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Effect of alginate coating enriched with 6-gingerol on the shelf life and quality changes of refrigerated red sea bream (*Pagrosomus major*) fillets

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Abstract

The study aimed to determine the shelf life and quality changes of red sea bream which is coated by using enriched sodium alginate (SA) with 6-gingerol (GR) during 20 days of refrigerated storage (4 ± 1 ℃). Fish total volatile basic nitrogen (TVB-N), thiobarbituric acid value (TBA), *K*-value and pH value, sensory evaluation, TCA-soluble peptide, texture, and microbiological analyses were measured. The results indicated that alginate coating combined with 6-gingerol (SAGR) treatment delayed lipid oxidation, protein degradation, nucleotide breakdown, and inhibited microbial growth compared with the control. The efficiency was better than that of SA or GR treatment. Sensory evaluation proved the efficacy of SAGR coating by maintaining the overall quality of red sea bream during storage. Additionally, SAGR maintained better textural characteristics. Our study suggests that the use of alginate coating enriched with 6-gingerol has the potential to maintain red sea bream quality and extend its shelf life to 20 d.

Keywords

Red sea bream; sodium alginate; 6-gingerol; shelf life; quality

1. Introduction

Red sea bream (*Pagrosomus major*) is one of the most important cultured marine fish species and widely cultured throughout the coastal areas of the Pacific and the Indian Ocean. It is well-known for its taste and healthy-eating properties. Nevertheless, raw fish are highly perishable commodities and start to deteriorate during processing and transportation. The spoilage of fish is a complicated process in which chemical, physical and microbiological changes interact, including the protein degradation (TCA-soluble peptides), ATP breakdown (*K*-value), lipid oxidation (TBA) and undesirable compounds production as the low molecular weight volatile bases (TVB-N). Activities of the fish's endogenous enzymes and chemical reactions are usually responsible for the initial loss of fish freshness, whereas the metabolic activities of microorganisms are involved in the whole spoilage.[1] In this context, it is of interest to evaluate the use of edible coatings to improve the quality and shelf life of red sea bream fillets during the storage period.

Application of edible coatings can be considered as a potential approach to preserve fish quality by keeping microbial safety and stability while assuring nutritional and sensory characteristics.[2] In the last decade, many ingredients have been used in edible coating formulations to satisfy increasing consumers' demand for natural and safe products. Polysaccharide-based coatings, owing to its low oxygen permeability, have been widely used for prolonging the shelf life of fish. These coatings allow enough gas exchange by modifying the internal atmosphere of the products to prevent an anaerobic environment, and then delay rancidity and

deterioration. Commonly, chitosan,[2] gelatin,[3] starch and derivates,[4] have been proposed for coating fish to reduce moisture loss, improve fish quality and extend storage life. Alginate is a polymer of D-mannuronic acid and L-guluronic acid, and is produced from brown algae. The ability of alginate presents advantages due to its unique colloidal properties that can form strong gels or insoluble polymers through cross-linking with divalent metal cations and create thick aqueous solutions.[5,6] Alginate is a generally recognized as safe (GRAS) substance, and has been used to enhance the antioxidant activity in sweet cherry,[7] and to keep the quality and prolong the shelf life of bream[8] and rainbow trout fillets.[9]

Further improvements could be obtained by incorporating antimicrobial compounds into the solution to provide protection against microbial contamination, thus enhancing food safety and stability. There are many varieties of antimicrobial agents such as enzymes and organic acids that have potential to be used into edible coating. Among them, natural plant extracts seem to have gained the most attention from researchers due to their strong antimicrobial activity against a broad-spectrum of microorganisms. As an alternative to chemical and synthetic preservatives, plant extracts can be used in any food, meet the demands of consumers for natural products. Ginger (*Zingiber officinale* Roscoe) is one of the commonly used spices belonging to the Zingiberaceae family and is widely used in processed food, such as chutneys, jams, pickles, beverages and bakery products, as well as in other industrial sectors. 6-Gingerol extracted from rhizome of the ginger is reported to possess various bioactive properties such as anticancer, anti-inflammation, antimicrobial, and

anti-oxidation.[10] In particular, 6-gingerol could reduce bacteria biofilm formation and virulence via quorum sensing inhibition.[11] Assessment of anti-oxidation potential of 6-gingerol has also been verified, which makes it important to apply it in pharmaceutical, agronomic, and food industries, as food preservers and additives and as natural remedies.[12]

However, to the best of our knowledge, the use of 6-gingerol as a natural antimicrobial agent, either individually or in combination with alginate, has not been studied to date, in fresh red sea bream fillets. Thus, the objective of the present study was to determine the effect of the alginate and 6-gingerol, applied individually and/or in combination, on the quality change and shelf-life of red sea bream during refrigerated storage.

