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Functional properties of protein-rich ingredients recovered from brown crab side streams via mechanical separation vs pH-shift processing

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ABSTRACT

The effects of steam cooking, cleaning (i.e., carapace and viscera removal), mechanical separation (MS), and alkaline pH-shift processing on mass yield from clawless, poorly filled brown crab were evaluated together with the structural, dynamic rheological, and functional properties of the resulting protein-enriched ingredients. Mechanical separation of cooked crab resulted in the highest mass yield (57-60% w/w based on the input biomass) but yielded the lowest protein functionality. In contrast, pH-shift processing enabled more efficient protein concentration (10-14% wet weight basis) than MS (8-9% wet weight basis) due to improved shell removal; however, this method was only applicable to raw crab due to the heat-induced protein denaturation reducing its solubility. Protein denaturation in cooked material and proteolytic degradation in non-cleaned crab significantly impaired gel-forming capacity in ingredients obtained by both processing routes. Ingredients produced by pH-shift processing of cleaned raw crab exhibited a threefold higher gel-forming capacity (164 g vs 54 g, respectively) and significantly improved water-holding capacity compared with MS-derived proteins. Overall, valorization of clawless, poorly filled brown crab into protein-enriched ingredients represents a viable strategy to reduce biomass losses in crab fisheries. Nevertheless, appropriate combinations of biomass pretreatment and processing methods must be selected according to the intended ingredient functionality.

1. Introduction

Crustaceans and crustacean-based products are among the most popular and high-value seafood products due to their high-quality protein and attractive taste (Nanda et al., 2021). Edible crab, also known as brown crab (*Cancer pagurus*), is one of the most economically important decapod crustaceans in Europe, with annual landings of approximately 40,000–60,000 tons and a total value reaching around €100 million (McDermott et al., 2018; Mesquita et al., 2025). Crab claws have become increasingly popular for consumption because of their unique taste and mild flavor. However, the crab bodies remaining after claw removal are commonly discarded, despite accounting for up to 90% of the initial biomass. The residual material, including muscle and hepatopancreas tissue, is rich in valuable nutrients such as proteins with a well-balanced amino acid profile, long-chain n-3 (LC n-3) fatty acids, as well as minerals and vitamins (Velazquez et al., 2021). The issue of brown crab body wastage is particularly pronounced during winter, when crabs are poorly filled, i.e., they contain little to no meat in the carapace, and are sometimes referred to as “hollow” crabs. This is mainly linked to reduced feeding activity and metabolic slowdown at low water temperatures, resulting in the discarding of a large proportion of these crabs at sea. Recovery of protein from such residues could significantly enhance the sustainability of crab fisheries. However, this is technically challenging, as the rigid exoskeleton restricts access to residual meat located beneath the carapace, within the legs, and at the lamellar attachment regions. Therefore, processing technologies that enable efficient meat separation while preserving protein functionality and sensory quality are needed to improve the utilization of crab resources.

Mechanical separation (MS) of muscle residues into mince from bones and exoskeletons is considered an inexpensive, simple, and highly efficient technology that has been successfully applied in the poultry industry for decades. More recently, interest in its application for the upcycling of seafood processing rest raw materials has increased rapidly (Abdollahi et al., 2021). In this method, residual meat is separated from bones or exoskeletons using either low ($<10^4$Pa) or high ($>10^4$Pa) pressure. In belt–drum mechanical separators, the raw material is compressed between a moving belt and a perforated rotating drum, whereby soft muscle tissue is forced through the drum perforations,

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while harder components such as bones and shells are retained or fragmented and the muscle fiber structure is minced during this process (Secci et al., 2017). Although there are no reports on the application of mechanical separation (MS) to poorly filled brown crab, previous studies have evaluated its use for recovering minced meat from green (Galetti et al., 2017), Jonah (Baxter & Skonberg, 2008), and blue (Gates & Parker, 1994) crabs. Due to the strong compressive action of the belt and the low selectivity of the drum, MS can yield relatively high mass recovery from crab residues. However, the resulting mince may contain substantial shell contamination, which can negatively affect nutritional composition (Abdollahi et al., 2025), functional properties, and, consequently, its applicability.

An alternative technique to the classic MS for value addition of seafood rest raw materials is the so-called pH-shift processing (Abdollahi & Undeland, 2019). The pH-shift technology involves selectively separating proteins from other insoluble materials at low temperature using water at a high or a low pH value (<3.5 and >10.5), followed by precipitation at the protein isoelectric point (pI) (Pezeshk et al., 2021). This technique has been extensively studied as a tool to extract high-quality gel-forming proteins from fish rest raw materials (Abdollahi & Undeland, 2019) and its potential for application on green crabs, which are small and invasive species, has been reported (Khiari et al., 2020). Generally, high selectivity of the pH-shift process allows efficient removal of unwanted materials, e.g., bone or shell, from highly complex seafood side streams, resulting in proteins with relatively high purity and good functionality (Abdollahi et al., 2017). However, compared to MS, the pH-shift method is a sophisticated technology involving higher levels of investment and processing and may result in lower mass yield (Abdollahi et al., 2021). To the best of our knowledge, a side-by-side comparison of MS and pH-shift processing for valorization of brown crab side streams and their effects on protein yield and functionality has not yet been reported.

Gel formation is one of the most important functional properties of proteins, defining their technological applications in different products. Proteins with good gelling ability are particularly valuable for the production of structured seafood products such as surimi-based items, restructured seafood analogues, and other processed foods where texture and water-holding capacity are critical quality attributes. The main muscle proteins involved in the network formed during gelation are myosin and actin, which are responsible for the textural and mechanical properties of the gels (Hernández-Robledo et al., 2017). To obtain gels with suitable mechanical attributes, the mentioned myofibrillar proteins must be in their native state. Denaturation/aggregation of muscle proteins generally prevents the formation of the three-dimensional structure responsible for the functional and mechanical attributes of gels (Wu et al., 2021). However, crab myofibrillar proteins have been shown to retain their gel-forming capacity even after heating, a treatment commonly applied to facilitate the removal of meat from the shell. For instance, Baxter and Skonberg (2008) reported that thermal pre-treatment of Jonah crab before manual meat separation improved the gel strength and water-holding capacity (WHC) of produced gels. Similarly, Martínez et al. (2014) found that blue crab meat pre-cooked at 50-120 °C for 30 min was able to form a gel with a relatively good quality. However, there is no report comparing the effect of pre-cooking on the efficiency of MS and pH-shift processing of brown crab and its subsequent effect on the gel-forming capacity of the recovered protein-enriched ingredients.

The present study aimed to comparatively evaluate the efficiency of MS and pH-shift processing for mass yield from clawless poorly filled brown crab residues, considering variations such as whole versus gutted and cooked versus uncooked raw materials. In addition, the effects of pre-cooking, gutting, and different processing technologies on the crude composition, structural properties, and gel-forming capacity of the recovered protein-rich ingredients were quantified and assessed. The findings contribute to a better understanding of how processing conditions built on different principles may influence crustacean protein recovery and quality, providing information relevant for potential industrial applications while considering both functional performance and food safety.

