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Effects of chitosan edible coating containing grape seed extract on the shelf-life of refrigerated rainbow trout fillet

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Abstract

In recent years, use of edible coatings as carriers of food additives and antimicrobial compounds has been considered in fishery products. This study was carried out to evaluate the effects of 2.00% chitosan coating singly and combined with 0.10% grape seed extract (GSE) on microbial (mesophils and psychrophils counts), chemical (thiobarbituric acid; TBA), pH and peroxide value (PV) and sensorial properties of rainbow trout fillet stored at 4 ˚C over a period of 15 days. The coating had a significant effect in reducing aerobic mesophilic and psychrophilic bacteria counts. The TBA, PV and pH of samples of chitosan coating alone and with GSE were lower than control ones indicating a significant influence of coating on fillet shelf-life. Moreover, chitosan coating represented an equal sensorial quality with controls. It can be concluded that chitosan coating containing GSE can help to maintain the sensorial quality and increase the shelf-life of rainbow trout fillets at refrigerated conditions.

اثرات پوشش خوراکی کیتوزان حاوی عصاره دانه انگور بر ماندگاری فیله ماهی قزل آلا در شرایط نگهداری در یخچال

چکیده

در سال‌های اخیر، استفاده از پوشش‌های خوراکی به عنوان حامل افزودنی‌های غذایی و ترکیبات ضد میکروبی در محصولات شیلاتی مورد توجه قرار گرفته است. این مطالعه با هدف بررسی آثار پوشش ۲/۰۰ درصد کیتوزان به تنهایی و حاوی ۰/۱۰ درصد عصاره دانه انگور روی خصوصیات میکروبی (شمارش مزوفیل‌ها و سرماگراها) شیمیایی (اسید تیوباربیتوریک، pH و شاخص پراکسید) و حسی فیله ماهی قزل آلای رنگین کمان نگهداری شده در دمای ۴ درجه سانتیگراد به مدت ۱۵ روز انجام گردید. پوشش‌دهی، اثر معنی‌داری در کاهش شمارش باکتری‌های مزوفیل و سرماگرا داشت. اسید تیوباربیتوریک، شاخص پراکسید و pH نمونه‌های پوشش داده شده با کیتوزان به تنهایی و حاوی عصاره دانه انگور کمتر از نمونه‌های شاهد بود که بیانگر اثر معنی‌دار پوشش‌دهی بر مدت ماندگاری فیله بود. همچنین، نمونه‌های پوشش داده شده با کیتوزان، کیفیت حسی مشابهی را با نمونه‌های شاهد نشان دادند. چنین برمی آید که پوشش‌دهی با کیتوزان حاوی عصاره دانه انگور می تواند به حفظ کیفیت حسی و افزایش مدت ماندگاری فیله ماهی قزل آلای رنگین کمان در شرایط یخچال کمک کند.

واژه های کلیدی: بسته بندی فعال، ضد میکروبی، عصاره دانه انگور، فیله، کیتوزان

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Introduction

Today, there are increasing awarenesses and interests to improve physicochemical, microbiological and sensorial properties of food using modern preservation methods. Among food products, meat and meat products are well known for their susceptibility to microbial and chemical contamination, so finding a cost-effective preservative with antioxidant and antimicrobial properties is vital for these products.[1]

The use of antioxidants in food, due to their ability to control fat oxidation and increase the shelf-life of food is highly appreciated by food authorities. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are among the most-used synthetic antioxidants found in many foods. Despite the effectiveness and good stability, use of these compounds is limited due to their toxicities.[2] In many countries around the world, a particular tendency to use a healthy and environmentally friendly compound in food has arisen. In recent years, special attention to use of grape by-products and in particular, grape seed extract (GSE; *Vitis vinifera*) has emerged. The GSE has approved by Food and Drug Administration as a generally recognized safe substance and it is commercially available as a dietary supplement and additive.[3] Strong antioxidant and antimicrobial activities which are actually related to high levels of flavonoids and phenolic acid, recommend GSE as a potential additive for food preservation.[4]

