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Low-Field NMR and MRI to Analyze the Effect of Edible Coating Incorporated with MAP on Qualities of Half-Smooth Tongue Sole (Cynoglossus Semilaevis Günther) Fillets during Refrigerated Storage

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Featured Application: The monitoring of quality characteristics of Half-smooth tongue sole (HTS, Cynoglossus semilaevis Günther) is essential during refrigerated period based on the edible coating incorporated with MAP treatment, which has not displayed in the previous literatures. Meanwhile, the low-field nuclear magnetic resonance (LF-NMR) and magnetic resonance imaging (MRI) technology has a potential development to assess the quality of aquatic products, which is applied to assess the water distribution and migration of HTS fillets during 24-day storage. This study demonstrate that results of LF-NMR and MRI have the close connection with the physicochemical parameter of HTS fillets and can be applied to monitor water migration in a noninvasive and fast way.

Abstract: Nondestructive and fast measurement and characterization of fish is highly desired during various processing treatments. This research investigated the effectiveness of low field LF-NMR and MRI as fast monitoring techniques to estimate the qualities of half-smooth tongue sole fillets treated with edible coating combined with modified atmosphere packaging during refrigeration. T_2 relaxation spectra showed three peaks representing bound water (T_{21}), immobile water (T_{22}), and free water (T_{23}), respectively. pT_{22} accounted for the largest proportion of three types of water, followed by pT_{23}. The weighted MRI provided the internal structure information associated with different samples, indicting the combination of edible coating and MAP (70% CO_2 + 30% N_2) is the best performance in the maintenance of qualities and freshness of HTS fillets. All results demonstrated that the combination of LF-NMR and MRI as fast and nondestructive methods have great potential to monitor qualities deterioration and predict shelf life in of HTS fillets during refrigerated storage.

Keywords: half-smooth tongue sole; LF-NMR; edible coating; modified atmosphere packaging

1. Introduction

Fish fillets is a highly perishable product due to its high water activity and protein content, neutral pH, and presence of autolytic enzymes, resulting in deterioration of organoleptic properties and nutritional values, and eventually losing edibility [1]. Edible coating as an effective fresh-keeping method has been considered in aquatic products [2–4]. Rosmarinic acid (RA) is a natural polyphenol

present in Lamiaceae herbs like *Perilla frutescens* with antioxidant, anti-inflammatory, and antimicrobial effect and has beneficial applications in improving shelf-life and imparting a desirable flavor to foodstuffs. ε-Polylysine (ε-PL) as a natural antimicrobial polypeptide is recognized as safe preservative in foods [5]. Our previous studies have shown edible coating containing 30 mg/kg RA incorporated with 0.1% ε-PL could effectively maintain the quality of HTS fillets during refrigeration. In addition, modified atmosphere packaging (MAP) is the process of filling with specific proportions of CO_2, N_2, and O_2 around a food product and then sealing that product in an impermeable package. Many published works indicated that MAP could be very proficient in extending the shelf-life of perishable foods especially fish products [6–8].

Different nondestructive analysis methods have been widely adopted to characterize the quality changes of raw fillets, such as near-infrared spectroscopy, X-ray computer tomography, hyperspectral imaging, frontface fluorescence spectroscopy, and so on [9]. However, low-field NMR (LF-NMR) and magnetic resonance imaging (MRI) techniques have more advantages in decreasing cost, saving time, and reducing damage to samples compared with the above instruments [4,10,11]. In addition, LF-NMR and MRI are able to assess the quality changes by providing the distribution as well as structural information of protons from water and lipid [12–14].

Half-smooth tongue sole (HTS, *Cynoglossus semilaevis* Günther) is rich in high-quality protein and polyunsaturated free fatty acids, and is emerging as one of the most valuable marine aquatic species in Northern China [12]. With the development of electronic commerce, the convenient and fast consumption mode is pursued by residents, and fresh fish fillets will become a main marketing form of fish products [1]. However, HTS decompose easily by environmental factors due to nutrient-rich character, and eventually lose edibility. Therefore, the principal objectives of the present study were to evaluate the quality effects of composite edible coating incorporated with MAP on HTS fillets during cold storage, and then investigated the effectiveness of low field LF-NMR and MRI as fast monitoring techniques to estimate the qualities of half-smooth tongue sole fillets during refrigerated storage (4 ± 0.5 °C).

2. Material and Methods

2.1. Preparation of Edible Coating Solution

Bioactive edible coating solutions were prepared containing RA (Aladdin Biochemistry Technology Co., Ltd., Shanghai, China) and ε-PL (Sinopharm Chemical Reagent Co., Ltd., Shanghai, China). Certain amounts of RA and PL were separately dissolved in distilled water to prepare RA solution (60 mg/kg) and ε-PL solution (2%, *w*/*v*). Equal volumes of RA solution and ε-PL solution were mixed and homogenized with magnetic stirring (Shanghai Siro Instruments Co., Ltd., Shanghai, China) to dissolve completely. The final concentration of RA was 30 mg/kg, and the final concentration of ε-PL was 0.1% (*w*/*v*).

