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Preservative Effects of Gelatin Active Coating Enriched with Eugenol Emulsion on Chinese Seabass (*Lateolabrax maculatus*) during Superchilling (−0.9 °C) Storage

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Abstract: This research was to evaluate the effects of gelatin (G) active coating containing eugenol/β-cyclodextrin (βCD) emulsions combined with superchilling (−0.9 °C) on physicochemical, microbiological, and organoleptic properties of Chinese seabass samples during 30 days of storage. Results showed that seabass samples dipped in G-βCD coatings containing 0.15% or 0.3% eugenol combined with superchilling could significantly lower the total volatile basic nitrogen, K value, total viable count, H_2S-producing bacteria, *Pseudomonas* spp. and Psychrophilic counts, and free fatty acids. Further, G-βCD coatings containing eugenol with superchilling (−0.9 °C) were more effective in retarding the water migration by low field NMR and MRI results, maintaining quality of seabass during storage according to organoleptic evaluation results.

Keywords: Chinese seabass; superchilling; active coating; eugenol; shelf life

1. Introduction

Chinese seabass (*Lateolabrax maculatus*) is recognized as one of the most important mariculture fish in China, which has an annual production exceeding 12 million tons [1,2]. Its high protein and low fat content make it a value-added seafood product with increasing demand [3]. However, gutted seabass is extremely susceptible to endogenous enzymes and exogenous spoilage microflora, resulting in lipid oxidation, protein degradation, or decomposition [4]. A low temperature is very important to retard bacterial growth and reduce enzyme activity to extend the shelf life of fish for some days [5]. Superchilling is the process of lowering the temperature of a product just below its initial freezing temperature and the proportion of water frozen is preserved approximately 5–30% within the food product [6–9]. Superchilling has been used in the fish processing to significantly increase the shelf life and has been successfully applied in preservation of Atlantic mackerel [10], hairtail [11], olive flounder [12], seabream [13], as well as other seafood products.

Besides the temperature control, active coating is considered to be an effective and environmentally friendly method of keeping aquatic products fresh [14,15]. Gelatin is widely used in

preparing the active coating, but the pure gelatin coating has poor antioxidant and antimicrobial abilities and does not extend the shelf life of seafood [16]. In recent years, some natural antioxidant or antibacterial compounds have been researched for their preservative activities for seafood. Eugenol (E) is a natural phenolic compound found as a major compound in clove essential oil with antioxidant and antimicrobial properties [17]. However, eugenol is associated with strong flavor altering the original food flavor and high hydrophobicity posing a great challenge to its direct incorporation into foods [18]. To minimize its adverse effects, eugenol has been encapsulated in systems suitable for food application [19,20].

Therefore, the objective of our research was to evaluate the preservative effects of gelatin active coating containing eugenol/β-cyclodextrin (βCD) emulsions on Chinese seabass during superchilling storage at −0.9 °C, in the aspects of pH, water distribution and migration, total volatile basic nitrogen (TVB-N), thiobarbituric acid (TBA) value, microbiological analysis, *K*-values, free amino acids (FAAs) analysis, and sensorial characteristics.

2. Material and Methods

2.1. Preparation of Gelatin Active Coating Containing Eugenol/βCD Emulsions

The eugenol/βCD emulsions were prepared in the same method as described by Sun et al. [21] and Shao et al. [22] with some modifications. Eugenol (0.075%, 0.15% and 0.3%) and 750 mg βCD were stirred mechanically in a beaker, respectively, and 5 g Tween 80 was added to make them homogeneously dispersed. Then 40 mL ultrapure water was added and stirred for 8 h at room temperature and more ultrapure water was added to a final emulsion volume of 100 mL. The emulsion was obtained by continuous stirring for another 6 h. The corresponding concentrations of eugenol in the eugenol/βCD emulsions were 0.075%, 0.15%, 0.3%. Gelatin (G, 6% *w*/*w*, Bloom value at 240–270, BBI Life Science, Shanghai, China) and glycerol (1.5% *v*/*w*) were dissolved in prepared eugenol/βCD emulsions (100 mL) at 45 °C and stirred for 2 h. Then, the mixture was treated with an ultrasonic homogenizer (XEB-1000-P, Xiecheng Ultrasonic Equipment co. LTD, Dongguan, China) at 20 KHz with the powder of 800 W for 10 min to obtain homogeneous coating solutions. The final active coating solutions were marked as G-βCD, G-βCD-0.075%E, G-βCD-0.15%E, and G-βCD-0.3%E, respectively.

2.2. Preparation of Seabass and Immersion Sample Treatment

A total of 72 live Chinese seabass (*Lateolabrax maculatus*) with an average weight of 800 ± 10 g were supplied by a local market in Luchao Port town (Shanghai, China). They were stunned with ice for 15 min then killed and the gill and viscera of seabass were removed. Then they were thoroughly washed with sterilized 1% NaCl solutions and 2 random seabass samples were taken to determine the basic quality profiles at initial sampling point (day 0). The remaining seabass samples were divided into 5 batches (14 fish per batch) for (1) CK; (2) G-βCD; (3) G-βCD-0.075%E; (4) G-βCD-0.15%E; and (5) G-βCD-0.3%E. Different batches of seabass samples were immersed in the corresponding freshly prepared coating solutions for 10 min at 4 °C with a solution ratio of 1:3 (*w*/*v*), then the seabass samples were taken out and put into a sterile biochemical incubator with air flow at 4 °C for 60 min to form the coating. After that, each seabass sample was individually packed in a sterile polyethylene bag and stored at −0.9 ± 0.1 °C in a refrigerator (BPS-250CB, Yiheng Thermostatic Chamber, Shanghai, China). Fish samples were selected randomly for analysis at 0, 5, 10, 18, 18, 21, 24, and 27 days.

