ANTIOXIDANT AND ANTIBACTERIAL EFFECTS OF *NIGELLA SATIVA* L. SEED AND *ECHINOPHORA PLATYLOBA* DC. LEAF EXTRACTS ON RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) FILLETS DURING REFRIGERATION STORAGE

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ABSTRACT

This study was performed to evaluate the effects of *Nigella sativa* L. Seed and *Echinophora platyloba* DC. Leaf Extracts as natural preservatives on microbiological [total viable count (TVC) and psychrophilic bacterial counts (PTC)], chemical [total volatile base nitrogen (TVB-N), peroxide values (PV) and thiobarbituric acid (TBA) values], sensory changes of rainbow trout (*Oncorhynchus mykiss*) fillets stored at 4°C±1. The present results showed that there was a significant delay in lipid oxidation and spoilage of the fish fillet samples in treated samples with a plant extracts in comparison with the control ($P < 0.05$).For the control, *N. sativa* (2.0 %), *N. sativa* (4.0 %), *E. platyloba* (2.0 %) and *E. platyloba* (4.0 %) treatments, The PTC and TVC values were above the acceptability limit (7 log10 colony-forming unit/g) at 6, 9, 12, 9 and 12th day of storage, respectively. The best antioxidative and antimicrobial activities and sensory scores, up to 12 days of storage were observed *E. platyloba* (4.0 %) extract. The results showed that *E. platyloba* extract had higher quality and enhanced the sensory attributes. This study showed that the *N. sativa* and *E. platyloba* can increase the shelf-life extension of Rainbow trout

fillet stored at 4°C±1 up to 9-12 days so could be used for increasing shelf-life extension of fish products.

Keywords: Antimicrobial, Antioxidant, *Echinophoraplatyloba*, Fish, *Nigella sativa*, shelf-life

INTRODUCTION

Rainbow trout (*Oncorhynchusmykiss*) belongs to the Salmonidae and is a species with high commercial value and much appreciated by consumers. It is sold as either whole fresh fish or in fillet form. Due to its desirable characteristics (taste, aroma, white flesh) and nutritional value, demand for rainbow trout in Iran and other country markets significantly increased [1]. Fish and fishery products are excellent food, especially in terms of protein, trace elements and vitamin D. Fish is one of the most perishable foods and is very susceptible to chemical and microbial deterioration such as hydrolysis, protein denaturation and lipid oxidation etc.[2]. Various spoilage mechanisms expressed to be involved in this quality loss including microbial development, non-enzymatic lipid oxidation, enzymatic browning and endogenous enzyme activity [3, 4] therefore, reducing the fish shelf-life extension. Fish is a very good culture media so contamination of spoilage microorganisms is almost unavoidable. The fish spoilage is a complicated process in which physical, chemical and microbiology changes interact. Oxidation of lipid is one of the primary sources of quality deterioration in seafood products. This process is a decisive parameter in the decreasing of the food products shelf-life because it is responsible for the production of texture deterioration, decrease in nutritional value, undesirable odors and formation of potentially toxic substances [5].The lipid oxidation prevention of seafood products is important for keeping their high quality and safety. Oxidation of lipid is a major deal of muscle food deterioration [6], succeeding off-flavors, toughening, unpleasant odors and muscle discoloration and nutritious value reduce[7]. Antioxidant addition of with synthetic or natural origin is one of the strategies to retard or decrease oxidation and prevent the quality loss and sensory properties[8].Natural aromatic plants have been widely used in many food products as seasoning and flavoring agents [9] for preservation and their medicinal value [10]. Medical plants, such as *N. sativa* and *E. platyloba*, have been used as condiments and spices in foods in the past. In last years, many researchers have investigated the application of these plants as natural sources of antimicrobial and antioxidant components

[11,12]. Therefore, these species can play an important role as natural preservative in the food industry.

Nigella sativa L. is an annual herbaceous plant which belongs to family Ranunculaceae. The plant commonly grows in the Eastern Europe, Middle East and Central and Western Asia. Due to its notable pharmacological properties **[13]**, this plant has been have been extensively investigated in last years.*N. sativa* Seed oil is considered as health beneficial. This seed oil has been expressed to possess antioxidant activity **[14]** and antibacterial activity **[15]**.

Echinophora platyloba D. C species from *Echinophora* genus is (Umbelliferaean) aromatic, mid-summer plant that wildly grows and mainly used for imparting taste and flavor to the food in Iran **[16]**. *E. platyloba* is used in folk medicine and as a food seasoning. Many studies have shown that *Echinophora* genus plants have significant antimicrobial and antioxidant activities **[11]**.

