Antimicrobial Activity of Edible Surface-active Xanthan Gum Coating Enriched with Different Extracts and Effect on Quality of Rainbow Trout (*Onchorhyncus mykiss*)

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Abstract

The aim of this study was to determine the antibacterial effects of daphne (*Laurus nobilis* L.) and basil (*Ocimum basilicum* L.) in coating formulation edible film-based xanthan gum on the shelf-life of rainbow trout (Oncorhynchus mykiss) for 10 days storage at 4 ± 1°C. The most effective concentration of the extracts used in the edible coating against seven fish pathogenic bacteria was determined as 1 mg ml^{-1}. While the use of antimicrobial edible film in trout significantly inhibited bacterial growth; the lactic acid bacteria increased during storage. It was clear that the best fish and plant odor were detected in the coated groups, coated with daphne extract groups were also judged as stronger than basil extract groups. Although the control groups deteriorated on the 7th day, the edible film-coating groups were finished sensory and microbiological on the 10th day. Throughout the storage period in this study, 14 different bacteria and 3 different yeasts were identified via Bacterial Identification API Kits.

Keywords: Edible surface-active coating, xanthan gum, plant extract, antimicrobial coating, *Oncorhynchus mykiss*

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INTRODUCTION

The rainbow trout (*Oncorhynchus mykiss*) contains a high percentage of polyunsaturated fatty acids (PUFAs), resulting in rapid microbial spoilage and chemical oxidation (Baron et al., 2007). Considering that rainbow trout fillets have a short shelf-life, but there is a growing market for fresh fish, new preservation methods need to be developed to extend the shelf-life of fresh fish (Aspevik et al., 2021; Hosseini et al., 2016). In order to meet consumers demands for more disposable, biodegradable, natural and recyclable food package material, nowadays, research have focused on the incorporation of natural antimicrobial compounds such as bacteriocins and plant extracts in the bio-based sustainable package materials in place of petroleum based plastic films (Díaz-Montes et al., 2021; Irkin and Esmer, 2015; Weber et al., 2002).

Therefore, it has recently become important to edible films/coatings with essential oils or plant extracts to extend the shelf life of fish and fishery products (Bensid et al., 2014; Gao et al. 2014; Gomez-Estaca et al., 2007; Mei et al., 2019; Ong et al., 2021).

Antimicrobial edible films made from biopolymers can improve food quality and increase shelf life by providing a slower respiration rate and controlled microbial growth (Garcia et al., 2010). Xanthan gum (XG), one of the most important commercial microbial hydrocolloids used in the food industry, is a high molecular weight extracellular polysaccharide produced by the bacterium *Xanthomonas campestris* (Zheng et al., 2019). Bio-based food packaging with antimicrobial properties derived from xanthan gum is a promising material for food preservation application (Sapper et al., 2019). Although there are many studies on the antibacterial activities of plant essential oils and extracts, the use of xanthan in edible film coatings in fish has not been seen. This is the main constituents of medicinal plants vary greatly depending on nutritional status, geographical origins, extracted plants (stem, leaf and flower), genetic factors and/or extraction methods (Burt, 2004; Costa et al., 2015; Lang and Buchbauer, 2012). Basil species contain 0.2 - 1% extract, the main ingredients are linalool and estragole (methyl chavicol), and camphene, o-cymene, beta-pinene, citral, alpha-pinene, geraniol (Sakkas and Papadopoulou, 2017). Furthermore, daphne species have been reported in previous studies to contain various secondary metabolites such as flavonoids, coumarins, diterpenes lignans and triterpenoids. It has been determined that these plant species have many biological activities including antimicrobial, antioxidant, cytotoxic, antitumor and anti-inflammatory activities (Asong et al., 2019; Balkan et al., 2017; Biswas et al., 2014; Hashemi and Khaneghah, 2017).

The aim of this study was (1) to evaluate the antibacterial potential of xanthan gum based edible coating containing extracts from daphne (*Laurus nobilis* L.) and basil (*Ocimum basilicum* L.) against fish pathogens and (2) to determine effectiveness of these extract containing surface-active coating on microbiological characteristics of rainbow trout (*Oncorhynchus mykiss*) fillets during refrigerated storage (4 ± 1°C).

MATERIAL and METHOD

Materials

Rainbow trout fillets were transfered to Ege University Laboratory of Fisheries Processing Technology via frigorific transport by Bağcı Alabalık A.Ş. (Izmir). Food grade biopolymers: Xanthan gum, glycerol and ethanol and other reagents were purchased from Merck (Germany). Extracts were prepared from two natural plants as daphne (*L. nobilis*) and basil (*O. basilicum*).

Preparation of plant extracts

A total 50,0 ± 0,1 g of dried/granulated daphne or basil was extracted with 300 mL of organic ethanol by shaking (Dragon Lab SK-330 model, Beijing, China) for 24 h at room temperature (Baytop, 1999). Afterwards the obtained solution was extracted using rotary evaporator (Stuart, RE300DB) at 45 °C.

Preparation of xanthan coating solution

Edible coating solution was prepared according to Sothornvit (2011) with slight modifications. Xanthan gum powder samples (1 g 100 ml^{-1} distilled water) were hydrated at 25 °C for 1 hour. The edible coating solutions were homogenized by stirring for 30 min at 90 °C.

Then glycerol (30 % w w^{-1} of the total hydrocolloid content) was added as a plasticiser. Plant extracts (0,5 % w v^{-1}), dispersed in an aqueous solution, were stirred for 15 min (Hotplate stirrer, Wissestir MSH-20A, Kore) at 25 °C. The most appropriate formulation for coating fish by the tested xanthan gum hydrocolloid(s) was detected when the latter's concentration was equivalent to 0.5 % (w w^{-1}).

Antibacterial activity of extracts and edible coating

Microbial strains

Gram-positive bacteria (*Bacillus cereus* ATCC 7064, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 43300, *Staphylococcus epidermidis* ATCC 12228, Gram-negative (*Escherichia coli* ATCC 29998, *Escherichia coli* 0157:H7 RSKK 234 and *Salmonella typhimurium* CCM 5445) as well as *Candida albicans* ATCC 10231 were used for the antimicrobial activity studies. Yeast and the lyophilized bacteria were obtained from Ege University, Faculty of Science, Department of Basic and Industrial Microbiology.

Minimum inhibitory concentration (MIC) by micro-dilution susceptibility test

MICs were determined according to the National Committee for Clinical Laboratory Standards (CLSI, 2011). Test microorganisms had grown in Mueller Hinton Broth (MHB) for 5 h (exponential phase) and adjusted to 0.5 McFarland. These test organisms and MHB were transferred to each well of a standard 96-well microplate. MIC values were determined after 20-24 hours of incubation at 37ºC. Positive control consisted of different Ampicillin (AMP) (Sigma) and flucytosine (FC) (Sigma), which are standard antibacterial and antifungal agents respectively, at concentrations 0.5 – 256 μg ml^{-1}. The same procedure was repeated three times.

