The recovery of protein hydrolysate during enzymatic isolation of chitin from shrimp *Crangon crangon* processing discards

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Abstract

Shell waste from shrimp *Crangon crangon* processing is a good source of chitin and proteins, contained on a dry basis of the offals in amounts 17.8% and 40.6%, respectively. The digestion of the shells with proteolytic enzymes allow to recovery of the chitin and nutritionally valuable protein hydrolysate. These products were prepared from the shells preliminarily demineralized with 10% HCl solution at 20°C for 30 min using commercially available Alcalase at 55°C and pH 8.5. Recovered protein hydrolysate contained, on a dry basis, 64.3% of protein (N×6.25), 6.24% lipids and 23.4% of sodium chloride and had, at pH 4.0, a minimum solubility, and 81.7% of total nitrogen in the product. The PER value of the obtained product was 2.99 as compared with that for hydrolysates from capelin (2.64) and beef *longissimus dorsi* muscle proteins (2.81). The charcoal decolorization of the product decreased the PER and amino acid index (EAA) values from 2.99 and 125.4 up to 2.74 and 123.2, respectively. The total amount of residual small peptides and amino acids directly attached to chitin molecules and resistant to enzymatic hydrolysis depends on degree of hydrolysis (DH) and was about 4.4% at DH value of 30%. Such purity of chitin is sufficient for many purposes. © 1999 Elsevier Science Ltd. All rights reserved.

Keywords: *Crangon crangon*; Protein hydrolysate; Chitin; Shell waste

1. Introduction

The economy of industrial processing of crustaceans can be improved by the full utilization of chitin and proteins included on a dry basis of the shell waste in amounts ranging from 14 to 32% and from 18 to 42%, respectively (Shahidi & Synowiecki, 1991). Chitin and its deacetylated derivative, chitosan, have unique properties, which make them useful for a variety of applications (Muzzarelli, 1989; Sandford, 1989; Synowiecki & Al-Khateeb, 1997). For deproteinization of the shells during isolation of the chitin from shrimp, Antarctic krill, crab and lobster processing discards, a simple base extraction is usually employed. However, this process produces waste liquid containing base, proteins and protein degradation products. The enzymatic digestion and separation of the shell waste proteins should allow the recovery of the protein hydrolysate with a well balanced amino acid composition.

The present study employed an enzymic deproteinization of the shells for production of protein hydrolysate during isolation of chitin from shrimp *Crangon crangon* processing discards.

2. Materials and methods

2.1. Demineralized material

Shell wastes from processing of shrimp *C. crangon* obtained from Crabpol Co., Lebork, Poland were ground using a meat grinder Model KU-2, Predom Mesko, Skarżysko Kam., Poland) through a 5 mm grind plate. Portions (about 100 g) of the ground shells were packed into polyethylene pouches and kept frozen at −20°C for up to 6 months until used. The demineralization of about 100 g of the shells was conducted at 20°C for 30 min using a 10% (w/v) HCl solution, at a ratio of shells to acid of 1:20 (w/v). The optimal conditions of the demineralization process were established elsewhere (Shahidi & Synowiecki, 1991). Demineralized shells

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were washed on a coarse sintered glass funnel (G-4) with distilled water to pH 7.0.

2.2. Enzymatic deproteinization

Protein hydrolysate from the demineralized shells was prepared using Alcalase 2.4L (Novo Industri, Denmark) according to the method presented in the flowsheet given in Fig. 1. A suspension of the demineralized shells in water (1:2, w/v) was prepared and the resultant mixture was subjected to protein hydrolysis at pH 8.5 and 55°C, by concentration of enzyme in reaction media up to 20 AU/kg of protein. Degree of hydrolysis (DH) was calculated from the consumption of 4 M NaOH solution required to maintain a constant pH during the hydrolysis (Olsen, 1983). The reaction was stopped by lowering pH for 30 min to 4.0 using 4 M HCl solution. The hydrolysate was then centrifuged at $3000 \times g$ in order to remove chitin residue and then decolorized with charcoal (4%, w/v), neutralized and lyophilized. Crude chitin was washed on a coarse-sintered glass funnel (G-4) with water, ethanol and acetone and dried for 1 h at 60°C.

3. Analyses

3.1. Proximate composition

Water content was determined by oven-drying of approximately 1 g of the sample at 105°C until a constant weight was obtained (AOAC, 1990). Total nitrogen and ash contents were determined according to the AOAC methods. Total lipids were extracted from the shell wastes according to the method of Bligh and Dyer (1959), using a chloroform–methanol–water (1:2:0.8, v/v/v) system.