Despite the above mentioned facts, the application of *N. sativa* seeds and *E. platyloba* leaf extract as natural preservatives has not been studied in depth in food product particularly fish products. So, the aim of this study was to investigate the effects of *N. sativa* seeds and *E. platyloba*leaf extract as antioxidant and antimicrobial compounds on shelf-life and quality of rainbow trout (*Oncorhynchus mykiss*) fish fillet during a storage trial at 4±1°C.

2. MATERIAL AND METHODS

2.1. Preparation of fish fillet

Rainbow trout (*Oncorhynchusmykiss*) with an average weight of 550 ± 60 g were purchased from a farm in Bandar-e Gaz, Golestan, Iran. Then, they were gutted and filleted with sterile equipment and placed at temperatures less than 10°C, then were packed in polystyrene boxes containing ice and transferred to the laboratory in 2 h at the same condition.

2.2. Preparation of Plant Extract

N. sativa seed were purchased from a local herbal store, Gorgan, Iran and fresh leaves of *E. platyloba* was collected (July2014) from Sistan rangeland, Iran. *N. sativa* seeds and *E. platyloba* leaves were dried in a hot air oven (Behdad 3490, Zanjan, Iran) at 50ºC. Then the samples were ground into a fine powder with a kitchen blender (Pars khazar, Iran).After that, the samples (20 g) were mixed with 200 ml of 85% ethanol using a shaking water bath for 24 h at room temperature. Through Whatman No. 1 filter paper, the extracts were separated from the solid concentrate by filtering. Then by using a rotary vacuum evaporator (Laborota 4000, Heidolph, Germany), the solvent was removed under

vacuum at 30°C. These extracts were kept at -80°C until used.

2.3. Treatment of Fish Samples

Fish samples were divided into five treatment batches consisting of: 1) control sample (dipped in distilled water), the other batches were dipped in *N. sativa* (2 and 4 %) and *E. platyloba* (2 and 4 %) extracts for 30 min. Then all of the treatments were drained well and were wrapped in polyethylene package in short time and stored at temperature of 4 ± 1°C in refrigerator. Chemical, microbiological and sensory characteristics were investigated at predetermined time intervals, namely, 0, 3, 6, 9 and 12 days.

2.4. Chemical Analysis

2.4.1. Lipid Oxidation Indicators

2.4.1.1. Determination of peroxide value (PV)

The peroxide value (PV) was measured according to the Egan *et al*. [17] method. Results were expressed as mill equivalent peroxide per kilogram of sample.

2.4.1.2. Determination of Thiobarbituric Acid Reactive Substances (TBARS)

The Thiobarbituric Acid Reactive Substances (TBARS) value was measured according to the Porkony and Dieffenbacher method as described by Ojagh *et al*.[18]. The results were expressed as milligram of

malondialdehyde equivalent per kilogram of tissue.

2.4.2. Fish Freshness Indicators

2.4.2.1. Determination of total volatile basic nitrogen (TVB-N)

Total volatile basic nitrogen (TVB-N) value was investigated by the method of micro-diffusion [19]. Results were expressed as milligram TVB-N per 100 g fish muscle.

2.4.3. Microbiological Analysis

Fish samples (10 g) were transferred and homogenized into stomacher containing 90 mL of 0.85% NaCl solution. Other decimal dilutions were prepared from this dilution. TVC and PTC were measured using plate count agar (Merck, Darmstadt, Germany). Plates of total viable aerobic bacteria (TVC) were incubated at 25°C for 2 days and plates of psychrotrophic bacteria (PTC) were incubated at 7°C for 10 days. All counts were indicated as log colony-forming unit (cfu)/g and were carried out in duplicate [18].

2.4.4. Sensory Evaluation

For flavor sensory investigation, the fish fillet samples (100 g) were separately cooked about 10–20 min at 98°C. In this step, 1.5% of salt was added to the samples [18].The sensory analysis of fish samples was evaluated by ten-member trained panelists (25-30 years old, male). Panelists scored texture, odor, flavor, color and general acceptability using a 10-

point scale, with excellent quality (10–9), good quality (8–7), fair and acceptable quality (6–5), poor quality (4–3) and very poor quality (2–1) **[20]**.

2.4.5. Statistical Analysis

All the experiments were carried out in triplicate and mean value ± standard deviation were indicated for each case. Analysis of variance was performed on the data to determine if there was any significant difference ($P < 0.05$) between treatments. *Post hoc* test (Duncan's multiple range test) was conducted using the Statistical Analysis System (SPSS 11.5, IBM SPSS, New York, USA).