2. Materials and Methods

2.1. Preparation of coating solutions

Food-grade alginate as the primary ingredient used in the edible coating formulations was purchased from Qingdao Haizhilin Biotechnology Co., Ltd. (Qingdao, China). Glycerol (Shengyue import & export trade Co., Ltd, Guangzhou, China) was added as plasticizer for polysaccharide-based edible coatings and stirred thoroughly to increase coatings strength and flexibility as well as oxygen permeability. 6-Gingerol was purchased from Chengdu PureChem-Standard Co., Ltd. (Chengdu, China). Coating solution was prepared by blending sodium alginate (SA) (2%, w/v) with distilled water and stirred on a hot plate at 70 °C for 20 min until the mixture became clear. Glycerol (1.5%, w/v) was added into the prepared alginate solution and

stirred for 8 min. Finally, 6-gingerol (GR) (0.5% w/v) was added to the alginate solution and then stirred using a magnetic stirrer for 15 min.

2.2. Sample treatment

Red sea bream varying from 550 g to 650 g in body weight were taken from a local aquatic market in Jinzhou, China. After being transferred to the laboratory, the fish were decapitated after stunned, filleted by hand. The fillets were divided into four treatment groups: (1) sodium alginate coating; (2) 6-gingerol immersion (0.5%, w/v); (3) sodium alginate coating combined with 0.5% 6-gingerol (SAGR); (4) control. The fish in control group were immersed in sterile distilled water for 5 min at 20 °C. Other fish were dipped into the above solution for 5 min at 20 °C, respectively. The ratio of fish to immersing solution was maintained as closely as possible to one part by weight of fish to four of solution. After that, samples were individually packed in air-proof polyethylene pouches and stored at 4 ± 1 °C. Fifteen replicates were included in each treatment group, and subsequently every 5 days, three replicates from each treatment group were analysed.

The experiment was carried out at the College of Food Science and Technology of Bohai University (Jinzhou, China). All procedures were approved by the Animal Care Committee of Bohai University and conducted according to the guidelines of the Liaoning Province Committee on Animal Care.

2.3. TVB-N value and TBA value

TVB-N value was determined with a Kjeltec 8400 (Foss, Sweden) using steam distillation for extraction volatile bases from fish samples.[13] Briefly, 10 g of fish flesh

from a mixture of both fillets was homogenised with 50 mL of distilled water on a Kjeldahl distillation tube. After homogenisation, 3 mL of silicone anti-foaming agent and 1 g of MgO were added. The distillate was collected into 10 mL of 0.1 M hydrochloric acid solution with an indicator solution (methyl red). The distillate was titrated with 0.0167 M sodium hydroxide solution, and the results were expressed in mg nitrogen per 100 g sample.

The TBA values of fish samples were evaluated by measuring the concentration of malonaldehyde (MDA) with some modification.[14] Samples (200 mg) were homogenized with 4.8 mL of a 5% solution of potassium chloride. To 0.5 mL of homogenate, 3 mL of 1% phosphoric acid and 1 mL of 0.6% TBA aqueous solution were added. The mixture was incubated in boiling water for 90 min followed by an ice bath for 10 min. Then 4 mL of 1-butanol was added. The tubes were shaken and the supernatant was removed after centrifugation. The absorbance (As) of the resulting pigment was recorded at 532 nm using a UV-Vis spectrophotometer (UV-2550, Shimadzu). A reagent blank was run and the absorbance (Ab) recorded. Three replicates were made for each test sample and the absorbance values were converted to the TBA value (mg MDA /kg tissue) using Eq. (1):

$$TBA = 50 \times (As - Ab)/200 \quad (1)$$

2.4. *K*-value and pH

Determination of ATP content and its related products were carried out by a reverse phase high-performance liquid chromatography method.[1] The identification of nucleotides, nucleosides, and bases was made by comparing their retention times with

145 those of commercially available standards, which were obtained from Sigma
146 Chemical Co. (St Louis, USA). The *K*-value was calculated as the ratio of the
147 percentage amounts of inosine (HxR) and hypoxanthine (Hx) to the sum of
148 adenosine-5'-triphosphate (ATP), adenosine-5'-diphosphate (ADP),
149 adenosine-5'-monophosphate (AMP), inosine-5'-monophosphate (IMP), HxR and Hx
150 as follows:

151 $$K\text{-value } (\%) = (HxR + Hx) / (ATP + ADP + AMP + IMP + HxR + Hx) \times 100 \quad (2)$$

152 The values of pH were determined by blending the fish samples (10 g) with 90
153 ml distilled water and the mixture was stirred for 30 min. After filtering, the pH
154 values of the filtrate were measured using a digital pH meter (FE20, Mettler Toledo,
155 Shanghai, China).