3.2. Proteins

Protein contents in the shells and crude chitin were determined (in three replicates) by extracting (2–3 g) samples with 10% (w/v) NaOH solution for 2 h at 90°C, separating the insoluble matter on a coarse sintered-glass funnel and dilution to 100 ml with distilled water. The extract (5–10 ml) was used for protein determination ($N \times 6.25$) according to the Kjeldahl procedure (AOAC, 1990).

3.3. Amino acid composition

Individual amino acids were determined (in two replicates) after digestion of the samples in 6 M HCl at 110°C as described by Blackburn (1968). The HCl was then removed under vacuum and dried samples were reconstituted with a pH 2.2 lithium citrate buffer. The amino acids were then quantified in the Department of Chemistry University of Gdańsk, using a Beckman 121 MB amino acid analyser (Beckman Instruments, Palo Alto, CA). Cysteine and methionine were determined by performic acid oxidation prior to their digestion in 6 M HCl and were measured as cysteic acid and methionine sulphone, respectively (Blackburn). Analysis of tryptophan was performed by hydrolysis of the samples under vacuum in 3 M mercaptoethane sulphonic acid at 110°C, as described by Penke, Ferenczi and Kovacs (1974).

Essential amino acid index (EAA) (FAO/WHO/UNU Report, 1985) and protein efficiency ratio (PER) values were then calculated by consideration of the content of 10 designated amino acids from the equation developed by Lee, Elliot, Richansrud and Hugberg (1978), as described elsewhere (Shahidi & Synowiecki, 1993).

3.4. Solubility

The solubility of protein hydrolysate was determined (in three replicates) using 0.2 g of the sample suspended in 20 ml of distilled water. Adjustment of pH values of the mixtures was achieved using 0.1 M solutions of NaOH or HCl. After standing for 10 min at room temperature, the mixtures were then centrifuged at $12,000 \times g$ for 15 min. The supernatants were diluted to 50 ml with water and 20 ml of aliquots were used for Kjeldahl analysis (AOAC, 1990). The percentage of solubility (X) was expressed as: $X(\%) = $ (Nitrogen content in the supernatant/Nitrogen in the hydrolysate) $\times 100\%$.

3.5. Taste evaluation

Taste evaluation of a 20% aqueous solution of the samples was conducted using a five-member trained panel and a 5-point scale (1, no bitterness; 2, weakly bitter; 3, mildly bitter; 4, strongly bitter; 5, very strongly bitter).

3.6. Degree of acetylation

The degree of acetylation (DA) of chitin was determined according to Roberts (1992) from the infrared spectra recorded on a Brucker IFS66 apparatus using the absorbances ratio at 1655 and 3450 cm^{-1}. The DA values were calculated using the equation: $DA(\%) = (A_{1655}/A_{3450}) \times 115$

