Shrimp paste is one of the traditional foods in eastern China, even in East Asia (Phewpan et al., 2020). The fresh shrimps are fermented naturally by adding high concentration of salt. The fermentation would take over one month normally to form flavor and nutrition (Zhu et al., 2019). Proteases could hydrolysis protein effectively by breaking down the peptide bond that links amino acid to poly-peptide chain, and they account for over half of the total world enzyme markets in the world (Amin, 2018; Moradi, Sun, Song, & Hu, 2019; Raval, Pillai, Rawal, & Singh, 2014; Uttatree & Charoenpanich, 2018). Protease from microorganisms have been studied due to their excellent characteristic and highly yield, and bacterial proteases have been exploited the most widely compared with fungi, plants, and animal (Olajuyigbe & Falade, 2014; Rekik et al., 2019).

1 | INTRODUCTION

Shrimp paste is one of the traditional foods in eastern China, even in East Asia (Phewpan et al., 2020). The fresh shrimps are fermented naturally by adding high concentration of salt. The fermentation would take over one month normally to form flavor and nutrition (Zhu et al., 2019). Proteases could hydrolysis protein effectively by breaking down the peptide bond that links amino acid to poly-peptide chain, and they account for over half of the total world enzyme markets in the world (Amin, 2018; Moradi, Sun, Song, & Hu, 2019; Raval, Pillai, Rawal, & Singh, 2014; Uttatree & Charoenpanich, 2018). Protease from microorganisms have been studied due to their excellent characteristic and highly yield, and bacterial proteases have been exploited the most widely compared with fungi, plants, and animal (Olajuyigbe & Falade, 2014; Rekik et al., 2019).
Microorganisms secreted protease to hydrolyze the fish proteins have important functions during traditional Chinese shrimp pastes fermentation (Lv et al., 2020). Different kinds of free amino acids, peptides, oligopeptides, and ammonia were produced, and that was the key to different nutrition and flavors (Mohamed, Man, Mustafa, & Manap, 2012; Yuan, Wang, Jia, Wang, & Xu, 2017). Proteases can improve the quality and yield of shrimp oil and extend the shelf life of food (Mizapour-Kouhdasht & Moosavis-Nasab, 2019; Wang et al., 2019). Although many microorganisms are known to produce protease, novel enzymes having specific properties are still required for different applications (Sperranza et al., 2015; Jayakumar, Jayashree, Annapurna, & Seshadri, 2012).

Three strains of *Pediciacoccus* were used to ferment fish sauce under laboratory conditions (Zhang, Wang, & Mou, 2017). The fermentation method not only shortened the fermentation time, but also improved the product quality. The halophilic protease-producing bacterium *Bacillus* isolated from shrimp paste plays a crucial role in the fermentation process. Bacterial protease contributed to the flavor formation greatly in a fermentation. Lian et al isolated a strain of *Aspergillus niger* with the ability to hydrolyze proteins from traditional fermented shrimp paste, and molds often produced good flavor in fermented foods (Lian et al., 2014). However, Lu et al. found that *Staphylococci* and *Bacillus* were the main microbial community that produced protease in shrimp paste (Lv et al., 2016). Due to a variety of harsh environments, such as osmotic pressure, pH, temperature of the natural shrimp paste fermentation (Singh & Chhatpar, 2011; Jeong, Jung, Lee, Jin, & Jeon, 2013), the separation of protease-producing salt-tolerance, special flavor producing bacteria in shrimp paste was of great significance for the shrimp paste processing (Ly, Mayrhofer, & Domig, 2018).

In the traditional shrimp paste fermentation, the microbial flora works together, and the dynamics of microbial diversity happens in the processing (Phewpan et al., 2020). However, the contribution of each of bacteria did not clarified. In modern industrial production, it was important to know the role of each bacterium (Lv et al., 2020). The nutrition and flavor of shrimp paste came from proteases. The different peptides or oligopeptides were produced, and they made the food with new function (Chokswangkarn, Phiphattananuhoon, Jaresitthikunchai, & Roytrakul, 2018).

