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Effect of vacuum impregnated fish gelatin and grape seed extract on metabolite profiles of tilapia (*Oreochromis niloticus*) fillets during storage

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ABSTRACT

Traditional methods evaluating fish quality do not involve comprehensive qualification and quantification of quality-related components. The objective of this study was to investigate the effect of vacuum impregnated fish gelatin (FG) and grape seed extract (GSE) on metabolites of tilapia fillets during storage using nuclear magnetic resonance (NMR). Totally 42 metabolites were identified, 36 of which were quantified. The multivariate analysis results demonstrated distinct separations between fresh and stored fillets, indicating significant metabolite changes during storage. Some metabolites like choline and trimethylamine oxide were closely related to freshness while organic acids were associated with spoilage. Combined FG and GSE reduced the formation of undesirable metabolites like trimethylamine and histidine significantly ($P < 0.05$). Traditional freshness indexes indicated preserved quality after combined coating and further verified NMR results. This study reveals the potential of NMR to analyse metabolites that determine fish quality and to monitor their changes during storage.

1. Introduction

Tilapia (*Oreochromis niloticus*) is one of the most abundant freshwater fish species in the world and occupies a significant position in the worldwide fishery market (Shi et al., 2018). However, tilapia is highly susceptible to putrefaction during storage due to the activities of endogenous enzymes and microorganisms. The deterioration of tilapia results in organoleptic and nutritional quality loss, generates harmful components which may even cause health issues for consumers (Sun, Sun, Thavaraj, Yang, & Guo, 2017).

To maintain freshness of fish during storage, bio-based coatings such as apple polyphenol and oregano essential oils have been applied as effective and non-toxic preservative choices. These edible coatings have been tested for preservation of various food products like red pitaya fruit and Bologna sausage, and proven effective in extending shelf-life and preserving quality (Fan et al., 2018; Nikmaram et al., 2018). Fish gelatin (FG), as a substitute of mammalian one, has gained the popularity in food industry due to its barrier function against gases and moisture, great film-formation ability and biodegradability (Feng, Ng, Mikš-Krajnik, & Yang, 2017). However, the major issues restricting the application of FG are the relatively limited antioxidant and antimicrobial properties and high water sensitivity (Lin, Regenstein, Lv, Lu, & Jiang, 2017). Combining antioxidant and antimicrobial agents with FG is a promising strategy to overcome these limitations and has been applied to preserve seafood products (Feng et al., 2017). Grape seed extract (GSE) is a natural nutraceutical product containing abundant polyphenols such as catechin, epicatechin, proanthocyanidins, and monomeric flavanols (Maestre, Micol, Funes, & Medina, 2010). It possesses not only significant antioxidant ability by scavenging free radicals, but also antimicrobial property due to the core structures with 3,4,5-trihydroxyphenyl groups (Shen & Su, 2017). In addition, emerging technology like vacuum impregnation (VI) is being applied to introducing various functional substances including vitamins, antioxidant agents, antimicrobials, and anti-browning compounds into porous tissues in a direct and rapid way. By affecting the capillary structures of foods, VI can accelerate the two-way mass transfer between foods and impregnation solutions (Zhang et al., 2019).

Quality and freshness changes of fishery products can be determined by sensory evaluation and other traditional standard methods. However, traditional methods for fish quality evaluation do not involve comprehensive qualification and quantification of quality-related components. Moreover, these methods are time-consuming, resource-demanding and environmentally unfriendly. Thus, more advanced and sensitive techniques are in desperate need. High resolution nuclear

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magnetic resonance (NMR) is a powerful technique for food quality evaluation (Castejón, García-Segura, Escudero, Herrera, & Cambero, 2015). It has been certified that the relationship between metabolite profiles and food quality could be uncovered by NMR-based metabolic characterization (Shumilina, Ciampa, Capozzi, Rustad, & Dikiy, 2015). Moreover, the quality indicators such as K-value and related characteristic biomarkers could be determined using NMR (Xiao, Ge, Zhou, Zhang, & Liao, 2019). Thus, the understanding of metabolite profiles during storage may help to gain deep insight on actively regulating the freshness status of fish products.

In this study, FG, GSE and the combined coatings were introduced to tilapia fillets via VI process. The aims of this study were to early detect metabolites as biomarkers of fish freshness and study the effect of vacuum impregnated coating solutions on metabolic changes during storage by high resolution NMR spectroscopy coupled with multivariate analysis. The principal component analysis (PCA) was applied to highlight the principal metabolites responsible for different treatments while the orthogonal partial least squares discriminant analysis (OPLS-DA) was further conducted for pairwise comparisons. Last, freshness indexes including pH, total volatile basic nitrogen (TVB-N), and K-value were tested to further verify the metabolic results.

2. Materials and methods

2.1. Preparation and VI treatment of tilapia fillets

All the reagents for the coating solutions were of food grade. Commercial tilapia (Oreochromis niloticus) fish gelatin (200 bloom) was purchased from Jiangxi Kesheng Organism Co., Ltd (Yingtan, Jiangxi, China) and GSE was obtained from Tianjin Jianfeng Natural Product Co., Ltd (Tianjin, China). Commercial tilapia was obtained from a local market in Singapore. The average weight was 500 ± 10 g. The tilapia with head, bone, viscera and skin removed were transported to the laboratory within one hour in an ice bag. The fillets were cut into 10 g pieces and then randomly divided into four groups: the control group treated with deionised water, the FG group (3%), the GSE group (0.9%) and the combination group (3% FG + 0.9% GSE).

VI was conducted in a jacketed chamber connected to a vacuum pump controlled by a vacuum controller (IKA VC10, Guangzhou, Guangdong, China). Fillets were impregnated with different coating solutions. The mass ratio of fillets to coating solution was kept at 1: 3. A vacuum pressure of 5 kPa was applied for 15 min. After that the atmospheric pressure was restored within 10 s and the samples were maintained for 10 min (Zhang et al., 2019). The adhering coating solution was then removed from the surface and the fillets were drained for 5 min. All the samples were kept in ziploc bags and stored at 4 ± 1 °C for 12 days for further analyses.

2.2. Metabolites extraction

Metabolites extraction was finished according to Chen, Ye, Chen, Zhan, and Lou (2017) with some minor modifications. Tilapia samples (2 g) were extracted via homogenization with 5 mL of water: methanol (1: 2, v/v) for 2 min and discontinuous ultrasonication in ice bath for 7 min (2 s ultrasonication followed by a 2 s break). After centrifugation at 12,000 × *g* for 10 min (4 °C), the supernatants were collected and the methanol was removed via rotatory evaporator. The resultant extracts were redissolved into 600 μL phosphate buffer (0.1 M, pH 7.4) containing 50% deuterium oxide (D_2O) and 0.01% (w/v) sodium 3-trimethylsilyl [2, 2, 3, 3-d_4] propionate (TSP). After centrifugation at 12,000 × *g* under 4 °C for 10 min, 550 μL of supernatants was transferred to a 5 mm NMR tube for further NMR analysis. TSP was used as an internal chemical shift reference. The metabolite profiles were measured in triplicate.

2.3. NMR spectroscopy analysis

All the prepared tubes were tested on a Bruker DRX-500 NMR spectrometer (Bruker, Rheinstetten, Germany) at 500.23 MHz frequency via a Triple Inverse Gradient probe with probe temperature at 25 °C (Liu et al., 2017). For metabolic profile of each sample, a one dimensional ^{1}H spectrum was obtained by using a first increment of standard Bruker Nuclear Overhauser Effect SpectroscopY (NOESY) pulse sequence (noesypr1d). All the data were collected using a spectral width of 20 ppm with an acquisition time of 3.3 s. Furthermore, the spectrum was recorded by 128 scans and relaxation delay of 2 s. A 90° pulse length was modified for each sample by using the automatic pulse calculation experiment (pulsecal) in TopSpin 3.6.0 (Bruker, Rheinstetten, Germany). All free induction decays were multiplied by an exponential function with a line broadening (1-Hz) factor prior to Fourier transformation. For the objective of signal assignment, standard two dimensional NMR spectra of a representative sample was acquired by using the Bruker hsqcedetgpsisp2.3 pulse sequence including ^{1}H–^{1}H correlation spectroscopy (COSY), ^{1}H–^{1}H total correlation spectroscopy (TCOSY) and ^{1}H–^{13}C heteronuclear single quantum correlation (HSQC). The ^{1}H and ^{13}C were tested in the F2 channel with a 10 ppm spectrum width and F1 channel with a 180 ppm spectrum width, respectively (Chen et al., 2019).

2.4. Spectral processing and statistical analysis

The NMR spectra of fillets were adjusted by manually phase correction and baseline distortions by the software TopSpin 3.6.0. The metabolites corresponding to the peaks were identified by 1D ^{1}H and 2D ^{1}H–^{13}C NMR spectra referring to the Human Metabolome Database (http://www.hmdb.ca/), Madison Metabolomics Consortium Database (http://mmcd.nmrfam.wisc.edu/), Biological Magnetic Resonance Data Bank (http://www.bmrb.wisc.edu/metabolomics/) and some related references (Chen et al., 2019; Shumilina et al., 2015). Furthermore, the metabolites proton signals in the range of 0–10 ppm were integrated and normalised to TSP signal by the software Mestrenova (Mestreab Research SL, Santiago de Compostela, Spain). The water region (4.6–4.8 ppm) was excluded and the obtained standardised data were applied for multivariate analysis by the SIMCA software (version 13.0, Umetrics, Sweden). PCA results was shown as score and loading plots representing an individual sample and metabolite to classification respectively. The binned data were further analysed by OPLS-DA to identify the dissimilarities between different treatments (Chen, Tan, Zhao, Yang, & Yang, 2019). Furthermore, the variable importance in projection (VIP) was analysed and the metabolites with a VIP value > 1 were recognized as the most influenced factors in the extracted OPLS models (Liu et al., 2018).