Agar well diffusion test

The antimicrobial activity of xanthan coating solutions containing daphne and basil extracts were tested against eight food pathogen microorganisms using agar well diffusion assay. Test organisms were activated in Mueller-Hinton Agar (MHA) (Oxoid) for 18-24 hours. Test organisms established according to the 0.5 McFarland standard were inoculated into the media. 30 μl of extracts and control antibiotics were absorbed into discs and placed in petri dishes. The petri dishes were kept at 4 ^{o}C for 2 hours before incubation, and then after 24 hours of incubation at 37 ^{o}C. The effect of the extract rich xanthan surface-active coating was compared to that without extract. Antimicrobial activity was assessed by measuring the inhibition zone (mm). Ceftazidime (Oxoid, 30μg) and Nystatin (Oxoid, 30μg) were used as positive controls. The inhibition zone result represents the average of 3 trials (CLSI, 2011).

Edible coating application and fish fillets quality analysis

Treatment of fish fillets

Samples were coated with daphne (DE) and basil (BE) extracts. Xanthan gum coated sample without extract was used as control (C). The trout fillets were coated by dipping xanthan gum solution for 2 min at room temperature (25 °C), and then dried for 2 min. A total 50 ± 5 g of fish fillets were aseptically placed into trays and covered with stretch film. The coated and uncoated trout fillets were stored at 4 ± 1 °C for 10 days. Analysis was carried out on the 1st, 3rd, 5th, 7th and 10th day of storage.

Microbiological analysis and bacteria identifications

Three fish were evaluated from each designated sampling point and 10 g samples were taken from each group. Samples (10 g) were added aseptically to 90 mL of sterile Buffered Peptone Water solution (1 g mL^{-1} bacteriological peptone, Merck, Germany) and mixed in a Stomacher (IUL, Barcelona, Spain) at high speed for 1 min. Plate Count Agar (PCA, Merck, Germany) was used to evaluate the total viable count (TVC) and and total aerobic psychrophilic bacteria (TAP). Violet Red Bile Dextrose Agar (VRBD-A, Merck, Germany), Baird Parker Agar (BPA, Merck, Germany) and De Man Rogosa Sharpe Agar (MRS, Merk, Germany) were used to assess *Enterobacteriaceae* (ENT), *Staphylococcus* (SPH), and lactic acid bacteria (LAB) respectively. On the other hand, Yeast Extract Glucose Chloramphenicol Agar (YGC, Merck, Germany) were used to grow Molds and yeasts (MY). Plates were incubated at 30 °C for 24 h; 37 °C for 24 h; 30 °C for 72 h respectively for TVC and ENT, SPH, LAB. TAP were proceeded after incubation at 7 °C for 10 d. Yeasts and fungi were incubated at 25 °C for 72 h (Harrigan and Mc Cance, 1976). After incubation, the means of counts with standard deviations, were reported as logarithms of the number of colonies forming units (log CFU g^{-1}). Furthermore, the isolated microorganisms were identified with API test kits with 20 NE (30 °C for 24 h), 20 E (36 °C for 24 h), 29 C AUX (30 °C for 48 h), 50 CH and 50 CHL (30 °C for 24 h) (Biomérieux, France) from bacterial flora. These kits were used according to the instructions of the manufacturer and the database provided by BioMerieux.

Sensory evaluation of raw and cooked fish fillets

Sensory evaluation was performed by seven trained panellists. Two different sensory analyses were carried out as raw and cooked trout. The fillets samples were cooked at 170 °C for 15 min in a preheated conventional electric oven. A scale ranging from 1 (bad) to 9 (perfect) was used by the panellists to evaluate the three groups according to hedonic scale parameters which include colour, odour, texture and general acceptability. However, taste was not evaluated in raw products. Sensory analyses were also performed on the 1st, 3rd, 5th, 7th and 10th days of storage. The panellists were also asked on daily basis to evaluate the fish whether they were still fit for consumption or not (Bonilla et al., 2007).

pH analysis

The pH value was measured directly from the homogenate (5 g fish 5 ml^{-1} distilled water) using a Hanna 211 model pH meter (Cluj-Napoca, Romania). The experiment was proceeded in triplicate (Lima Dos Santos et al., 1981).

Statistical analysis

The data were analysed by one-way ANOVA. Tukey's multiple range test was applied for determining group differences at 95% significance level. Analysis was performed using SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS and DISCUSSION

Antimicrobial assays

Antimicrobial activity by MIC

The production yield of daphne and basil extracts was 5.8 % and 7.8 % respectively. The effect of different extraction methods solvent systems on plant extracts yields and their antimicrobial activity have been reported in many studies (Dirar et al., 2019; Do et al., 2014; Hayouni et al., 2007). Moreover, the antimicrobial activity can be exploited in edible coating to protect seafood. The antimicrobial activity result of the extracts against some target microorganisms were given by using MIC and disk diffusion method (Table 1 and 2).

Table 1. The minimum inhibitory concentration (MIC) of daphne and basil extracts against pathogen bacteria. Data shown are the means ± standard deviation.

Test bacteria	MIC (mg ml^{-1})			
	Daphne	**Basil**	**AMP**	**FC**
***E. coli* ATCC 29998**	0.90 ± 0.8^{aA}	0.73 ± 0.5^{aB}	0.78 ± 0.2^{aB}	R
***E. coli* O157:H7 RSKK 234**	0.45 ± 0.25^{bB}	0.73 ± 0.2^{aA}	R	R
***S. aureus* ATCC 43300**	0.23 ± 0.3^{cC}	0.73 ± 0.1^{aA}	0.39 ± 0.3^{bB}	R
***S. typhimurium* CCM 5445**	0.23 ± 0.12^{cB}	0.73 ± 0.5^{aA}	0.78 ± 0.4^{aA}	R
***E. faecalis* ATCC 29212**	0.11 ± 0.55^{dC}	0.73 ± 0.35^{aA}	0.39 ± 0.15^{bB}	R
***B.cereus* ATCC 7064**	0.11 ± 0.4^{dC}	0.73 ± 0.25^{aA}	0.39 ± 0.12^{bB}	R
***S. epidermidis* ATCC 12228**	0.23 ± 0.33^{cB}	0.73 ± 0.1^{aA}	0.19 ± 0.5^{cB}	R
***C. albicans* ATCC 10231**	0.11 ± 0.3^{dC}	0.73 ± 0.1^{aB}	R	0.78 ± 0.8^{A}

AMP: Ampicillin, FC: Flucytosine, R: No inhibition zone.
*Lowercase letters indicate significant differences in a column ($P < 0.05$). Uppercase letters indicate significant differences in a row ($P < 0.05$).

