Nutritional Quality, Functionality, and In-vitro Protein Digestibility of Protein Concentrates Extracted from Selected Shellfish Species

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ABSTRACT

Background: *Underutilized sources of high-quality protein such as shellfish have the potential to be useful components in food compositions. Processing them into protein concentrates may significantly improve their nutritional and functional applications.*

Objective: *This study investigated the nutritional quality and in-vitro protein digestibility of protein concentrates extracted from selected shellfish species.*

Methodology: *Protein concentrates and flours were prepared from clam, shrimp, oyster, periwinkle, and whelk using standard methods, each separated into two parts, edible portion (flesh) and the shell except for shrimps which was taken wholly. The samples were oven-dried at 50°C for 8 hours using a Nuve FN 400P / FN 500P Oven, pulverized into fine powder with a Philips HR2061/02, and sieved through a 200-mesh screen. The proximate composition and mineral content of the flour and concentrates were determined, and in vitro digestibility was assessed for the concentrates using standard analytical laboratory methods. Protein digestibility (IVPD) was determined using an in vitro enzymatic spectrophotometric method.*

Results: *Shellfish protein concentrates had higher protein (62.88–77.44%) and lower fat (2.00–4.00%) and ash (1.00–4.50%) than the flours. Clam concentrate had the highest protein (77.44%), while whelk had the highest ash (4.50%) and carbohydrate (11.94%). Shellfish flours were rich in the mineral magnesium. In vitro digestibility of the concentrates indicated high protein bioavailability, with clam protein concentrate showing the highest digestibility.*

Conclusion: *Shellfish protein concentrates, especially from shrimp and clam, possess some favourable nutritional and functional characteristics, suggesting their potential use as high-quality protein ingredients in formulated foods.*

Keywords: Shellfish; Protein concentrates; Oyster; Periwinkle; Shrimp; Whelk

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INTRODUCTION

Protein deficiency is common due to poor dietary habits, as protein and other macronutrients are essential for the growth and maintenance of the body. By the year 2050, it is estimated that more than 40% of women and children in Sub-Saharan Africa will be malnourished due to protein deficiencies [1]. Protein shortages may occur if demand continues to rise faster than supply. Every demographic indicator points to demand for protein increasing further in the years ahead, as the world's growing and increasingly wealthy population consumes more of it [2]. Many protein sources require large amounts of land and produce significant amounts of greenhouse gases; a more sustainable alternative is needed to meet demand. Therefore, food manufacturers and scientists are actively seeking novel sources of protein [3].

Generally, shellfish are widely consumed as low calorie-alternative digestible proteins, providing up to one-fifth of the average animal protein intake of nearly 50% of the world's population [4]. Shellfish are of two groups: mollusks (clams, periwinkles, oysters, whelks) and crustaceans (shrimps, lobsters, crabs, and crayfish) [5]. A study showed that the shellfish nutritive value varies widely. For example, [5] reported the protein content and digestibility quality of smooth and rough periwinkles, whelk, oysters, and clams to vary between 12.01% and 18.01% and 8.62% and 16.75%, respectively. Proteins from shellfish are highly nutritious, containing a high quantity of essential and non-essential amino acids [6]. Despite the exceptional nutritional qualities of shellfish, they are underutilized in the food industry mainly due to their high perishability in nature [7]. Utilization of shellfish becomes necessary to process them into a shelf-stable, ready-to-use form that can be incorporated into food products. Protein concentrates, especially from shellfish, are suitable for use as ingredients in the food industry because of their high nutritional value [8].

Generally, digestion of protein concentrates from shellfish releases bioactive peptides that have proven to possess exceptional health benefits, such as anti-diabetic, anti-inflammatory, and cardio-protection [9]. Furthermore, multiple technologies are employed in the production of protein concentrates, encompassing milling of flour and drying to dehydrate the protein extracts into a powder during the execution of wet extraction. Nevertheless, at the core of this procedure lies protein extraction and the subsequent separation. The choice of the most suitable protein extraction and separation process hinges on various factors, including the desired protein levels in the ingredients [10]. Alkaline extraction-isoelectric precipitation stands as the most frequently employed technique for isolating proteins from plant-derived foods. In comparison to alternative approaches, it presents cost-effectiveness and a relatively straightforward procedural framework [11]. This study aimed to compare the physicochemical and functionality of five selected shellfish (periwinkle, whelk, clam, shrimp, and oyster) protein concentrates.