2. Materials and methods

2.1. Preparation of brown crab samples

Poorly filled brown crabs were caught off the Swedish West coast in March 2020, specifically for this project and transported to Sweden Shellfish AB (Bua, Sweden) while kept alive. Fig. 1 shows how the cooked and raw crabs had barely any meat in their carapace area. Then, 30 kg of the crabs were subjected to an industrial steam cooking system at 95–100 °C for 5 min at Sweden Shellfish, which is currently used for shrimp and crayfish steaming. The cooked samples and around 60 kg of raw crabs still alive were thereafter transported to Fisk Idag AB for MS. Before MS, the raw crabs were humanely killed by piercing the central nerve ganglia using a sharp tool. The crabs were processed in three independent experimental batches using pooled samples of >30 individuals to minimize biological variability. All crabs were in a comparable physiological condition and were sampled during the intermolt stage with no visible signs of molting or recent molting. Individual carapace width and the exact number of crabs per batch were not recorded. Based on their commercial size and visual assessment, the crabs were generally within the typical adult size range reported for *C. pagurus*; however, these biometric characteristics were not quantitatively determined.

Both raw and cooked clawless samples were then divided into two parts where one half was used directly for processing and the second half was subjected to pre-removal of carapace as well as pre-emptying of viscera, including e.g., brownish belly content, egg and hepatopancreas (hereafter referred to as with or without carapace) to investigate their effect on quality of separated meat/protein isolate (See Fig. 1). The experimental design therefore consisted of four raw material conditions (raw whole, raw gutted, cooked whole, cooked gutted) subjected to either mechanical separation or pH-shift processing.

2.2. Mechanical separation of crab meat

To separate meat, a pre-trial with both raw and cooked crabs was conducted using a BAADER mechanical separator (BAADER 600) equipped with a band specifically provided by BAADER for crab processing. A drum with a 2 mm hole diameter was used based on a recommendation from the supplier. The crabs were manually pre-crushed with a hammer to facilitate feeding to the separator and passed through the mechanical separator, whereafter the separated meat was collected, packed in plastic zip lock bags and frozen at −80 °C until further studies (See Fig. 1).

For the raw samples dedicated pH-shift processing at Chalmers University of Technology, the mechanical separator was used as a grinder where one portion of the samples with and one without carapace was remixed with the separated shell in a correct ratio (1 to 1). The separated shell fraction was remixed back with the minced biomass to reconstruct the original crab composition. The ratio was determined based on the average shell-to-meat proportion obtained during preliminary separation trials.

2.3. pH-shift processing of crab

A pre-trial evaluating the solubility of raw and cooked crab proteins at different pH values (2-12) showed that only raw crab can be used for pH-shift processing due to the very low solubility of heat-denatured proteins (see Fig. S1). The crushed raw crabs (500 g) were mixed with cold distilled water at a ratio of 1:6 (w/v) and then homogenized using a Silverson L5M homogenizer (Silverson Machines Ltd, England) for 3 min (7000 rpm). The pH of the homogenate was adjusted to 10 using 2 mol/L

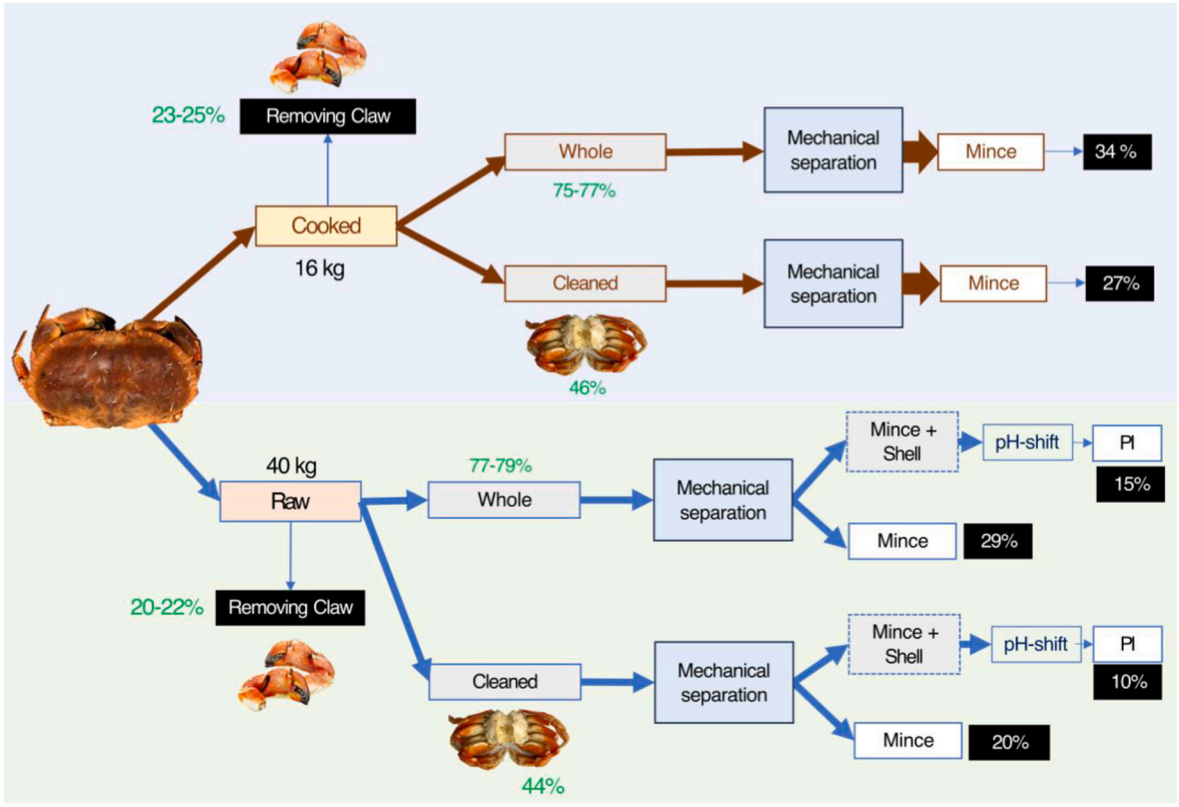

Fig. 1. Schematic overview of the study plan and mass yield obtained after each step or type of processing. The data in green and in black boxes show the mass yield after pre-cutting, mechanical separation (MS) and pH-shift processing based on the weight of the initial whole crab. Removal of the carapace also included the removal of viscera (cleaned). PI means protein isolate.

NaOH, and after 10 min incubation, the homogenate was centrifuged at 5000 × g in a centrifuge (Thermo Scientific, Germany) for 10 min at 4 °C. Thereafter, the supernatant containing the solubilized proteins was separated from the insoluble shell residue using a metal sieve. The pH of the supernatant was readjusted to pH 5 using 2 mol/L HCl. After 10 min incubation, a second centrifugation step followed (5000 × g, 4 °C, 10 min) to separate processing water from the protein isolate. The protein was dewatered using a third centrifugation step at 8000 × g for 10 min (4 °C), and the moisture content was adjusted to 80%. The moisture content of the protein was measured using an IR moisture analyzer (Mettler Toledo, HE73, Switzerland) and adjusted by an extra centrifugation cycle or addition of water, depending on the achieved moisture content after the first cycle. Lastly, the pH of the protein isolate (PI) was adjusted to pH 7 using 2 mol/L NaOH. The entire process was conducted on ice. The PI was stored at −80 °C until further use.