2.2. Immersion Treatment of Fillets

Fresh HTS (weight: 0.75 ± 0.05 kg; length: 40 ± 2 cm) were purchased from the wholesale aquatic market of Luchao Port (Shanghai, China) and immediately transported to the laboratory with ice within 30 min. The fresh HTS had excellent physical properties and the head, bone, and skin were removed. Fillets with $6 \times 4 \times 1$ cm, weighing approximately 40 g were cut and prepared. Fillets were randomly separated into five treatments: the first one without any treatment was used as control (CK1) and other four groups were immersed in freshly prepared bioactive edible coating solution (ratio of coating solution to fish samples, 2:1, *v*/*w*) for 30 s, then removed and allowed to drain at 4 °C around 30 min in order to form the coatings on the surface of fillets. All the samples were packed in polyethylene bags and coated groups submitted to the following modified atmosphere packaging (MAP) treatment.

2.3. MAP Treatment of Fillets

Coated samples were treated with four packaging conditions: air packaging (CK2) and modified atmosphere treatment (60% CO_2/40% N_2: C1; 65% CO_2/35% N_2: C2; 70% CO_2/30% N_2: C3) using a thermos-sealing equipment (DQB-360W, Qingba Food Packaging Machinery Co., Ltd., Shanghai, China) with a gas-product ratio 2:1. Extraction time was 15 s, aeration time was 5 s, electrothermal sealing temperature was 140 °C, gas mixing accuracy < 2%, power gas source was 0.714 MPA, mixed fresh gas pressure was 0.408 MPa, and fresh gas source pressure was 0.51 MPa. All samples were stored at 4 °C for subsequent assessments at 4-day intervals.

2.4. LF-NMR Analysis

Analysis was performed according to the method proposed by Huang et al. [11] with following modifications. Portions of 3 × 3 × 1 cm (about 5 g) were cut from the dorsal part of fillets and sealed with polyethylene films. The samples were placed in NMR tubes (70 mm diameter). Transverse relaxation T_2 measurements were recorded on a LF NMR analyzer (MesoMR23-060H.I, Niumag Corporation, Suzhou, China) with a proton resonance frequency of 20 MHz, corresponding to the pulse sequence of Carr–Purcell–Meiboom–Gill (CPMG) and the primary parameters were as follows. SW (the receiver bandwidth frequency) = 100 kHz, RFD (the parameter to control the first data point that acquired) = 0.08, the number of the scans NS = 4, P1 = 18 μs, P2 = 36 μs, RG1(analog gain) = 20 db, DRG1 (digital gain) = 6 db, PRG (preamplifier gain) = 0, delay DL1 = 0.2 ms, and TW (the duration between successive scans) = 2000 ms. Three pieces of fillet samples were measured for every sample group.

2.5. MRI Analysis

MRI experiments of all samples were also implemented by the LF-NMR analyzer (MesoMR23-060H.I, Niumag Electronic Technology Co., Ltd., Shanghai, China) to obtain proton density weighted images. Acquisition parameters were showed as follows. Slice width = 3 mm, TR (time repetition) = 2000, and TE (time echo) = 15 ms. Three images were taken for each sample and three pieces of fillet samples were measured for each group.

2.6. Water Content

The water content were determined according to the Chinese standard (GB 5009.3-2016). Specifically, 2–10 g sample was precisely weighted and dried at 105 °C to constant weight. The percentage of water content was calculated according to the weight before drying and after drying. Three replicates were made from every group and mean value was considered as the final result.

2.7. Fat Content

The fat content were determined according to the Chinese standard (GB 5009.6-2016). Briefly, 2.0 g of drying sample was dripped in 40 mL anhydrous ether and refluxed at 65 °C for 8 h and constantly solvent was removed at 65 °C for 3 h in the vacuum environment. The percentage of fat content was determined according to the weight before and after extraction. Three replicates were carried out of every group and the mean value was considered as the final result.

2.8. TVB-N Value

The total volatile basic nitrogen (TVB-N) values were determined according to the Chinese standard (GB 5009.228-2016). The TVB-N values of fish samples were evaluated using an automatic Kjeldahl nitrogen-determination instrument (Kjeltec8400, Foss, Denmark) and were expressed in mg N/100g. Three replicates were carried out of every group and the mean value was considered as the final result.

2.9. K Value

The determinations of nucleotides and related compounds were carried out referred to the method as proposed by Su et al. [15], using a RP-HPLC procedure (Waters 2695, Milford, CT, USA). The K value was calculated as follows:

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

2.10. Ca^{2+}-ATPase Activity

The extraction of actomyosin was performed as described by Sun et al. [14], with slight modifications. Briefly, fillet samples of 1 g were mixed and homogenized with 10 mL precooled KCl solution (0.6 mol/L, pH 7.0) and then the homogenate was centrifuged at 10,000 r/min for 20 min in 4 °C, then the supernatant was added to three fold volume cooled distilled water and centrifuged at 10,000 r/min for 20 min. The supernatant was discarded and precipitate was added to the same volume precooled KCl solution (1.2 mol/L, pH 7.0) to dissolve completely the actomyosin. The solution was centrifuged at 8000 r/min for 20 min to remove the insoluble protein. The supernatant was actomyosin and Ca^{2+}-ATPase activity was measured using Ca^{2+}-ATPase kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Three replicates were carried out of every group and the mean value was considered as the final result.