MATERIALS AND METHODS

Sample collection and preparation

Tympanotonus fuscatus (Periwinkle), *Macrobachium vollenhoveni* (Shrimp), *Crassostrea gasar* (Oyster), *Egeria radiata* (Clam) and *Buccinum undatum* (Whelk) of maturity were procured alive from Creek road market, Port Harcourt Local Government Area in Rivers State (Nigeria). The shellfish samples, after being meticulously washed with distilled water to eliminate any loose and soiled parts, were then carefully arranged inside an ice box to maintain their freshness. For every 10 kilograms of shellfish, 20 kilograms of ice were added to ensure proper chilling. The samples were subsequently transported to the laboratory without delay. Oyster, whelk, periwinkle, and clam, contributing to a total weight of 22kg, were put into a stainless pot and boiled for 5 minutes at 100 °C. After boiling, the samples were poured into a perforated basket to drain and allowed to cool at room temperature (28 ± 2 °C). The edible portion (meat) was extracted from the shell with the aid of a sterile pin in the case of the periwinkle and whelk, and a sharp knife in the case of the oyster and clam. Fresh shrimp were cooked for just 2 minutes at 100 °C, drained, and then cooled at room temperature.

Specifically, periwinkle samples were standardized to an average individual weight of approximately 18 g, with multiple specimens pooled to obtain a total composite sample weight of 3.5 kg. Clam (*Egeria radiata*) specimens were selected at a mean individual weight of about 35 g, contributing to a total sample weight of 2.2 kg. Shrimp samples consisted of individuals with an average weight of 10 g, pooled to achieve a total sample weight of 4.0 kg. Oyster specimens were standardized at an average weight of approximately 80 g per individual, yielding a total sample weight of 6.8 kg, while whelk samples were collected at a mean individual weight of about 50 g, with a cumulative sample weight of 5.5 kg.

Preparation of defatted shellfish flour

Periwinkles, clams, oyster, and whelk were each separated into two (2) parts: edible portion (flesh) and the shell, except for shrimps, which were taken wholly. The samples were dried in a Nuve FN 400P / FN 500P Oven at 50 °C for 8 h and ground to fine powder using a blender (HR2061/02) and sieved using a 200-mesh screen, packed in bags, and stored in a box until further analysis. About 100 g of shellfish flour was sieved into an 80-mesh sieve and was soaked in

600 mL of 95 % ethanol for 20-hours period. After soaking, the solvent was decanted, and defatted shellfish flour was dried in an oven at 60 °C for 12 hours. Thereafter, the defatted shellfish flour was packed in air- tight plastic bags.

Extraction of shellfish protein concentrate

Shellfish protein concentrate was produced using the technique reported by [12]. A hundred grams of defatted flour were mixed with 1 L of distilled water, resulting in a final flour-to-water ratio of 1:10. The mixture was then gently agitated on a magnetic stirrer for 10 minutes to form a suspension. Subsequently, the pH of the resulting slurry was adjusted to pH 4 (a value determined from preliminary solubility tests of the defatted flour) using 1.0 M HCl to precipitate the least soluble proteins. The precipitation process continued for 4 hours with constant stirring while maintaining the pH. To eliminate soluble carbohydrates (oligosaccharides) and minerals, the mixture was subjected to centrifugation at 3500×g for 30 minutes using a Bosch centrifuge (TDL-5, United Kingdom). The resulting precipitate (concentrate) was then washed twice with distilled water to remove any remaining minerals and soluble carbohydrates. The pH was adjusted to 7.0 using 1.0 M NaOH for neutralization and subsequently centrifuged at 3500×g for 10 minutes. The resulting precipitate (concentrate) was collected and dried in an oven (Uniscope SM9053, Singerfriend, England) at 45 °C for 8 hours and then sieved to obtain fine powder. It was then stored for further analysis. The protein yield in the concentrate was determined as the ratio of the protein content in the dried material to that in the defatted flour.