Additionally, proteases with novel catalytic properties and their hydrolysates could be applied in various industries such as detergent, surfactant, antibacterial substances, and oxidizing agent (Akiyama, Yamazaki, Tada, Ito, & Akiyama, 2014; Duan et al., 2014; He, Nguyen, Su, & Zhang, 2016; Hu, Ren, Zhou, & Ye, 2019; Zhang & Kim, 2010).

In this study, we selected strains which could secret protease from the shrimp paste which was very popular in local area. The growth characteristics of strain and the protease properties were investigated. Moreover, we analyzed the shrimp paste, and the hydrolysates had quantitative flavoring amino acids and antioxidant activity. The result suggested the strain and the protease were candidates for the further application in the shrimp paste producing and food industries.

### 2 MATERIALS AND METHODS

#### 2.1 Sample of shrimp paste and medium

Shrimp paste and Fresh shrimp were collected from Haiwa food company located at Haizhou Bay, Lianyungang, Jiangsu Province. Samples were kept in an icebox and brought back to the laboratory within one hour for next experiments. Broth medium: beef powder 3 g/L, peptone 10 g/L, NaCl 100 g/L, distilled water, and pH 8.0; broth solid medium: beef powder 3 g/L, peptone 10 g/L, NaCl 100 g/L, Agar 20 g/L, distilled water, and pH 8.0; casein liquid medium: beef powder 3 g/L, peptone 10 g/L, casein 10 g/L, NaCl 100 g/L, distilled water, and pH 8.0; screening medium: beef powder 3 g/L, peptone 10 g/L, casein 10 g/L, NaCl 100 g/L, Agar 20 g/L, distilled water, and pH 8.0.

#### 2.2 Screening and identify the strains produced protease

One gram shrimp paste was added to 20 ml of broth medium for enrichment culture at 25°C, 180 rpm for 12 hr. After slightly precipitating, 100 μl of the supernatant was spread on screening medium, and the plates were inverted in 25°C incubators. Colony which was around with a clear zone was selected and streak plate to pure the strain. Individual bacteria were isolated and stored. The strains were inoculated in casein liquid medium at 25°C and shaken at 180 rpm for 36 hr. The fermentation broth was centrifuged at 15,777 g for 10 min, and the supernatant was taken as a crude enzyme solution.

The purified cultured strain ST-1 was subjected to morphological observation and physiological and biochemical identification according to the "Common Bacterial System Identification Manual" and "Berger’s Bacterial Identification Manual."

The genome of the strain was extracted using a bacterial genome extraction kit, and amplification of 16S rDNA was performed. The PCR universal primer was as follows: 27F: 5’-AGAGTTTGATCCTGGCTCAG-3’ 1492R: 5’-GGTTACCTTGTTACGACTT-3’, and the reaction system was as follows: PCR mix (20 μl), upstream and downstream primers (1 μl each), and DNA template 4 μl. Reaction procedure: denatured at 94°C for 5 min; denatured at 94°C for 30 s; annealing at 55°C for 30 s; extension at 72°C for 90 s, 34 cycles; final extension at 72°C for 5 min (Lai et al., 2019). The amplified products were sent to Shenggong company located at Haizhou Bay, Lianyungang, Jiangsu Province. Samples were kept in an icebox and brought back to the laboratory within one hour for next experiments. Broth medium: beef powder 3 g/L, peptone 10 g/L, NaCl 100 g/L, Agar 20 g/L, distilled water, and pH 8.0; broth solid medium: beef powder 3 g/L, peptone 10 g/L, NaCl 100 g/L, Agar 20 g/L, distilled water, and pH 8.0; screening medium: beef powder 3 g/L, peptone 10 g/L, casein 10 g/L, NaCl 100 g/L, Agar 20 g/L, distilled water, and pH 8.0.

