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RESEARCH

Effect of Fractionation Technology on Nutrient Profile of Protein-Rich Ingredients from Hollow Brown Crab (*Cancer pagurus*)

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Abstract

The sustainable utilization of seafood side streams is increasingly recognized as a key strategy to improve resource efficiency and deliver high-quality nutrients. Brown hollow crab (*Cancer pagurus*) represents one such underutilized resource, as the body is typically discarded after claw removal due to its complex composition. Here, we investigated how pretreatments (thermal processing, cleaning) and two separation technologies, i.e., mechanical separation (MS) and alkaline pH-shift processing, affect the nutritional composition of protein-rich products recovered from brown hollow crab. Both techniques successfully recovered residual tissue that was enriched in essential amino acids (203–395 mg/100 g dw), vitamin D (0.048–0.140 µg/100 g dw), and zinc (19–30 mg/100 g dw), with a favorable n-3/n-6 fatty acid ratio (2.9–3.6). The pH-shift method proved more effective than MS in concentrating protein and vitamin D, as it removed the ash-rich shells more efficiently, resulting in products with lower levels of sodium, calcium, magnesium, potassium, and arsenic. Contrary, MS gave higher n-3 PUFA and lower mercury levels; for whole crab, also higher B12 levels. Products produced from whole crabs generally contained higher amounts of total lipids, unsaturated fatty acids, vitamin D, vitamin B12, copper, and cadmium, but had lower essential amino acid content compared to those from cleaned crabs. Overall, this study demonstrates that both biomass pretreatments and valorization technologies significantly influence the macro- and micronutrient profile of products derived from brown hollow crab, providing critical insights for their potential use in sustainable food applications.

Keywords Mechanical separation · Alkaline solubilization/isoelectric precipitation · Nutritional value · Marine proteins · Side streams

Introduction

Seafood can play a significant role in our transition to a sustainable future food system and is experiencing a fast-growing global demand. Compared to many terrestrial animal proteins, seafood production can have a lower environmental footprint in terms of land use, feed requirements, and greenhouse gas emissions. Moreover, seafood provides high-quality proteins, long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA), and micronutrients that are critical for global nutrition. Harnessing both wild-caught and responsibly farmed or novel aquatic resources therefore represents an important pathway towards healthier diets and more sustainable food systems.

Brown crab (*Cancer pagurus*), also referred to as edible crab, is one of the economically important decapod crustaceans in Europe (McDermott et al., 2018). Crab claws have become increasingly popular for consumption because of their mild flavor. However, the claws only constitute < 10% (w/w) of the crab and their sole use results in the wasting of 90% of the harvested crab biomass. This problem is especially pronounced during winter when the crabs have no or very little meat in their carapace section due to improper feeding; then almost all these poorly filled, or "hollow," crabs are wasted at sea. A more sustainable option would be their landing to recover residual tissue. Both muscle and hepatopancreas residues are rich in valuable nutrients including proteins with a balanced profile of amino acids, long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA), minerals, and vitamins (Velazquez et al., 2021). Recovery

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of meat from hollow crabs is however challenging due to the exoskeleton or shell limiting access to the meat remaining under the carapace area or in the legs, both of which are very good sources of high-value nutrients (Umer et al., 2021). Considering the sensitive nature of aquatic tissue, the development of mild valorization technologies that enable efficient meat/shell separation while retaining nutrients and product-forming capacity is crucial.

Mechanical separation (MS) is perceived as an inexpensive, simple, and highly efficient separation technology (Palmeira et al., 2016; Yuste et al., 1999). It has been successfully applied within the poultry industry for decades and also, interest in its use for upcycling of seafood processing side streams has rapidly increased (Abdollahi et al., 2021; Bacelar et al., 2023). In this method, residual meat from bones or shells is recovered via a belt and perforated metal drum using low (< 104 Pa) or high (> 104 Pa) pressure. Among challenges described are modifications of the muscle structure and exposure of muscle components to released proteolytic enzymes and pro-oxidants, e.g., heme pigments, which can negatively impact the nutrient stability and sensorial attributes of the mechanically separated meat (MSM) (Secci et al., 2017). Although there are no publications of MSM production from brown hollow crab, a few studies have comprised green (Galetti et al., 2017), Jonah (Baxter & Skonberg, 2008), and blue (Gates & Parker, 1992) crabs. Results have revealed relatively good mass yield, but considering the crushing effects of the belt and the low selectivity of the drum, high shell-contamination may affect the nutritional value of the meat by increasing its ash level, an angle which is poorly explored. In addition, no studies to date have evaluated the outcome of using whole crab, versus crab with its viscera, brown meat, and carapace removed. The same is true for pre-boiled versus raw crab.

A more sophisticated alternative technology for the value addition of complex marine side streams is the so-called pH-shift process (Abdollahi & Undeland, 2019). This technology involves selective separation of proteins from other insoluble materials at low temperature using a high or a low pH values (< 3.5 and > 10.5), followed by precipitation at their 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 side streams (Abdollahi & Undeland, 2019) and its potential for application on green crab (*Carcinus maenas*), a small and invasive species, has been recently reported (Kang et al., 2019; Khiari et al., 2020). Generally, the high selectivity of the pH-shift process towards proteins allows efficient removal of unwanted materials such as bone or shell, resulting in proteins with relatively high purity and good functionality (Abdollahi et al., 2017). However, compared to MS, the pH-shift method involves a higher level of investment (at least five–tenfold higher) and induces the generation of large volumes of effluents (acid and alkaline solutions) and more extensive processing which may result in lower mass yield (Abdollahi et al., 2021). Its economic feasibility depends strongly on the raw material, scale, and desired product quality. Compared to simpler mechanical separation, pH-shifting involves multiple unit operations (alkali solubilization, centrifugation, acid precipitation, washing, and neutralization), which increases both capital and operational costs. Energy and water use are also higher, and the need for chemicals and wastewater treatment can represent additional limitations. From a nutritional perspective, the high selectivity of this technique towards proteins and the utilization of relatively large water volumes can affect residual amounts of both nutrients and unwanted compounds in the final product, depending on their affinity for water versus protein and lipids (Abdollahi et al., 2021). In general, protein-bound molecules are expected to be up-concentrated, while the opposite has been documented for aqueous or lipophilic compounds (Trigo et al., 2023; Abdollahi et al., 2021). It was hypothesized that this could possess both opportunities and risks when it comes to crab, particularly with respect to the hepatopancreas tissue which is known to accumulate, e.g., cadmium (Wiech et al., 2020), but also high levels of, e.g., LC n-3 PUFA and vitamins B12, D, and E (Reksten et al., 2024).

To the best of our knowledge, a side-by-side comparison of MS and pH-shift processing for the valorization of brown crab side streams and its effect on macronutrients, micronutrients, and heavy metals of the final protein-enriched product has not yet been reported. This knowledge gap was addressed in the present study which comprised brown hollow crab residues after removal of the claws. The crabs were processed as whole and cleaned, as well as in cooked and raw form. Among nutrients, fatty acids, amino acids, minerals, and vitamins in the recovered protein-enriched products were evaluated. On the heavy metal side, we monitored cadmium, lead, mercury and arsenic.

