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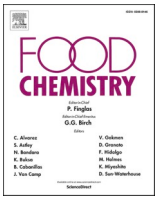

Changes in texture, rheology and volatile compounds of golden pomfret sticks inoculated with *Shewanella baltica* during spoilage

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ABSTRACT

Shewanella baltica has a high spoilage ability to decompose nutrients in fish. To investigate the role of *S. baltica* in fish protein and flavour during spoilage, the texture, rheology, protein patterns and volatile compounds of golden pomfret inoculated with *S. baltica* during 10-day storage were tested. During storage, *S. baltica* reduced the hardness of fish sticks by 29.73–49.24 %. Compared to the control (G_0': 20.27 ± 2.15 kPa), inoculated samples showed lower moduli (G_0': 16.71 ± 0.82–17.50 ± 1.80 kPa). Their myosin heavy chains, myosin-binding protein C and actin were decomposed into smaller proteins, which was validated by the lower intensities of molecules with M_w 160–176 kDa. Furthermore, *S. baltica* generated volatile spoilage markers, including dimethyl sulfide, 2-methyl-butanal and 3-methyl-butanal. This study reveals the mechanism of fish texture and flavour changes induced by *S. baltica*, and provides insights into controlling bacterial spoilage of seafood.

1. Introduction

Golden pomfret (*Trachinotus blochii*) is a marine fish with a slightly sweet taste and firm texture. It has been successfully spawned by Singapore Food Agency (SFA, previously known as Agri-Food & Veterinary Authority of Singapore) in tropical waters to enhance food security in Singapore and the annual supply of golden pomfret was expected to be over 80 tonnes (Feng, Zhu, Liu, Lai, & Yang, 2017). As with most other fish species, fresh golden pomfret can keep freshness at 4 °C for only 4–6 days, and the refreeze-rethaw process may generate negative effects on sensory perception, which pose a great challenge to commercial marketing.

Fish spoilage during chilled storage is mainly due to the activities of autolytic enzymes and spoilage microorganisms (Ghaly, Dave, Budge, & Brooks, 2010). Proteins make up 17–20 % of fish meat, which accounted for 17 % of the total intake of animal proteins (FAO, 2020). During spoilage, damage to muscle fibre occurs and myofibrillar proteins are degraded. The level of protein degradation can reflect the fish freshness and determine the quality of fish products.

Bacterial spoilage is dominating in the later period of fish spoilage (Ghaly et al., 2010). Zhou, Zheng, Liu, and Ma (2020) indicated that microbial flora is associated with the firmness of fish slices and the integrity of myofibrils. The specific spoilage organisms (SSOs) of fish have great potential to impair fish texture, since they could secrete various proteases and consume protein degradation products (Lou, Hai, Le, Ran, & Yang, 2023). As one of the SSOs of golden pomfret, *Shewanella baltica* has been reported to increase the levels of amino acids, biogenic amines and other metabolites during refrigerated storage, some of which were possibly derived from the degraded proteins (Lou, Zhai, & Yang, 2021). In addition, Leyva-Díaz, Poyatos, Barghini, Gorrasi, and Fenice (2017) reported that *S. baltica* KB30 showed the highest respiratory activity on proteolytic pathways, compared to the lipolytic and glycolytic pathways. Hence, it is essential to explore the effect of *S. baltica* on the protein degradation of golden pomfret during storage.

Moreover, the spoilage potential of SSOs refers to their ability to produce unpleasant volatile compounds, which are considered as the off-flavour (Gram & Dalgaard, 2002; Huang, Zhou, Chen, Huang, Li, & Hu, 2021). The specific volatile compounds that change significantly during spoilage can be evaluated as the freshness or spoilage markers of fish (Wierda, Fletcher, Xu, & Dufour, 2006). Vogel, Venkateswaran, Satomi, and Gram (2005) reported that *S. baltica* is the dominant H_2S producer in iced Danish marine fish. Up to now, little information is available on the flavour changes of golden pomfret sticks induced by *S. baltica* during spoilage.

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The objectives of this report were to investigate the role of *S. baltica* in the texture damage and off-flavour generation of fish spoilage, and to provide information for seafood spoilage controlling. Three *S. baltica* strains were inoculated on sterile golden pomfret sticks and samples were stored at 4 °C for up to 10 days. The changes in texture, rheology, protein patterns and volatile compounds of fish sticks during storage were analysed.

2. Materials and methods

2.1. Chemicals and reagents

Chemicals were bought from Sigma-Aldrich (St. Louis, MO, USA) with analytical grade. Luria-Bertani (LB) agar and broth were purchased from Oxoid (Basingstoke, UK). Laemmli sample buffer and precast gel for electrophoresis were purchased from Bio-Rad Laboratories (Hercules, CA, USA).

2.2. Preparation of sterile fish sticks

Golden pomfret (*Trachinotus blochii*) were bought from Song Fish Dealer Pte Ltd in Singapore and were transported to the laboratory within 1 h in an ice bath. Sterile fish sticks of golden pomfret were prepared according to our previous work (Lou et al., 2021). All sterile sticks were used within 1 h.

2.3. Inoculation and enumeration of S. baltica

Three *S. baltica* strains (ABa4, ABe2, and BBe1) were previously separated from golden pomfret. The cryopreserved bacteria were activated, plated and re-cultured in LB broth and on LB agar, which was the same as the description in the study of Lou et al. (2021). Around 4 log CFU/g of *S. baltica* cells were inoculated onto fish sticks. Samples were dried for 5 min and stored at 4 °C for 10 days.

The bacterial counts of inoculated fish sticks on days 0, 4 and 10 were determined (Lou et al., 2021). Total viable count (TVC) was determined by plating on LB agar and expressed as log CFU/g.

2.4. Texture profile analysis (TPA)

Fish sticks were cut into small cubes ($1 \times 1 \times 1$ cm^3) and analysed by a TA-XT2i Texture analyser (Stable Micro Systems, Surrey, UK) with a P35 probe, according to Zhao, Zhou, Zhao, Chen, He, and Yang (2019). The cubes were set on the platform and compressed to 50 % of deformation with the following parameters: pre-test speed: 2.0 mm/s; test speed: 1.0 mm/s; trigger force: 0.05 N; post-test speed: 1.0 mm/s. Four characteristics including hardness, springiness, gumminess, and chewiness were obtained from TPA curves.

2.5. Rheological analysis

2.5.1. Extraction of protein isolate

The protein isolate of fish sticks was extracted according to the method of Zhou and Yang (2019) with slight modifications. In short, minced fish sticks were homogenised with cold DI water at a ratio of 1:3 (w/v) for 1.5 min and kept in an ice bath for 15 min, followed by centrifugation at 10,000×*g* for 10 min at 4 °C. This procedure was repeated twice. Then the precipitate was stirred with chilled 0.5 % NaCl solution at a ratio of 1:3 (w/v) and centrifugated at 12,000×*g* for 10 min at 4 °C. The precipitate was obtained as the protein isolate.

2.5.2. Rheological measurement

The rheological properties of protein isolate were performed according to Sow, Tan, and Yang (2019) and Liu, Zhang, Xue, and Xue (2019) with slight modifications. An Anton Paar MCR 102 controlled-stress rheometer (Anton Paar, Graz, Austria) with a 25 mm diameter parallel plate was applied and each sample was put onto the sample platform at 25 °C for 10 min for equilibrium. The gap between the plate and sample platform was 1 mm, and silicone oil was used to prevent moisture evaporation.