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#### 2.3 Growth characteristics of strain ST-1

The seed broth of strain ST-1 was prepared by inoculated strain into 50 ml of liquid medium incubated at 25°C, 180 rpm for 16 hr. Inoculate...
2% seed liquid in broth medium, pH 8.0, rotation speed 180 rpm, liquid volume 20%, cultured at different temperatures (0–40°C) for 24 hr. Cells concentration was determined at OD600 nm. pH range 5.0–11.0, to prevent pH changes during the culture, a final concentration of 10 mM buffer was added: pH 5.0–6.0 (MES buffer), pH 6.5–7.0 (PIPS buffer), and pH 7.5–8.0 (HEPES buffer). pH 9.0–11.0 is adjusted directly with 0.1M NaOH. The medium was prepared with distilled water, the NaCl range was from 1% to 13%. Under the optimum conditions of temperature, pH, and NaCl concentration, different carbon sources were added to study the growth of the strain. The common carbon source of 5 g/L was used to replace the beef power in the basic medium. Similarly, the common nitrogen source 10 g/L common carbon source was used to replace the peptone in the basic medium, and the culture was carried out for 20 hr under the optimal conditions.

2.4 | The properties of the protease

The properties of the protease was expressed as follows:

\[ W(\%) = \left( 1 - \frac{A_i - A_j}{A_0} \right) \times 100\% \]  

where \( A_i \) was the absorbance of the mixture of DPPH solution and LP solution, \( A_j \) was the absorbance of the mixture of LP solution and absolute ethanol, and \( A_0 \) was the absorbance of the mixture of DPPH solution and water.

2.6 | Amino acid composition of fermented shrimp paste

Fifty grams thawed shrimp was weighed, and 3% the strain ST-1 broth was added. Then, the fermentation was carried out at 25°C for 7 days. The cultured products were centrifuged to collect the supernatant. To analyze the amino acid, 6 M HCl was added in the supernatant and hydrolyzed at 110°C for 24 hr under nitrogen. The reactive solution was cooled to room temperature and filtered. The filtered solution was mixed with ultrapure water to hold 50 ml of solution. One mL of the solution was taken and mixed with 4 ml 100 mM HCl solution to analyze the amino acid compositions using HITACHI High Speed Amino Acid Analyzer (Model L-8900, JAPAN) at a loading amount of 20 μl.
Where $A_i$ was sample + Tris-HCl buffer + pyrogallol solution; $A_j$ was sample + Tris-HCl buffer + HCl solution; and $A_0$ was water + Tris-HCl buffer + pyrogallol solution.

The scavenging capability of the hydroxyl radical was calculated according to following equation:

$$W(\%) = \frac{A_s - A_n}{A_b - A_n} \times 100\%$$

(2)

where $A_s$ was the absorbance of a sample, $A_b$ was the absorbance of the control without a sample, and $A_n$ was the absorbance of the reagent blank.

Determination of reducing power, in brief, 0.1 ml of LP solution was mixed with 0.1 ml of 0.2 M pH 6.6 PBS solution and 0.1 ml of 1% (w/v) $K_3$Fe(CN)$_6$, and the mixture was incubated at 50°C for 20 min. Then, 0.1 ml 10% trichloroacetic acid was added into the mixture. The mixture was centrifuged at 5,000 g for 10 min, and 0.05 ml of supernatant was mixed with 0.25 ml of distilled water and 0.01 ml 0.2% FeCl$_3$. The mixture was then incubated at room temperature for 10 min and measured the absorption at 700 nm. All samples were in triplicate.

2.9 Statistical analysis

All the experiments were performed in triplicate for each sample. The data were subjected to an analysis of variance, and significance of the difference between means was determined with Duncan’s multiple range test ($p < .05$) using SPSS (SPSS Statistics 20, International Business Machine).

3 | RESULTS

3.1 Protease-producing strains and identification

The shrimp paste liquid shaken to 16 hr was applied to casein solid agar plates, and the strains circled a transparent zone was picked. The strain numbered ST-1 was found to produce the largest transparent zone on the casein solid plate (Figure 1a). The colony was milky white, opaque, smooth and moist, round, with neat edges, slightly protruding at the center, and easy to pick up colonies. The strain ST-1 was observed by Gram staining, and the strain was Gram-positive. The colony was small, round, low convex, microtransparent to opaque, and the cells were rod-shaped (Figure 1b).