2.11. Malondialdehyde (MDA) Value

The MDA value was determined by MDA kit according to the instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China). Three replicates were carried out of every group and the mean value was considered as the final result.

2.12. Sensory Scores

The organoleptic properties of HTS fillets samples were evaluated and a panel of ten trained members (five woman and five men, aged 20–30 years) from the laboratory staff participated. Panelists were asked to evaluate the color, odor, tissue morphology, elasticity and mucus using a five-point scale: 5 corresponded to 'most liked' and 1 to 'most disliked'. Shelf life criteria assumed that rejection would occur when the sensory attributes declined below 5.0.

2.13. Statistical Analysis

Distributed multiexponential fitting of CPMG and IR decay curves was performed in MultiExp Inv analysis software (Niumag Electric Corporation, Shanghai, China). Experimental data were analyzed using SPSS software (version 19.0, Statistical Package for the Social Sciences Inc., Chicago, IL, USA). The one-way ANOVA procedure followed by Duncan's multiple range tests was adopted to determine the significant difference ($p < 0.05$) between treatment means, and the results were expressed as means $\pm$ SD.

3. Results and Discussion

3.1. Quality Attributes

The changes of sensory and physicochemical qualities of HTS fillets were evaluated during storage. Organoleptic properties play great role in consumer acceptance and marketability of food products. Fresh fish is among the most perishable foods due to lipid content, microbiological changes, and external factors [15]. Thereby maintaining its organoleptic characteristics becomes very important. As showed in Figure 1a, sensory scores decreased significantly with storage time in CK1, however, C2 and C3 had relatively slower reduction. This fact indicted that bioactive edible coating combined with MAP had promising effects on the maintenance of sensory attributes.

