

# Effects of Gelatin-Based Edible Films Enriched with Laurel Essential Oil on the Quality of Rainbow Trout (*Oncorhynchus mykiss*) Fillets During Refrigerated Storage

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## Summary

The effects of gelatin films enriched with laurel leaf essential oil on the quality of rainbow trout (*Oncorhynchus mykiss*) during refrigerated storage at  $(4\pm 1)$  °C were examined over a period of 26 days. Fish fillets were wrapped with 8 % gelatin films containing 0, 0.1 and 1 % (by volume per mass) of laurel essential oil and vacuum packaged. Sensory (raw and cooked fish), microbiological (total viable counts, psychrotrophic bacteria counts, Enterobacteriaceae and lactic acid bacteria) and chemical (proximate composition, pH, total volatile base nitrogen (TVB-N), thiobarbituric acid (TBA), free fatty acid (FFA) and peroxide value (PV)) analysis, and colour measurement were carried out during the storage period and shelf-life was determined periodically. The obtained results showed that the gelatin film enriched with laurel essential oil was suitable for the preservation of rainbow trout fillet and the ability of laurel essential oil to preserve the film depended on its ratio. Combined effects of gelatin film and laurel essential oil (1 % by volume per mass) was efficient in maintaining the quality characteristics at an acceptable level up to 22 days of storage, while the control and gelatin film without the essential oil reached an unacceptable level at 15 and 20 days, respectively.

*Key words:* gelatin films, laurel essential oil, rainbow trout, *Oncorhynchus mykiss*, shelf-life

## Introduction

Fish and fishery products have been recognized as a nutrition source due to their high protein content. Moreover, they contain considerable amounts of unsaturated fatty acids, especially  $\omega$ -3 fatty acids, which are regarded as health-promoting compounds. However, the shelf-life of seafood is limited due to biochemical and microbiological changes (1). Rainbow trout is a fatty farmed fish, and it is highly demanded among consumers. Quality deterioration of fatty fish species is primarily caused by microorganisms and lipid oxidation during storage (2). Food safety and quality are the major concerns in food industry because of the direct effect of food on human health and quality of life. Edible films and coatings made

from polysaccharides, proteins and lipids can extend the shelf-life of foods by acting as moisture, oxygen, carbon dioxide or vapour barriers and as enhancers of mechanical properties (3). Edible films and coatings have a range of advantages, such as edibility, biodegradability, biocompatibility and barrier properties, as well as being nontoxic and non-polluting (4). In addition, films and/or coatings may act as carriers of antimicrobial agents, antioxidants and other food additives and have been particularly considered in food preservation because of their ability to extend the shelf-life (5). The incorporation of essential oils (EOs) into polymeric matrices gives them interesting antimicrobial/antioxidant properties. Although the use of bioactive coatings enriched with EOs appears to be a promising technology in fish and meat preservation, few

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studies have been published to date (6). Gelatin is a protein obtained by physical, chemical or biochemical denaturation and hydrolysis of collagen. Gelatin-based edible film coatings have already been proposed to extend the shelf-life of various meat products.

*Laurus nobilis* L. (Lauraceae) is an aromatic plant used as a spice in seafood cooking and as a traditional medicine for the treatment of some infectious diseases due to its skin care properties. Its dried leaves and essential oil are used in Italy, France, Turkey, Algeria, Morocco, Spain, Portugal and Mexico as a valuable spice in the culinary and food industry (7). Nevertheless, there is no information on the development of gelatin films enriched with laurel leaf essential oil.

The aim of this work is thus to determine whether the shelf-life of refrigerated rainbow trout fresh fillets can be extended by the use of a gelatin film enriched with laurel essential oil as an antioxidant and antimicrobial agent.

## Materials and Methods

### *Plant materials*

Fresh bay laurel leaves were collected from Mugla (Turkey) in May 2012. Leaves were separated from their stem parts and air-dried until use.

### *Extraction and analysis of essential oil*

The essential oil (EO) was obtained by hydrodistillation, using a Clevenger apparatus (Edutek Instrumentation, Haryana, India) with 150 g of dry plant material and 1500 mL of water. The oil was obtained after 3 h of distillation at boiling temperature and stored at  $(4\pm 1)$  °C in airtight glass vials covered with aluminium foil. The gas chromatography-mass spectrophotometry (GC-MS) analysis of the obtained essential oil was conducted at the ARGEFAR-ÇEG Laboratory of Aegean University (İzmir, Turkey), using an Agilent gas chromatograph model 6890 equipped with an Agilent mass selective detector (MSD) model 5973 (Agilent Technologies, Santa Clara, CA, USA). Identification of components in the essential oil was carried out with the Wiley 275 mass spectral library (NIST, Wiley, New York, NY, USA).

### *Preparation of fish samples*

Fresh aquacultured rainbow trout (*Oncorhynchus mykiss*, average mass and length:  $(250\pm 10)$  g and  $(200\pm 5)$  mm, respectively) were obtained from a local fish farm. The samples were placed in polystyrene foam boxes with flake ice and transferred to the Fish Processing Laboratory at the Faculty of Fisheries at Mugla Sıtkı Kocman University (Mugla, Turkey) within 2 h, decapitated, filleted by hand and washed in tap water. Two fillets were obtained from each fish. In total, 216 rainbow trout fillets (average mass:  $(100\pm 10)$  g) were prepared for analysis.