Within packaging sector, the market for novel food packaging has experienced remarkable growth over the last decade. Coating of food with edible materials is a type of active packaging acting as a barrier to the exchange of gasses and moisture as well as microorganisms between food and environment and extends the shelf-life of commodities from manufacturing until received by the consumer.[5] The advantages such as biodegradability and product shelf-life improving have caused edible coatings to be used widely in food industry and pharmaceutical sciences. Edible coatings could act as carriers for different functional agents including antimicrobial and antioxidant agents. In this case, microbial and chemical qualities of food are specifically improved.[6,7]

The base for the edible coating could be a protein (soy protein, whey protein and zein), carbohydrate (chitosan, carrageenan and cellulose) and lipid (waxes and fatty acids). Chitosan is a promising commercially produced polysaccharide from chitin, a versatile long-chain polymer in the world. Indeed, it has been proposed by many researchers that chitosan could serve as a suitable eco-friendly material for antimicrobial coating development due to its good film-forming properties as well as antibacterial, antioxidant and antifungal activities.[6,8]

Fish is a more important source of high-quality proteins for human than other meat products and a subject to high spoilage compared to fresh commodities.[9] Incorporation of compounds such as GSE into chitosan solution could improve the functional properties of coating and lead to extended stability of the fish fillet.

The main aim of this research was to prepare chitosan coating containing GSE and investigate the effects of active coating on chemical, microbial and overall sensorial acceptabilities of rainbow trout fillet maintained in a refrigerated condition.

Materials and Methods

Materials. Commercial GSE powder including extraction solvent: water, total phenolics (gallic acid equivalents, dry basis) ≥ 90 g GAE per 100 g and < 10 CFU mL^{-1}, was provided kindly from Mega Natural Inc. (Madera, USA). Chitosan (medium molecular weight, 75.00 to 85.00% degree of deacetylation) was purchased from Sigma Aldrich Company (St. Louis, USA). Peptone water, Plate Count Agar and all chemical regents were supplied by Merck Company (Darmstadt, Germany).

Fillets preparation. Rainbow trouts were obtained from Tabriz central fish market (Tabriz, Iran) on the day of the experiment and delivered in insulated polystyrene boxes on ice flakes and filleted (ca. 100.00 ± 15.00 g each) under an aseptic condition. Fillets were subsequently kept under refrigeration in a cooling incubator (4.00 ± 0.50 ˚C) before coating.

Proximate composition analyses. The fillets were separately homogenized using tissue homogenizer (IKA Works, Wilmington, USA) and analyzed for protein (%), fat (%), moisture (%) and ash (%), according to Association of Official Analytical Chemists methods.[10]

Preparation of GSE and chitosan coating solution. Chitosan solution (20 g L^{-1}) was prepared by dissolving 2 g of chitosan in 100 mL of 10 g L^{-1} acetic acid with constant agitation overnight at room temperature.[11] Glycerol as a plasticizer was added to the solution at 0.50 mL g^{-1} concentration of chitosan.

Treatment of fillet samples. Fillet samples were divided into four groups: (i) uncoated (control, C); (ii) immersed in chitosan solution (CH); (iii) immersed in 0.10% GSE solution (GSE) and (iv) immersed in chitosan solution containing 0.10% GSE (GSE-CH). Samples were glazed in each freshly and pre-homogenized (13000 rpm for 1 min) 200 mL coating solution for 1 min and then drained for 30 min at room temperature and packed in sterile polyethylene bags and maintained at 4.00 ± 0.50 ˚C for 15 days. Sampling for microbiological, chemical and sensorial evaluations was done at days 0, 3, 6, 9 and 15.