The 16S rDNA PCR products were sequenced to obtain a sequence of 1529 bp. After sequence alignment, the similarity between strain ST-1 and Virgibacillus halodenitrificans was found to be 98.9%, and the phylogenetic tree is shown in Figure 2.

ST-1 and V. halodenitrificans have highly similarities in physiological and biochemical characteristics (Table 1). It was different on the branch characteristics, such as oxidase and decarboxylase. Combined with results of the physiological, biochemical characteristics, and molecular identification, the ST-1 strain is V. halodenitrificans.
3.2 | Growth characteristics of strain ST-1

The strain ST-1 was growth well at the temperature between 25°C and 30°C. With increasing temperature, the growth of ST-1 decreased sharply (Figure 3a). As shown in Figure 3b, the optimum growth pH of the strain ST-1 was 8.0. The results showed the strain ST-1 was very sensitive to the change of pH. The strain ST-1 could tolerant higher concentration of sodium chloride. The optimum growth was in 7% NaCl medium. When the concentration of sodium chloride was higher than 9%, the growth ability of the strain decreased significantly (Figure 3c).

The growth of the strain was measured after cultured with different carbon source, and the sucrose could promote the growth significantly (Figure 4a). For the nitrogen source, the yeast extract and beef extract could enhance the growth of the strain ST-1 significantly (Figure 4b). The results showed the strain ST-1 preferred to grow in the medium that contained more protein (Table 1).

3.3 | Characteristics of the protease

The optimum temperature of protease activity was 50°C. It was sensitive to higher temperature, but the activity combined with temperature from 25 to 50°C, as linear (Figure 5a). pH 6.0 was optimum condition for the protease. The enzyme activity remained above 80% between pH 5.5 and pH 7.5 (Figure 5b). The protease activity decreases with the increase of NaCl concentration (Figure 5c). The protease could keep 50% activity in 8% NaCl condition. The results showed activity can remain above 80% in the concentration range of 4% NaCl.

The effects of 10 and 50 mM metal ions on the protease activity are shown in Table 2. Ca²⁺, Mg²⁺, K⁺, Ba²⁺, Co²⁺, Mn²⁺, Si²⁺, and Na²⁺ could promote the activity; on the contrast, Cu²⁺, Fe³⁺, Zn²⁺, Li¹, and Cd²⁺ could inhibit the enzyme activity to a large extent. The Cu²⁺ could denature the protease on the both concentrations specially.

### TABLE 1 Differences between strain ST-1 and Virgibacillus halodenitrificans

| Characters                      | ST-1 | V. halodenitrificans |
|---------------------------------|------|----------------------|
| G⁺/G⁻                           | +    | V                    |
| 0°C                             | –    | –                    |
| Temperature (°C)                | 10–45| 10–45                |
| NaCl (%)                        | 1–15 | 2–23                 |
| pH                              | 6.0–10.0| 5.8–9.6              |
| Casein                          | +    | +                    |
| Gelatin                         | –    | –                    |
| Urea                            | –    | –                    |
| Methyl red                      | –    | –                    |
| Oxidase                         | –    | +                    |
| V-P                             | –    | –                    |
| Arabinose                       | –    | –                    |
| H₂S                             | –    | –                    |

Note: 1. Strain ST-1; 2. V. halodenitrificans.

**FIGURE 3** The strain growth was affected by temperature (a), pH (b), and NaCl concentration (c)

**FIGURE 4** Effect of (a) carbon source and (b) nitrogen source on the growth of strain ST-1
The protease could tolerate organic solvent such as Tween 80, methanol, PMST, DMSO, acetonitrile, and acetone, and the residual activities were 113%, 97%, 96%, 95%, 89%, and 80%. However, some of organic solvent could affect the activities that shown in Table 3. When the EDTA and EGTA were mixed with protease, the activity was inhibited totally. It may suggest the protease was a metal enzyme. The highest activity was performed when the substrate was casein. Also, the protease could hydrolyze different substrates such as power of skim milk, gelatin, BSA, azocasein, and hemoglobin (Table 4).

### 3.4 Amino acid composition of fermentation product

The amino acid of fermentation products was analyzed (Figure 6a), and the composition of amino acid is shown in Figure 6b. After 7 days of fermentation, the protease could release different kind of amino acid. As showed in the figure, glutamic acid was more than the others significantly.