The frequency sweep was done with 1–100 rad/s at 0.1 % strain. The strength of protein isolate (A_n) was obtained after the fitness of the following power law models (Eqs. (1), (2), (3)) (Liu et al., 2019).

$$G' = G'_0\omega^{n'} \tag{1}$$

$$G'' = G''_0\omega^{n''} \tag{2}$$

$$G^* = \sqrt{G'^2 + G''^2} = A_n\omega^n \tag{3}$$

where G' is the storage modulus; G" is the loss modulus; G* is the complex modulus; A_n is the complex moduli at 1 rad/s; n', n" and n are the dynamic power-law factors for G', G' and G*, respectively; ω is the angular frequency.

The temperature sweep was performed from 5 to 90 °C at 3.4 °C/min with 0.1 % strain and 1 rad/s frequency. The creep-recovery sweep was performed at 25 °C after the temperature sweep. A constant shear stress of 200 Pa was applied from 0 to 177 s as creep phase. Then the stress was removed and the change was measured from 177 to 352 s as recovery phase. The results were fitted into Burgers model for creep and recovery phases using Eqs. (4) and (5), respectively (Sow et al., 2019).

$$J(t)_c = J_{0c} + \sum_{i=1}^{3}\left[Jm_{ic}\left(1 - e^{\frac{-t}{\lambda_{ic}}}\right)\right] + \frac{t}{\eta_0} \tag{4}$$

$$J(t)_r = J_{max} - J_{0r} - \sum_{i=1}^{3}\left[Jm_{ir}\left(1 - e^{\frac{-t}{\lambda_{ir}}}\right)\right] \tag{5}$$

where J(t) is the compliance; J_0, Jm and J_{max} are the instantaneous, viscoelastic and maximum compliance, respectively; t is the time; λ is the retardation time; η_0 is the zero-shear viscosity.

2.6. Extraction of myofibrillar protein

The extraction of myofibrillar protein was using the method according to Zhao et al. (2019) with slight modifications. In short, 2 g fish stick was minced and homogenised with 20 mL of solution A (20 mmol/L Tris-HCl buffer containing 0.10 mol/L KCl, 1 mmol/L phenylmethylsulfonyl fluoride, 0.02 % NaN_3, pH 7.5), followed by incubation at 4 °C for 15 min and centrifugation at 10,000 × *g* for 10 min. The pellet was homogenised with 10 mL of solution B (20 mmol/L Tris–maleate buffer containing 0.45 mol/L KCl, 5 mmol/L β-mercaptoethanol, 1 mmol/L ATP,1 mmol/L N,N,N,*N*-tetraacetic acid (EGTA), 0.2 mol/L Mg $(CH_3COO)_2$, pH 6.8), and recentrifuged at 10,000 × *g* for 10 min. The supernatant was myofibrillar protein extract, which was stored at 4 °C and used within 48 h.

2.7. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE)

The degradation degree of myofibrillar proteins was analysed by SDS-PAGE according to Zhao et al. (2019). Samples were loaded into a Miniprotein® TGX™ precast gel, and the electrophoresis was performed on a Bio-Rad mini-PROTEAN tetra Cell (Hercules, CA, USA). The image was photographed by ChemiDoc MP imaging system (Bio-Rad, Hercules, CA, USA). The images were analysed using ImageJ for band intensity.

2.8. Volatile compounds analysis

Volatile compounds were determined according to Feng, Zhu, et al. (2017) using headspace solid-phase microextraction-gas chromatography-mass spectrometry (HS/SPME/GC–MS) with slight modifications. Two grams of minced fish sticks were put in a 20 mL flat

bottomed headspace vial and 3 mL of 10^{-5} mol/L of 2-methylpentanal (internal standard) in saturated NaCl solution was added and mixed with the fish meat. Shimadzu GCMS-QP200 Ultra Gas Chromatography-Mass Spectrometer coupled with a Shimadzu AOC-5000 Autosampler (Shimadzu Corporation, Kyoto, Japan) was applied with a DB-WAX column (60 m × 0.25 mm × 0.25 mm, Agilent Technologies, Santa Clara, CA, USA). The SPME fibre (Supelco 57298-U, Sigma-Aldrich, St. Louis, MO, USA) was used to adsorb volatile compounds for 30 min and introduced into the injector port at 250 °C with a splitless injection mode for 1 min. The chromatographic parameters were listed as follows: initial oven temperature was set at 35 °C for 5 min, then ramped to 100 °C at 3 °C/min and kept for 4 min. Then the oven temperature was ramped at 5 °C/min to 210 °C and held for 20 min. The compounds identification was performed by the comparison of mass spectra in the databases of NIST08, NIST12, NIST21, NIST62 and NIST147. The retention index (RI) of each compound was calculated according to the retention times of the compound and *n*-alkanes standard (C8-C20). The semi-quantification was calculated via the ratio of the target peak area to the peak area of the internal standard.

2.9. Matrix-assisted laser desorption ionisation time-of-flight mass spectrometry (MALDI-TOF-MS)

MALDI-TOF-MS was performed according to Feng, Bansal, and Yang (2016) with slight modifications. The matrix was saturated sinapic acid in 60 % acetonitrile at room temperature. The myofibrillar protein samples from Section 2.6 were serially diluted (1/4, v/v) with the matrix on a MALDI target plate and air-dried in a fume hood at room temperature before analysing using a Bruker autoflex MALDI-TOF/TOF Mass Spectrometer with a smart beam laser (Bruker Daltonics, Billerica, MA, USA). Mass spectra from 10,000 to 180,000 *m/z* were obtained and analysed using flexAnalysis software (Bruker Daltonics, Billerica, MA, USA).

2.10. Statistical analysis

All tests were done independently in triplicate. Analysis of variance (ANOVA) was performed by using Duncan's multiple range test in XLSTAT (AddinSoft, New York, NY, USA). The difference of $P < 0.05$ was set as the significant difference.

3. Results and discussion

3.1. Microbial enumeration

The TVC of fish sticks inoculated with *S. baltica* during storage is shown in Fig. S1. All of the initial TVCs of three strains were about 3.6 log CFU/g, which was congruent with the report that the TVCs of fresh seafood were 3–4 log CFU/g (Zhang, Ding, Gu, Zhu, Zhou, & Ding, 2020). The final TVCs of strain ABa4, ABe2 and BBe1 were 8.35, 8.30, and 8.12 log CFU/g, respectively. It is regarded that the sensory rejection limit is reached when the TVC is over 7 log CFU/g (López-Caballero, Huidobro, Pastor, & Tejada, 2002). According to Lou et al. (2021), TVCs of all strains were higher than 7 log CFU/g after day 4. In our result, the storage time from day 0 to day 4 could be regarded as the period of early spoilage, while the later spoilage occurred during day 4–10.