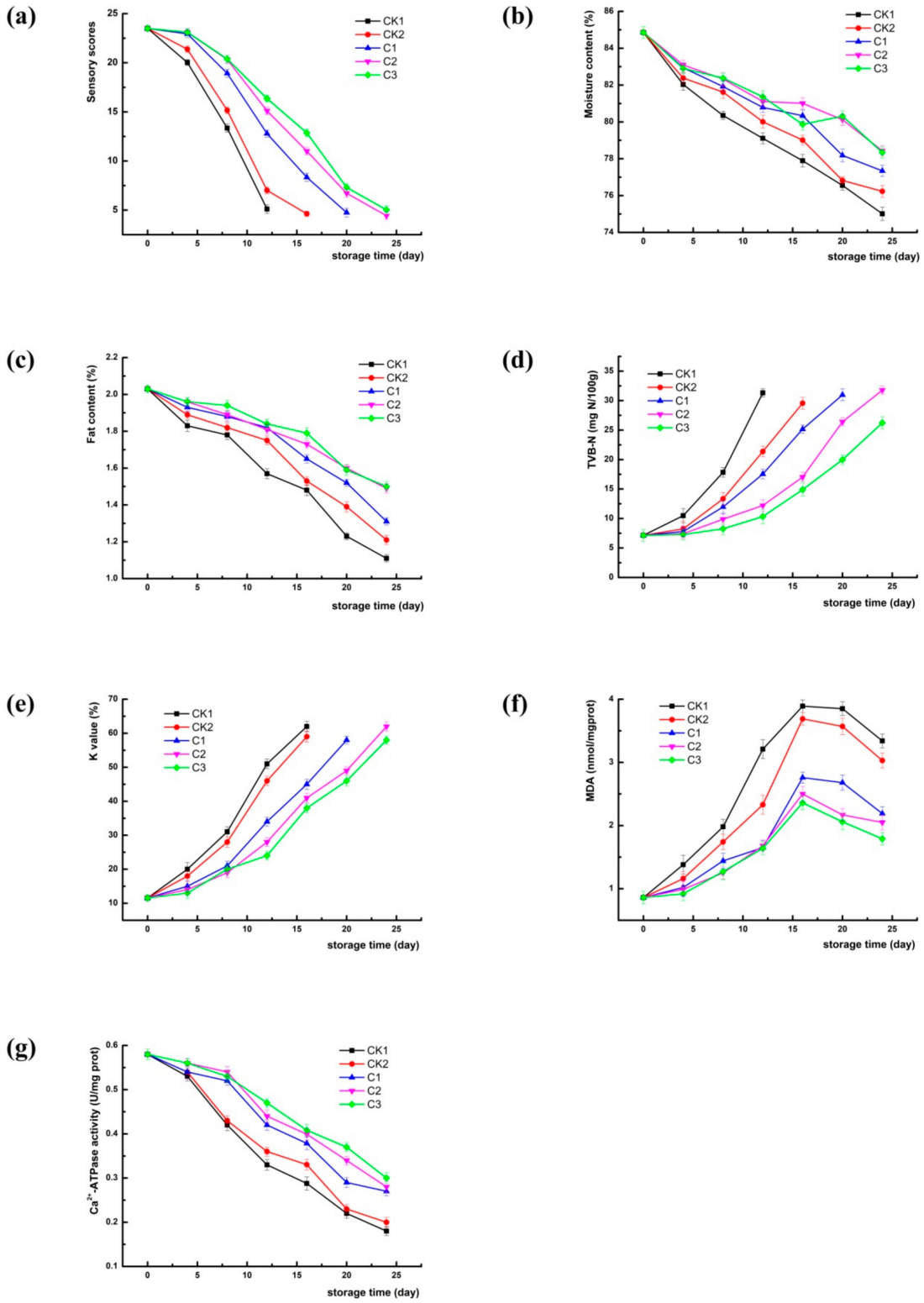

Figure 1. Changes in sensory scores (**a**), moisture content (**b**), fat content (**c**), and TVB-N value (**d**), K value (**e**), MDA (**f**) and activity of Ca_2+-ATPase (**g**) of samples during refrigerated storage (CK1: distilled water + air packing; CK2: edible coating + air packing; C1: edible coating + MAP (60% CO_2/40% N_2); C2: edible coating + MAP (65% CO_2/35% N_2); C3: edible coating + MAP (70% CO_2/30% N_2)).

Figure 1b suggested that there was a gradual moisture content reduction in all samples during 24-day storage. Then the decrease rate of edible coating treated group combined with MAP was slower than that of CK1 and CK2. That may be the MAP provides an effective inbuilt water vapor barrier and prevents loss by evaporation [7]. The fat content with different treatments showed a similar trend with moisture content during storage (Figure 1c). Interestingly, there were no obvious differences between C2 and C3 during storage, indicating that 65% CO_2/35% N_2 or 70% CO_2/30% N_2 were suitable treatments of MAP combined with edible coating applied refrigerated HTS fillets.

TVB-N value is usually considered as an important index to assess the quality and shelf life of aquatic products [16]. As shown in Figure 1d, the initial at day 0 was 7.14 mg/100g and there was an increase in all the samples during storage. The TVB-N value in CK1 reached an unaccepted level (31.32 mg/100 g) on the 12th day and the samples with MAP incorporated with bioactive edible coating had lower TVB-N values compared with CK1 and CK2, especially 70% CO_2/30% N_2 of MAP. The dominant reason was that the high concentration of CO_2 could retard bacterial action in the conversion of trimethylamine oxide (TMAO) into trimethylamine (TMA), which was one of the main substrates for the production of volatile bases [17].

The K value is defined as the ratio of inosine (HxR) plus hypoxanthine (Hx) to the total adenosine triphosphate (ATP) and related compounds (ADP, AMP, IMP, HxR, and Hx) in fish muscle extract, reflecting the degree of nucleotide degradation [18]. As shown in Figure 1e, the initial K value of HTS fillets was 11.56%. K values increased progressively ($p < 0.05$) over time for different treated samples because of the degradation of ATP to derivative compounds [1]. Generally, a K value of 60% fish muscle was taken as the highest acceptable level; as proposed by the European Commission [19]. Briefly, the K value of CK1 reached 62% at day 12, while the CK2, C1, and C2 group exceeded this limitation at day 16, 20, and 24, respectively. The fact suggested that a high concentration of CO_2 in the MAP environment could effectively retard the increased rate of K value and extend the shelf life of HTS fillets. Reports showed that the transformation process from IMP to Hx can be affected by spoilage bacteria [20]. Therefore, a high concentration of CO_2 can suppress ATP breakdown via retarding the growth of microbial metabolism.

The amount of malonaldehyde (MDA) is commonly used to measure the level of rancidity and is mainly related to the accumulation of secondary products via liquid oxidation [20]. In the present study, the MDA value of CK1 increased gradually from 0.86 nmol/mgprot at day 0 to 3.89 nmol/mgprot at day 16 (Figure 1f). However, the samples treated with edible coating combined with MAP had lower values at the corresponding time, especially the gas composition of 65% CO_2/35% N_2 or 70% CO_2/30% N_2. Then the MDA value of all samples had a decrease at the end of storage as a consequence of the interaction between malonaldehyde and protein degradation products [21]. In general, the combined effect of edible coating and MAP (65% CO_2/35% N_2 or 70% CO_2/30% N_2) has positive effects on maintaining quality characteristics of HTS fillets.

The activity of Ca^{2+}-ATPase is broadly used to assess the actomyosin integrity, reflecting the degradation of myofibrillar protein [14]. In the current study, a decreasing trend was detected no matter what kind of treatment was carried out during refrigerated storage (Figure 1g), which was concordance with the results with Pavap [22]. In comparison with the initial value, the activity of Ca^{2+}-ATPase of CK1 group was diminished by 68.97% at the end of storage, while other samples showed more excellent influence on reduction of the activity of Ca^{2+}-ATPase, especially the C3 group. This results may be related with conformation and aggregation of myosin globular head [23].

