Isolation and Characterization of Nutrients and Value-Added Products from Snow Crab (*Chinoecetes opilio*) and Shrimp (*Pandalus borealis*) Processing Discards

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Byproducts from shrimp and different parts of crab contained, on a dry basis, from 17.0 to 32.2% of chitin and from 3.4 to 14.7 mg/100 g of carotenoid pigments, mostly astaxanthin and its esters. Chitin was produced at a yield of about 86%, and carotenoids were extracted in cod liver oil at a yield of up to 74%. Optimal deproteinization involved treatment with 1% KOH solution for shrimp and 2% KOH for crab shells, at 90 °C for 2 h, with a shells to alkali solution ratio of 1:20 (w/v). Optimal conditions of demineralization were 2.5% HCl at 20 °C for 1 h with a solid to solvent ratio of 1:10 (w/v). The chitin preparations isolated from shell waste without or with the initial extraction of carotenoids were not different. They contained 6.29% nitrogen in shrimp and 6.42% nitrogen in crab chitins. The residual protein contents in chitin from shrimp and crab shells were 2.3 and 0.4%, respectively. The ash content in both cases did not exceed 0.11%.

INTRODUCTION

The growing quantities of shell wastes from processing of crustaceans have become a major concern for seafood-processing plants, since their biodegradation is very slow. The shell proteins putrefy, and their enzyme components are therefore rendered inactive. These processing byproducts, however, may serve as an important source for production of chitin, its deacetylated derivative, chitosan, and carotenoid pigments.

Global annual production of shell wastes from crab, lobster, shrimp, krill, and clam/oyster, calculated on a dry basis, is estimated at 1.44 million metric tons (Knorr, 1991). Furthermore, it is estimated that crab and shrimp shell wastes in the United States alone could provide annually about 6000 and 39 000 metric tons of chitin, respectively (Knorr, 1991).

Two main approaches for utilization of chitin/chitosan presently exist. One is the bioconversion of chitinous polymers to single-cell proteins by the use of *Pichia kudriavzevii* yeast, which grows on the hydrolysates obtained from chitin by chitinase digestion (Carroad and Tom, 1978; Cosio et al., 1982). The other method is the utilization of chitin/chitosan for the removal and recovery of other waste materials such as heavy metals (Muzzarelli et al., 1989; Ramachandran et al., 1982), dyes (McKay et al., 1982; Venkatrao et al., 1986), pesticides (Thomé and Van Daele, 1986), amino acids (No and Meyers, 1989), and proteins (Knorr, 1991). Chitosan could also be used for removal of microorganisms (Popper and Knorr, 1990), clarification of juices (Imeri and Knorr, 1988), and production of biodegradable packaging films (Mayer et al., 1989). Chitosans may be employed in cosmetics, pharmaceuticals, and agriculture. Their use as a component of toothpaste, hand and body creams, and shampoo in cosmetics and toiletries as well as pharmaceuticals for lowering of serum cholesterol has been documented. Furthermore, their application in enzyme and cell immobilization, drug carrier, material for production of contact lens, or eye bandages as well as seed coats, etc., has been reviewed (Sandford, 1988; Muzzarelli, 1988).

Carotenoid pigments isolated from shell wastes are suitable for incorporation into fish feed for aquaculture industries of salmonid species. Although carotenoids are biologically important compounds, fish like other animals are unable to perform their de Novo synthesis. The presence of carotenoid pigments in fish feed influences red coloration of salmonid fish flesh and favors consumer acceptability of the product (Saito and Regier, 1971; Spinelli et al., 1974; Kuo et al., 1976; Chen et al., 1984; Long and Haard, 1988). The isolation of carotenoids from shell wastes in suitable forms for fish feeding could be achieved by oil extraction (Chen and Meyers, 1982, 1983; Chen et al., 1984; Meyers et al., 1985).

This study is part of a program to investigate the nutrient and chemical composition and utilization of crustacean offals, namely snow crab (*Chinoecetes opilio*) and pink shrimp (*Pandalus borealis*), in Atlantic Canada and specifically in Newfoundland and Labrador. Thus, proximate composition of shells, amino acid composition of proteins, fatty acid composition of lipids, and chitin and pigment content of Atlantic snow crab and shrimp discards were investigated.