Materials and Methods

Preparation of Brown Crab Samples

A schematic overview of the study design is illustrated in Fig. 1. Hollow brown crabs were harvested in March 2020 on the Swedish west coast specifically for this project and transported to Sweden Shellfish AB (Bua, Sweden) while kept alive. The cooked and raw hollow crabs having no meat in their carapace area are illustrated in Supplementary Fig. 1. Thirty kilograms of crabs was subjected to steam cooking at 95–100 °C for 5 min using an industrial steam cooking system currently used for shrimp and crayfish steaming at Bua Shellfish. Then, the cooked samples and around 60 kg

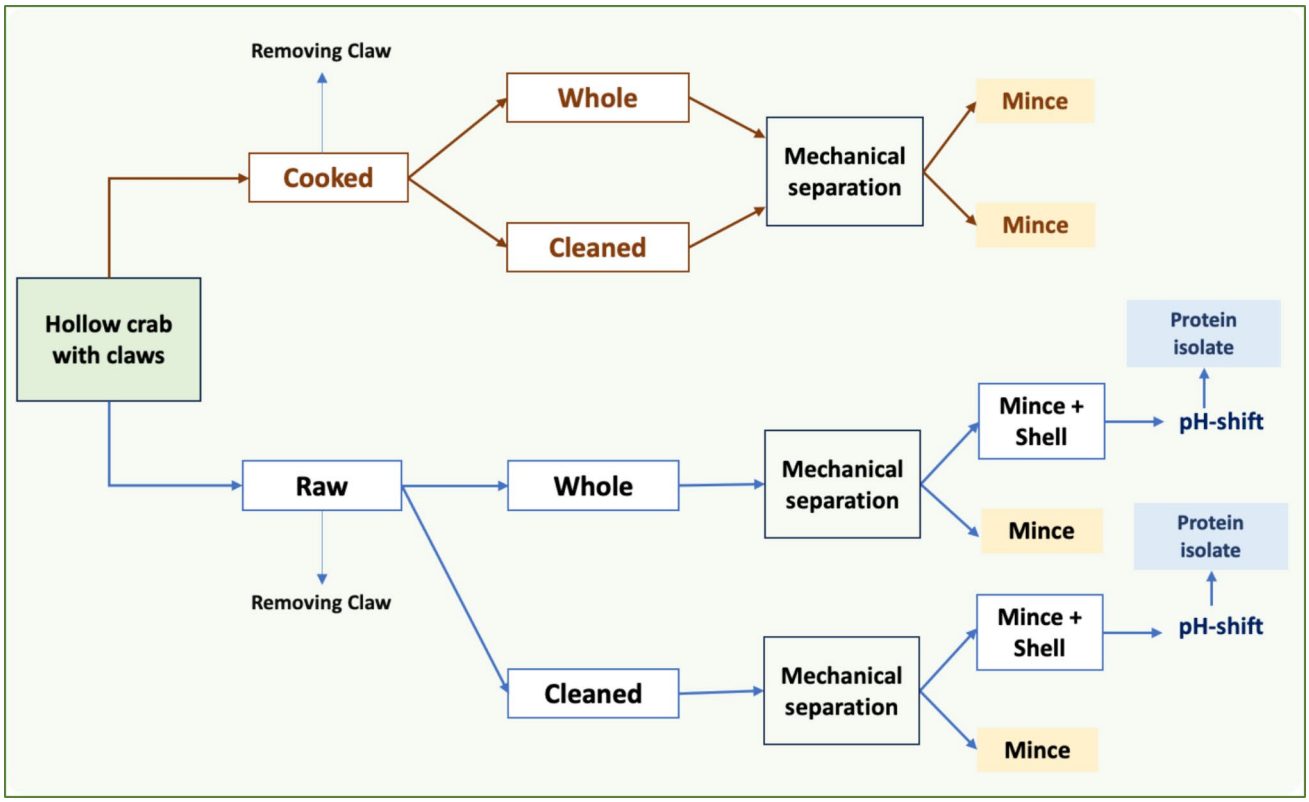

Fig. 1 Schematic overview of the study plan and mass yield obtained after each step or type of processing. The data in the black boxes show the mass yield for mechanically separated meat (MSM) and protein isolates based on the weight of initial whole crab. "Whole" and "Cleaned" denote whether the crabs were used intact or after removal of carapace, abdominal segment, viscera, and brownish belly content

of raw crabs, still alive, were transported to Fisk Idag AB for MS before which they were killed by piecing. For both raw and cooked crabs, the claw was manually separated prior to MS and pH-shift processing since it is marketed as a high-quality product in both Sweden and abroad.

After removal of the claws, both raw and cooked samples were divided into two parts where one part was used directly for MS. The second half was subjected to removal of carapace, abdominal segment, viscera, and brownish belly content (referred to as "cleaned") to investigate its effect on color and quality of the separated meat (see Supplementary Fig. 1).

Mechanical Separation of Crab Meat

To extract 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. After two trials with drums having 5- and 3-mm hole diameters which resulted in too high amount of shell in the meat, a drum with 2-mm hole diameter was used based on recommendation of the supplier. The crabs were first slightly crushed with a hammer and passed through the mechanical separator and the mechanically separated meat (MSM) was collected, packed in plastic zip lock bags, and frozen at −80 °C until further studies (see Fig. 1).

For the raw crab, both whole and cleaned, the mechanical separator was used as grinder where one portion of the mince was remixed with its separated shell in a correct ratio to later be used for pH-shift processing at Chalmers University of Technology.

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 based on the very low solubility obtained after heat-denaturation of proteins. The crushed raw crab biomass (500 g) was mixed with 6 ratios of cold distilled (4 °C) water 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 NaOH and after 10-min incubation, it was centrifuged at 5000 × g in a Thermo Scientific Sorvall LYNX 6000 Centrifuge (Thermo

Scientific, Germany) for 10 min at 4 °C. Thereafter, the middle phase (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 M 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 further dewatered using a third centrifugation step at 8000 × g for 10 min (4 °C) and the moisture content was adjusted to 80% ± 1. Lastly, the pH was adjusted to pH 7 using 2 M NaOH. The entire process was conducted on ice. The protein isolate was stored at −80 °C until further use.

Crude Composition Analysis

The total crude protein of MSM and protein isolates was analyzed using a nitrogen analyzer (TruMac-N, LECO Corp., St. Joseph, MI, USA) according to the Dumas method with a conversion factor of 5.58 (Mariotti et al., 2008). Crude lipid content of all samples was determined using the solvent extraction method described by Lee et al. (1996) with a chloroform:methanol solvent mixture at a ratio of 2:1. The moisture content and crude ash were measured at 105 °C and 550 °C, respectively (AOAC, 2023).

Amino Acid Composition

Amino acid composition was carried out according to the method described by Abdollahi et al. (2021). Briefly, the samples were hydrolyzed for 24 h in 6 M HCl at 110 °C. Then, the hydrolyzed samples were washed with water and evaporated, and the residual contents were dissolved in citric acid and analyzed by a HPLC-MS (Agilent 1100 HPLC, Waldbron, Germany). Finally, quantification was done against an amino acid standard mixture (Sigma-Aldrich, St. Louis, MO, USA).

Fatty Acid Composition Measurement

To determine the fatty acid composition, total lipids were extracted from the different crab samples according to the Bligh and Dyer method (Bligh and Dyer, 1959) and their fatty acid composition was measured as described by Tullberg et al. (2016). To produce fatty acid methyl esters (FAMEs), esterification was done by following the method of Cavonius et al. (2014). FAMEs were eventually dissolved in methanol and subjected to analysis on a gas chromatograph (GC-MS-QP2010; Shimadzu) fitted with a polar capillary column (SPB-50, 60 m length, 0.25 mm I.D., 0.25 lm film thickness). Injection volume and split ratio were 1 µL and 15:1, respectively, at an initial temperature of 275 ℃.

Vitamin D and Vitamin B12

Content of vitamin D was measured as described by Standal et al. (2018). Briefly, 0.4 g of lyophilized and homogenized sample was blended with ethanol:methanol (50/50 v/v) with 0.5% (w/v) pyrogallol, 1 g KOH, and internal standard D6–25(OH)D3, covered by N_2 gas, and vibrated in ambient temperature overnight. Five milliliters toluene was then added to the sample which was treated for an extra 30 min whereafter 2 mL H_2O was added, and the upper organic phase was moved to a test tube. The sample was thereafter extracted twice with 2 mL petroleum ether:diethyl ether (80:20 v/v). The recovered organic phases were merged, evaporated to a volume of 1 mL, and washed with distilled water until neutral pH was achieved. The evaporated organic phase was dissolved in 2 mL 1% 2-propanol in heptane. Then, the extracts were subjected to solid phase extraction (TELOS Silica, Kinetics, St Neots, Cambridgeshire, UK) according to the method described method by Jäpelt et al. (2011). Determination of vitamin D3 was done by injecting the samples to an LC–MS system (Agilent 1200 series system with an Agilent 6120 MSD single quadrupole, Agilent Technologies, Santa Clara, CA, USA). The results are stated as µg/g ww.