3.2. Changes in texture of fish sticks inoculated with S. baltica during spoilage

Fig. 1 demonstrates the texture profiles of fish sticks inoculated with *S. baltica* on days 0, 4 and 10. The hardness of fish sticks without inoculation decreased from 1,682.39 g on day 0 to 1,055.28 g on day 10 (Fig. 1a). In inoculated samples, the hardness decreased more dramatically than in uninoculated sticks on day 10, down to 556.76–741.58 g (decrement 29.73–49.24 % compared to the control). The hardness of the control was significantly higher than that of inoculated sticks on both day 4 and day 10 ($P < 0.05$), while there was no significant

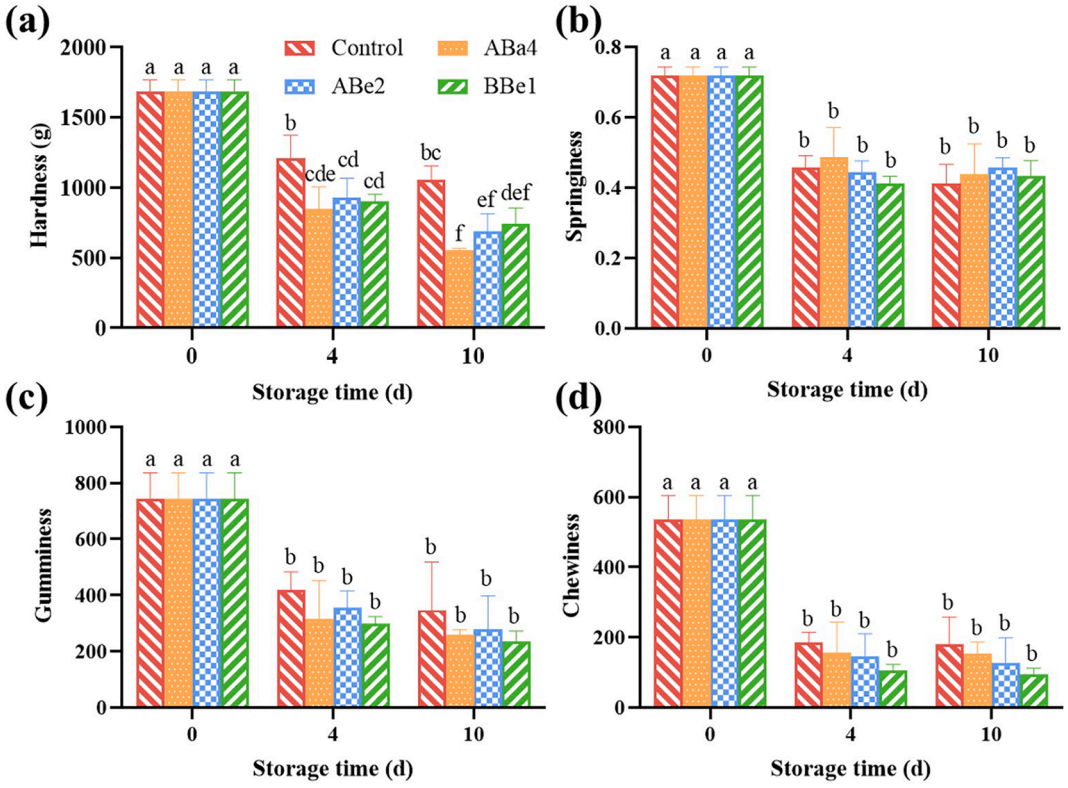

Fig. 1. Changes of texture of golden pomfret sticks inoculated with *S. baltica* during spoilage (a) Hardness; (b) Springiness; (c) Gumminess; (d) Chewiness. Different letters indicate a significant difference ($P < 0.05$).

difference in the hardness of sticks inoculated with different strains on the same day. It has been reported that the hardness loss was attributed to the weakness of connective tissue and muscle fibres (Lakshmanan, Piggott, & Paterson, 2003). The autolytic enzymes accelerated the protein denaturation, destroyed the connective tissue structure and the activity of spoilage microorganisms promoted the development of the structural breakdown of muscle (Viji et al., 2015). Feng, Ng, Mikš-Krajnik, and Yang (2017) reported that the height, weight and diameter of myofibrillar proteins decreased during storage, which was related to the decrease in fillet hardness. Lou et al. (2023) found that *S. baltica* could produce Lon protease during growth. In our results, the hardness decrease of uninoculated sticks was mainly due to the enzymatic autolysis, while the hydrolysis of proteins was promoted by the extracellular proteases secreted by *S. baltica*.

Springiness demonstrates the elasticity of the sample after compression. The springiness of fish sticks on day 0 was 0.72 (Fig. 1b). It decreased significantly after storage in both uninoculated and inoculated fish sticks. No significant difference was observed between all groups on day 4 and day 10 ($P > 0.05$), indicating that springiness might be mainly affected by enzymatic autolysis during early spoilage. Gumminess and chewiness in the fish sticks rapidly decreased after storage ($P < 0.05$), compared to those in the sample on day 0 (Fig. 1c and 1d). In addition, the longer storage after 4 days did not change the gumminess and chewiness in all fish sticks ($P > 0.05$). These results showed that little influence on springiness, gumminess and chewiness of golden pomfret sticks was caused by *S. baltica* during storage. Similar trends were also observed by Viji et al. (2015), who reported decreased springiness, gumminess and chewiness in gutted sutchi catfish during the first 12-day storage.

3.3. Changes in rheology of protein isolate from fish sticks inoculated with *S. baltica* during spoilage

The rheological behaviours of protein isolate from golden pomfret sticks inoculated with *S. baltica* during spoilage are shown in Fig. 2. The storage modulus (G') represents the elastic portion of the material, which means the stored mechanical energy after a force, while the loss modulus (G") characterises the viscous portion, showing the dissipated energy as heat. In both frequency and temperature sweeps, G" was always much lower than G' and showed the same trend as G', and thus only G' was shown in Fig. 2a and 2b.

As the frequency (ω) increased, G' increased continuously (Fig. 2a). The control on day 0 had the highest G', suggesting that the protein isolate before storage presented the greatest elasticity and the relatively complete myofibrillar protein structure could withstand higher shearing force, which was consistent with the texture results of fish sticks. Table S1 shows the parameters of frequency sweep after the fitness of Eqs. (1), (2) and (3). All protein isolates showed low n', n'', and n values, indicating that the systems were closer to an elastic solid, since when n = 0, the material is a pure elastic solid (Sow et al., 2019). During storage, G_0', G_0'', and A_n decreased significantly, while n', n'', and n increased ($P < 0.05$). The values of G_0'' in inoculated sticks were lower than those in uninoculated sticks on day 10 ($P < 0.05$). However, no significant difference was observed between the values of G_0' and A_n of inoculated and uninoculated samples after storage ($P > 0.05$). These changes illustrated that storage and *S. baltica* could impair the structural strength of the protein isolate network. Protein isolate inoculated with strain BBe1 on day 10 showed the lowest values of G', G", and A_n, which reflected that less energy was stored and lost during shear (Liu et al., 2019). On the other hand, the values of n', n'' and n in sticks inoculated with strain ABa4 on day 4 and day 10 were the highest, suggesting a high viscoelasticity (Campo-Deaño, Tovar, Pombo, Solas, & Borderías, 2009). The significant difference in parameters of frequency sweep indicated protein denaturation and degradation induced by *S. baltica*.

As shown in Fig. 2b, G' of inoculated protein isolate under the same temperature decreased during storage, especially the sample inoculated with strain ABa4 on day 10. Zhou and Yang (2019) reported that there were 4 stages of surimi protein during temperature sweep: (1) first increase, (2) first decrease (gel weakening), (3) second increase (gel strengthening), and (4) second decrease. However, only the last three stages were observed in our temperature sweep (Fig. 2b). The temperatures of the inflection points between stages (2) and (3), and between stages (3) and (4) were named as T_1 and T_2, respectively. The values of T_1 and T_2 as well as their G' values are shown in Table S2.