2.5. Quality and freshness analysis of tilapia fillets

2.5.1. Determination of pH value

Minced fillets were dissolved in deionised water at a ratio of 1:10. The mixture was thoroughly homogenized via magnetic stirrer for 15 min and centrifuged at 10,000 × *g* for 5 min (4 °C). The supernatant was used to detect pH value with a digital pH meter (Feng et al., 2017).

2.5.2. Determination of TVB-N

Sample supernatant from pH test and MgO solution (1%) were equally mixed and transferred into a Kjeldahl tube. Distillation was lasted for 5 min using a Kjeldahl nitrogen apparatus and the distillate was collected with 10 mL of boric acid (0.02 g/L) containing indicator. The absorption solution was titrated with 0.01 M HCl until the solution turned to blue purple. A blank test was carried out using deionised water instead (Yu et al., 2018). TVB-N value was calculated using the following equation:

$$\mathrm{TVB-N}\ (mg/100\ g) = \frac{(V_1 - V_2) \times C \times 14}{m \times 5/50} \times 100$$

where V_1 and V_2 are the volume (mL) of HCl used for the sample and the blank, respectively, C represents the HCl concentration (mol/L), and m is the sample mass (g).

2.5.3. Determination of K-value

ATP (adenosine triphosphate) and ATP related degradation products including ADP (adenosine diphosphate), AMP (adenosine monophosphate) IMP (inosine monophosphate), HxR (inosine) and Hx (hypoxanthine) were extracted by 0.6 M perchloric solution (Chen et al., 2018). The extraction mixture was centrifuged at 3000 × *g* for 10 min (4 °C) and then the pH was adjusted to 6.5–6.8. After filtration through a 0.45 ìm membrane, the solution was stored under −20 °C for further analysis. High-performance liquid chromatography (HPLC) system (Alliance 2695, Waters, MA, USA) with a UV detector at 254 nm was used to monitor the ATP related components. The separation was carried out on a Luna C18 column (150 mm × 4.6 mm) using phosphate buffer (0.05 M, pH 6.8) pumped at a flow rate of 1.2 mL/min. The content of each compound was calculated using standard curves (0.5–20 μg/mL, $R^2 > 0.99$). K-value was calculated using the following equation:

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

2.6. Statistical analysis

Data were statistically analysed by analysis of variance (ANOVA) of at least three independent replicates. Means were compared using the least significant difference (LSD) method with software IBM SPSS Statistics Version 23 (International Business Machines Co. Armonk, NY, USA) to evaluate the effect of different treatments on fillets. P value < 0.05 was regarded as statistically significant.

3. Results and discussion

3.1. ^{1}H NMR spectra of the extracts of tilapia fillets

The metabolite profile of tilapia fillets under different treatments during refrigerated storage is shown in Fig. 1. Multiple signals were recorded from 0.5 to 9 ppm with different detections of metabolites in tilapia fillets stored for 6 and 12 days. For all groups, most signals were located in the region of 3.0–5.0 ppm. With prolonged storage time, intensities of most ^{1}H signals in the region of 8.0–9.0 ppm underwent obvious changes, which indicated that some metabolites were formed while others degraded during refrigerated storage. In total, 42 metabolites were unambiguously identified based on the chemical shifts of ^{1}H NMR spectra and 2D ^{1}H–^{13}C NMR spectra (Fig. 2 and Table S1) and 36 of them were quantified. The metabolome of fillets was composed of 18 amino acids (isoleucine, leucine, valine, etc.), 1 dipeptide (anserine), 3 organic acids (succinic acid, lactic acid, and acetic acid), 5 carbohydrates (maltose, glycogen, α-glucose, etc.), 2 alcohols (ethanol and 2, 3-butanediol), 5 nucleotides (ADP, AMP, IMP, etc.), 5 biogenic amines (tyramine, putrescine, trimethylamine, etc.) and 3 other components (betaine, choline, and creatinine). In the region of 0.5–3 ppm, the signals belonged to aliphatic groups, especially amino acids and some organic acids. Various carbohydrates and ATP degradation metabolites were identified in the region of 3–5.5 and 5.5–9 ppm, respectively (Shumilina et al., 2015).

3.2. Effect of VI coating on the metabolite profiles of tilapia fillets

To elucidate the effect of different treatments on the metabolite profiles of tilapia fillets during chilled storage, PCA was conducted and the results are shown in Fig. 3. PC1 and PC2 explained 91.1% while the PC1 explained 81.4% of the total variances. In the score plot (Fig. 3A), the fresh fillets (day 0, I) were located in the positive side of PC1 which was distinctly separated from the fillets after storage (day 12, II-V). Obvious difference in PC1 values of fresh (I) and stored fillets (II-V) indicated the dramatic differences in metabolic profiles. Moreover, fillets under different treatments on day 12 were separated into three clusters, the control group (II), the combination group (V), and FG or GSE treated groups (III and IV). The metabolite profiles of the treatment groups tended to the positive side of PC2 compared with that of the control group which was negatively influenced by PC2. The combination group on day 12 tended to the positive side of PC1 compared to the other three groups. The loading plot (Fig. 3B) responsible for the group separation was further conducted, which revealed that metabolites such as creatine, choline, valine and acetic acid characterised PC1 while metabolites such as lactic acid, glycine, and trimethylamine oxide (TMAO) represented PC2. Moreover, some metabolites like creatine, TMAO and choline were closely related to fresh fillets. Such results agreed with a previous study (Shumilina et al., 2015). On the other hand, organic acids like lactic acid and acetic acid were identified in stored fillets. These metabolites are all closely associated with glycolysis pathway (Wang et al., 2017).

To further understand the effect of different treatments on metabolites during chilled storage, pairwise comparisons from the fresh fillets (day 0, I) and control group on day 12 (II), control group on day 12 (II) and combination group on day 12 (V) were conducted by OPLS-DA, respectively. In the cross-validated score plots, the paired groups I-II and II-V were distinctively differentiated by PC1 (Fig. 4A and C). R^2 and Q^2 were 0.978 and 0.999 for paired group I-II, 0.945 and 0.998 for paired group II-V, respectively. High R^2 and Q^2 values indicate significant difference between paired groups and overall predictabilities of these fit models. The loading S-line plots were applied to distinguish metabolites of different groups. The metabolites with upward trend indicated higher relative concentration in the latter samples while those with downward tendency showed larger content in the former groups. Based on the results of I-II (Fig. 4B), the relative higher concentrations of metabolites like creatine, choline, IMP, and glycine of the fresh fillets indicating a significant decrease during the refrigerated storage while metabolites such as lactic acid, hypoxanthine, and histamine increased after 12 days. For the pairwise II-V (Fig. 4D), the combination group contained relative higher contents of metabolites such as creatine, choline, IMP and glutamate. Nevertheless, metabolites such as lactic acid, histamine and taurine showed downward tendencies indicating higher contents in the control group compared to the combination group on day 12. Moreover, the hotter the colour of metabolites, the greater the significance between paired groups. The S-line between pairwise II-V revealed that combined FG and GSE reduced the production of organic acid via glycolytic pathway and biogenic amines, and inhibited the degradation of adenine nucleotides.

Coefficient plots (Fig. 4E and F) were statistically applied to identify significant metabolites in paired groups during storage. The results revealed that during 12-day storage, 19 metabolites (lactic acid, alanine, hypoxanthine, etc.) with increased relative concentrations were recorded (Fig. 4E). The rest of 15 quantified metabolites presented decreased relative contents after storage. The results of VIP test further revealed that metabolites such as choline, lactic acid, and creatine were of significant difference of paired I-II groups, meaning that they were significantly affected during chilled storage (VIP value > 1, $P < 0.05$). Accumulated organic acids, especially lactic acid in stored fillets, indicated the major sugar of fish muscle, glycogen, suffered anaerobic glycolysis during storage (Choe et al., 2008). In addition, the abundant histamine and tyramine in stored fillets were transformed from the degradation of corresponding amino acid precursors (Romero-González, Alarcón-Flores, Vidal, & Frenich, 2012). On the other hand, higher relative levels of choline, creatine and TMAO in fresh fillets as compared to the stored fillets indicate a certain extent of degradation or