### *Preparation of film-forming solution and treatment of fish fillets*

Preparation of edible films was slightly modified from Gómez-Estaca *et al.* (8). Food grade gelatin powder

(8 g; Doga Drug and Raw Material Co. Ltd., Ankara, Turkey) was dissolved in 100 mL of distilled water (at room temperature) and the mixture was stirred until the gelatin completely dissolved (approx. 15 min). Glycerol (0.1 mL per g of gelatin) and D-sorbitol (0.15 g per g of gelatin) were then added to the gelatin solution, which was kept at 45 °C for additional 15 min. Laurel essential oil (LEO) in a ratio of 0.1 and 1 % (by volume per mass of gelatin) was then added to the film-forming solution. To stabilize the emulsion, Tween-20 was also added at 15 % (by volume), depending on the LEO content. One of the gelatin groups was prepared without the addition of laurel essential oil (0 % LEO).

The film-forming solution with the added LEO was homogenized with an Ultraturrax T25 basic blender (21 500 rpm, position 5, for 1 min; IKA-Werke GMBH & Co. KG, Staufen, Germany). The films were obtained by casting 40 mL of film-forming solution into square polystyrene foam dishes (inner dimension of the foam dish was  $10.5\times 19$  cm) and drying at room temperature for 48 h at  $(57\pm 5)$  % relative humidity (RH). Film thickness was measured using a digital micrometer (model MDC-25M, Mitutoyo, Kanagawa, Japan), averaging nine different locations ( $210\ \mu\text{m}$  ( $p<0.05$ )).

Three different types of films were then obtained: gelatin film with 0 % LEO, and gelatin films with 0.1 and 1 % LEO. Wrapping method was slightly modified from Ahmad *et al.* (9). Dried film samples were peeled off aseptically from the foam dish. Prior to analysis, both sides of the films were subjected to UV for 2 min to be sterilized. To wrap the fillets, one piece of film was put into a sterile foam dish, the rainbow trout fillet was put onto the film and another sterile film was put onto the fillet. Thus, each fillet was coated completely on both sides. The control group was not wrapped in any kind of gelatin film. Then, each sample (including the control group) was vacuum packaged (Culinary, ATM Machinery BV, Haaksbergen, The Netherlands) and stored at  $(4\pm 1)$  °C.

### *Sensory evaluation*

Sensory evaluation of the control and gelatin-wrapped groups (0, 0.1 and 1 % LEO) was conducted by six trained persons. Panellists gave scores for sensory characteristics, such as appearance, colour, odour, texture and general acceptability using a 9-point descriptive scale. On this scale, scores between 7.0 and 9.0 indicated extremely like; scores between 4.0 and 6.9 indicated like, and 3.9 was the limit of acceptability (10).

### *Microbiological analysis of fish samples*

The following groups of microflora were monitored: total viable count (TVC), psychrotrophic bacteria count, *Enterobacteriaceae* and lactic acid bacteria (LAB). A sample of 10 g was removed aseptically from the fillet using a scalpel and forceps, transferred to a stomacher bag containing 90 mL of sterile peptone water (PW) solution (0.1 %), and homogenized at room temperature. For each sample, further serial decimal dilutions were prepared in PW solution (0.1 %). The appropriate dilutions were subsequently used for enumeration and differentiation of

microorganisms. TVC was determined using plate count agar (PCA, Merck code 1.05463, Merck, Darmstadt, Germany) after incubation for 2 days at 37 °C, and psychrotrophic bacteria counts were determined after incubation at 7 °C for 10 days with the same medium (11). *Enterobacteriaceae* were determined using double layer Violet Red Bile Agar (VRB Agar, Merck code 1.01406) after incubation for 2 days at 37 °C (12). Lactic acid bacteria were determined using double layer de Man-Rogosa-Sharpe agar (MRS, Merck code 1.10660) after incubation for 2 days at 37 °C (13).

### Chemical analysis of fish samples

The fish samples were analysed in triplicate for proximate composition: lipid content of fish by the Bligh and Dyer method (14), moisture and ash content by AOAC method (15) and total crude protein by Kjeldhal method (16). The pH values were recorded by a digital pH meter (InoLab, WTW, Weilheim, Germany) after homogenization of each 10 g of fish muscle sample in 100 mL of distilled water (17). Determination of total volatile base nitrogen (TVB-N) was carried out as described by Antonacopoulos (18). Homogenized fish samples were steam-distilled and the TVB-N value (in mg of nitrogen per 100 g of fish) was determined according to the consumption of 0.1 M HCl. Thiobarbituric acid (TBA) reactive substances were determined according to Tarladgis *et al.* (19) to evaluate the oxidation stability during chilled storage and the results were expressed as TBA value in mg of malondialdehyde per kg of fish meat. Free fatty acid (FFA) content, expressed in percentage of oleic acid, was determined by acidometric titration of extracts according to Bligh and Dyer (14), after the addition of ethanol and with phenolphthalein as an indicator, following the AOCS method (20). Peroxide value (PV), expressed in millimole of peroxide per kilogram of fat, was determined according to AOAC (21).

### Colour measurement

The colour of samples was measured by a lab colour meter (Pen Color Art 1L model, Artoksi MSM, Istanbul, Turkey) and was in accordance with the recommendations of the International Commission on Illumination (22). The measured  $L^*$ ,  $a^*$  and  $b^*$  colour parameters indicated lightness/brightness, redness/greenness and yellowness/blueness, respectively. The colourimeter was calibrated with a white standard and the colour measurement was repeated 5 times on different parts of the surface.

### Statistical analysis

Experiments were performed in triplicate ( $N=3$ ) for three independent samples and a completely randomized design (CRD) was used. Data were presented as mean values  $\pm$  standard deviations and a probability value of  $p>0.05$  was considered significant. Analysis of variance (ANOVA) was performed and the mean comparisons were done by Duncan's multiple range tests. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS for Windows, SPSS Inc., Chicago, IL, USA).

