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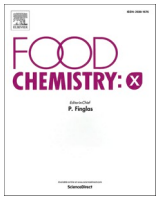

Edible portions of precooked blue swimming crab: Chemical composition and effect of chitooligosaccharide conjugate and high-pressure processing on microbial inactivation

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ABSTRACT

Different edible portions including meat (lump, claw and backfin) and roe of blue swimming crab (*Portunus pelagicus*) were analyzed. Both meat and roe had high protein content, but a greater fat content was found in roe. All meats showed higher levels of polyunsaturated fatty acids than roe. Myosin heavy chain and actin constituted as key proteins in meat, but the protein pattern of roe was completely different. Glutamic acid/glutamine were dominant in lump meat, while leucine was prevalent in roe. Meats were fibrous, while roe had a granular structure. *Moraxellaceae* were dominant in both samples and roe had higher microbial diversity than lump meat. When chitooligosaccharide-catechin (COSC) conjugate and high-pressure processing (HPP) were applied for both lump meat and roe, COSC conjugate (200 ppm) reduced *Vibrio* spp., and HPP at 500 MPa eliminated all detectable bacteria in both samples. Developed method was promising for enhancing food safety and maintaining quality of precooked crab products.

1. Introduction

Blue swimming crab (BSC, *Portunus pelagicus*) is a crustacean, which is popular among seafoods, especially in Thailand. In 2020, Thailand exported over 5233 tons of frozen and canned blue swimming crab meat with the value of 25.15 million US dollars to different countries such as the United States, China, Taiwan, and Hong Kong (Rattanarat et al., 2024). Crustacean meat can be converted to several products, e.g., canned crab meat, chilled crab meat packed in containers, such as crab meat soaked in salt water, cooked crab meat and crab meat packed in a vacuum container (Cocito et al., 2024). In Thailand, most consumers prefer to consume it in the form of precooked crab meat, which is ready-to-cook or ready-to-eat. Thus, safety is one of the major concerns. BSC is perishable and has profound microbiological risk. Processing environment and hygiene during the hand-picking process of crab meat are very crucial in determining the microbial load (Olatunde & Benjakul, 2021). In general, BSC meat sold in the market is commonly packed in plastic bags and tied with rubber band.

Hurdle technology plays a crucial role in food preservation globally. Via a strategic combination of hurdles such as controlled redox potential, temperature management, reduced water activity, preservatives, competitive flora, etc., the microbiological hazards can be better controlled, ensuring the safety and storage stability of food products. Widespread adoption of hurdle technology is essential, guaranteeing the high-quality and safety of foods for consumers (Pal et al., 2017).

Chitooligosaccharides (COS), a low molecular weight derivative of chitosan, demonstrates notable non-toxicity and biodegradability. Its antimicrobial efficacy is enhanced through modifications such as polyphenol grafting (Singh et al., 2020), which augmented its activity against both Gram-negative and Gram-positive bacteria. The antimicrobial effects of COS and its polyphenol conjugates are primarily attributed to their interactions with bacterial cell walls, leading to disruption and eventual cell death (Mittal et al., 2022; Singh et al., 2020) Additionally, high-pressure processing (HPP) offers a promising non-

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thermal method for extending the shelf-life of aquatic products by inactivating microorganisms and enzymes without compromising the nutritional quality of the food (Olatunde & Benjakul, 2018; Wu & Yang, 2023). The efficacy of HPP depends on processing parameters, such as pressure, temperature, and time (Aganovic et al., 2021).

Sincethe limited information regarding chemical composition and microbiological abundance of precooked BSC (P-BSC) meats and roe exists, this study aimed to compare the chemical compositions of P-BSC meats across different anatomical portions (lump, claw, and backfin) as well as roe and to investigate the impact of HPP and chitooligosaccharide-catechin (COSC) conjugate on the microbiological quality of P-BSC lump meat and roe.

2. Materials and method

2.1. Chemicals and microbial media

Chemicals for analyses were sourced from Sigma-Aldrich (St. Louis, MO, USA), and microbial media were obtained from Oxoid™ (Thermo Fischer Scientific, Hampshire, England).

2.2. Preparation of chitooligosaccharide-catechin (COSC) conjugate

Chitooligosaccharide (COS) was synthesized using the free radical grafting method outlined by Mittal et al. (2022). Chitosan from shrimp shell (MW: ~2100 kDa and DDA: 85 % as analyzed by gel permeation chromatography and ^{1}H NMR, respectively) was acquired from Marine Bio Resources Co., Ltd., Samutsakhon, Thailand. Catechin (99 % purity) was procured from Xi'an Julong Bio-Tech Co., Ltd. (Xi'an, China). Firstly, COS solution (1 %, *w/v*) was adjusted to pH 5.0 using acetic acid (1 M). Simultaneously, 4 mL of 1 M hydrogen peroxide containing 100 mg ascorbic acid were incubated (40 °C, 10 min) to generate hydroxyl radicals. Thereafter, both solutions were mixed, and the mixture was incubated at room temperature for 1 h with continuous stirring. Catechin (10 %, *w/w* of COS) was then added into the mixture and the incubation was performed for another 24 h in the dark at room temperature. Finally, the reaction mixture was dialyzed using a dialysis bag (MW cut-off: 500 Da) against 20 volumes of distilled water to remove the unbound catechin. COSC conjugate powder was obtained after lyophilization of the dialysate.

2.3. Collection and precooking of BSC

Precooked BSC (P-BSC) was prepared from whole crab with the aid of steam at 100–120 °C for 20 min. The crab meats and roe were manually collected by hand-picking. Meats from three different parts, namely lump, claw and backfin as well as roe, were donated by All Crab Company, Mueang district, Nakhon Sri Thammarat province, Thailand. All the samples were packed in polyethylene bags and imbedded in crushed ice using an insulated box as the container for transportation to the laboratory within 3 h.

2.4. Characterization of P-BSC meats and roe

2.4.1. Appearance and microstructure analysis

The photo showing the appearance was captured by the smartphone (iPhone model 12 Pro, Apple Inc., CA, USA). The P-BSC meats and roe were fixed in 0.2 M sodium phosphate buffer containing 2.5 % glutaraldehyde, and the fixed samples were washed in 0.1 M sodium phosphate buffer. The samples were dehydrated in a series of ethanol with varying concentrations (50 %, 60 %, 70 %, 80 %, 90 % and 100 %). The dehydrated sample was coated with gold–palladium and viewed with a scanning electron microscope (FEI Quanta 400-ESEM FEG, Hillsboro, OR, USA).