156 2.5. Sensory evaluation

157 The sensory attributes of fish samples were measured by a panel of 8 trained
158 assessors, aged 25-35 years (4 female and 4 male) from the teachers and students of
159 seafood group. All the treatments were evaluated every five day. The samples for
160 sensory evaluation were prepared by steaming for 30 min at 98 °C. The sensory
161 evaluation was rated on a five-point Hedonic scale to evaluate the taste (1, very poor;
162 2, poor; 3, not bad or not good; 4, good; 5, excellent) of the samples.[15]

163 2.6. Trichloroacetic acid (TCA)-soluble peptides

164 The fish flesh samples (3 g) were homogenised with 27 mL of cold 5 % (w/v)
165 TCA. The homogenate was kept in ice for 30 min and centrifuged at 10,000 g for 5
166 min at 4 °C. The soluble peptides in the supernatant were measured according to the

Lowry method[16] and expressed as μmol tyrosine/g muscle.

2.7. Texture profile analysis (TPA)

The texture properties of fish samples were evaluated at room temperature using a TA-XT plus texture analyser (Stable Micro Systems Ltd, Godalming, UK) equipped with a 5 mm diameter cylindrical probe (P/5). TPA was performed using the dorsal muscle above the lateral line of each fish (1.5 cm × 1.5 cm × 1.0 cm) which was compressed twice to 75 % of its original height. The speed of probe was 2 mm s^{-1} during penetration. The parameters (hardness, cohesiveness, adhesiveness, springiness, chewiness, gumminess, and resilience) were calculated from published definitions.[17]

2.8. Microbiological analysis

The fillet samples (25 g) were obtained aseptically and transferred to 225 mL of sterile 0.1% peptone water solution. The mixture was homogenized for 60 s using a BagMixer (Model 400, Interscience, France). For microbial count, 0.1 mL samples of serial dilutions (1:10) of flesh homogenates were spread on the plates of various agar materials. Six serial decimal dilutions were applied for microbiological evaluation of fillet samples. Mesophilic bacteria was determined on plate count agar (PCA, Aoboxing Bio-Tech, Beijing, China) by counting the number of colony-forming units after incubation at 35 °C for 48 h. Psychrophilic bacteria (PTC) was performed on PCA after incubation at 7 °C for 10 days. Pseudomonas growth was determined on cephaloridin fucidin cetrimide agar (Aoboxing Bio-Tech, Beijing, China) and incubated at 30 °C for 48 h. *Shewanella putrefaciens* were counted from the black colonies grown on iron agar (Aoboxing Bio-Tech, Beijing, China) at 20 °C for 72 h

and a representative number of colonies were confirmed by using API 20NE (Biomerieux, France). *Enterobacteriaceae* were enumerated in violet red bile glucose agar (Aoboxing Bio-Tech, Beijing, China) with a double layer at 30 °C for 24 h. Lactic acid bacteria (LAB) were enumerated on de Man Rogosa Sharpe agar (Aoboxing Bio-Tech, Beijing, China) incubated at 25 °C for 5 days under anaerobic conditions. Three replicates were made for each sample and four appropriate dilutions were used for each replicate. Microbiological data were transformed into logarithms of the number of colony forming units (CFU/g).

2.9. Statistical analysis

All experiments were based on a completely randomized design and were performed in triplicate. Data were subjected to one-way analysis of variance (ANOVA). Mean separations were assessed by Duncan's multiple range test (SAS Version 8.1). Differences at $p < 0.05$ were considered significant.

3. Results and discussion

Effect of alginate coating enriched with 6-gingerol on TVB-N

Changes in the TVB-N value of red sea bream fillets during storage are shown in Fig.1A. TVB-N values in all samples increased along with the storage time. The TVB-N value of control samples reached 26.37 mg N/100 g on day 10, while coated samples did not exceed 25 mg N/100 g on day 20. A TVB-N value of 25 mg N/100 g fish muscle is considered as an unacceptable value in fish and fish products.[18] The TVB-N values of SA and GR samples were lower than that of control samples. Meanwhile, there were significant differences in TVB-N value between SA and GR

samples throughout the storage period ($p < 0.05$). Additionally, SAGR sample had a significantly lower TVB-N value compared with SA, GR, and control samples from day 10 to day 20 ($p < 0.05$). TVB-N was produced primarily by the activity of spoilage bacteria in fish meat,[18] suggesting that the combination of SA and GR was more effective at inhibiting microbial activity than each treatment alone. Similar superior effects of edible coating combined with other bioactive substances were observed in other fishes (Rainbow trout, bream, and Japanese sea bass).[3,8,19]