### 3.5 Enzymatic specificity of the protease

The release rate of the amino acid could be used to predict the cleavage site under certain conditions. In the experiment, the free amino...
acid content had a good linear relationship when the shrimp was hydrolyzed by the protease. The release rate is shown in Table 5. The fastest release rates are Glu, Asp, Gly, Leu, and Lys. It was suggested that the protease produced by *V. halodenitrificans* ST-1 preferentially hydrolyzed the peptide bonds of Glu-, Asp-, Gly-, Leu-, and Lys- (Figure 6).

### Table 5 | The rate of release of AAs from shrimp hydrolyzed by the protease

| AA  | V       | $R^2$  | AA  | V       | $R^2$  |
|-----|---------|--------|-----|---------|--------|
| Asp | $7.13 \times 10^{-2}$ | 0.9573 | Ile | $3.22 \times 10^{-2}$ | 0.9921 |
| Thr | $2.36 \times 10^{-2}$ | 0.9858 | Leu | $5.37 \times 10^{-2}$ | 0.943  |
| Ser | $1.72 \times 10^{-2}$ | 0.9893 | Tyr | $2.74 \times 10^{-2}$ | 0.9349 |
| Glu | $7.52 \times 10^{-2}$ | 0.9337 | Phe | $3.56 \times 10^{-2}$ | 0.9971 |
| Gly | $6.90 \times 10^{-2}$ | 0.9816 | Lys | $4.70 \times 10^{-2}$ | 0.9706 |
| Ala | $3.96 \times 10^{-2}$ | 0.9312 | His | $1.47 \times 10^{-2}$ | 0.9192 |
| Cys | $9.60 \times 10^{-3}$ | 0.9948 | Arg | $3.70 \times 10^{-2}$ | 0.9961 |
| Val | $3.66 \times 10^{-2}$ | 0.995  | Trp | $2.26 \times 10^{-2}$ | 0.9987 |
| Met | $2.88 \times 10^{-2}$ | 0.9897 |

Note: V and $R^2$ represented the slope and correlation of the linear relationship between free amino acid content and hydrolysis time, respectively.

### 3.6 | Purification of hydrolysates and antioxidant activity

0.5 mg/ml of peak 3 (Figure 7) could reach 50% of DPPH radical scavenging (Figure 8a); 1.5 mg/ml of peak 3 could reach 50% of superoxide anion radical scavenging (Figure 8b). Also, the higher increase the concentration of peak 3, the stronger reducing power (Figure 8d).

### 4 | DISCUSSION

After screening and identification the morphological, physiological, and biochemical characteristics, phylogenetic analysis (Ghauri, Khalid, Grant, Grant, & Heaphy, 2006), we fund the highest copy strains and highest protease activity was *V. halodenitrificans*. So, we decided to study it deeply. *V. halodenitrificans* RSK CAS1 was performed to determine the optimal medium and optimal culture conditions. Its optimum NaCl concentration is 15.32 g/L (Sathishkumar, Ananthan, & Raghunathan, 2015). *V. halodenitrificans* SK1-3-7 isolated from the fish sauce could produce protease which could tolerant 0.5 M NaCl (Montriwong, Rodtong, & Yongsawatdigul, 2015). *V. halodenitrificans* ST-1 could grow in a halobiotic condition, and the
optimal NaCl concentration was 70 g/L. Meanwhile, the protease of *V. halodenitrificans* ST-1 can maintain the enzyme activity above 80% in the concentration range of 0%-3% NaCl. Compared with *Virgibacillus* sp. P-4, it can shorten the fermentation time of shrimp paste (Zhang et al., 2017). The strain ST-1 which could grow well in 1%-15% NaCl was more suitable for fermentation of marine products.

The protease of *V. halodenitrificans* ST-1 could not be affected high-concentration divalent metal ions (Ba$^{2+}$, Ca$^{2+}$, Mg$^{2+}$, Co$^{2+}$, Mn$^{2+}$, Si$^{2+}$, and Na$^{+}$) and almost unaffected by some organic solvents such as Methanol and Dimethyl sulphoxide. The characteristic makes the protease expand its application (Fang et al., 2009).