2.4.2. Determination of proximate compositions

Proximate compositions of P-BSC meats and roe were determined using AOAC method (Paez et al., 2016). Moisture content was determined by drying in an oven at 105 °C (analytical no. 952.08, 2016). Total fat content was measured (analytical no. 948.15, 2016) using the Soxhlet apparatus. Crude protein content was determined using the Kjeldahl method (conversion factor: 6.25) (analytical no. 992.23, 2016). Ash content was examined by incineration at 550 °C (analytical no. 930.30, 2016). Total carbohydrate was determined by subtracting the content (%) of other components from 100. Energy was calculated based on the contents of lipid (9 kcal/g) and protein and carbohydrate (4 kcal/g) (Europe & Commerce, 2016).

2.4.3. SDS-polyacylamide gel electrophoresis (SDS-PAGE)

Protein patterns of all precooked samples were determined by SDS-PAGE according to the method of Laemmli (1970) using 4 % stacking gel and 12 % running gel. Before analysis, the samples were solubilized in 5 % SDS at 85 °C for 1 h as detailed by Sinthusamran et al. (2013). After staining and destaining, the molecular weights (MWs) of protein bands were computed from the plot of log (MW) of protein standards vs. relative mobility (Rf).

2.4.4. Determination of fatty acid profiles

P-BSC meats and roe were subjected to lipid extraction using a chloroform/methanol mixture (2:1, *v*/v) (Bligh & Dyer, 1959). Subsequently, the lipid was converted into fatty acid methyl esters (FAME) before being separated, quantified, and characterized using a gas chromatograph (GC) (Agilent 7890B, Santa Clara, CA, USA) equipped with a flame ionization detector (FID). A capillary column (30 m × 0.32 mm × 0.25 μm) was used. The fatty-acid content (g/100 g lipid) was reported.

2.4.5. Determination of amino acid profiles

Lump meat and roe of P-BSC were rich in protein content and were selected for amino acid profile analysis. Sample (1 g) was transferred into a 100-mL extraction bottle and 10 mL of 6 M HCl was added and capped tightly. For tryptophan analysis, 10 mL of 4.2 M NaOH was used with the aid of an autoclave at 121–123 °C for 3 h. After neutralization with 2 M NaOH, the digest was filtered through filter paper no. 42, followed by filtering through a syringe filter (0.45 μm). Digest was analyzed using an GC–MS (QP-2010SE, Scientific Instrument, Inc., Columbia, MA, USA). The amino acids were categorized into essential amino acids (EAAs) and non-essential amino acids (NEAAs) (Li et al., 2021).

2.4.6. Determination of microbial diversities

P-BSC lump meat and roe were rich in protein content, which were prone to microbial spoilage. Spoilage microorganisms were able to use proteinaceous substances as the nutrients for their growth. Microbial diversity analysis was then performed in these two samples. Briefly, sample (1 g) was carefully chopped using an aseptic technique to prevent contamination. Thereafter, the samples were mixed with 3 mL of DNA/RNA Shield™ agent (Zymo Research, Irvine, CA, USA) and stored at 4 °C until analysis. Lump meat and roe were used for next-generation sequencing (NGS) analysis. All samples were processed and analyzed with the ZymoBioMICs® Service (Zymo Research, Irvine, CA, USA), following the method tailored by Chayanupatkul et al. (2022).

2.5. Effect of chitooligosaccharide-catechin conjugate (COSC) and high-pressure processing (HPP) on microbiological quality of P-BSC lump meat and roe

2.5.1. Treatment of P-BSC lump meat and roe using chitooligosaccharide-catechin (COSC) conjugate

Thirty grams of lump meat or roe samples were spread on the sterilized stainless-steel tray. One hundred μL of COSC conjugate solution at concentrations of 0 and 200 ppm was sprayed evenly over the surface of

each sample to ensure uniform coverage. The samples were then mixed manually using the plastic gloves and the treated samples were transferred into the polyethylene bag before being sealed under vacuum condition (Palamae et al., 2023).

2.5.2. HPP of P-BSC lump meat and roe without and with COSC conjugate treatment

The vacuum-packed P-BSC lump meat and roe containing COSC conjugate at 0 and 200 ppm were placed in a commercial HPP machine with a pressure transmission medium (5 L of water) (Model HPP600 MPa/5 L, Jiujiu, Baotou KeFa High Pressure Technology Co., Ltd., Baotou City, Inner Mongolia Autonomous Region, China). The treatment was carried out for 1.5 and 3 min at 0, 100, and 300 MPa. A sample without any treatment was used as the control.

2.5.3. Microbiological analysis

The treated samples and the control (10 g) for both lump meat (L) and roe (R) were added with 90 mL of 0.85 % NaCl solution (*w/v*) and homogenized (230 rpm for 30 s) using a stomacher (Stomacher 400 Seaward Medicals, Worthing, UK). The microbiological determination was performed as tailored by Olatunde et al. (2019). Standard plate count agar was employed for the enumeration of aerobic plate count (APC) and psychrophilic bacteria count (PBC) in the sample after being incubated at 37 °C for 3 days and 4 °C for 10 days, respectively. *Pseudomonas* spp. count (PSC) and hydrogen sulfide (H_2S) producing bacteria count (H_2SPBC) were evaluated using *Pseudomonas* isolation agar and triple sugar iron agar, respectively, with the incubation at 25 °C for 72 h. Thiosulfate citrate bile sucrose (TCBS) agar was used for determination of *Vibrio* spp. at 37 °C for 18 h.