2.4. Mass yield measurement

Mass yield for pH-shift processing and MS was determined according to equations (1) and (2) (Abdollahi et al., 2021).

$$\text{Mass yield } (\%) = \frac{\text{Weight of recovered MS meat or protein isolate}}{\text{Weight of processed whole crabs}} * 100 \quad (1)$$

In Equation (1), the weight of processed whole crabs refers to the initial whole-crab weight before any removal or processing. The pH-shift process was applied exclusively to raw (uncooked) crab; therefore, the corresponding yields relate only to raw crab materials since pH-shift method could not be applied on cooked crab.

2.5. Crude composition analysis

The total crude protein was analyzed using a nitrogen analyzer (TruMac-N, LECO Corp., St. Joseph, MI, USA) according to the Dumas method. A species-specific conversion factor of 5.58 was applied for crab proteins, as suggested by previous studies, to more accurately reflect the nitrogen-to-protein ratio for crustacean muscle (Kang et al., 2019). The fat content of all samples was determined using the method of Goulas and Kontominas (2007) using liquid-liquid extraction. The moisture content and crude ash were measured at 103 °C and 550 °C in an oven, respectively (AOAC, 1984).

2.6. Gel preparation from crab PI and mince

Crab PI and crab mechanically separated meat of each treatment were thawed in tight plastic bags under running tap water until their core temperature reached 0 °C. The samples were then cut into small pieces, and their moisture was adjusted to ~80 (g/hg) by adding melted ice before chopping for 5 min in the presence of 2 g/hg of NaCl to form a homogeneous paste. Then, the pastes were stuffed into plastic tubes with a diameter of 15 mm, both ends of the tubes were sealed, and they were cooked in two steps by heating in a water bath at 35 °C for 30 min and then at 90 °C for 20 min. After that, the gels were placed in iced water for 30 min to stop cooking and stored overnight at 4 °C before being analyzed (Chaijan et al., 2010).

2.7. Measurement of water holding capacity (WHC)

WHC was determined using the method used by (Cardoso et al., 2009). A 5 g (X) of each gel samples was positioned between two layers of pre-weighed (Y) filter paper (Whatman No. 1), centrifuged for 10 min at 3000×g and 20 °C, and weighed again (Z). WHC (g/hg) was

calculated using Equation (2):

$$WHC = \frac{X * \left(\frac{M}{100}\right) - (Z - Y)}{X - \left(\frac{M}{100}\right)} * 100 \quad (2)$$

where M is initial moisture (%).

2.8. Rheological analysis

The rheological properties of crab PI and crab MS minces were measured by a dynamic rheometer (Paar Physica Rheometer MCR 300, Anton Paar GmbH, Austria) as explained previously by Berlo et al. (2023). Briefly, 1.5-2 g of each sample paste was prepared for gel production (see section 2.5) was placed between two parallel geometries (25 mm in diameter) and spaced 1 mm apart. The exposed edges of the cover were smeared with mineral oil to prevent evaporation. The storage modulus (G′) was recorded in the predefined linear viscoelasticity region of the samples with a frequency of 0.1 Hz and a constant strain of 1% while heating the sample to induce the samples to *in-situ* thermal gelation on the rheometer. The in situ gelation was done in three steps in a viscoelastic region (1% strain, 0.1 Hz frequency). First, the temperature increased from 20 °C to 90 °C at a constant heating rate of 5 °C/min, after which the highest temperature was held for 30 min, before ramping the temperature down to 20 °C at a constant cooling rate of 5 °C/min.

2.9. Texture analysis of gels

Textural properties of gels (15 mm in diameter and 15 mm in height) were measured using a puncture test with the aid of a texture analyser TVT 6700 (Perten Instruments, PerkinElmer company, Australia) as described by Sajib et al. (2023). Using a spherical probe (Perten Instruments, N672040, TVT, diameter 5 mm, stainless steel), the gel was once subjected to compression (40%) at a depression speed of 60 mm/min. Five replicates were made for each gel (n = 5), and both deformation (cm) and breaking force (g) were recorded. The samples had an equal diameter and height of 15 mm.

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

The molecular weight distribution of polypeptides in samples was determined by SDS-PAGE according to the method of Laemmli (Laemmli, 1970) as explained by Abdollahi and Undeland (2019). Briefly, protein isolates and MS minces were solubilized in an SDS solution (5 g per hg solution) and homogenized using an IKA Polytron (T18 digital ULTRA-TURRAX®, Germany) at 6000 rpm for 1 min. The proteins were then dissolved by heating the homogenate in a water bath at 85 °C for 1 h. Protein content was determined using the Lowry method modified by Markwell (Markwell et al., 1978), as described above, and adjusted to 4 mg mL^{-1}. Subsequently, 50 μL of the sample was mixed with an equal volume of Laemmli buffer (65.8 mM Tris-HCl, pH 6.8, 2.1% SDS, 26.3% (w/v) glycerol, 0.01% bromophenol blue) (Bio-Rad, USA) containing 50 mL β-mercaptoethanol per liter of buffer, heated at 95 °C for 5 min, cooled, and centrifuged for 5 min at 5000 ×g (Heraeus Fresco 17, Thermo Fisher Scientific, Germany). Aliquots containing approximately 20 μg protein and protein molecular weight markers were separated by electrophoresis at voltages up to 200 V. As a standard, 5 μL of protein ladder (Precision Plus Protein™ Dual Color Standards, 10–250 kDa, Bio-Rad, USA) was used. The SDS–PAGE gel was stained for 30 min with a solution containing 0.2 g L^{-1} Coomassie® Brilliant Blue G-250 (Bio-Rad, USA) in 500 mL L^{-1} methanol and 75 mL L^{-1} acetic acid, followed by two destaining steps (30 min each) using 500 mL L^{-1} methanol and 75 mL L^{-1} acetic acid. The gel was stored at 4 °C overnight prior to scanning and band analysis using a GS-800 Calibrated Densitometer (Bio-Rad, USA).

2.11. Statistical analysis

All experiments were carried out in triplicate, except the mechanical analyses, which were performed in 5 replicates. Statistical analyses were conducted using SPSS 26.0 (SPSS Inc., Chicago, IL, USA) and one-way ANOVA followed by Duncan's multiple range test were used to identify differences between the variables. If $P < 0.05$, the differences were considered significant.

3. Results and discussion

3.1. Mass yield during pH-shift processing and MS

Fig. 2A and B shows mass yield (% w/w) calculated relative to whole crab biomass and to the input materials used for MS and pH-shift processing, respectively. As can be seen, the effect of removing the carapace was more pronounced for MS, resulting in up to a 25% reduction in yield, compared to only a 5% decrease for the pH-shift process. This difference is likely due to the prior removal of brown meat (hepatopancreas) and gills before applying the separation processes. The pronounced difference observed in MS suggests that the lipid-rich hepatopancreas contributes a substantial fraction to the final mince mass recovered through size-based separation, an effect less critical in the solubility-driven pH-shift process. When calculating the yield based on input material to the processes (Fig. 2b), differences in yield due to carapace removal were only visible for the raw crabs.