MATERIALS AND METHODS

Raw Materials. Different parts of shell wastes separated during the commercial processing of snow (queen) crab (*C. opilio*) at Quinlan Brothers Plant, Bay de Verde, Newfoundland, and wastes from processing of shrimp (*P. borealis*) obtained from Fishery Products International were used as starting materials. The shell wastes were ground in a Waring blender (Model 33 BL73, Dynamics Corp., New Hartford) vacuum packed in plastic bags, and kept frozen at −20 °C until used.

Isolation of Chitin and Carotenoids. Isolation of chitin and carotenoid extracts involved basic operations such as carotenoid extraction, deproteinization, demineralization, and decolorization of chitin by acetone. The overall process is summarized in Figure 1.

The influences of temperature and ratio of shell waste to cod liver oil on the recovery of carotenoids (in triplicate determinations) were investigated. The deproteinization and demineralization variables examined were shell to reagent ratio of 1:2 to 1:20, reaction period of 0.5–3 h, temperature of deproteinization of 20–90 °C, concentration of KOH solution of 0.1–28.0% (w/v),

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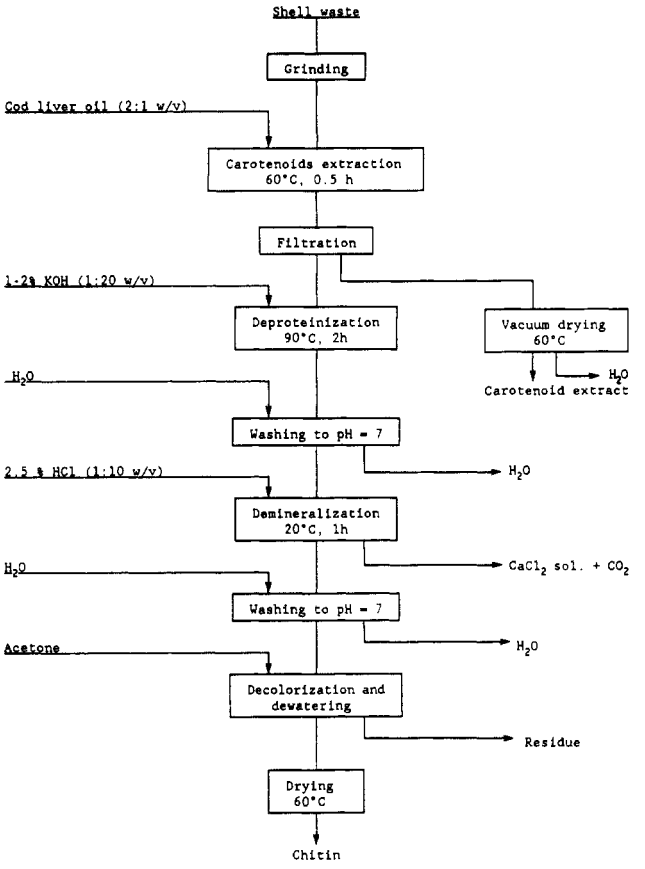

Figure 1. Flow sheet for the preparation of chitin and carotenoid extracts from crustacean shell wastes.

and concentration of HCl of 1.0–22.0% (w/v). These determinations were made in four replicates. Optimal conditions were designated as those giving a minimum protein and ash residue in chitin by using optimum amounts of reagents and reaction time/temperature.

Methods. Moisture content in shell wastes and chitin was determined by oven-drying of approximately 1 g of the sample at 105 °C overnight or until a constant weight was obtained (AOAC, 1980). Total nitrogen and ash contents were determined according to AOAC (1980) methods.

Protein content in shell wastes was determined (in four replicates) after a 2-h extraction of a known amount of sample with a 2.5% (w/v) KOH solution at 90 °C. The protein extract was separated from insoluble matters in KOH by using a coarse sintered glass funnel and was then diluted to 250 mL with deionized water. The protein content of the solution was determined according to the Kjeldahl (AOAC, 1980) method.

Residual protein in chitin preparations was determined according to the method of Lowry et al. (1951) using bovine serum albumin (Sigma Chemical Co., St. Louis, MO) as a protein standard. The protein residue in chitin was extracted from about 0.1 g of a chitin sample with 5 mL of 2 M KOH solution for 1 h at 90 °C. The content of amino acids in proteins extracted from shell waste by a 2% (w/v) KOH solution (20 °C, 2 h) and then precipitated with a 10% acetic acid solution, pH 4.5, was assayed. The individual amino acids in freeze-dried samples were determined (in triplicate) after their hydrolysis with 6 M HCl for 24 h at 110 °C (Blackburn, 1968). Amino acids were then separated and identified using a Beckman 121 MB amino acid analyzer. Tryptophan was determined separately according to the method of Penke et al. (1974).