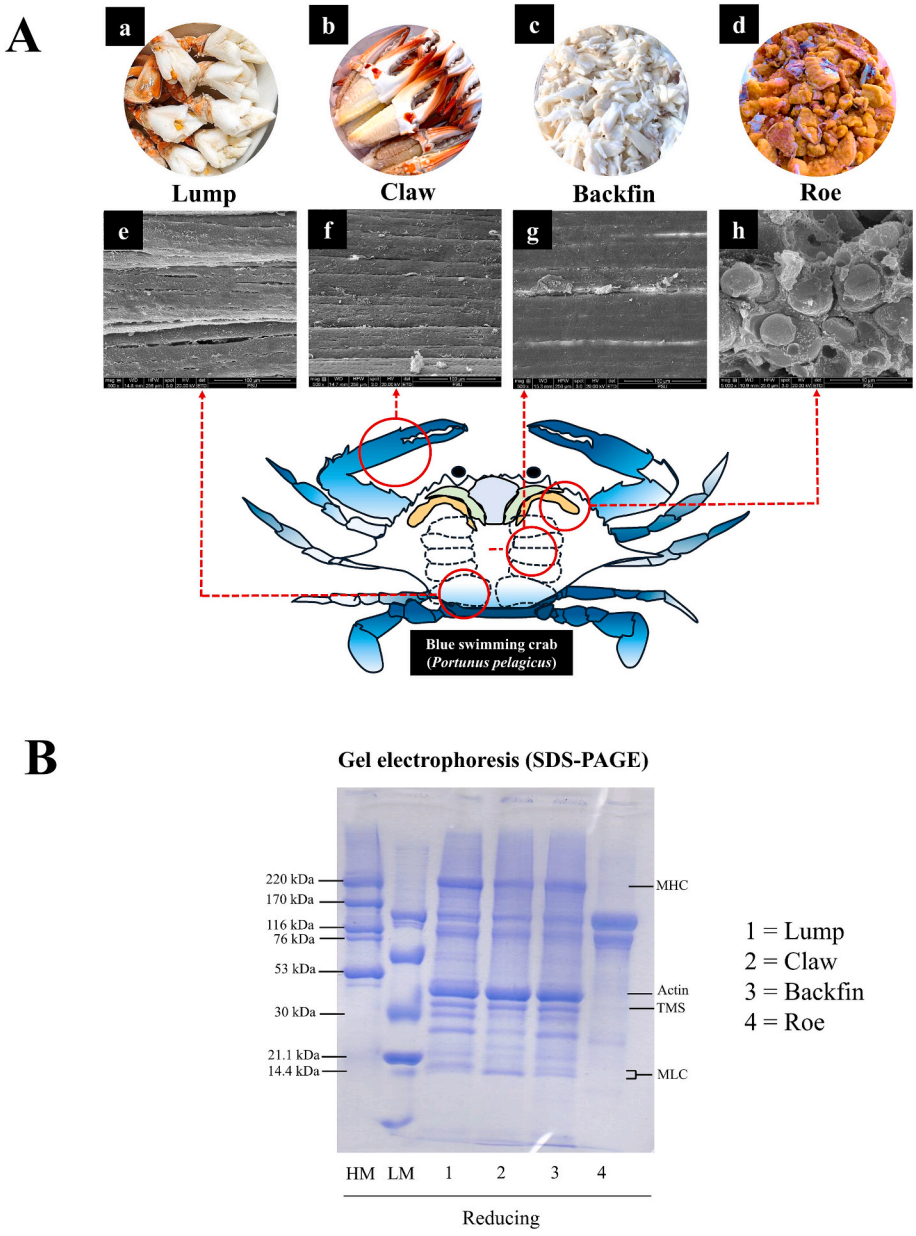

Fig. 1. (A) Appearance surface (a-d) and microstructures (e-h) (SEM: Magnification of 500×) of lump, claw, backfin meats and roe of precooked blue swimming crab (*Portunus pelagicus*). (B) Protein patterns of lump, claw, backfin meats and roe of precooked blue swimming. HM: high molecular weight, LM: Low molecular weight, MHC: Myosin heavy chain, AC: Actin, TMS: Tropomyosin, MLC: Myosin light chain. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.6. Data processing and statistical analysis

A completely randomized design (CRD) was used for the entire study. All experiments and analyses were performed in triplicate. Data were displayed as the mean ± standard deviation (SD). All analyses were done with a Statistical Package for the Social Sciences (SPSS) package (SPSS 14.0 for Windows, SPSS Inc., Chicago, IL, USA). One-way analysis of variance was employed for the analysis of the data and comparisons of means were done using Duncan's Multiple Range Test (DMRT).

The operational taxonomic unit (OTU) statistics were performed using Usearch 7 software; OTU clustering was done using Uparse 7.0.1090 software; alpha diversity analysis was carried out using Mothur 1.30.2 software. All were analyzed through the ZymoBioMICs® Service (Zymo Research, Irvine, CA, USA). Principal component analysis (PCA) was used to assess variation in microbial counts across samples. Log transformed microbial counts (log CFU/g), including APC, PBC, VSC, H_2SPBC and PSC, were used for this analysis. PCA was performed using the prcomp function in R v4.4.1 (Team, 2020), and PCA scores were visualized with ggplot2 v3.5.1 (Wickham, 2011). PERMANOVA with Euclidean distance was used to test significant effects of sample type, COSC conjugate concentration, and HPP pressure level. An alpha = 0.05 was used throughout the analysis.

3. Results and discussion

3.1. Appearances and microstructure of P-BSC meats and roe

The appearance and microstructure visualized by scanning electron microscopy (SEM) revealed distinct differences between the crab meat from various parts and the roe (Fig. 1A; a–h). For lump meat (Fig. 1A; a and e), the elongated or striated muscle fibers arranged in a relatively parallel orientation, contributing to the dense appearance. For claw meat (Fig. 1A; b and f), the muscle had thicker muscle fibers, compared to backfin meat. Backfin had the tight microstructure due to the dense orientation of muscle fibers (Fig. 1A; c and g). This might be related to its function in movement. For crab roe (Fig. 1A; d and h), it possessed granular and irregular microstructure, compared to meat, regardless of anatomical parts. Individual egg was visible and the eggs were clustered together, forming a gelatinous matrix. Overall, SEM analysis revealed that lump, claw, backfin, and roe exhibited distinct microstructural characteristics. The claw meat feature was described as well-organized fibers linked with connective tissues, contributing to very dense and compact appearance, while the roe possessed a unique granular feature.

3.2. Proximate composition of P-BSC meats and roe

The proximate compositions were significantly different among the meats from various parts of P-BSC (Table 1). The lump meat from three different parts had similar moisture content. The roe possessed the lowest moisture content ($P < 0.05$). Protein content was highest in the roe and lump meat, while the claw and backfin meats had lower contents ($P < 0.05$). In terms of fat content, roe showed the highest content ($P < 0.05$), followed by backfin, claw, and lump meats, respectively. The ash content was also highest in the roe, followed by lump meat. Lower ash contents were found in claw, and backfin meats. The results indicated that BSC meat and roe could serve as good sources of nutrients. Therefore, different edible portions of P-BSC had varying chemical compositions, which could influence their culinary uses and market value. The high protein content in meat and roe could support their uses in dishes that require high protein. Claw and backfin meat with their comparatively lower protein content might be more suitable for less protein-intensive dishes. The fat content was another crucial component, especially in the roe. The roe might deliver richer flavors and could be more desirable in some cuisines (Mei et al., 2023). Furthermore, the ash content, which represents the mineral content, was highest in the roe ($p < 0.05$), suggesting that it could serve as an essential source of numerous minerals. The variations in ash content among the different parts may also be related to the specific anatomical and biological functions (Sarower et al., 2013). Roe was mineral-rich, which is related to reproductive purpose (Wang et al., 2022).

Table 1
Proximate compositions of lump, claw, backfin meat and roe of precooked blue swimming crab (*Portunus pelagicus*).