3.7. Specific gravity

The specific gravity of chitin preparations was measured (in four replicates) for particle size fraction 16-35 mesh separated after 30s of grinding with a laboratory grinder (Model WZ, Spomasz, żnin, Poland).
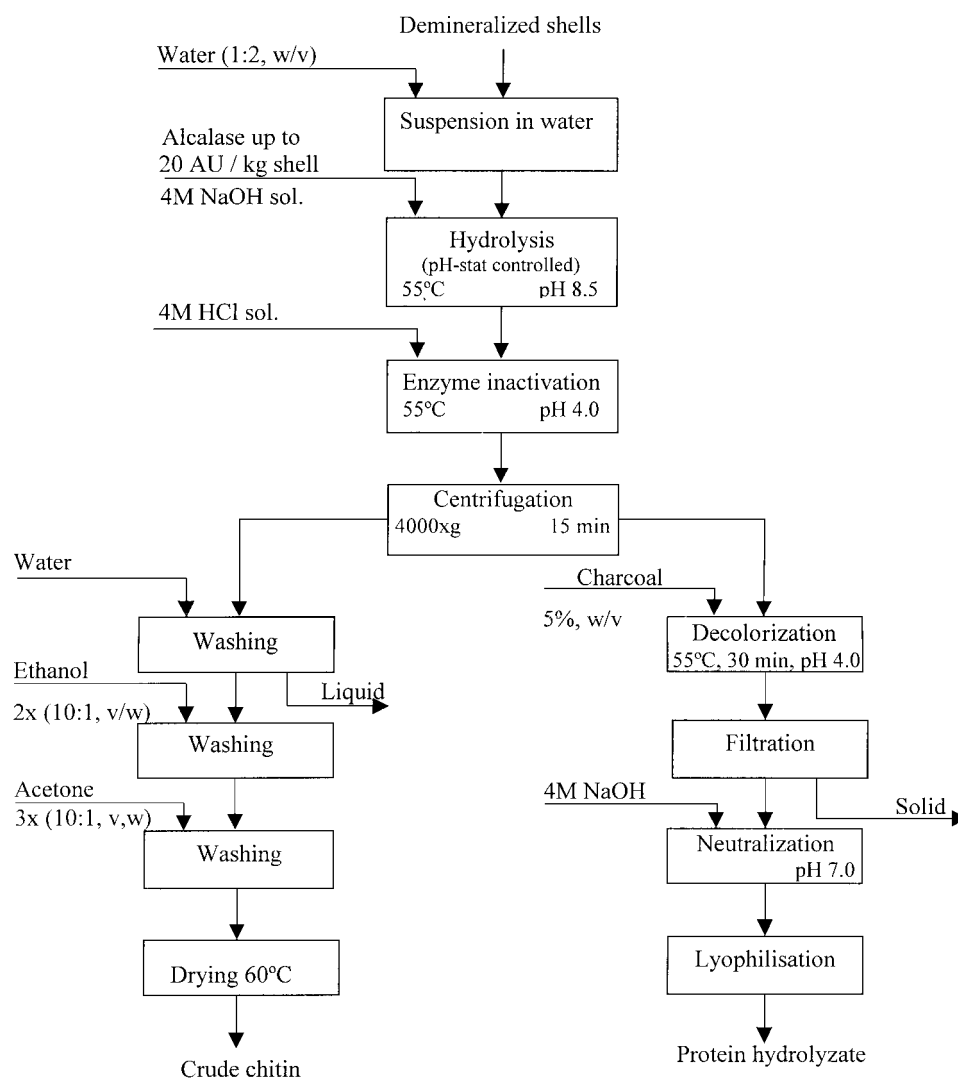
Fig. 1. Flowsheet for production of chitin and protein hydrolysate from shrimp *C. Crangon* shell waste.

4. Statistical analysis

Analysis of variance and Tukey's studentized range test (Snedecor & Cochran, 1980) were used to determine differences in mean values of the data from three to four replicates. Significance was determined at 95% probability.

5. Results and discussion

The main unit operations for production of protein hydrolysate from demineralized *C. crangon* shells (PHCS) were: enzymatic hydrolysis at a constant pH adjusted by addition of base, inactivation of enzyme under acidic conditions and decolorization by charcoal followed by neutralization and lyophilization (Fig. 1). The application of Alcalase allows an easy control of the degree of hydrolysis, calculated from the consumption

of base required for maintenance of a constant pH during the process. The choice of this enzyme was also made based on its specifity for terminal hydrophobic amino acids, which generally leads to the production of non-bitter hydrolysate (Adler-Nissen, 1986), and the hydrolysis conditions established previously (Shahidi, Synowiecki & Balejko, 1994).

The protein content, on a dry basis, of *C. crangon* processing discards was 40.6%, similar to that for shell waste from shrimp, *Pandalus borealis* (Table 1). The yield of PHCS depended on the degree of hydrolysis (DH) of the shell proteins. The best nitrogen recovery was 69.2% of that present in the protein fraction of *C. crangon* processing discards at DH about 30%. This yield was achieved after 4 h reaction at 55°C using an enzyme concentration 20 AU/kg of protein. The bitterness of this product (DH 30%) was 4.5 ± 0.3 of sensory scores and drop up to 3.7 ± 0.3 for hydrolysates with DH value of 19%. However, at DH 19% the nitrogen

Table 1
Composition of the shells from shrimps *C. crangon* and *P. Borealis*[a]

Component	*Crangon crangon*	*Pandulas borealis*[b]
Water (%)	71.12 ± 1.21	75.61 ± 0.20
Proteins (%) (db)[c]	40.6 ± 5.43	41.9 ± 0.20
Lipids (%) (db)	9.95 ± 0.22	10.23 ± 0.41
Chitin (%) (db)	17.8 ± 0.91	17.0 ± 0.25
Minerals (%) (db)	27.5 ± 0.13	29.2 ± 0.20

[a] Results are mean values of three replicates ± standard deviation.
[b] From Shahidi and Synowiecki (1991).
[c] db, dry basis.

recovery decreased up to 64.6%. The observed relationship between the PHCS yield and DH values is in a good agreement with the experimental data reported by Tybor, Dill and Lundmann (1975) for protein hydrolysates from bovine blood. The final yield of the hydrolysate was reduced by partial extraction of the proteins during preliminary demineralization of the shells. The PHCS hydrolysed up to DH 30% contained on a dry basis: 64.3% of protein (N×6.25), 6.24% lipids and 23.9% of minerals, mainly sodium chloride synthesized during pH adjustment and neutralization of the hydrolysate. The lipid content of PHCS preparations was decreased about 1.5-times, when compared to that in the starting material (Table 1). Retention of lipids reduces the storage stability of the hydrolysate and may thwart its use in some food applications.