The content of chitin in the offal was determined (in triplicate) by demineralization of 0.5–1.0 g of shells, deproteinized with a 5% (w/v) KOH solution for 2 h at 100 °C, and then treated with 20 mL of 5% (w/v) HCl for 2 h at room temperature. Chitin was collected on a coarse sintered glass funnel and washed with deionized water to pH 7.0 and then with 3 × 25 mL of acetone followed by oven-drying at 105 °C.

Table I. Chitin, Total Carotenoid Pigments, and Protein Content of Shrimp and Different Parts of Snow Crab Shells[a]

shells from	crude chitin, % dry basis	proteins, % dry basis	total carotenoids, mg % dry basis
shrimp	17.01 ± 0.25	41.90 ± 0.20	14.77 ± 0.25
crab backs[b]	22.32 ± 0.45	17.79 ± 0.11	13.99 ± 0.20
backs	18.70 ± 0.13	18.61 ± 0.14	11.96 ± 0.25
claws	23.70 ± 0.33	17.21 ± 0.18	1.64 ± 0.01
legs	32.25 ± 0.11	15.68 ± 0.11	3.43 ± 0.02
shoulders	26.90 ± 0.14	23.95 ± 0.16	2.68 ± 0.01
tips	27.91 ± 0.22	18.01 ± 0.12	1.98 ± 0.11

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

Total lipids were extracted from shell wastes according to the method of Bligh and Dyer (1959) using a chloroform–methanol–water system. Fatty acid composition of lipids, extracted according to the method of Bligh and Dyer (1959), was determined as methyl esters, by gas chromatography using a Perkin-Elmer Model 8500 gas chromatograph. Results are for duplicate injections of two samples. A 30 m × 0.25 mm SP 2330 fused silica capillary column (Supelco Inc., Bellefonte, PA) was used, and methyl heptadecanoate served as an internal standard. The initial oven temperature was 180 °C and then ramped to 200 °C after 12 min at 20 °C/min and kept there for 8 min. The injection port and the flame ionization detector oven temperatures were set at 230 °C.

Total carotenoids in the lipid fraction of the offal were determined according to the method of Saito and Regier (1971). The concentration of carotenoid pigments was calculated from the equation

$$C_{ppm} = A_{468nm} V_{extract} (\text{dilution factor}) / 0.2 W_{sample}$$

where the absorbance of 1 μg/mL of a standard astaxanthin solution at 468 nm was 0.2. The concentration of carotenoids in the oil extract was calculated according to the equation given by Chen and Meyers (1982).

Carotenoid fractions were then separated (in four replicates) by using thin-layer chromatography. Silica gel TLC plates (Analtech, Inc., Newark, DE) were spotted with the carotenoid extracts in chloroform and were then developed by using a benzene–petroleum ether–acetone (10:3:2 v/v/v) mixture. Each component was then extracted three times in 3 mL of chloroform. The absorbance of carotenoids in the centrifuged solution, diluted to 10 mL, was then read on a Beckman DU-8 spectrophotometer. Concentrations of carotenoids were then calculated on the basis of linear equations of standard curves prepared for individual carotenoid standard (Hoffmann La-Roche, Etobicoke, ON) solutions in chloroform.

Specific gravity of chitin preparations was measured for particle size fraction 16–35 mesh separated after 30 s of grinding with a French-made Moulinex coffee grinder.

The content of free amino groups in the chitin fraction was determined by a titration method as described by Hayes and Davies (1978).

The tristimulus color parameters, L (lightness/darkness, 100 for white and 0 for black), a (red, +; green, −), and b (yellow, +; blue, −), of ground chitin samples were measured by using a standard Colormet colorimeter (Instrumar Engineering Limited, St. John's, NF). The unit was standardized with a B-143 white calibration tile with a Hunter L value of 94.5 ± 0.2, a value of −1.0 ± 0.1, and b value of 0.0 ± 0.2.