In our study, the MIC values of daphne and basil extracts against test microorganisms ranged between 0.90 mg ml^{-1} and 0.11 mg ml^{-1}. 0.90 mg ml^{-1} demonstrated the maximum antimicrobial activity, therefore, a value of 1 % (mg 100 ml^{-1}) was added to the edible coating solution. The highest antibacterial activity exhibited by daphne was recorded against *E. faecalis, B.cereus* and *C. albicans* with MIC value 0.11 µg ml^{-1} ($P < 0.05$). Basil extract exhibited the same antimicrobial activity (MIC = 0,73 µg ml^{-1}) against all the tested strains ($P > 0.05$). The antibiotics and extracts had higher microbial activity on Gram-positive bacteria when compared to that on Gram-negative (*E. coli* ATCC 29998, *E. coli* 0157:H7 RSKK 234 and *S. thyphimurium* CCM 5445). Tayoub et al,. (2012) were determined the leaves ethanolic extract had the highest effect on pathogenic bacteria, compared with other plant parts extracts. Other studies on the genus Daphne and basil showed that the ethanol extract contains compounds with antibacterial properties (Adamczak et al., 2020; Manojlovic et al., 2012).

Agar well diffusion test

The xanthan-daphne coating displayed significant antimicrobial activity with inhibition zone ≥ 15 mm when a concentration was used 500 µl ml^{-1}. Inhibition zones measured against the tested strains were as follows: *S. epidermidis* (21.6 mm), *S. aureus* (16.2 mm), *E. coli* O157:H7 (16.1 mm), *B.cereus* (15.2 mm) and *E.faecalis* (15.0 mm).

At the same time, the xanthan-basil coating displayed significant antimicrobial activity against *S. typhimurium* (17.5 mm) and *E. coli* O157:H7 (15.7 mm).

However, DE and BE did not show any activity against *S. typhimurium* at any of the employed concentrations expect to Ceftazidime chemical agent (12.2 mm). All the extracts obtain edible coating displayed significant zone of inhibition against *S. aureus* and *S. epidermidis* at all concentrations (Table 2).

Table 2. The inhibition zone diameter of xanthan surface-active coating with daphne and basil extract.

Test bacteria	Agar well diffusion (mm)							
	Daphne			Basil			Standard antibiotics	
	0.50 mg ml^{-1}	0.25 mg ml^{-1}	0.10 mg ml^{-1}	0.50 mg ml^{-1}	0.25 mg ml^{-1}	0.10 mg ml^{-1}	CF	NYS
***E. coli* ATCC 29998**	12.9 ± 0.3^{cC}	R	R	15.7 ± 0.1^{bA}	14.0 ± 0.2^{aB}	R	14.2 ± 0.2^{dB}	R
***E. coli* O157:H7 RSKK 234**	16.1 ± 0.1^{bA}	14.3 ± 0.1^{aB}	10.1 ± 0.3^{cC}	R	R	R	R	R
***S. aureus* ATCC 43300**	16.2 ± 0.2^{bB}	15.6 ± 0.4^{aC}	13.2 ± 0.2^{aD}	14.0 ± 0.4^{cD}	13.1 ± 0.4^{bD}	12.7 ± 0.1^{aDE}	20.5 ± 0.3^{aA}	R
***S. typhimurium* CCM 5445**	R	R	R	R	R	R	12.2 ± 0.1^{eA}	R
***E. faecalis* ATCC 29212**	15.0 ± 0.0^{bB}	12.8 ± 0.2^{bC}	R	12.2 ± 0.4^{cdC}	R	R	17.0 ± 0.2^{bA}	R
***B.cereus* ATCC 7064**	15.2 ± 0.2^{bA}	14.4 ± 0.3^{aAB}	11.4 ± 0.2^{bCD}	13.1 ± 0.1^{cB}	12.1 ± 0.2^{bC}	10.4 ± 0.2^{bD}	15.4 ± 0.2^{cA}	R
***S. epidermidis* ATCC 12228**	21.6 ± 0.2^{aA}	14.2 ± 0.0^{aC}	11.6 ± 0.2^{bD}	17.5 ± 0.1^{aB}	14.5 ± 0.2^{aC}	R	14.5 ± 0.5^{cdC}	R
***C. albicans* ATCC 10231**	13.0 ± 0.2^{cB}	11.9 ± 0.1^{cC}	R	11.2 ± 0.3^{dC}	R	R	R	18.5 ± 0.1^{A}

CF:Ceftazidime, NYS: Nystatine, R: No inhibition zone.

* Lowercase letters indicate significant differences in a column ($P < 0.05$). Uppercase letters indicate significant differences in a row ($P < 0.05$).

Bodini et al., (2013) had studied gelatine based edible films with ethanol propolis extract at different (0, 5, 40 and 200 g of extract 100 g^{-1} of gelatine) concentrations. The study showed only the films with propolis extracts, whose concentrations ranged between to 40 and 200 g, inhibited the growth of *S. aureus*.

Such results can be possibly attributed to the increase in polyphenol concentrations. Fish covered with edible film caused the inhibition of the microbial growth because of being created a good barrier of oxygen transfer to the fish (Song et al., 2011). However, Flores et al., (2010) reported that xanthan gum surface-active coating material had no positive effect on the inhibition of the microbial activity.

Microbiological quality

On first day was detected 3.0 (log CFU g^{-1}) and 3.5 (log CFU g^{-1}) from control group in total viable count (TVC) and and total aerobic psychrophilic bacteria (TAP). On the other hand, 3.05 (log CFU g^{-1}), 2.01 (log CFU g^{-1}) 2.5 (log CFU g^{-1}), 2.5 (log CFU g^{-1}) were detected for ENT, SPH, MY and LAB respectively. There was not statistically significant (*P>0.05*) difference between the groups on the first day of storage. During storage time, TVC and TAP of basil and daphne xanthan coated samples demonstrated an increase and reached to the maximal levels at day 10 of chilled storage. The uncoated fish samples from experiment group were completely spoiled at day 7. The microbial growth in trout fillets coated with extracts was lower than uncoated samples (*P > 0.05*). Extract coatings were effective in slowing down the bacterial growth, but the two extracts (daphne and basil) were not differences (*P > 0.05*). On the other hand, DE and BE resulted in good preservation in coated samples when compared with uncoated samples after 7 days of storage. Therefore, the odour and appearance of xanthan coated samples were not only better than the uncoated samples but also the inhibition of the microbial growth was in xanthan coated samples.