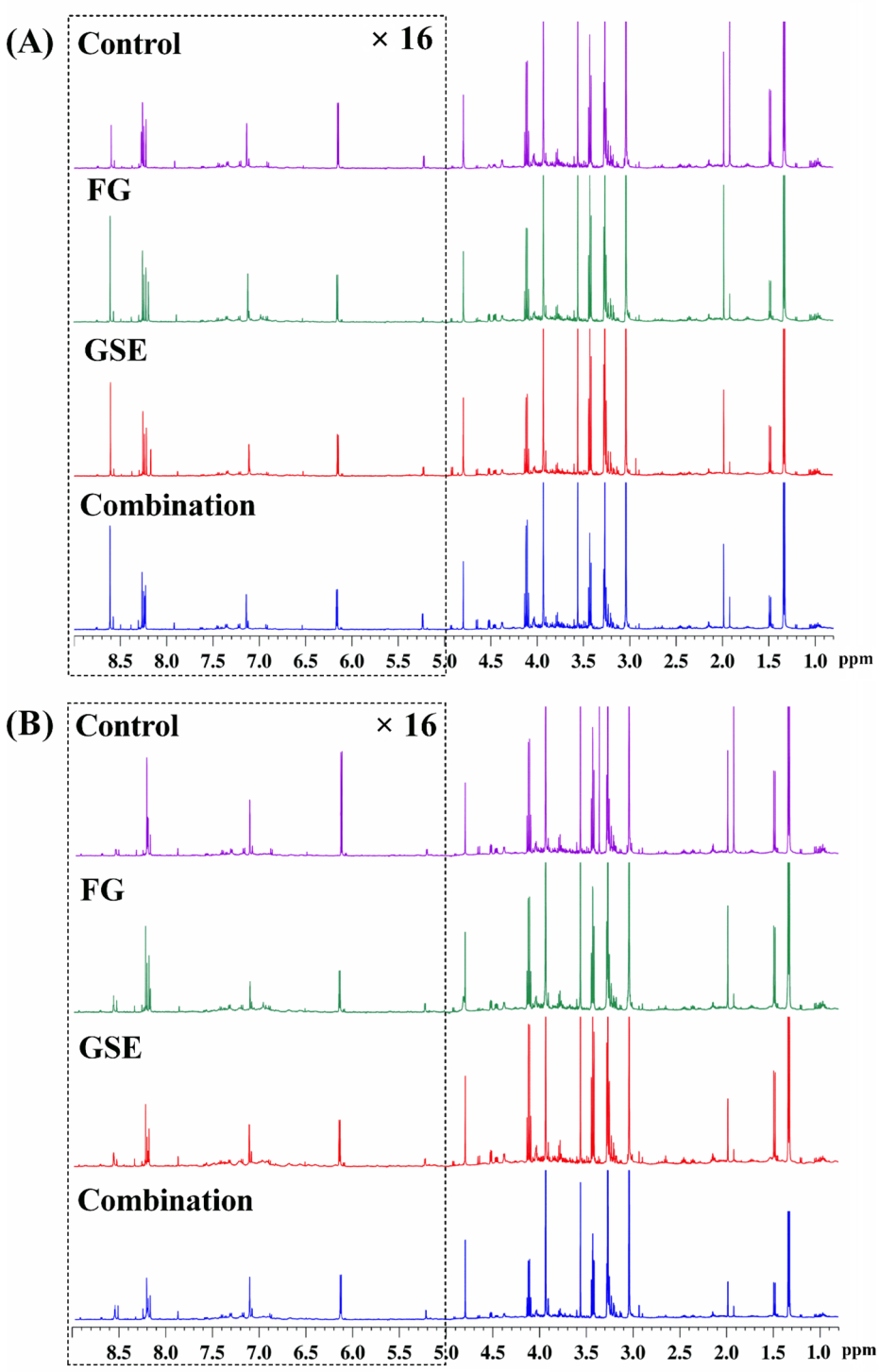

Fig. 1. ^{1}H NMR spectra of stored tilapia fillets on day 6 (A) and day 12 (B).

transformation during storage (Shumilina et al., 2015).

The coefficient plot of II-V shows the difference of metabolic profiles under FG-GSE coating (Fig. 4F). The combined FG and GSE resulted in lower relative levels of metabolites such as alanine, histamine and lactic acid and higher relative levels of metabolites including choline, creatine, glycine, arginine, etc. Based on the results of VIP test, 15 metabolites (alanine, histamine, creatine etc.) were significant variants for the pair separation. Under FG-GSE treatment, the relative contents of some carbohydrates (maltose, and glycogen) and amino acids (creatine, arginine and glutamate) were kept at higher levels as compared to that of the control group. On the contrary, the coating treatment induced lower contents of compounds which are related with poor quality status of fish, such as histamine and tyramine. The results probably indicate the potential of combined FG and GSE in preserving fish quality and freshness.

3.3. Metabolic changes of tilapia fillets under different treatments during storage

To obtain more detailed information of the metabolic changes in

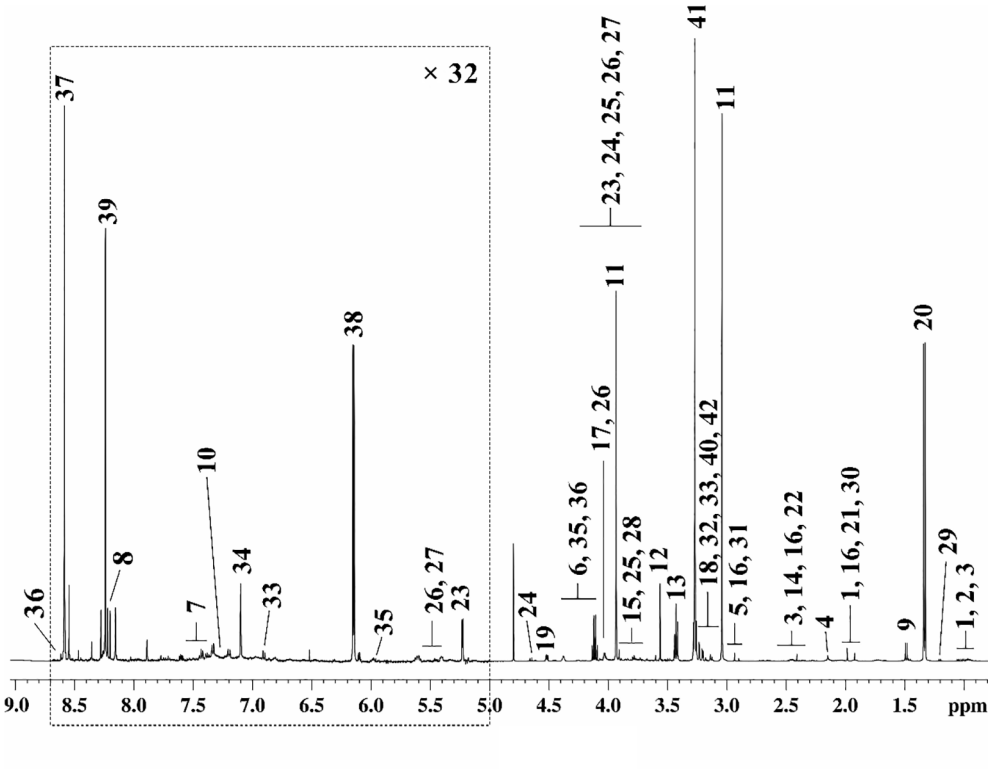

Fig. 2. Representative 1D ^{1}H NMR spectra (A) and 2D ^{1}H–^{13}C NMR spectra (B) of fresh tilapia fillet. Note: 1. Leucine; 2. Isoleucine; 3. Valine; 4. Methionine; 5. Lysine; 6. Threonine; 7. Phenylamine; 8. Histidine; 9. Alanine; 10. Tyrosine; 11. Creatine; 12. Glycine; 13. Taurine; 14. Glutamine; 15. Glutamate; 16. γ-aminobutyric acid; 17. Aspartic acid; 18. Arginine; 19. Anserine; 20. Lactic acid; 21. Acetic acid; 22. Succinic acid; 23. α-glucose; 24. β-glucose; 25. Glycogen; 26. Maltose; 27. Sucrose; 28. Ethanol; 29. 2, 3-butanediol; 30. Putrescine; 31. TMA; 32. TMAO; 33. Tyramine; 34. Histamine; 35. ADP; 36. AMP; 37. IMP; 38. HxR; 39. Hx; 40. Betaine; 41. Choline; 42. Creatinine.

chilled fillets with different treatments during storage, absolute quantifications of metabolites with no overlapping chemical shifts were conducted (Table 1).

3.3.1. Changes of amino acids and peptides

Amino acids and peptides were major components in tilapia fillets. Among total 18 identified amino acids, leucine, alanine and methionine were the most abundant essential amino acids while creatine, taurine and glycine were the most abundant non-essential amino acids. In addition, among all these free amino acids, alanine, threonine and glycine impart sweetness to fish, glutamate contribute to umami taste while the rest of amino acids contribute to bitterness. For most amino acids, increased trends were observed with different rising rate for different groups. It was due to the protein hydrolysis mediated by spoilage bacteria during chilled storage, resulting in release of free amino acids in muscle tissues (Moczkowska, Półtorak, Montowska, Pospiech, & Wierzbicka, 2017). Moreover, considering that FG is insoluble at low temperature (4 °C) and the FG used in this study has a relatively high gel strength (200 bloom), we assume that the increase of these amino acids was irrelevant to the degradation of peptide structure of FG (Hanani, Roos, & Kerry, 2014). However, the concentrations of histidine, glutamate, tyrosine and creatine decreased during storage for all groups. The reduced contents were probably revealed that the rate of protein hydrolysis was lower than that of enzymatic degradation. Moreover, as creatine is an important component associated with energy delivery and ATP formation, higher content in the combination group indicated less disturbed energy-generating process during storage (Sundekilde, Rasmussen, Young, & Bertram, 2017).