3. RESULTS AND DISCUSSION

3.1. Chemical Analysis

3.1.1. Lipid oxidation indicators

3.1.1.1. Peroxide values (PV)

Oxidation of lipid is an important problem in seafood products particularly in fish because it includes high amount of polyunsaturated fatty acids **[19, 21]** and it causes quality deterioration and formation of unpleasant odor **[22]**.PV values Changes is shown in Fig. 1. The results showed that the PV value significantly increased during storage period for control and treated samples ($P < 0.05$). PV value for control samples during all storage time was higher than treated samples. This different could be related to antioxidant activity of plant extracts. The antioxidant activity of *N. sativa* and *E. platyloba*is related to phenolic components **[15,11, 23]**. Huss **[24]**reported that the maximum acceptable limit of PV value 10-20 meq O_2 kg^{-1} oil for food stuffs. In this study, the PV value for control, *N. sativa* (2%) and *N. sativa* (4%) and *E. platyloba* (2%) and *E. platyloba* (4%) was upper than acceptable limit in days 6, 9, 12, 9 and 12, respectively.According to the results, the plant extracts showed the best performance in retardation of primary lipid oxidation.

3.1.1.2. TBA Reactive Substances (TBARS)

The value of TBA is considered as an indicator for the malonaldehyde(MDA) content; the most predominant secondary oxidation products for food lipids, therefore it is considered a good chemical constant for measuring the secondary oxidation extent of edible lipids during storage period**[25]**. The results of TBARS formation indicated in Fig. 2.The initial TBARS of fish samples were found to be 0.72 ± 0.01 to 0.76 ± 0.00 mg MDA per kilogram of tissue.According to the results, TBA value was significantly increased during storage time for control and other treatments ($P < 0.05$). According to the previews reports, acceptable limit of TBA is 1-2 mg MDA kg^{-1}. In present study, the TBARS value for control, *N. sativa* (2%) and

N. sativa (4%) and *E. platyloba* (2%) and *E. platyloba* (4%) was upper than acceptable limit in days 6, 9, 12, 9 and 12, respectively. It can be related to antioxidant activity of plant materials and their ability to break free radicals and chelate metal cations[18].

3.1.2. Fish freshness indicators

3.1.2.1. Total volatile basic nitrogen (TVB-N)

The TVB-N is one of the most widely used for seafood quality determination [26]. TVB-N value change is shown in Fig 3. The results showed that the TVB-N values significantly increased during storage time for treated and untreated samples ($P < 0.05$) but with a more rapid increase in the untreated samples (control).Maximum acceptable limit for fatty fish such as trout is 20 mg N/100 g sample [27]. The TVB-N values were upper than acceptability limit in days 6, 9, 12, 9 and 12 for control, *N. sativa* (2%) and *N. sativa* (4%) and *E. platyloba* (2%) and *E. platyloba* (4%), respectively. In present study, TVB-N formation delaying in treated samples can be associated with a more rapidly reduced in bacterial population and bacterial capacity decrease for oxidative deamination of non-protein nitrogen compound [28].

3.2. Microbiological analyses

Results of TVC value is shown in Fig.4.The recommended limit of TVC is $\log 10^7$cfu/g for freshwater fish [29].During storage, TVC values significantly increased for all samples ($P < 0.05$). For control, the TVC value was higher than the acceptable limit on day 6 of storage (7.26 $\log_{10}$cfu/g). However, the TVC values for treated samples with *N. sativa* (2%) and *N. sativa* (4%) and *E. platyloba* (2%) and *E. platyloba* (4%)was remained lower than the proposed values at 6, 9, 12, 9 and 12th day of storage time, respectively. The psychrotrophic bacteria (PTC) are well known as the main cause of refrigerated fish spoilage. So, the inhibitory effect of *N. sativa*,*E. platyloba* extracts on psychrotrophic bacteria growth in fish fillets during cold storage was measured. The results are indicated as in Fig. 5. Results showed that PTC was significantly increased for all samples during storage time ($P < 0.05$). According to the results, the PTC was lower in treated samples with higher concentration of plant material. Also the results showed that the TVC and PTC were lower in treated samples with *E. platyloba*leafextract. It is related to higher antibacterial activity of *E. platyloba* in comparison with *N. sativa.*

3.3. Sensory Evaluation

The results of sensory evaluation that included Tenderness, color, odor, taste and overall acceptability of fish fillets are presented in Table1. According to the table,

the acceptability limit of both for all factors was reached on day 6, 9, 12, 9 and 12 for control, *N. sativa* (2%) and *N. sativa* (4%) and *E. platyloba* (2%) and *E. platyloba* (4%), respectively.