It has been reported that the first increase in the early stage was owing to myosin head-head interactions, and the first decrease was related to the breaking of reversible polar interactions, which caused the denaturation of light meromyosin and the dissociation of actomyosin (Campo-Deaño et al., 2009). The first increase in this study was not distinct, which might be because the degree of heat-induced protein

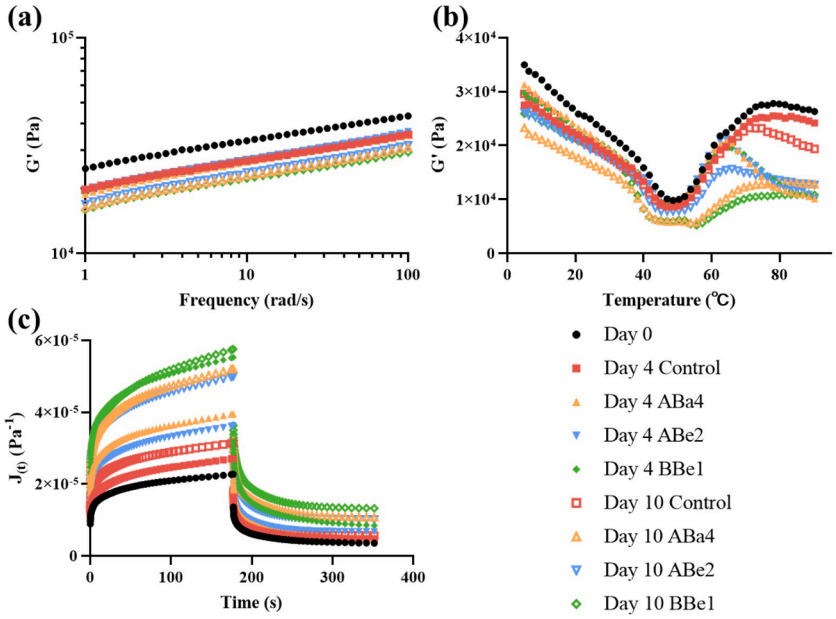

Fig. 2. Changes of rheology of protein isolate from golden pomfret sticks inoculated with *S. baltica* during spoilage. (a) Frequency sweep; (b) Temperature sweep; (c) Creep-recovery sweep.

denaturation was stronger than myosin head-head interaction in our protein isolate. Yin and Park (2015) demonstrated that the second increase in both G' and G" was due to the thermal aggregation of myofibril. The lower T_2 of protein isolate inoculated with *S. baltica* might be due to fewer bonds would be formed at high temperatures. The second decrease was attributed to the denaturation and aggregation of more thermostable protein than myosin (Zhou & Yang, 2019). Protein isolates before storage showed the highest G_1' and G_2' at 9.82×10^3 and 27.94×10^3 Pa, respectively. The values of G_1' and G_2' decreased in uninoculated protein isolate during storage ($P < 0.05$), suggesting that the endogenous proteases in fish tissue were still activated to impair the completeness of fish muscle during chilled storage. Compared to those of the control, G_1' and G_2' of inoculated protein on day 4 and day 10 were lower, which demonstrated that a weaker gel system was built in inoculated samples during heating (Sow et al., 2019). It might be related to the further degradation of myofibrillar proteins caused by microorganisms, leading to a decrease in the gelling ability with lower intramolecular and intermolecular interactions. Our results were in line with Mehta, Balange, Lekshmi, and Nayak (2017), who reported that the G' of Indian squid mantle proteins in temperature sweep decreased as the storage time increased.

In the creep-recovery test (Fig. 2c), *S. baltica* increased the compliance (J) of protein isolates during spoilage. The parameters for the creep and recovery phases are presented in Tables S3 and S4, respectively. A strong negative relationship between hardness and J was reported in a previous study (Jingjing Huang, Zeng, Xiong, & Huang, 2016). J(t) increased as the storage time increased, suggesting that storage induced a softer gel structure to resist deformation. J_{0c} and J_{0r} presented the instantaneous deformation when the shear stress was applied and removed, respectively; Jm_c and Jm_r reflected the cohesive force within the surimi network structure under (J_c) or without (J_r) shear stress, respectively; λ reflected the elastic response of gels; J_{max} showed the maximum deformation at the end of creep (Juszczak, Witczak, Ziobro, Korus, Cieślik, & Witczak, 2012). In our results, the control showed the lowest J_{0c}, J_{max} and J_{0r} at 8.07×10^{-6} Pa^{-1}, 22.62×10^{-6} $Pa^{-1}$and 8.50×10^{-6} Pa^{-1} (Tables S3 and S4), respectively. These parameters were significantly higher in inoculated protein than in the control on the same day ($P < 0.05$) and were highlighted in the sticks inoculated with strain BBe1. Jm for both creep and recovery phases were observed with a similar trend to J_0, indicating a tender network of protein isolate inoculated with *S. baltica* during these phases.

3.4. Changes in protein pattern of myofibrillar protein from fish sticks inoculated with S. baltica during spoilage

As shown in Fig. 3, myofibrillar proteins in golden pomfret sticks were mainly composed of myosin heavy chain (MHC), myosin-binding protein C (MyBP-C), actin, troponin-T (Tn-T) and myosin light chain (MLC). The intensities of these bands are presented in Table S5. Compared to those of the control, MHC bands of inoculated samples were lighter (Fig. 3) and the MHC band intensities were lower (Table S5), indicating that MHC was decomposed by *S. baltica* during storage. The molecular masses around 140 kDa could be deemed to be MyBP-C, which showed a similar trend as MHC (Heling, Geeves, & Kad, 2020). It has been reported that full-length actin was associated with the protein with molecular masses of 42 kDa in myofibrillar protein (Koubassova & Tsaturyan, 2011). The degradation of actin was obvious, although the Tn-T band appeared under actin at 36 kDa (Lametsch, Roepstorff, Møller, & Bendixen, 2004), became darker during early spoilage and turned lighter after further storage. Tn-T is a regulator of the actin-myosin interaction and is regarded as a marker of myosin degradation in animal muscle (Li, Xu, & Zhou, 2012). However, the degradation level of MLC by *S. baltica* was not significant.

3.5. Changes in volatile compounds of fish sticks inoculated with S. baltica during spoilage

Table 1 shows the semi-quantification results of different volatile compounds from fish sticks inoculated with *S. baltica* during spoilage. A total of 31 volatile compounds of golden pomfret were identified and quantified. All volatile compounds were divided into six groups according to their types, including 7 aldehydes, 2 ketones, 10 alcohols, 5 hydrocarbons, 4 esters/acids and 3 other compounds.

In uninoculated fish sticks, the contents of volatile compounds decreased during storage, except ethanol, hexane and 2-pentyl-furan during early spoilage, as well as 1-pentanol, 1-hexanol, and 1-octen-3-ol during later spoilage. In addition, several volatile compounds, such as 2-methyl-pentanol and 2-methyl-butanoic acid ethyl ester, were not detected before storage but appeared on day 10. These results showed that storage time could affect the volatile profile in fish sticks, which could be attributed to autolytic spoilage and flavour release.

Compared to the control on day 4, *S. baltica* induced a significant increase in most alcohols, accompanied with a decline of ketones, hydrocarbons and most aldehydes. Such an enhancement was prominent in the stick inoculated with strain BBe1, approximate 5-fold in 2-methyl-butanal, 7.5-fold in 3-methyl-butanal and 19-fold in 3-methyl-butanol. Among declined compounds in sticks inoculated with different strains, propanal, pentanal, hexanal and nonanal decreased by 43.39–92.08 %; 2,3-pentanedione and hydrocarbons decreased by 66.49–88.66 %. Furthermore, 1-penten-3-one totally disappeared in inoculated sticks. In the period of later spoilage, almost all volatile compounds in inoculated sticks showed decreased concentrations, except 3-methyl-butanal and dimethyl disulfide, compared to the control.