Effect of alginate coating enriched with 6-gingerol on TBA value

As shown in Fig. 1B, TBA values increased as the storage period progressed in all treatments. The highest TBA values were observed in the control samples; it reached 0.75 mg MDA/kg flesh at the end of storage, followed by 0.62 mg MDA/kg flesh at the 15th day of storage, indicating that TBA value as index of lipid oxidation is a reliable parameter in quality loss of red sea bream fillet during post-mortem storage. For SA, GR, and SAGR samples, the TBA values were 0.56, 0.61, and 0.44 mg MDA/kg flesh on day 20, respectively, which suggested that the coated fillets maintained freshness during refrigerated storage. Since the increase in TBA value can be greatly favored by the presence of O_2, the incorporation of SA to coating formulations may reduce O_2 diffusion, slow down the oxidation rate, and consequently better retard quality deterioration in fish. Additionally, the SA and GR samples had lower TBA values than the control sample, but there were no significant differences in TBA values between SA and GR samples throughout the storage period ($p > 0.05$), indicating that SA and GR had the equal inhibited effects on lipid

oxidation of red sea bream fillets during storage. Some studies showed that the gas barrier properties of polysaccharides-based and protein-based coatings were crucial for extending the shelf life of seafood.[3,20,21] Other researchers reported that antioxidant activity of 6-gingerol played an important role in food preservation, either reducing free radical or decreasing lipid oxidation.[10,12] In the present study, SAGR treatment led to a significantly lower TBA value than that in other samples from day 15 to day 20 ($p < 0.05$), due to a synergistic effect of SA and GR.

Effect of alginate coating enriched with 6-gingerol on *K*-value

Variations in *K*-value during 4 °C storage are shown in Fig. 2A. Generally, the initial *K*-value was around 5% for freshly caught fish, and the *K*-values of lower than 20% are considered as "sashimi" quality, and values ranging from 20% to 60% have been considered to be within the acceptance range for most fish species, with higher than 60% as the rejection point.[22] In the present study, the initial *K*-value of red sea bream was 5.51%, indicating fish samples that could be considered very fresh, indeed of "sashimi" quality. The *K*-value of red sea bream increased fast from day 0 to day 10, this suggesting that microbial enzyme could not be crucial factor for nucleotide catabolism, and the degradation enzymes may be primarily endogenous,[23] which resulted in the rapid increase in *K*-value during the initial 10 days storage. After 10 days of storage, the *K*-values of the SA, GR, SAGR and control samples were 25.85%, 31.44%, 19.22% and 47.58%, respectively. Among them, the SAGR sample had a significant lower *K*-value than SA and GR samples ($p < 0.05$), this could be explained by the stronger synergistic effect of SA and GR treatments to minimize the activity of

255 5-nucleotidase, thus inhibiting the decomposition of inosine monophosphate. Similar
256 results were reported by Li et al.[24] who found that the chitosan coating combined with
257 tea polyphenol retarded the nucleotide degradation of large yellow croaker during
258 chilled storage and Ojagh et al.[25] who found that the chitosan coating incorporated
259 with cinnamon oil had the same effect in rainbow trout slices.

260 Effect of alginate coating enriched with 6-gingerol on pH

261 Fig. 2B shows changes in the pH value of red sea bream fillets during storage.
262 The pH values decreased in the initial period due to the decomposition of glycogen in
263 fish flesh, but some researchers attributed it to the dissolution of CO_2 in the fish
264 fillets.[20,26] The pH values of control samples increased after 5 days of storage whilst
265 treated samples experienced a slight increase during the same period, these can be
266 attributed to the production of volatile basic components, such as ammonia and the
267 formation of dimethylamine from trimethylamine oxide.[27] Similar results were
268 obtained by Chamanara et al.,[2] who reported an increase in pH in rainbow trout coated
269 using chitosan assisted with thyme essential oil stored at 5 ± 1°C. The lower levels of
270 pH value were recorded with the alginate coating fillets at the end of the storage, and
271 showed that the SA coatings provided an excellent semi-permeable film around the
272 fillets, modifying the internal atmosphere by isolating O_2. The cooperation of SA and
273 GR showed a lower pH value than SA coating alone from day 10 to day 20.

274 Effect of alginate coating enriched with 6-gingerol on sensory evaluation

275 Changes in the sensory score of red sea bream over the entire storage are shown
276 in Table 1. The results clearly showed that the prepared coatings did not produce

277 unfavorable change in taste, and concentration of SA and GR used for coating was
278 suitable. The observed shelf life of fish, as determined by panelists who indicated that
279 the fish were acceptable, was 10 days for control, 15 days for SA and GR, and 20 days
280 for SAGR. The result was in accordance with Song et al.[8] who found that the shelf life
281 of untreated bream was less than 12 days according to sensory score, and the fish with
282 alginate-calcium coating were still considered to be acceptable during the storage
283 period. The fish were rejected in SA and GR samples at the end of storage even
284 though the microbial counts did not exceed the limit of 7 log CFU/g. These indicated
285 that not only microbial load played a role in the shelf life of fish, but also other factors
286 such as microbial types, autolytic enzyme activity, physiochemical properties of fish
287 and storage conditions should be considered.[28] The SAGR samples were acceptable
288 and in marketable condition and recorded a sensory scores of 3.54 after 20 days of
289 storage. This result was in accordance with TVB-N value, TBA value, *K*-value and
290 microbial changes, suggesting that SAGR was effective in retarding red sea bream
291 sensory deterioration.