Fish samples were proposed to be acceptable for human consumption until the sensory score decreased from 10 to 4 **[20]**. At beginning of storage time, all samples had excellent quality. Fish quality significantly decreased during storage period ($P < 0.05$). Also the result showed that treated sample with plant extracts had higher sensory score than untreated samples. According to the results, treated samples with higher concentration (4 %) of plant material indicated better sensory qualities and extended shelf-life in comparison with treated samples with 2% plant extracts. Plant extract addition exhibit antimicrobial and antioxidant activity and can increase the shelf-life extension of fish samples while maintaining its quality.

4. CONCLUSIONS

Generally, the tested plant extracts treatments caused a significant extending of the shelf-life, also improvement the physico-chemical, microbiological and sensory quality properties for fish fillets during cold storage. In addition, fish samples treated with *E. platyloba* (4%) followed by samples treated

with *N. sativa* (4%) attained the highest scores for sensory quality attributes and indicated the better acceptability than the control and other treated fish samples. So, the present findings suggested that the *N. sativa* and *E. platyloba* extracts can be utilized for extending the shelf-life and improving quality properties of fish fillets during cold storage.

ACKNOWLEDGMENTS

Conflict of interest

The authors declare no financial or other conflicts of interest.

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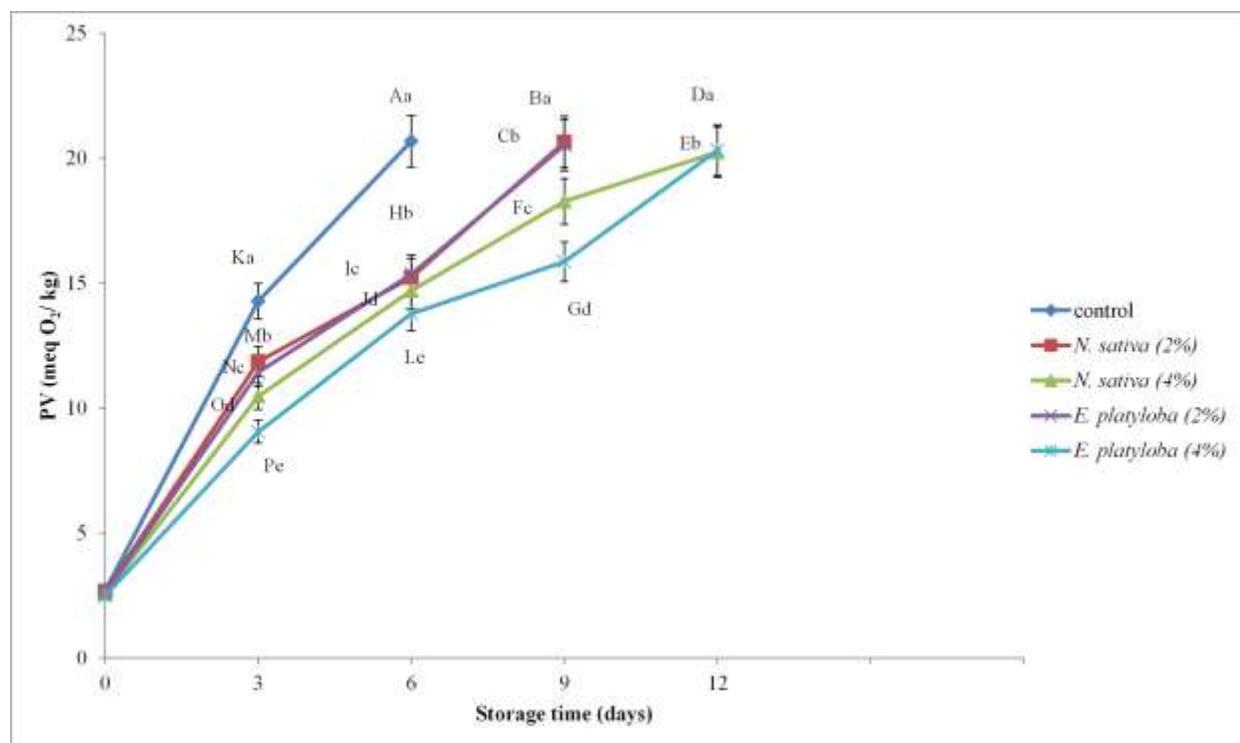
Figure 1: Changes in peroxide values (PV) of fish samples during refrigerated storage. Different letters represent significant difference at *P < 0.05*. The capital and lower case letters indicate the differences among different treatments and the variation of treatment during different days, respectively.
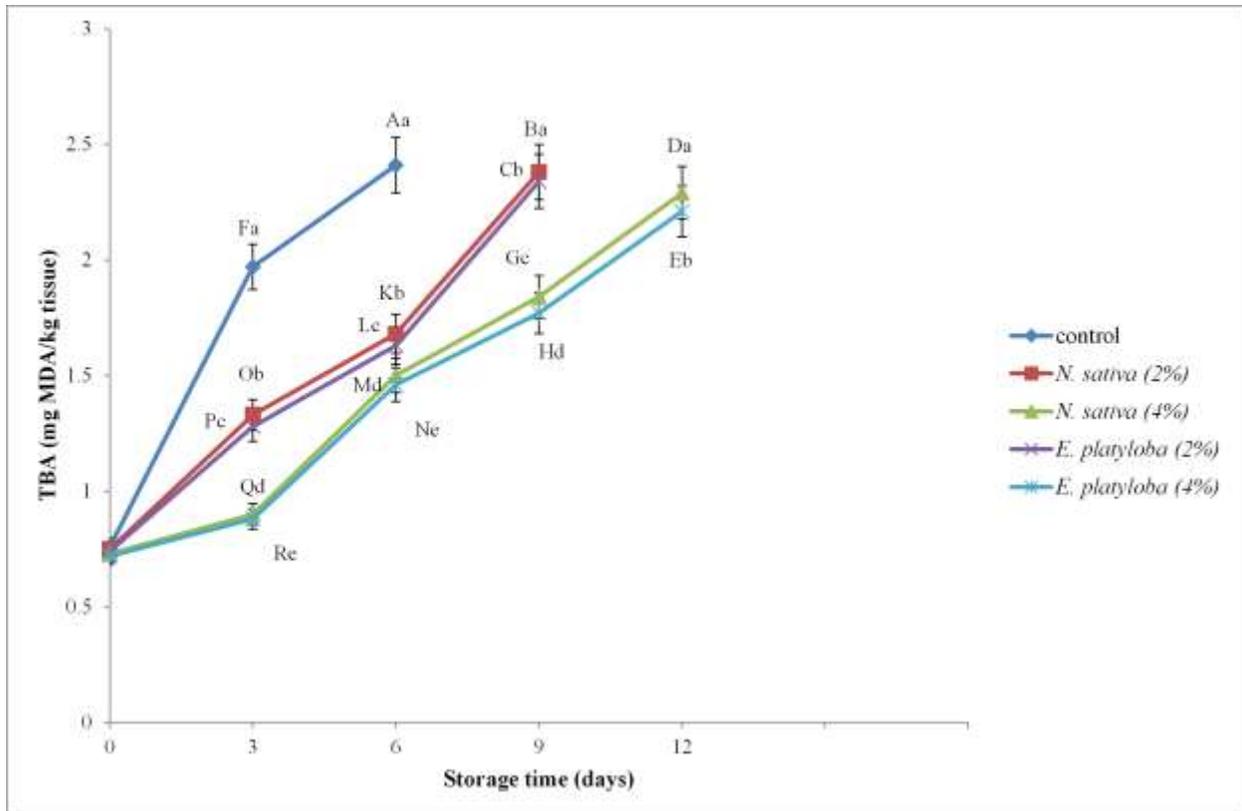
Figure 2: Changes in thiobarbituric acid (TBA) values of fish samples during refrigerated storage. Different letters represent significant difference at *P < 0.05*. The capital and lower case letters indicate the differences among different treatments and the variation of treatment during different days, respectively.