Histidine as well as histidine-peptides like anserine normally exist in fish tissues. They are of great importance because of the antioxidant and buffering properties. In our study, histidine and anserine were found in relatively low contents (0.39 and 2.13 mg/100 g, respectively) in fresh tilapia fillets. A significant higher concentration of anserine was observed in the combination group as compared to the control group, which could be due to the inhibited hydrolysis process via anserinase. It was reported in a previous study that the decreased anserine was related to the increased levels of anserine degradation products such as 1-methyl-histidine and β-alanine (Shumilina et al., 2016).

3.3.2. Changes of nucleotides

Nucleotides are also important components in fish muscles which are primarily derived from metabolism of ATP. A general degradation of ATP is shown in the following sequence: ATP → ADP → AMP → IMP → HxR → Hx → xanthine → uric acid (Hong, Regenstein, & Luo, 2017). As major intermediate nucleotides, IMP is regarded as a flavour enhancer while HxR and Hx impart bitterness and off-odour. Accumulation of HxR and Hx in fish muscles correlates well with the loss of freshness, which can be expressed as K-value. Recent reports revealed that the nucleotides catabolism in fish fillets, indicating autolysis, may result in the transformation of ATP towards IMP, while microorganisms may assume an important responsibility for hydrolysing HxR to Hx (Li et al., 2017).

In this study, detectable ATP breakdown products included ADP, AMP, IMP, HxR and Hx. For all the samples, the contents of ADP, AMP and IMP decreased in the first six days and became undetectable on day 12. No significant differences for ADP and AMP levels were found among different treatment groups during storage ($P > 0.05$). As for IMP, the content in the control group was only 0.55 mg/100 g on day 6, which was significantly lower than the values of the coated samples (1.27–1.69 mg/100 g) ($P < 0.05$). The finding suggests that the relevant enzymatic activities were inhibited by the coating treatments (Yu et al., 2018). In addition, relatively higher IMP contents were found in GSE and combination groups as compared to the FG group. One possible reason might be that IMP degradation was further restrained by GSE addition.

On the other contrary, HxR and Hx were detected for all time points with various increasing rates during storage. The initial level of HxR (0.88 mg/100 g) was higher than that of Hx (0.18 mg/100 g), which was in accordance with a previous study (Li et al., 2012). The HxR level in four groups showed a rising tendency as a result of IMP conversion. The highest concentration of HxR in the control group (1.96 mg/100 g) was detected on day 12. As comparison, coating treatments reduced the

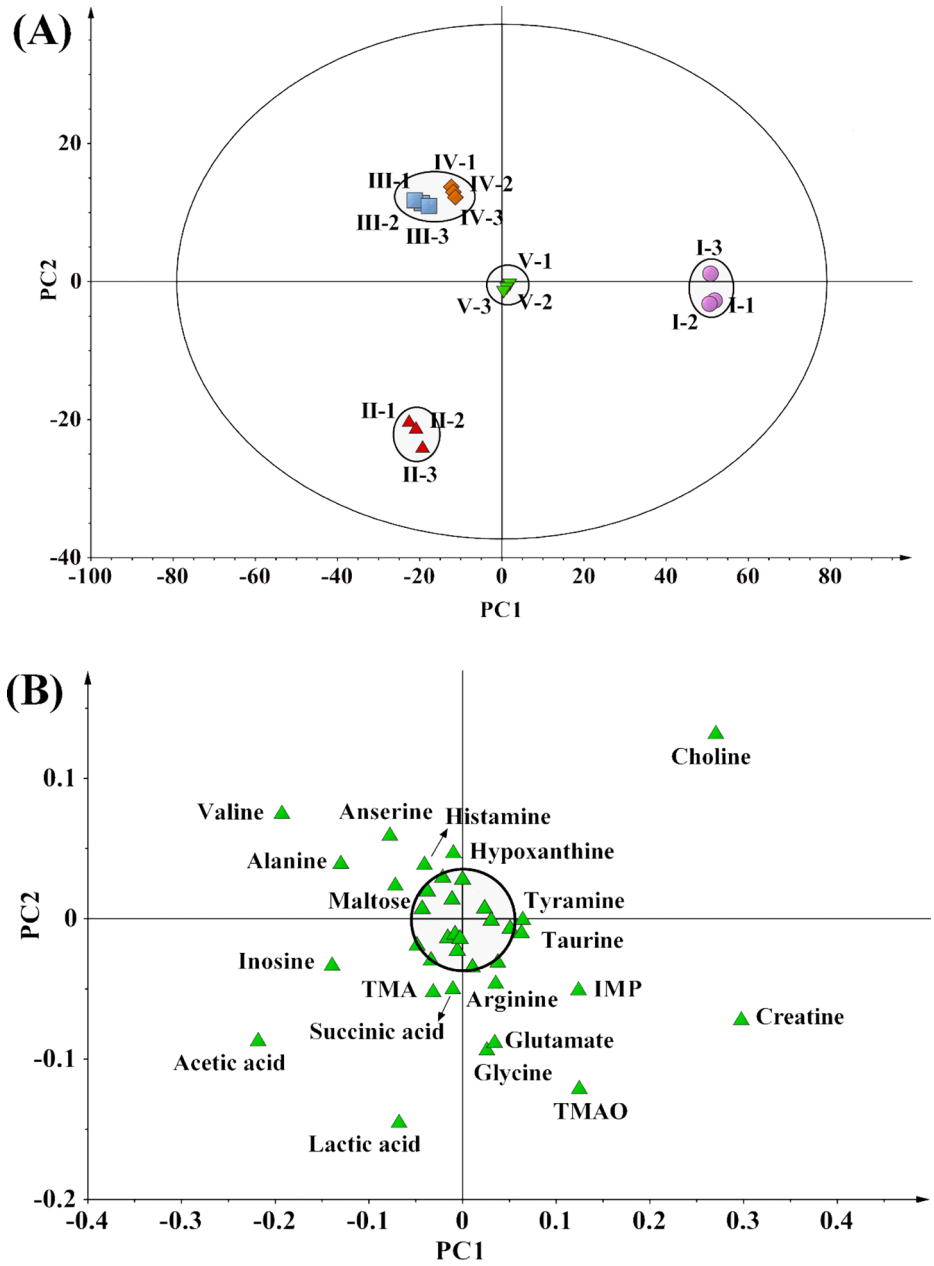

Fig. 3. The PCA score plot (A) and loading plot (B) for the metabolite profile of tilapia extracts. Note: I, fresh tilapia fillet on day 0; II, III, IV and V represent stored tilapia fillets on day 12 under DI water, FG, GSE and combined FG-GSE treatments, respectively.

formation of HxR at a rate of 49.49–56.12% for coated samples. In addition, a mild increase of Hx levels was observed in the first six days with no significant difference among groups, which might be due to relatively low amounts of HxR and microorganisms. However, rapid increase of Hx concentration was noted after 6 days, and finally reached around 2.4 mg/100 g in control and FG groups, while the corresponding values in GSE and combination groups were around 1.2 mg/100 g. As has been confirmed that the degradation pathway from IMP towards Hx was considerably mediated by spoilage bacteria, a possible reason explaining the lower concentration of Hx in GSE and combination groups may be due to the antibacterial ability of GSE (Nikmaram et al., 2018). It can be concluded that inhibited accumulation of HxR and Hx in GSE-coated groups led to positive effect on fillets' edibility by improving flavour quality during refrigeration from nucleotides assessment.

3.3.3. Changes of biogenic amines

The quality and freshness of fish were affected by the formation of biogenic amines (Papageorgiou et al., 2018). In this study, the identified biogenic amines included trimethylamine (TMA), TMAO, tyramine, histamine, and putrescine. TMAO, as an important osmoprotectant, is commonly present in various fish species. During fish spoilage, TMAO is degraded into TMA via N-oxide demethylase, causing unpleasant fishy odour in low concentrations. TMA can also be formed by the breakdown of other compounds such as choline, betaine and carnitine (Shumilina et al., 2015). With a gradual decrease in TMAO during storage came an increase of TMA contents in all groups. At the end of the storage, a significant lower content of TMA was observed in the combination group as compared to the control group ($P < 0.05$), followed by the GSE group. This may be due to the antimicrobial activities of GSE against spoilage bacteria as some of them are responsible for the formation of TMA (Fan, Luo, Yin, Bao, & Feng, 2014).