Microbiological evaluation. At each time, meat samples (25 g) were homogenized in 225 mL of sterile 0.10% peptone water for 1 min using pulsifier (Microgen Bioproducts Ltd., Surrey, UK). Decimal dilutions (1:10) in

0.10% peptone water solution were prepared and appropriate diluents were poured on plates of the following agars in duplicate: Plate count agar (PCA) for the total mesophilic viable count incubated at 35 ˚C for 24 hr; PCA for the total psychrotrophic viable count incubated at 7 ˚C for 5 to 7 days. Results were expressed as log CFU g^{-1}.

Chemical quality evaluation. For pH measurement, 10 g of sample was homogenized with 90 mL of distilled water for 30 sec and pH value of homogenized meat was measured using a pH-meter (model E520; Metrohm, Herisau, Switzerland).

Peroxide Value (PV) of trout fillets was determined using the method of Egan *et al*.[12] First, 0.10 g of each sample was mixed with 25 mL of acetic acid: chloroform (Merck) mixture, followed by addition of an aliquot of saturated potassium iodide solution (1 mL). The mixture was then allowed to stand in the dark for 10 min. Distilled water (20 mL) and 1 mL of 1.00% starch solution were transferred into the mixture and titrated with 0.01 N sodium thiosulphate (Sigma-Aldrich). The PV was calculated using the following equation:

$$Peroxide\ value\ (mEq\ kg^{-1}\ sample) = (a-b) \times N \times 100/w$$

where, a and b are the volume (mL) of sodium thiosulphate using for the blank and sample titration, respectively, N is the concentration of sodium thiosulphate and w is a sample weight (g).

Lipid oxidation progress in samples was evaluated using a method described by Pikul *et al*. with minor modification.[13] Ten grams of samples were individually homogenized in 35 mL of a cold (4 ˚C) extraction solution containing 4.00% perchloric acid and 1 mL of BHT (1 mg mL^{-1}) at 13500 rpm for 1 min. The preparation was mixed and filtered, then the filtrate was adjusted to 50 mL with 4.00% perchloric acid and 5 mL aliquots of the filtrate was added to 5 mL of 0.02 M thiobarbituric acid (TBA). The mixture was vortexed and then incubated in a 100 ˚C water bath for 60 min for color development. The absorbance at 532 nm was measured with a spectrophotometer (Novaspec II; Amersham Pharmacia Biotech Inc., Buckinghamshire, UK). The TBA values were expressed as mg malondialdehyde (MDA) per kg of sample.

Sensorial evaluation. Fish samples were evaluated by 10 sensory panel participants from our faculty according to a simple and unstructured sensory performance. Panelists evaluated odor, appearance and overall acceptability using a nine-point hedonic scale, 1 to 3 (spoiled), 4 to 6 (good) and 7 to 9 (excellent).[14]

Statistical analysis. All experiments had three replications for each treatment and measurement. The ANOVA and Tukey's test with a significance set at $p < 0.05$ were used to compare means of the groups using SPSS (version 13.0; SPSS Inc., Chicago, USA).

Results

Microbiological analysis. Changes in the microbial population of samples during storage are presented in Figs. 1 and 2. The initial mesophilic and psychrotrophic counts were found to be 4.10 and 4.47 log CFU g^{-1}, respectively. Population of both types of bacteria increased over the time for all groups. Mesophilic counts (Fig. 1) indicated that control and GSE coated samples passed spoilage index (7.00 log CFU g^{-1}) from day 6, whereas chitosan and CH-GSE coated samples crossed the index after day 9, indicating that chitosan coating, both in single or combination with GSE showed better inhibitory activity against this type of bacteria. Moreover, no significant differences were found between the GSE 0.10% and uncoated samples over the storage period.

A difference of less than 1.20 log cycle was demonstrated between control and CH-GSE coated samples and less than 0.50 log cycle between GSE and chitosan containing GSE coated fillets. Regarding the viable numbers of psychrotrophic microorganisms (Fig. 2), similar trends were observed for the control compared to all treatments except CH-GSE coated samples.