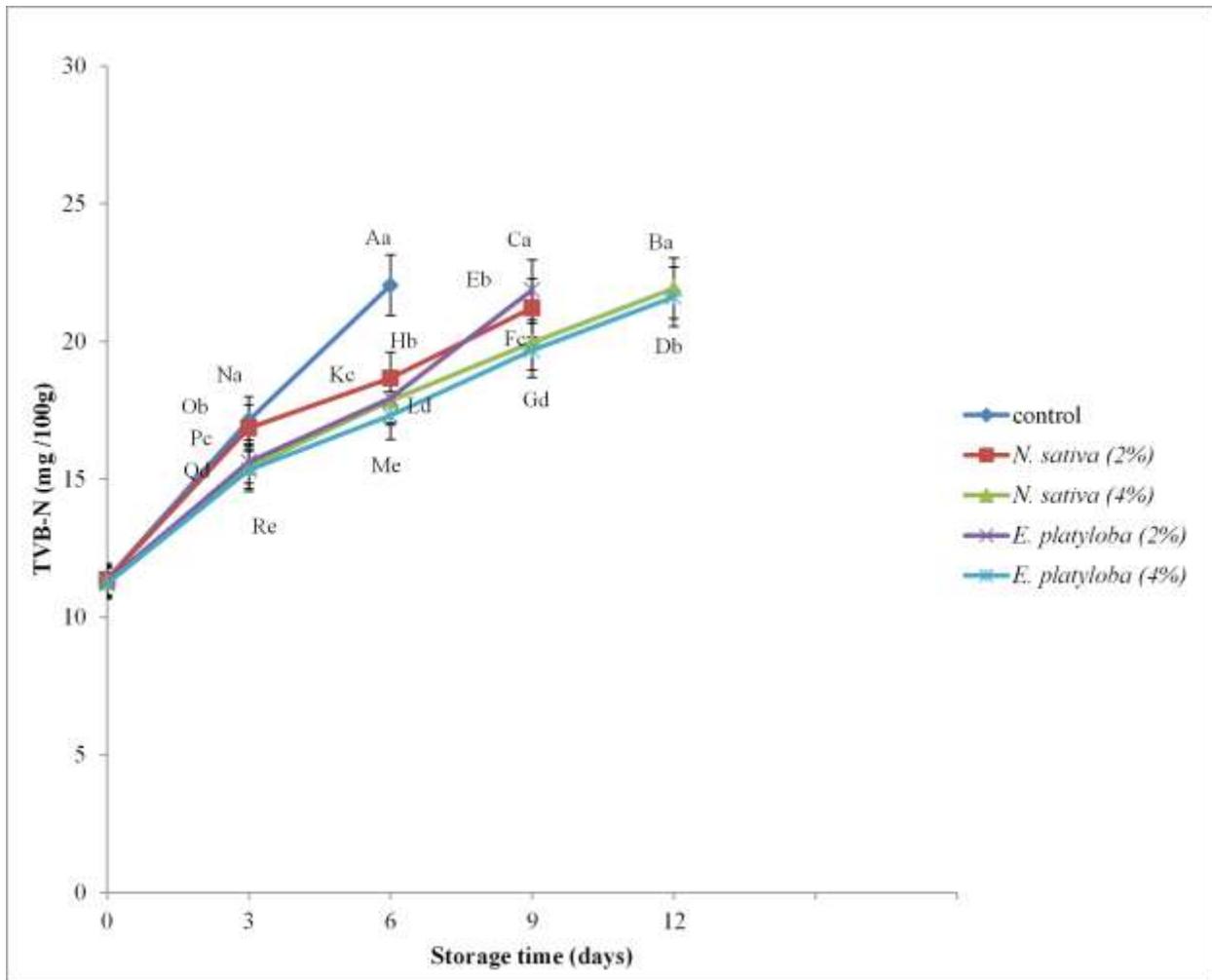
Figure 3: Changes in total volatile base nitrogen (TVB-N) values of fish samples during refrigerated storage. Different letters represent significant difference at *P < 0.05*. The capital and lower case letters indicate the differences among different treatments and the variation of treatment during different days, respectively.
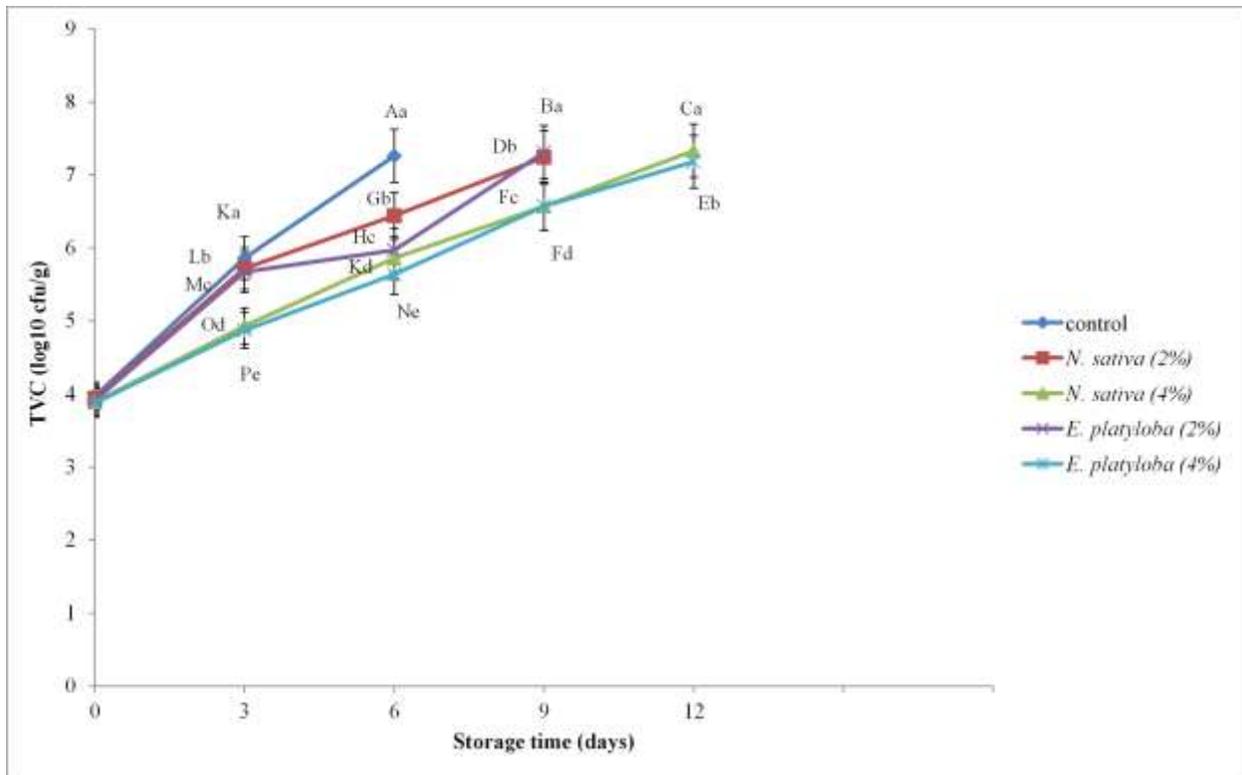
Figure 4: changes in total viable count (TVC) values of fish samples during refrigerated storage. Different letters represent significant difference at *P < 0.05*. The capital and lower case letters indicate the differences among different treatments and the variation of treatment during different days, respectively
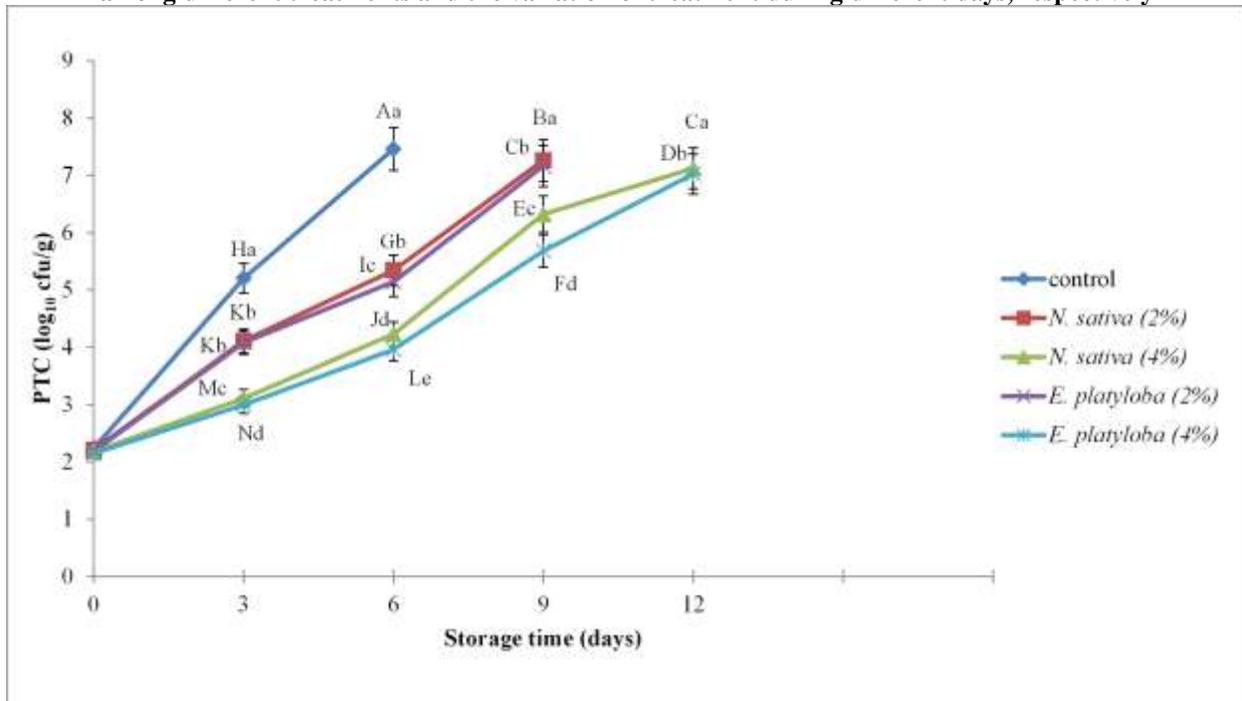