When applying MS to the crabs, mass yields based on both whole crab and direct input material were higher for cooked than raw crab. A

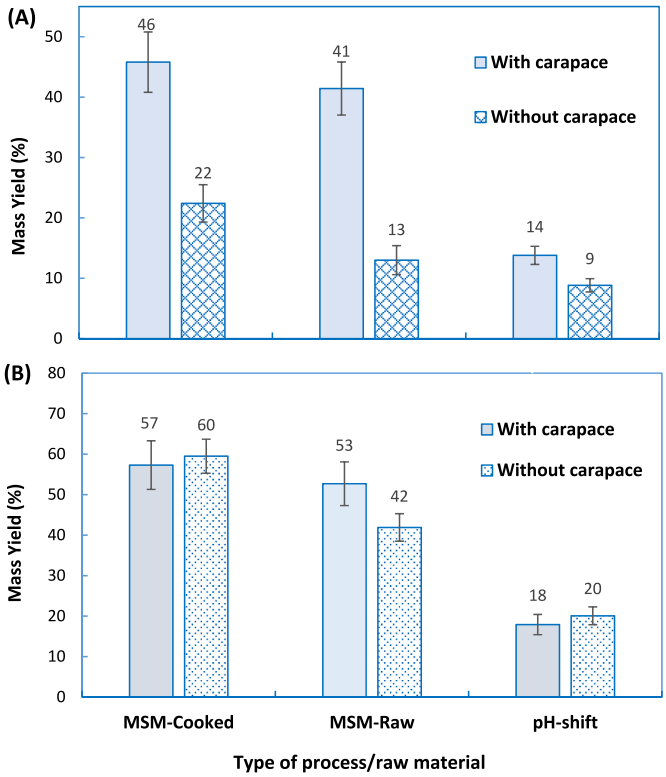

Fig. 2. (A) Mass yield based on whole crab biomass, and (B) based on input raw material to each type of process, for pre-cooked brown crab subjected to mechanical separation (MS, "MSM cooked") or raw poorly filled crab subjected to MS ("MSM raw") or pH-shift processing ("pH-shift"), with or without carapace. MSM = mechanically separated meat. pH-shift method was only applied on raw (uncooked) crab biomass since it was not applicable on the cooked biomass.

plausible explanation is that cooking altered water retention in the crab biomass, which may have influenced processing losses and thus apparent mass yield. Since moisture content was not determined, this should be interpreted with caution. More critically, this is related to the structural changes induced by thermal treatment. Heat denaturation of the myofibrillar proteins (e.g., myosin, paramyosin and actin) facilitates a protein network formation, which contributes to the more self-supporting texture of the muscle tissue. The mechanical stability of the cooked muscle preserves fiber integrity during high-pressure processing, which minimizes fragmentation and fluid loss, thereby enhancing solid recovery (Vaskoska, 2024, pp. 189–194). It can also be related to the more self-supporting texture of the muscle tissue of the cooked crabs induced by heat denaturation, facilitating their separation during mechanical pressing. Similarly, Kang et al. (2019) reported that thermal processing could improve the yield of minced meat from crabs using MS. However, despite the higher total yield, the denaturation of proteins during thermal processing reduces the functional properties of the minced tissue obtained from cooked crab, making it less suitable for applications that require high gelation or water-holding capacity, but more appropriate for culinary uses where textural modifications are desirable (Cortés Morales et al., 2024; Gillman & Skonberg, 2002).

A preliminary trial was conducted to evaluate the applicability of pH-shift processing to both raw and cooked crab biomass by determining protein solubility over a broad pH range (Fig. S1). The raw crab exhibited a pronounced pH-dependent solubility profile, with protein solubility decreasing from approximately 60% at pH 2–3 to a minimum of approximately 7% at pH 5, followed by a progressive increase under alkaline conditions. At pH 10–12, approximately 60–65% of the protein was solubilized, demonstrating that a substantial proportion of the proteins in the raw biomass could be brought into solution at extreme pH values and subsequently recovered by isoelectric precipitation. In contrast, proteins in the cooked crab showed consistently low solubility across the entire pH range, generally remaining below approximately 15%, with no substantial increase even at highly acidic or alkaline pH values (Fig. S1). The markedly reduced solubilization following cooking can most likely be attributed to heat-induced protein denaturation, aggregation, and formation of irreversible intermolecular interactions, which limit protein dissociation and subsequent solubilization during pH adjustment. Thus, although extreme pH conditions efficiently solubilized proteins from raw crab, they were unable to reverse the structural changes induced by cooking. These preliminary results demonstrated that conventional pH-shift processing was not suitable for the cooked crab biomass under the conditions investigated. Consequently, subsequent pH-shift trials were performed only with raw crab, whereas mechanical separation was evaluated for both raw and cooked materials.

Compared with MS of raw crabs, the mass yield obtained using the pH-shift process was almost 50% lower ($P < 0.05$), which can be attributed to the fundamentally different separation mechanisms of the two technologies. MS is essentially a size-exclusion process, allowing recovery of any soft or hard tissue with a particle size smaller than the perforation diameter of the drum (2 mm in this study) under the applied belt pressure. Consequently, MS shows limited selectivity and allows shells, hepatopancreas, other visceral tissues, and muscle to enter the recovered mince. In contrast, the pH-shift process separates components based on pH-dependent solubility and density. Under highly alkaline conditions, mainly alkali-soluble proteins are recovered, while insoluble materials such as lipids, collagenous components, and cellular debris are removed during centrifugation. This high chemical selectivity inevitably reduces the overall mass yield but results in a protein fraction with higher purity and improved functional quality (Nolsoe et al., 2011). The high selectivity was also reflected in similar yields obtained when processing crabs with or without carapace. Similar trends have previously been reported when comparing MS and pH-shift processing of herring and salmon frames (Abdollahi et al., 2021).

3.2. Proximate composition

The proximate compositions (on wet weight, ww, basis) of crab mince and crab PI are presented in Fig. 3. As presented in Fig. 3A, protein was more concentrated in the PI than in the MS minces, especially the PI recovered from crabs without a carapace, which had 35% higher protein content compared with its MS-derived counterpart. This reinforces the higher protein selectivity and efficiency of the pH-shift process compared with the MS.

Regardless of the used separation technology, minces and PI recovered from crabs with carapace had 2-3-fold higher fat content compared with their counterparts from crabs without carapaces. This could be due to the higher fat content of starting materials comprising the viscera, including e.g., belly contents, testis/eggs, gills and/or hepatopancreas, which are the most fatty tissues in crabs, in contrast to the lean muscle (Galetti et al., 2017). The fat content was 1.6 and 4.3% higher in the PI derived from crab without and with carapace, respectively, compared with their MS counterparts (Fig. 3B). There was no fat/emulsion layer formed during pH-shift processing of the crab samples, which is otherwise the main source of fat-removal when applying the process on fatty raw materials. When applying the pH-shift process on lean resources such as crab, it is mainly the solid residues, such as shell or bone residues, which are removed along with some of the phospholipids (Shi et al., 2017), resulting in an up-concentration of fat rather than removal. This up-concentration occurs because most lipids in lean crab muscle are structural phospholipids which, due to their amphipathic nature, form stable emulsified particles under alkaline conditions and are not fully removed during centrifugation (Lu et al., 2020). This higher content of fat in crab PI is in line with the up-concentration of fat found when the pH-shift process was applied to cod rest raw materials (Abdollahi et al., 2021). Other studies of edible crab species (Rangasamy et al., 2024) report significant intra-species variation in proximate composition depending on anatomical parts and physiological condition, reflecting fat distribution differences that influence overall fat content in recovered materials.