3.2. Measurements of LF-NMR

LF-NMR undoubtedly provided excellent prospects in predicting of quality characteristics and shelf life of aquatic products. Previous researches demonstrated that there was a close relationship between transverse relaxation T_2 and traditional physicochemical parameters of the quality of muscle products [9]. Figure 2 showed the change trends of CPMG relaxation signal intensity at the beginning, middle, as well as the last stage of storage with different treatments. As mentioned in Figure 2, there

was an obvious decreasing intensity in the curves with the storage time regardless of treated methods and no significant changes was not observed beyond 1000 ms of relaxation time. Meanwhile, the decreased rate of echoes of CK1 was higher than that of other treatments, especially samples treated with edible coating incorporated with 70% CO_2/30% N_2 MAP. But Zang et al. reported that the curves of signal intensity could rarely provide a descending trend of signal amplitude and reflect information about the status and distribution of different types of water [9]. In fact, the information obtained from CPMG echoes as raw date was limited, and further analysis needs to be carried out to fully explain the diversification of the three types of water.

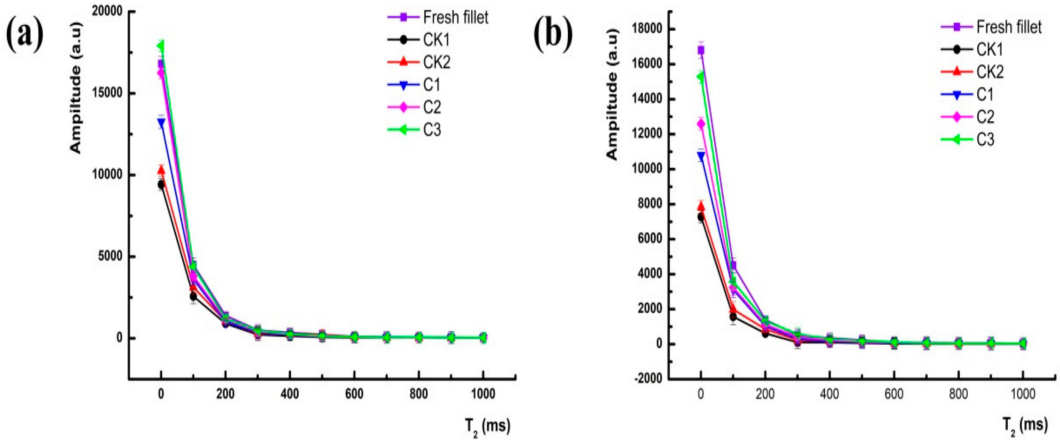

Figure 2. Changes in CPMG echoes of samples under different treatments on the 12th (**a**) and 24th day of the refrigerated period (CK1: distilled water + air packing; CK2: edible coating + air packing; C1: edible coating + MAP (60% CO_2/40% N_2); C2: edible coating + MAP (65% CO_2/35% N_2); C3: edible coating + MAP (70% CO_2/30% N_2)).

The multiexponential fitting of transverse relaxation T_2 distribution was carried out by Origin software and the protons associated with three types of water were identified. These results were consistent with some researches [14,16]. As shown in Table 1, the shortest relaxation time T_{21} in the range of 1.32–6.14 ms was detected, representing the bound water entrapped within tertiary and quaternary protein structures. Besides, the intermediate population of T_{22} with a relaxation time of 28.48–117.38 ms considered as immobile water within the myofibril. Finally the third population, T_{23} in the range of 403.7–3274.55 ms corresponds to free water extramyofibrillar space [24]. Briefly, no significant differences were detected in T_{21} time among different treated samples in the current experiment, indicating transverse time T_{21} was not affected by treated methods as well as storage time. Wang previously pointed out that T_{22} diminished while T_{23} increase with time in muscle [16]. But the conclusions disagreed with the results in the present study. Briefly, T_{22} time of all samples fluctuated without regular trends; while T_{23} firstly declined steadily and increased constantly. This fact was also indicated that the changes of free water were more obvious than those of bound and immobile water with the extension of storage time. Interestingly, the range of relaxation time for the three types of water was slightly different among all samples during storage, which may related to the migration of moisture during storage. This prediction is further verified by the changes trends of peak area proportion of certain type of water. Furthermore, the pT_{21}, pT_{22}, and pT_{23} were corresponded to the areas of relaxation time T_{21}, T_{22}, and T_{23}. Obviously, pT_{21} almost rarely changed during the entire storage period, ranging from 1.74 to 1.96. This behavior occurred due to the viewpoint that the water entrapped within highly organized myofibril structures and water in tertiary and quaternary protein structures were stable [25]. The pT_{22} diminished progressively while pT_{23} increased during storage, and pT_{22} accounted for the largest proportion of three types of water, followed by pT_{23}. In the present study, The CK1 had significant lower immobilized water (from an initial value of 87.63% to 69.29%)

than that of other samples. No significant difference was observed of the amounts of the immobilized water in C3 ($p < 0.05$), probably owing to the conclusion that the combination effects of edible effects and MAP (gas composition of 70% CO_2/30% N_2) enormously decreased the rate of diffusion of the immobilized water into free water and maintained excellent quality characteristics of the HTS fillets. Besides, this synergetic effect was well reflected in the fact that pT_{23} increased obviously during storage period. Furthermore, the samples treated with bioactive edible coating combined with MAP had lower content of free water than the control and edible coating group alone. Above all, we could conclude that the synergetic effects of the edible coating combined with MAP could effectively retard water located within the myofibrillar macromolecules to release or translate to free water based on the destruction of the muscle fiber and keep excellent quality attributes of the HTS fillets [14].