Compositions*	Lump	Claw	Backfin	Roe
Moisture (%)	76.59 ± 0.75^{a}	77.36 ± 1.45^{a}	77.50 ± 0.57^{a}	67.60 ± 1.18^{b}
Protein (%)	19.50 ± 0.66^{a}	17.73 ± 0.43^{b}	16.97 ± 0.84^{b}	20.48 ± 0.13^{a}
Fat (%)	1.46 ± 0.56^{c}	3.12 ± 1.00^{b}	3.89 ± 0.47^{b}	8.98 ± 0.67^{a}
Ash (%)	1.91 ± 0.12^{b}	1.56 ± 0.09^{c}	1.26 ± 0.25^{c}	2.42 ± 0.23^{a}
Carbohydrate (%)	0.53 ± 0.02^{a}	0.23 ± 0.00^{c}	0.38 ± 0.02^{b}	0.52 ± 0.01^{a}

* Data are expressed as mean ± SD ($n = 3$). Different lowercase superscripts within the same row indicate significant difference ($p < 0.05$).

3.3. Protein patterns of P-BSC meats and roe

The protein patterns of meat from different parts and roe from P-BSC, examined under reducing conditions using 4 % stacking gel and 12 % separating gel, are illustrated in Fig. 1B. In the present study, the predominant proteins identified in lump, backfin, and claw meats were myosin heavy chain (MHC: 220 kDa), actin (45 kDa), and tropomyosin (TMS: 35–41 kDa) (Fig. 1B) (Tan et al., 2017). Myofibrillar proteins are majorly involved in contraction and relaxation, related to the movement of crabs or other animals (Perry et al., 2009). Moreover, the lump meat treated with sous vide had actin as the dominant protein (Olatunde & Benjakul, 2021). In contrast, the crab roe displayed different protein patterns, in which the bands having MWs of 144 kDa and 109 kDa were predominant. No MHC and actin were found in roe samples. These findings highlighted the differences in protein composition between meat and roe, emphasizing the significance of myofibrillar proteins in muscle tissue, whereas the roe constituted the unique proteins.

3.4. Fatty acid profiles of P-BSC meats and roe

Variations in fatty acid composition were observed among different samples ($p < 0.05$) (Table 2). For saturated fatty acids (SFA), the roe exhibited the highest content, while the claw meat had the lowest content. Other parts, such as the lump and backfin meat, had relatively similar amounts. Monounsaturated fatty acid (MUFA) content was highest in the roe ($p < 0.05$). Claw and backfin meat showed similar levels. The lump meat contained the lowest amount of MUFA. For polyunsaturated fatty acids (PUFA), the claw meat had the highest content, followed by the lump meat and backfin meat. The roe had the lowest PUFA content. The results highlighted the differences in individual fatty acids among the samples. The lump and claw meats were rich in eicosapentaenoic acid (EPA). Docosahexaenoic acid (DHA) was most abundant in the backfin meat, followed by the claw meat. The essential fatty acids, such as EPA and DHA, are vital for cognitive function, reducing inflammation, and supporting overall cardiovascular health (Calder, 2021). The claw, lump, and backfin meats possessed substantial quantities of these fatty acids, making them a valuable source of these essential fatty acids. Furthermore, the significant variations in fatty acid profiles across edible portions of P-BSC might be attributed to their physiological roles and lipid storage mechanisms. The roe, being the reproductive organ, contains higher energy storage in the form of SFAs, while other parts like the lump and claw meat, which are involved in movement and structural functions, are richer in PUFAs (Sreelakshmi et al., 2016). Therefore, the lump and claw meats, with their high PUFA content and substantial levels of EPA and DHA, are

Table 2
Fatty acid composition of lump, claw, backfin meats and roe in precooked blue swimming crab (*Portunus pelagicus*).

Fatty acid (g/100 g) *	Lump	Claw	Backfin	Roe
C14:0 (Myristic)	1.36±0.03^{b}	1.01±0.01^{c}	1.29±0.00^{b}	3.27±0.04^{a}
C15:0 (Pentadecanoic)	1.45±0.03^{b}	1.32±0.01^{d}	1.38±0.01^{c}	2.16±0.01^{a}
C16:0 (Palmitic)	20.12±0.21^{b}	19.23±0.14^{c}	20.18±0.20^{b}	29.54±0.06^{a}
C16:1 (Palmitoleic)	0.10±0.02^{b}	0.07±0^{b}	0.08±0.01^{b}	9.33±0.03^{a}
C17:0 (Heptadecanoic)	3.00±0.03^{b}	3.06±0.01^{a}	3.02±0.00^{b}	2.83±0.00^{c}
C17:1 cis 10 (cis-10-Heptadecanoic)	0.86±1.15^{ab}	1.84±0.01^{a}	1.60±0.01^{ab}	0.07±0.01^{b}
C18:0 (Stearic)	14.34±0.10^{a}	14.24±0.00^{a}	14.31±0.04^{a}	12.50±0.04^{b}
C18:1 cis 9 (Oleic)	4.37±0.02^{b}	4.16±0.02^{c}	4.21±0.02^{c}	5.00±0.04^{a}
C18:3 cis 6,9,12 gamma (gramma-Linolenic)	0.72±0.01^{b}	0.36±0.01^{d}	0.66±0.01^{c}	1.95±0.01^{a}
C18:3 cis 9,12,15 alpha (alpha-Linolenic)	0.09±0.00^{c}	0.74±0.00^{a}	0.06±0.00^{c}	0.37±0.06^{b}
C20:0 (Arachidic)	0.43±0.04^{b}	0.27±0.00^{c}	0.37±0.05^{bc}	1.21±0.04^{a}
C20:0 (Docosanoic)	0.27±0.06^{c}	0.36±0.06^{bc}	0.39±0.00^{b}	0.91±0.00^{a}
C20:1 cis 11 (cis-11-Eicosenoic)	0.20±0.01^{b}	0.11±0.00^{b}	0.17±0.00^{b}	0.39±0.12^{a}
C20:3 cis 8,11,14 (cis-8,11,14-Eicosatrien)	0.31±0.11^{b}	0.21±0.01^{b}	0.26±0.00^{b}	0.73±0.05^{a}
C20:4 cis 5,8,11,14 (cis-5,8,11,14-Eicosatetraenoic)	14.36±0.23^{b}	16.49±0.04^{a}	13.99±0.01^{c}	9.23±0.01^{d}
C20:5 cis 5,8,11,14,17 EPA (cis-5,8,11,14,17-Eicosapentaenoic)	21.97±0.42^{a}	20.25±0.04^{b}	20.70±0.04^{b}	8.54±0.03^{a}
C22:6 cis 4710,13,16,19 DHA (cis-4710,13,16,19-Docosahexaenoic)	15.78±0.08^{c}	16.24±0.01^{b}	17.13±0.18^{a}	11.12±0.01^{d}
C23:0 (Tricosanoic)	0.31±0.03^{b}	0.07±0.01^{d}	0.26±0.02^{c}	0.90±0.00^{a}
Saturated Fatty Acid (SFA)	41.28±7.66^{b}	39.55±7.46^{c}	41.18±7.68^{b}	53.32±10.00^{a}
Monounsaturated Fatty Acid (MUFA)	5.51±2.02^{c}	6.17±1.93^{b}	6.05±1.93^{b}	14.78±4.38^{a}
Polyunsaturated Fatty Acid (PUFA)	53.21±9.66^{b}	54.28±9.54^{a}	52.79±9.52^{c}	31.93±4.82^{d}

* Data are expressed as mean ± SD ($n = 3$). Different lowercase superscripts within the same row indicate significant difference ($p < 0.05$).

particularly beneficial from a nutritional standpoint, thereby promoting heart and brain health (Swanson et al., 2012). Although the roe was rich in SFAs, it still offered benefits due to its high MUFA content. Thus, different crab edible parts could achieve distinct dietary purposes, depending on health priorities such as cardiovascular or cognitive health.