2.3. pH Measurement

The pH measurement determination were determined according to Kim et al. [23]. A precise amount of minced seabass muscle (5 g) homogenized with 45 mL distilled water, then filtered after 30 min. The filtrate pH was measured by pH meter.

2.4. Water Distribution and Migration

The proton relaxation experiments were performed proposed by Li et al. [24]. The fish samples from the seabass muscle were cut into small squares (2.5 × 2 × 1.3 cm^3, about 5 g) and sealed with polyethylene films. Transverse relaxation T_2 measurements were executed on a LF-NMR analyzer (Niumag MesoMR23-060H.I, Suzhou, China) with a proton resonance frequency of 20 MHz. For each measurement 16 scans were performed with 3000 echoes, the relative content of three water components was obtained from the iterative inversion with analytical software of T_2 transverse relaxation time. Acquisition parameters were showed as followed: slice width = 3 mm, TR (time repetition) = 2000 and TE (time echo) = 15 ms. MRI experiments were performed to get proton density weighted images and the echo time, repetition time and slice width were 18.2 ms, 850 ms, and 2 mm, respectively.

2.5. Total Volatile Basic Nitrogen (TVB-N)

For TVB-N determinations, the distillation method of a deproteinized sample as recommended by Neira et al. [25] was used. A precise amount of minced seabass muscle (5 g) was placed into a distilling flask and blended with 1.5 g of MgO. Then steam distillation was performed with Kjeldahl nitrogen-determination apparatus (Kjeltec8400, Foss, Hillerød, Denmark) and TVB-N was expressed as mg N/100 g of seabass sample.

2.6. Evaluation of TBA Value

Lipid oxidation in seabass samples was monitored by the evaluation of thiobarbituric acid reactive substances (TBA-RS) by the method portrayed by Cheng et al. [26] with some modifications. Then, 5 g of seabass muscle was homogenized in 25 mL of 20% TBA solution, centrifuged at 8000 g for 10 min at 4 °C after stand still for 1 h and the supernatants filtered with Whatman No. 3 qualitative filter paper. The filtrate was diluted with ultrapure water to 50 mL. After that, 10 mL diluent and 10 mL TBA solution was mixed and heated at 100 °C for 15 min and then cooled to 30 °C. The absorbance of the supernatant was measured at 532 nm using a spectrophotometer (Evolution 220, Thermo Fisher Scientific, Waltham, MA, USA) and the TBA-RS values were expressed as mg of malonaldehyde (MDA)/100 g of seabass sample.

2.7. K-Values

The ATP-related compounds were determined by a RP-HPLC procedure (Waters 2695, Milford, MA, USA) proposed by Li et al. [27] The minced seabass muscle (5 g) homogenized with 10 mL 10% perchloric acid (PCA) and centrifuged at 8000 g for 15 min at 4 °C. The precipitate was stirred with 10 mL 5% PCA and centrifuged at 8000 g for 10 min at 4 °C for 2 times. The supernatant pH was adjusted to 6.5 after the supernatant was merged and added with 15 mL distilled water. After 30 min, take the supernatant fixed in 50 mL volumetric bottle with ultrapure water. Finally, the supernatant was filtered with 0.22 μm membrane and applied to RP-HPLC procedure. The *K*-value was calculated as the ratio of the percentage amounts of inosine (HxR) and hypoxanthine (Hx) to the sum of adenosine-5′-triphosphate (ATP), adenosine-5′-diphosphate (ADP), adenosine-5′-monophosphate (AMP), inosine-5′-monophosphate (IMP), HxR and Hx was calculated as follows:

$$K\ \text{value}\ (\%) = \frac{\text{HxR} + \text{Hx}}{\text{ATP} + \text{ADP} + \text{AMP} + \text{IMP} + \text{HxR} + \text{Hx}} \times 100 \quad (1)$$

2.8. Free Amino Acid (FAA) Analysis

FAAs were determined according to Abdelhedi et al. [28] The minced samples (2.0 g) were homogenized with 10 mL of 5% trichloroacetic acid and centrifuged at 8000 g for 15 min at 4 °C. The supernatant was centrifuged again under the same conditions, then the combined supernatant was

diluted to 25 mL. The 1 mL extract was filtered with a 0.22 μm disposable filter for reserve, and automatic amino acid analysis was used. The diluted solution was filtered using a 0.22 μm filter membrane and applied to Hitachi L-8800 amino acid analyzer (Tokyo, Japan).

2.9. Microbiological Analysis

Representative 5 g portions of seabass samples were blended with 45 mL of sterilized normal saline (0.85% NaCl) fully homogenized and then subjected to serial dilutions. The following microbiological analyses were performed: (i) determination of the mesophile bacteria on plate count agar medium (Hopebio, Qingdao, China) with 0.4 mg/mL nystatin (Aladdin, Shanghai, China) were incubated at 30 °C for 48 h; (ii) determination of H_2S-producing bacteria on iron agar medium (Hopebio, Qingdao, China) were incubated at 30 °C for 72 h; (iii) determination of *Pseudomonas* spp. on cetrimide agar medium (Hopebio, Qingdao, China) were incubated at 30 °C for 72 h; and (iv) determination of psychrophilic bacteria on plate count agar medium were incubated at 4 °C for 7 days.