Table 1. Changes of transverse relaxation time and relative content of three water component in HTS fillets with different treatments during the refrigerated period.

Storage Time (Day)	T_{21} (ms)	T_{22} (ms)	T_{23} (ms)	pT_{21}	pT_{22}	pT_{23}
0d	0.079 ± 0.002	75.65 ± 2.79	1232.84 ± 5.4	1.96 ± 0.13	87.63 ± 3.26	10.41 ± 0.56
12d						
CK1	0.15 ± 0.02	79.38 ± 1.84	932.60 ± 3.82	1.78 ± 0.21	77.83 ± 2.18	20.39 ± 0.93
CK2	0.064 ± 0.003	65.79 ± 3.27	717.13 ± 4.92	1.74 ± 0.09	79.19 ± 2.84	19.12 ± 1.13
C1	0.166 ± 0.046	75.83 ± 2.64	705.48 ± 4.04	1.84 ± 0.34	81.23 ± 3.16	16.93 ± 0.92
C2	0.194 ± 0.032	69.79 ± 2.18	533.67 ± 1.84	1.83 ± 0.22	84.98 ± 2.21	13.19 ± 1.26
C3	0.141 ± 0.027	73.93 ± 2.35	642.84 ± 3.15	1.74 ± 0.13	85.13 ± 2.19	13.11 ± 1.17
24d						
CK1	0.15 ± 0.026	65.79 ± 4.19	1072.27 ± 4.2	1.84 ± 0.08	69.29 ± 3.17	28.87 ± 1.04
CK2	0.132 ± 0.025	73.19 ± 2.83	1232.85 ± 6.8	1.86 ± 0.13	70.54 ± 2.56	27.6 ± 1.22
C1	0.116 ± 0.011	86.97 ± 3.62	1417.48 ± 3.2	1.79 ± 0.36	73.19 ± 3.9	25.02 ± 1.47
C2	0.102 ± 0.027	68.59 ± 2.91	1069.57 ± 5.6	1.85 ± 0.32	79.92 ± 2.89	18.23 ± 1.02
C3	0.107 ± 0.034	74.27 ± 3.15	1243.38 ± 3.9	1.92 ± 0.24	80.18 ± 3.04	17.9 ± 0.93

3.3. Analysis of MRI

MRI provides visual information on the spatial, internal morphological organization, and molecular distribution of in food matrix and has drawn great attention because of its rapid, direct, and accurate imaging characteristics in comparison with LF-NMR [26]. It is well-known that a red color represents high proton density while a blue color represents low proton density in the pseudo-color images. The T_2 proton density weighted images of samples with different treatments during refrigeration are shown in Figure 3. No significant difference was detected in images brightness of samples treated with edible coating combined with MAP at the beginning stage, while obvious changes occurred in the control group, especially CK1. From the 15th day, the color of CK1 was bluer and darker than that of other samples, as demonstrated by Wang et al. [16], which indicated the degradation of the myofibrils and destruction of the microstructure in CK1. Those results were consistent with the changes of transverse relaxation of LF-NMR. Above all, MRI is also a complementary technology for understanding water migration of HTS fillets during storage. On the other hand, results also confirmed that water distribution was correlated with the quality properties of HTS fillets.

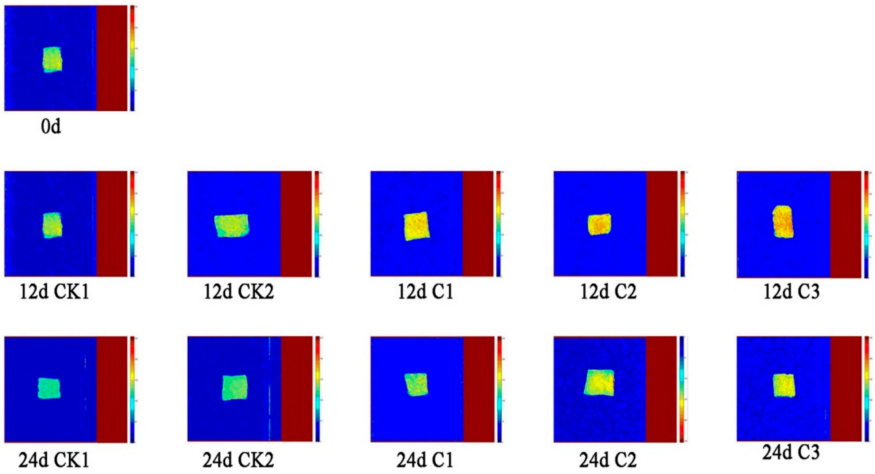

Figure 3. Results of magnetic resonance imaging (MRI) of samples under different treatments during refrigerated storage (CK1: distilled water + air packing; CK2: edible coating + air packing; C1: edible coating + MAP (60% CO_2/40% N_2); C2: edible coating + MAP (65% CO_2/35% N_2); C3: edible coating + MAP (70% CO_2/30% N_2)).