On the other hand, the ash contents obtained in crab PIs (0.5–0.7%) were remarkably lower than those found in MS mince (2.1–2.9%) (Fig. 3C), reflecting the high efficiency of the pH-shift process in the removal of shell residues. This efficient removal is attributable to the high chemical selectivity of the pH-shift process. The major mineral components of the shell, primarily calcium salts, are largely insoluble in the alkaline aqueous phase and are consequently retained in the solid waste fraction (Özcan & Gürel, 2022). The higher ash in MS minces is attributed to the recovery of small shell pieces due to the low selectivity of mechanical processing towards protein, and its high pressure in crushing the shells. Still, the MS was very effective in removing shell from the meat, as illustrated by the significantly lower ash content in the MS minces compared with the start materials. Our data are in agreement with previous reports indicating that MS-derived green crab mince exhibits relatively high ash content (Kang et al., 2019). Using cooked samples without the carapace, or raw crabs, resulted in slightly lower ash content in the MS minces compared with cooked crabs including the carapace. Recent proximate composition data from harbour crab (*Liocarcinus depurator*) show that inclusion of shell material markedly lowers apparent protein content and substantially increases ash levels, highlighting the strong influence of shell removal efficiency on the compositional quality of recovered crab protein ingredients (Calvo et al., 2025).

3.3. Polypeptide pattern of the MS minces and protein isolates

The electrophoretic patterns of initial raw biomasses, MS minces and isolated protein from the crab are shown in Fig. 4. The muscle tissue of the raw crab without carapace showed intense bands which could be tentatively identified based on several earlier studies of fish muscle proteins using SDS-PAGE (Stefansson & Hultin, 1994). Based on their

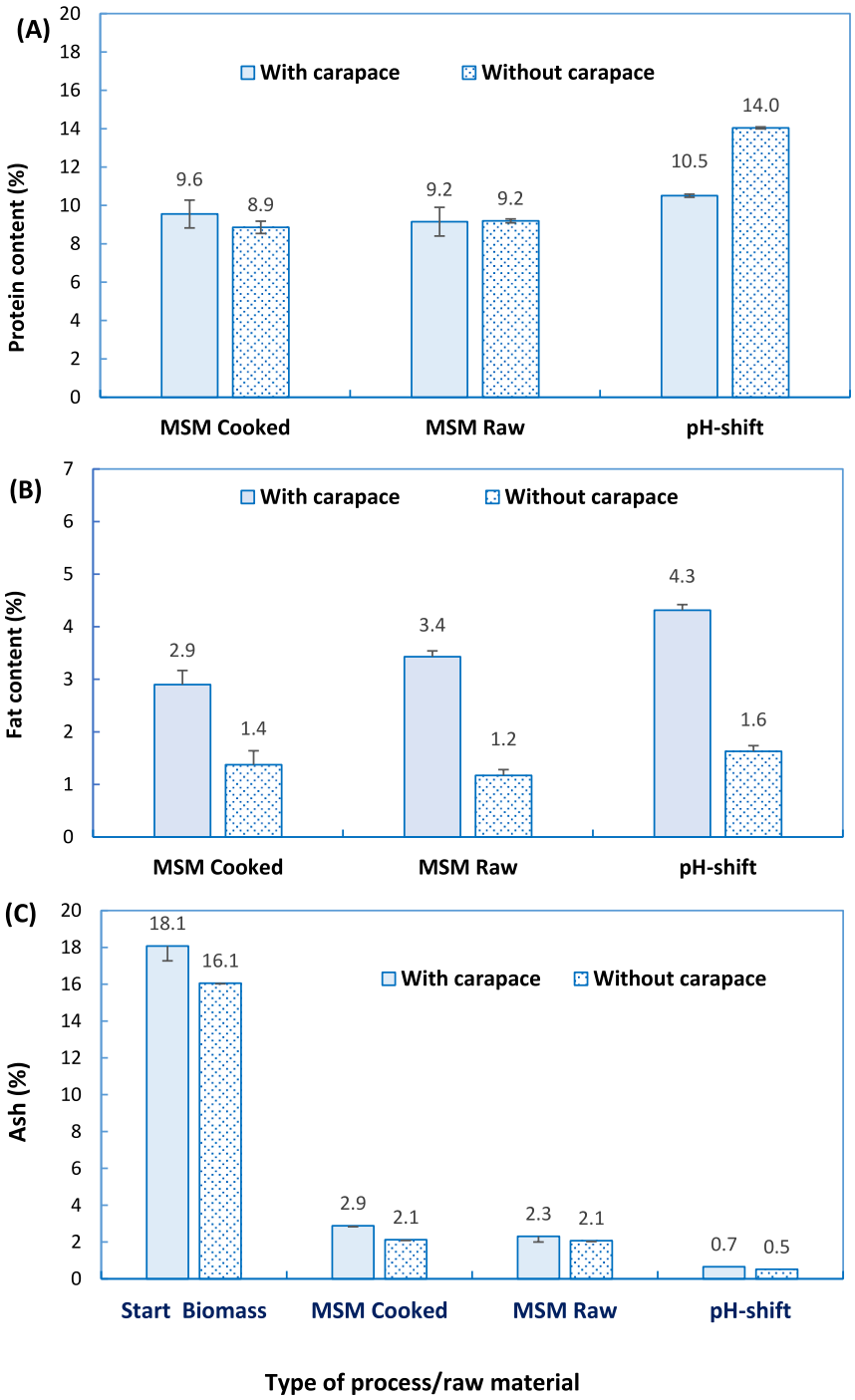

Fig. 3. Crude composition including (A) protein (B) fat and (C) ash content of minces from cooked brown crab subjected to mechanical separation (MS) ("MSM cooked") or raw crab subjected to MS ("MSM raw") or pH-shift processing ("pH-shift") without or with carapace on wet weight (ww) basis. MSM = mechanically separated meat.

molecular weights, the most dominant polypeptides were tentatively assigned as 220 kDa (myosin heavy chain, MHC), 100–80 kDa (paramyosin and glycogen phosphorylase), ~43 kDa (actin), ~40 kDa (putatively troponin T), ~37 kDa (tropomyosin), ~27–30 kDa (troponin I), and below 25 kDa (myosin light chains and troponin C) (Lin & Park, 1996). This polypeptide pattern was similar to those reported for proteins from blue crab (Martínez-Maldonado et al., 2020), green crab (Kang et al., 2019) and Jonah crab (Baxter & Skonberg, 2008). The dense band at ~60–70 kDa could not be unambiguously identified based on the literature, but it may correspond to miniparamyosin, an invertebrate-specific thick-filament protein of ~60 kDa. However, confirmatory proteomic analyses (e.g., mass spectrometry or immunoblotting) are required to unequivocally identify this band. This protein has previously been described in insects such as Drosophila (Cervera et al., 2006, pp. 76–85) and in Antarctic mussel shrimp Acetabulastoma sp. (*Crustacea, Ostracoda*) (Royuela et al., 1997). Other tentative candidates could be calcium-dependent cysteine proteinase (CDP) IIa (Mykles & Skinner, 1982), involved e.g. in molting-related muscle atrophy, or heat shock protein (Hsp) 70. While CDP IIa usually occurs in small amounts, Hsp70 expression can be elevated in response to various stressors (Banfi et al., 2025). The crab biomasses, which were crushed and stored in the presence of carapace, belly content and intestines,