Tyramine is an aromatic amine and formed from tyrosin via decarboxylation. It is noted that tyramine increased with the storage time in all the samples with a different rising rate; however, the amount of

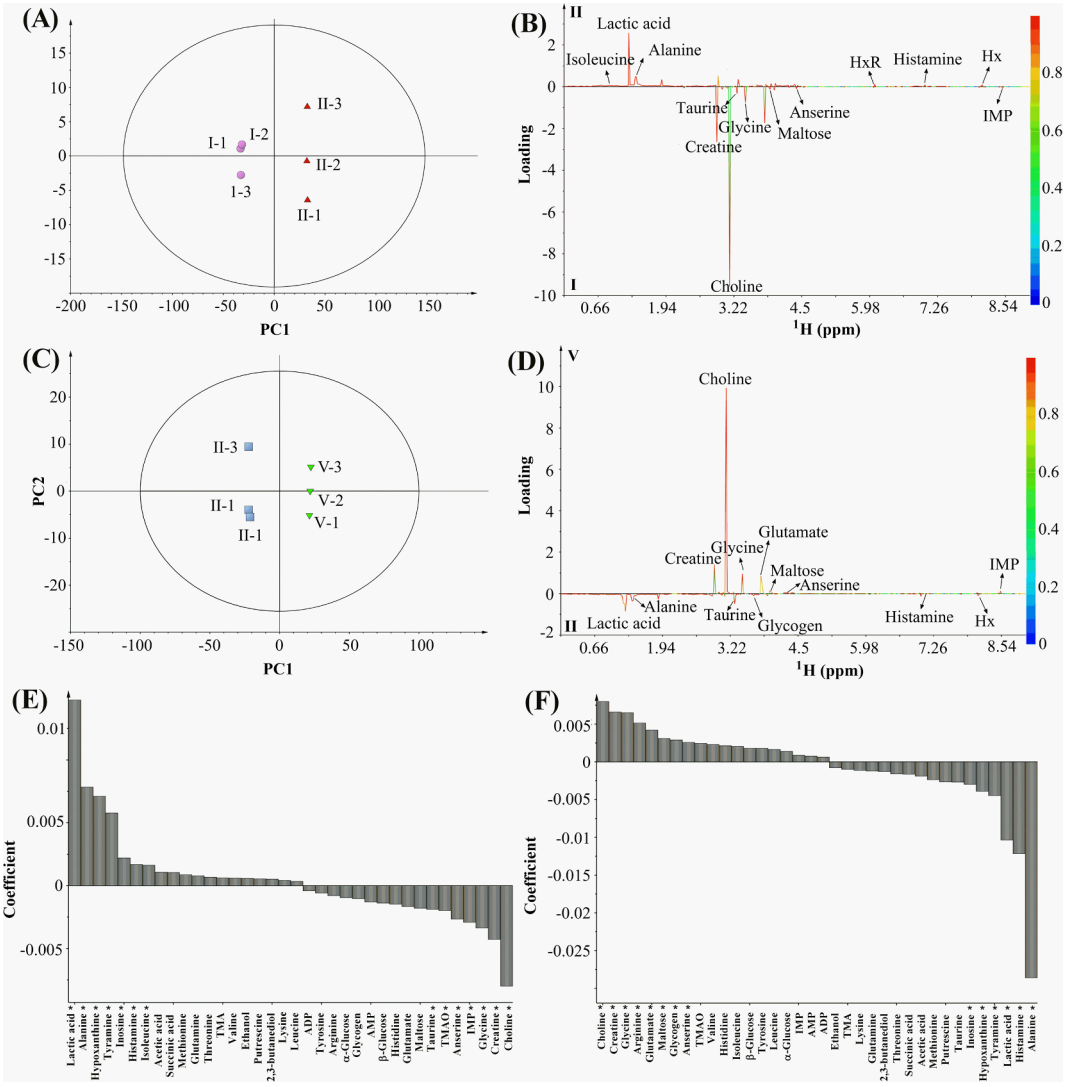

Fig. 4. OPLS-DA comparison results of pairwise groups. OPLS-DA score plot of I-II, $R^2 = 0.978$, $Q^2 = 0.999$ (A); loading S-line of I-II (B); OPLS-DA score plot of II-V, $R^2 = 0.945$, $Q^2 = 0.998$ (C); loading S-line of II-V (D); coefficient plot of I-II (E); coefficient plot of II-V (F), the metabolite marked with * means the variable importance in projection (VIP) value > 1, indicating significant contribution to OPLS-DA models. Note: I, fresh tilapia fillet on day 0; II and V, stored tilapia fillets on day 12 under DI water and combined FG-GSE treatments, respectively.

tyramine did not reach the maximum level (95 mg/100 g) (Paulsen, Grossgut, Bauer, & Rauscher-Gabernig, 2012). The amount of histamine (a histidine derivative) in the all treated tilapia fillets stored at 4 °C did not exceed the threshold allowed by current regulation (20 mg/100 g) (Commission, 2007). It is interesting that the histamine level in the combination group was significantly lower than that in the control group, followed by the GSE group ($P < 0.05$). This may be attributed to the effect of GSE on inhibiting *Enterobacteriaceae* species responsible for histidine decarboxylation (Fan et al., 2014).

Compared with tyramine and histamine, putrescine is less active toxicodynamically. However, it is of great importance due to its reinforcement of the toxicity of other compounds like histamine. In the analysed tilapia fillets the content of putrescine did not exceed the limitation (17 mg/100 g) after 12-day storage (Rauscher-Gabernig et al., 2012).

3.3.4. *Changes of carbohydrates and their corresponding glycolytic products*

The main metabolites assigned to carbohydrates in the tilapia fillets were glycogen, maltose, α-glucose and β-glucose. It is noted that during post-mortem storage, a decrease in content of glycogen was observed, especially at the latter storage period (Shumilina et al., 2015). A mild increase of maltose was observed for all the samples in the first six days followed by a decrease trend. The end product of glycolytic pathway is lactic acid, which in our study was abundantly present and tended to increase along with the whole refrigerated storage with the decrease of glycogen and the maltose. As indicated in a previous report, there was a negative correlation between lactic acid concentration, tenderness and water-holding capacity of meat (Choe et al., 2008). This may help explain the difference of quality and pH value among different fillets. Moreover, the reduced lactate formation may be related to the higher creatine concentration in the combination group, which was in accordance with previous findings (Sundekilde et al., 2017).

The decrease in the content of sugars in the fish muscle finally resulted in the loss of the sweetness and meaty flavour (Shumilina et al., 2015). In this study, gradual decrease in α-glucose and β-glucose was observed for all samples with different changing rates. It was probably resulted from alcohol fermentation which led to the formation of some organic acids and alcohol. Organic acids like acetic acid and succinic acid as well as some alcohols including ethanol and 2,3-butanediol were detected with a notable increase during refrigeration. A significant lower formation rate of these components, especially lactic acid and acetic acid, was observed in the coating groups with prolonged storage

Table 1
The contents (mg/100 g) of metabolites in fresh and stored tilapia fillets.