3.4. Linear Regression Analysis

The potential of LF-NMR as predictor of quality characteristics has received substantial research interest and it has been perceived as an efficient technique to assess the freshness of aquatic products. Previous researches have also confirmed that LF-NMR measurements were associated with quality parameters, such as WHC, drip loss, color, and so on [16,27,28]. In the current study, the relationship between LF-NMR relaxation parameters and quality indexes was investigated during refrigerated storage (Table 2). Obviously, no relationship was detected among pT_{21} and the physicochemical parameters, suggesting that the value of pT_{21} was barely high enough to predict the quality characteristics of HTS fillets. However, a good correlation between pT_{22}, pT_{23}, and physicochemical parameters were detected. Briefly, the pT_{22} showed excellent correlation with R^2 in the range of 0.79631–0.98873 with water contents, fat contents, and the activities of Ca^{2+}-ATPase. While the TVB-N values and MDA values showed relatively poor relationship correlation with pT_{22}. It also can be concluded that there was no relationship between pT_{22} and K values in all samples. By comparison, there was a significantly good correlation between pT_{23} and TVB-N, K value and MDA ($p < 0.01$). However, a poor relationship was detected between pT_{23} and water content or the activity of Ca^{2+}-ATPase. No correlation was detected between pT_{23} and fat content. In general, pT_{22} plays a more important role in the evaluation of water contents, fat contents, and the activities of Ca^{2+}-ATPase changes, while pT_{23} is more effective in assessing the qualities of TVB-N, K value, and MDA.

Table 2. Linear regression analysis of HTS fillets with different treatment during refrigerated storage.

Groups	T_2		TVB-N	K Value	Moisture Content	Fat Content	Activity of Ca^{2+}-ATPase	MDA
CK1	pT_{21}	R	−0.116	−0.252	0.376	0.257	0.334	−0.356
		R^2	0.01346	0.06351	0.14138	0.02146	0.111556	0.12674
		p	ns	ns	ns	ns	ns	ns
	pT_{22}	R	0.95119	0.29514	0.92732	0.98812	0.98214	0.99312
		R^2	0.90476	0.08711	0.85992	0.97638	0.9646	0.98629
		p	0.05	ns	0.01	0.01	0.01	0.05
	pT_{23}	R	0.86271	0.94352	0.84978	0.22136	0.94675	0.94978
		R^2	0.74427	0.89023	0.72213	0.0449	0.89634	0.90208
		p	0.01	0.01	0.05	ns	0.05	0.01

Table 2. *Cont.*

Groups	T_2		TVB-N	K Value	Moisture Content	Fat Content	Activity of Ca^{2+}-ATPase	MDA
CK2	pT_{21}	R	0.56437	0.34126	0.39643	0.23168	0.41154	0.28746
		R^2	0.31851	0.11646	0.15716	0.05367	0.16937	0.08263
		p	ns	ns	ns	ns	ns	ns
	pT_{22}	R	0.38324	0.29435	0.93293	0.97317	0.97224	0.89146
		R^2	0.3705	0.08644	0.87036	0.94706	0.94525	0.7947
		p	ns	ns	0.01	0.01	0.01	0.05
	pT_{23}	R	0.90213	0.97568	0.82514	0.28246	0.87412	0.93562
		R^2	0.81384	0.95195	0.68086	0.07978	0.76173	0.87539
		p	0.01	0.01	0.05	ns	0.05	0.01
C1	pT_{21}	R	0.54873	0.28534	0.42674	0.32143	0.37892	0.29836
		R^2	0.30111	0.08142	0.18211	0.10332	0.14358	0.08902
		p	ns	ns	ns	ns	ns	ns
	pT_{22}	R	0.88412	0.38423	0.96612	0.97412	0.97733	0.88911
		R2	0.78167	0.14763	0.93339	0.94628	0.94731	0.79052
		p	0.05	ns	0.01	0.01	0.01	0.05
	pT_{23}	R	0.93215	0.97346	0.79354	0.58421	0.74278	0.96542
		R^2	0.8689	0.94762	0.62971	0.3413	0.55172	0.93204
		p	0.01	0.01	0.05	ns	0.05	0.01
C2	pT_{21}	R	0.32145	0.31238	0.50156	0.16973	0.45672	0.37829
		R^2	0.10333	0.0969	0.25156	0.02881	0.20859	0.1431
		p	ns	ns	ns	ns	ns	ns
	pT_{22}	R	0.82356	0.49324	0.94582	0.96374	0.91214	0.62569
		R^2	0.67825	0.24329	0.89458	0.9288	0.832	0.39149
		p	0.05	ns	0.01	0.01	0.01	ns
	pT_{23}	R	0.96739	0.95678	0.94718	0.50047	0.81248	0.45768
		R^2	0.93584	0.91543	0.89715	0.25047	0.66012	0.20947
		p	0.01	0.01	0.05	ns	0.05	ns
C3	pT_{21}	R	0.45362	0.37288	0.51118	0.19463	0.47284	0.32145
		R^2	0.20577	0.13904	0.26131	0.03789	0.22358	0.10333
		p	ns	ns	ns	ns	ns	ns
	pT_{22}	R	0.73246	0.30123	0.94378	0.94178	0.89236	0.64113
		R^2	0.53649	0.09074	0.89072	0.88695	0.79631	0.41105
		p	0.05	ns	0.01	0.01	0.01	0.05
	pT_{23}	R	0.98275	0.95467	0.79932	0.5101	0.80213	0.95467
		R^2	0.9658	0.9114	0.79389	0.2602	0.64341	0.9114
		p	0.01	0.01	0.05	ns	0.05	0.01