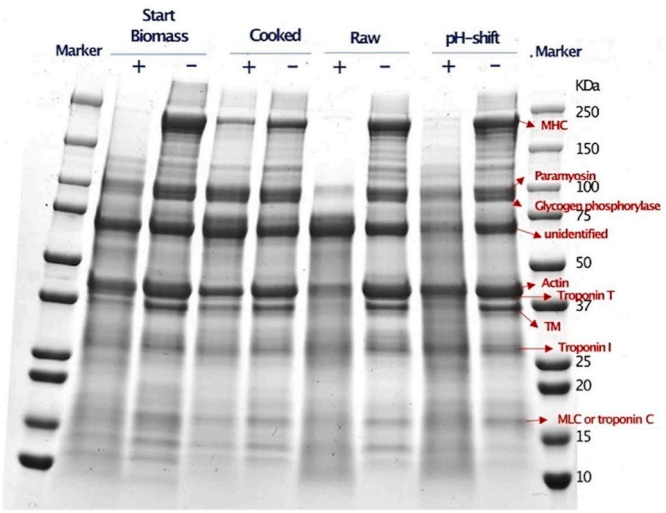

Fig. 4. Polypeptide patterns of the starting brown crab biomass, mince recovered by mechanical separation (MS) from cooked or raw crab, and protein isolate (PI) recovered by the pH-shift process from raw crab with (+) or without (−) carapace. Red labels indicate the tentative identities of the most dominant polypeptides. The band at ~60–75 kDa remained unidentified, as its definitive assignment requires confirmatory proteomic analysis (e.g., mass spectrometry). MHC, myosin heavy chain; TM, tropomyosin; MLC, myosin light chain.

showed almost no MHC band and only a low-intensity tropomyosin band. This pattern suggests proteolytic degradation of myofibrillar proteins, likely associated with endogenous digestive proteases present in the intestinal tract or released from the hepatopancreas following cell lysis, particularly cathepsins (Hu & Leung, 2007). The mince recovered with MS from cooked crab showed all the bands found in the initial crab biomass without carapace, but with lower intensity of MHC, especially in the mince from crabs with carapace. This means that the heating was able to partially inactivate the proteolytic enzymes in the crab viscera, but, further to this, heat denaturation may have reduced the extractability of MHC into the sample buffer used in preparation for SDS-PAGE. The reduced extractability is a chemical consequence of heat-induced unfolding followed by aggregation, which leads to the formation of stabilized intermolecular networks. These aggregated structures are less accessible to the SDS buffer, thereby decreasing protein solubilization (Sheard et al., 2007). Harsh proteolytic degradation was also seen in the MS mince and PI recovered from raw crab samples with a carapace, but with a higher intensity in the PI. The latter could be due to intensification of enzyme activities during the pH-adjustment to alkaline and acid pH-values, as has been reported before (Abdollahi et al., 2017). Generally, the pH-shift process was able to recover almost all the muscle components of the crab samples without carapace, providing a polypeptide pattern similar to that for the MS mince. This result shows that removal of crab carapace and its viscera components is necessary to avoid proteolytic degradation of the crab proteins, regardless of the applied separation technology, especially when using raw crabs.

While both MS and pH-shift processing can recover protein from brown crab residues, our results highlight some important differences. MS, particularly when applied to whole crabs including viscera, can lead to partial denaturation of proteins due to increased temperature and activity of proteolytic enzymes, which may reduce functional properties (Boye, Ma, & Harwalkar, 1997). Additionally, this method poses a higher risk of microbial contamination from intestinal microorganisms. In contrast, pH-shift processing better preserves protein functionality and mitigates hygienic concerns. These findings guide selection of processing methods for potential industrial applications, considering both protein quality and food safety.

3.4. Rheological properties and gel-forming capacity of the recovered protein-rich ingredients

Gelation of myofibrillar proteins follows from the conversion of an amorphous sticky solution to a three-dimensional elastic network, which is normally induced by thermal treatment. Therefore, modifications in relationships of stress–strain during the gelation process can be evaluated using rheological parameters (Karthikeyan et al., 2006). As can be seen in Fig. 5, storage modulus (G′) generally increased with increasing temperature in all the sample pastes during the temperature ramp up to 90 °C (heating step) and during the cooling step down to 20 °C, but not during the isotherm at 90 °C. An increase in G′ values normally indicates a conformational-forming reaction related to arranged aggregation requiring thermic energy (Karthikeyan et al., 2006). However, in all pastes, the storage modulus showed a declining trend right after starting the heating and up to around 28-30 °C, followed by an increasing trend. The initial reduction could be due to softening of the samples by heating, which breaks weak hydrogen bonds in the initial paste. A similar trend in G′ was reported for myofibrillar proteins isolated from other aquatic species such as threadfin bream meat (Karthikeyan et al., 2006), and for silver carp protein isolate derived from pH-shift processing (Abdollahi et al., 2017). Some authors have attributed such softening to light meromyosin denaturation, resulting in enhanced fluidity (Egelandsdal et al., 1986). The subsequent G′ increase might be related to protein-protein interactions, mainly involving disulfide bonds, resulting from heat-induced denaturation of myosin rods, which could produce an extended three-dimensional network (Smyth et al., 1996). More fundamentally, the sharp increase above 30 °C is consistent with the progressive unfolding of myosin heads and the exposure of hydrophobic regions, which together drive the transition from a viscous paste to an elastic gel through extensive hydrophobic interactions (Visessanguan et al., 2000). However, all the samples produced from crabs with carapace showed another softening around 60 °C, which could be related to potential degradation of the protein structure induced by heat-activated enzymes or reduced protein extractability. Temperatures around ~60 °C coincide with the partial activation range of certain endogenous cathepsins (e.g., B and L) before their full denaturation, allowing them to transiently cleave myofibrillar proteins and weaken the developing gel matrix (Kwon & Chang, 2021). The MS mince from cooked crab with carapace showed the lowest final G′ among all samples after the cooling-induced physical structure formation. This could be related to the already mentioned heat-induced denaturation, impaired protein extractability, or proteolytic degradation, low protein content or impurities. While protein denaturation refers to the unfolding and structural disruption of native proteins caused by thermal or chemical treatments, gel formation is a functional process that requires partially unfolded proteins to reassociate through intermolecular interactions into a three-dimensional network upon heating. Excessive denaturation or proteolytic degradation can thus impair this network formation by reducing molecular integrity and reactive sites necessary for aggregation. The finding is in line with the physical appearance of the gel made from this sample (Fig. 7), which was very loose and not self-supporting. The highest final storage modulus was seen in the PI from raw crab without carapace, which is in line with its high content of both total protein and MHC, the latter observed in the SDS-PAGE results, allowing better structure formation during the heating and cooling step. The superior final G′ of this PI likely reflects both its higher myosin content and the reduced presence of interfering lipids or endogenous enzymes, conditions that favor uniform network development and stronger gel elasticity. The other PIs and MS minces were located between the two mentioned samples in terms of the final G'.