Vitamin B12 was determined by an accredited laboratory (National Food Agency of Sweden) and was performed using the microbiological assay and turbidimetric detection of the growth of *Lactobacillus casei* subsp. *Rahmnosus* (*L. rahmnosus*, equivalent to *L. casei* American Type Culture Collection, ATCC 7469) (AACC, 2000).

Minerals and Heavy Metals

Levels of minerals (sodium, potassium, calcium, magnesium, iron, selenium, copper, zinc, chromium, molybdenum, iodine, cadmium, lead, mercury, and arsenic) in crab samples subjected to different treatments were measured according to the method described by Abdollahi et al. (2021). The organic matrix was subjected to acidic microwave digestion by milestone microwave laboratory system (Ethos Plus and a laboratory terminal 800 controller, Sorisole, Italy). The remaining ash were diluted and used for measurement of the minerals using atomic absorption spectroscopy (AAS).

Statistical Analysis

All analyses were carried out in triplicate. Statistical analyses were conducted using SPSS 26.0 (SPSS Inc., Chicago, IL, USA) and one-way ANOVA followed by Duncan's multiple range test was used to find the differences between

the variables. If $p < 0.05$, the differences were considered significant.

Results and Discussions

Crude Composition of Recovered Crab MSM and Protein Isolates

The proximate compositions (on a wet weight basis) of mechanically separated meat (MSM) and protein isolate from brown crab are presented in Table 1. As can be seen, the protein content was higher in protein isolates compared to MSM. In particular, the protein isolate recovered from cleaned crabs had 53% higher protein content than its MSM counterpart, indicating the higher selectivity of the pH-shift process towards proteins compared with the MS method. This selectivity can be attributed to the fundamental principles of the two technologies. In mechanical separation, tissues are physically pressed and forced through sieves or screens, which results in the recovery of both soft tissues and small fragments of shell and connective material. Consequently, the MSM is not only enriched in proteins but also contains a relatively high proportion of ash and minerals originating from shell residues. In contrast, the pH-shift process relies on the solubilization of proteins at alkaline pH, followed by their selective precipitation at the isoelectric point. During solubilization, most non-protein components such as shell particles, connective tissues, and insoluble minerals remain in the sediment phase and are subsequently discarded. Similarly, low-molecular-weight components (e.g., salts, water-soluble minerals) remain in the supernatant and are removed during the centrifugation and washing steps. This biochemical and physicochemical selectivity allows the pH-shift process to yield products with a substantially higher protein concentration and lower ash content compared to MSM.

This finding aligns with previous reports where pH-shift processing achieved more efficient protein up-concentration from fish frame residues than MS, confirming its superior ability to discriminate proteins from non-proteinaceous materials (Abdollahi et al., 2021). In the case of brown crab, this selectivity is particularly valuable, as the shell is highly mineralized and rich in contaminants which are difficult to remove mechanically. Therefore, while MS provides a fast and simple recovery route with high yield, the pH-shift process offers a refined product with enhanced protein purity and reduced levels of undesirable shell residue, making it more suitable for high-value food applications.

Different small letters in each column show a significant difference ($p < 0.05$).

MSM and protein isolates obtained from whole crabs, regardless of the separation technology used, exhibited two–threefold higher crude lipid content compared to the corresponding samples from cleaned crabs. This difference can be attributed to the higher lipid content of the starting material; here shown in terms of total fatty acid levels (FA) (Table 3), which contained viscera, testis/eggs, and hepatopancreas, tissues that are typically the major fat reservoirs in crabs. In contrast, crab muscle tissues generally have very low fat content (Galetti et al., 2017).

The protein isolates derived from whole and cleaned crabs contained 26% and 38% more crude lipids, respectively, than their MSM counterparts on a wet weight (ww) basis; 25% and 20% when normalized to the same moisture basis. Notably, the pH-shift process did not generate any oil/emulsion layer during processing, which is the main mechanism for lipid removal from fatty raw materials. A secondary lipid removal mechanism is the loss of membranes during the first centrifugation or into the processing water after the second centrifugation (Shi et al., 2017). Phospholipids constitute the main lipid class in lean resources such as crab muscle. However, instead of yielding a lower lipid content than MSM, the pH-shift process actually showed higher lipids to a greater extent. This effect was likely due to the more pronounced removal of ash derived from shell and bone residues. The ash content in the protein isolates was only 0.37–0.38% ww, compared to 3.24–6.12% ww in MSM. Similar results were observed when the pH-shift process was applied to cod side streams alongside MS (Abdollahi et al.,

Table 1 Crude composition of MSM from cooked (MSM cooked) or raw (MSM raw) hollow brown crab, together with protein isolate from pH-shift processed (pH-shift) raw hollow brown crab. Both processes were applied to whole or cleaned crab. Data are given on a wet weight basis, and numbers represent mean values ± SD ($n = 3$)

	Moisture (%)	Crude protein (%)	Lipid (%)	Ash (%)
MSM cooked (whole)	80.22 ± 0.18^{c}	9.55 ± 0.72^{c}	2.9 ± 0.26^{c}	6.12 ± 0.34^{a}
MSM cooked (cleaned)	83.52 ± 0.2^{b}	8.85 ± 0.32^{e}	1.37 ± 0.02^{e}	4.16 ± 0.5^{b}
MSM raw (whole)	83.68 ± 0.75^{b}	9.15 ± 0.74^{d}	3.42 ± 0.11^{b}	3.24 ± 0.45^{c}
MSM raw (cleaned)	85.59 ± 0.06^{a}	9.19 ± 0.1^{d}	1.17 ± 0.01^{f}	3.25 ± 0.21^{c}
pH-shift raw (whole)	83.39 ± 0.45^{b}	10.5 ± 0.07^{b}	4.31 ± 0.1^{a}	0.37 ± 0.02^{d}
pH-shift raw (cleaned)	83.26 ± 0.29^{b}	14.04 ± 0.05^{a}	1.62 ± 0.0^{d}	0.38 ± 0.01^{d}

2021). The difference stems from the pressure applied in MS, crushing the shells to small pieces, combined with its lower selectivity for protein compared to pH-shift processing. These findings are consistent with the relatively high ash content previously reported for crab MSM by Kang et al. (2019). It is worth mentioning that using raw crab resulted in MSM with slightly, yet significantly less ash content compared to cooked crab, likely due to the softer texture of the raw meat, creating a larger differentiation between meat and shell, thus aiding separation.

Amino Acid Composition of the Recovered Crab MSM and Protein Isolates

The results presented in Table 2 indicate that brown crab is an aquatic protein source possessing an amino acid composition rich in essential amino acids. In general, crustaceans exhibit high levels of essential amino acids (EAA), comparable to other marine proteins.

The amino acid profiling demonstrates a significant ($p < 0.05$) increase in EAA per gram of protein in both MSM and protein isolates compared to the hollow brown crab raw material. Further, the protein isolate derived from cleaned crabs exhibited a higher level of total EAA (TEAA) and a higher ratio of EAA to total AA (TAA) in comparison to the MSM. This outcome reflects the successful elimination of collagenous proteins and other impurities during pH-shift processing, which efficiently up-concentrates the EAA-rich myofibrillar and sarcoplasmic proteins. This aligns with the findings of Abdollahi et al. (2021), who discovered that protein isolates obtained from fish frames contained a greater quantity of EAA compared to their MSM counterpart and their original by-product raw materials.