Proximate composition of the shellfish and shellfish protein concentrates

The AOAC (2012) methods were used to determine the proximate composition of the shellfish flour and shellfish protein concentrates. Mineral analysis was carried out by the dry ashing method according to the procedure outlined by AOAC [13]. Briefly, known weights of the samples were placed in pre-weighed porcelain crucibles and charred gently on a hot plate to remove excess moisture and organic matter. The samples were then ashed in a muffle furnace at 550 °C until a light grey or white ash was obtained

In-vitro Protein Digestibility (IVPD)

In vitro protein digestibility (IVPD) explains how easily a protein is broken down by digestive enzymes in a controlled laboratory setting, thereby providing insights into its nutrient availability [14,15]. Notwithstanding of how simple in vitro models are [16], they are usually very beneficial in forecasting outcomes of the in vivo digestion [17, 18].

A known weight of each sample, equivalent to 16 mg nitrogen (approximately 100 mg crude protein), was weighed in triplicate and digested with 1 mg pepsin (Cat. No. P6887, Sigma Chemicals Ltd., USA) in 15 mL of 0.1 N HCl at 37 °C for 2 h. The mixture was then quantitatively filtered through the Whatman No. 1 filter paper. The TCA soluble fraction was assayed for Nitrogen using the Micro-Kjeldahl method, AOAC.

Statistical analysis

Results were expressed as mean values, and the standard deviation of the triplicate was determined. Data obtained for all the analyses carried out were subjected to statistical analysis using the software SPSS for Windows version 21.0 statistical package (SPSS Inc.). Analysis of variance (ANOVA) tables were constructed and evaluated to determine significant differences ($P \leq 0.05$). Least significant difference (LSD) was used to separate the means. Standard deviation was used to show the difference in the composition.

RESULTS

Proximate composition of the shellfish flour and concentrate

Table 1 shows the proximate composition of shellfish flours and their protein concentrates. Moisture content of the flours ranged from 5.78% in oyster flour (OF) to 8.63% in whelk flour (WF). WF had significantly ($p<0.05$) higher moisture than other samples, while periwinkle flour (PF), shrimp flour (SF), and clam flour (CF) showed similar values (7.34–7.54%). Ash content varied between 4.03% (OF) and 11.21% (SF), with significant differences observed across samples. Fat content ranged from 5.79% in PF to 10.76% in OF; PF and SF had comparable values ($p>0.05$), as observed in samples CF and WF. Protein content was highest in CF (65.56%) and OF (64.75%), which were statistically similar, while WF had the lowest protein (54.25%). Crude fibre ranged from 9.60–10.79% with no significant differences. Carbohydrate content ranged from 1.26% in SF to 10.04% in WF; shrimp, clam, and oyster were similar (1.26–3.22%), while WF was significantly higher ($p<0.05$).

For protein concentrates, moisture ranged from 8.27% (SPC) to 9.25% (PPC), with no significant differences. Fat content was lowest (2.00%) in PPC, SPC, and WPC, and highest (4.00%) in OPC. Protein values ranged from 62.88% (WPC) to 77.44% (CPC). PPC, SPC, and OPC had statistically similar protein levels (around 70.44–73.50%). Crude fibre content ranged from 7.41% to 9.50% without significant variation. Carbohydrate content ranged widely, from 0.10% in CPC to 11.94% in WPC; periwinkle and shrimp concentrates (6.32% and 8.58%, respectively) were statistically similar, while whelk was significantly higher ($p<0.05$). Shellfish flours and concentrates were rich in protein, with CF and CPC recording the highest values. Variations were more pronounced in carbohydrate and ash contents, while crude fibre showed little difference among samples.

Table 1: Proximate composition (%) of selected defatted shellfish and shellfish protein concentrates