3.5. Amino acid profiles of P-BSC lump meat and roe

Amino acid profiles of both samples contained a substantial amount of essential amino acids (EAAs) (Table 3). Roe had a slightly higher content of EAA, than lump meat ($p < 0.05$). The main amino acids like glutamic acid, leucine, and aspartic acid were prominent in both portions. Glutamic acid was highest in the lump portion and comparatively lower in the roe. This might contribute to the different levels of umami taste between lump meat and roe. However, roe had a higher content of leucine than lump. Interestingly, cystine level was notably higher in roe than lump meat, whereas lump meat had higher aspartic acid content than roe. The difference in amino acid composition between the lump meat and roe reflected the distinct biological functions of these tissues. Lump meat is largely involved in movement and thus requires more glutamic acid, which plays a key role in muscle metabolism and energy production (Li et al., 2021). In contrast, the higher cystine and leucine contents of roe aligned well with its role in reproduction, as leucine is crucial for protein synthesis, supporting growth and development (Harlıoğlu et al., 2021).

Table 3
Amino acid profiles of lump meat and roe of precooked blue swimming crab (*Portunus pelagicus*).*

Types of amino acid	Amino acid content (%)	
	Lump	Roe
Aspartic Acid/Asparagine	9.38±0.04^{a}	8.02±0.03^{b}
Cystine	2.53±0.04^{b}	4.65±0.08^{a}
Glutamic Acid/Glutamine	12.95±0.08^{a}	6.18±0.04^{b}
Glycine	3.45±0.07^{a}	2.75±0.01^{b}
Histidine	3.60±0.06^{b}	5.38±0.03^{a}
Hydroxylysine	0.98±0.01^{b}	1.84±0.01^{a}
Hydroxyproline	0.13±0.03^{b}	0.42±0.06^{a}
Isoleucine	4.11±0.09^{b}	4.51±0.04^{a}
L-Alanine	5.59±0.05^{a}	4.71±0.04^{b}
L-Arginine	10.07±0.12^{a}	7.71±0.01^{b}
Leucine	9.69±0.07^{b}	11.04±0.01^{a}
Lysine	8.37±0.05^{a}	6.02±0.03^{b}
Methionine	4.33±0.04^{a}	4.39±0.04^{a}
Phenylalanine	4.49±0.01^{b}	4.92±0.01^{a}
Proline	4.16±0.06^{b}	4.96±0.01^{a}
Serine	3.55±0.04^{b}	5.11±0.01^{a}
Threonine	3.68±0.03^{b}	4.84±0.01^{a}
Tryptophan	0.74±0.00^{b}	1.45±0.08^{a}
Tyrosine	3.76±0.02^{b}	4.94±0.04^{a}
Valine	4.49±0.01^{b}	6.20±0.07^{a}
Essential Amino Acid (EAA)	**53.55±0.52^{b}**	**56.48±0.33^{a}**
Non-Essential Amino Acid (NEAA)	**46.45±0.45^{a}**	**43.52±0.31^{b}**

* Data are expressed as mean ± SD ($n = 3$). Different lowercase superscripts within the same row indicate significant difference ($p < 0.05$).

3.6. Next generation sequencing of P-BSC lump meat and roe

The taxonomic analysis of both lump meat and roe provided a comprehensive view of the microbial composition at multiple taxonomic levels, as illustrated in Fig. 2. The lump sample had certain bacterial families. *Moraxellaceae* was the most abundant, followed by *Pseudoalteromonadaceae* and *Planococcaceae* (Fig. 2A–C). Other families were present but at lower proportions. In contrast, the roe sample displayed a slightly different microbial composition, in which *Moraxellaceae* was still the most abundant. *Vibrionaceae* and *Pseudoalteromonadaceae* were important microbial populations in roe (Fig. 2D–F). These results reflected the distinct microbial communities associated with the lump and roe samples. Crab meat products are highly perishable owing to the high abundant nutritional components and moisture (Anupama et al., 2018) and the spoilage is largely caused by the metabolic activities of microorganisms (McDermott et al., 2018).

At the genus level, lump meat had *Pseudoalteromonas* as the dominant genus. *Psychrobacter* was the second most prevalent genus, followed by *Planococcus* and *Vibrio*. Interestingly, *Pseudoalteromonas* and *Psychrobacter* were both genera associated with marine environments (Kothe et al., 2020). Thus, the result was consistent with the habitat of the samples studied. On the other hand, the roe sample was dominated by *Psychrobacter*, followed by *Pseudoalteromonas*, *Fusobacterium*, and *Vibrio*.

The species-level analysis revealed further variations between the two samples. In the lump meat, *Pseudoalteromonas* sp. was the most abundant species, followed by *Planococcus halocryophilus* and