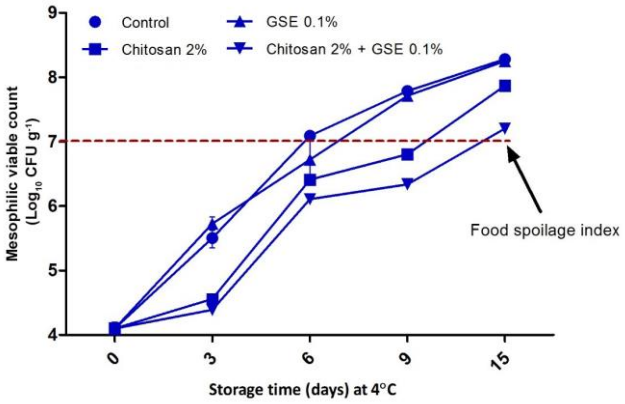

Fig. 1. Mesophilic viable counts of trout fillets during refrigerated storage. GSE: Grape seed extract.

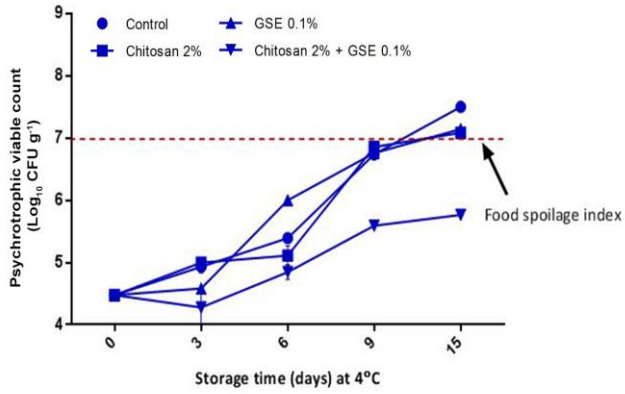

Fig. 2. Psychrotrophic viable counts of trout fillets during refrigerated storage. GSE: Grape seed extract.

The population of psychrotrophic bacteria in samples treated with GSE was gradually increased to 4.14 log CFU g^{-1} after 15 days of storage, whereas mesophilic bacteria increased to 8.20 log CFU g^{-1} in the same samples during similar storage time.

Biochemical analysis. Percentage of protein, fat, moisture and ash contents of the control samples were analyzed on the first day of study and results were 22.11 ± 0.71%, 2.10 ± 0.43%, 71.12 ± 0.22% and 4.40 ± 0.42%, respectively. The pH values of fillet samples during storage at refrigerated condition are presented in Table 1. The initial pH values of samples were between 6.05 and 6.35.

The results of PV of fish lipids are presented in Table 2. The PV of all samples increased during storage time. Significant differences in PV of all treatments were also found. The highest rate of peroxide formation was detected in control samples while CH-GSE showed the lowest rate of oxidation. The TBA values of trout fillets during refrigerated storage are given in Table 3. The initial TBA values for samples were in the range of 0.21 to 49.00 mg MDA kg^{-1}.

Sensorial analysis. The effects of chitosan and GSE coating alone and in combination on the sensorial profile of trout fillets are presented in Fig. 3. Uncoated trout samples began to show some spoilage signs including off-odor and discoloration after 9 days refrigerated storage, whereas, coating with chitosan and CH-GSE minimized the spoilage.

Discussion

Mesophilic and psychrotrophic micro-organisms are the main microflora of fish meat and they are known as potential candidates for the spoilage. Chitosan reveals unique adhesiveness properties towards biological surfaces because of its positive charge and the negative charge of biological membranes and, therefore, it reveals to form a stable films.[15] The polymer significantly suppresses the growth of various types of spoilage bacteria because of its unique potential to bind water and inhibit various enzymes and through its capability to absorb nutrients normally used by bacteria. In this study, mesophilic and psychrotrophic counts were relatively higher than the bacterial load stated in some researches for rainbow trout.[14,16]

The antimicrobial mechanism of action of phenolic compounds found in plant extract such as GSE is related to the sensitization of phospholipid membrane surrounding the core of bacteria. This phenomenon increases the permeability of membrane and leads to the leakage of intracellular compounds such as vital enzymes to outside of cell.[17]

Jasour *et al.*[14] have demonstrated that control and 1.50% chitosan coated trout samples reached the values of 6.00 to 7.00 logs CFU g^{-1} in mesophilic and psychrotrophic bacteria on the days 12 and 16 of refrigerated storage, while samples coated by chitosan incorporated by the

Table 1. The pH values of trout fillets during refrigerated storage time 0 - 15 days.