## Results and Discussion

### Sensorial characteristics of fish samples

The results of the sensory assessment of samples are given in Table 1. The fish samples were considered to be acceptable for human consumption until the sensory score reached 4 (3,23). Texture, odour, colour and overall acceptability of control samples received unacceptable scores by the 15th day according to the panellists' evaluation, samples wrapped in gelatin films with 0 and 0.1 % LEO received unacceptable scores by the 20th day, whereas that with 1 % LEO was evaluated as unacceptable after 22 days. The sensory evaluation results appeared to be correlated with microbial and chemical value analysis. Changes in the sensory characteristics of control group were observed as off-odour and softening of the flesh that were probably caused by fish enzyme activation. The antioxidant, antimicrobial and gas barrier effects of the coating were shown to minimise the oxidative effects, prolonging the product shelf-life while maintaining quality. Adding laurel oil to gelatin coating enhanced significantly ( $p<0.05$ ) the beneficial effects on the colour and overall acceptability of the fish in final days.

### Microbial flora of rainbow trout fillets

Microbial flora changes of rainbow trout fillets after the treatment with gelatin film containing LEO are shown in Figs. 1a–d. The initial total viable count (TVC) of the fish fillets (Fig. 1a) was 4 log CFU/g. TVC reached 7.9 log CFU/g after 18 days of storage in all other groups except for the one containing 1 % LEO (6.1 log CFU/g). Initial TVC load of the fresh trout samples may be associated with contamination of samples during filleting. Considering the microbiological upper limit of total viable count of  $5 \cdot 10^5$  CFU/g in fresh fish proposed by ICSMF (24), our result indicates good fish quality. Chytiri *et al.* (25) reported that the total initial mesophilic load of the rainbow trout fillets was 3.8 log CFU/cm<sup>3</sup>. Higher initial counts of 4.5 log CFU/g in sea bass slices were reported by Ahmad *et al.* (9). During storage, the TVC observably increased in control, and the groups without or containing 0.1 % LEO. The differences between the latter two groups were not statistically significant ( $p>0.05$ ). The combination of both the gelatin film and the laurel essential oil showed an additive inhibitory effect on the microflora of rainbow trout fillets.

Bacterial spoilage in fish and fish products stored under chilled and aerobic conditions is caused by Gram-negative psychrotrophic bacteria such as *Pseudomonas*, *Alteromonas*, *Shewanella* and *Flavobacterium* spp. (26). Changes in total psychrotrophic bacteria of rainbow trout fillets are shown in Fig. 1b. During storage, samples wrapped in gelatin film containing 1 % LEO had the lowest psychrotrophic bacteria count when compared to the control and samples wrapped in gelatin film with 0 % LEO ( $p<0.05$ ).

Enterobacteriaceae, regarded as a hygiene indicator, are also one of the spoilage microorganisms of fresh rainbow trout (27). Enterobacteriaceae counts in fresh samples were approx. 4.0 log CFU/g at the beginning the storage (Fig. 1c). By day 24 of storage, Enterobacteriaceae counts increased to 7.6 log CFU/g in control group,

Table 1. Changes in attribute scores of raw rainbow trout fillets wrapped in gelatin films containing LEO during refrigerated storage