The most abundant aldehydes in golden pomfret were hexanal and nonanal, which contributed to the grassy and fatty aroma (Li, Wu, Li, & Dai, 2013). Most C6-C10 carbonyl compounds and alcohols were involved in the enzymic catabolism of unsaturated fatty acids, and some were related to the autoxidation of polyunsaturated fatty acids (Zhang et al., 2020). Additionally, ethanol and 1-octen-3-ol were reported to be produced by bacteria (Parlapani, Mallouchos, Haroutounian, & Boziaris, 2017). Wierda et al. (2006) mentioned that 1-penten-3-ol, hexanal, and octanal were identified as markers for fish freshness because they

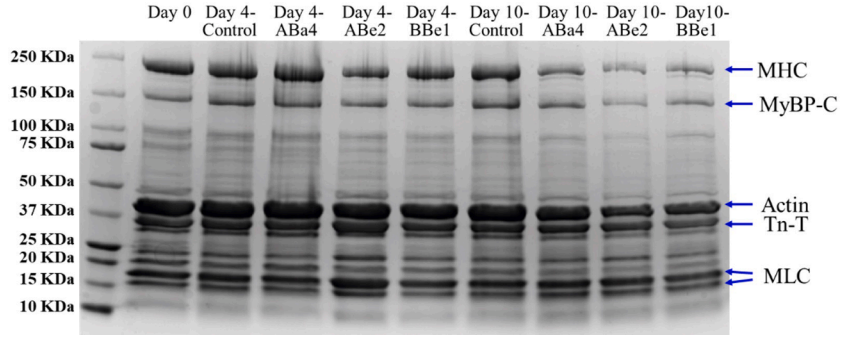

Fig. 3. Protein patterns of myofibrillar proteins in golden pomfret sticks inoculated with *S. baltica* during spoilage.

Table 1
Volatile compounds of golden pomfret sticks inoculated with *S. baltica* during spoilage.

Compound (μg/kg)	RI[1]	Threshold (μg/kg)[2]	Day 0	Day 4				Day 10			
				Control	ABa4	ABe2	BBe1	Control	ABa4	ABe2	BBe1
Aldehydes											
Propanal	792	15.1	24.27^{a}	19.64^{b}	4.99^{cd}	3.91^{cd}	6.08^{c}	5.26^{cd}	1.73^{cd}	3.39^{cd}	0.36^{d}
2-Methyl-butanal	905	1	3.32^{b}	7.23^{b}	16.57^{b}	12.46^{b}	41.91^{a}	1.92^{b}	8.25^{b}	9.60^{b}	9.99^{b}
3-Methyl-butanal	910	0.2	2.54^{c}	3.38^{c}	10.43^{bc}	7.42^{bc}	29.35^{a}	1.17^{c}	9.27^{bc}	16.97^{b}	10.00^{bc}
Pentanal	967	12	40.71^{a}	41.60^{a}	12.64^{bcd}	13.19^{bcd}	19.49^{bc}	15.94^{bcd}	8.57^{cd}	23.92^{b}	5.63^{d}
Hexanal	1055	5	1787.66^{a}	1419.27^{a}	471.68^{bcd}	596.46^{bc}	803.39^{b}	471.25^{bcd}	228.92^{cd}	433.74^{bcd}	66.93^{d}
Heptanal	1147	2.8	26.72^{ab}	28.50^{a}	23.26^{ab}	23.67^{ab}	25.83^{ab}	21.25^{ab}	12.55^{ab}	17.07^{ab}	6.00^{b}
Nonanal	1403	1.1	119.21^{a}	89.60^{a}	103.37^{a}	7.10^{b}	25.82^{b}	29.86^{b}	18.33^{b}	12.46^{b}	16.93^{b}
Ketones											
1-Penten-3-one	1004	1.3	8.21^{a}	2.57^{b}	ND	ND	ND	0.86^{b}	ND	ND	ND
2,3-Pentanedione	1037	5.13	102.06^{a}	75.24^{b}	25.22^{cd}	17.11^{cd}	23.60^{cd}	62.20^{b}	11.24^{cd}	29.20^{c}	5.35^{d}
Alcohols											
Ethanol	932	100,000	54.87^{c}	110.20^{b}	263.74^{a}	136.71^{b}	130.73^{b}	15.20^{c}	0.70^{c}	2.46^{c}	1.52^{c}
1-Penten-3-ol	1137	358.1	176.58^{a}	122.37^{abc}	47.80^{d}	16.96^{d}	67.50^{cd}	168.76^{ab}	40.33^{d}	87.00^{bcd}	15.74^{d}
2-Methyl-butanol	1174	15.9	6.42^{c}	7.55^{c}	24.11^{b}	67.18^{a}	62.32^{a}	2.50^{c}	0.67^{c}	0.84^{c}	0.46^{c}
3-Methyl-butanol	1176	4	5.12^{d}	4.24^{d}	32.35^{c}	67.79^{b}	85.69^{a}	8.06^{d}	2.96^{d}	4.66^{d}	6.28^{d}
1-Pentanol	1220	150.2	44.63^{a}	17.56^{b}	8.52^{b}	12.48^{b}	14.76^{b}	69.81^{a}	6.40^{b}	14.92^{b}	2.56^{b}
2-Methyl-pentanol	1275		ND	ND	ND	ND	ND	25.96^{b}	22.56^{b}	59.30^{a}	30.36^{b}
2-Penten-1-ol	1297	720	33.93^{a}	0.88^{b}	0.97^{b}	0.78^{b}	1.17^{b}	4.06^{b}	2.59^{b}	2.82^{b}	0.40^{b}
1-Hexanol	1336	5.6	20.19^{b}	1.21^{c}	1.04^{c}	10.78^{bc}	6.37^{bc}	42.83^{a}	8.93^{bc}	47.14^{a}	3.10^{bc}
1-Octen-3-ol	1444	1	90.10^{c}	37.83^{def}	20.43^{ef}	6.54^{f}	35.32^{def}	197.25^{b}	62.72^{cd}	225.02^{a}	45.54^{de}
2-Ethyl-hexanol	1489	270,000	ND	ND	1.12^{d}	19.52^{a}	53.26^{a}	44.93^{a}	10.12^{bcd}	7.71^{cd}	16.02^{bc}
Hydrocarbons											
Hexane	–		2.83^{b}	8.76^{a}	1.37^{b}	1.33^{b}	1.94^{b}	1.05^{b}	1.07^{b}	0.89^{b}	1.00^{b}
Heptane	700	50,000	5.85^{a}	7.15^{a}	0.94^{b}	1.52^{b}	1.28^{b}	1.40^{b}	1.39^{b}	1.65^{b}	0.46^{b}
Octane	800		17.72^{a}	24.06^{a}	4.15^{b}	3.29^{b}	2.73^{b}	2.80^{b}	4.23^{b}	6.12^{b}	1.96^{b}
Nonane	900	10,000	12.34^{a}	14.55^{a}	2.00^{b}	4.83^{b}	2.14^{b}	3.87^{b}	3.11^{b}	3.83^{b}	1.59^{b}
Decane	1000	10,000	28.71^{a}	27.15^{a}	7.40^{bc}	8.78^{bc}	7.02^{bc}	12.72^{b}	8.37^{bc}	12.69^{b}	5.13^{c}
Esters/acids											
2-Methyl-butanoic acid ethyl ester	1032		ND	ND	ND	ND	ND	3.18^{b}	0.97^{c}	4.09^{a}	1.67^{c}
3-Methyl-butanoic acid ethyl ester	1045		ND	ND	ND	ND	ND	4.41^{b}	0.89^{c}	6.79^{a}	1.81^{c}
Hexanoic acid	1890	3000	ND	ND	ND	3.99^{abc}	7.79^{a}	7.46^{ab}	2.09^{c}	3.47^{abc}	2.49^{bc}
Nonanoic acid	–	3000	27.79^{ab}	46.81^{a}	11.29^{ab}	39.23^{ab}	26.19^{ab}	2.71^{b}	5.44^{b}	2.13^{b}	3.20^{b}
Others											
2-Ethyl-furan	943	2.3	19.78^{a}	18.20^{a}	3.37^{b}	4.46^{b}	7.60^{b}	7.74^{b}	2.43^{b}	8.43^{b}	1.42^{b}
Dimethyl disulfide	1047	1.1	ND	ND	ND	ND	ND	6.46^{c}	4.95^{c}	49.00^{a}	24.16^{b}
2-Pentyl-furan	1194	6	12.06^{b}	19.45^{a}	4.84^{c}	4.42^{c}	3.43^{c}	9.40^{c}	3.80^{c}	5.14^{c}	3.66^{c}