Treatments	Day 0	Day 3	Day 6	Day 9	Day 15
Control	6.35 ± 0.26^{a}	6.67 ± 0.23^{a}	6.58 ± 0.23^{a}	8.24 ± 0.36^{a}	NA
CH	6.05 ± 0.04^{bc}	6.02 ± 0.09^{c}	6.21 ± 0.22^{b}	6.98 ± 0.17^{b}	7.27 ± 0.28^{b}
GSE	6.14 ± 0.07^{b}	6.29 ± 0.15^{b}	6.58 ± 0.42^{a}	6.91 ± 0.45^{b}	7.81 ± 0.24^{a}
CH-GSE	6.11 ± 0.06^{b}	6.24 ± 0.27^{b}	6.56 ± 0.26^{a}	6.76 ± 0.30^{bc}	6.94 ± 0.19^{c}

CH: 2.00% chitosan solution, GSE: 0.10% grape seeds extract solution; GSE-CH: 2.00% chitosan solution containing 0.10% GSE NA: Fillets were not analyzed due to samples spoilage.
^{abc} Different letters for each time indicate a statistically significant differences ($p < 0.05$).

Table 2. The peroxide value (mEq per kg sample) of trout fillets during refrigerated storage time 0 - 15 days.

Treatments	Day 0	Day 3	Day 6	Day 9	Day 15
Control	0.035 ± 0.004^{a}	0.086 ± 0.005^{a}	0.159 ± 0.005^{a}	0.694 ± 0.022^{a}	NA
CH	0.033 ± 0.004^{a}	0.046 ± 0.004^{c}	0.102 ± 0.008^{c}	0.143 ± 0.006^{c}	0.197 ± 0.013^{c}
GSE	0.032 ± 0.004^{a}	0.072 ± 0.007^{ab}	0.126 ± 0.004^{b}	0.201 ± 0.008^{b}	0.221 ± 0.009^{b}
CH-GSE	0.030 ± 0.001^{a}	0.036 ± 0.006^{cd}	0.074 ± 0.004^{d}	0.093 ± 0.006^{d}	0.143 ± 0.101^{d}

CH: 2.00% chitosan solution, GSE: 0.10% grape seeds extract solution; GSE-CH: 2.00% chitosan solution containing 0.10% GSE NA: Fillets were not analyzed due to samples spoilage.
^{abcd} Different letters for each time indicate a statistically significant differences ($p < 0.05$).

Table 3. The thiobarbituric acid values (mg malondialdehyde per kg of sample) of trout fillets during refrigerated storage time 0 - 15 days.

Treatments	Day 0	Day 3	Day 6	Day 9	Day 15
Control	0.49 ± 0.10^{a}	0.91 ± 0.29^{a}	1.12 ± 0.28^{a}	1.68 ± 0.31^{a}	NA
CH	0.37 ± 0.11^{b}	0.49 ± 0.09^{b}	0.62 ± 0.16^{b}	0.91 ± 0.32^{b}	0.90 ± 0.16^{b}
GSE	0.21 ± 0.10^{cd}	0.35 ± 0.07^{c}	0.53 ± 0.16^{c}	0.85 ± 0.27^{bc}	0.93 ± 0.22^{b}
CH-GSE	0.29 ± 0.09^{c}	0.41 ± 0.07^{bc}	0.59 ± 0.03^{b}	0.85 ± 0.12^{bc}	1.13 ± 0.37^{a}

CH: 2.00% chitosan solution, GSE: 0.10% grape seed extract solution; GSE-CH: 2.00% chitosan solution containing 0.10% GSE. NA: Fillets were not analyzed due to samples spoilage.
^{abcd} Different letters for each time indicate a statistically significant differences ($p < 0.05$).