RESULTS AND DISCUSSION

Composition of Shell Offals. The protein, chitin, and carotenoid content in snow crab (*C. opilio*) and shrimp (*P. borealis*) processing discards is given in Table I. Shells from different parts of crab contained more chitin (18.7–32.2%, on a dry basis) than shrimp offals (17.0%, on a dry basis). The observed differences were mainly due to the fact that crab offals contained less protein than shrimp shell wastes. Muzzarelli (1977) and Johnson and Peni-

Table II. Composition of Carotenoids in Crustacean Offals[a]

carotenoid, % of total	shrimp	hard-shelled crab	soft-shelled crab
astaxanthin	3.95 ± 0.19	21.16 ± 1.15	31.04 ± 1.30
astaxanthin monoester	19.72 ± 0.19	5.11 ± 0.23	1.94 ± 0.06
astaxanthin diester	74.29 ± 0.38	56.57 ± 1.60	56.47 ± 1.58
astacene		3.26 ± 0.47	2.24 ± 0.47
canthaxanthin			2.66 ± 0.73
lutein		8.24 ± 0.30	1.11 ± 0.82
zeaxanthin	0.62 ± 0.05	4.64 ± 0.76	4.00 ± 0.69
unidentified		0.22 ± 0.05	0.16 ± 0.07

[a] Results are mean values of four replicates ± standard deviation. Values for crab are for backs only.

ston (1982) have demonstrated that chitin represents from 14 to 27% and from 13 to 15%, on a dry basis, of shrimp and whole crab processing wastes, respectively. A higher content of chitin in shell wastes was observed in our studies, and this perhaps resulted from different shrimp processing conditions and/or the presence of a smaller amount of residual meat in the wastes. Investigations of the chitin content in five different segments of snow crab shell wastes, namely backs, claws, legs, shoulders, and tips, were performed. The best sections of crab for chitin isolation were legs, shoulders, and tips. The chitin content in these parts of crab was comparable with the amount of chitin in Louisiana crawfish (*Procambarus clarkii*) shell waste which, on a dry basis, contained 23.5% chitin (No et al., 1989).

The content of carotenoid pigments in shrimp offals was 14.7 mg/100 g on a dry basis (Table I). This was somewhat more than the content of carotenoids in pink shrimp (11.1 mg/100 g, on a dry basis) caught in the Maine coastal area of the United States (Saito and Regier, 1971). Such differences in the amount of pigment depend on the carotenoid content available in the feed as well as the living condition and region. For crab, the greatest amount of pigments was present in the back shell segments as compared with other shell parts. A total of 11.9–13.9 mg/100 g of pigments was determined in hard and soft crab back shells, respectively. These data are comparable with the content of carotenoids in Louisiana crawfish shell waste (15.3 mg/100 g, on a dry basis) (No et al., 1989). Other segments of crab shells contained 1.6–3.4 mg/100 g carotenoids. Qualitative and quantitative data of isolated carotenoids from shrimp and crab shell wastes are given in Table II. In all cases, astaxanthin diester was the major carotenoid present. Astaxanthin and its monoester, however, were present in smaller amounts. In addition, shrimp and crab shell wastes contained a small amount of zeaxanthin. Crab offals also contained minor traces of astacene, canthaxanthin (soft-shelled crab only), lutein, and an unidentified compound.

The water-extractable proteins, with potent flavor characteristics, accounted for 1.4 ± 0.2 and 1.6 ± 0.1% of the total proteins from crab and shrimp offals, respectively. Table III summarizes the essential amino acid composition of proteins extracted from shrimp and crab shells by a 2% (w/v) KOH solution at 20 °C. Except for a lower content of lysine and tryptophan, the content of essential amino acids of protein from snow crab offals was similar to that in pulp from whole red crab, *Pleuroncodes planipes* (Spinelli et al., 1974). These results indicate that shrimp shell proteins are well-balanced in their essential amino acid composition and, as such, they may serve as an excellent component of starter feed for animals and for aquaculture industries. The crab shell proteins, however, were of a lesser quality and contained smaller amounts of lysine, leucine, and isoleucine. The proteins removed by