2.10. Organoleptic Properties

The organoleptic properties of seabass samples were assessed with the method described by Cai et al. [4]. Nine experienced judges received some training about the superchilling storage seabass samples, focusing on odor, color, mucus, tissue morphology, and elasticity using ten-grade marking system: 10.0–9.0 (excellent), 8.9–7.0 (good), 6.9–5.0 (fair), and 4.9–1.0 (rejectable).

2.11. Statistical Analysis

The one-way ANOVA procedure followed by Duncan test was used for multiple comparisons by the SPSS 22.0, and the results were expressed as means ±SD.

3. Results and Discussions

3.1. Microbiological Results

Figure 1 shows the counts corresponding to the growth of TVC, H_2S-producing bacteria, *Pseudomonas* spp., and psychrophile of seabass samples during superchilling storage. The initial mesophiles population was 2.30 log CFU/g (Figure 1a), which was at a low microbial level for the starting seabass samples. The mesophile number increased during storage for all seabass samples and the G-βCD-3.00E group had significantly lower mesophile number than other samples during whole storage period. At day 21, CK and G-βCD should be removed due to exceeding the allowed maximum limit of 7.0 log CFU/g [29].

Eugenol could prolong the shelf life of seabass samples because of its remarkable inhibitory effect. Dimitrijević et al. [30] also demonstrated that eugenol showed good antimicrobial activity on retarding TVC growth, similar results also shown towards the increase of H_2S-producing bacteria, *Pseudomonas* spp., and psychrophile in the current research. H_2S-producing bacteria is one of the specific spoilage organisms in seabass samples during cold storage [31]. An initial count of H_2S-producing bacteria in seabass samples was approximately 2.3 log CFU/g (Figure 1b). At the end of storage, samples treated with eugenol presented lower counts. *Pseudomonas* spp. had a similar growth pattern with that of mesophilic microbes (Figure 1c), which suggests that the aerobic spoilage bacteria dominated for seabass samples during supperchilling storage. Psychrotrophic bacteria could lead to seabass samples' quality deterioration in odor, texture, and flavor through the production of metabolic compounds including ketones, aldehydes, volatile sulfides, and biogenic amines [32]. At the end of storage, the total psychrophilic bacteria counts for CK, G-βCD, G-βCD-0.075%E, G-βCD-0.15%E, and G-βCD-0.3%E were 8.01, 7.66, 6.95, 6.77, and 6.51 log CFU/g (Figure 1d), respectively, indicating that the seabass samples quality was retained by eugenol addition.

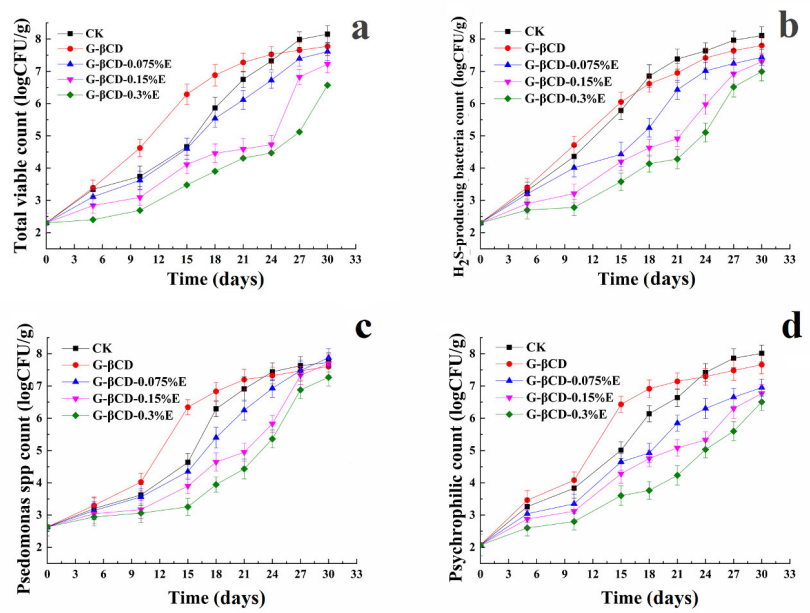

Figure 1. Changes in total viable count (**a**), H_2S-producing bacteria (**b**), *Pseudomonas* spp. (**c**), and Psychrophilic counts (**d**) of Chinese seabass during superchilling storage at −0.9 °C. (CK: uncoated; G-βCD: gelatin-β-cyclodextrin coating without eugenol; G-βCD-0.075%E: gelatin-β-cyclodextrin coating with 0.075% eugenol; G-βCD-0.15%E: gelatin-β-cyclodextrin coating with 0.15% eugenol; G-βCD-0.3%E: gelatin-β-cyclodextrin coating with 0.3% eugenol).