In general, the protein isolate obtained from cleaned crabs contained significantly higher levels of all EAA compared to the recommended requirements for adults set by WHO/FDA/UNN (World Health Organization, & United Nations University, 2007), revealing a superior nutritional value. Nevertheless, it is worth noting that the levels of methionine and phenylalanine in the protein isolate did not meet the recommended intakes for infants, which are actually higher than those for adults. MSM did not fulfill the recommended

Table 2 Amino acid composition (mg/100 g sample, dw) of MSM from cooked (MSM cooked) or raw (MSM raw) hollow brown crab, together with protein isolate from pH-shift processed (pH-shift) raw hollow brown crab. Both processes were applied to whole or cleaned crab, and data are also shown for the uncooked raw materials. On the right side, recommended levels of essential amino acids (EAA) are shown for the purpose of comparison

Amino acid (mg/100 g sample, dw)	Raw whole crab	Raw cleaned crab	MSM cooked (whole)	MSM cooked (cleaned)	MSM raw (whole)	MSM raw (cleaned)	pH-shift (whole)	pH-shift (cleaned)	FAO/WHO Adult (infant) (mg/g protein)
Valine *	10.8 ± 0.59^{g}	14.1 ± 1.07^{f}	28.1 ± 2.22^{e}	31.9 ± 1.57^{cd}	29.3 ± 1.71^{de}	32.5 ± 1.08^{c}	43.8 ± 0.80^{b}	49.0 ± 2.49^{a}	39 (55)
Threonine *	10.2 ± 1.06^{e}	13.7 ± 1.34^{e}	27.0 ± 4.03^{b}	31.7 ± 2.33^{b}	27.9 ± 4.05^{b}	32.3 ± 3.92^{b}	44.3 ± 5.37^{a}	48.3 ± 7.15^{a}	23 (31)
Isoleucine *	9.3 ± 0.03^{f}	11.8 ± 0.48^{e}	23.8 ± 1.35^{d}	28.8 ± 1.08^{c}	25.3 ± 0.65^{d}	30.4 ± 1.37^{c}	41.0 ± 0.89^{b}	46.3 ± 1.40^{a}	30 (32)
Leucine *	13.9 ± 0.21^{f}	18.4 ± 0.83^{e}	38.6 ± 1.39^{d}	46.5 ± 2.45^{c}	39.9 ± 0.97^{d}	49.1 ± 1.60^{c}	69.4 ± 0.85^{b}	77.3 ± 2.53^{a}	59 (66)
Lysine *	15.0 ± 1.47^{g}	20.3 ± 1.51^{f}	39.9 ± 2.68^{e}	51.9 ± 2.58^{e}	41.0 ± 1.81^{d}	57.0 ± 1.51^{c}	70.1 ± 2.42^{b}	82.9 ± 4.16^{a}	45 (57)
Methionine *	0.9 ± 0.51^{e}	3.6 ± 1.06^{e}	7.3 ± 2.43^{d}	11.2 ± 1.25^{c}	7.0 ± 2.22^{d}	11.7 ± 0.80^{c}	16.2 ± 2.58^{b}	21.2 ± 1.52^{a}	17 (42)
Histidine *	5.6 ± 0.22^{c}	6.5 ± 0.62^{c}	15.2 ± 0.57^{b}	16.7 ± 0.66^{b}	16.6 ± 0.57^{b}	15.7 ± 2.86^{b}	25.6 ± 1.75^{a}	26.3 ± 1.57^{a}	15 (20)
Phenylalanine *	9.7 ± 0.33^{f}	11.8 ± 0.74^{f}	23.9 ± 1.20^{e}	27.6 ± 1.43^{cd}	25.4 ± 1.60^{de}	28.5 ± 1.04^{c}	40.8 ± 1.40^{b}	44.4 ± 2.62^{a}	19 (72)
Glycine	12.7 ± 0.22^{g}	19.1 ± 0.36^{f}	24.7 ± 1.42^{e}	33.0 ± 2.52^{b}	24.4 ± 0.78^{e}	36.2 ± 1.04^{a}	26.8 ± 0.37^{d}	30.2 ± 1.06^{c}	
Alanine	11.9 ± 0.44^{e}	14.3 ± 1.48^{e}	25.0 ± 1.73^{d}	32.9 ± 2.01^{c}	27.2 ± 1.55^{d}	39.3 ± 1.29^{b}	39.0 ± 0.84^{b}	44.2 ± 1.40^{a}	
Serine	9.4 ± 0.96^{d}	12.2 ± 1.47^{d}	24.4 ± 3.25^{c}	28.5 ± 1.46^{bc}	25.3 ± 2.06^{bc}	29.4 ± 2.72^{b}	40.2 ± 2.82^{a}	43.1 ± 4.33^{a}	
Proline	10.3 ± 0.55^{e}	12.8 ± 0.65^{f}	22.4 ± 1.26^{d}	25.9 ± 1.18^{c}	22.6 ± 1.31^{d}	28.6 ± 0.97^{b}	30.5 ± 0.66^{b}	34.5 ± 2.47^{a}	
Aspartic acid	21.9 ± 2.56^{d}	29.8 ± 2.55^{d}	57.3 ± 7.48^{c}	73.6 ± 3.58^{b}	59.4 ± 8.34^{c}	77.4 ± 6.39^{b}	110.5 ± 9.79^{a}	120.4 ± 12.63^{a}	
Glutamic acid	29.8 ± 3.83^{f}	43.1 ± 3.07^{f}	76.8 ± 11.27^{e}	105.2 ± 4.69^{d}	81.4 ± 8.58^{e}	123.7 ± 12.57^{c}	144.3 ± 8.18^{b}	169.2 ± 19.92^{a}	
Arginine	13.9 ± 0.61^{d}	17.2 ± 1.92^{d}	35.5 ± 1.56^{c}	50.7 ± 2.33^{b}	39.2 ± 2.87^{c}	53.7 ± 7.92^{b}	53.0 ± 3.40^{b}	61.1 ± 6.58^{a}	
Tyrosine	9.4 ± 0.55^{d}	10.7 ± 1.21^{d}	23.4 ± 3.52^{c}	25.2 ± 1.39^{c}	24.7 ± 3.36^{c}	24.2 ± 2.71^{c}	39.6 ± 4.16^{b}	41.7 ± 4.81^{a}	
TAA	**194.8^{h}**	**259.4^{g}**	**493.1^{f}**	**621.2^{c}**	**516.6^{e}**	**669.7^{d}**	**835.3^{b}**	**940.3^{a}**	
TEAA	**75.5^{h}**	**100.1^{g}**	**203.7^{f}**	**246.3^{d}**	**212.4^{e}**	**257.2^{c}**	**351.3^{b}**	**395.8^{a}**	
TEAA/TAA	**0.39**	**0.39**	**0.41**	**0.40**	**0.41**	**0.38**	**0.42**	**0.42**	

Data are shown as mean ± standard deviation ($n = 3$). The asterisk (*) indicates essential amino acids (EAA). TEAA represents the total amount of essential amino acids, and TAA represents the total amount of amino acids. Significant differences between rows are denoted by different small letters ($p < 0.05$)

requirements for adults set by WHO/FDA/UNN for the EAA leucine, isoleucine, valine, and methionine, which could stem from a combination of raw material composition and processing methods. Taken together, these findings highlight that while both methods improve the nutritional value of crab side streams relative to the raw material, the pH-shift process clearly outperforms MSM in selectively enriching high-quality proteins, particularly those rich in EAAs, thereby offering a nutritionally superior product. The deficiency in methionine and phenylalanine for infants highlights the need for complementary protein sources in formulations, a strategy already common in food protein blending to overcome limiting amino acids. Moreover, the enrichment of myofibrillar proteins through pH-shift processing could provide added functionality in food structuring applications, strengthening the case for protein isolates as dual-purpose ingredients.