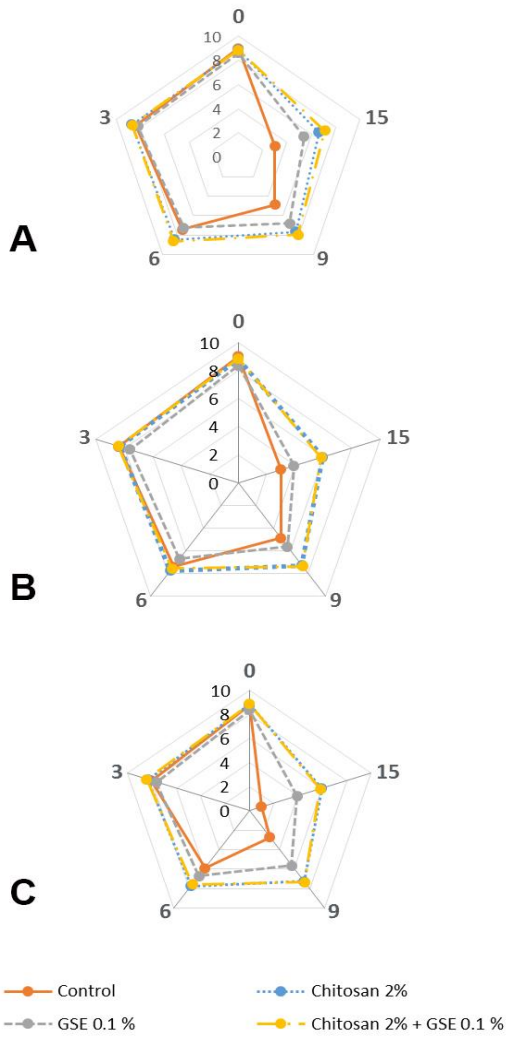

Fig. 3. The spider diagram of descriptive sensory analysis of fillets during refrigerated storage. A: Color; B: Odor and C: Overall acceptability. Treatments: Control (C), 2.00% chitosan solution (CH), 0.10% grape seed extract solution (GSE) and 2.00% chitosan solution containing 0.10% GSE (GSE-CH).

lactoperoxidase system did not reach the value at the end of storage. The significant differences ($p < 0.05$) between coated and uncoated samples and among different coated samples are related to the type and concentration of antimicrobial compounds incorporated into the polymer solution.[18] The counts were the lowest for psychrotrophic bacteria until day 15. This was probably due to the combined antimicrobial effects of chitosan and GSE on the spoilage bacteria on the meat surface.

Previously, it has been proposed that GSE is more effective on gram positive than gram-negative bacteria and some groups of spoilage bacteria including gram-positive lactic acid bacteria show a high resistance to the GSE.[6] It appeared that psychrotrophs were more sensitive than mesophilic microflora of fish fillets to 0.10% concentration of GSE.

Similar results have also demonstrated in a recent study on the trout fillets coated with carboxymethyl cellulose containing GSE.[18] The authors have reported that incorporating GSE at 0.50 and 1.00% concentrations into biopolymer solution did not significantly ($p < 0.05$) affect the bacterial load compared to control during the storage at 4 ˚C.