The data given in Table 2 show that the product obtained according to the procedure outlined in Fig. 1 is particularly rich in glutamic and aspartic acids. The essential amino acid index (EAA) value for unpurified PHCS was 125.4, similar to that for carotenoproteins from shrimp, *P. borealis* and beef muscle proteins and higher than those for hydrolysate from capelin, *Mallotus villosus* (Table 3). These results indicate that the hydrolysate obtained by Alcalase digestion of *C. crangon* processing discards can be used as a good source of essential amino acids in food products. The full nutritional quality of the hydrolysate was also confirmed by high protein efficiency ratio (PER) values, calculated according to equations developed by Lee et al. (1978). The PER value for PHCS was 2.99, as compared with that for protein hydrolysate from capelin (2.64), carotenoproteins from shrimp, *P. borealis* (2.82) and beef *longissimus dorsi* muscle proteins (2.81). The charcoal purification step slightly decreased the PER and EAA values of PHCS (Table 3). This was probably caused by selective adsorption of some hydrophobic amino acids and peptides (Table 2) as reported also by Pedersen (1994). The treatment of PHCS with charcoal for a period of 30 min at 50°C and pH 4.0 allowed removal of coloured impurities and most of the compounds responsible for off-flavour in the product. The solubility of the investigated hydrolysate, expressed as the percentage of the soluble nitrogen compounds and mea-

Table 2
Effect of charcoal treatment on amino acid composition (% N×6.25) of protein hydrolysate from *C. crangon* shell waste[a]

Amino acid	Hydrolysate not treated with charcoal	Hydrolysate purified by charcoal
Ala	5.21 ± 0.02	5.15 ± 0.05
Arg	4.94 ± 0.10	2.91 ± 0.07
Asp + Asn	11.0 ± 0.13	10.6 ± 0.05
Cys	0.90 ± 0.04	0.87 ± 0.02
Glu + Gln	12.4 ± 0.10	14.3 ± 0.17
Gly	4.32 ± 0.22	4.69 ± 0.07
His	5.01 ± 0.08	4.10 ± 0.12
Ile + Leu	13.2 ± 0.04	11.5 ± 0.09
Lys	6.60 ± 0.02	6.99 ± 0.05
Met	2.99 ± 0.10	3.78 ± 0.08
Phe	4.93 ± 0.065	4.44 ± 0.08
Pro	4.68 ± 0.08	5.47 ± 0.06
Ser	5.09 ± 0.02	5.19 ± 0.03
Thr	5.19 ± 0.03	5.51 ± 0.07
Trp	1.20 ± 0.03	1.21 ± 0.05
Tyr	5.18 ± 0.03	4.91 ± 0.04
Val	5.89 ± 0.10	5.37 ± 0.12

[a] Results are mean values of three replicates ± standard deviation.

Table 3
Charcteristics of nutritional value of *C. crangon* shell protein hydrolysates as compared with the products from other sources

Source	PER	EAA	Essential amino acids as % of (N×6.25)
Proteins extracted from *C. crangon* shell waste at pH 8.5	1.82	119	46.8
Not purified hydrolysate from *C. crangon* shell proteins	2.99	125	46.6
Protein hydroysate from *C. crangon* shells purified with charcoal	2.74	123	46.0
Protein hydroysate from capelin (*mallotus villosus*)[a]	2.64	99.0	43.4
Carotenoproteins from shrimp *P. borealis*[b]	2.82	117	45.5
Beef muscle proteins from *longissimus dorsi*[c]	2.81	102	—

[a] According to Shahidi et al. (1995).
[b] According to Simpson and Haard (1985).
[c] According to Synowiecki, Jagiełła and Shahidi, (1996).

sured in the pH range 2.0–10.0, attained a minimum value of 81.7 ± 1.25% at pH 4.0 (Fig. 2). The appearance of minimum solubility within the region of isoelectric points was also reported by Shahidi, Syowiecki et al. (1994) and Shahidi, Xiao-Qing Han and Synowiecki (1995) for hydrolysates from muscle proteins.