292 Effect of alginate coating enriched with 6-gingerol on TCA-soluble peptide

293 TCA-soluble peptide contents of red sea bream during 20 days of refrigerated
294 storage are shown in Fig. 3. In the initial phase of storage, TCA-soluble peptides may
295 primarily be composed of the endogenous oligopeptides and free amino acids
296 generated during post-mortem processing. On day 5, a significant increase in
297 TCA-soluble peptide content was observed for the control sample ($p < 0.05$), but only
298 slight increases were noticeable in the treated samples. The result suggested that the

control sample might contain higher activity of proteases, especially trypsin-like proteases, which resulted in an increase in muscle-derived nitrogenous degradation products, thereby favoring the proliferation of bacteria and rapid decomposition.[29] At the same storage period, TCA-soluble peptide contents of control sample were generally higher than those of samples treated with SA, GR and SAGR throughout the storage period ($p < 0.05$). This was in agreement with the higher pH of the control sample in comparison with other samples (Fig. 2B). From day 10 to day 20, the SAGR sample had a lower increase in TCA-soluble peptides than the SA and GR samples. This result suggested that SAGR treatment better inhibited protein degradation than SA or GR treatment alone, due to the strongly synergistic antioxidant activity of alginate and 6-gingerol in the inhibition of protein oxidation.[30]

Effect of alginate coating enriched with 6-gingerol on TPA

In this study, different textural properties of the red sea bream fillets were measured (Table 2). During the storage period, values of hardness, gumminess, chewiness, adhesiveness, cohesiveness, resilience and springiness changed significantly within each treatment group. Moreover, values of hardness, gumminess and chewiness in control samples decreased to 51.28%, 51.42% and 57.73% of their initial values at the end of storage. The fish death triggers autolysis and then the muscle becomes softer and less elastic, where the process can be accelerated by microbial activity.[27] The above three property values of fish treated with SA, GR and SAGR were significant higher than that of the control group ($p < 0.05$). In this study, SA and GR have the ability to slow down the loss of hardness, gumminess and

chewiness by inhibiting microbial activity. Although groups treated with SA and SAGR had much higher values of springiness from day 10 to day 20, treatment groups and the control were almost equal until the 10th day. In addition, there was no significant difference ($p > 0.05$) between treatment groups and the control in adhesiveness, cohesiveness and resilience during the storage period. Texture properties (especially for hardness, gumminess and chewiness) were correlated significantly with *K*-value, which might be affected by microbial activity,[19] so we suggested that texture properties also might be closely related with microbial activity, and can be improved by SA, GR and SAGR treatments under refrigerated condition.

Effect of alginate coating enriched with 6-gingerol on microbiological characteristics

As shown in Table 3, the samples coated with SA or GR exhibited the slower growth rates in mesophilic bacteria counts than the control samples due to the high antimicrobial activities. The control samples after 15 days of storage showed higher mesophilic bacteria counts to exceed 7 log CFU/g, the recommended acceptable limit for the fish and fish products.[31] The samples treated with SAGR possessed the significantly lower mesophilic bacteria counts than SA or GR samples from day 15 to day 20 ($p < 0.05$). PTC cause most of changes in odor and flavor as a result of production of different metabolic compounds such as aldehydes, ketones, volatile sulphides and biogenic amines.[25] The use of SA, GR and SAGR in red sea bream fillets also reduced the PTC. The counts of Pseudomonas were higher compared with those of other microbial classes, which are to some extent resistant to low temperature due to a special cell membrane structure and the presence of cold resistant compounds.

The initial population of *Shewanella putrefaciens* was 1.85 log CFU/g, and on day 20 of storage *S. putrefaciens* reached 6.43 log CFU/g in the control sample while in the presence of SA, GR and SAGR coating their counts were reduced by 5.47, 5.21 and 4.32 log CFU/g, respectively. In addition, Enterobacteria was found to grow fast in the latter stages of spoilage of red sea bream, a finding consistent with results reported for different fish species, including meager,[32] and golden gray mullet.[33] The LAB counts increased throughout the storage period, the low LAB count in this study were expected since LAB tends to grow slowly at refrigeration temperatures.

The antibacterial effect found with alginate coating solutions containing 6-gingerol was to be expected. In the present study, 6-gingerol possesses significant antibacterial effects. Treatment with SAGR was therefore more effective in reducing bacterial counts than SA and GR, and it exhibited a synergistic function with regards to inhibiting microorganism growth. The synergistic effect may be due to the SA coating, which isolates the products from environments, reducing loss of GR, rendering it more effective in inhibiting microbial growth and maintaining the keeping quality of red sea bream.

4. Conclusions

Successful inhibition of microbial growth in refrigerated red sea bream was possible with an alginate coating (2%) + 6-gingerol (0.5%) treatment, as together they kept the texture profile and overall sensory quality within acceptable limits throughout storage. This suggests that alginate coating enriched with 6-gingerol not only delayed lipid oxidation, protein degradation but also retarded nucleotide breakdown during storage,

365 and also suggests that SAGR is promising as an antioxidant, antimicrobial and gas
366 barrier coating for use in commercial applications for prolonging the storage life of
367 red sea bream.