3.2. Chemical Results in Seabass Samples

A tendency for initially decreasing and then increasing pH values could be observed in all samples (Figure 2a). The pH reduction at the beginning was caused by the accumulation of lactic acid during glycolysis reactions and the increased pH values due to the production of volatile basic components as a result of bacterial propagation [33]. CK showed significantly ($p < 0.05$) higher average pH values when compared with the seabass samples dipped in G-βCD coatings containing eugenol during storage. Furthermore, smaller pH changes was observed in G-βCD-0.3%E samples and it could be concluded that G-βCD-0.3%E combined with superchilling could be have a positive synergistic effects on the inhibition of bacterial spoilage.

The TVB-N value of fresh seabass sample was 8.65 mg N/100 g fish muscle (Figure 2b) indicating satisfactory freshness of seabass samples at the beginning. TVB-N values slowly increased at the beginning and had a sharp increase from day 21. The increase TVB-N formation was especially obvious in CK and G-βCD seabass samples, however, seabass samples with G-βCD coatings containing eugenol significantly reduced the formation of TVB-N during storage comparing with the CK and G-βCD samples. The TVB-N values of CK, G-βCD, G-βCD-0.075%E, G-βCD-0.15%E, and G-βCD-0.3%E seabass samples reached to 55.41, 47.29, 35.49, 30.69, and 27.66 mg N/100 g fish muscle, respectively, at the end of storage, which signified only G-βCD-0.3%E samples did not exceed the upper limit (30 mg N/100 g) throughout superchilling storage [34]. High TVB-N values in aquatic products preservation indicates that nitrogen-containing substances accumulated from the nitrogen-containing molecules, such as protein and nucleic acid, degraded by proteolytic bacteria [35].

Changes in IMP, Hx, and *K* value of seabass samples during superchilling storage are presented in Figure 2c–e. IMP offers sweet and meaty flavor enhancing fish quality, however, it could be conversed to Hx representing for unpleasant bitterness in aquatic products [36]. The concentration of IMP at day 0 was 34.75 mg/100 g and decreased in all samples during superchilling (Figure 2c). At the end of storage, the concentrations of IMP in the CK, G-βCD, G-βCD-0.075%E, G-βCD-0.15%E, and G-βCD-0.3%E seabass samples were 0.17, 1.25, 2, 2.94, and 4.27 mg/100 g, respectively. The IMP concentrations in seabass samples dipped in G-βCD coatings containing eugenol were significantly

($p < 0.05$) higher than that of CK and G-βCD samples during superchilling storage, which demonstrated that G-βCD coatings containing eugenol could inhibit bacteria IMP degradation resulting from spoilage organism and microbial enzymes. The initial Hx concentration was 1.36 mg/100 g and increased irregularly in all samples during superchilling (Figure 2d). The CK and G-βCD seabass samples showed significantly higher ($p < 0.05$) Hx concentration than that of seabass samples dipped in G-βCD coatings containing eugenol, which demonstrated that eugenol addition could be efffective in controlling the microbial growth and inhibiting the formation of Hx [37].

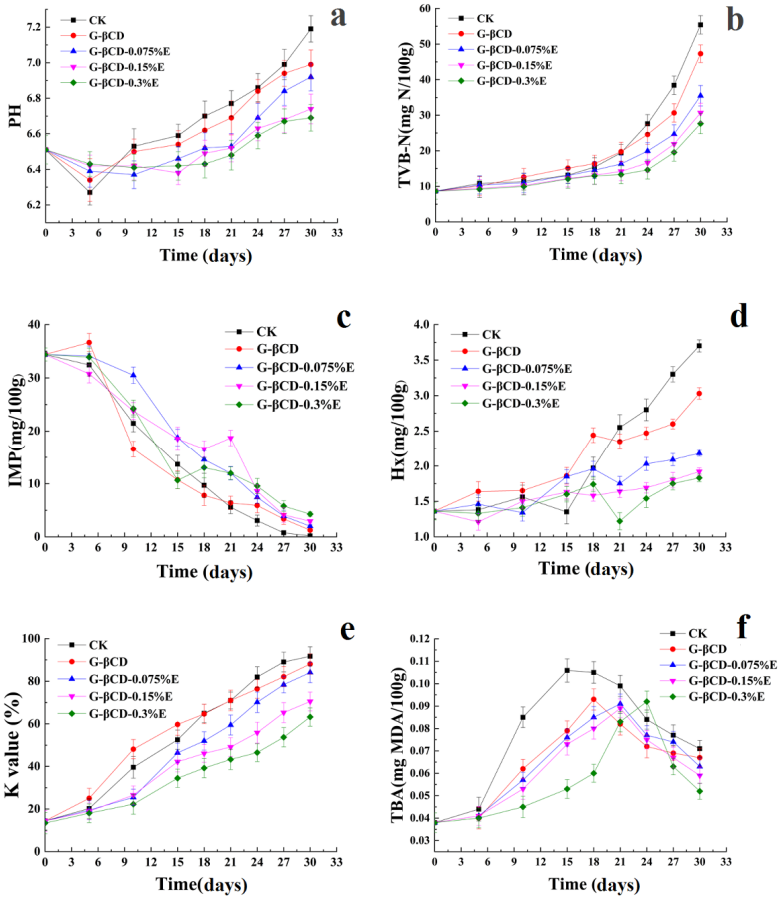

Figure 2. Changes in pH values (**a**), total volatile basic nitrogen values (TVB-N, **b**), inosine 5′-monophosphate (IMP, **c**), hypoxanthine (Hx, **d**), *K* value (**e**), and Thiobarbituric acid (TBA, **f**) of Chinese seabass during superchilling storage at −0.9 °C. (CK: uncoated; G-βCD: gelatin-β-cyclodextrin coating without eugenol; G-βCD-0.075%E: gelatin-β-cyclodextrin coating with 0.075% eugenol; G-βCD-0.15%E: gelatin-β-cyclodextrin coating with 0.15% eugenol; G-βCD-0.3%E: gelatin-β-cyclodextrin coating with 0.3% eugenol).