Table III. Amino Acid Composition, as Percent of Proteins Removed from Shrimp and Crab Offals by Alkaline (2% Aqueous KOH) Extraction[a]

amino acid	shrimp proteins	snow crab proteins	proteins from whole red crab pulp[b]
arginine	6.13 ± 0.07	6.66 ± 0.02	7.63
histidine	2.24 ± 0.09	3.58 ± 0.01	2.62
isoleucine	5.78 ± 0.13	2.67 ± 0.02	3.62
leucine	7.01 ± 0.02	5.14 ± 0.02	5.95
lysine	6.58 ± 0.07	2.51 ± 0.07	6.65
methionine	2.41 ± 0.08	1.93 ± 0.00	2.06
phenylalanine	5.13 ± 0.07	5.98 ± 0.01	5.05
threonine	4.14 ± 0.20	4.74 ± 0.02	3.95
tryptophan	1.19 ± 0.07	0.78 ± 0.01	1.58
valine	5.95 ± 0.06	7.07 ± 0.10	7.90

[a] Results are mean values of three replicates ± standard deviation. Proteins were extracted by 2% (w/v) KOH solution (20 °C) and then precipitated with a 10% acetic acid solution, pH 4.5. [b] Spinelli et al. (1974).

Table IV. Major Fatty Acids of Shellfish Discard Lipids, Reported as Area Percent of Total Lipids[a]

fatty acid	shrimp	snow crab	pulp from whole red crab[b]
16:0	11.88 ± 0.2	9.36 ± 0.0	18.7 ± 23.9
18:1 ω9	21.82 ± 0.1	25.76 ± 0.2	14.1 ± 17.3
20:1 ω9	10.00 ± 0.1	13.33 ● 0.1	0.5 ± 1.4
22:1 ω9	7.63 ± 0.0	7.38 ± 0.1	3.1 ± 4.2
20:5 ω3	13.16 ± 0.1	12.72 ± 0.0	11.4 ± 15.2
22:6 ω3	10.57 ± 0.1	5.59 ± 0.1	17.9 ± 32.1

[a] Results are mean values of duplicate injection of two samples ± standard deviation. [b] Spinelli et al. (1974).

mild alkaline treatment had higher aspartic and glutamic acids, leucine, lysine, and tyrosine contents than water-extracted proteins. Aspartic and glutamic acids were the major amino acids present. On the other hand, the water-extracted proteins had an intense flavor corresponding to the species utilized and as such may serve as flavorants in Kamaboko products.

The lipid content of shrimp shells ranged from 1.1 to 2.3% and exceeded that of crab shell segments (0.1–1.4%). The major fatty acids given in Table IV indicate that monounsaturated fatty acids are the major components of shell waste lipids of shrimp and snow crab. However, the amount of monounsaturated fatty acids in red crab lipids reported by Spinelli et al. (1974) was smaller than those in this study (Table IV). Shrimp and snow crab shell waste lipids contained 23.73 and 18.31% ω − 3 type fatty acids, respectively. Corresponding data for red crab, which depended on the season and place of catch, varied from 29.3 to 47.3% of the total fatty acids (Spinelli et al., 1974). The significance of ω − 3 fatty acids in the diets of salmonids has been demonstrated by Yu and Sinnhuber (1972) and Castell et al. (1972). These authors showed that certain physiological changes in rainbow trout such as increased liver respiration rate, lower hemoglobin content, and increased muscle water were related to dietary deficiencies of this group of fatty acids. The contents of eicosapentaenoic acid (EPA) for shrimp and crab shells were similar, while shrimp lipids had a larger content of docosahexaenoic acid (DHA).

Extraction of Carotenoids with Cod Liver Oil. Sequential extraction of carotenoid pigments and chitin from shell wastes was attempted in this study. To avoid their decomposition, carotenoid pigments were extracted from the offals before isolation of chitin. Different methods of isolating carotenoids from shell wastes and their possible incorporation into fish feed have been reported. Chen and Meyers (1982) extracted astaxanthin pigments in soybean oil at a 1:1 (v/w) ratio of oil to crawfish

Table V. Recovery, as Percent of Total Carotenoids from Shrimp Shells by Cod Liver Oil at Different Ratios of Shells to Oil (*R*)[a]

	R (w/v)		
temp, °C	2:1	1:1	1:2
50	25.62 ± 0.18	39.39 ± 3.51	41.72 ± 1.23
60	30.25 ± 1.90	52.89 ± 0.99	74.23 ± 5.04
80	28.33 ± 1.05	45.06 ± 2.35	60.19 ± 0.58

[a] Results are mean values three replicates ± standard deviation. Total carotenoids in shell offals was 3.32 mg/100 g.

waste. Additional hydrolysis of shell waste by proteases increased the proportion of extractable carotenoids by up to 58% (Chen and Meyers, 1982; Simpson and Haard, 1985; Manu-Tawiah and Haard, 1987). Acid treatment, at pH 4.5–5.5, of the shell waste before extraction resulted in decalcification as well as an increase in the release of carotenoids (Chen and Meyers, 1983; Omara et al., 1985; Meyers et al., 1985).