Metabolites	Day 0	Day 6				Day 12			
		Control	FG	GSE	Combination	Control	FG	GSE	Combination
Leucine	3.95 ± 0.08 a	5.79 ± 0.07 c	4.89 ± 0.01 b	5.39 ± 0.14 c	4.59 ± 0.14 b	5.58 ± 0.02 c	5.76 ± 0.20 c	5.25 ± 0.13 c	5.75 ± 0.19 c
Isoleucine	0.90 ± 0.02 a	1.16 ± 0.02 b	1.13 ± 0.03 b	1.41 ± 0.04 c	1.32 ± 0.03 c	1.55 ± 0.01 d	1.66 ± 0.05 d	1.33 ± 0.03 c	1.61 ± 0.04 d
Valine	1.08 ± 0.02 a	1.28 ± 0.03 b	1.22 ± 0.01 b	1.58 ± 0.06 c	1.38 ± 0.05 b	1.38 ± 0.02 b	1.93 ± 0.07 e	1.43 ± 0.05 bc	1.44 ± 0.08 bc
Alanine	3.42 ± 0.06 a	7.94 ± 0.04 d	5.80 ± 0.02 b	7.53 ± 0.08 d	6.87 ± 0.07 c	10.04 ± 0.07f	9.04 ± 0.06 e	8.77 ± 0.04 e	6.33 ± 0.15 c
Methionine	4.09 ± 0.13 a	4.41 ± 0.01 b	4.18 ± 0.05 a	4.48 ± 0.03 b	4.40 ± 0.05 b	5.47 ± 0.15 c	4.97 ± 0.15 b	4.55 ± 0.11 b	4.88 ± 0.16 b
Lysine	0.30 ± 0.12 a	0.41 ± 0.02 ab	0.54 ± 0.05 b	0.62 ± 0.22 c	0.54 ± 0.13 b	0.71 ± 0.20 d	0.76 ± 0.04 d	0.61 ± 0.24 c	0.56 ± 0.22 ab
Threonine	0.65 ± 0.12 a	0.73 ± 0.01 a	0.79 ± 0.01 a	0.94 ± 0.02 b	0.77 ± 0.02 a	1.24 ± 0.13 c	0.99 ± 0.09 b	0.94 ± 0.08 b	0.87 ± 0.08 ab
Histidine	0.39 ± 0.02 a	0.13 ± 0.01 c	0.16 ± 0.02 c	0.16 ± 0.01 c	0.23 ± 0.06 b	0.06 ± 0.01 d	0.12 ± 0.01 c	0.14 ± 0.02 c	0.14 ± 0.10 c
Glutamine	1.10 ± 0.04 a	1.12 ± 0.01 a	1.30 ± 0.11 b	1.66 ± 0.01 c	1.46 ± 0.03 b	1.71 ± 0.05 c	1.71 ± 0.03 c	1.38 ± 0.03 b	1.49 ± 0.03 b
Glutamate	1.22 ± 0.03 a	0.82 ± 0.01 b	1.07 ± 0.01 ab	0.78 ± 0.01 b	1.11 ± 0.04 ab	0.60 ± 0.02 c	0.77 ± 0.03 b	0.76 ± 0.02 b	1.01 ± 0.02 ab
Taurine	19.58 ± 0.13 a	12.48 ± 0.01 e	14.59 ± 0.02 c	14.98 ± 0.33 c	13.77 ± 0.04 d	17.36 ± 0.20 b	17.75 ± 0.13 b	13.29 ± 0.11 d	13.26 ± 0.01 d
Glycine	6.89 ± 0.41 a	4.13 ± 0.07 d	5.54 ± 0.12 b	6.07 ± 0.10 a	6.56 ± 0.07 a	3.77 ± 0.32 e	5.70 ± 0.41 b	4.44 ± 0.20 c	5.85 ± 0.36 b
Tyrosine	0.34 ± 0.02 a	0.22 ± 0.04 b	0.24 ± 0.01 b	0.24 ± 0.01 b	0.28 ± 0.01 b	0.15 ± 0.02 c	0.18 ± 0.02 c	0.26 ± 0.03 b	0.25 ± 0.02 b
Creatine	20.33 ± 0.05 a	17.22 ± 0.03 c	17.70 ± 0.18 c	17.90 ± 0.06 c	20.52 ± 0.07 a	14.70 ± 0.13 d	17.22 ± 0.18 c	19.16 ± 0.26 a	18.33 ± 0.15 b
Arginine	5.14 ± 0.10 a	4.76 ± 0.17 a	4.83 ± 0.52 a	4.77 ± 0.17 a	4.64 ± 0.13 a	3.88 ± 0.24 b	4.43 ± 0.37 ab	4.95 ± 0.50 a	4.76 ± 0.31 a
Anserine	2.13 ± 0.01 a	1.71 ± 0.03 b	1.91 ± 0.06 a	1.77 ± 0.02 b	2.02 ± 0.02 a	1.41 ± 0.04 d	1.51 ± 0.06 d	1.56 ± 0.05 c	1.74 ± 0.06 b
Glycogen	37.06 ± 0.96 a	33.00 ± 0.07 b	35.49 ± 0.88 ab	36.19 ± 0.03 a	36.47 ± 0.13 a	9.69 ± 0.94 d	14.85 ± 1.00 c	14.79 ± 0.66 c	15.55 ± 0.89 c
Maltose	7.34 ± 0.10 a	8.74 ± 0.02 b	8.89 ± 0.11 b	9.84 ± 0.22 bc	8.34 ± 0.14 b	7.08 ± 0.05 a	7.65 ± 0.08 a	7.46 ± 0.19 a	7.86 ± 0.08 ab
α-glucose	0.51 ± 0.07 a	0.38 ± 0.01 b	0.34 ± 0.07 b	0.46 ± 0.01 a	0.48 ± 0.01 a	0.39 ± 0.02 b	0.39 ± 0.04 b	0.40 ± 0.05 b	0.44 ± 0.04 a
β-glucose	0.27 ± 0.02 a	0.13 ± 0.03 c	0.11 ± 0.04 c	0.20 ± 0.01 b	0.16 ± 0.03 b	0.14 ± 0.01 c	0.18 ± 0.02 b	0.14 ± 0.02 c	0.18 ± 0.03 b
Lactic acid	45.13 ± 2.31 a	69.64 ± 0.49 d	54.69 ± 0.07 b	59.70 ± 0.67 bc	50.49 ± 2.29 b	103.79 ± 2.28f	75.97 ± 1.11 e	64.50 ± 0.80 c	61.49 ± 0.54 c
Acetic acid	0.46 ± 0.11 a	3.47 ± 0.04 d	0.67 ± 0.02 b	0.80 ± 0.01 c	0.69 ± 0.09 b	3.55 ± 0.01 d	0.77 ± 0.02 bc	0.65 ± 0.01 b	0.58 ± 0.03 b
Succinic acid	0.63 ± 0.01 a	0.73 ± 0.05 b	0.73 ± 0.03 b	0.75 ± 0.02 b	0.68 ± 0.04 a	1.18 ± 0.01 d	0.82 ± 0.01 bc	0.74 ± 0.01 b	0.62 ± 0.01 a
2, 3-butanediol	0.60 ± 0.07 a	0.89 ± 0.01 c	0.70 ± 0.04 b	0.77 ± 0.04 b	0.74 ± 0.01 b	1.10 ± 0.03 d	0.75 ± 0.05 b	0.83 ± 0.07 bc	0.75 ± 0.01 b
Ethanol	0.31 ± 0.01 a	0.45 ± 0.02 b	0.44 ± 0.01 b	0.41 ± 0.01 b	0.42 ± 0.04 b	0.53 ± 0.01 c	0.49 ± 0.01 bc	0.45 ± 0.01b	0.44 ± 0.03b
ADP	0.32 ± 0.01 a	0.14 ± 0.04 c	0.15 ± 0.03 c	0.22 ± 0.02 b	0.19 ± 0.02 b	–	–	–	–
AMP	3.29 ± 0.04 a	0.42 ± 0.02 d	0.56 ± 0.07 c	0.71 ± 0.02 b	0.61 ± 0.01 c	–	–	–	–
IMP	2.49 ± 0.03 a	0.55 ± 0.03 d	1.27 ± 0.05 c	1.69 ± 0.07 b	1.55 ± 0.03 b	–	–	–	–
HxR	0.88 ± 0.01 a	1.20 ± 0.03 c	1.06 ± 0.03 c	0.94 ± 0.01 a	1.02 ± 0.04 b	1.96 ± 0.04 d	0.99 ± 0.01 b	0.86 ± 0.02 a	0.90 ± 0.03 a
Hx	0.18 ± 0.01 a	0.26 ± 0.01 b	0.26 ± 0.03 b	0.29 ± 0.04 b	0.27 ± 0.01 b	2.45 ± 0.43 d	2.43 ± 0.22 d	1.21 ± 0.06 c	1.23 ± 0.03 c
Putrescine	2.66 ± 0.12 a	4.17 ± 0.05 c	3.50 ± 0.02 b	3.67 ± 0.01 b	2.77 ± 0.03 a	4.78 ± 0.14 d	4.58 ± 0.11 d	3.85 ± 0.09 b	3.77 ± 0.15 b
Tyramine	0.64 ± 0.10 a	0.94 ± 0.07 b	0.66 ± 0.05 a	0.67 ± 0.16 a	0.68 ± 0.06 a	1.12 ± 0.04 c	0.94 ± 0.03 b	0.73 ± 0.06 a	0.69 ± 0.04 a
Histamine	0.21 ± 0.04 a	0.36 ± 0.01 b	0.41 ± 0.03 c	0.44 ± 0.05 c	0.37 ± 0.01 b	0.61 ± 0.05 d	0.49 ± 0.04 c	0.36 ± 0.02 b	0.33 ± 0.01 b
TMA	13.07 ± 1.55 a	16.09 ± 0.32 d	14.99 ± 1.01 b	14.82 ± 0.33 b	14.31 ± 0.25 b	20.02 ± 0.47 e	19.74 ± 0.73 c	16.13 ± 0.97 d	15.12 ± 0.61 c
TMAO	9.53 ± 0.53 a	7.77 ± 0.04 c	8.27 ± 0.28 b	7.61 ± 0.30 c	9.26 ± 0.29 a	5.35 ± 0.08 d	8.60 ± 0.24 b	7.70 ± 0.22 c	8.29 ± 0.31 b
Choline	16.96 ± 0.42 a	5.86 ± 0.01 c	5.27 ± 0.07 c	6.82 ± 0.02 d	13.24 ± 0.03 b	4.14 ± 0.09 e	6.86 ± 0.15 d	6.27 ± 0.20 d	10.37 ± 0.18 c

Within the same row, values with different lowercase letters are significantly different ($P < 0.05$). Note: FG: fish gelatin; GSE: grape seed extract; ADP: adenosine diphosphate; AMP: adenosine monophosphate; IMP: inosine monophosphate; HxR: inosine; Hx: hypoxanthine; TMA: trimethylamine; TMAO: trimethylamine oxide.