According to International Commission for Microbiological Specifications for Foods (ICMSF, 1986) the limits for mesophilic bacteria counts should not exceed equivalent to 10^7 CFU g^{-1} in fresh fish for human consumption. According to Figure 1A uncoated samples attained 7 log CFU g^{-1} on day 7, thus this time was taken as the maximum shelf-life of C group. However, coating with xanthan delayed and restricted the growth of microorganisms. The antibacterial extracts on fish samples can function as a barrier against oxygen diffusion and bacterial proliferation. The lowest microbial count, after 7 days of storage was recorded with DE coated fish. In fact, the TVC of fish coated with DE was equivalent to 5.3 log CFU g^{-1}, which is significantly lower (*P < 0.05*) than that recorded for both the control group (7.95 log CFU g^{-1}) and fish coated with BE group (5.51 log CFU g^{-1}). Andevari and Rezaei (2011) indicated that a cinnamon oil coated gelatine films extended the shelf-life of fresh rainbow trout fillets in cold storage. Gelatine coatings with cinnamon oil reduced the total bacteria growth for 15 days.

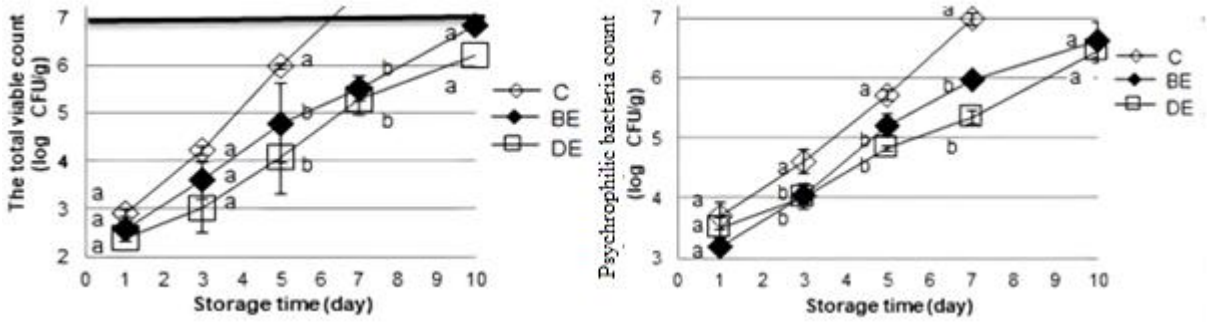

Figure 1. Changes in (A) total viable counts (log CFU g^{-1}) and (B) psychrophilic microorganisms (log CFU g^{-1}) during cold storage (4 °C) of uncoated and coated rainbow trout fillets. Lowercase letters difference between groups in the same day.

The changes in TAP of the uncoated and coated fish fillets are presented in (Figure 1B). Especially in the control group, the number of psychrophilic bacteria increased in the storage time and had the fastest bacterial growth rate ($P < 0.05$). Although extract coatings were effective in slowing down the bacterial growth on fillets, but no significant difference was recorded between the two extracts (DE and BE) ($P > 0.05$). In general, the most effective inhibition of psychrophilic bacteria was obtained when coated fillets by DE.

Ojagh et al. (2010) reported that the counts of psychrophilic bacteria obtained from coated trout fillets with chitosan enriched with cinnamon (2.88 log CFU g^{-1}) and chitosan (3.85 log CFU g^{-1}) were very close to each other at the beginning of storage. The study also mentioned the growth and cell counts in PVC and TVC were similar.

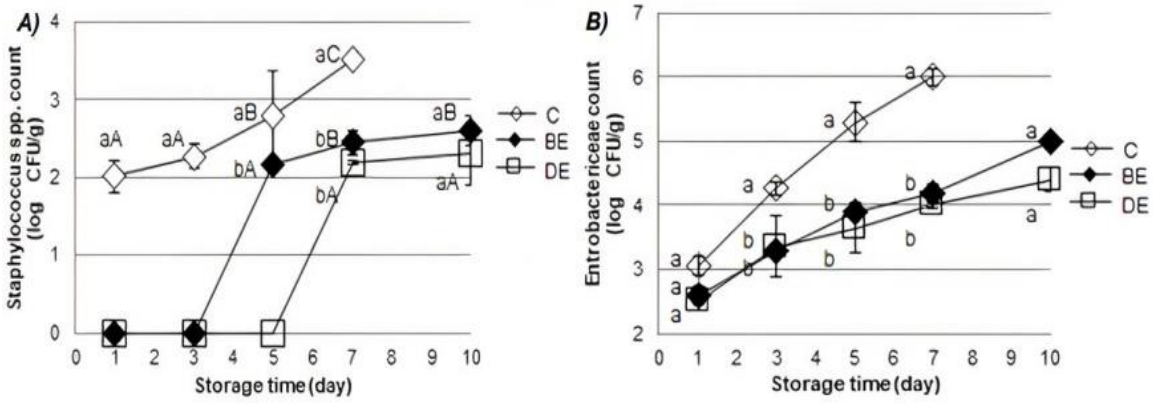

Figure 2. Changes in (A) *Staphylococcus* spp. (SPH) (log CFU g^{-1}); (B) *Enterobacteriaceae* (ENT) microorganisms (log CFU g^{-1}) during cold storage (4 °C) of uncoated and coated rainbow trout fillets. Lowercase letters difference between groups in the same day. Uppercase letters difference in the same group during storage.

At the beginning of storage period, SPH were not detected in DE and BE (<10 CFU g^{-1}), yet an increase was recorded during the last days of storage (Figure 2A). The growth of *Staphylococcus* bacteria started after the 5th (2.16 log CFU g^{-1}) and 7th (2.8 log CFU g^{-1}) days in the samples coated with BE and DE. The highest slope of increment in population of *Staphylococcus* bacteria observed was uncoated samples ($P > 0.05$). The lowest growth of *Staphylococcus* bacteria was that of the sample DE ($P > 0.05$). Stojanovic-Radic et al., (2018) reported that treatment of meat pieces with basil essential oils (BEO) demonstrated bactericidal effect against the entire bacteria population and resulted in reduced the growth of *S. aureus* by 2.74 log CFU g^{-1}.

Significant reduction (more than 2 log CFU g^{-1}) in the *Enterobacteriaceae* count (ENT) was observed in treatments with extracts ($P < 0.05$) (Figure 2B). Both DE and BE samples resulted in a significant reduction in bacterial counts equivalent to (4.00 log CFU g^{-1}) and (4.20 log CFU g^{-1}) respectively when compared to the control samples (6.00 log CFU g^{-1}) at the 7th day ($P < 0.05$). In daphne treatment also demonstrated bactericidal effect (almost 1 log) which was higher than that recorded by basil coated samples at the end of storage ($P > 0.05$).

This result is agreement with that of Volpe et al., (2015) regarding the *Enterobacteriaceae* counts. The *Enterobacteriaceae* counts at the end of the experiment were equivalent to 2 log CFU g^{-1}, 3.5 log CFU g^{-1} and 5 log CFU g^{-1} when the trout fillets were coated with carrageenan film containing lemon essential oil (1 % g ml^{-1}), carrageenan film and uncoated samples respectively. Frangos et al. (2010) reported that addition of 0.4 % (v w^{-1}) oregano essential oil in vacuum-packaged rainbow trout fillets significantly decreased the lactic acid bacteria, *Enterobacteriaceae*, *Pseudomonas* spp. and H_2S producing bacteria during storage at 4°C.