Sensory attributes	V(LEO) m(gelatin) %	t(storage)/day								
		0	5	10	15	18	20	22	24	26
Appearance	control	(8.6±0.1) <sup>a</sup>	(8.4±0.4) <sup>a</sup>	(6.6±1.0) <sup>b</sup>	(5.5±1.4) <sup>b</sup>	(3.8±1.4) <sup>c</sup>	(2.7±0.8) <sup>b</sup>	(2.6±1.0) <sup>c</sup>	(1.6±0.4) <sup>d</sup>	(0.9±0.1) <sup>d</sup>
	0	(8.6±0.1) <sup>a</sup>	(7.8±1.1) <sup>b</sup>	(7.1±0.9) <sup>ab</sup>	(6.6±1.2) <sup>a</sup>	(6.3±0.7) <sup>a</sup>	(5.6±0.9) <sup>a</sup>	(3.9±1.0) <sup>b</sup>	(2.3±0.8) <sup>c</sup>	(1.7±0.6) <sup>c</sup>
	0.1	(8.6±0.1) <sup>a</sup>	(7.7±1.0) <sup>b</sup>	(7.3±0.8) <sup>a</sup>	(6.5±0.8) <sup>a</sup>	(5.3±0.8) <sup>b</sup>	(5.2±0.9) <sup>a</sup>	(4.1±1.3) <sup>ab</sup>	(3.0±1.0) <sup>b</sup>	(2.1±0.6) <sup>b</sup>
	1	(8.6±0.1) <sup>a</sup>	(7.8±1.0) <sup>b</sup>	(7.5±0.6) <sup>a</sup>	(6.0±0.7) <sup>b</sup>	(5.7±0.5) <sup>b</sup>	(5.3±1.0) <sup>a</sup>	(4.5±1.2) <sup>a</sup>	(3.2±0.8) <sup>a</sup>	(3.0±0.6) <sup>a</sup>
Colour	control	(8.7±0.1) <sup>a</sup>	(8.3±0.3) <sup>a</sup>	(6.7±1.2) <sup>b</sup>	(4.6±1.7) <sup>b</sup>	(4.0±1.6) <sup>c</sup>	(3.2±0.5) <sup>c</sup>	(2.7±1.0) <sup>c</sup>	(1.7±0.1) <sup>c</sup>	(0.9±0.3) <sup>b</sup>
	0	(8.7±0.1) <sup>a</sup>	(7.7±1.1) <sup>b</sup>	(7.1±0.7) <sup>ab</sup>	(6.8±1.0) <sup>a</sup>	(5.9±1.2) <sup>a</sup>	(5.2±0.6) <sup>a</sup>	(4.2±1.2) <sup>ab</sup>	(2.4±0.5) <sup>b</sup>	(2.7±0.7) <sup>a</sup>
	0.1	(8.7±0.1) <sup>a</sup>	(7.5±1.5) <sup>b</sup>	(7.4±0.6) <sup>a</sup>	(6.6±0.8) <sup>a</sup>	(5.2±1.0) <sup>b</sup>	(4.8±1.5) <sup>b</sup>	(3.9±1.3) <sup>b</sup>	(2.9±1.1) <sup>b</sup>	(2.7±0.6) <sup>a</sup>
	1	(8.7±0.1) <sup>a</sup>	(7.6±1.4) <sup>b</sup>	(7.6±0.6) <sup>a</sup>	(6.3±0.9) <sup>a</sup>	(6.0±0.7) <sup>a</sup>	(4.9±1.0) <sup>b</sup>	(4.7±1.1) <sup>a</sup>	(3.4±1.0) <sup>a</sup>	(2.9±0.4) <sup>a</sup>
Odour	control	(8.7±0.2) <sup>a</sup>	(7.8±0.8) <sup>a</sup>	(5.4±0.9) <sup>c</sup>	(4.0±1.0) <sup>c</sup>	(2.7±0.4) <sup>c</sup>	(2.1±1.2) <sup>c</sup>	(1.4±0.3) <sup>c</sup>	(0.9±0.3) <sup>d</sup>	(0.8±0.3) <sup>b</sup>
	0	(8.7±0.2) <sup>a</sup>	(7.6±0.7) <sup>a</sup>	(6.6±0.5) <sup>bc</sup>	(5.9±0.8) <sup>b</sup>	(5.1±0.8) <sup>b</sup>	(4.5±0.7) <sup>b</sup>	(3.9±1.3) <sup>b</sup>	(1.8±0.4) <sup>c</sup>	(2.0±0.7) <sup>a</sup>
	0.1	(8.7±0.2) <sup>a</sup>	(8.0±0.5) <sup>a</sup>	(6.9±0.8) <sup>ab</sup>	(6.0±0.9) <sup>ab</sup>	(5.0±1.1) <sup>b</sup>	(4.9±1.2) <sup>b</sup>	(4.5±0.8) <sup>a</sup>	(2.9±0.8) <sup>b</sup>	(2.2±0.4) <sup>a</sup>
	1	(8.7±0.2) <sup>a</sup>	(7.9±0.4) <sup>a</sup>	(7.1±0.7) <sup>a</sup>	(6.2±1.1) <sup>a</sup>	(5.9±0.6) <sup>a</sup>	(5.6±1.7) <sup>a</sup>	(4.6±1.2) <sup>a</sup>	(3.8±1.3) <sup>a</sup>	(3.0±0.7) <sup>a</sup>
Texture	control	(8.8±0.1) <sup>a</sup>	(8.9±0.3) <sup>a</sup>	(5.7±0.8) <sup>c</sup>	(4.0±1.0) <sup>b</sup>	(2.9±0.1) <sup>b</sup>	(2.3±0.9) <sup>b</sup>	(1.8±0.6) <sup>c</sup>	(1.3±0.4) <sup>c</sup>	(0.9±0.1) <sup>c</sup>
	0	(8.8±0.1) <sup>a</sup>	(8.1±0.5) <sup>a</sup>	(6.4±0.6) <sup>b</sup>	(6.0±0.7) <sup>a</sup>	(5.5±0.7) <sup>a</sup>	(4.7±0.3) <sup>a</sup>	(3.2±1.0) <sup>b</sup>	(2.6±1.0) <sup>b</sup>	(2.3±0.9) <sup>b</sup>
	0.1	(8.8±0.1) <sup>a</sup>	(8.0±0.5) <sup>a</sup>	(6.8±0.6) <sup>ab</sup>	(6.1±0.8) <sup>a</sup>	(5.3±0.6) <sup>a</sup>	(4.5±0.3) <sup>a</sup>	(3.9±1.2) <sup>ab</sup>	(3.5±0.6) <sup>a</sup>	(2.4±1.0) <sup>b</sup>
	1	(8.8±0.1) <sup>a</sup>	(7.9±0.5) <sup>a</sup>	(7.0±0.7) <sup>a</sup>	(5.6±0.5) <sup>a</sup>	(5.3±0.7) <sup>a</sup>	(4.6±0.5) <sup>a</sup>	(4.6±0.9) <sup>a</sup>	(3.9±0.9) <sup>a</sup>	(3.1±0.9) <sup>a</sup>
General acceptability	control	(8.7±0.1) <sup>a</sup>	(8.3±0.3) <sup>a</sup>	(6.0±1.0) <sup>c</sup>	(4.3±0.9) <sup>c</sup>	(2.9±0.2) <sup>b</sup>	(2.3±0.9) <sup>b</sup>	(2.0±0.8) <sup>c</sup>	(1.2±0.2) <sup>c</sup>	(1.0±0.1) <sup>c</sup>
	0	(8.7±0.1) <sup>a</sup>	(8.2±0.3) <sup>a</sup>	(6.7±0.6) <sup>b</sup>	(6.4±1.0) <sup>a</sup>	(5.6±1.2) <sup>a</sup>	(5.0±0.8) <sup>a</sup>	(3.7±0.6) <sup>b</sup>	(2.4±0.8) <sup>b</sup>	(2.2±0.7) <sup>b</sup>
	0.1	(8.7±0.1) <sup>a</sup>	(8.1±0.3) <sup>a</sup>	(6.9±0.7) <sup>ab</sup>	(6.3±0.7) <sup>ab</sup>	(5.2±0.5) <sup>a</sup>	(4.7±1.0) <sup>a</sup>	(3.8±0.7) <sup>b</sup>	(3.1±0.5) <sup>a</sup>	(2.5±0.4) <sup>ab</sup>
	1	(8.7±0.1) <sup>a</sup>	(8.2±0.2) <sup>a</sup>	(7.2±0.8) <sup>a</sup>	(6.0±0.5) <sup>b</sup>	(5.6±0.8) <sup>a</sup>	(5.0±0.5) <sup>a</sup>	(4.5±0.6) <sup>a</sup>	(3.6±0.7) <sup>a</sup>	(2.9±0.8) <sup>a</sup>