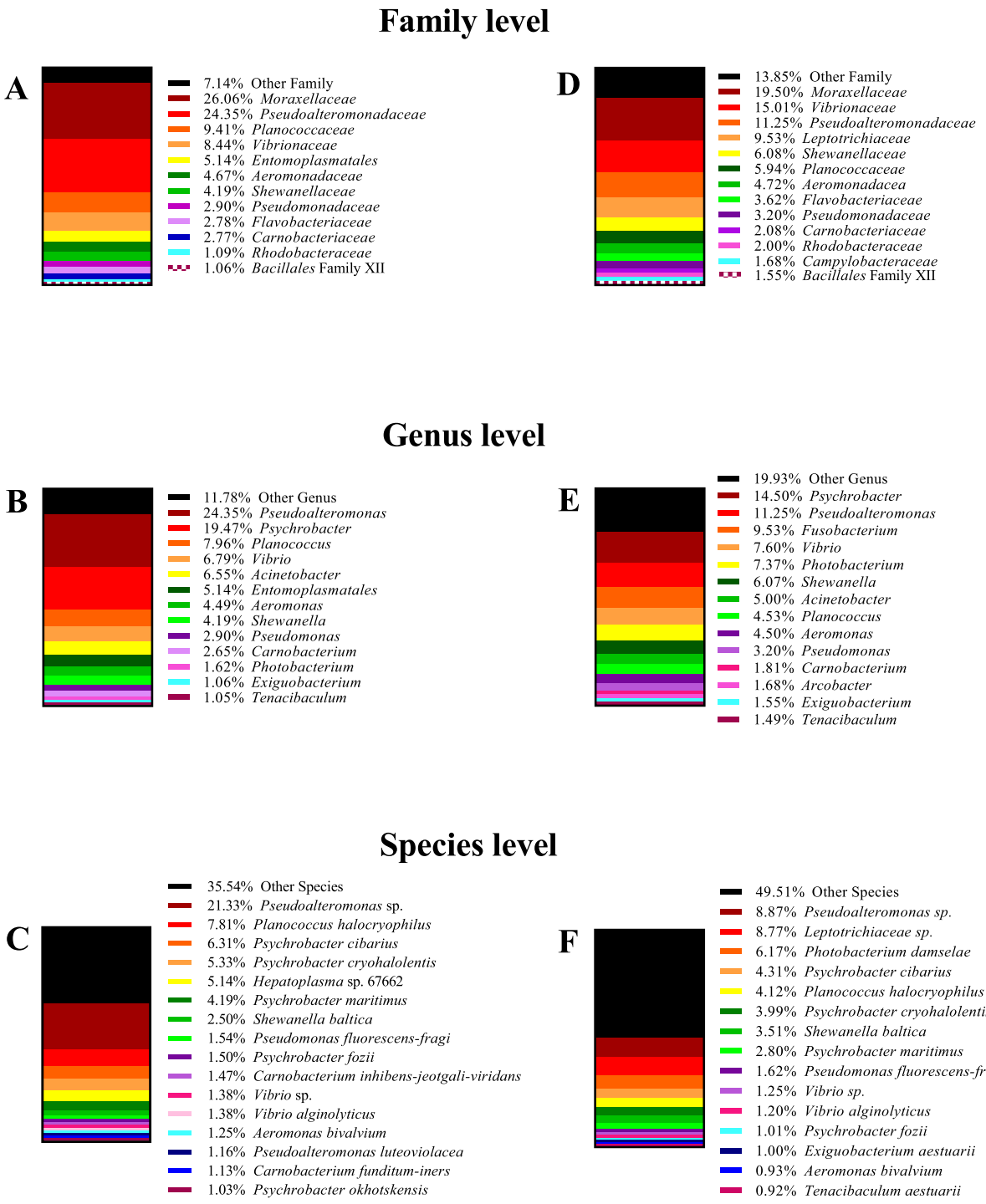

Fig. 2. Relative abundance (%) of the taxonomies of lump meat sample (A-C) and roe (D—F) with family level, genus level and species level. Unassigned and low abundant (<1 %) microorganisms were summarized in the group "Others".

Psychrobacter cibarius. Meanwhile, in the roe sample, *Psychrobacter cibarius* was one of the prevalent species, and *Photobacterium damselae* and *Shewanella baltica* were also detected.

The differences between the lump meat and roe samples highlighted the influence of environmental and physiological factors on microbial composition. The roe, for example, showed a higher proportion of *Vibrionaceae* compared to the lump meat. *Vibrionaceae*, specifically *Vibrio* species, are often associated with nutrient-rich environments (Sampaio et al., 2022), which may explain their higher abundance in roe due to its high nutrient content. The presence of *Vibrio alginolyticus*, which has been linked to fish spoilage and marine diseases, raises concerns about the potential implications for food quality maintenance. Further research in the spoilage potential and safety risks posed by *Vibrio* species in roe could provide valuable insights for management and mitigation of these risks in BSC or other crustaceans.

Furthermore, the presence of *Flavobacteriaceae* and *Carnobacteriaceae* in both samples, at lower abundances, suggested that these families play secondary roles in the microbial ecosystem. Despite their lower prevalence, members of these families are known for their ability to degrade complex organic materials, which could indicate their involvement in the breakdown of crab tissues during spoilage or decomposition (Anacleto et al., 2011). The differences observed between the genus and

species compositions in the lump meat and roe samples were also related with specific ecological roles. For example, *Psychrobacter cibarius* was found at higher concentrations in the roe sample, which could be related to its specific chemical composition. In contrast, the dominance of *Pseudoalteromonas* in the lump meat indicated that the bacteria in this part of the crab were more adapted to environmental stressors, as many species in this genus produce antimicrobial compounds (Zote et al., 2018). When compared to the lump meat, the relatively high proportion of *Vibrionaceae* in the roe sample was observed. This finding could suggest that the roe provides a unique microenvironment that supports the growth of *Vibrio* species. The nutrient content of roe likely played a role in determining its microbial community. Contamination by bacteria generally occurs during production, particularly through hand-picking, as microorganisms from both the surrounding processing environment, handlers and processors can transfer to aquatic roe (Miettinen et al., 2003). Roe more likely offered an abundance of substrates for microbial metabolism that differs from the lump meat. Moreover, the presence of *Fusobacterium* in the roe sample was particularly noteworthy. Members of this genus are often associated with anaerobic environments and are known for their ability to metabolize amino acids and peptides (Robinson et al., 2020). This could indicate that the roe provided an anaerobic condition, while the aerobic marine environment contributed to the growth of other organisms.

Furthermore, the alpha diversity indices revealed that roe possessed a higher microbial diversity than lump meat. The roe displayed an observed species count of 807.90 ± 10.30, with a Shannon diversity index of 6.66 ± 0.02, and an Inverse Simpson index of 0.97 ± 0.00. Conversely, the lump meat showed lower diversity values, including an observed species count of 712.70 ± 7.90, a Shannon index of 6.28 ± 0.02, and an Inverse Simpson index of 0.95 ± 0.00. These results implied that crab roe could support a richer microbial community than lump meat, which could negatively affect its safety and quality.

3.7. Effect of COSC conjugate and HPP treatment on microbiological quality of P-BSC lump meat and roe

The microbial quality of P-BSC lump meat and roe is shown in Table 4. In the control lump meat sample (LC0H0), all microbial counts were observed. However, the control roe sample (RC0H0) showed slightly higher values of APC, PBC, VSC, H_2SPBC, and PSC (Table 4). These results aligned with the NGS data (Fig. 2), highlighting higher initial contamination levels in roe.

For lump meat and roe treated with 200 ppm COSC conjugate without HPP (LC200H0 and RC200H0), a slight reduction in microbial counts was attained. The APC for lump meat and roe dropped by 0.09 and 0.10 log CFU/g, respectively, compared to that found in the control samples, indicating the antimicrobial effect of COSC conjugate. PBC, VSC, H_2SPBC, and PSC were also decreased to some extent, though overall microbial populations remained relatively high (Table 4). The result suggested that COSC conjugate alone possessed antimicrobial properties but was insufficient to significantly reduce microbial counts in crab meat or roe. Typically, the CHOS-CAT conjugate demonstrates bactericidal and bacteriostatic effects due to its multiple hydroxyl groups and an amino group (Mittal et al., 2022). It was also noted that the use of COSC conjugate above 200 ppm led to a color change in the lump meat from a white color to a darker color, which was undesirable and unacceptable for consumers (data not shown).