To evaluate the suitability of cod liver oil for extracting carotenoids, a direct extraction from shell wastes without previous acid or enzyme treatment was attempted (Figure 1). Results of the extraction of carotenoids from shrimp shell wastes with cod liver oil are given in Table V. Different temperatures and proportions of oil to shell wastes were used. The best recovery of carotenoids (74.23%) was obtained when the ratio of offals to oil was 1:2 (w/v) and the extraction temperature was adjusted to 60 °C. At higher temperatures, the recovery yield decreased perhaps due to thermal decomposition of some pigments. According to Chen and Meyers (1982), 40.5–52.2% of the total pigments in crawfish shell wastes were removed by soybean oil in a one-stage extraction using an oil to waste ratio of 1:1 (v/w). The present study shows that cod liver oil is a good extractant of carotenoids. Use of fish oils for extraction of carotenoid pigments has the added advantage of offering feeds rich in $\omega-3$ fatty acids. The effect of antioxidants on the stability of carotenoids in the extracts was not studied; however, it will be the subject of further investigations.

Chitin Isolation. To establish optimal conditions of the process, information on the best deproteinization and demineralization conditions was required. It has already been demonstrated (Table I) that shrimp and crab shells contained, on a dry basis, about 42 and 18–24% proteins, respectively.

Deproteinization was achieved by using KOH treatments of the shells under different experimental conditions. Effects of alkali concentration and temperature of deproteinization are given in Figure 2. A KOH solution at a shell to base ratio of 1:20 (w/v) removed almost all of the proteins from shrimp and crab shell wastes at 90 °C when a 1.0 or 2.0% (w/v) KOH solution, respectively, was used. Results in Table VI indicate that a minimum shell to KOH solution ratio of 1:4 (w/v) was required to extract over 90% of the proteins. However, when this ratio was increased to 1:10 (w/v), the protein residue in chitin from shrimp and crab offals was less than 2.3 and 0.5%, respectively. An increase in shell weight to KOH volume ratio above 1:4 (w/v) had only a minor effect on the deproteinization efficiency of crustacean offals.

In another set of experiments, the extraction period of deproteinization of crab and shrimp shell wastes was varied (Figure 3). A minimum period of 60 min was deemed necessary to extract over 90% of the proteins from the offal. However, a 2-h extraction period was required for removal of all proteins present in the offals. The extraction efficiency of proteins depended on not only the concentration of alkali and the temperature of the deproteinization process but also the species. It was found that deproteinization of shrimp offals was achieved with a lesser base concentration than that required for crab shell wastes [1 vs 2% (w/v) KOH; Figure 2]. Deproteinization conditions of crab and shrimp offals in this study were similar to those of No et al. (1989) for deproteinization of crawfish waste. These authors used a 3.5% (w/v) NaOH solution at 65 °C for 2 h with a solid to solvent ratio of 1:10 (w/v).

The efficiency of demineralization is generally followed by measuring the residual ash content of the isolated chitin.

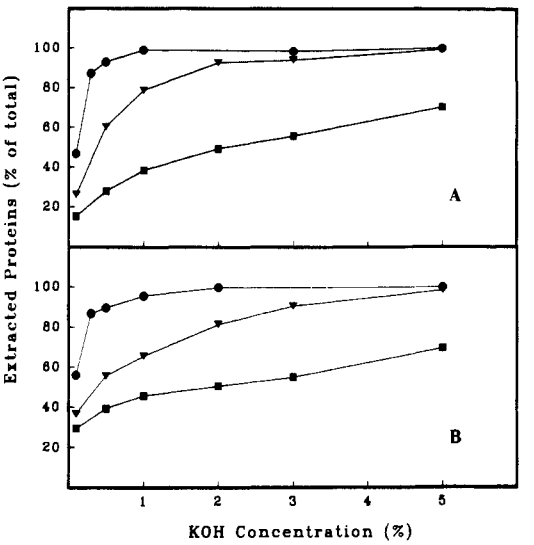

Figure 2. Effect of KOH concentration and temperature on the amount of protein extracted in 2 h from (A) shrimp and (B) crab offals. Shell to KOH solution ratio of 1:20 (w/v) at 20 (■), 60 (▼), and 90 °C (●). Results are mean values of four replicates, and standard deviations did not exceed 1.6% of the recorded values.