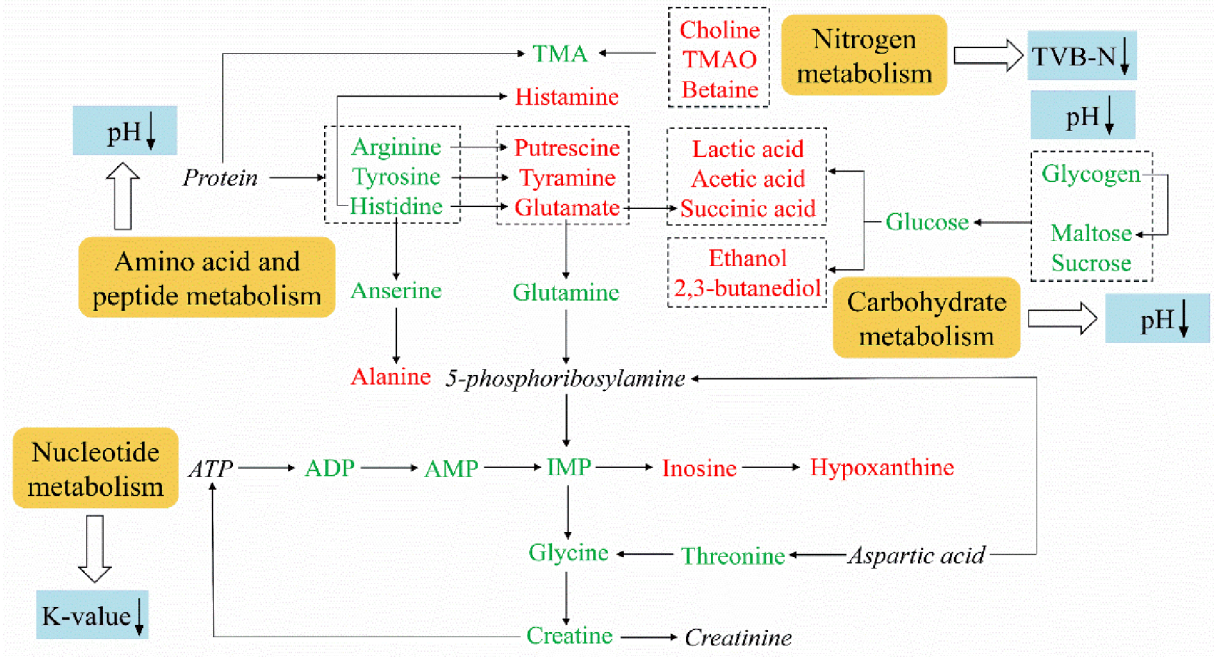

Fig. 5. Proposed schematic of the effect of vacuum impregnated FG and GSE coatings on the metabolic changes in chilled fillets during storage. Note: Metabolite in an italic font is not identified or quantified in the present study. Metabolites coloured in green and red represent a higher and lower level in tilapia fillets treated with vacuum impregnated FG and GSE coatings as compared to the control group, respectively.

time as compared to the control group ($P < 0.05$). It was in accordance with a previous study, which showed the addition of GSE into fish balls reduced the formation of alcohol and acid, contributing to unfavourable fishy odour, and thus maintaining the flavours during storage (Guan, Ren, Li, & Mao, 2019).

3.4. Effect of VI coating on quality and freshness of tilapia fillets

To better understand the effect of FG and GSE coatings on the quality and freshness of fillets, pH, TVB-N and K-value were analysed. Fig. S1A shows decreased pH values in the first several days followed by increase tendencies during storage which was consistent with a previous study (Qian et al., 2018). The initial decrease may be due to the degradation of glycogen or dissolution of CO_2 in fish fillets. The following increase was attributed to the formation of volatile basic amines like TMA by the activity of spoilage microbes or endogenous enzymes. The significant lower pH values of GSE and combined groups may be due to the antimicrobial abilities of GSE, which restrain microbial spoilage and thus inhibit the degradation of amino acid and formation of biogenic amines ($P < 0.05$) (Haskaraca, Juneja, Mukhopadhyay, & Kolsarici, 2019). These results were in accordance with a previous report on golden promfret coated with gelatin incorporating tea polyphenol (Feng et al., 2017).

Fig. S1B shows the increase trends of TVB-N values for all groups during refrigerated storage. The increase is mainly associated with the decomposition of amino acids, protein and some nitrogen-containing substances and corresponding formation of volatile bases like TMA by spoilage bacteria and endogenous enzymes (Yu et al., 2018). Based on a previous report, 15 mg/100 g was the level of rejection (Sun et al., 2019). For the control group, TVB-N value sharply increased from 2.92 mg/100 g on day 0 to 21.76 mg/100 g on day 12, suggesting the freshness of control group declined rapidly and gradually turned unacceptable. Additionally, combination group shows the lowest TVB-N value, followed by FG and control groups, and the difference between groups became significant at the end of storage ($P < 0.05$). The result suggested that vacuum impregnated FG containing GSE was more effective in restraining the increase of TVB-N values.

As shown in Fig. S1C, an immediate increase of K-value was observed in the first three days and then the rising trend became slower for all the samples. It can be noticed that the K-value was significantly reduced for the fillets treated with coating treatments (38.36–52.21% *vs.* 72.80% for the control). The relatively lower K-value may be due to the ability of FG and GSE to inhibit the activity of 5-nucleotidase to decompose IMP (Shi, Cui, Yin, Luo, & Zhou, 2014). Moreover, the lowest increase rate of K-value was found in the combination group, suggesting that the combined FG and GSE after VI-coating possessed the best preventive effect against nucleotide degradation.

3.5. Schematic illustration

Based on the results of multivariate statistical analyses and quantification, a schematic of the effect of vacuum impregnated FG and GSE on the metabolic changes of fillets during chilled storage was proposed, as shown in Fig. 5. The variations of metabolites can be divided into four major metabolic pathways, namely the amino acid and peptide pathway, nucleotide pathway, nitrogen pathway and carbohydrate pathway. Amino acids and peptides were released via protein hydrolysis, which were further transformed to other metabolites such as histamine (Moczkowska et al., 2017). The nitrogen metabolic pathway mainly refers to the formation of TMA and some other biogenic amines (histamine, tyramine etc.) via microbial decarboxylation of free amino acids (Jasour et al., 2018). The increase in the TVB-N level is mainly caused by the transformation of TMAO, choline and betaine towards TMA, which can cause fishy odour at low concentration and is regarded as an indicator of fish spoilage (Shumilina et al., 2015). Sugars (glycogen, maltose etc.) in fish underwent degradation by the hydrolysis and the glycolysis (Wang et al., 2017). The major by-products of carbohydrate metabolism detected were organic acids like lactic acid and alcohols like ethanol. These metabolic pathways jointly contributed to the changes of pH values. Adenine nucleotides undergo a series of gradual degradation from ATP, ADP, etc. towards inosine and hypoxanthine by the activities of endogenous enzymes and microorganisms during storage especially in the initial stage (Hong et al., 2017). The changes of 5 identified nucleotides using NMR was in accordance with

the K-value result even though ATP was not identified in this study. The vacuum impregnated FG and GSE collectively reduced the formation of biogenic amines, degradation of nucleotides, glycolysis of carbohydrates, hydrolysis of proteins and transformation of amino acids. The inhibited metabolic variations of chilled tilapia together with the reduced changes in pH, TVB-N and K-value indicate the potential of vacuum impregnated FG-GSE coating in preserving fish quality.

4. Conclusion

In conclusion, this study investigated the effect of vacuum impregnated FG and GSE on the metabolite profile of tilapia fillets during refrigerated storage. The NMR analysis provides comprehensive information about the changes that different metabolites suffered during storage and how these changes had been affected by different coatings. The NMR spectral data indicated that combined FG and GSE coating exerted significant protective effects against metabolic changes as compared to the control, FG and GSE groups. The combined coating significantly reduced changes in the contents of the main metabolites as well as inhibited the formation of harmful substances or undesirable compounds with off-odour. In addition, the inhibited changes of pH, TVB-N and K-value further verified the NMR results. These findings indicate that the combination of FG and GSE was effective in preserving the freshness and quality of tilapia fillets by reducing metabolic changes. This study provides insights to understand the relationship between metabolic changes and fish quality, and extends the applicability of NMR spectroscopy for fish by-products analysis. In the future, applying edible coating based on natural sources may be a trend as it satisfies consumers' increasing demands for healthy, high-quality and stable foods with clean label.

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Conflict of interest

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with this manuscript. We have no financial and personal relationships with other people or organizations that can inappropriately influence our work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2019.05.001.

References

Castejón, D., García-Segura, J. M., Escudero, R., Herrera, A., & Cambero, M. I. (2015). Metabolomics of meat exudate: Its potential to evaluate beef meat conservation and aging. *Analytica Chimica Acta, 901*, 1–11.

Chen, L., Tan, G. J. T., Pang, X., Yuan, W., Lai, S., & Yang, H. (2018). Energy regulated nutritive and antioxidant properties during the germination and sprouting of broccoli sprouts (*Brassica oleracea* var. *italica*). *Journal of Agricultural and Food Chemistry, 66*(27), 6975–6985.

Chen, L., Tan, J. T. G., Zhao, X., Yang, D., & Yang, H. (2019). Energy regulated enzyme and non-enzyme-based antioxidant properties of harvested organic mung bean sprouts (*Vigna radiata*). *LWT-Food Science and Technology, 107*, 228–235.

Chen, L., Wu, J. E., Li, Z., Liu, Q., Zhao, X., & Yang, H. (2019). Metabolomic analysis of energy regulated germination and sprouting of organic mung bean (*Vigna radiata*) using NMR spectroscopy. *Food Chemistry, 286*, 87–97.

Chen, D., Ye, Y., Chen, J., Zhan, P., & Lou, Y. (2017). Molecular nutritional characteristics of vinasse pike eel (*Muraenesox cinereus*) during pickling. *Food Chemistry, 224*, 359–364.

Choe, J. H., Choi, Y. M., Lee, S. H., Shin, H. G., Ryu, Y. C., Hong, K. C., et al. (2008). The relation between glycogen, lactate content and muscle fiber type composition, and their influence on postmortem glycolytic rate and pork quality. *Meat Science, 80*(2), 355–362.