3.5. Texture properties of gels

As shown in Fig. 6A, the puncture force representing the firmness was highest (163.9 g) for gels made of PI produced by subjecting crab

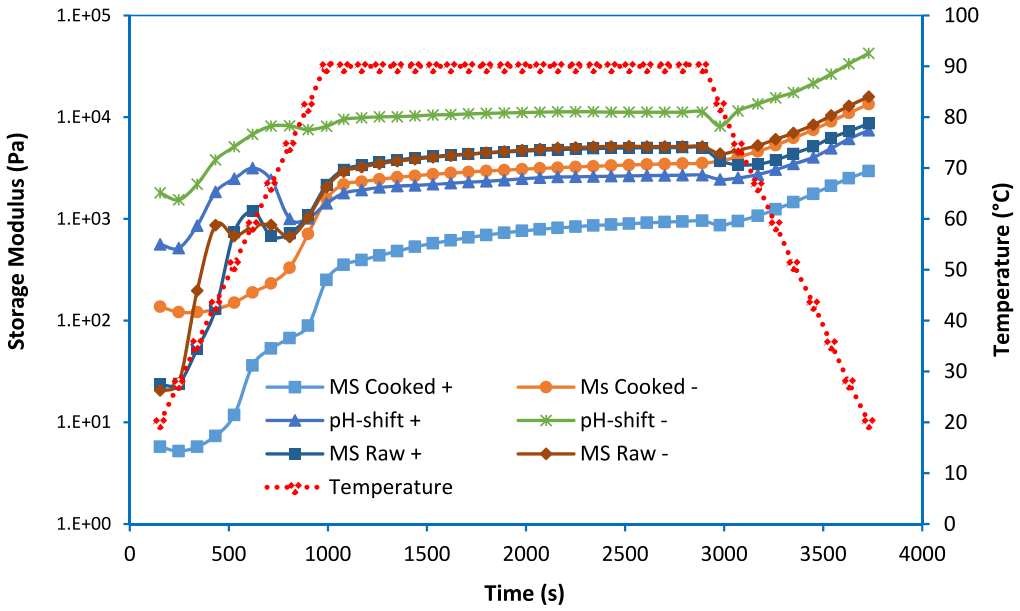

Fig. 5. Rheological properties of minces recovered from brown cooked ("Cooked") or raw ("Raw") crab by mechanical separation (MS) ("Cooked" and "Raw") or pH-shift process ("pH-shift") without (−) or with (+) carapace.

without carapace to alkaline pH-shift processing. This shows that pH-shift processing of crab without the carapace results in a firmer gel. The higher firmness is likely due to better removal of impurities, allowing concentration of myofibrillar proteins. The elasticity of the muscle protein gels is mostly attributed to myofibrillar proteins, particularly MHC and tropomyosin. Efficient up-concentration of those proteins by elimination of impurities (e.g. shell) and to some extent sarcoplasmic proteins during the pH-shift process thus created a stronger protein network (Yongsawatdigul & Park, 2004). The high purity of the PI maximizes the concentration of the primary gel-forming agent (MHC), thereby promoting a denser three-dimensional network with an increased number of effective protein–protein cross-links, particularly those mediated by hydrophobic and ionic interactions. Also, proteins isolated using the alkaline pH-shift process are partially denatured and unfolded. This could lead to rather exposed sulfhydryl groups resulting in disulfide bond formation and a more stabilized gel structure at high temperatures (Pan et al., 2024; Sano et al., 1990). However, the PI made from crab with carapace was subjected to harsh proteolysis (Fig. 4), minimizing its three-dimensional gel structure formation. The weaker gel properties of this sample could also be related to its lower amount of protein and higher amount of fat, which could interfere with MHC and tropomyosin during the cross-linking-dependent formation of a gel matrix (Baxter & Skonberg, 2008). Specifically, the retained lipids physically hinder protein–protein interactions necessary for gelation, acting both as a network diluent and as a blocker of cross-linking sites. In general, the lower purity of the MS minces from crabs without carapace resulted in lower gel forming capacity compared with the pH-shift processed sample without carapace. However, among the MS minces, those from cooked crab showed the lowest puncture force and the mince from cooked crab with carapace was not self-supporting enough to even be analyzed (see Fig. 7). As could be seen in the rheological results, gels from cooked crab samples in which proteins were already heat coagulated and denatured showed the lowest structure formation during the reheating step. This inability to re-gel results from the irreversible nature of primary heat-induced aggregation (coagulation) during the initial cooking step, which prevents the proteins from undergoing the sequential unfolding and re-aggregation required to form a new, robust thermal gel in the subsequent heating cycle (Brodkorb et al., 2016). This differs from the denaturation induced by pH-shift processing, which primarily exposes functional groups and enhances their participation in gel formation upon heating, whereas cooking-induced denaturation leads to irreversible crosslinking and aggregation. This was most pronounced in the sample derived from crab with carapace that had already experienced some proteolytic degradation and had a higher fat content. Deformation in the crab mince gels was affected both by the presence of carapace and the cooking process (Fig. 6B). MS mince gels derived from raw crab with carapace and from cooked crab without carapace exhibited significantly ($P < 0.05$) less deformation than either of the gels of raw samples without carapace.

Overall, protein ingredients recovered using the pH-shift method, exhibiting high gel-forming capacity, could be promising for applications requiring structure and moisture retention, such as restructured seafood products, and hybrid or plant-seafood formulations. In contrast, mechanically separated crab proteins with lower gel strength but higher yield may be more suitable for applications where emulsification, flavour contribution, or nutritional enrichment is desired, such as soups, sauces, spreads, and formulated ready meals.

3.6. *Water-holding capacity of gels*

Water-holding capacity (WHC) refers to the ability of a gel to retain water when subjected to centrifugation. The WHC values of the different crab gels are shown in Fig. 6C. In line with the textural properties, the gel made of PI from crabs without carapace showed the highest values of WHC. This could be related to the mentioned better removal of impurities during pH shift processing of crabs compared with MS, and consequently to higher up-concentration of myofibrillar proteins (Chaijan et al., 2010). The high purity and concentration of the myofibrillar proteins facilitate the formation of a dense, three-dimensional gel network that effectively traps water within its fine capillary structures. Again, the proteolytic degradation and probably the higher fat content in PIs and MS minces from crabs with carapace resulted in lower WHC compared with their carapace-free counterparts (Wang et al., 2022). Proteolytic degradation shortens protein chains, impairing the formation of continuous, space-filling networks necessary for effective water retention, while the presence of lipids acts as a network diluent, lowering the overall gel matrix density (Zhang et al., 2025). The WHC value for the gel made from cooked crab with carapace recovered by MS could not be determined because the gel was too weak for reliable testing. However, for the other samples, the results showed that the