Table VI. Proteins (N × 6.25) Extracted at Different Solid to KOH Solution Ratios (*R*) from Crab Shoulder Shells [2% (w/v) KOH] or Shrimp Shells [1% (w/v) KOH][a]

	extracted proteins as % of	
R (w/v)	shrimps	crab shoulders
1:2	5.61 ± 0.06	9.70 ± 0.04
1:4	6.73 ± 0.04	10.94 ± 0.03
1:5	7.05 ± 0.04	11.32 ± 0.04
1:10	7.14 ± 0.04	11.99 ± 0.25
1:20	7.05 ± 0.06	12.09 ± 0.07

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

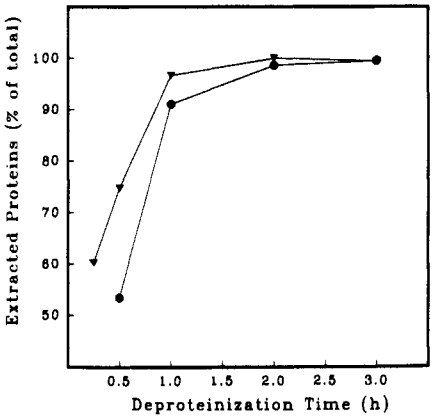

Figure 3. Effect of extraction time on the percentage of total proteins at 90 °C from crab (▼) (2% KOH) and shrimp (●) (1% KOH) at a solid to extraction solution ratio of 1:20 (w/v). Results are mean values of four replicates, and standard deviations did not exceed 1.4% of the recorded values.

Table VII. Characteristics of Chitins Prepared from Crab and Shrimp Shells[a]

specification	shrimp		crab	
	A	B	A	B
moisture, %	3.00 ± 0.32	2.47 ± 0.27	0.60 ± 0.19	0.46 ± 0.31
nitrogen, %, (db)	6.29 ± 0.05	6.24 ± 0.06	6.42 ± 0.10	6.48 ± 0.05
ash, % (db)	0.09 ± 0.02	0.09 ± 0.02	0.10 ± 0.03	0.11 ± 0.02
free amino groups, μmol/g (db)	58.91 ± 0.13	55.31 ± 0.99	47.2 ± 1.06	45.94 ± 0.54
protein residue, % (db)	2.32 ± 0.02	2.07 ± 0.03	0.49 ± 0.01	0.45 ± 0.02
lipids, % (db)	0.00	0.00	0.00	0.00
specific gravity, g/cm^3, of fraction (16–35 mesh)	0.06 ± 0.00	0.06 ± 0.00	0.17 ± 0.01	0.20 ± 0.01
Hunter color parameters				
L	83.58 ± 0.39	83.52 ± 1.08	92.62 ± 0.55	91.84 ± 0.28
a	5.26 ± 0.18	5.08 ± 0.37	3.54 ± 0.27	2.80 ± 0.25
b	10.06 ± 0.30	10.66 ± 1.21	4.06 ± 0.54	6.02 ± 0.90
particle size fraction after 30 s of grinding, %				
>7 mesh	0.00	0.83 ± 0.12	0.00	0.00
7–16 mesh	17.76 ± 0.20	18.02 ± 0.35	28.05 ± 0.20	28.61 ± 0.30
16–35 mesh	73.61 ± 0.50	74.67 ± 0.65	54.11 ± 0.70	53.15 ± 1.20
<35 mesh	7.31 ± 0.15	5.04 ± 0.12	15.58 ± 0.25	16.07 ± 0.23

[a] Results are mean values of four determinations ± standard deviation. Shells: A, without prior carotenoid extraction; B, after carotenoid extraction by cod liver oil. db, dry basis.

For demineralization, it is important that the amount of acid be stoichiometrically equal to or greater than all minerals present in the shells to ensure complete reaction with them. This depends on both the concentration of the HCl solution and the ratio of the shells to HCl. The time and temperature variables of the process are less important.