Samples	Defatted Shellfish Flour					
	Moisture	Ash	Fat	Crude protein	Crude fibre	Carbohydrate
PF	$7.34^{b}±0.00$	$8.27^{b}±0.18$	$5.79^{c}±0.27$	$62.13^{ab}±1.24$	$9.60^{a}±1.41$	$5.89^{b}±1.51$
SF	$7.54^{b}±0.64$	$11.21^{a}±0.82$	$7.18^{c}±0.01$	$60.22^{b}±0.14$	$10.79^{a}±0.34$	$2.06^{c}±0.85$
CF	$7.34^{b}±0.23$	$5.87^{c}±0.34$	$9.03^{b}±1.26$	$65.56^{a}±1.33$	$9.95^{a}±0.55$	$1.26^{c}±0.83$
OF	$5.78^{c}±0.11$	$4.03^{d}±0.46$	$10.76^{a}±0.01$	$64.75^{a}±2.47$	$10.48^{a}±0.98$	$3.22^{c}±0.45$
WF	$8.63^{a}±0.03$	$7.12^{bc}±0.58$	$8.96^{b}±0.25$	$54.25^{c}±1.23$	$10.01^{a}±0.71$	$10.04^{a}±0.11$
	Shellfish protein concentrates					
PPC	$9.25^{a}±0.35$	$1.50^{c}±0.71$	$2.00^{b}±0.00$	$70.44^{b}±0.62$	$9.50^{a}±0.71$	$6.32^{b}±0.45$
SPC	$8.27^{a}±0.34$	$1.00^{c}±0.00$	$2.00^{b}±0.00$	$70.44^{b}±0.62$	$8.72^{a}±0.40$	$8.58^{b}±2.09$
CPC	$8.56^{a}±0.64$	$2.50^{bc}±0.71$	$3.00^{ab}±1.41$	$77.44^{a}±1.86$	$7.41^{a}±1.88$	$0.10^{c}±0.05$
OPC	$8.68^{a}±0.82$	$3.50^{a}±0.71$	$4.00^{a}±0.00$	$73.50^{b}±2.47$	$9.10^{a}±0.71$	$0.22^{c}±0.24$
WPC	$8.79^{a}±0.32$	$4.50^{a}±0.71$	$2.00^{b}±0.00$	$62.88^{c}±0.18$	$8.91^{a}±0.43$	$11.94^{a}±0.77$

Mean values are of triplicate determinations. Mean values within a column with different superscripts are significantly different at ($p<0.05$). Keys: PF= Periwinkle flour; OF= Oyster flour; SPC= Shrimp protein concentrate; SF= Shrimp flour; WF= Whelk flour; CPC= Clam protein concentrate; CF= Clam flour; PPC= Periwinkle protein concentrate; OPC= Oyster protein concentrate; WPC= Whelk protein concentrate

Mineral composition of shellfish flour

Table 2 shows the mineral composition of shellfish. Zinc content ranged from 3.37 mg/100 g in shrimp flour (SF) to 262.33 mg/100 g in oyster flour (OF), with significant ($p<0.05$) differences observed. Iron ranged from 14.17 mg/100 g (SF) to 50.92 mg/100 g (clam flour, CF). Calcium varied from 616.01 mg/100 g in OF to 4506.24 mg/100 g in shrimp protein concentrate (SPC). Magnesium ranged from 249.88 mg/100 g (CF) to 1697.60 mg/100 g (whelk flour, WF).

Table 2. Mineral composition (mg/100g) of selected defatted shellfish flour

Samples	Zinc	Iron	Calcium	Magnesium
PF	$7.25^{d}±0.07$	$28.08^{c}±0.78$	$2636.29^{b}±0.32$	$1548.61^{b}±0.52$
SF	$3.37^{e}±0.02$	$14.17^{e}±0.92$	$4506.24^{a}±0.25$	$375.67^{d}±0.61$
CF	$55.83^{b}±0.22$	$50.92^{a}±0.06$	$751.49^{d}±0.11$	$249.88^{e}±0.06$
OF	$262.33^{a}±0.34$	$28.32^{b}±0.13$	$616.01^{e}±0.78$	$631.65^{c}±0.57$
WF	$34.39^{c}±0.25$	$18.21^{d}±0.03$	$2225.77^{c}±0.30$	$1697.60^{a}±0.50$

Mean values are of triplicate determinations. Mean values within a column with different superscripts are significantly different at ($p<0.05$). Keys: SF= Shrimp flour; CF= Clam flour; OF= Oyster flour; WF= Whelk flour