Figure 5: Changes in psychrotrophic count (PTC) values of fish samples during refrigerated storage. Different letters represent significant difference at *P < 0.05*. The capital and lower case letters indicate the differences among different treatments and the variation of treatment during different days, respectively

Table 1: Sensory properties rainbow trout fillet samples stored at 4°C±1

Storage duration (day)	control	*N. sativa* (2 %)	*N. sativa* (4 %)	*E. platyloba*(2%)	*E. platyloba*(4 %)
Color					
0	9.55±0.02^{Aa}	9.55±0.02^{Aa}	9.55±0.01^{Aa}	9.55±0.00^{Aa}	9.55±0.01^{Aa}
3	6.83±0.02^{He}	7.32±0.00^{Fd}	7.82±0.03^{Cb}	7.51±0.01^{Dc}	8.02±0.01^{Ba}
6	5.1±0.03^{Oe}	6.41±0.02^{Jd}	7.3±0.02^{Gb}	6.54±0.02^{Ic}	7.43±0.02^{Ea}
9		5.5±0.01^{Ld}	6.41±0.00^{Jb}	5.61±0.00^{Kc}	6.56±0.00^{Ia}
12			5.27±0.02^{Nb}		5.41±0.01^{Ma}
Taste					
0	9.1±0.02^{Ba}	9.5±0.2^{Aa}	9.49±0.00^{Aa}	9.52±0.01^{Aa}	9.5±0.02^{Aa}
3	6.56±0.01^{Ke}	7.4±0.03^{Gd}	8.1±0.03^{Cb}	7.51±0.01^{Fc}	8.32±0.01^{Ba}
6	5.2±0.02^{Pe}	6.52±0.01^{Ld}	7.66±0.00^{Eb}	6.63±0.01^{Jc}	7.84±0.03^{Da}
9		5.33±0.00^{Od}	6.83±0.02^{Ib}	5.52±0.02^{Mc}	6.91±0.00^{Ha}
12			5.43±0.01^{Nb}		5.62±0.02^{La}
Odor					
0	9.01±0.00^{Ed}	9.85±0.02^{Aa}	9.73±0.01^{Bb}	9.53±0.01^{Cc}	9.8±0.02^{Aa}
3	7.69±0.01^{Ed}	7.52±0.02^{Fe}	8.01±0.00^{Cb}	7.78±0.03^{Dc}	8.1±0.04^{Ba}
6	5.2±0.03^{Oe}	6.51±0.00^{Jd}	7.52±0.02^{Fb}	6.66±0.02^{Hc}	7.55±0.00^{Fa}
9		5.22±0.02Nd	6.61±0.00^{Ib}	5.53±0.01^{Lc}	6.77±0.02^{Ga}
12			5.44±0.03^{Mb}		5.64±0.01^{Ka}
Tenderness					
0	9.02±0.01^{Bb}	9.5±0.28^{Aa}	9.62±0.01^{Aa}	9.63±0.01^{Aa}	9.65±0.02^{Aa}
3	7.5±0.02^{Fe}	7.53±0.01^{Ed}	8.00±0.02^{Cb}	7.65±0.03^{Dc}	8.12±0.02^{Ba}
6	5.3±0.00^{Qe}	6.51±0.03^{Ld}	7.2±0.01^{Hb}	6.64±0.00^{Kc}	7.32±0.01^{Ga}
9		5.36±0.00^{Pd}	6.66±0.00^{Kb}	5.52±0.03^{Nc}	6.84±0.02^{Ja}
12			5.43±0.00^{Ob}		5.66±0.01^{Ma}
Overall acceptability					
0	9.66±0.01^{Aa}	9.63±0.01^{Aa}	9.63±0.01^{Aa}	9.66±0.01^{Aa}	9.66±0.01^{Aa}
3	6.64±0.03^{Ie}	7.52±0.03^{Gd}	8.0±0.03^{Cb}	7.64±0.02^{Fc}	8.3±0.03^{Ba}
6	5.34±0.02^{Pe}	6.51±0.00^{Kd}	7.79±0.01^{Eb}	6.64±0.00^{Ic}	7.92±0.00^{Da}
9		5.42±0.02^{Od}	6.6±0.01^{Gc}	5.64±0.02^{Mb}	6.73±0.01^{Ha}
12			5.56±0.00^{Nb}		5.72±0.00^{La}

The capital letters represent the differences between various treatments during storage trial while the lower case letters indicate the variation of treatment for different samples at the same day.