When examining the effects of HPP alone, 100 MPa HPP for 1.5 min (LC0H100–1.5 and RC0H100–1.5) resulted in a reduction of APC by 0.27 and 0.40 log CFU/g, respectively, with corresponding decreases in other microbial counts. Extending HPP treatment to 3 min (LC0H100–3 and RC0H100–3) further decreased microbial levels (Table 4). The addition of 200 ppm COSC conjugate along with 100 MPa HPP led to slightly greater reductions, compared to the sample treated with 100 MPa HPP without COSC treatment. LC200H100–3 and RC200H100–3 with the longer HPP time showed higher reduction in microbial load.

Table 4
Changes in microbial count of lump meat and roe of precooked blue swimming crab (*Portunus pelagicus*) treated with COSC conjugate or HPP and their combination.*

Treatments	Microbial count of precooked blue swimming crab				
	APC (Log CFU/g)	PBC (Log CFU/g)	VSC (Log CFU/g)	H_2SPBC (Log CFU/g)	PSC (Log CFU/g)
Lump meat sample (L)					
Control (LC0H0)	6.06 ± 0.10^{a}	5.10 ± 0.07^{a}	5.35 ± 0.12^{a}	4.26 ± 0.24^{a}	5.80 ± 0.18^{a}
LC0H100–1.5	5.79 ± 0.10^{bc}	4.88 ± 0.06^{b}	4.69 ± 0.09^{c}	3.84 ± 0.06^{b}	5.36 ± 0.10^{b}
LC0H100–3	5.41 ± 0.05^{e}	4.36 ± 0.10^{c}	4.36 ± 0.10^{d}	3.36 ± 0.32^{cd}	4.59 ± 0.11^{c}
LC0H300–1.5	4.52 ± 0.07^{g}	ND	ND	ND	ND
LC0H300–3	4.26 ± 0.24^{hi}	ND	ND	ND	ND
LC0H500–1.5	ND	ND	ND	ND	ND
LC0H500–3	ND	ND	ND	ND	ND
LC200H0	5.97 ± 0.07^{ab}	5.02 ± 0.06^{a}	5.22 ± 0.10^{b}	4.20 ± 0.17^{a}	5.76 ± 0.15^{a}
LC200H100–1.5	5.69 ± 0.09^{cd}	4.82 ± 0.04^{b}	4.42 ± 0.10^{d}	3.72 ± 0.10^{b}	5.20 ± 0.17^{b}
LC200H100–3	5.20 ± 0.17^{f}	4.20 ± 0.17^{d}	4.20 ± 0.17^{e}	3.16 ± 0.28^{d}	4.46 ± 0.15^{c}
LC200H300–1.5	4.40 ± 0.03^{gh}	ND	ND	ND	ND
LC200H300–3	4.10 ± 0.17^{i}	ND	ND	ND	ND
LC200H500–1.5	ND	ND	ND	ND	ND
LC200H500–3	ND	ND	ND	ND	ND
Roe sample (R)					
Control (RC0H0)	6.33 ± 0.03^{a}	5.24 ± 0.06^{a}	5.69 ± 0.09^{a}	4.49 ± 0.20^{a}	5.99 ± 0.09^{a}
RC0H100–1.5	5.93 ± 0.08^{b}	5.00 ± 0.04^{ab}	5.11 ± 0.12^{c}	4.16 ± 0.28^{b}	5.59 ± 0.11^{b}
RC0H100–3	5.67 ± 0.06^{de}	4.51 ± 0.52^{c}	4.52 ± 0.07^{e}	3.59 ± 0.11^{c}	5.10 ± 0.17^{c}
RC0H300–1.5	4.99 ± 0.11^{f}	ND	ND	ND	ND
RC0H300–3	4.59 ± 0.11^{h}	ND	ND	ND	ND
RC0H500–1.5	ND	ND	ND	ND	ND
RC0H500–3	ND	ND	ND	ND	ND
RC200H0	6.23 ± 0.08^{a}	5.19 ± 0.06^{ab}	5.46 ± 0.15^{b}	4.46 ± 0.15^{a}	5.88 ± 0.06^{a}
RC200H100–1.5	5.88 ± 0.06^{bc}	4.95 ± 0.10^{b}	4.82 ± 0.30^{d}	4.10 ± 0.17^{b}	5.49 ± 0.20^{b}
RC200H100–3	5.59 ± 0.11^{e}	4.42 ± 0.10^{c}	4.46 ± 0.15^{ef}	3.46 ± 0.15^{c}	4.68 ± 0.14^{d}
RC200H300–1.5	4.75 ± 0.05^{g}	ND	ND	ND	ND
RC200H300–3	4.42 ± 0.10^{i}	ND	ND	ND	ND
RC200H500–1.5	ND	ND	ND	ND	ND
RC200H500–3	ND	ND	ND	ND	ND

* Data are expressed as mean ± SD ($n = 3$). Different lowercase superscripts within the same column indicate significant difference ($p < 0.05$). ND: not detected; APC: Aerobic plate count; PBC: Psychrophilic bacteria count; VSC: *Vibrio* spp. count; H_2SPBC: Hydrogen sulfide producing bacteria count and PSC: *Pseudomonas* spp. count. Control denotes the crab meat without COSC conjugate and HPP; C0H0 and C200H0: sample treated with COSC conjugate at concentrations of 0, 200 ppm, respectively, without HPP; C0H100–1.5, C0H300–1.5, C0H500–1.5: sample treated with COSC conjugate at concentrations of 0 ppm, followed by HPP at 100, 300, 500 MPa, respectively, for 1.5 min; C200H100–1.5, C200H300–1.5, C200H500–1.5: sample treated with COSC conjugate at concentrations of 200 ppm, followed by HPP at 100, 300, 500 MPa, respectively, for 1.5 min; C0H100–3, C0H300–3, C0H500–3: sample treated with COSC conjugate at concentrations of 0 ppm, followed by HPP at 100, 300, 500 MPa, respectively, for 3 min; C200H100–3, C200H300–3, C200H500–3: sample treated with COSC conjugate at concentrations of 200 ppm, followed by HPP at 100, 300, 500 MPa, respectively, for 3 min.