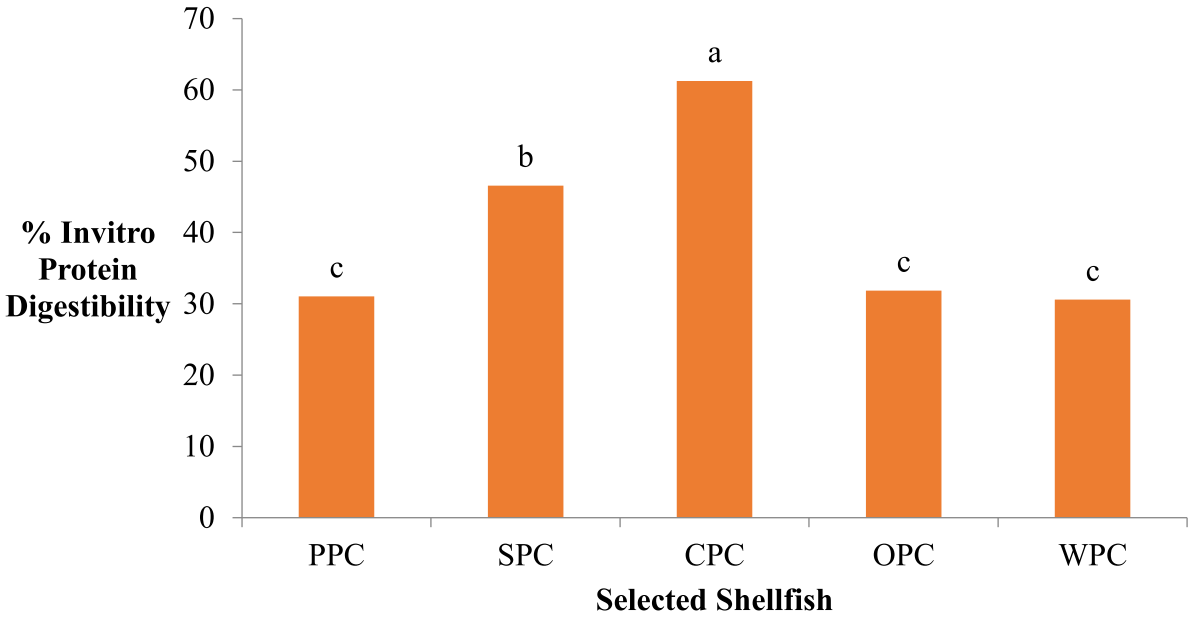

Fig 1. In vitro protein digestibility of protein concentrates extracted from selected shellfish

Keys: PPC= Periwinkle protein concentrate; SPC= Shrimp protein concentrate; CPC= Clam protein concentrate; OPC= Oyster protein concentrate; WPC= Whelk protein concentrate

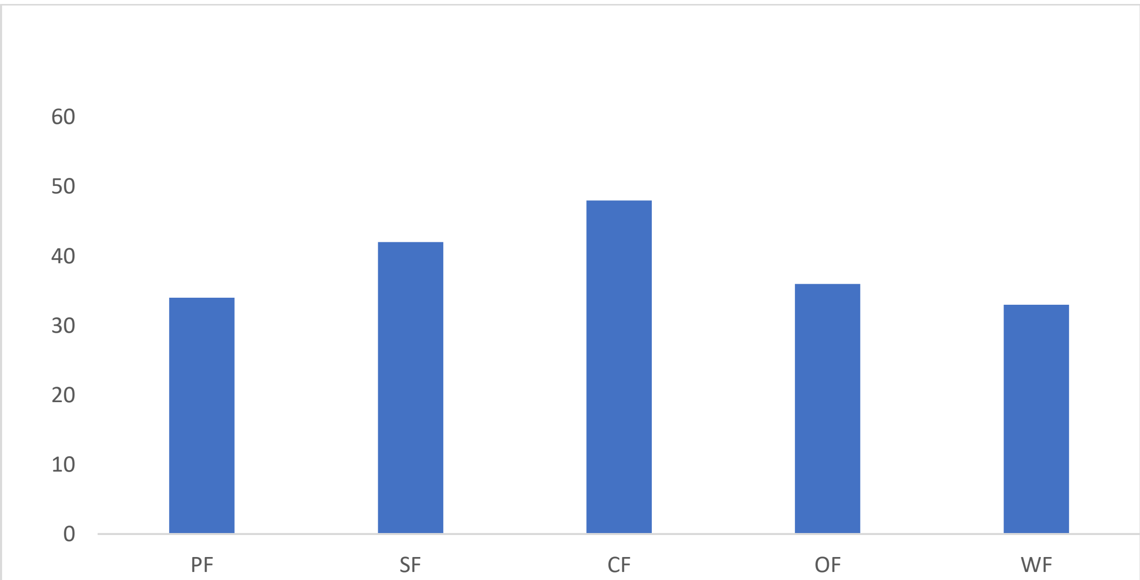

Fig. 2. In vitro protein digestibility of defatted shellfish flour.