The *K* value of fresh seabass samples was 14.39% and increased during superchilling storage (Figure 2e). CK and G-βCD seabass samples exceeded the maximum permissible level on day 15. The unacceptable limit for K value is 60% [38] and the CK and G-βCD seabass samples exceeded the maximum permissible level on day 15, however, the seabass samples dipped in G-βCD coatings containing eugenol remained below the rejection limit on day 21. These results indicated that G-βCD coatings containing eugenol combined with superchilling could suppress the degradation of ATP and keep good quality of seabass samples during superchilling storage.

TBARS could indicate the peroxidation of polyunsaturated fatty acids in fish and be measured as MDA equivalent [39]. The TBARS value in fresh seabass sample was 0.038 mg MDA/100 g and then increased from day 0 to day 15. Afterwards, the TBARS values decreased slightly due to MDA

reacting with aldehydes and ketones [40]. TBARS occurred in rather different rates with increasing storage time, as expected, the CK seabass samples had the highest TBARS values, and TBARS could be significantly affected ($p < 0.05$) by eugenol concentration in the active coating solutions. The TBARS values for G-βCD-0.075%E, G-βCD-0.15%E, and G-βCD-0.3%E were 0.091, 0.089, and 0.092 mg MDA/100 g, respectively, which were lower than that of CK and G-βCD seabass samples and there were no significant differences among the treated samples. Therefore, eugenol as the antioxidant in the active coatings and coatings barrier against oxygen penetration resulting in delayed lipid oxidation [41–43].

The concentrations of FAAs in seabass samples on the 1st, 15th, and 30th day of superchilling storage are shown in Table 1. From Table 1, the alanine and aspartic acid concentrations in all samples sharply increased and then decreased during superchilling storage. The major amino acids in seabass samples were glycine, alanine, and histidine, which accounted for 42.86%–65.26% of total FAAs contents. As an off-taste amino acid, histidine accounted for 8.70%–12.66% of total FAA contents among all seabass samples. The content of histidine increased from 17.81 mg/100 g to 45.64 mg/100 g on day 30 in the CK samples, however, the corresponding contents in G-βCD-0.075%E, G-βCD-0.15%E, and G-βCD-0.3%E were only 36.64, 33.88, and 33.47 mg/100 g on day 30, respectively, which was caused by the oxidation process from trimethylamine oxide and led to a reduction in the bitterness for seabass samples during superchilling storage [44]. Glycine, alanine, glutamic acid, and aspartic acid are responsible for the characteristic flavor of fish [45]. Glycine contents in CK samples increased from 55.63 mg/100 g to 75.80 mg/100 g on day 15 and then decreased to 68.56 mg/100 g on day 30. Glycine contents in G-βCD-0.075%E, G-βCD-0.15%E, and G-βCD-0.3%E had similar trends comparing with CK samples, however, their final contents were significantly ($p < 0.05$) higher than that of CK and G-βCD. In addition, seabass samples dipped in G-βCD coatings containing eugenol significantly increased alanine content from day 0 to day 15 and the CK, G-βCD, and G-βCD-0.15%E decreased from day 15 to day 30, however, the alanine content in G-βCD-0.15%E and G-βCD-0.3%E samples increased at that time. In general, seabass samples with G-βCD coatings containing eugenol could accumulate the partial umami-associated FAAs and total FAAs and reduce off-tasting histidine, which improved flavor quality of seabass samples during superchilling storage. Active coating effectively controlled water loss and microbial metabolism to keep excellent quality parameters of seabass samples.

3.3. Water Distribution by LF NMR Analysis

T_{21} representing for bound water varied, ranging from 0.15% to 3.30% during superchilling storage (Figure 3a), indicating the bound water in seabass samples could be free from the influence of treated methods and storage time [46]. T_{22} known as immobile water within the myofibril and decreased gradually during superchilling storage ($p < 0.05$). T_{23} known as free water increased constantly. In the current research, the CK seabass samples had lower immobilized water than that of other seabass samples. Some researchers also demonstrated that water located within myofibrillar macromolecules could release or translate to free water on account of destruction of muscle fiber during storage [25,47–49]. At the same time, G-βCD coatings containing eugenol retarded the change rates of T_{22} and T_{23} to hinder water migration, especially for G-βCD-0.15%E and G-βCD-0.3%E samples.

MRI provides visual information for seabass samples during superchilling storage. Red color stands for high proton density and blue color stands for low proton density in the pesudo-color images. As shown in Figure 3b, the brightness of images varied obscure in the early storage and the brightness of samples were darker and bluer with the time increasing. On day 30, the color of CK seabass samples was bluer and darker than others, demonstrating that the degradation of myofibill and destruction of microstructure in CK seabass samples [50]. The brightness of seabass samples dipped in G-βCD coatings containing eugenol is lighter compared to CK and G-βCD samples on day 30, which indicated that the G-βCD coatings containing eugenol are more suitable for quality maintenance of seabass samples. The result was consistent with the variation of LF-NMR transverse relaxation.