The cleavage site was predicted by analyzing the release rate of free amino acids in the enzymatic hydrolysate. Yanjie Zhang et al. studied the cleavage sites of *Virgibacillus* sp. P-4 as Phe-, Tyr-, Lys-, His-, Pro-, and Leu- (Zhang et al., 2017). The protease produced by *V. halodenitrificans* SK1-3-7 preferably hydrolyzed Suc-Ala-Ala-Pro-Phe-pNA (Montriwong et al., 2015). Our research found the cleavage sites of the protease of *V. halodenitrificans* ST-1 were Glu-, Asp-, Gly-, Leu-, and Lys-. The quantity of glutamic acid, umami amino acid, was released significantly higher, and the flavor will be enhanced.

The DPPH scavenging and superoxide anion radical scavenging were higher than the products from marine animal *Tergillarca granosa* (Ganesan et al., 2020), *Eupolyphaga sinensis* walker (Zhang et al., 2019). The antioxidant activity came from peptides or oligopeptide of hydrolysates (Hu et al., 2019; Liang, Wang, Li, Chu, & Sun, 2019).

CONCLUSION

*Virgibacillus halodenitrificans* ST-1 was isolated from shrimp paste of Haizhou Bay, Lianyungang. The characteristic of protease produced by ST-1 was studied. The optimal activity pH and temperature were 8.0 and 30°C, respectively. The protease was stable at a wide range of pH (3.0–11.0) and temperature (15–45°C). More than 90% residual activity was observed when the enzyme was incubated with determined organic solutions. The protease activity could be enhanced by divalent cations such as Ba$^{2+}$, Ca$^{2+}$, Mg$^{2+}$, K$^{+}$, Mn$^{2+}$, Si$^{2+}$, and Na$^{+}$ and inhibited by Cu$^{2+}$, Fe$^{2+}$, and Zn$^{2+}$. The protease could hydrolyze various native proteinaceous substrates such as BSA, casein, skim milk, gelatine, azocasein, and hemoglobin. The protease preferentially hydrolyzed the peptide bonds of Glu-, Asp-, Gly-, Leu-, and Lys. The hydrolysates of the protease had antioxidant activity, especially for DPPH and superoxide anion radical scavenging. The strain ST-1 and the protease both were have a high application prospect in food industry.

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CONFLICT OF INTEREST

The authors confirm that there is no conflict of interests regarding this paper.

ETHICAL APPROVAL

This article does not contain any studies with animals performed by any of the authors.
REFERENCES

Akiyama, T., Yamazaki, T., Tada, A., Ito, Y., & Akiyama, H. (2014). Classification of microbial α-amylases for food manufacturing using proteinase digestion. Food Science & Nutrition, 2, 571–577.

Amin, M. (2018). Marine protease-producing bacterium and its potential use as an abalone probiotic. Aquaculture Reports, 12, 30–35.

Chokswangkarn, W., Phiphatthananukoon, S., Jaresitthikunchai, J., & Roystrakul, S. (2018). Antioxidative peptides from fish sauce by-product: Isolation and characterization. Agriculture and Natural Resources, 52, 460–466.

Duan, S., Hu, X., Liao, G., Wu, Y., Kuang, H., Yang, L., & Zhou, X. (2014). Effects of salinity on proteases composition, bacterial population and quality of shrimp sauce during fermentation. Modern. Food Science & Technology, 30, 38–43 and 204.

Fang, Y., Liu, S., Wu, S., Lyu, M., & Li, C. (2009). Screening and identification of an organic-solvent-stable protease-producing strain. Food Science, 30, 197–201.

Ganesan, A., Mohan, M., Balasubramanian, B., Kim, I., Seedevi, P., Mohan, K., ... Ignacimuthu, S. (2020). Marine invertebrates’ proteins: A recent update on functional property. Journal of King Saud University - Science, 32, 1496–1502.