Keys: SF= Shrimp flour; CF= Clam flour; OF= Oyster flour; WF= Whelk flour

DISCUSSION

As a result of their unique structure, shellfish have widely varied maximum moisture contents [19]. The values of moisture content of the defatted shellfish flour from this study (5.78-8.63%) were comparable to those (6.50-13.96%) reported by [20] for clam, whelk, oyster, and periwinkle. The low moisture content of oyster flour over other shellfish samples indicates a good shelf life, since moisture content affects its stability and overall quality [21]. Ash content of the flour samples was high in shrimp and higher when compared to the ash content of periwinkle, whelk, oyster, and clam (1.32-2.92%) reported by Kiin-Kabari [5]. It is also higher than the values (0.75-0.87%) obtained by [22] for wild and cultured prawns (*Macrobrachium rosenbergii*) and shrimps (*Penaeus monodon*). The presence of ash in food indicates the availability of minerals.

The values of the fat content in this study are higher than the findings of Kiin-Kabari [5], who reported fat content of oyster, clam, periwinkle, and whelk to be in the range of 0.53-1.75%. It is slightly higher than the values (3.38-5.62%) obtained by [20] for clam, whelk, oyster, and periwinkle. The differences observed in the fat content could be attributed to the variation in the location of the shellfish sampled. The flour protein content of whelk (64.75%) and clam (65.56%) obtained from this study is higher than that reported by [20] for whelk (47.34%) and clam (46.90%). Crude fibre values obtained for oyster (10.48%) and clam (9.95%) are comparable to those reported by [20] for oyster (10.60%) and clam (10.07%). The carbohydrate content of shrimp from this study (2.06%) was higher than that obtained by [22] for cultured and wild shrimps.

The Recommended Dietary Allowance (RDA) for zinc varies by age, sex, and condition. Infants require 2–3 mg/day, children 3–5 mg, and adolescents 8–11 mg. The RDA for zinc for adult males is 11 mg/day, while females require 8 mg/day. During pregnancy and lactation, zinc needs increase to 11–12 mg and 12–13 mg, respectively to support growth and development [23]. PF can contribute 90.63%, SF 42.13%, CF 697.88%, OF 3279.13%, and WF 429.88% of zinc to RDA. Recommended dietary intake (RDI) of iron for children 9-13 years/day is 8 mg [24]. PF can contribute 351.00%, SF 177.13%, CF 636.50%, OF 354.00%, and WF 227.63% of iron to RDA. Iron is important for red blood cell formation; the selected shellfish under study can be recommended for pregnant women and children [25]. Hence, consumption of shellfish could adequately meet the iron needs of children in these age brackets.

Calcium values obtained from this study (616.01-4506.24 mg/100g) were higher than those (40.27-493.31mg/100g) reported by [5] for oyster, clam, periwinkle, and whelk flour. The Recommended Dietary Allowance (RDA) for calcium is 1000 - 1200 mg/day for adults [26]. Shrimp, whelk and periwinkle can contribute a meaningful amount of dietary calcium, which is required for growth, maintenance of bone, teeth, and muscle, by eating significant quantities.

Magnesium content of oyster and clam flours from this study was higher than that (55.76% and 40.27%, respectively) reported by [5]. The study suggests that the selected shellfish, mainly whelk, oyster, and periwinkle, are a rich source of magnesium required for the proper functioning of the muscles.

Moisture content of the shellfish protein concentrates (5.78-8.63%) was lower than the values obtained [27] for protein concentrate extracted from ribbon fish in percent of wet basis (10.78-13.88%). The moisture content of a food product imparts its shelf life; lower moisture content indicates a longer shelf life. Based on generally accepted classifications, foods are grouped according to their moisture content into low-moisture foods, intermediate-moisture foods, and high-moisture foods. Low-moisture foods typically contain less than 15 % moisture and are characterized by extended shelf stability due to reduced water availability for microbial activity. Intermediate-moisture foods contain approximately 15–40 % moisture, while high-moisture foods possess greater than 40 % moisture and are generally more susceptible to spoilage. In the present study, the shellfish protein concentrates, with moisture contents below 15 %, are classified as low-moisture foods, indicating enhanced storage stability and a longer shelf life compared with fresh or minimally processed shellfish, which are high-moisture and more prone to spoilage. [28 and 29]. Ash content ranged from 1.00% to 4.50%, with the lowest value obtained in SPC, while the highest value was obtained in WPC. PPC, SPC, and CPC exhibited similar ash content (1.00-2.50%). Ash content from this study (1.00-4.50%) is higher when compared to that (1.87-3.54%) obtained by [27] for ribbon fish protein concentrate.