368

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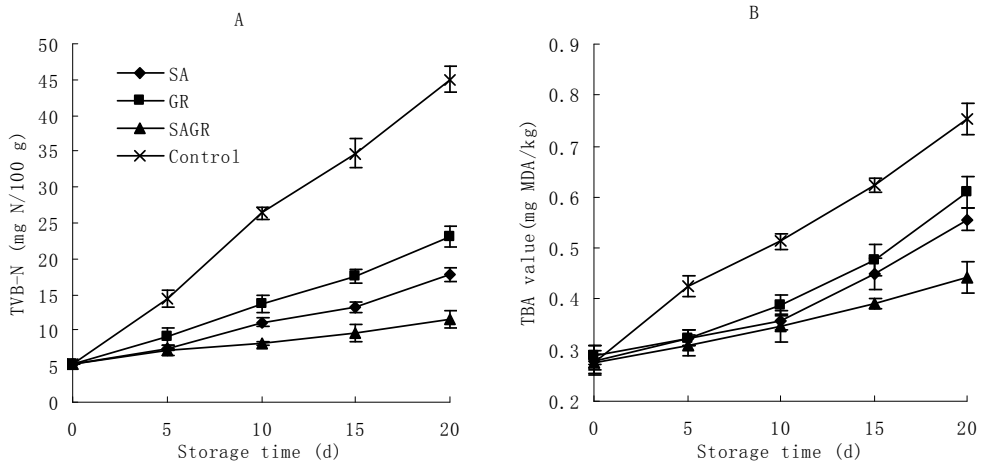

Fig. 1 Changes in TVB-N (A) and TBA (B) of red sea bream fillets treated with control (×), SA (◆), GR (■), and SAGR (▲) stored at 4 ℃ for 20 days. Each data point is the mean of three replicate samples. Vertical bars represent standard deviation of means.

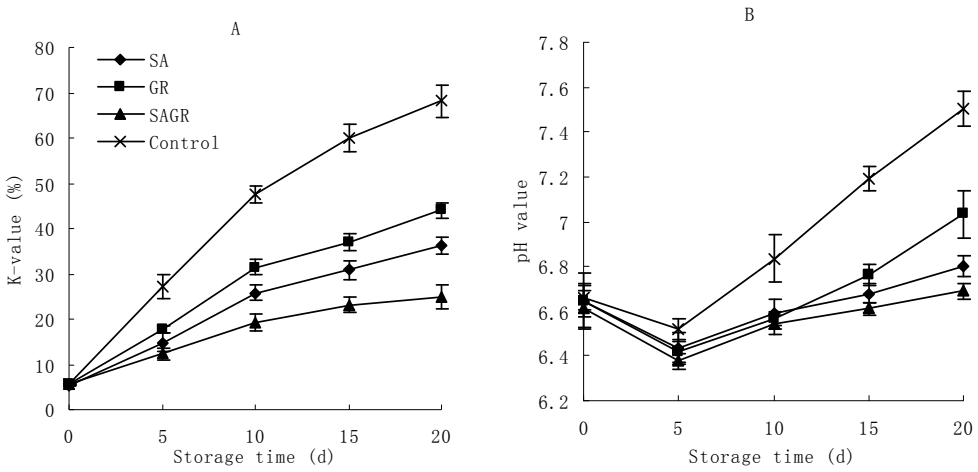

Fig. 2 Changes in *K*-value (A) and pH value (B) of red sea bream fillets treated with control (×), SA (◆), GR (■), and SAGR (▲) stored at 4 °C for 20 days. Each data point is the mean of three replicate samples. Vertical bars represent standard deviation of means.

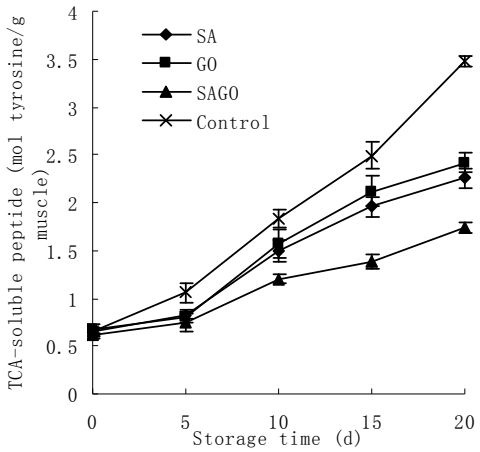

Fig. 3 Changes in TCA-soluble peptide of red sea bream fillets treated with control (×), SA (◆), GR (■), and SAGR (▲) stored at 4 ℃ for 20 days. Each data point is the mean of three replicate samples. Vertical bars represent standard deviation of means.

