAB (Burt, 2004; Emir Çoban and Patır 2013). Kostaki et al (2009), reported that the treatment of fresh fish fillets (sea bass) with a mixture of thyme oil reduced the final LAB counts by approximately 1 log, compared with control group. Likewise, Kykkidou et al. (2009) reported that thyme–oregano oil treatment was effective in eliminating the growth of TVC and LAB in modified atmosphere packaged fish under refrigerated storage. They are also in agreement with those reported by other researcher (Erkan 2007; Chouliali et al.2007; Kykkidou et al. 2009; Atrea et al. 2009; Emir Çoban and Patır 2013).

In the present study, the number of yeast–mold of control and clove oil treated samples were determined as 1.26 and 0.95 log10 cfu/g, respectively at the beginning of storage. Yeast–mold amount increased in all groups during storage (Fig 1). Yeast–mold populations were highest in the control samples, as expected, compared to the added clove oil samples. Clove oil causes a decrease in the number of bacterial cells, demonstrating they can be used to protect a food matrix such as fish, prolonging the bacteria lag period and that’s why prolonging its shelf life. Similar findings have been found by Suhr and Nielsen (2003) and Emir Çoban and Patır (2013).

**Sensorial changes during frozen storage**

The acceptability of fish products during frozen storage depends on the changes in their sensory attributes. Fish products were considered to be acceptable for human consumption until the sensory score reached 4 (Amerine et al.1965; El-Hanafy et al. 2011). The sensory scores of frozen trout fillet with clove oil are presented in Fig 2.
Figure 2. The results of the sensory evaluation of rainbow trout fillets during frozen storage. Control: without clove oil vacuum packaged. 0.5 Co: added clove oil 0.5 % v/wt, vacuum packaged. 1 Co: added clove oil 1 % v/wt, vacuum packaged.

All the samples at 1st months had high odour scores ranged from 8.5 to 8.7, which means that all samples at initially were in excellent quality. During the storage period, there was a significant loss in the fish quality for all samples. Odour score of control, 0.5Co and 1 Co groups were determined as 5, 6.4, 7.2, respectively at 6th months. Similar findings were reported by other researcher (Özyilmaz 2007; Chouliara et al. 2007; Emir Çoban and Patır 2013). Plant extract applied groups were more preferred than control groups like in our study.

Initially the best odour score were found in control samples, however the score significantly decreased at the end of the storage period. The samples treated with 0.5% clove oil were the most preferred fillets followed by the samples with 1% clove oil. (Fig 2).

Chemical and physical changes of proteins occurring during frozen storage may result in texture deterioration. This is an important problem because it negatively affects sensory quality. Texture modification is mainly a result of damage caused to the protein structure. In Fish, most of these changes are caused by the production of formaldehyde in the muscle (Sotelo et al. 1995). The formaldehyde liberated reacts with proteins to form crosslinks. These changes also causes loss of taste and odor. The rainbow trout fillets treated with clove oil were the most preferred samples in terms of sensory characteristics, whereas control samples had low sensory scores.

CONCLUSION

Consumer demands for foods with a prolonged shelf life are of major importance. Ensuring food safety and at the same time meeting demands for retention of nutrition and quality attributes has resulted in increased interest in alternative preservation techniques for inactivating microorganisms.
This study has clearly shown that preservation of frozen fish applying clove oil have longer shelf life than normal. Moreover, as fish has good sensory quality, a proper preservation technique suggested in this paper is highly relevant.

REFERENCES


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