[1]RI: retention index. RIs of compounds were validated according to NIST Chemistry WebBook (https://webbook.nist.gov/chemistry/).
[2]Thresholds were mainly obtained from the literatures and online databases: https://www.leffingwell.com/odorthre.html; https://www.flavornet.org/flavornet.html.
[a–f] Different letters mean significant difference among different samples ($P < 0.05$, along the rows).

decreased dramatically after storage. In our results, aldehydes, ketones, and some alcohols were the crucial compounds to fish flavour, while hydrocarbons and esters/acids contributed little to the overall odour due to their high threshold (Table 1). The decrease of some carbonyl compounds and alcohols in inoculated sticks indicated the loss of fish freshness induced by *S. baltica*. Our results were in agreement with Edirisinghe, Graffham, and Taylor (2007), who found that hexanal, heptanal and nonanal showed high amounts in fresh fish, but decreased as the storage time increased.

On the other hand, dimethyl sulfide, 2-methyl-butanal and 3-methyl-butanal could be regarded as the spoilage markers of fish (Leduc et al., 2012). Dimethyl sulfide could be derived from the transformation of dimethylsulfoniopropionate, Met and S-methylmethionine, which could be enhanced by bacterial activity and cell lysis (Curson, Sullivan, Todd, & Johnston, 2010; He et al., 2018). Giri, Osako, and Ohshima (2010) reported that 2-methyl-butanal and 3-methyl-butanal mainly originated from the biodegradation of amino acids, like Leu and Ile. In this study, dimethyl sulfide was undetectable in the period of early spoilage but was found in samples on day 10, especially in the stick inoculated with ABe2. The high contents of dimethyl sulfide, 2-methyl-butanal and 3-methyl-butanal in inoculated sticks indicated that *S. baltica* was able to produce spoilage-related volatile compounds during storage.

3.6. Schematic illustration

Based on the above results, an assumptive schematic (Fig. 4) is proposed to show the effect of *S. baltica* on the muscle fibre and flavour compounds of golden pomfret during storage. Before storage, the fish muscle fibre and myofibril were intact, and the myosin and actin regularly formed the thick and thin filaments, respectively. After 10-day storage, the myofibril of uninoculated sticks unfolded to a minor degree, which could be attributed to the activity of autolytic enzymes in fish muscle cells (Viji et al., 2015). The fish sticks inoculated with *S. baltica* after 10-day storage were significantly different from the control. The muscle fibre and myofibril structures were destroyed dramatically. Myosin and actin filaments were depolymerised, and

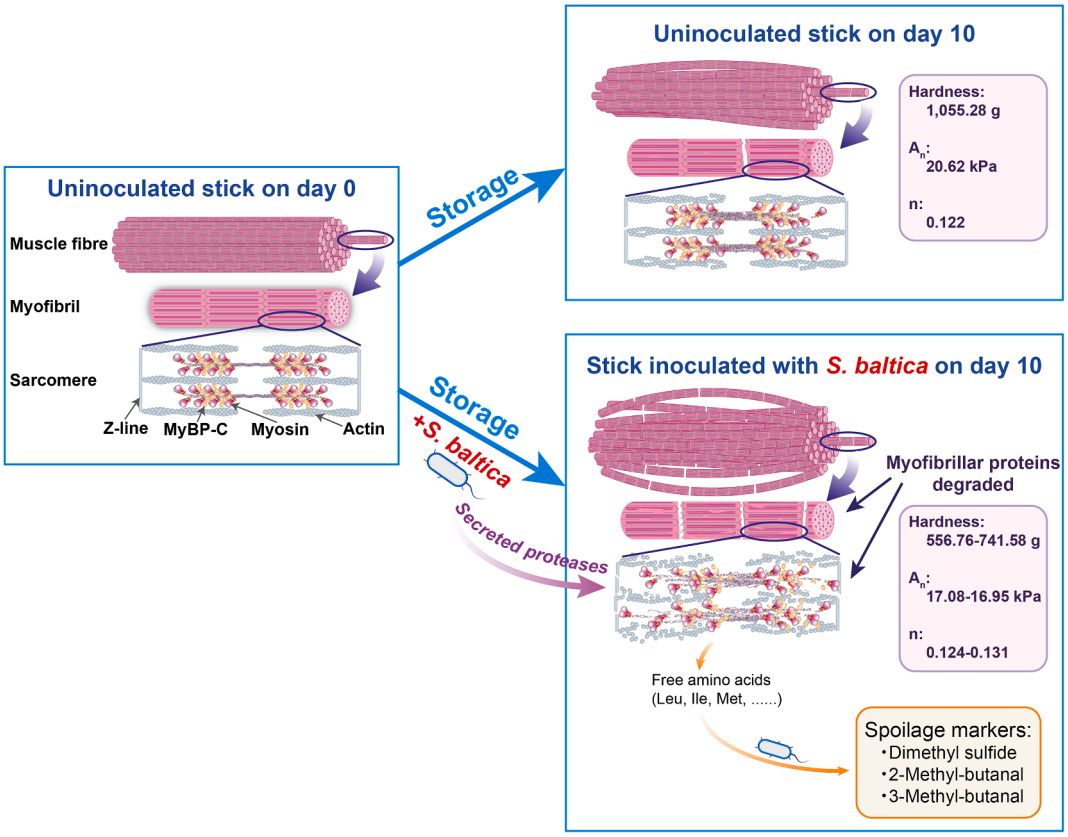

Fig. 4. Proposed schematic on the effect of *S. baltica* on the protein and flavour compounds of golden pomfret sticks during storage.

proteins were degraded by *S. baltica* (Fig. 3). The contents of most volatile compounds in fish sticks decreased after storage, while *S. baltica* specifically produced dimethyl sulfide, 2-methyl-butanal and 3-methyl-butanal (Table 1).

3.7. Validation - MALDI-TOF-MS

To validate the effect of *S. baltica* on golden pomfret sticks, MALDI-TOF-MS was applied to acquire the precise molecular weight of proteins and peptides (Feng, Ng, et al., 2017). Fig. S2 shows the spectra and Table 2 summarises molecular weights with the intensity > 20 (a.u.) of each sample. As shown in Fig. S2, a lot of peaks with different molecular weights from 24 to 176 kDa were detected on day 0. The largest protein on day 0 in the spectrum had a molecular weight of 176 kDa, which might be a part of MHC (Reichelt & Kendrick-Jones, 2000). The intensities of peaks were lower in stored controls than those in the sticks on day 0, suggesting the disintegration of myofibril by autolytic enzymes during storage. After 4-day storage, the molecule with 176 kDa disappeared in all sticks (Table 2), while the intensities of peaks around 115–120 kDa increased in the groups inoculated with strains ABa4 and BBe1. This revealed that *S. baltica* promoted the fragmentation of large molecules of MHC during early spoilage. On the other hand, *S. baltica* further induced the degradation of smaller proteins during days 4–10. On day 10, there were several peaks at 168, 153, 137, 124, 111, and 103 kDa in uninoculated sticks, whereas the molecules with 100–170 kDa disappeared or showed extremely low intensities in inoculated sticks (Fig. S2). The molecular weight at 120–140 kDa could be regarded as MyBP-C (Heling et al., 2020). In addition, the 48–52 kDa fragments, which were hypothesised to be tropomyosin (Tm), also disappeared in inoculated groups on day 10 (Claeys, Uytterhaegen, Buts, & Demeyer, 1995). The loss of Tm might contribute to the fragmentation of myofibrillar protein, since it played an important role in regulating the interactions between actin filaments and other proteins, including myosin and actin-binding proteins (Feng et al., 2016; Gunning, Hardeman, Lappalainen, & Mulvihill, 2015).