For medium pressure HPP (300 MPa), both lump meat and roe exhibited significant microbial reductions, in which PBC, VSC, H_2SPBC, and PSC were undetectable in both COSC-treated and untreated samples. A similar result was documented for blood clams treated with HPP (Palamae, Patil, et al., 2024). The combination of COSC conjugate and 300 MPa HPP for 3 min was particularly effective, nearly eliminating all microorganisms. APC reductions in lump meat and roe were 32.0 % and 30.0 % (LC200H300–3 and RC200H300–3) and 29.7 % and 27.5 % (LC0H300–3 and RC0H300–3), compared to that of the control. At the highest pressure used (500 MPa), microbial populations in both lump meat and roe were undetectable (ND) after both 1.5 and 3 min of treatment. Likewise, the combination of 200 ppm COSC conjugate with 500 MPa HPP resulted in no detectable microbial counts for all microorganisms tested. HPP at 500 MPa alone was sufficient to ensure microbial safety, and no additional benefit from COSC conjugate was found when HPP at this pressure level was employed. HPP is effective in killing or damaging bacterial cells by disrupting various cellular structures and functions (Palamae, Temdee, et al., 2024). The intense pressure causes the leakage of intracellular substances, such as ATP, which leads to cell death (Smelt et al., 1994). Structural disruptions include changes in the cell membrane's permeability, stability, and the formation of pores, as well as the denaturation of proteins and membrane-bound enzymes. HPP also affects other cellular components, including the cytoskeleton, nucleus, and organelles, potentially inducing genetic changes (Nikparvar et al., 2021). These combined effects, particularly the rupturing of cell membranes and release of intracellular contents, are key contributors to the reduction in bacterial populations (Lee et al., 2020). Additionally, HPP treatment plausibly enhanced the penetration of the CHOS-CAT conjugate into the lump meat and roe, thereby causing more effective damage to the bacterial cell membranes.

Therefore, HPP at 500 MPa or the combination of COSC conjugate and HPP treatment effectively reduced microbial loads in both crab lump meat and roe. HPP at higher pressure levels and longer treatment times showed the highest efficacy in inactivating microorganisms in crab meat or roe. COSC conjugate alone showed some antimicrobial activity but was most effective when combined with HPP. These findings suggested that HPP at 500 MPa was a promising approach for enhancing the microbial safety of precooked seafood products, such as precooked crab products. However, COSC conjugate could be used in combination with HPP at lower pressure levels to avoid the drastic disruption of the structure of both meat and roe caused by the high pressure introduced.

Fig. 3 illustrates the relationship between sample type, COSC conjugate concentration, and HPP pressure levels on the microbial counts of P-BSC lump meat and roe. The PCA results revealed that the two principal components accounted for 99.94 % of the total variance in microbial counts. PERMANOVA analysis revealed that HPP pressure level was the only factor significantly influencing microbial counts ($p < 0.05$). In contrast, sample type and COSC conjugate concentration had no significant effect on microbial composition (sample type: $p > 0.05$ and COSC conjugate concentration: $p > 0.05$).

4. Conclusion

This study highlighted significant findings regarding the differences in appearance, microstructure, and chemical compositions between the lump, claw, backfin meats, and roe of the P-BSC. Lump meat was rich in protein with high content of glutamic acid, while roe had high fat and protein contents, with notable amounts of saturated fatty acids and essential amino acids. Both portions were susceptible to microbial spoilage. Roe had more diverse microbial community, which might affect its spoilage. The high-pressure processing (HPP) at 500 MPa completely reduced microbial contamination in both the lump meat and roe. HPP at 300 MPa in combination of COSC conjugate eradicated microbial loads to high degree. However, HPP pressure level was the only factor significantly influencing microbial counts. Further study on quality changes of HPP or COSC conjugate treated samples and the use of combined COSC conjugate and HPP at lower pressure under the hurdle concept during the extended storage will be carried out.

Ethics and consent

This study does not involve any human or animal testing.

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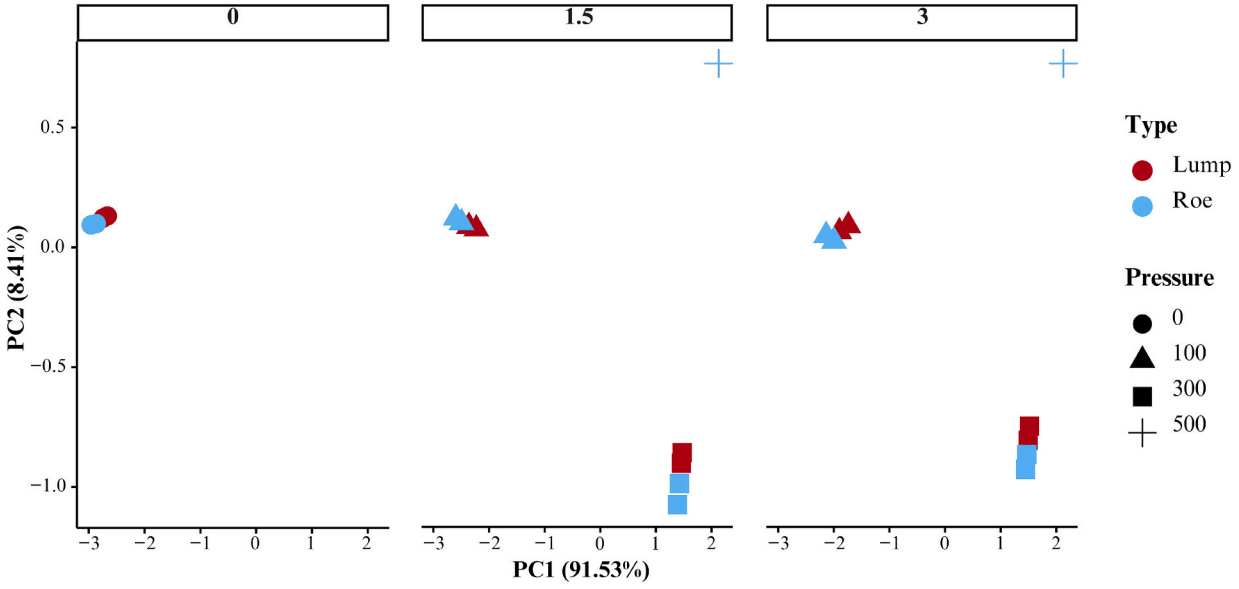

Fig. 3. Principal component analysis (PCA) for changes in microbial count of lump meat and roe of precooked blue swimming crab (*Portunus pelagicus*), considering the effects of sample type, COSC conjugate concentration, and HPP pressure levels. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

CRediT authorship contribution statement

Khaettareeya Pimsannil: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Suriya Palamae:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Xinru Fan:** Writing – review & editing. **Qiancheng Zhao:** Writing – review & editing. **Bin Zhang:** Writing – review & editing. **Soottawat Benjakul:** Writing – review & editing, Validation, Supervision, Resources, Investigation, Funding acquisition, Conceptualization.

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.

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Data availability

Data will be made available on request.

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