Commission, E. (2007). Commission Regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. Off. J. Eur. Union L 322.

Fan, P., Huber, D. J., Su, Z., Hu, M., Gao, Z., Li, M., et al. (2018). Effect of postharvest spray of apple polyphenols on the quality of fresh-cut red pitaya fruit during shelf life. *Food Chemistry, 243*, 19–25.

Fan, H., Luo, Y., Yin, X., Bao, Y., & Feng, L. (2014). Biogenic amine and quality changes in lightly salt-and sugar-salted black carp (*Mylopharyngodon piceus*) fillets stored at 4 °C. *Food Chemistry, 159*, 20–28.

Feng, X., Ng, V. K., Mikš-Krajnik, M., & Yang, H. (2017). Effects of fish gelatin and tea polyphenol coating on the spoilage and degradation of myofibril in fish fillet during cold storage. *Food and Bioprocess Technology, 10*(1), 89–102.

Guan, W., Ren, X., Li, Y., & Mao, L. (2019). The beneficial effects of grape seed, sage and oregano extracts on the quality and volatile flavor component of hairtail fish balls during cold storage at 4 °C. *LWT-Food Science and Technology, 101*, 25–31.

Hanani, Z. A. N., Roos, Y. H., & Kerry, J. P. (2014). Use and application of gelatin as potential biodegradable packaging materials for food products. *International Journal of Biological Macromolecules, 71*, 94–102.

Haskaraca, G., Juneja, V. K., Mukhopadhyay, S., & Kolsarici, N. (2019). The effects of grapefruit seed extract on the thermal inactivation of *Listeria monocytogenes* in sous-vide processed döner kebabs. *Food Control, 95*, 71–76.

Hong, H., Regenstein, J. M., & Luo, Y. (2017). The importance of ATP-related compounds for the freshness and flavor of post-mortem fish and shellfish muscle: A review. *Critical Reviews in Food Science and Nutrition, 57*(9), 1787–1798.

Jasour, M. S., Wagner, L., Sundekilde, U. K., Larsen, B. K., Rasmussen, H. T., Hjermitslev, N. H., et al. (2018). Fishmeal with different levels of biogenic amines in aquafeed: Comparison of feed protein quality, fish growth performance, and metabolism. *Aquaculture, 488*, 80–89.

Li, T., Li, J., Hu, W., Zhang, X., Li, X., & Zhao, J. (2012). Shelf-life extension of crucian carp (*Carassius auratus*) using natural preservatives during chilled storage. *Food Chemistry, 135*(1), 140–145.

Li, D., Zhang, L., Song, S., Wang, Z., Kong, C., & Luo, Y. (2017). The role of micro-organisms in the degradation of adenosine triphosphate (ATP) in chill-stored common carp (*Cyprinus carpio*) fillets. *Food Chemistry, 224*, 347–352.

Lin, L., Regenstein, J. M., Lv, S., Lu, J., & Jiang, S. (2017). An overview of gelatin derived from aquatic animals: Properties and modification. *Trends in Food Science & Technology, 68*, 102–112.

Liu, Q., Wu, J. E., Lim, Z. Y., Aggarwal, A., Yang, H., & Wang, S. (2017). Evaluation of the metabolic response of *Escherichia coli* to electrolysed water by ^{1}H NMR spectroscopy. *LWT-Food Science and Technology, 79*, 428–436.

Liu, Q., Wu, J. E., Lim, Z. Y., Lai, S., Lee, N., & Yang, H. (2018). Metabolite profiling of *Listeria innocua* for unravelling the inactivation mechanism of electrolysed water by nuclear magnetic resonance spectroscopy. *International Journal of Food Microbiology, 271*, 24–32.

Maestre, R., Micol, V., Funes, L., & Medina, I. (2010). Incorporation and interaction of grape seed extract in membranes and relation with efficacy in muscle foods. *Journal of Agricultural and Food Chemistry, 58*(14), 8365–8374.

Moczkowska, M., Półtorak, A., Montowska, M., Pospiech, E., & Wierzbicka, A. (2017). The effect of the packaging system and storage time on myofibrillar protein degradation and oxidation process in relation to beef tenderness. *Meat Science, 130*, 7–15.

Nikmaram, N., Budaraju, S., Barba, F. J., Lorenzo, J. M., Cox, R. B., Mallikarjunan, K., et al. (2018). Application of plant extracts to improve the shelf-life, nutritional and health-related properties of ready-to-eat meat products. *Meat Science, 145*, 245–255.

Papageorgiou, M., Lambropoulou, D., Morrison, C., Kłodzińska, E., Namieśnik, J., & Płotka-Wasylka, J. (2018). Literature update of analytical methods for biogenic amines determination in food and beverages. *TrAC Trends in Analytical Chemistry, 98*, 128–142.

Paulsen, P., Grossgut, R., Bauer, F., & Rauscher-Gabernig, E. (2012). Estimates of maximum tolerable levels of tyramine content in foods in Austria. *Journal of Food & Nutrition Research, 51*(1), 52–59.

Qian, P., Zhang, Y., Shen, Q., Ren, L., Jin, R., Xue, J., et al. (2018). Effect of cryogenic immersion freezing on quality changes of vacuum-packed bighead carp (*Aristichthys nobilis*) during frozen storage. *Journal of Food Processing and Preservation, 42*(6), e13640–e13646.

Rauscher-Gabernig, E., Gabernig, R., Brueller, W., Grossgut, R., Bauer, F., & Paulsen, P. (2012). Dietary exposure assessment of putrescine and cadaverine and derivation of tolerable levels in selected foods consumed in Austria. *European Food Research and Technology, 235*(2), 209–220.

Romero-González, R., Alarcón-Flores, M. I., Vidal, J. L. M., & Frenich, A. G. (2012). Simultaneous determination of four biogenic and three volatile amines in anchovy by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry. *Journal of Agricultural and Food Chemistry, 60*(21), 5324–5329.

Shen, X., & Su, Y. C. (2017). Application of grape seed extract in depuration for decontaminating *Vibrio parahaemolyticus* in Pacific oysters (*Crassostrea gigas*). *Food Control, 73*, 601–605.

Shi, C., Cui, J., Yin, X., Luo, Y., & Zhou, Z. (2014). Grape seed and clove bud extracts as natural antioxidants in silver carp (*Hypophthalmichthys molitrix*) fillets during chilled storage: Effect on lipid and protein oxidation. *Food Control, 40*, 134–139.

Shi, C., Qian, J., Han, S., Fan, B., Yang, X., & Wu, X. (2018). Developing a machine vision system for simultaneous prediction of freshness indicators based on tilapia (*Oreochromis niloticus*) pupil and gill color during storage at 4 °C. *Food Chemistry, 243*,

134–140.
Shumilina, E., Ciampa, A., Capozzi, F., Rustad, T., & Dikiy, A. (2015). NMR approach for monitoring post-mortem changes in Atlantic salmon fillets stored at 0 and 4 °C. *Food Chemistry, 184*, 12–22.
Shumilina, E., Slizyte, R., Mozuraityte, R., Dykyy, A., Stein, T. A., & Dikiy, A. (2016). Quality changes of salmon by-products during storage: Assessment and quantification by NMR. *Food Chemistry, 211*, 803–811.
Sun, X., Guo, X., Ji, M., Wu, J., Zhu, W., Wang, J., et al. (2019). Preservative effects of fish gelatin coating enriched with CUR/βCD emulsion on grass carp (*Ctenopharyngodon idellus*) fillets during storage at 4 °C. *Food Chemistry, 272*, 643–652.
Sun, L., Sun, J., Thavaraj, P., Yang, X., & Guo, Y. (2017). Effects of thinned young apple polyphenols on the quality of grass carp (*Ctenopharyngodon idellus*) surimi during cold storage. *Food Chemistry, 224*, 372–381.
Sundekilde, U. K., Rasmussen, M. K., Young, J. F., & Bertram, H. C. (2017). High resolution magic angle spinning NMR spectroscopy reveals that pectoralis muscle dystrophy in chicken is associated with reduced muscle content of anserine and carnosine. *Food Chemistry, 217*, 151–154.
Wang, X., Li, J., Cong, J., Chen, X., Zhu, X., Zhang, L., et al. (2017). Preslaughter transport effect on broiler meat quality and post-mortem glycolysis metabolism of muscles with different fiber types. *Journal of Agricultural and Food Chemistry, 65*(47), 10310–10316.
Xiao, Z., Ge, C., Zhou, G., Zhang, W., & Liao, G. (2019). ^{1}H NMR-based metabolic characterization of Chinese Wuding chicken meat. *Food Chemistry, 274*, 574–582.
Yu, D., Xu, Y., Regenstein, J. M., Xia, W., Yang, F., Jiang, Q., et al. (2018). The effects of edible chitosan-based coatings on flavor quality of raw grass carp (*Ctenopharyngodon idellus*) fillets during refrigerated storage. *Food Chemistry, 242*, 412–420.
Zhang, L., Wang, P., Chen, F., Lai, S., Yu, H., & Yang, H. (2019). Effects of calcium and pectin methylesterase on quality attributes and pectin morphology of jujube fruit under vacuum impregnation during storage. *Food Chemistry, 289*, 40–48.