The ash content for deproteinized shell wastes from shrimp and crab, on a dry basis, was 41.96 ± 0.52 and 45.77 ± 0.53%, respectively. After 30 min of demineralization at 20 °C by a sufficient amount of acid [2.5% (w/v) HCl solution, at a ratio of shells to acid of 1:20 (w/v)], the ash content in chitin from shrimp and crab decreased to 0.10 ± 0.04 and 0.25 ± 0.05%, respectively. Addition of more HCl solution or longer time for demineralization showed no beneficial effects. According to No et al. (1989), similar conditions were necessary for demineralization of crawfish waste [3.6% (w/v) HCl, 20 °C, 30 min at a waste to HCl solution ratio of 1:15 (w/v)].

Characterization of Chitin Preparations. To characterize the quality of chitin products prepared from shrimp and crab shell wastes with and without extraction of carotenoid pigments by oil, physicochemical properties of chitins isolated were determined (Table VII). For both crab and shrimp offals, extraction of carotenoids did not influence the physicochemical properties of chitin preparations. Chitins from shrimp and crab shells contained 6.29 and 6.42% nitrogen, respectively. This is in good agreement with data by Muzzarelli (1977). The low ash content of chitin, from 0.09 to 0.11%, indicates the effectiveness of the method used for the removal of calcium carbonate and other minerals from shells. Furthermore, chitin prepared from shrimp shells had a greater protein residue, about 2.3%, as compared to chitin from crab, which contained less than 0.5% proteins. Lipids in all preparations were undetectable.

Shrimp and crab chitin prepared without prior extraction of carotenoids from shell wastes contained, on a dry basis, 58.91 and 47.21 μmol of free amine groups/g of sample. The amount of free NH_2 groups strongly depends on concentration of the alkali solution used for the deproteinization process. In krill chitin deproteinized with a 10 or 28% (w/v) KOH solution, the concentration of free amino groups varied from 190 to 408 μmol/g of chitin (Synowiecki, 1986). Smaller amounts of amino groups liberated by deacetylation were perhaps due to mild alkali treatments used during the deproteinization process in this study.

The color characteristics of the chitin so prepared were also examined. The Hunter *a* value of chitin prepared from offals after extraction by oil was slightly lower and the *b* value slightly higher than those for chitin from offals without extraction of carotenoids. These differences were greater in crab chitin than shrimp chitin and showed that chitin without oil extraction of carotenoids is more red in appearance. Greater changes of Hunter *a* values caused by oil extraction of crab shells indicated that effectiveness of carotenoid removal was better in the case of crab than shrimp shell wastes. In addition, high Hunter *L* values indicated that chitin preparations had almost a white color and that chitin prepared from crab shells was somewhat more white compared to that prepared from shrimp shells.

The specific gravity of chitin fraction (16–35 mesh) from shrimp and crab was 0.06 and 0.17 g/cm^3, respectively. Thus, shrimp chitin was more porous and, for this reason, more suitable as an adsorbant and enzyme support. After 30 s of grinding, the dominant (74%) particle size fraction of shrimp chitin was 16–35 mesh. Crab chitin, however, had a smaller amount of this fraction (53–54%). Instead, the content of less fine fraction (7–16 mesh) was increased (Table VII). This was caused by a different degree of hardness and presence of structural differences between shrimp and crab shell waste chitins.

In conclusion, crab and shrimp shell wastes were an excellent source of chitin and yielded about 84 and 86% chitin, respectively. Yields from particle sizes smaller than 0.5 mm were not determined, since loss of fine particulates during washing on a filter occurred. Incorporation of oil extraction of carotenoids to the process did not result in any difficulties in deproteinization and demineralization reactions; lipids in chitin preparations were not detectable.

Optimal deproteinization involved treatment with a 1% (w/v) KOH solution for shrimp and 2% (w/v) KOH for crab shells, carried for 2 h at 90 °C with a shell to KOH ratio of 1:20 (w/v). Optimal conditions of demineralization were 2.5% (w/v) HCl at 20 °C for 1 h with a solid to solvent ratio of 1:10 (w/v). The oil extraction of carotenoids had a minimal additional cost, and fish oils proposed as extractants were a rich source of nutritionally important ω-3 fatty acids. After extraction of carotenoids, almost 74% of total pigments present in the wastes were removed. This yield is quite acceptable since shell waste raw material is almost free, except for collection and transportation costs; thus, the total cost of carotenoid preparation depends mainly on processing expenses.

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Registry No. Chitin, 1398-61-4; astaxanthin, 472-61-7; astacene, 514-76-1; canthaxanthin, 514-78-3; lutein, 127-40-2; zeaxanthin, 144-68-3.