Chemical compositions of chitins deproteinized by enzymatic digestion of *C. crangon* processing discards or by base extraction of the proteins are given on Table 4. The purity of these preparations depends on the efficiency of demineralization and deproteinization of the shells. The effectiveness of deproteinization was limited

by residual small peptides and amino acids directly attached to chitin molecules and resistant to enzymatic hydrolysis. Their amounts in crude chitin depend on the process conditions and ranged from 4.4 ± 0.5 to $7.9 \pm 0.8\%$, when the DH values of obtained hydrolysates were 30 and 19%, respectively. This caused the chitin deproteinized by Alcalase digestion to contain about a twice higher protein residue than commercial product traditionally treated with NaOH solution (Table 4). The low ash contents of chitin, from 0.31 to 1.56%, indicate the suitability of the method of preliminary removal of calcium carbonate and other minerals from the shells.
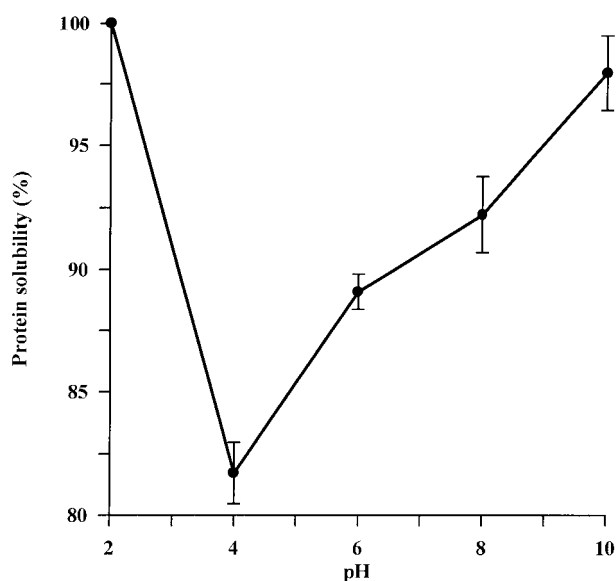

Fig. 2. Solubility of protein hydrolysate from shrimp *C. crangon* shell waste at different pH values.

Table 4
Characteristics of chitins from shrimps (*C. crangon* and *P. borealis*) shells deproteinized by Alcalase or 10% NaOH solution at 90°C for 2 h

Specification	Alcalase		10% NaOH solution
	Crangon crangon		*Pandalus borealis*[a]
Moisture (%)	4.92 ± 0.19a[b]	6.17 ± 0.31b	3.00 ± 0.32
Nitrogen (%) (db)[c]	6.89 ± 0.06a	6.58 ± 0.05b	6.29 ± 0.05
Ash (%) (db)	1.56 ± 0.48a	0.31 ± 0.07b	0.09 ± 0.02
Protein residue (%) (db)	4.45 ± 0.53a	2.44 ± 0.81b	2.32 ± 0.02
Degree of acetylation (%)	88.9 ± 0.25a	83.8 ± 0.11b	Not determined
Specific gravity (g/cm^3)	0.05 ± 0.01a	0.07 ± 0.01a	0.06 ± 0.01

[a] From Shahidi and Synowiecki (1991).
[b] Results are mean values of three determinations ± standard deviation. Values in each row with the same letter are not significantly different ($p > 0.05$) from one another.
[c] cd, dry basis.

Chitin, from *C. crangon* shells deproteinized by Alcalase or by base solution, contained 6.89 and 6.58% of nitrogen, respectively. However, the nitrogen content in the preparations isolated using Alcalase digestion, expressed on an ash-free basis of polysaccharide (7.00%) was slightly higher than the theoretical value of 6.9% for pure chitin. This is probably due to the presence of some protein impurities. Determined amount of nitrogen is in good agreement with the data reported by No, Meyers and Lee (1989) for chitin isolated from crawfish shell waste using mild alkali treatment (3.5% NaOH solution at 65°C). The degree of acetylation (DA) calculated according to Roberts (1992) from the infrared spectra of chitin isolated by enzymatic digestion (88.9%) was higher than that for the samples (83.8%) deproteinized with 10% NaOH solution at 90°C. Observed differences in the DA values were caused by deacetylation of polysaccharide occurring in the base solution during the deproteinization process.

In conclusion, the enzymatic deproteinization of the shrimp shells is suitable for isolation of the chitin containing only about 4% of protein impurities and also for production of protein hydrolysate with good values of essential amino acid index and protein efficiency ratio.

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