Fatty Acid Composition of the Recovered Crab Minces and Protein Isolates

Based on previous studies, the white meat from crab (i.e., the muscle) generally has a low lipid content, typically around 0.6–0.7% (w/w) (Maulvault et al., 2012). However, out of the fatty acids present, there are notable amounts of LC n-3 PUFA, such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (Umer et al., 2021), rendering crab meat an excellent dietary choice (Kanwal & Saher, 2016). The so-called brown meat (i.e., hepatopancreas tissue) is known for having a higher lipid content, 9.5–14% w/w (Maulvault et al., 2012), with the higher level derived from crabs caught in the summer. This relation between white and brown meat was also visible through the present data (Table 3), revealing more than double the amount of total fatty acids in whole crab compared to cleaned crab, with, e.g., hepatopancreas removed.

When the shells of whole crabs were separated from the meat using MS or pH-shift processing, the total amount of fatty acids (FA), saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and PUFA of the n-3 and n-6 families significantly increased (Table 3). Further, in MSM and protein isolates recovered from the cleaned crabs, the levels were significantly lower than in corresponding samples from whole crabs, again reflecting that brown meat and viscera are the primary sources of lipids in crabs, including n-3 PUFA.

MSM from cooked crabs exhibited a higher content of n-3 PUFA compared to MSM from raw crabs and the protein isolates. Further, the ratio of n-3 PUFA to n-6 PUFA was higher in the products recovered from cleaned than whole crabs, suggesting that the lipids of the brown meat have a lower relative content of n-3 PUFA compared to lipids in crab muscle, something which was earlier found in summer-caught crabs, but not in those caught during winter (Maulvault et al., 2012). The crab protein isolates from whole crab exhibited slightly but significantly lower absolute quantities of all six fatty acid groups compared to both the MSM and the whole crab raw material ($p < 0.05$), especially EPA and DHA. For total fatty acids, isolates were around 8.5% lower than in the corresponding non-cooked MSM. This observation contradicts the higher crude lipid content of the protein isolates compared to MSM, as indicated in Table 1, on a wet weight basis which could be due to carotenoids' contribution to the measured

Table 3 Fatty acid composition (mg/100 g sample, dw) of MSM from cooked (MSM cooked) or raw (MSM raw) hollow brown crab, together with protein isolate from pH-shift processed (pH-shift) raw hollow brown crab. Both processes were applied to whole or cleaned crabs; the pH-shift process only to the raw ones. Data are also shown for the uncooked raw materials

Fatty acids (mg/g dw)	Raw whole crab	Raw cleaned crab	MSM cooked (whole)	MSM cooked (cleaned)	MSM raw (whole)	MSM raw (cleaned)	pH-shift (whole)	pH-shift (cleaned)
ΣFA	19.87 ± 4.83^{e}	8.88 ± 0.51^{f}	106.13 ± 1.57^{a}	42.62 ± 0.50^{d}	82.30 ± 8.29^{b}	20.61 ± 4.69^{e}	75.15 ± 1.93^{c}	26.48 ± 0.67^{e}
ΣSFA	3.85 ± 0.87^{f}	1.61 ± 0.26^{g}	21.33 ± 1.03^{a}	8.41 ± 0.25^{d}	18.13 ± 0.60^{b}	3.90 ± 0.89^{f}	13.91 ± 0.17^{c}	5.11 ± 0.08^{e}
ΣMUFA	8.00 ± 1.90^{d}	2.82 ± 0.08^{f}	43.85 ± 0.84^{a}	15.08 ± 0.31^{c}	32.67 ± 5.21^{b}	5.85 ± 1.89^{de}	29.50 ± 0.65^{b}	8.64 ± 0.09^{d}
ΣPUFA	8.03 ± 2.07^{e}	4.45 ± 0.21^{f}	40.95 ± 0.61^{a}	19.14 ± 0.20^{c}	31.50 ± 2.49^{b}	10.87 ± 2.10^{d}	31.74 ± 1.52^{b}	12.73 ± 0.82^{d}
C20:5n3 (EPA)	2.25 ± 0.58^{e}	1.86 ± 0.07^{e}	11.29 ± 0.15^{a}	7.13 ± 0.16^{c}	9.78 ± 0.18^{b}	4.60 ± 0.86^{d}	8.65 ± 1.71^{b}	5.57 ± 0.03^{d}
C22:5n3 (DPA)	0.37 ± 0.10^{e}	0.13 ± 0.03^{f}	2.92 ± 0.15^{a}	1.02 ± 0.03^{d}	2.46 ± 0.07^{b}	0.33 ± 0.05^{e}	1.54 ± 0.13^{c}	0.48 ± 0.05^{e}
C22:6n3 (DHA)	3.13 ± 0.78^{e}	1.39 ± 0.07^{f}	14.31 ± 0.28^{a}	6.12 ± 0.32^{d}	10.87 ± 2.30^{c}	3.55 ± 0.69^{e}	12.65 ± 0.26^{b}	3.72 ± 0.80^{e}
Σn-3 PUFA	**5.98 ± 1.52^{e}**	**3.47 ± 0.18^{f}**	**30.55 ± 0.47^{a}**	**14.71 ± 0.20^{c}**	**24.28 ± 2.14^{b}**	**8.63 ± 1.66^{d}**	**23.70 ± 1.59^{b}**	**9.96 ± 0.83^{d}**
Σn-6 PUFA	**2.05 ± 0.55^{f}**	**0.97 ± 0.03^{g}**	**10.40 ± 0.15^{a}**	**4.43 ± 0.09^{d}**	**7.22 ± 0.35^{c}**	**2.23 ± 0.44^{ef}**	**8.04 ± 0.38^{b}**	**2.77 ± 0.02^{e}**
n-3/n6 ratio	**2.92 ± 0.08^{c}**	**3.56 ± 0.1^{ab}**	**2.93 ± 0.005^{c}**	**3.32 ± 0.09^{b}**	**3.35 ± 0.13^{b}**	**3.86 ± 0.13^{a}**	**2.95 ± 0.27^{c}**	**3.60 ± 0.32^{ab}**

Data are shown as mean ± standard deviation ($n = 3$). Different small letters in each row show a significant difference ($p < 0.05$)

lipid content. As stated above, there was no typical emulsion layer observed during the first centrifugation step when pH-shift processing crab biomass. Likely, lipids were rather removed via sedimentation of phospholipids during the first centrifugation and/or during the second centrifugation step, which can be seen as a washing phenomenon. Although the phospholipids are recognized for their higher content of n-3 PUFA compared to the triglycerides (Zeng et al., 2010), the n-3/n-6 ratio was not significantly changed in the protein isolates compared to the raw materials. Oppositely, the crab protein isolates from cleaned crab exhibited significantly higher amounts of all classes of studied fatty acids compared with the corresponding MSM. This could indicate that the distribution of fatty acids originating from the crab viscera and the crab muscle have been different during the pH-shift processing.

Overall, cleaning and processing with both MS and pH-shift processing led to a substantial increase in total fatty acids, particularly EPA and DHA, compared to whole crabs, underlining that the brown meat and viscera are the major lipid reservoirs. Interestingly, MSM retained higher levels of n-3 PUFA than protein isolates, and the n-3/n-6 ratios were generally more favorable in products derived from cleaned crabs. From a nutritional standpoint, this suggests that while crab-based protein isolates may provide a leaner ingredient with reduced lipid content, they could be less favorable as a source of health-promoting LC n-3 PUFA. In contrast, MSM, especially from cooked crabs, could offer a more balanced nutritional profile by retaining a higher proportion of these n-3 fatty acids, which are particularly connected with cardiovascular disease protection through their anti-inflammatory effects (Djuricic and Calder., 2021).