while they were 5.3 log CFU/g in the group containing 1 % LEO ( $p < 0.05$ ). Gelatin film without LEO (0 % LEO) did not show an inhibitory effect on Enterobacteriaceae counts and 7.5 log CFU/g were obtained by the end of the 26th day. The contribution of Enterobacteriaceae to the microflora of fish and its spoilage potential must be taken into consideration (28).

Lactic acid bacteria (LAB) are facultative anaerobic bacteria that can grow under both anaerobic and aerobic conditions (29). The initial LAB count in the control group was approx. 3.8 log CFU/g (on day 0) and reached 6.6 log CFU/g on day 24 of storage (Fig. 1d). On the same day, the use of gelatin film with 1 % laurel essential oil resulted in a reduction of LAB counts by 2.9 log CFU/g ( $p < 0.05$ ). Our findings are in agreement with the results of Mexis *et al.* (27), who reported a reduction of LAB by 2.3 log CFU/g in Greek cod roe paste after the addition of oregano oil at 0.4 % (by volume per mass).

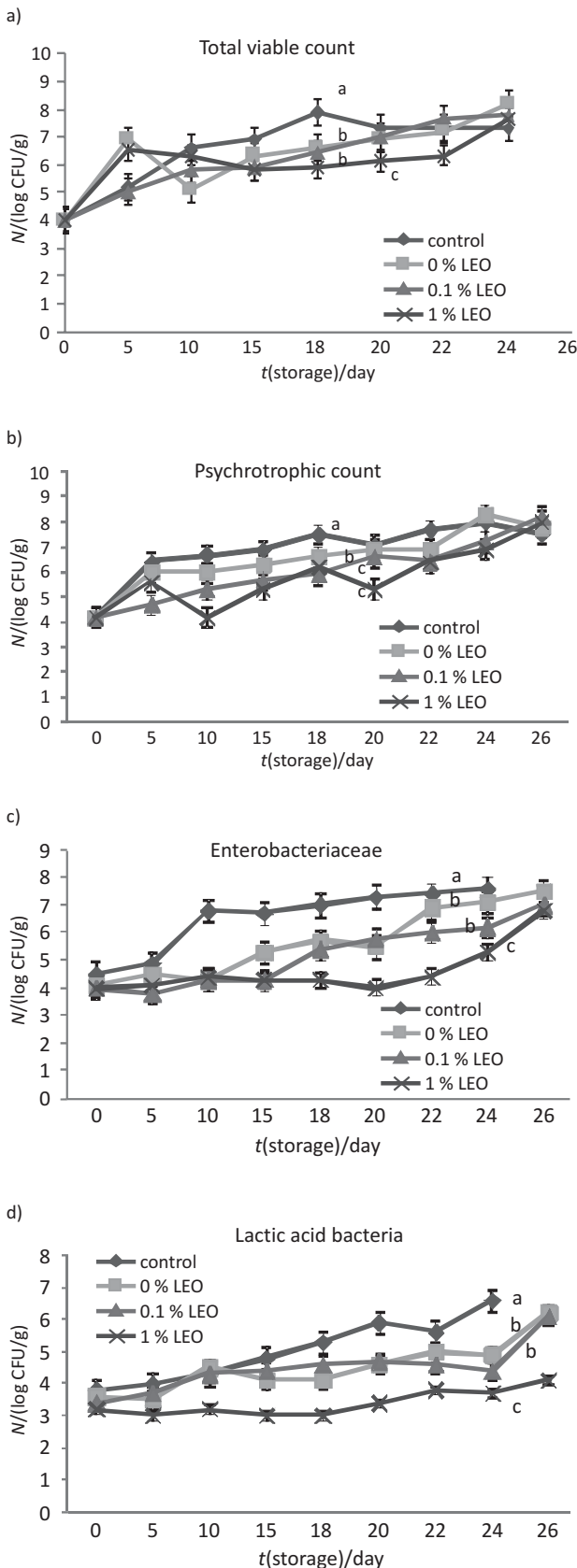
Ramos *et al.* (30) also reported that bay laurel essential oil and its ethanolic extract can be used as natural alternatives to synthetic food preservatives, in order to enhance food safety and increase food shelf-life. In their study, the EO exhibited strong antibacterial activity against all tested foodborne spoilage and pathogenic bacteria. There are some other effective studies dealing with the antimicrobial properties of films based on proteins, chitosan or alginates incorporated with various essential oils

such as oregano, rosemary, garlic, pimento or cinnamon (31).