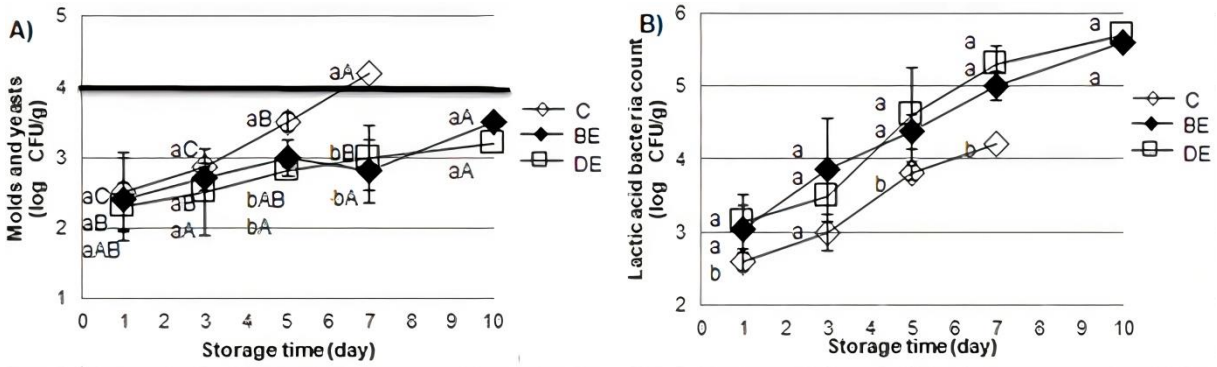

Figure 3. Changes in (A) Molds and yeasts (MY); (B) Lactic acid bacteria (LAB) (log CFU g^{-1}) during cold storage (4 °C) of uncoated and coated rainbow trout fillets. Lowercase letters difference between groups in the same day. Uppercase letters difference in the same group during storage.

Molds and yeast(s) exhibited similar growth trends during storage (Figure 3A). The increment in the Molds and yeast populations of uncoated sample was equivalent to 2.50 (log CFU g^{-1}), 2.85 (log CFU g^{-1}), 3.50 (log CFU g^{-1}), 4.20 (log CFU g^{-1}) during the storage period ($P > 0.05$). Molds and yeast count of daphne and basil extract coated fillets recorded 1.5 log lower than control samples in the last day of storage ($P < 0.05$). Xanthan film coating reduces contact with oxygen of microorganisms. The microbial population were observed reduction in the trout presented in this study agrees with other studies that used plant essential oils and/or extracts (Ojagh et al., 2010, Pyrgotou et al., 2010).

The LAB count was equivalent to 2.60 log CFU g^{-1} (on day 1) which increased to 4.20 log CFU g^{-1} on day 7 of storage for the uncoated samples (Figure 3B). On the same day, the use of daphne and basil extracts resulted in a rise of LAB by 0.55 and 0.44 log CFU g^{-1}, respectively ($P > 0.05$). The increment of LAB was also observed (López de Lacey et al., 2014) in hake fillet coated agar green tea film and agar probiotic green tea film during storage. The study was reported that green tea and probiotic edible agar films not only increased the shelf-life of hake fish for 1 week but also increased the number of beneficial lactic acid bacteria in fish. Similar results were reported by Raeisi et al., (2015) when rainbow trout treated with carboxymethyl cellulose-based coatings incorporated with *Zataria multiflora Boiss.* essential oil and grape seed extract. Previous studies also reported the enzyme produced by lactic acid bacteria can reduce the number of food spoilage microorganisms and inhibit the growth of pathogenic microorganisms (Adams et al., 1987; Gómez-Estaca et al., 2010; Ostergaard et al., 1998).

Bacterial strain identification

Dominant bacteria definitions in fish consumed as human food determine the bacteriological quality. Throughout the storage period in this study, 14 different bacteria and 3 different yeasts were identified via Bacterial Identification API Kits. The identified yeast isolates were *Candida calliculosa* (84.5 %), *Candida zeylanoides* (98.3 %), *Trichospora inkin* (92.7 %). These microorganisms are generally of soil origin. It is stated that it can be transmitted from plant extracts used in fish or from tools and materials used after catching. The lactic acid bacterial isolates were *Lactobacillus salivarius* (99.9 %), *Lactobacillus brevis* (99.6 %), *Lactobacillus acidophilus* (89.1 %), *Lactococcus lactis* ssp. (81.8 %). *Serratia liquefaciens* (98 %), *Serratia marcescens* (98 %), *Erwinia* spp. (85 %) which belong to *Enterobacteriaceae*, weren't detected only in xanthan-coated groups.

Serratia and *Erwinia* species are opportunistic pathogens and can be isolated from water, soil, plants, and air (Mossad, 2000). In our study, the identification of these species in coated trout samples with daphne and basil extract was thought that these species were come from the plants. On the other hand, *Moraxella* (76 %) was detected all groups. Mesophilic bacterial species were also identified those includes *Pseudomonas fluorescens* (99.9 %), *Pseudomonas luteola* (99.7 %), *Vibrio metschnikovii* (99.4 %), *Pasteurella multocida* (98%), *Pseudomonas putida* 96.8% and *Ralstonia picketti* (91.1%).

Sensory evaluation

Figure 4 shows the scores of the treated fish fillets in the sensory evaluation. Appearance and colour, odour, texture and in general acceptability were considered to evaluate the total acceptance (Figure 4).

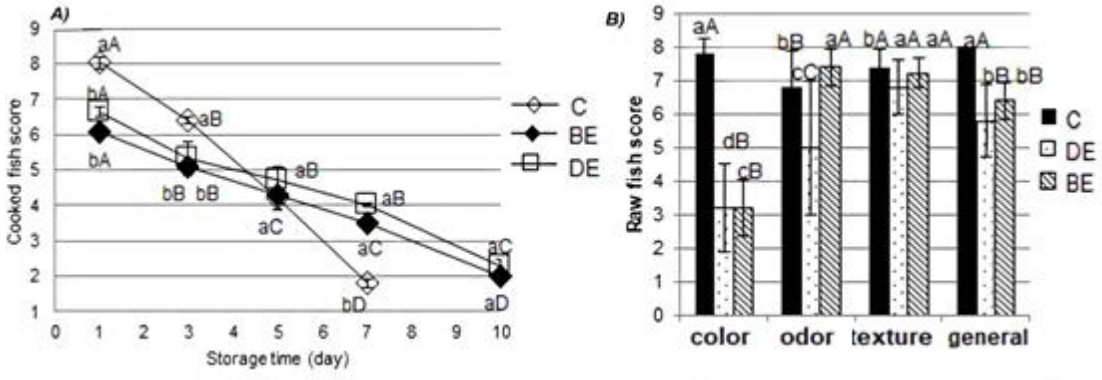

Figure 4. The sensory scores of cooked (A) and raw (B) fish fillets uncoated and coated with xanthan surface-active coating daphne and basil extract. Lowercase letters difference between groups in the same day. Uppercase letters difference in the same group during storage.