Different authors have reported varied proximate composition for rainbow trout.[1,14] These differences would be related to the factors such as nutrition program, sexual differences, size of fish and living area which directly influence the chemical, microbiological and sensorial characteristics of fillets obtained from fish.[18]

In agreement with previous reports,[19,20] overall pH values showed an increasing trend during two weeks of cold storage which directly correlated with accumulation of basic substances such as ammonia and trimethylamine as consequences of utilizing low molecular weight compounds such as amino acids by spoilage-related bacteria of fish muscle.[21] Hence, in a similar trend to growth kinetics of spoilage bacteria (Figs. 1 and 2), pH value of CH-GSE coated samples was increased slowly, reaching to 6.94 at the end of storage, which was significantly ($p < 0.05$) lower than others. According to Fan *et al.*, lower pH of dip coated silver carp with tea polyphenol, inhibited microbial growth and extended the shelf-life of samples.[22] Moreover, significant differences were observed between the pH values of chitosan- and GSE-coated samples during 15 days of storage.

In comparison, chitosan was more effective than GSE to retard peroxides formation. These findings are in agreement with those reported by other researchers.[16,23]

The TBA method is the most widely used test for measuring the extent of lipid oxidation in meat due to its speed and simplicity.[24] In TBA method, the concentration of MDA, a marker of oxidative rancidity in meat products, was determined. The MDA reacts with TBA to form a stable pink chromophore with maximal absorbance at 532 nm.[25]

The results of TBA are in agreement with Jasour *et al.* and Kilinc *et al.*, reported 44.00 and 43.00 mg MDA kg^{-1} in fresh rainbow trout, respectively.[15,26] The TBA values of all samples were increased with storage time; while treated samples had significantly ($p < 0.05$) lower TBA values than control ones. However, there were no significant differences between treated samples. The antioxidant activity of GSE is contributed to polyphenolic compounds including gallic acid, monomeric flavan-3-ols catechin, epicatechin, gallocatechin, epigallocatechin and epicatechin 3-*O*-gallate as well as more highly polymerized procyanidin.[27] During the storage, an increment in lipid oxidation of samples containing GSE is possibly related to development of phenolic aldehydes due to degradation of some phenolic compounds of phenolic rich agents.[6]

The scores of sensorial evaluation represented a progressive increasing in unacceptability for all control and coated samples. Similar results were found in a study on the application of chitosan or chitosan-lactoperoxidase system coating on trout fillets during storage at 7 ˚C.[15] Regarding hedonic scores for color, odor and overall acceptability, samples treated only by chitosan and in combination with GSE presented moderate to high acceptability compared to uncoated and GSE coated fillets until day 6. After six days of storage, controls started to present spoilage symptoms represented by hedonic scales lower than five. Similar findings were found in samples coated with GSE, giving this fact that GSE coating was not pleasant to sensorial panel members. On the other hand, when GSE was added into chitosan solution, significant changes in sensorial scores were reported by the members until day 9. After 15 days of storage, panelists have noted that only GSE incorporated chitosan coating represents good scores according to the hedonic scales.

At the end of the storage, all chitosan- and CH-GSE coated samples were accepted by sensory members with good scores, while the bacterial loads in the same days were above the limited level. This phenomenon may be due to the unique activity of chitosan coating in meat masking some deteriorative signs including off-flavour and off-odor developments as well as textural and sensory defects.[15] This observation agrees with the finding of Ojagh *et al*., declared a significant increase in an overall acceptability of fish fillets using a natural biopolymer based coating. [16]

In conclusion, the growth of microorganisms can cause spoilage of fresh food and shorten the overall shelf-life. Edible coating and film is a newly emerging method of chemical and microbial preservations of food commodity. Generally, chitosan coating showed antimicrobial activities against different types of spoilage bacteria of fillet with acceptable sensorial attributes until the day 15. Despite the good antioxidant activity, GSE coating had no significant activity on microbiological properties of fillets, but negatively influenced consumer overall acceptance. Incorporation of GSE into chitosan solution improved the antioxidant effectiveness of chitosan solution, but also masked the adverse sensorial effects of GSE. From a practical point of view, our results demonstrated that chitosan coating alone or incorporated with GSE increases the shelf-life of trout fillet by 3-5 days during storage at 4 ˚C.

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Conflicts of Interest

The authors declare no conflicts of interest.

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