Vitamins of the Recovered Crab Minces and Protein Isolates

Marine products are widely recognized as an essential dietary source of fat- and water-soluble vitamins, such as vitamins D and B12. Vitamin D plays multiple pivotal roles within our bodies, acting as a hormone that regulates calcium and phosphorus metabolism. Vitamin D is particularly vital for maintaining bone health, and its importance cannot be overstated (Schmid & Walther, 2013). In seasons of low sunlight and in cultures where clothing regimes prevent skin exposure to sunlight, vitamin D deficiency is widespread (Alagöl et al., 2000). Among the various treatments, the whole crab processed with the pH-shift process exhibited the highest level of vitamin D, measuring 0.14 µg/100 g DW, revealing an up-concentration throughout the process. This was followed by a value of 0.099 µg/100 g DW in MSM from whole crabs (Fig. 2A). MSM production from raw cleaned crabs (without carapace) resulted in a decrease in vitamin D content, while conversely, MS of cooked and whole crab concentrated vitamin D. The vitamin D content of the minces and protein isolates correlate with their crude lipid content which could be due to the lipid soluble nature of vitamin D. The observed differences in vitamin D content between MSM and pH-shift-based protein isolates can largely be attributed to the distinct processing mechanisms. The higher up-concentration of vitamin D during pH-shift processing compared to MS aligns with the slightly higher content of crude lipids of the former process, reflecting the

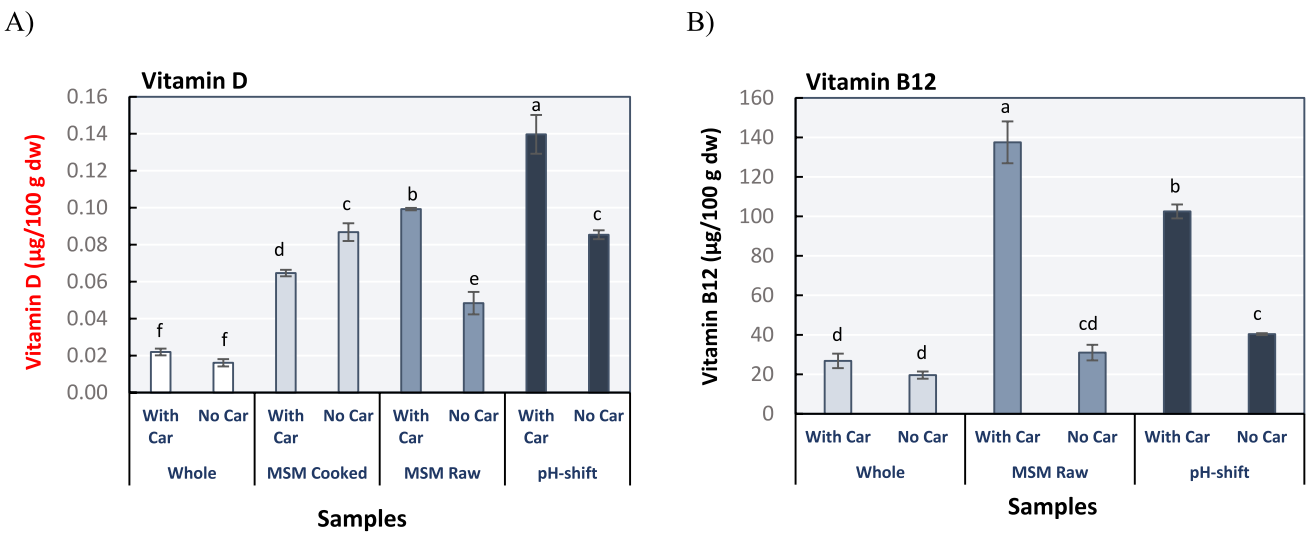

Fig. 2 Vitamin D (**A**) and vitamin B12 (**B**) content in hollow brown crab subjected to mechanical separation (MSM) or pH-shift processing (pH-shift) with or without being cleaned. The raw material is shown in its raw/cooked state. Data are shown on a dry weight (dw) basis and represent mean ± standard deviation ($n = 3$ for vitamin D and $n = 3$ for vitamin B12)

lipophilic nature of this vitamin and its compartmentalization in fat-rich crab tissues. Interestingly, cooking prior to MS of cleaned crab appeared to enhance vitamin D retention, possibly by disrupting tissue matrices and releasing lipid droplets, facilitating their recovery into the MSM. From a nutritional perspective, while the absolute amounts of vitamin D measured in crab fractions may appear modest (0.08–0.14 µg/100 g DW), regular inclusion of such products in the diet could contribute to meeting daily requirements (5–15 µg/day), particularly in populations at risk of deficiency due to low sun exposure. Importantly, the recovery of vitamin D alongside high-quality proteins in pH-shift isolates highlights a dual nutritional benefit, suggesting that this processing route may provide superior raw material for designing functional foods compared with MSM.

Based on the 2016 report from the Food and Agriculture Organization (FAO, 2016), it is evident that crab and lobster typically exhibit a higher vitamin B12 content (also known as cobalamin) within their muscle tissues compared to in brown meat. Regarded as one of the most indispensable vitamins essential for the well-being of all organisms, vitamin B12 possesses paramount importance. It serves as a catalyst for cell proliferation, particularly in blood formation, while also playing a fundamental role in maintaining optimal functionality of the nervous system (Santos et al., 2023). MS of raw whole crab led to a significantly higher vitamin B12 content in the MSM compared to its original raw materials ($p < 0.05$), and MSM obtained from whole crabs had the highest content of vitamin B12 out of all samples (137.5 µg/100 g DW). For cleaned crab, there was however no significant up-concentration of B12 during MS. Differently, pH-shift processing increased vitamin B12 content in both whole and cleaned crab, however, more so in whole crab, where the protein isolate reached 102.5 µg/100 g DW (Fig. 2B). Although the isolate from whole crab did not reach the same B12 levels as MSM from whole crab, the up-concentration pattern is noteworthy. Unlike MS, which relies mainly on physical disintegration and tissue co-recovery, the pH-shift method facilitates selective solubilization and precipitation, enabling it to recover protein-bound micronutrients. Although there were no significant differences between whole and cleaned crab raw materials, likely due to the large dilution from the carapace in the former, the drastic up-concentration of B12 when processing whole crab reveals that brown meat and viscera have higher B12 content than muscle. This aligns with the recent study of Reksten et al. (2024) reporting a 19-fold difference in vitamin B12 between crab claw meat and brown meat.

Minerals of the Recovered Crab MSM and Protein Isolates

Many minerals play a crucial role in supporting various physiological and biochemical functions within the human body, making their dietary intake at the required levels essential. Crustaceans, like crabs, are highly abundant in both macro- and microminerals. Notably, some reports have revealed that crab meat is particularly rich in manganese, calcium, zinc, copper, and selenium (Kwoczek et al., 2006).

The content of different minerals analyzed in crab MSM and protein isolates and their key variations can be seen in Tables 4 and 5, presented alongside recommended daily intake (RDI) or adequate intake (AI) values to facilitate nutritional evaluation of the samples. Recorded levels were more affected by the separation technology than the pre-removal of brown meat, carapace, and guts during the cleaning. The washing effect of the pH-shift processing, together with the more efficient removal of shell residues, resulted in substantially lower content of sodium, potassium, calcium, magnesium, iron, selenium, and iodine in the protein

Table 4 Mineral contents (mg/100 g sample, dry weight, DW) of hollow brown crab cooked and subjected to mechanical separation (MSM cooked) or raw subjected to mechanical separation (MSM) or pH-shift process (pH-shift) without being cleaned (with carapace) or cleaned (without carapace). Data are shown as mean values ± SD ($n = 3$)