This study selected natural extracts such as daphne and basil due to their theoretically compatible organoleptic properties (odour, taste) with fish products. Even though the counts of treated samples were different from those of the control ($P < 0.05$), the sensory scores, which were analysed daily, demonstrated almost no significant differences between the treated groups ($P > 0.05$). In general, initial sensorial quality was good for all fish samples. Aroma and flavour due to treatment with plant extracts was evaluated by panellists. Superficial moisture was given negative evaluation when the control fish fillets were assessed at the 5th day. Yet severe liquid loss wasn't detected xanthan surface-active coating samples during the same storage period.

The colour scores, of the control groups at the last day of storage, were significantly lower when compared to those coated with DE and BE ($P < 0.05$) (Figure 4A). Samples of all groups tended to slowly deteriorate in their sensory scores up to day 5 ($P > 0.05$), after which a sudden and significant drop in sensory scores was recorded on the day 7 ($P < 0.05$). The texture parameters were stable over the entire storage period, with no significant difference between all groups during the first 7 days of storage. The C groups were evaluated as unsuitable for consumption on the 7th day, while the treated groups got discarded on the 10th day of storage (Figure 4B). Significant differences between the plant extracts became more evident in the end of week. It was clear that the best fish and plant odour were detected in the coated groups, DE were also judged as stronger than BE. Higher deterioration of sensory characteristics was detected by the panellists in control groups when compared to the treated groups on the 7th day. Subsequently, lower score in “cooked fish flavour” was recorded for the xanthan coated samples.

In a study performed by Mexis et al. (2009) the shelf-life of trout fillets was determined as 4 days for the control samples when stored at 4 ºC, 7–8 days for samples containing oregano essential oil, 13–14 days for samples containing O_2 absorber and 17 days for samples containing O_2 absorber and oregano oil. They reported that both odour and taste are determinant factors effecting the quality of rainbow trout fillet ($P < 0.05$).

pH

Figure 5 presents the pH values of the xanthan coated (DE and BE) and uncoated control samples (C) for 10 days storage at 4 °C. The initial pH of all samples was founded 6.2. Coating the surface of fish samples by xanthan gum was resulted in decrease of pH values ($P > 0.05$), it's, as low of pH in xanthan coating and plant extracts. The values of pH in coated samples were no significant difference ($P > 0.05$) between the groups. Similarly, change in pH values were also reported in other previous studies (Arashisar et al., 2004; Chytiri et al., 2004; Kakaei and Shahbazi, 2016).

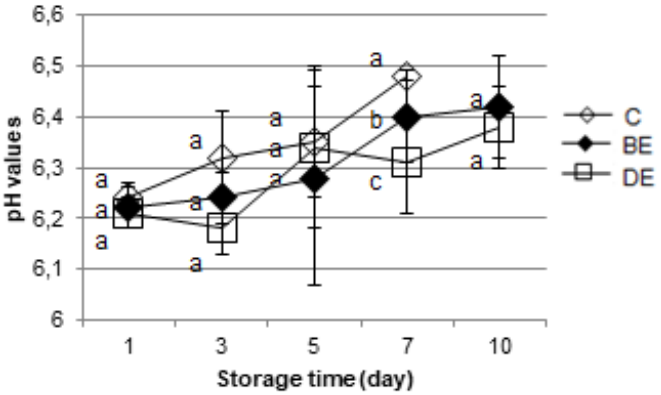

Figure 5. Changes in pH values of uncoated and coated rainbow trout fillets. Lowercase letters difference between groups in the same day.

CONCLUSION

The results of the study proved that xanthan gum-based edible film coatings with antimicrobial extracts are a successful and effective method to extend the shelf life of fish fillets for 10 days storage at 4 °C. All the coated with basil and daphne extract samples showed lower degradation in total viable counts, total aerobic psychrophilic bacteria, Molds – yeasts and pH as opposed to coated without extract samples. Significant delays were also found in weight loss, *Staphylococcus* spp. and *Enterobacteriaceae* microorganisms. The use of 1% daphne and basil extracts in the edible coating combination shows that it is the best coating combination as it preserves the microbial quality in the fish. The lactic acid bacteria were more increased in all coated with extract samples due to anaerobic condition and plant extracts. While the samples coated with the extract were less preferred due to the different colour formation in the fish, especially the products with daphne extract were appreciated in terms of flavour and texture properties. In general, all coated with extract samples were acceptable at the end of the 10-day storage run, while only xanthan gum coated samples were acceptable up to day 7. In future studies, it is necessary to be improved the colours of the extracts used in fish edible surface coatings, containing natural antimicrobials. Thus, BE and DE could be used as an active coating to maintain the quality and extend the shelf-life of the rainbow trout fillets under refrigerated storage.

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REFERENCES

Adamczak A., Ożarowski, M. & Karpiński, T.M. 2019. Antibacterial activity of some flavonoids and organic acids widely distributed in plants, *Journal of Clinical Medicine*, 9(1), 109. https://doi.org/10.3390/jcm9010109

Adams M.R., Cooke R.D. & Twiddy D.R. 2007. Fermentation parameters involved in the production of lactic acid preserved fish-glucose substrates, *International Journal of Food Science & Technology*, 22(2), 105-114. https://doi.org/10.1111/j.1365-2621.1987.tb00464.x

Andevari G.T. & Rezaei M. 2011. Effect of gelatin coating incorporated with Cinnamon oil on the quality of fresh rainbow trout in cold storage, *International Journal of Food Science & Technology*, 46(11), 2305-2311. https://doi.org/10.1111/j.1365-2621.2011.02750.x

Arashisar Ş., Hisar O., Kaya M. & Yanik T. 2004. Effects of modified atmosphere and vacuum packaging on microbiological and chemical properties of rainbow trout (*Oncorhynchus mykiss*) fillets, *International Journal of Food Microbiology*, 97(2), 209-214. DOI: 10.1016/j.ijfoodmicro.2004.05.024

Asong J.A., Amoo S.O., McGaw L.J., Nkadimeng S.M., Aremu A.O. & Otang-Mbeng W. 2019. Antimicrobial activity, antioxidant potential, cytotoxicity and phytochemical profiling of four plants locally used against skin diseases, *Plants*, 8(9), 350. DOI: 10.3390/plants8090350