Table 1. Changes in FAAs content (mg/100 g) in different treated Chinese seabass samples during superchilling storage.

Time	Groups	FAAs								
		Asp	**Thr**	**Ser**	**Glu**	**Gly**	**Ala**	**Val**	**Met**	–
Day 0	–	2.36 ± 0.36	5.47 ± 0.55	7.28 ± 0.48	8.06 ± 0.43	55.63 ± 0.59	34.20 ± 0.32	4.21 ± 0.25	2.63 ± 0.17	–
Day 15	CK	9.82 ± 0.26a	8.86 ± 0.56c	9.50 ± 0.34c	12.03 ± 0.53b	75.80 ± 0.47d	47.11 ± 0.47c	6.16 ± 0.22c	3.40 ± 0.20b	–
–	G-βCD	9.68 ± 0.22a	12.22 ± 0.71a	13.94 ± 0.38a	18.87 ± 0.95a	82.56 ± 1.13c	59.61 ± 1.43a	10.24 ± 0.16a	6.16 ± 0.26a	–
–	G-βCD-0.075%E	8.90 ± 0.41b	8.09 ± 0.65d	10.56 ± 0.44b	9.28 ± 0.49c	84.28 ± 0.64c	47.78 ± 0.75c	6.05 ± 0.19c	3.04 ± 0.16c	–
–	G-βCD-0.15%E	8.49 ± 0.19c	9.10 ± 0.53c	9.15 ± 0.37c	7.37 ± 0.38d	96.87 ± 0.68b	48.97 ± 0.83c	7.43 ± 0.14b	3.66 ± 0.43b	–
	G-βCD-0.3%E	7.75 ± 0.28d	9.64 ± 0.42b	13.86 ± 0.27a	7.13 ± 0.33d	104.98 ± 0.49a	52.24 ± 0.28b	7.00 ± 0.27bc	3.45 ± 0.32b	–
Day 30	CK	2.64 ± 0.14a	8.69 ± 0.43c	10.17 ± 0.52c	14.07 ± 0.47bc	68.56 ± 0.63c	41.42 ± 0.43d	7.88 ± 0.57d	4.55 ± 0.32c	–
–	G-βCD	2.54 ± 0.75a	17.28 ± 0.58a	12.96 ± 0.59a	27.50 ± 0.48a	63.37 ± 0.67d	58.44 ± 52a	19.93 ± 0.73a	11.25 ± 0.48a	–
–	G-βCD-0.075%E	2.36 ± 0.35a	10.47 ± 0.35b	12.72 ± 0.0.93ab	15.26 ± 0.45b	81.34 ± 0.76bc	54.75 ± 0.48bc	10.06 ± 0.63b	5.78 ± 0.37b	–
–	G-βCD-0.15%0E	2.39 ± 0.18a	11.06 ± 0.84b	11.83 ± 0.62ab	12.30 ± 0.44d	82.22 ± 0.68b	47.49 ± 0.42cd	9.07 ± 0.59	5.51 ± 0.31b	–
–	G-βCD-0.3%E	2.27 ± 0.32a	10.19 ± 0.41bc	11.69 ± 0.47b	13.37 ± 0.46c	86.06 ± 0.62a	52.85 ± 0.48b	10.25 ± 0.68b	5.16 ± 0.35bc	–
–	–	**Ile**	**Leu**	**Tyr**	**Phe**	**Lys**	**His**	**Arg**	**Pro**	**Total**
Day 0	–	2.60 ± 0.18	3.83 ± 0.29	1.20 ± 0.21	2.82 ± 0.28	10.34 ± 0.34	17.81 ± 0.46	2.76 ± 0.22	3.37 ± 0.27	164.62 ± 4.37
Day 15	CK	3.81 ± 0.24c	5.98 ± 0.32c	2.13 ± 0.18b	3.76 ± 0.23b	9.59 ± 0.38c	27.28 ± 0.77a	3.80 ± 0.25d	3.76 ± 0.28c	232.81 ± 4.76c
–	G-βCD	6.73 ± 0.75a	11.64 ± 0.43a	6.10 ± 0.36a	7.33 ± 0.46a	22.18 ± 0.44a	26.94 ± 0.32a	8.80 ± 0.63a	7.28 ± 0.36a	310.31 ± 5.84a
–	G-βCD-0.075%E	3.63 ± 0.29c	5.57 ± 0.28c	1.94 ± 0.17b	3.70 ± 0.26b	18.24 ± 0.48b	26.42 ± 0.53a	4.62 ± 0.18c	5.29 ± 0.23b	247.45 ± 4.28c
–	G-βCD-0.15%E	5.00 ± 0.88b	7.66 ± 0.25b	2.60 ± 0.23b	4.21 ± 0.31b	18.79 ± 0.37b	21.50 ± 0.38b	5.81 ± 0.35b	5.10 ± 0.34b	261.75 ± 4.98b
–	G-βCD-0.3%E	4.53 ± 0.27b	6.97 ± 0.31b	2.30 ± 0.25b	4.11 ± 0.27b	22.37 ± 0.36a	19.52 ± 0.53c	6.01 ± 0.23b	7.29 ± 0.26a	279.20 ± 5.22b
Day 30	CK	4.35 ± 0.87d	7.81 ± 0.34d	4.79 ± 0.69b	5.22 ± 0.48c	22.81 ± 0.74c	45.64 ± 0.83a	6.40 ± 0.63b	4.69 ± 0.74c	259.69 ± 4.28c
–	G-βCD	12.00 ± 1.32a	22.40 ± 0.75a	14.71 ± 1.06a	12.72 ± 0.74a	32.77 ± 0.97a	35.62 ± 0.59b	12.56 ± 01.22a	11.49 ± 0.63a	367.57 ± 5.74a
–	G-βCD-0.075%E	5.90 ± 0.59bc	9.64 ± 0.44c	4.74 ± 0.64b	6.52 ± 0.48b	20.43 ± 0.56d	36.64 ± 0.48b	5.95 ± 0.49b	6.78 ± 0.72b	289.36 ± 4.92b
–	G-βCD-0.15%E	5.28 ± 0.99c	9.21 ± 0.63bc	4.86 ± 0.65b	5.75 ± 0.39bc	26.42 ± 0.64b	33.88 ± 1.30b	5.82 ± 0.69b	6.38 ± 0.83b	279.51 ± 4.73bc
–	G-βCD-0.3%E	6.46 ± 0.76b	11.07 ± 0.48b	5.16 ± 1.59b	6.12 ± 0.57b	27.08 ± 0.73b	33.47 ± 0.88b	6.17 ± 0.53b	6.23 ± 0.77b	293.62 ± 5.07b