Minerals (mg/100 g DW)	MSM Cooked (with carapace)	MSM Cooked (without carapace)	MSM Raw (with carapace)	MSM Raw (without carapace)	pH-shift (with carapace)	pH-shift (without carapace)	RDI/AI
Sodium	2127.88 ± 3.99^{b}	**2780.12 ± 81.76^{a}**	1953.38 ± 16.74^{c}	2157.71 ± 6.8^{b}	**784.82 ± 12.91^{d}**	**719.92 ± 0.77^{d}**	23 00
Calcium	902.65 ± 1.69^{c}	**1142.58 ± 1.92^{a}**	951.77 ± 8.15^{b}	525.68 ± 15.68^{d}	**71.0 ± 0.44^{e}**	**86.39 ± 1.02^{e}**	950-1000 (RDI)
Potassium	123.38 ± 0.98^{b}	117.9 ± 1.51^{c}	113.8 ± 0.97^{d}	**156.66 ± 3.22^{a}**	**11.15 ± 0.34^{e}**	**12.63 ± 0.01^{e}**	3.4-3.5 (AI)
Iodine	0.67 ± 0.001^{c}	**1.17 ± 0.04^{a}**	1.13 ± 0.009^{a}	0.94 ± 0.002^{b}	**0.49 ± 0.03^{d}**	**0.50 ± 0.01^{d}**	0.15-0.2 (RDI)
Selenium	0.69 ± 0.001^{bc}	0.73 ± 0.02^{b}	0.67 ± 0.005^{c}	**0.93 ± 0.002^{a}**	**0.44 ± 0.02^{d}**	**0.37 ± 0.004^{e}**	0.075-0.09 (AI)
Zinc	25.75 ± 2.09^{b}	21.79 ± 0.47^{c}	**19.97 ± 1.41^{c}**	**30.32 ± 0.78^{a}**	28.53 ± 0.04^{a}	28.88 ± 0.34^{a}	9.3-12.7 (RDI)
Copper	**8.42 ± 0.01^{f}**	14.81 ± 0.02^{b}	13.68 ± 0.52^{c}	9.13 ± 0.02^{e}	**23.64 ± 0.07^{a}**	10.97 ± 0.13^{d}	0.9-1.3 (RDI)
Iron	35.8 ± 1.81^{a}	**37.19 ± 0.06^{a}**	27.55 ± 0.23^{b}	26.84 ± 0.3^{b}	**14.03 ± 0.08^{d}**	20.35 ± 0.24^{c}	7-26 (RDI)
Magnesium	252.68 ± 8.07^{b}	**289.21 ± 0.48^{a}**	229.09 ± 1.96^{c}	222.95 ± 9.22^{c}	**37.14 ± 0.23^{d}**	41.99 ± 0.49^{d}	300-350 (AI)
Chromium*	0.11 ± 0.0^{d}	**1.3 ± 0.0^{a}**	**0.09 ± 0.0^{f}**	0.11 ± 0.0^{e}	0.17 ± 0.0^{c}	0.22 ± 0.0^{b}	0.025-0.035 (AI)
Molybdenum	0.02 ± 0.0^{a}	0.08 ± 0.0^{a}	0.04 ± 0.0^{a}	0.01 ± 0.0^{a}	0.03 ± 0.0^{a}	0.02 ± 0.0^{a}	0.065 (AI)

*A naturally occurring heavy metal

Different small letters in each row show a significant difference ($p < 0.05$). The highest and lowest values for each mineral are shown in bold and highlighted in green and yellow respectively

isolates compared with the MSM ($p < 0.05$). Among the minerals differentiating MSM and protein isolates, the most are those essential for bone health, such as calcium, magnesium, and potassium. Further, there was a tenfold lower content of potassium in the protein isolates compared with the MSMs, reflecting its high solubility in water and thus extraction into the second supernatant during the pH-shift process. The processing-induced redistribution of water-soluble minerals such as potassium, sodium, magnesium, iodine, and selenium also helps explain the observed patterns. These minerals are readily leached into the aqueous phase during homogenization and alkaline solubilization and are subsequently lost with the supernatant during washing. Their depletion was particularly pronounced for potassium, which is almost exclusively present in soluble form and thus poorly retained in the isolates (Lee et al., 2016). However, the content of zinc, copper, and molybdenum was nearly equal in the two product types derived from cleaned crab, while copper and zinc were significantly higher in protein isolates of whole crab. This can be linked to their stronger affinity to amino acid side chains (e.g., histidine, cysteine, glutamate), which allows them to remain partially bound to metalloproteins even after repeated washing steps. This phenomenon has previously been observed when recovering protein isolates from gutted herring with the pH-shift process (Marmon & Undeland, 2010). The same observation was not done in protein isolates from cleaned crabs that could be due to the presence of more copper in brown meat, which is removed during the cleaning process, along with the carapace. Generally, the impact of mechanical separation and pH-shift processing on the mineral content of protein-enriched products obtained from crabs varies depending on the minerals' presence in either the carapace or muscle fraction. Very pronounced reduction of calcium and magnesium in the protein isolates compared with MSMs could be associated with the more effective removal of shell residues during the pH-shift processing. Additionally, it is influenced by the minerals' affinity for binding to proteins as opposed to their solubility in water.

Altogether, the pronounced reduction of calcium, magnesium, potassium, and selenium in the crab protein isolates highlights a nutritional trade-off of the pH-shift process. While the removal of shell residues and soluble minerals yields a purer protein fraction with improved functionality for food applications, it simultaneously reduces the contribution of these isolates to essential mineral intake. In particular, the tenfold reduction of potassium is nutritionally relevant, as seafood is often promoted as a dietary source of electrolytes and minerals beneficial for cardiovascular health. Similarly, the lower levels of calcium and magnesium compromise the potential role of crab-derived proteins in bone-supportive nutrition. Conversely, the relatively well-retained levels of zinc, copper, and molybdenum, together with their biological roles in enzymatic and immune functions, suggest that not all trace minerals are equally affected by the process.

Heavy Metal Content of the Recovered Crab Minces and Protein Isolates

The buildup of toxic heavy metals like lead, cadmium, mercury, and other heavy metals in the body's tissues can result in significant health hazards such as neurological issues, liver damage, and disruption of red blood cells, gastrointestinal disturbances, hypertension, kidney malfunction, and reproductive abnormalities (Ahmed et al., 2023). Contents of heavy metals including cadmium, mercury, lead, and arsenic in the crab MSM and protein isolates are summarized in Fig. 3 and Table 5. Removal of the brown meat during pre-processing cleaning caused up to a tenfold reduction of cadmium in the derived MSM and protein isolates (Fig. 3A). A likely reason for this is that the brown meat of crab predominantly comprises its hepatopancreas, which is the primary location for environmental toxins, especially cadmium (Bolam et al., 2016). The cadmium levels observed here are discussed in the context of recent permissible limits reported for brown crab by Lordan and Zabetakis (2022). Pre-cooking resulted in significantly ($p < 0.05$) higher cadmium levels in the derived MSM, and pH-shift processing resulted in slightly, yet significantly ($p < 0.05$), higher content of cadmium than MS. This could be attributed to the previously described selective nature of pH-shift processing towards proteins. Cadmium, which binds strongly to protein thiols and metallothioneins, thus remains associated with the recovered protein fraction. Additionally, the removal of water and water-soluble compounds during precipitation further concentrates cadmium per unit protein. Therefore, pH-shift processing does not remove cadmium but can slightly increase its concentration in the protein isolate. On the other hand, pre-cooking or the type of separation technology did not have a significant effect on the lead content of the recovered protein-rich products (Fig. 3B). This suggests that lead was more evenly distributed among exoskeleton, proteins, lipids, and soluble phase, thereby demonstrating an equal partitioning following processing.