Aspevik T., Thoresen, L., Steinsholm, S., Carlehög, M. & Kousoulaki, K. 2021. Sensory and chemical properties of protein hydrolysates based on mackerel (*Scomber scombrus*) and salmon (*Salmo salar*) side stream materials, *Journal of Aquatic Food Product Technology*, 30(2), 176-187. DOI: https://doi.org/10.1080/10498850.2020.1868644

Balkan İ.A., Taşkın T., Doğan H.T., Deniz İ., Akaydın G. & Yesilada E. 2017. A comparative investigation on the in vitro anti-inflammatory, antioxidant and antimicrobial potentials of subextracts from the aerial parts of *Daphne oleoides* Schreb. subsp. Oleoides, *Industrial Crops and Products*, 95, 695-703. DOI: https://doi.org/10.1016/j.indcrop.2016.11.038

Baron C.P., KjÆrsgård I.V., Jessen F. & Jacobsen C. 2007. Protein and lipid oxidation during frozen storage of rainbow trout (*Oncorhynchus mykiss*), t.,55(20), 8118-8125. DOI: https://doi.org/10.1021/jf070686f

Baytop T. 1984. Türkiyede bitkiler ile tedavi (geçmişte ve bugün). İstanbul Üniversitesi Eczacılık Fakültesi, İstanbul, 550.

Bensid A., Ucar Y., Bendeddouche B. & Özogul F. 2014. Effect of the icing with thyme, oregano and clove extracts on quality parameters of gutted and beheaded anchovy (*Engraulis encrasicholus*) during chilled storage, *Food Chemistry*, 145, 681-686. DOI: 10.1016/j.foodchem.2013.08.106

Biswas N.N., Saha S. & Ali M.K. 2014. Antioxidant, antimicrobial, cytotoxic and analgesic activities of ethanolic extract of Mentha arvensis L, *Asian Pacific Journal of Tropical Biomedicine*, 4(10), 792-797. https://doi.org/10.12980/APJTB.4.2014C1298

Bodini R., Sobral P., Favaro-Trindade C. & Carvalho R. 2013. Properties of gelatin-based films with added ethanol–propolis extract, *LWT - Food Science and Technology*. 51(1), 104-110. https://doi.org/10.1016/j.lwt.2012.10.013

Bonilla A. C., Sveinsdottir K. & Martinsdottir E. 2007. Development of Quality Index Method (QIM) scheme for fresh cod (Gadus morhua) fillets and application in shelf life study, *Food control*, 18(4), 352-358. DOI: https://doi.org/10.1016/j.foodcont.2005.10.019

Burt S. 2004. Essential oils: their antibacterial properties and potential applications in foods—a review, *International journal of food microbiology*, 94(3), 223-253. DOI: https://doi.org/10.1016/j.ijfoodmicro.2004.03.022

Chytiri S., Chouliara I., Savvaidis I. N. & Kontominas M. G. 2004. Microbiological, chemical and sensory assessment of iced whole and filleted aquacultured rainbow trout, *Food Microbiology*, 21(2), 157-165. DOI: https://doi.org/10.1016/S0740-0020(03)00059-5

Clinical Laboratory Standards Institute, CLSI 2011. Performance standards for antimicrobial susceptibility testing: Twenty-first informational supplement. Clinical & Laboratory Standards Institute.

Costa D.C., Costa H., Albuquerque T.G., Ramos F., Castilho M. & Sanches-Silva A. 2015. Advances in phenolic compounds analysis of aromatic plants and their potential applications, *Trends in Food Science & Technology*, 45(2), 336-354. DOI: https://doi.org/10.1016/j.tifs.2015.06.009

Díaz-Montes E. & Castro-Muñoz R. 2021. Edible films and coatings as food-quality preservers: An overview, *Foods*, 10(2), 249. doi: 10.3390/foods10020249

Dirar A. I., Alsaadi D. H. M., Wada M., Mohamed M. A., Watanabe T. & Devkota H. P. 2019. Effects of extraction solvents on total phenolic and flavonoid contents and biological activities of extracts from Sudanese medicinal plants, *South African Journal of Botany*, 120, 261-267. DOI: https://doi.org/10.1016/j.sajb.2018.07.003

Do Q. D., Angkawijaya A. E., Tran-Nguyen P. L., Huynh L. H., Soetaredjo F. E., Ismadji S. & Ju Y. H. 2014. Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*, *Journal of food and drug analysis*, 22(3), 296-302. DOI: https://doi.org/10.1016/j.jfda.2013.11.001

Flores S. K., Costa D., Yamashita F., Gerschenson L. N. & Grossmann M. V. 2010. Mixture design for evaluation of potassium sorbate and xanthan gum effect on properties of tapioca starch films obtained by extrusion, *Materials Science and Engineering: C*, 30(1), 196-202. DOI: https://doi.org/10.1016/j.msec.2009.10.001

Frangos L., Pyrgotou N., Giatrakou V., Ntzimani A. & Savvaidis I. N. 2010. Combined effects of salting, oregano oil and vacuum-packaging on the shelf-life of refrigerated trout fillets, *Food microbiology*, 27(1), 115-121. DOI: https://doi.org/10.1016/j.fm.2009.09.002

Gao M., Feng L., Jiang T., Zhu J., Fu L., Yuan D. & Li J. 2014. The use of rosemary extract in combination with nisin to extend the shelf-life of pompano (*Trachinotus ovatus*) fillet during chilled storage, *Food Control*, 37, 1-8. DOI: https://doi.org/10.1016/j.foodcont.2013.09.010

Garcia L. C., Pereira L. M., de Luca Sarantópoulos C. I. & Hubinger M. D. 2010. Selection of an edible starch coating for minimally processed strawberry, *Food and Bioprocess Technology*, 3(6), 834-842. DOI: 10.1007/s11947-009-0313-9

Gómez-Estaca J., López de Lacey A., López-Caballero M., Gómez-Guillén M. & Montero P. 2010. Biodegradable gelatin–chitosan films incorporated with essential oils as antimicrobial agents for fish preservation, *Food Microbiology*, 27(7), 889-896. DOI: https://doi.org/10.1016/j.fm.2010.05.012

Gomez-estaca J., Montero P., Gımenez B. & Gomezguıllen M. 2007. Effect of functional edible films and high pressure processing on microbial and oxidative spoilage in cold-smoked sardine (*Sardina pilchardus*), *Food Chemistry,* 105(2), 511-520. DOI: https://doi.org/10.1016/j.foodchem.2007.04.006

Harrigan W.F. & McCance M.E. 1976. Laboratory methods in food and dairy microbiology. London: Academic Press Inc.