Different larger case letters in same group from different day indicate a significant difference ($p < 0.05$).

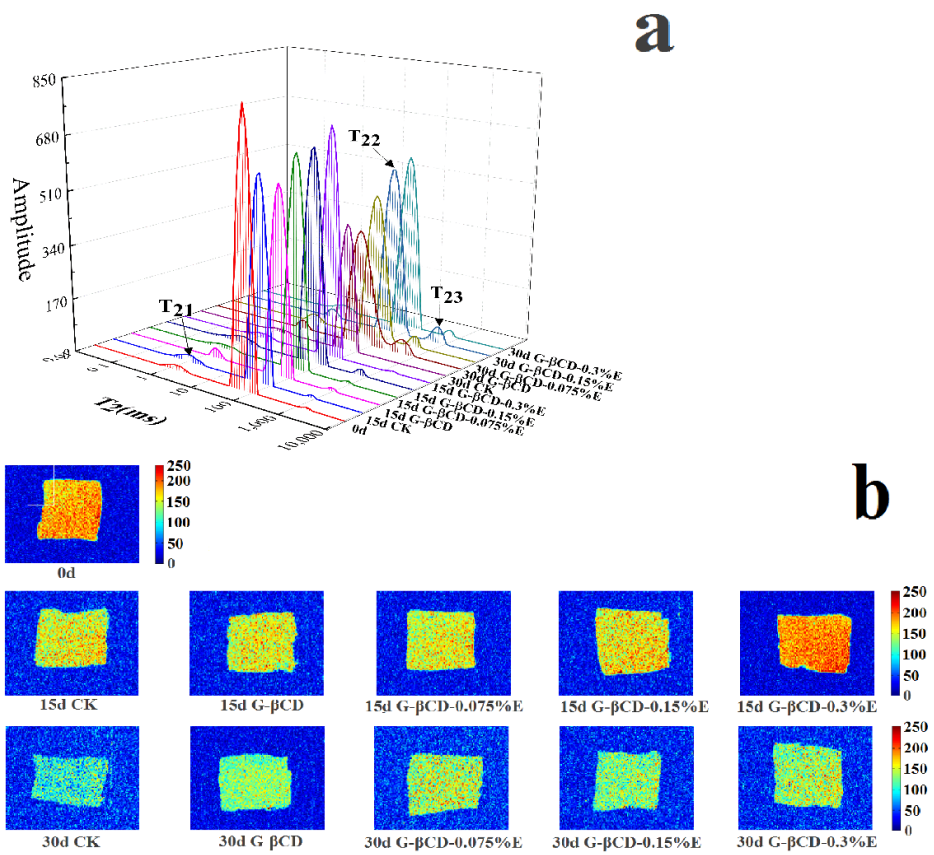

Figure 3. Changes in water distribution (**a**) and magnetic resonance imaging (**b**) of Chinese seabass during superchilling storage at −0.9 °C. (CK: uncoated; G-βCD: gelatin-β-cyclodextrin coating without eugenol; G-βCD-0.075%E: gelatin-β-cyclodextrin coating with 0.075% eugenol; G-βCD-0.15%E: gelatin-β-cyclodextrin coating with 0.15% eugenol; G-βCD-0.3%E: gelatin-β-cyclodextrin coating with 0.3% eugenol).

3.4. Organoleptic Properties

The acceptability of seabass samples during superchilling storage depends upon their changes in organoleptic characteristics. Figure 4a–e display the organoleptic evaluation results for the t seabass samples including smell, color, mucus, muscular tissue and elasticity during supperchilling storage. At the beginning, all seabass samples had high organoleptic scores and they were of excellent quality, and then a significant quality loss in all seabass samples was observed ($p < 0.05$) during superchilling storage. However, seabass samples dipped in G-βCD coatings containing eugenol had significant higher organoleptic scores than those of the CK and G-βCD samples ($p < 0.05$). The CK and G-βCD seabass samples were considered unacceptable by the panelists on day 21 and day 24, respectively, when the samples were spoiled with off-odor and loose elasticity. No significant difference ($p > 0.05$) was detected among G-βCD-0.075%E, G-βCD-0.15%E, and G-βCD-0.3%E, and G-βCD-0.075%E on day 24, G-βCD-0.15%E, and G-βCD-0.3%E both on day 24 were not suitable for consumption suggested by panelists. The best organoleptic evaluations were reported in G-βCD-0.15%E and G-βCD-0.3%E, which were also supported by microbial and chemical quality analyses.