Ghauri, M., Khalid, A., Grant, S., Grant, W., & Heaphy, S. (2006). Phylogenetic analysis of bacterial isolates from man-made high-pH, high-salt environments and identification of gene-cassette-associated open reading frames. Current Microbiology, 52, 487–492.

He, S., Nguyen, T., Su, P., & Zhang, W. (2016). Protein hydrolysates produced from rock lobster (Jasus edwardsii) Head: Emulsifying capacity and food safety. Food Science & Nutrition, 4, 869–877.

Hu, C., Ren, L., Zhou, Y., & Ye, B. (2019). Characterization of antimicrobial activity of three Lactobacillus plantarum strains isolated from Chinese traditional dairy food. Food Science & Nutrition, 7, 1997–2005.

Jayakumar, R., Jayashree, S., Annapurna, B., & Seshadri, S. (2012). Characterization of thermostable serum alkaline protease from an alkaliphilic strain Bacillus pumilus MCAS8 and its applications. Applied Biochemistry & Biotechnology, 168, 1849–1866.

Jeong, S., Jung, J. I., Lee, S., Jin, H., & Jeon, C. (2013). Microbial succession and metabolite changes during fermentation of dongchimi, traditional Korean watery kimchi. International Journal of Food Microbiology, 164, 46–53.

Kleekayai, T., Harnedy, P., O’Keeffe, M., Poyarkov, A., CunhaNeves, A., Sundornsuk, W., & FitzGerald, R. (2015). Extraction of antioxidant and ACE inhibitory peptides from Thai traditional fermented shrimp pastes. Food Chemistry, 176, 441–447.

Lai, X., Liu, X., Liu, X., Deng, T., Feng, Y., Tian, X., ... Wang, S. (2019). The Marine Catenovulum agarivorans MNH15 and dextranase: Removing dental plaque. Marine Drugs, 17, 592.

Lei, F., Cui, C., Zhao, Q., Sun-Waterhouse, D., & Zhao, M. (2014). Evaluation of the hydrolysis specificity of protease from marine Exiguobacterium sp. SWJS2 via free amino acid analysis. Applied Biochemistry & Biotechnology, 174, 1260–1271.

Lian, X., Yang, X., Xie, W., Yang, Y., Wu, S., Ji, H., ... Mao, W. J. (2014). Isolation, identification and salt-tolerance analysis of aroma-producing yeasts from the Chinese traditional shrimp paste. Modern Food Science & Technology, 30, 92–97.

Liang, L., Wang, C., Li, S., Chu, X., & Sun, K. (2019). Nutritional compositions of Indian Moringa oleifera seed and antioxidant activity of its polypeptides. Food Science & Nutrition, 7, 1–7.

Lowry, O., Rosebrough, N., Farr, A., & Randall, R. (1951). Protein measurement with the folin phenol reagent. Journal of Biological Chemistry, 193, 265–275.

Lv, X., Li, Y., Cui, T., Sun, M., Bai, F., Li, X., ... Yi, S. (2020). Bacterial community succession and volatile compound changes during fermentation of shrimp paste from Chinese Jinhzhou region. LWT, 122, 108998.

Lv, X., Li, Y., Ma, H., Miao, L., Du, J., Bai, F., ... Li, J. (2016). Isolation and identification of protease-producing halophilic bacteria in traditional Jinhzhou shrimp paste. Science & Technology of Food Industry, 37, 121–125.

Lv, D., Mayrhofer, S., & Domig, K. (2018). Significance of traditional fermented foods in the lower Mekong subregion: A focus on lactic acid bacteria. Food Bioscience, 26, 113–125.

Mirzapour-Kohdasth, A., & Moosavi-Nasab, M. (2019). Shelf-life extension of whole shrimp using an active coating containing fish skin gelatin hydrolysates produced by a natural protease. Food Science & Nutrition, 00, 1–10.

Mohamed, H., Man, Y., Mustafa, S., & Manap, Y. (2012). Tentative identification of volatile flavor compounds in commercial Budu, a Malaysian fish sauce, using GC-MS. Molecules, 17, 5062–5080.