Not only are the intrinsic properties of the food (fat/protein/water content, antioxidants, preservatives, pH, salt and other additives) relevant in this respect; the extrinsic determinants (temperature, packaging in vacuum/gas/air, characteristics of microorganisms) can also influence bacterial sensitivity. Generally, the susceptibility of bacteria to the antimicrobial effect of essential oils also appears to increase with a decrease in the pH of the food, the storage temperature and the amount of oxygen within the packaging (32). Thus, factors such as low pH, storage temperature, decreased O<sub>2</sub> concentrations and high salt content enhance the antimicrobial effect of the essential oil, while factors like high levels of protein and fat and low water activity seem to protect the bacteria (33).

#### Proximate composition of raw fish material

Proximate analysis results of raw material were: crude protein (20.16±0.10) %, moisture (73.97±0.01) %, crude fat (4.21±0.2) % and ash (1.61±0.04) %. The proximate composition of the rainbow trout fillet reported in different studies (3) showed some differences, especially in the lipid content. Such variations in the chemical composition of fish are strongly related to the nutrition, catching season, sexual variation, fish size, living area, as well as other environmental conditions (34). The composition of



**Fig. 1.** Effect of gelatin film enriched with laurel essential oil (LEO) on: a) total viable count, b) psychrotrophic count, c) Enterobacteriaceae population, and d) lactic acid bacteria population in rainbow trout fillets during refrigerated storage. Letters show significant differences among the groups at  $p < 0.05$

fish can affect the sensory characteristics, such as colour, texture, odour, taste and surface appearance, which strongly influence the acceptability of fish as food (3,26,35), as it may also affect the microbial growth (34).

### The results of chemical analysis

#### Chemical composition of the essential oil

Bay laurel essential oil was found to contain 1,8-cineole (29.37 %),  $\alpha$ -terpinene acetate (15.52 %), linalool (4.97 %), sabinene (5.29 %),  $\alpha$ -pinene (5.32 %) and  $\beta$ -pinene (4.03 %) as major constituents. It was reported that bay laurel leaves contained mostly 1,8-cineole (50 %), eugenol, acetyl eugenol, methyl eugenol and terpineol (36). In our study, 1,8-cineole was also found as the most abundant constituent. Most of  $\alpha$ -terpinene acetate (15.52 %) was thought to be particular to the location or caused by seasonal changes of the bay laurel tree. Different bay laurel essential oil compositions found in previous studies are generally related to several factors such as genetic, climatic, seasonal and geographic (37). The ability of prolonging the shelf-life of rainbow trouts may be affected by the synergistic effects with the constituents of laurel essential oil.

#### pH value of fresh fish samples

The pH value of fresh fish flesh is often between 6 and 6.5 (38). The acceptable upper limit for the pH of fish meat is 6.8–7.0 (39). The initial pH value of rainbow trout samples in this study was  $6.30 \pm 0.01$ . During storage, pH was found to be low in gelatin film-wrapped samples containing LEO and there was statistically significant difference among the groups ( $p < 0.05$ ). At the end of the storage (by day 26), pH increased and reached the level of  $6.61 \pm 0.01$ . The pH values of all fish samples used in the study did not exceed the acceptable limit. As reported in literature, the increase of pH value during storage is attributed to the production of basic compounds such as ammonia, trimethylamine and other biogenic amines by fish spoilage bacteria (40).

#### Total volatile base nitrogen content

Total volatile base nitrogen (TVB-N) was used as the indicator of the quality of fresh trout fillets. The TVB-N values of the samples during storage are presented in Fig. 2. The initial value of TVB-N content in fillets was 17.06 mg of N per 100 g of fish. The TVB-N values of both control and the coated samples increased significantly ( $p < 0.05$ ) with storage time. Its increase is related to the activity of spoilage bacteria and endogenous enzymes (40). There were no statistically significant differences between groups with 0.1 % and without any LEO added to the film ( $p > 0.05$ ). TVB-N content in freshly caught fish is typically between 5 and 20 mg of N per 100 g, and TVB-N content of 30–35 mg of N per 100 g is usually regarded as the limit of acceptability for fish (41). The limit TVB-N level of 35 mg per 100 g, which was specified by the European Commission (EC) guidelines (42), was exceeded in the control and the group containing 1 % LEO in the film after 18 and 26 days, respectively. In the groups without or with 0.1 % LEO in the film coating, the limit values were reached by day 24 of storage (Fig. 2). Tsai *et al.* (43) found that pretreatment of fish (*Oncorhynchus nerka*) fillets with 1 % of chitosan

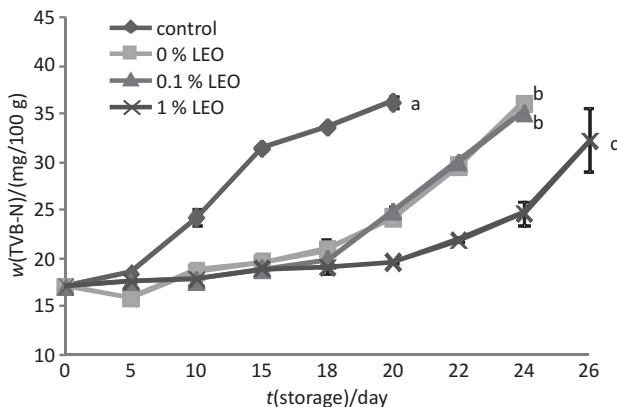


Fig. 2. Changes in total volatile base nitrogen (TVB-N) values of rainbow trout fillets wrapped in gelatin films containing laurel essential oil (LEO) during refrigerated storage. Letters show significant differences among the groups at  $p < 0.05$

solution for 3 h retarded the increase in the TVB-N. Also, López-Caballero *et al.* (44) found that the protective chitosan-gelatin coating lowered TVB-N values distinctly and hence slowed down the spoilage. Ahmad *et al.* (9) reported that the inhibitory effect of gelatin film containing lemongrass essential oil against microbial growth could retard or lower the production of microbial degradation products.