3.5. Analysis of PCA

Multi-way chemometric analysis based on LF-NMR measurements was used to investigate the relationship of LF-NMR parameters and quality characteristics; PCA were highly considered in aquatic products among those methods [29–31]. Figure 4 demonstrated the component plot of the first two principal component PC1 and PC2 scores of HTS fillets during storage. In this case, 84.12% and 10.69% of the variability was explained by principal components PC1 and PC2, respectively. It can been seen that pT_{22}, water content, fat content, and the activity of Ca^{2+}-ATPase were located on the positive axis of PC1, which was consistent with the correlation of pT_{22} and special physiochemical parameters. Meanwhile, the pT_{23}, TVB-N, K value, and MDA located on the negative axis of PC1 and also verified the results of linear regression analysis. Above all, the preliminary PCA showed that the pT_{22} parameter could be a candidate in characterizing the water and fat losses as well as enzyme activity of HTS fillets, while the pT_{23} parameter could replace the traditional TVB-N, K value, and MDA, although with some limitations. These results demonstrated that LF-NMR combined with PCA analysis has great potential in evaluate quality characteristics of HTS fillets during storage, which was confirmed previously [32–34].

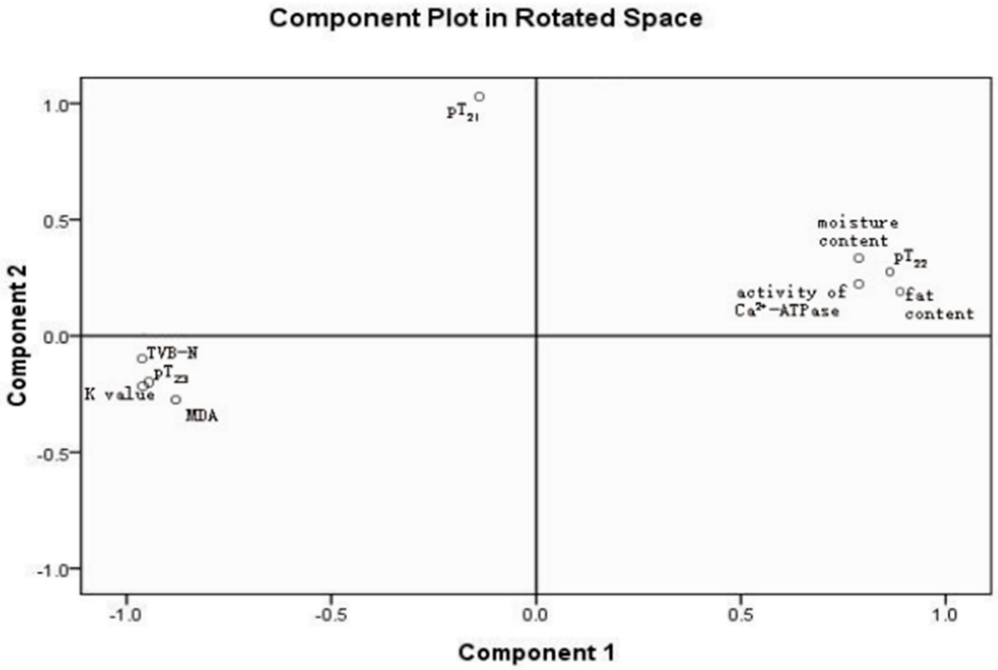

Figure 4. Principal component PC1 and PC2 score plot from the principal component analysis (PCA) of HTS fillets during refrigerated storage (CK1: distilled water + air packing; CK2: edible coating + air packing; C1: edible coating + MAP (60% CO_2/40% N_2); C2: edible coating + MAP (65% CO_2/35% N_2); C3: edible coating + MAP (70% CO_2/30% N_2)).

4. Conclusions

The edible coating incorporated with MAP (gas composition of 70% CO_2/30% N_2) has positive effects to maintain the excellent qualities/characteristics and extend shelf life for 8–12 days of HTS fillets during refrigerated storage. The water dynamic changes were evaluated by LF-NMR and MRI and three types of water were distinguished: bound water (T_{21}), immobile water (T_{22}), and free water (T_{23}). The pT_{22} diminished progressively while pT_{23} increased during storage, and pT_{22} accounted for the largest proportion of three types of water, followed by pT_{23}. The weighted MRI provided the internal structure information associated with different treated samples during storage. We also demonstrated the excellent correlation of LF-NMR measurements and quality parameters based on the linear regression analysis and PCA. Above all, the combination of LF-NMR and MRI was able to give more information about water distribution and migration, demonstrating the feasibility of LF-NMR pulse MRI to monitor quality deterioration and predict shelf life of HTS fillets during refrigerated storage.

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