Table 1 Effect of alginate coating combined with 6-gingerol treatment on sensory evaluation stored at 4 °C for 20 days [a,b,c]

Days at 4 °C	0	5	10	15	20
SA	5	4.38±0.38 aA	4.08±0.52 aA	3.63±0.45 abAB	2.79±0.51 bAB
GR	5	4.17±0.31 aAB	3.67±0.51 abAB	3.17±0.56 bcB	2.46±0.44 cBC
SAGR	5	4.58±0.40 aA	4.42±0.47 abA	4.33±0.31 abA	3.54±0.51 bA
Control	5	3.50±0.45 aB	3.04±0.51 abB	2.13±0.63 bcC	1.67±0.56 cC

[a]All values were means ± standard deviation of three values.

[b]Different small letters in the same row indicate significant differences between means ($p < 0.05$).

[c]Different capital letters in the same column indicate significant differences between means ($p < 0.05$).

Table 2 Effect of alginate coating combined with 6-gingerol treatment on texture profiles stored at 4 °C for 20 days [a,b,c]

Days at 4 °C	0	5	10	15	20
Hardness (N)					
SA	105.43±1.96 aA	96.05±1.48 bB	87.47±1.27 cB	81.81±1.05 dB	74.69±1.55 eB
GR	107.54±1.56 aA	95.80±1.51 bB	86.01±1.57 cB	80.12±1.93 dB	69.92±1.61 eC
SAGR	105.94±1.79 aA	99.75±1.76 bA	92.72±1.79 cA	86.43±2.16 dA	82.88±1.57 eA
Control	105.99±1.32 aA	91.44±0.72 bC	81.65±2.19 dC	68.72±1.61 dC	51.64±1.04 eD
Chewiness (N mm)					
SA	82.74±1.32 aA	77.56±1.80 bA	71.55±1.57 cAB	64.02±1.91 dA	54.91±2.55 eB
GR	83.09±1.98 aA	75.84±0.42 bA	69.34±2.26 cB	59.47±2.24 dB	47.21±1.41 eC
SAGR	81.55±1.39 aA	78.55±1.24 bA	73.63±0.80 cA	67.08±1.96 dA	61.02±1.90 eA
Control	82.25±1.38 aA	75.96±1.80 bA	63.70±0.89 cC	53.43±2.04 dC	39.96±1.92 eD
Gumminess (N)					
SA	91.55±0.87 aA	76.09±2.27 bB	67.82±0.60 cB	56.63±0.64 dB	48.58±0.92 eB
GR	92.56±0.91 aA	78.40±0.85 bB	68.75±1.46 cB	57.88±2.39 dB	48.20±0.84 eB
SAGR	91.82±0.95 aA	84.31±1.51 bA	74.64±1.60 cA	63.17±1.52 dA	54.61±2.40 eA
Control	92.16±0.57 aA	77.72±2.19 bB	60.15±2.12 cC	47.79±1.31 dC	38.96±1.48 eC
Adhesiveness (Ns)					
SA	-15.88±1.62 bA	-13.67±1.58 bAB	-10.55±1.51 aA	-14.48±1.19 bA	-18.45±1.13 cA
GR	-15.34±0.60 bA	-12.07±1.16 aA	-11.78±0.34 aA	-15.01±0.43 bA	-17.19±1.47 cA
SAGR	-16.06±1.83 bcA	-14.29±1.94 bAB	-10.99±0.56 aA	-14.60±1.57 bA	-18.51±1.04 cA
Control	-17.04±1.08 cA	-15.45±1.22 bcB	-12.15±0.50 aA	-14.67±1.68 bA	-19.44±1.14 dA
Cohesiveness					
SA	0.35±0.03 dA	0.43±0.01 cA	0.45±0.03 abA	0.45±0.02 bcA	0.47±0.01 aA
GR	0.36±0.03 cA	0.46±0.02 abA	0.44±0.02 abA	0.47±0.02 aA	0.44±0.02 bA
SAGR	0.36±0.01 cA	0.44±0.03 abA	0.42±0.02 bA	0.46±0.02 abA	0.47±0.02 aA
Control	0.37±0.02 cA	0.44±0.01 bA	0.44±0.02 bA	0.47±0.01 aA	0.45±0.01 abA
Resilience (mm)					
SA	0.16±0.01 aA	0.15±0.02 abA	0.13±0.02 bcA	0.13±0.02 abA	0.12±0.02 cA
GR	0.17±0.01 aA	0.15±0.01 bA	0.14±0.01 bcA	0.15±0.02 abA	0.13±0.01 cA
SAGR	0.18±0.02 aA	0.15±0.02 bA	0.12±0.02 cA	0.15±0.02 bA	0.13±0.01 bcA
Control	0.18±0.01 aA	0.13±0.01 bA	0.14±0.02 bA	0.13±0.01 cA	0.14±0.01 bA
Springiness (mm)					
SA	0.83±0.02 aA	0.78±0.03 bA	0.77±0.02 bA	0.74±0.01 cA	0.70±0.02 cA
GR	0.86±0.03 aA	0.80±0.02 bA	0.70±0.03 cB	0.63±0.02 dB	0.61±0.03 dB
SAGR	0.84±0.01 aA	0.77±0.02 bA	0.76±0.02 bA	0.73±0.01 cA	0.73±0.02 cA
Control	0.84±0.01 aA	0.79±0.02 bA	0.69±0.02 cB	0.62±0.02 dB	0.59±0.02 eB

[a]All values were means ± standard deviation of three values.