Table 2
Molecular weights (kDa) of proteins and peptides with the intensity > 20 (a.u.) from golden pomfret sticks inoculated with *S. baltica* during spoilage.

Day	Strain	Molecular weights (kDa)
0	Control	19, 24, 36, 44, 51, 62, 64, 70, 74, 91, 102, 111, 138, 152, 172
4	Control	11, 12, 13, 18, 20, 25, 29, 32, 34, 37, 38, 44, 51, 70, 73, 82, 97, 168
	ABa4	11, 12, 14, 15, 16, 21, 28, 35, 38, 38, 39, 44, 115, 126
	ABe2	12, 13, 17, 19, 31, 42, 51, 51, 53, 64, 66, 67, 76, 79, 82
	BBe1	10, 13, 16, 19, 24, 26, 31, 48, 67, 73, 75, 87, 119
10	Control	13, 21, 27, 31, 36, 48, 51, 53, 59, 61, 67, 111
	ABa4	10, 10, 13, 33, 41, 62
	ABe2	10, 12, 15, 21, 72
	BBe1	11, 13, 14, 17, 19, 21, 22, 25, 37

4. Conclusion

In this study, TPA, rheological analysis, SDS-PAGE, GC–MS and MALDI-TOF-MS were used to investigate the changes in textural, rheological and flavour profiles of golden pomfret sticks inoculated with *S. baltica* during storage. The hardness of fish sticks decreased dramatically during spoilage, especially in inoculated samples, which showed lower hardness than the control. No significant differences were observed among TPA characteristics of sticks inoculated with different strains on the same day. During storage, protein isolates inoculated with *S. baltica* showed lower G' and G" during shearing and heating. MHC, MyBP-C, and actin of inoculated sticks were degraded into smaller proteins. MALDI-TOF-MS results showed lower intensities of molecules

with high M_w in inoculated samples, validating the degradation of myofibrillar proteins. In addition, *S. baltica* induced a significant increase in most alcohols but decreased ketones, hydrocarbons and most aldehydes during early storage whereas the strains produced high amounts of dimethyl sulfide, 2-methyl-butanal and 3-methyl-butanal during later storage. This study demonstrates the effect of *S. baltica* on fish protein degradation and flavour changes during spoilage and provides information for controlling seafood spoilage.

CRediT authorship contribution statement

Xiaowei Lou: Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Visualization, Writing – original draft. **Xiaokang Wen:** Data curation, Resources, Formal analysis, Investigation. **Leijian Chen:** Resources, Validation. **Weichen Shu:** Software, Data curation. **Yue Wang:** Writing – review & editing. **Tung Thanh Hoang:** Validation. **Hongshun Yang:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data (Fig. S1, S2, and Tables S1-S5) to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.134616.

References

Campo-Deaño, L., Tovar, C. A., Pombo, M. J., Solas, M. T., & Borderías, A. J. (2009). Rheological study of giant squid surimi (*Dosidicus gigas*) made by two methods with different cryoprotectants added. *Journal of Food Engineering, 94*(1), 26–33. https://doi.org/10.1016/j.jfoodeng.2009.02.024

Claeys, E., Uytterhaegen, L., Buts, B., & Demeyer, D. (1995). Quantification of beef myofibrillar proteins by SDS-PAGE. *Meat Science, 39*(2), 177–193. https://doi.org/10.1016/0309-1740(94)P1819-H

Curson, A. R. J., Sullivan, M. J., Todd, J. D., & Johnston, A. W. B. (2010). Identification of genes for dimethyl sulfide production in bacteria in the gut of Atlantic Herring (*Clupea harengus*). *The ISME Journal, 4*(1), 144–146. https://doi.org/10.1038/ismej.2009.93

Edirisinghe, R. K. B., Graffham, A. J., & Taylor, S. J. (2007). Characterisation of the volatiles of yellowfin tuna (*Thunnus albacares*) during storage by solid phase microextraction and GC-MS and their relationship to fish quality parameters. *International Journal of Food Science & Technology, 42*(10), 1139–1147. https://doi.org/10.1111/j.1365-2621.2006.01224.x

FAO. (2020). *The state of world fisheries and aquaculture 2020*. Rome: Sustainability in action.

Feng, X., Bansal, N., & Yang, H. (2016). Fish gelatin combined with chitosan coating inhibits myofibril degradation of golden pomfret (*Trachinotus blochii*) fillet during cold storage. *Food Chemistry, 200*, 283–292. https://doi.org/10.1016/j.foodchem.2016.01.030

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. https://doi.org/10.1007/s11947-016-1798-7

Feng, X., Zhu, Y., Liu, Q., Lai, S., & Yang, H. (2017). Effects of bromelain tenderisation on myofibrillar proteins, texture and flavour of fish balls prepared from golden pomfret. *Food and Bioprocess Technology, 10*(10), 1918–1930. https://doi.org/10.1007/s11947-017-1963-7

Ghaly, A. E., Dave, D., Budge, S., & Brooks, M. (2010). Fish spoilage mechanisms and preservation techniques: Review. *American Journal of Applied Sciences, 7*(7), 859–877. https://doi.org/10.3844/ajassp.2010.859.877

Giri, A., Osako, K., & Ohshima, T. (2010). Identification and characterisation of headspace volatiles of fish miso, a Japanese fish meat based fermented paste, with special emphasis on effect of fish species and meat washing. *Food Chemistry, 120*(2), 621–631. https://doi.org/10.1016/j.foodchem.2009.10.036

Gram, L., & Dalgaard, P. (2002). Fish spoilage bacteria-problems and solutions. *Current Opinion in Biotechnology, 13*(3), 262–266. https://doi.org/10.1016/S0958-1669(02)00309-9

Gunning, P. W., Hardeman, E. C., Lappalainen, P., & Mulvihill, D. P. (2015). Tropomyosin - master regulator of actin filament function in the cytoskeleton. *Journal of Cell Science, 128*(16), 2965–2974. https://doi.org/10.1242/jcs.172502

He, R., Yao, X.-Z., Chen, M., Ma, R.-C., Li, H.-J., Wang, C., & Ding, S.-H. (2018). Conversion of sulfur compounds and microbial community in anaerobic treatment of fish and pork waste. *Waste Management, 76*, 383–393. https://doi.org/10.1016/j.wasman.2018.04.006

Heling, L. W. H. J., Geeves, M. A., & Kad, N. M. (2020). MyBP-C: One protein to govern them all. *Journal of Muscle Research and Cell Motility, 41*(1), 91–101. https://doi.org/10.1007/s10974-019-09567-1

Huang, J., Zeng, S., Xiong, S., & Huang, Q. (2016). Steady, dynamic, and creep-recovery rheological properties of myofibrillar protein from grass carp muscle. *Food Hydrocolloids, 61*, 48–56. https://doi.org/10.1016/j.foodhyd.2016.04.043

Huang, J., Zhou, Y., Chen, M., Huang, J., Li, Y., & Hu, Y. (2021). Evaluation of negative behaviors for single specific spoilage microorganism on little yellow croaker under modified atmosphere packaging: Biochemical properties characterization and spoilage-related volatiles identification. *LWT, 140*, Article 110741. https://doi.org/10.1016/j.lwt.2020.110741