Montriwong, A., Rodtong, S., & Yongswatdigul, J. (2015). Detergent-stable salt-activated proteinases from Virgibacillus halodenitrificans SK1-3-7 isolated from fish sauce fermentation. Applied Biochemistry & Biotechnology, 176, 505–517.

Moradi, M., Sun, Z., Song, Z., & Hu, H. (2019). Effect of proteases secreted from a marine isolated bacterium Bacillus vietnaminensis on the corrosion behaviour of different alloys. Bioelectrochemistry, 126, 64–71.

Olajuyigbe, F., & Falade, A. (2014). Purification and partial characterization of serine alkaline metalloprotease from Bacillus brevis MWB-01. Biosources & Bioprocessing, 1, 1–10.

Phewpan, A., Phuwprisirisan, P., Takahashi, H., Ohshima, C., Lopetcharat, K., Techaruvichit, K., & Keeratipibul, S. (2020). Microbial diversity during processing of Thai traditional fermented shrimp paste, determined by next generation sequencing. LWT, 122, 108989.

Raval, V., Pillai, S., Rawal, C., & Singh, S. (2014). Biochemical and structural characterization of a detergent-stable serine alkaline protease from seawater haloalkaliphilic bacteria. Process Biochemistry, 49, 955–962.

Rekik, H., Jaouadi, N., Gargouri, F., Bejar, F., Frikaa, F., Jmal, N., ... Jaouadi, B. (2019). Production, purification and biochemical characterization of a novel detergent-stable serine alkaline protease from Bacillus safensis strain RH12. International Journal of Biological Macromolecules, 121, 1227–1239.

Sathiashkumar, R., Ananthan, G., & Raghunathan, C. (2015). Production and characterization of haloalkaline protease from ascidian-associated Virgibacillus halodenitrificans RSK CAS1 using marine wastes. Annals of Microbiology, 65, 1481–1493.

Singh, A., & Chhatpar, H. (2011). Purification, characterization and thermodynamics of antifungal protease from Streptomyces sp. A6. Journal of Basic Microbiology, 51, 424–432.

Speranza, B., Racioppo, A., Bevilacqua, A., Beneduce, L., Sinigaglia, M., & Corbo, M. (2015). Selection of autochthonous strains as starter cultures for fermented fish products. Journal of Food Science, 80, M151–M160.

Uttatree, S., & Charoenpanich, J. (2018). Purification and characterization of a harsh conditions-resistant protease from a new strain of Staphylococcus saprophyticus. Agriculture and Natural Resources, 52, 16–23.

Wang, L., Yang, F., Rong, Y., Yuan, Y., Ding, Y., Shi, W., & Wang, Z. (2019). Effects of different proteases enzymatic extraction on the lipid yield and quality of Antarctic krill oil. Food Science & Nutrition, 7, 2224–2230.

Yuan, N., Wang, X., Jia, L., Wang, T., & Xu, H. (2017). Study on deodorization technology of shrimp paste by the response surface method. Food Products Processing, 1, 11–13.

Zhang, C., & Kim, S. (2010). Research and application of marine microbial enzymes: Status and prospects. Marine Drugs, 8, 1920–1934.
Zhang, N., Zhao, Y., Shi, Y., Chen, R., Fu, X., & Zhao, Y. (2019). Polypeptides extracted from *Eupolyphaga sinensis* walker via enzymic digestion alleviate UV radiation-induced skin photoaging. *Biomedicine & Pharmacotherapy, 112*, 108636.

Zhang, Y., Wang, J., & Mou, H. (2017). Isolation, identification and properties of protease-producing halophilic bacterium *Virgibacillus* sp. P-4 from traditional shrimp sauce. *Food Science, 38*, 102–108.

Zhao, C., Li, X., Miao, J., Jing, S., Li, X., Huang, L., & Gao, W. (2017). The effect of different extraction techniques on property and bioactivity of polysaccharides from *Dioscorea hemsleyi*. *International Journal of Biological Macromolecules, 102*, 847–856.

Zhu, W., Luan, H., Bu, Y., Li, X., Li, J., & Ji, G. (2019). Flavor characteristics of shrimp sauces with different fermentation and storage time. *LWT, 110*, 142–151.

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