[b]Different small letters in the same row indicate significant differences between means ($p < 0.05$).

[c]Different capital letters in the same column indicate significant differences between means ($p < 0.05$).

Table 3 Effect of alginate coating combined with 6-gingerol treatment on microbiological characteristics stored at 4 °C for 20 days [a,b,c]

Days at 4 °C	0	5	10	15	20
Mesophilic bacteria					
SA	1.90±0.05 eA	2.57±0.18 dB	3.63±0.19 cB	5.28±0.12 bB	6.65±0.11 aB
GR	1.95±0.09 eA	2.48±0.12 dB	3.39±0.12 cB	5.16±0.20 bB	6.40±0.17 aB
SAGR	1.98±0.08 eA	2.35±0.18 dB	3.48±0.23 cB	4.68±0.09 bC	5.66±0.16 aC
Control	1.93±0.14 eA	3.36±0.16 dA	5.42±0.19 cA	7.33±0.18 bA	8.41±0.14 aA
Psychrophilic bacteria					
SA	1.85±0.11 eA	2.95±0.21 dB	4.30±0.15 cB	5.38±0.22 bB	6.31±0.14 aB
GR	1.90±0.05 eA	2.79±0.19 dB	4.05±0.12 cB	5.02±0.16 bB	5.93±0.12 aC
SAGR	1.90±0.14 eA	2.38±0.08 dC	3.48±0.16 cC	4.34±0.15 bC	5.36±0.18 aD
Control	1.85±0.18 eA	3.69±0.06 dA	4.95±0.22 cA	6.52±0.29 bA	7.62±0.18 aA
Pseudomonads					
SA	1.60±0.07 eA	2.04±0.13 dA	2.48±0.16 cA	3.33±0.14 bA	3.98±0.15 aB
GR	1.65±0.09 eA	2.06±0.11 dA	2.46±0.27 cA	3.23±0.15 bA	3.87±0.10 aB
SAGR	1.70±0.14 eA	1.95±0.11 dA	2.45±0.07 cA	2.91±0.21 bB	3.36±0.14 aC
Control	1.70±0.13 eA	2.19±0.15 dA	2.77±0.07 cA	3.53±0.14 bA	4.77±0.22 aA
Shewanella putrefaciens					
SA	1.93±0.06 eA	2.83±0.11 dAB	3.69±0.23 cAB	4.60±0.22 bB	5.47±0.13 aB
GR	1.88±0.16 eA	2.66±0.18 dB	3.36±0.25 cB	4.37±0.13 bB	5.21±0.06 aB
SAGR	1.88±0.14 eA	2.35±0.08 dC	2.92±0.15 cC	3.58±0.15 bC	4.32±0.18 aC
Control	1.85±0.10 eA	2.94±0.11 dA	3.94±0.21 cA	5.11±0.17 bA	6.43±0.19 aA
Enterobacteria					
SA	1.74±0.07 dA	1.98±0.17 dB	2.50±0.14 cB	3.23±0.16 bB	3.83±0.12 aB
GR	1.78±0.13 dA	1.95±0.11 dB	2.47±0.21 cB	2.92±0.15 bBC	3.67±0.07 aB
SAGR	1.78±0.13 dA	1.88±0.13 dB	2.25±0.15 cB	2.79±0.19 bC	3.29±0.14 aC
Control	1.74±0.10 eA	2.36±0.20 dA	2.98±0.16 cA	3.68±0.20 bA	4.43±0.15 aA
Lactic acid bacteria					
SA	1.60±0.06 eA	2.02±0.22 dB	2.80±0.13 cAB	3.10±0.16 bB	3.67±0.17 aB
GR	1.54±0.19 dA	2.06±0.11 cB	2.68±0.18 bB	2.96±0.21 bB	3.49±0.12 aB
SAGR	1.60±0.14 cA	1.85±0.13 cB	2.33±0.09 bC	2.61±0.13 aC	2.73±0.24 aC
Control	1.54±0.03 eA	2.35±0.07 dA	3.11±0.24 cA	3.51±0.14 bA	4.06±0.10 aA

[a]All values were means ± standard deviation of three values.

[b]Different small letters in the same row indicate significant differences between means ($p < 0.05$).

[c]Different capital letters in the same column indicate significant differences between means ($p < 0.05$).