Hashemi S.M. & Mousavi Khaneghah A. 2017. Characterization of novel basil-seed gum active edible films and coatings containing oregano essential oil, *Progress in Organic Coatings*, 110, 35-41. DOI: https://doi.org/10.1016/j.porgcoat.2017.04.041

Hayouni E. A., Abedrabba M., Bouix M. & Hamdi M. 2007. The effects of solvents and extraction method on the phenolic contents and biological activities in vitro of Tunisian *Quercus coccifera* L. and *Juniperus phoenicea* L. fruit extracts, *Food Chemistry*, 105(3), 1126-1134. DOI: https://doi.org/10.1016/j.foodchem.2007.02.010

Hosseini S.F., Rezaei M., Zandi M. & Ghavi F.F. 2016. Effect of fish gelatin coating enriched with oregano essential oil on the quality of refrigerated rainbow trout fillet, *Journal of Aquatic Food Product Technology*, 25(6), 835-842. DOI: https://doi.org/10.1080/10498850.2014.943917

Irkin R. & Esmer O. K. 2015. Novel food packaging systems with natural antimicrobial agents, *Journal of food science and technology*, 52(10), 6095-6111. DOI: 10.1007/s13197-015-1780-9

Kakaei S. & Shahbazi Y. 2016. Effect of chitosan-gelatin film incorporated with ethanolic red grape seed extract and *Ziziphora clinopodioides* essential oil on survival of *Listeria monocytogenes* and chemical, microbial and sensory properties of minced trout fillet, *LWT-Food Science and Technology*, 72, 432-438. DOI: https://doi.org/10.1016/j.lwt.2016.05.021

Lang G. & Buchbauer G. 2012. A review on recent research results (2008-2010) on essential oils as antimicrobials and antifungals. A review, *Flavour and Fragrance Journal*, 27(1), 13-39. DOI: https://doi.org/10.1002/ffj.2082

Lima dos Santo C.A., James D. & Teutscher F. 1981. Guidelines for chilled fish storage experiments. Bernan Press(PA) FAO Fisheries Technical Papers.

López de Lacey A., López-Caballero M. & Montero P. 2014. Agar films containing green tea extract and probiotic bacteria for extending fish shelf-life, *LWT - Food Science and Technology*, 55(2), 559-564. DOI: https://doi.org/10.1016/j.lwt.2013.09.028

Manojlovic N., Maskovic P., Vasiljevic P., Jelic R., Juskovic M., Sovrlic M. & Radojkovic M. 2012. HPLC analysis, antimicrobial and antioxidant activities of *Daphne cneorum* L, *Chemical Industry*, 66(5), 709-716. DOI: 10.2298/HEMIND120114029M

Mexis S. F., Chouliara E. & Kontominas M. G. 2009. Combined effect of an oxygen absorber and oregano essential oil on shelf life extension of rainbow trout fillets stored at 4 °C, *Food microbiology*, 26(6), 598-605. DOI: https://doi.org/10.1016/j.fm.2009.04.002

Mossad S.B. 2000. The world's first case of Serratia liquefaciens intravascular catheter-related suppurative thrombophlebitis and native valve endocarditis. *Clinical microbiology and infection.* 6(10), 559. DOI: 10.1046/j.1469-0691.2000.00164.x

Ojagh S. M., Rezaei M., Razavi S. H. & Hosseini S. M. H. 2010. Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout, *Food chemistry*, 120(1), 193-198. DOI: https://doi.org/10.1016/j.foodchem.2009.10.006

Ong G., Kasi R. & Subramaniam R. 2021. A review on plant extracts as natural additives in coating applications, *Progress in Organic Coatings*, 151, 106091. DOI: https://doi.org/10.1016/j.porgcoat.2020.106091

Ostergaard A., Embarek P. K. B., Wedell-Neergaard C., Huss H. H. & Gram L. 1998. Characterization of anti-listerial lactic acid bacteria isolated from Thai fermented fish products, *Food Microbiology,* 15(2), 223-233. DOI: https://doi.org/10.1006/fmic.1997.0153

Pyrgotou N., Giatrakou V., Ntzimani A. & Savvaidis I. N. 2010. Quality assessment of salted, modified atmosphere packaged rainbow trout under treatment with oregano essential oil, *Journal of food science,* 75(7), 406-411. DOI: 10.1111/j.1750-3841.2010.01724.x

Raeisi M., Tajik H., Aliakbarlu J., Mirhosseini S. H. & Hosseini S. M. H. 2015. Effect of carboxymethyl cellulose-based coatings incorporated with Zataria multiflora Boiss. essential oil and grape seed extract on the shelf life of rainbow trout fillets, *LWT-Food Science and Technology,* 64(2), 898-904. DOI: https://doi.org/10.1016/j.lwt.2015.06.010

Sakkas H. & Papadopoulou C. 2017. Antimicrobial activity of basil, oregano, and thyme essential oils, *Journal of Microbiology and Biotechnology*, 27(3), 429-438. DOI: 10.4014/jmb.1608.08024

Sapper M., Talens P. & Chiralt A. 2019. Improving functional properties of cassava starch-based films by incorporating xanthan, gellan, or pullulan gums, *International Journal of Polymer Science,* 2019. DOI: https://doi.org/10.1155/2019/5367164

Song Y., Liu L., Shen H., You J. & Luo Y. 2011. Effect of sodium alginate-based edible coating containing different anti-oxidants on quality and shelf-life of refrigerated bream (*Megalobrama amblycephala*), *Food Control,* 22(3-4), 608-615. DOI: https://doi.org/10.1016/j.foodcont.2010.10.012

Sothornvit R. 2011. Edible coating and post-frying centrifuge step effect on quality of vacuum-fried banana chips, *Journal of Food Engineering,* 107(3-4), 319-325. DOI: https://doi.org/10.1016/j.jfoodeng.2011.07.010

Tayoub G., Abu Alnaser A. & Shamma M. 2012. Microbial inhibitory of the Daphne oleifolia lam. ethanolic extract, *International Journal of Medicinal and Aromatic Plants,* 2(1), 161-166. Corpus ID: 55592514

Volpe M., Siano F., Paolucci M., Sacco A., Sorrentino A., Malinconico M. & Varricchio E. 2015. Active edible coating effectiveness in shelf-life enhancement of trout (*Oncorhynchus mykiss*) fillets, *LWT - Food Science and Technology,* 60(1), 615-622. DOI: https://doi.org/10.1016/j.lwt.2014.08.048

Weber C.J., Haugaard V., Festersen R. & Bertelsen G. 2002. Production and applications of biobased packaging materials for the food industry, *Food Additives & Contaminants,* 19(sup1), 172-177. DOI: https://doi.org/10.1080/02652030110087483

Zheng M., Lian F., Zhu Y., Zhang Y., Liu B., Zhang L. & Zheng B. 2019. pH-responsive poly (xanthan gum-g-acrylamide-g-acrylic acid) hydrogel: Preparation, characterization, and application, *Carbohydrate polymers,* 210, 38-46. DOI: https://doi.org/10.1016/j.carbpol.2019.01.052