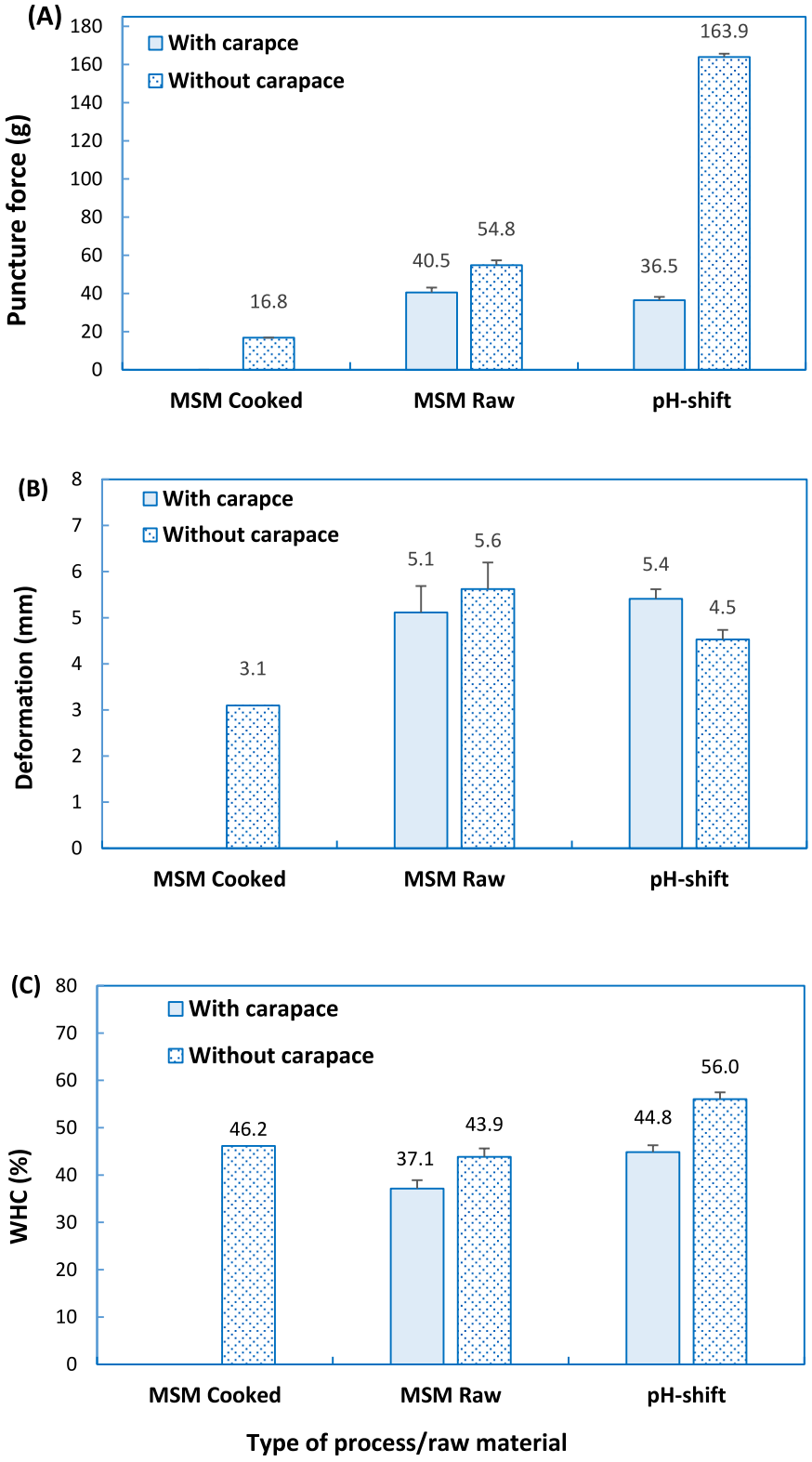

Fig. 6. Puncture force (A), deformation (B) and water holding capacity (WHC) (C) of gels made from protein-rich ingredients derived from cooked or raw brown crab subjected to mechanical separation (MS) ("MSM cooked" vs. "MSM") or from raw brown crab subjected to pH-shift processing ("pH-shift"). The raw crab was processed wither with or without carapace. The WHC value for the gel made from cooked crab with carapace and recovered by MS was not determined because the gel was too weak for reliable handling and testing. MSM = mechanically separated meat.

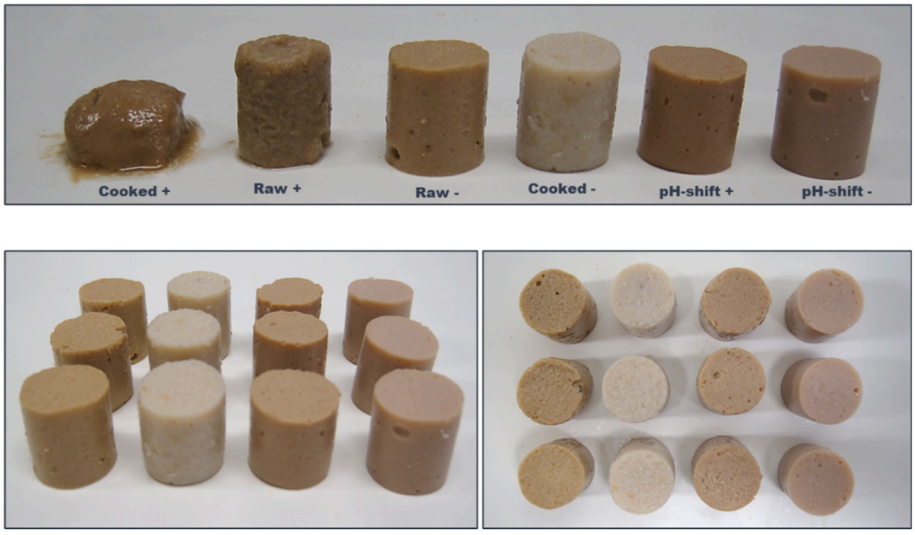

Fig. 7. Picture of gels made of mince recovered from poorly filled cooked/raw brown crab by mechanical separation (shown as "Raw" and "Cooked") or by the pH-shift process (pH-shift). In both cases, crabs were either with (+) or without (−) carapace.

heat-coagulated protein network, despite lower rigidity, can remain effective in retaining water during centrifugation (Jiang et al., 2022). Overall, the WHC findings are in agreement with those obtained by Martínez et al. (2014) for blue crab proteins.

4. Conclusions

Using poorly filled, clawless crabs with intact carapace as starting material, mechanical separation provided the highest mass yield; however, it resulted in extensive proteolytic degradation, low protein purity, and dark coloration, likely due to the inclusion of hepatopancreas and abdominal contents. Pre-cooking prior to mechanical separation increased mass yield and effectively inhibited proteolysis, but also induced heat-driven protein denaturation, leading to reduced solubility and gel-forming capacity. This thermal denaturation limited the applicability of pH-shift processing to raw biomass. In contrast, applying the pH-shift process to raw crab enabled efficient removal of shell residues and significant up-concentration of protein and lipids, while preserving functional properties such as gelation. These results highlight a trade-off between yield and functionality: pre-cooking improves process stability and yield but compromises protein functionality, whereas pH-shift processing of raw material produces higher-quality protein ingredients. However, proteolytic degradation remained an issue when whole crab (including carapace and viscera) was used as the starting material. Applying the pH-shift process to cleaned crab yielded highly purified proteins with a threefold higher gel-forming capacity compared to mechanically separated mince. Nevertheless, the resulting protein ingredient exhibited a pinkish color, possibly due to co-extraction of carotenoids. The novelty of this study lies in demonstrating how preprocessing conditions, specifically viscera and carapace removal, as well as cooking, interact with the choice of protein recovery method to determine both quality and applicability of the resulting ingredients. Overall, these preprocessing conditions should be carefully considered, as they strongly influence both fractionation efficiency and protein functionality in poorly filled crab. Mechanical separation appears suitable for recovering cooked crab meat for direct consumption, whereas pH-shift technology shows strong potential for producing high-value, functional crab protein ingredients from raw crab for use in restructured products.

CRediT authorship contribution statement

Mehdi Abdollahi: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Samaneh Pezeshk:** Writing – review & editing, Writing – original draft, Visualization, Data curation. **Ingrid Undeland:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ingird Undeland reports financial support was provided by European Maritime and Fisheries Fund. If there are other authors, they 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2026.119879.

Data availability

Data will be made available on request.

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