Removal of brown meat, viscera, and carapace in cleaned crabs resulted in significantly higher content of mercury in the derived MSMs (Fig. 3C). Mercury, particularly in the form of methylmercury, is known to bind strongly to thiol groups (especially cysteine) in proteins, which are abundant in muscle tissue, whereas brown meat and viscera contain higher levels of lipids and non-protein components that may sequester less mercury (Gong et., 2011). Consequently, selective removal of these tissues enriches the relative proportion of mercury-binding muscle proteins in the MSM fraction. Furthermore, the significantly higher mercury

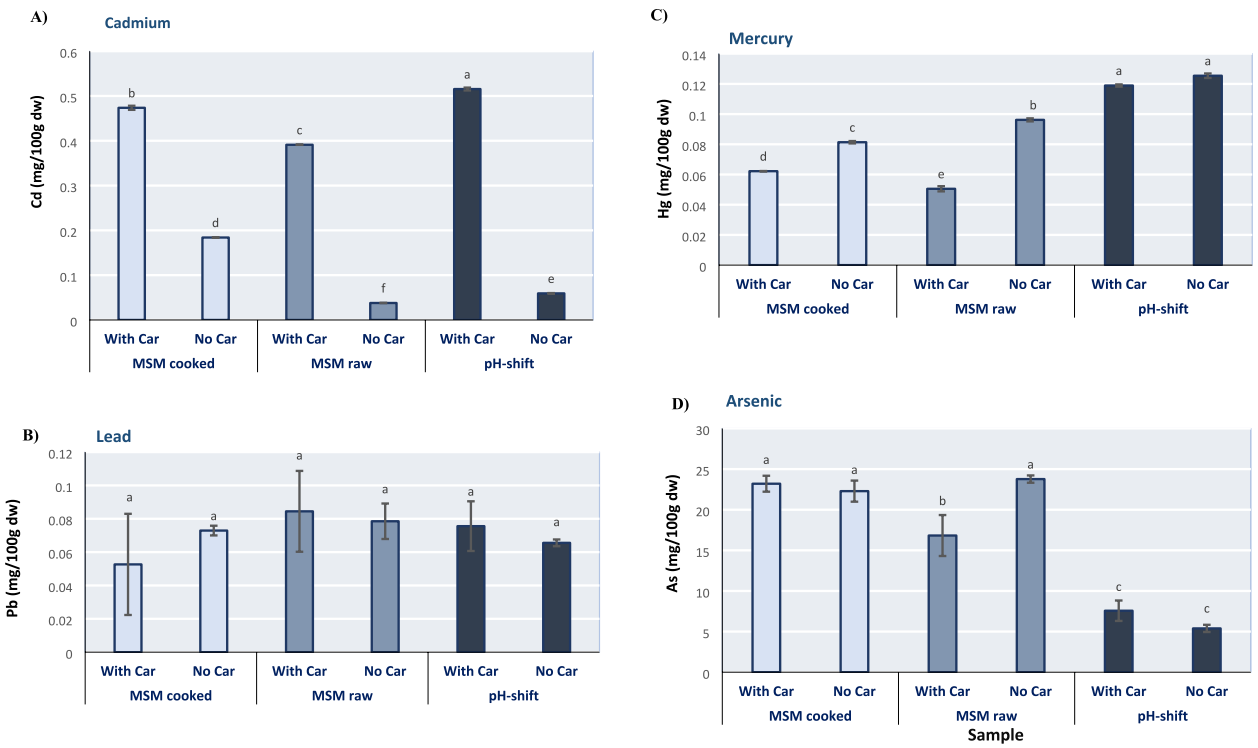

Fig. 3 Cadmium (**A**), lead (**B**), mercury (**C**), and arsenic (**D**) content (mg/100 g dw) of minces from hollow brown crab subjected to mechanical separation (MSM) or pH-shift process (pH-shift) without being cleaned (with carapace, viscera, and brown meat) or cleaned (without carapace, viscera, and brown meat) on dry weight (dw) basis. Data are shown as mean values ± SD ($n = 3$). EFSA regulatory limits (e.g., cadmium 0.5 mg/kg wet weight) are provided in the figure legends for comparison

Table 5 A summary diagram of the difference between mechanical separation (MS) and the pH-shift processing on macro and micronutrient content in protein-rich ingredients recovered from whole (not cleaned) and cleaned crabs

Treatment		Protein/ TAA	TFA	Ash	TEAA	n-3 PUFA	Vitamin D	Vitamin B12	Minerals	Heavy metals			
										Cd	Hg	As	Pb
MS	not cleaned	↑	↑	↓	↑	↓	↑↑	↑↑	→	↓	↓	↓	→
	cleaned	↑	↑	↓	↑	↓	↑	→	↓	↓↓	↑	→	→
pH-shift	not cleaned	↑↑	↑↑	↓↓	↑↑	↓↓	↑↑↑	↑↑	↓	↑	↑↑	↓↓	→
	cleaned	↑↑	↑↑	↓↓	↑↑	↓↓	↑	↑	↓	↓↓	↑↑	↓↓	→

TAA total amino acids, *TEAA* total essential amino acids, *TFA* total fatty acids, *Cd* cadmium, *Hg* mercury, *As* arsenic. Green colors mean positive effects and brown color means negative effects of each macro and micronutrient and its intensity. The arrows show the direction of the differences where ↑ means more and ↓means less and the number of arrows shown a rough estimate of the intensity of the changes

levels observed in protein isolates compared to MSMs, independent of whether the crabs were pre-cleaned or not, can likely be explained by the fact that mercury remains tightly bound to protein thiols during the solubilization and precipitation steps of the pH-shift process. This mechanism aligns with previous reports showing that processing methods that enrich muscle proteins, such as protein isolation or fractionation, often result in higher contaminant concentrations per unit of protein, even if the total tissue concentration remains unchanged (Gong et al., 2011).

In contrast to cadmium and mercury, using the pH-shift method could reduce the content of arsenic in the crab protein four–fivefold compared with the mechanical separation, regardless of the presence or removal of carapace, brown meat, and viscera. This is likely related to the water solubility of organic arsenic (mainly arsenobetaine), which is the dominant form in crabs (Liu et al., 2020) and the mentioned leaching effect from the pH-shift process, as shown previously for other biomasses subjected to this technology. Overall, the organic arsenic forms are far less toxic than the inorganic forms; and in Chinese mitten crab, total arsenic ranged from 0.25 to 1.66 mg/kg ww, while inorganic arsenic was only present at 0.01–0.21 mg/kg ww (Liu et al., 2020). Arsenic speciation should be a next step to further

understand the impact of MS and pH-shift processing on the distribution of this heavy metal in derived products.

Conclusions

Applying mechanical separation and pH-shift processing to whole or cleaned raw brown crab revealed that the latter technology yielded a protein-enriched product of superior purity, primarily attributable to the more efficient elimination of shell. Both technologies, for mechanical separation also with cooked crabs, successfully delivered products that were more abundant in essential nutrients, including, e.g., essential amino acids and vitamins D and B12 (for whole crab) compared to their corresponding raw materials. Both technologies also provided products with a balanced n-3/n-6 ratio and equal zinc levels. Protein isolates recovered using the pH-shift technology had significantly higher levels of vitamin D and essential amino acids, but lower levels of vitamin B12 (for whole crab), total PUFA, LC n-3 PUFA, certain minerals (sodium, calcium, magnesium, potassium, selenium, iodine, and iron), and total arsenic compared with the MSM. The specific composition of essential and non-essential nutrients present in the retrieved protein ingredients significantly relied on whether the crab was processed whole or cleaned, i.e., in the presence or absence of the viscera, carapace, and brown meat. The content of crude lipids, SFA, MUFA, PUFA, vitamin D, vitamin B12, copper, and cadmium was lower in products from cleaned compared to whole raw crab for both technologies. On the other hand, for example, total and essential amino acids were higher in products made from cleaned crabs, and mercury levels were also higher in the cleaned crab-derived MSM samples. Furthermore, the main effect of pre-cooking crabs before MSM processing was a higher retention of exoskeleton fragments, which led to increased ash and cadmium content in the final product. Overall, our study demonstrates that the choice of valorization technology significantly affects the macro- and micronutrient composition of protein-enriched ingredients obtained from brown crab, and that pretreatments applied to the raw material play an important role that requires careful consideration.

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Data Availability Data have been archived and will be available on request.

Declarations

Competing Interests The authors declare no competing interests.

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