Juszczak, L., Witczak, T., Ziobro, R., Korus, J., Cieślik, E., & Witczak, M. (2012). Effect of inulin on rheological and thermal properties of gluten-free dough. *Carbohydrate Polymers, 90*(1), 353–360. https://doi.org/10.1016/j.carbpol.2012.04.071

Koubassova, N. A., & Tsaturyan, A. K. (2011). Molecular mechanism of actin-myosin motor in muscle. *Biochemistry (Moscow), 76*(13), 1484–1506. https://doi.org/10.1134/S0006297911130086

Lakshmanan, R., Piggott, J. R., & Paterson, A. (2003). Potential applications of high pressure for improvement in salmon quality. *Trends in Food Science & Technology, 14*(9), 354–363. https://doi.org/10.1016/S0924-2244(03)00121-3

Lametsch, R., Roepstorff, P., Møller, H. S., & Bendixen, E. (2004). Identification of myofibrillar substrates for μ-calpain. *Meat Science, 68*(4), 515–521. https://doi.org/10.1016/j.meatsci.2004.03.018

Leduc, F., Krzewinski, F., Le Fur, B., N'Guessan, A., Malle, P., Kol, O., & Duflos, G. (2012). Differentiation of fresh and frozen/thawed fish, European sea bass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*), cod (*Gadus morhua*) and salmon (*Salmo salar*), using volatile compounds by SPME/GC/MS. *Journal of the Science of Food and Agriculture, 92*(12), 2560–2568. https://doi.org/10.1002/jsfa.5673

Leyva-Díaz, J. C., Poyatos, J. M., Barghini, P., Gorrasi, S., & Fenice, M. (2017). Kinetic modeling of *Shewanella baltica* KB30 growth on different substrates through respirometry. *Microbial Cell Factories, 16*(1), 189. https://doi.org/10.1186/s12934-017-0805-7

Li, C., Wu, J., Li, Y., & Dai, Z. (2013). Identification of the aroma compounds in stinky mandarin fish (*Siniperca chuatsi*) and comparison of volatiles during fermentation and storage. *International Journal of Food Science & Technology, 48*(11), 2429–2437. https://doi.org/10.1111/ijfs.12254

Li, S., Xu, X., & Zhou, G. (2012). The roles of the actin-myosin interaction and proteolysis in tenderization during the aging of chicken muscle. *Poultry Science, 91*(1), 150–160. https://doi.org/10.3382/ps.2011-01484

Liu, X., Zhang, T., Xue, Y., & Xue, C. (2019). Changes of structural and physical properties of semi-gel from Alaska pollock surimi during 4 °C storage. *Food Hydrocolloids, 87*, 772–782. https://doi.org/10.1016/j.foodhyd.2018.09.011

López-Caballero, M., Huidobro, A., Pastor, A., & Tejada, M. (2002). Microflora of gilthead seabream (*Sparus aurata*) stored in ice. Effect of washing. *European Food Research and Technology, 215*(5), 396–400. https://doi.org/10.1007/s00217-002-0574-2

Lou, X., Hai, Y., Le, Y., Ran, X., & Yang, H. (2023). Metabolic and enzymatic changes of *Shewanella baltica* in golden pomfret broths during spoilage. *Food Control, 144*, Article 109341. https://doi.org/10.1016/j.foodcont.2022.109341

Lou, X., Zhai, D., & Yang, H. (2021). Changes of metabolite profiles of fish models inoculated with *Shewanella baltica* during spoilage. *Food Control, 123*, 107697. https://doi.org/10.1016/j.foodcont.2020.107697

Mehta, N. K., Balange, A. K., Lekshmi, M., & Nayak, B. B. (2017). Changes in dynamic viscoelastic and functional properties of Indian squid mantle during ice storage. *Journal of Food Processing and Preservation, 41*(3), e12891. https://doi.org/10.1111/jfpp.12891

Parlapani, F. F., Mallouchos, A., Haroutounian, S. A., & Boziaris, I. S. (2017). Volatile organic compounds of microbial and non-microbial origin produced on model fish substrate un-inoculated and inoculated with gilt-head sea bream spoilage bacteria. *LWT-Food Science and Technology, 78*, 54–62. https://doi.org/10.1016/j.lwt.2016.12.020

Reichelt, S., & Kendrick-Jones, J. (2000). Myosins. In C. J. Staiger, F. Baluška, D. Volkmann, & P. W. Barlow (Eds.), *Actin: A dynamic framework for multiple plant cell functions* (pp. 29–44). Dordrecht: Springer, Netherlands.

Sow, L. C., Tan, S. J., & Yang, H. (2019). Rheological properties and structure modification in liquid and gel of tilapia skin gelatin by the addition of low acyl gellan. *Food Hydrocolloids, 90*, 9–18. https://doi.org/10.1016/j.foodhyd.2018.12.006

Viji, P., Tanuja, S., Ninan, G., Lalitha, K. V., Zynudheen, A. A., Binsi, P. K., & Srinivasagopal, T. K. (2015). Biochemical, textural, microbiological and sensory attributes of gutted and ungutted sutchi catfish (*Pangasianodon hypophthalmus*) stored in ice. *Journal of Food Science and Technology, 52*(6), 3312–3321. https://doi.org/10.1007/s13197-014-1358-y

Vogel, B. F., Venkateswaran, K., Satomi, M., & Gram, L. (2005). Identification of *Shewanella baltica* as the most important H_2S-producing species during iced storage of Danish marine fish. *Applied and Environmental Microbiology, 71*(11), 6689–6697. https://doi.org/10.1128/AEM.71.11.6689-6697.2005

Wierda, R. L., Fletcher, G., Xu, L., & Dufour, J.-P. (2006). Analysis of volatile compounds as spoilage indicators in fresh king salmon (*Oncorhynchus tshawytscha*) during storage using SPME-GC-MS. *Journal of Agricultural and Food Chemistry, 54*(22), 8480–8490. https://doi.org/10.1021/jf061377c

Yin, T., & Park, J. W. (2015). Textural and rheological properties of Pacific whiting surimi as affected by nano-scaled fish bone and heating rates. *Food Chemistry, 180*, 42–47. https://doi.org/10.1016/j.foodchem.2015.02.021

Zhang, Q., Ding, Y., Gu, S., Zhu, S., Zhou, X., & Ding, Y. (2020). Identification of changes in volatile compounds in dry-cured fish during storage using HS-GC-IMS. *Food Research International, 137*, 109339. https://doi.org/10.1016/j.foodres.2020.109339

Zhao, X., Zhou, Y., Zhao, L., Chen, L., He, Y., & Yang, H. (2019). Vacuum impregnation of fish gelatin combined with grape seed extract inhibits protein oxidation and degradation of chilled tilapia fillets. *Food Chemistry, 294*, 316–325. https://doi.org/10.1016/j.foodchem.2019.05.054

Zhou, R., Zheng, Y., Liu, Y., & Ma, M. (2020). Texture and quality assessment of ready-to-eat farmed obscure puffer fish (*Takifugu obscurus*) fillet by evaluating bacterial and myofibrillar degradation and biochemical changes during refrigerated storage. *Journal of Aquatic Food Product Technology, 29*(7), 604–615. https://doi.org/10.1080/10498850.2020.1781318

Zhou, Y., & Yang, H. (2019). Effects of calcium ion on gel properties and gelation of tilapia (*Oreochromis niloticus*) protein isolates processed with pH shift method. *Food Chemistry, 277*, 327–335. https://doi.org/10.1016/j.foodchem.2018.10.110