#### The results of thiobarbituric acid assay

During storage, a significant increase in thiobarbituric acid (TBA) content was observed in control samples as compared to the samples wrapped in gelatin film only or in gelatin film containing different amounts of LEO ( $p < 0.05$ ) (Fig. 3). The initial TBA value was 0.03 mg of malondialdehyde per kg of tissue. Sathivel (45) reported that the initial TBA value for the raw noncoated pink salmon fillets was 0.25 mg of malondialdehyde per kg of fish. In our study, TBA values of the control group and the samples with gelatin coating without LEO were higher than of the gelatin coatings with LEO ( $p < 0.05$ ). TBA is a widely used indicator of the degree of lipid oxidation (26,46). It has been proposed that the maximum level of TBA value indicating good quality of fish (frozen, chilled or stored in ice) is 5 mg of malondialdehyde equivalents per kg of tissue, while the fish may be consumed with up to the level of 8 mg of malondialdehyde equivalents per kg of tissue (26). In our study, TBA values in control and coated trout fillet samples were much lower than the recommended limits throughout the 26-day storage period (Fig. 3). TBA value was found to be lower in the samples containing 1 % LEO in comparison with control and other groups ( $p < 0.05$ ). As the LEO ratio increased, lower oxidation rate was obtained. In our study, all wrappings with or without LEO could inhibit the lipid oxidation of trout fillets, and gelatin film enriched with LEO had a better effect than the control group and gelatin alone. Our results suggested that lipid oxidation in rainbow trout fillets could be retarded by gelatin film enriched with LEO. This is probably due to the antioxidant characteristics of LEO as well as the low oxygen permeability of gelatin film (9). Antoniewski *et al.* (47) found that a gelatin coating had no effect on

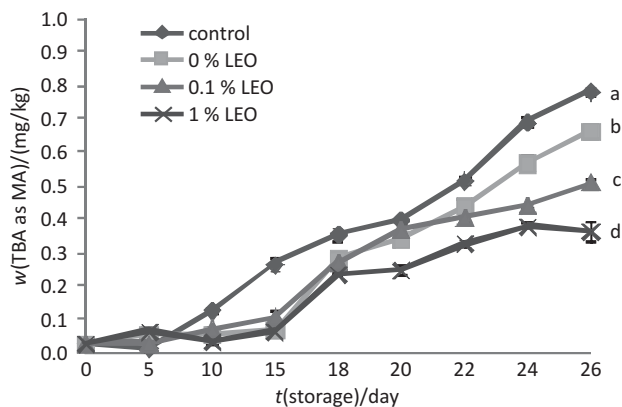


Fig. 3. Changes in thiobarbituric acid (TBA) values of rainbow trout fillets wrapped in gelatin films containing laurel essential oil (LEO) during refrigerated storage. Letters show significant differences among the groups at  $p < 0.05$ . MA=malondialdehyde

lipid oxidation in salmon fillet stored at 4 °C for 14 days. Adding different ratios of laurel essential oil to the gelatin film, therefore, probably had a synergistic effect. In the case of most herbal extracts, their antioxidant activity was attributed to their ability to break the free radical chain by donating a hydrogen atom (48).

#### Free fatty acid content of fish samples

The presence of free fatty acids (FFA) is caused by the hydrolysis of lipids. Therefore, quantifying the FFA percentage can be profitably used as an index of the degree of lipolysis, which in turn is an indicator of the fish freshness. The initial hydrolytic rancidity as evaluated by FFA value of the trout fillets was 2.44 (expressed as percent of oleic acid) on day 0. During storage, the FFA values increased significantly ( $p < 0.05$ ) in all samples. FFA values expressed as 8.31, 8.15, 6.74 and 6.41 % of oleic acid were observed in the control, and in samples wrapped in gelatin without LEO, or with 0.1 or 1 % LEO, respectively. Gelatin wrapping with 1 % LEO had a better effect on maintaining the quality of fish fillet than the one without LEO, while the quality of fish samples wrapped with gelatin with 0.1 % LEO was better than of the control group. Gomez-Guillén *et al.* (49) investigated the effect of gelatin-based films with incorporated aqueous extracts of either oregano or rosemary on cold-smoked sardine under high pressure, and recorded a significant decrease in FFA by day 20.

#### The results of peroxide value determination

Primary (peroxide value, PV) and secondary (TBA) oxidation products were determined as indicators of the degree of lipid oxidation, similar to Mexis *et al.* (27). The effect of film on the changes of PV of fish lipids is shown in Fig. 4. The initial PV in the analyzed fish fillets was  $(0.44 \pm 0.08)$  mmol per kg of fish sample. The PV of the controls and treated samples increased significantly ( $p < 0.05$ ) concurrent with storage time. When compared to samples treated solely with gelatin film, PV increase of the LEO-containing groups was found to be slower ( $p < 0.05$ ). In the control group, PV value exhibited a rapid increase by day 5 and reached 4.40 mmol/kg by day 20. During storage, PV value of 4.11 (on the 24th day), 3.36 (on the 26th day) and 2.47 mmol/kg (on the 26th day) was ob-