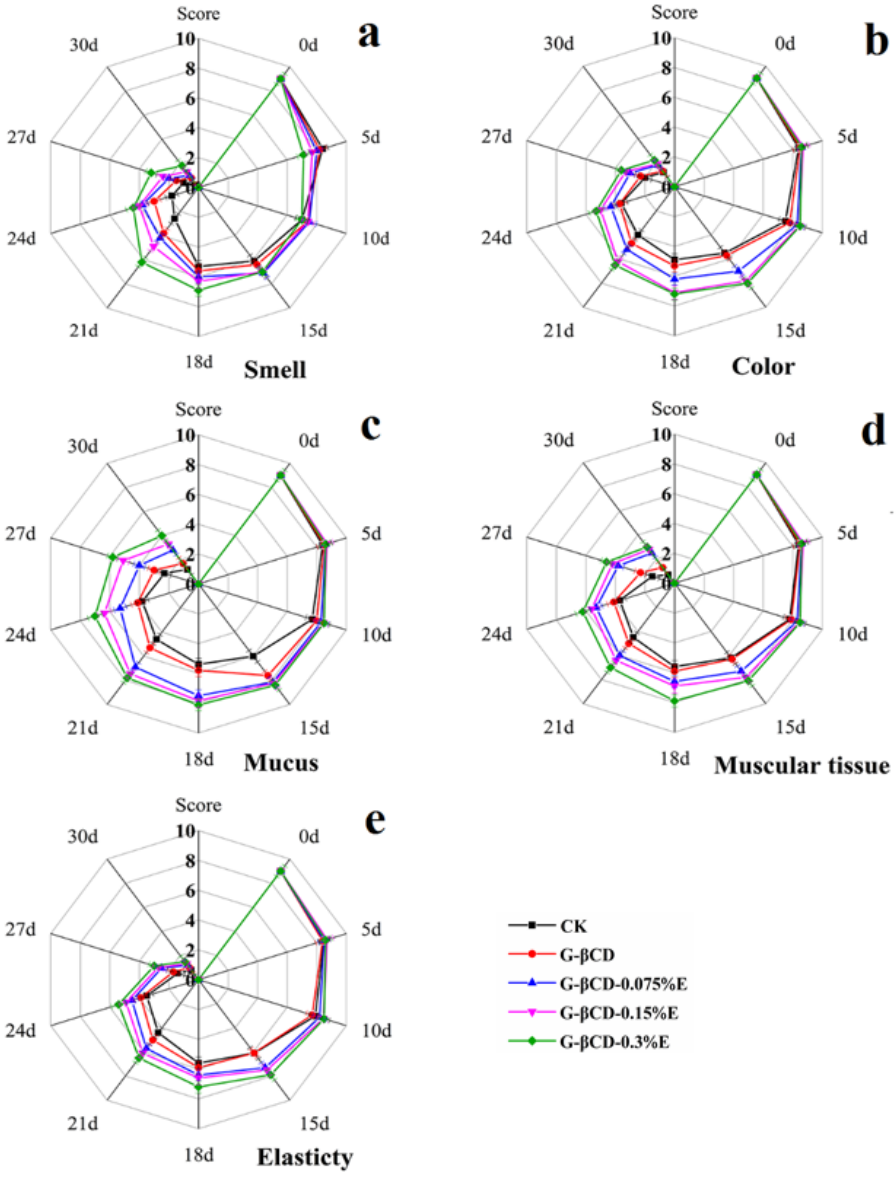

Figure 4. Changes in organoleptic properties results (smell: **a**; color: **b**; mucus: **c**; muscular tissue: **d**; elasticty: **e**) of Chinese seabass during superchilling storage at −0.9 °C. (CK: uncoated; G-βCD: gelatin-β-cyclodextrin coating without eugenol; G-βCD-0.0%75E: gelatin-β-cyclodextrin coating with 0.075%eugenol; G-βCD-0.15%E: gelatin-β-cyclodextrin coating with 0.15% μL mL^{-1} eugenol; G-βCD-0.3%E: gelatin-β-cyclodextrin coating with 0.3% eugenol).

4. Conclusion

Seabass samples dipped in G-βCD coatings containing 0.075%, 0.15%, and 0.3% eugenol combined with superchilling (−0.9 °C) could slow down the rate of seabass samples spoilage during storage. The results of physicochemical, microbiological and organoleptic properties indicated that the G-βCD-0.15%E and G-βCD-0.3%E seabass samples maintained better physico-chemical properties organoleptic evaluation results during superchilling storage, which mainly due to that eugenol could effectively suppress the growth of spoilage microorganisms. G-βCD-0.15%E and G-βCD-0.3%E had similar effects in slowing down seabass samples spoilage, however, 0.3% eugenol addition gave the active coating solution a strong flavor. Therefore, 0.15% eugenol addition combined with superchilling (−0.9 °C) could be suitable for maintaining the freshness of seabass samples and extended the shelf life.

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