Protein concentrates derived from fish and shellfish are classified into three categories based on their lipid content. "Category A" comprises protein concentrates with a fat content of 0.75% or lower, while "Category B" includes those with a fat content ranging from 0.76% to 3%. Finally, "Category C" consists of protein concentrates with a fat content exceeding 3%. This classification system helps evaluate and differentiate these concentrates based on their lipid levels, aiding in quality assessment and nutritional analysis [30 and 31]. Due to the notably higher fat content of the protein concentrates under study, they are classified under "category C" of fish protein concentrates. The relatively high lipid content observed in the protein concentrate is likely influenced by the extraction and processing procedure employed.. In a saturated solution, the concentration of the solute is equal to that of the solvent [29]. It is the polarity of the solvent that allows the fat to be extracted from the fish [31], however, [32] stated that changing the solvent every hour can aid maximum fat extraction during the shellfish protein concentrate purification process. The results of the fat content of PPC, CPC, and SPC are higher than those (7.40%) obtained by [28] for tilapia fish protein concentrate.

Protein is also an important parameter that determines the quality of fish protein concentrate. Fish protein with protein content >65% is a category A fish protein concentrate group [33]. The results showed that PPC, SPC, CPC, and OPC can be classified as category A fish protein concentrate, while whelk can be classified as category B fish protein concentrate (protein <65%). The results obtained were higher than the protein content of tilapia fish protein concentrate (56.93%) obtained by [28]. It is, however, similar to the value (72.38%) obtained by [34] for fish protein powder. Hence, the shellfish under study are highly proteinaceous ingredients with low fat and moisture content. The high fibre content of the shellfish protein concentrates from this study has several health benefits, as it will aid in digestion in the colon and reduce constipation.

The high digestibility of CPC (61.23%) as compared to other shellfish protein concentrates indicates that clams can be a suitable raw material for commercial protein concentrate manufacture. In-vitro protein digestibility is a useful method for estimating the nutritional value of proteins since it allows for a realistic and accurate prediction of protein quality. The present in-vitro protein digestibility results provided a ranking of protein quality for the shellfish protein concentrates. CPC was more digestible than SPC, PPC, WPC and OPC. The enzymes responsible for protein digestion in shellfish may account for the observed variation in digestibility. The amino acid composition and the efficiency with which digestive enzymes free the protein in meals are the two most important factors in determining the protein quality of food [35]. Any given protein's digestibility is based on how well the fish can process the nutrients it has consumed. The results of the protein digestibility of the shell fish protein concentrates are higher than the values (20.65%) reported by (36) for protein digestibility for shrimp meal.. It is also higher than the values (11.90-16.75%) for periwinkle, whelk, oyster and clam reported by [5]. In-vitro protein digestibility of oyster protein was reported within the range of 60.08-74.07% [9] which is higher than the values obtained in the present study. One possible explanation for these variations is that the processing technique employed in the study enhanced protein digestibility. The nutritional value of a protein depends on its amino acid composition, digestibility, and capacity to deliver the essential amino acids in proportions needed by the animal ingesting the protein [37].

CONCLUSION

The study demonstrated that the selected shellfish protein concentrates were rich sources of protein, with CPC containing the highest levels, followed by Oyster protein concentrate, Shrimp protein concentrate, and Oyster protein concentrate, while ash content was highest in OPC and WPC. Protein extraction yields were highest in PPC, and protein digestibility was similar across periwinkle protein concentrate, shrimp protein concentrate, Oyster protein concentrate, and whelk protein concentrate, with clam protein concentrate showing the highest value. These findings indicate that Shrimp protein concentrate, in particular, could serve as a functional protein ingredient in formulated foods. The protein concentrates offer potential for providing quality nutrition and supporting sustainable shellfish-based enterprises. Future research should explore novel extraction techniques to further improve the quality of shellfish protein concentrates.

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