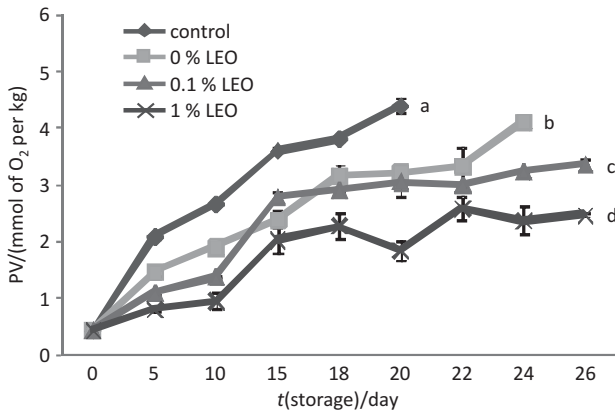


Fig. 4. Changes in peroxide value (PV) of rainbow trout fillets wrapped in gelatin films containing laurel essential oil (LEO) during refrigerated storage. Letters show significant differences among the groups at  $p < 0.05$

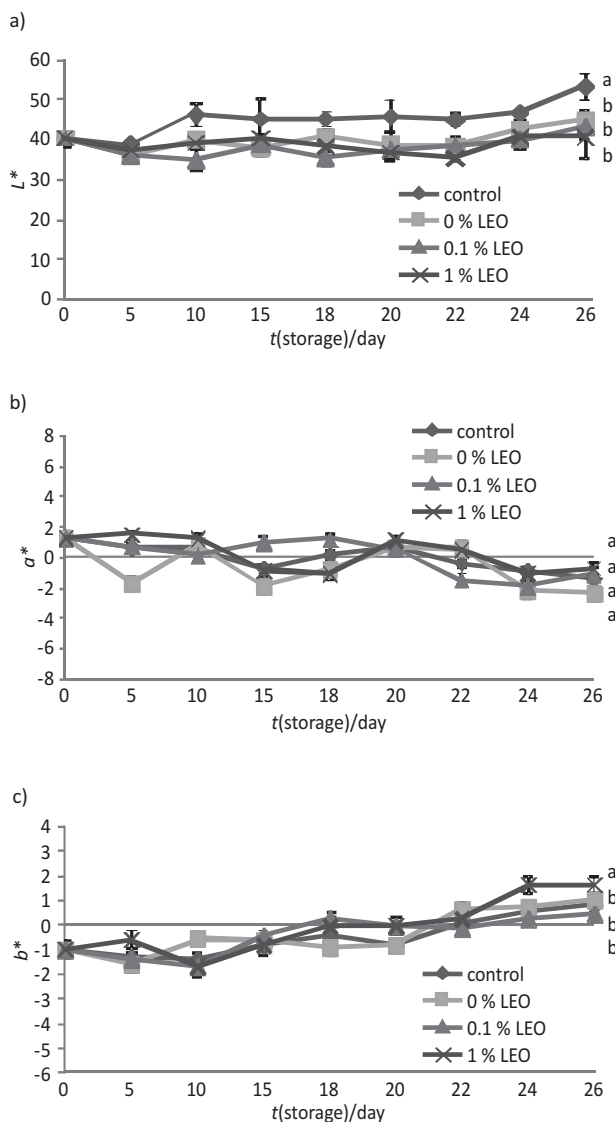


Fig. 5. Changes in the measured instrumental colour parameters of fish samples: a) lightness ( $L^*$ ), b) redness ( $a^*$ ) and c) yellowness ( $b^*$ ). Letters show significant differences among the groups at  $p < 0.05$

served in samples without and with 0.1 or 1 % LEO, respectively. According to the literature, a PV value of approx. 10 mmol/kg (20 meq) is considered as the upper limit for foodstuffs (50). Similar results for PV were obtained by Mohan *et al.* (51) in catfish. The major protective effect is probably due to the use of gelatin as an oxygen absorber and the antioxidant effect of laurel essential oil.

#### The results of colour determination

The results of colour measurement of the rainbow trout fillets are shown in Fig. 5. At the beginning of storage, lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ) values of samples were determined at  $40.5 \pm 1.9$ ,  $1.3 \pm 0.4$  and  $-1.0 \pm 0.4$ , respectively. The  $L^*$  value of the control group increased throughout the storage, while no significant differences ( $p > 0.05$ ) were determined in gelatin-wrapped samples without LEO, and with 0.1 or 1 % LEO (Fig. 5a). The  $a^*$  values decreased from red to green, while those of  $b^*$  increased from yellow to blue throughout the entire storage period (Figs. 5b and c). Ahmad *et al.* (9) reported that sea bass slices wrapped in gelatin film containing lemongrass essential oil showed fewer changes in colour values, probably due to the antioxidant and antimicrobial properties of lemongrass essential oil.

#### Conclusions

Based on the sensory evaluation, it can be concluded that the combination of gelatin film and laurel essential oil was effective in extending the shelf-life of fresh rainbow trout fillets to 22 days, whereas samples packaged without gelatin film had a shelf-life of 15 days. Therefore, the addition of laurel essential oil to gelatin films extended the shelf-life of rainbow trout fillets for 5–7 days more when compared to the noncoated group. Gelatin films with 0.1 and 1 % laurel essential oil improved the quality of fish during storage in terms of chemical indices (pH, TVB-N, TBA, FFA, PV). Fish fillets wrapped in gelatin film containing 1 % LEO had the best quality scores according to the sensorial, chemical and microbiological data. As the ratio of essential oil increased, the shelf-life parameters were beneficially affected throughout the analysis period. It is suggested that gelatin film treatment with laurel essential oil may be profitable for salted, dried and smoked seafood, which has a lower moisture content. In conclusion, gelatin film enriched with laurel essential oil provides an effective coating that prolongs the shelf-life and maintains the quality attributes of the packaged food.

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