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A seasonal study of the chemical composition and chitin quality of shrimp shells obtained from northern shrimp (*Pandalus borealis*)

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Abstract

The chemical composition of shrimp shells from the deep-water shrimp (*Pandalus borealis*) obtained from a local factory in northern Norway that were harvested from January to December (2000) in the Barents Sea was investigated. The average dry matter content of the samples of shrimp shells was $22 \pm 2\%$, with no significant seasonal variation. The protein content was found to vary between 33% and 40% of the dry weight, the chitin content varied between 17% and 20% and the ash content of dried shrimp shells was found to be relatively constant with an average value of $34 \pm 2\%$ of the dry weight and consisting mainly of calcium carbonate ($CaCO_3$). No clear seasonal variations were found for the content of these three main components of shrimp shells (protein, chitin and ash). The shrimp shells had a very low lipid content, varying from 0.3% to 0.5% of the dry weight. The content of astaxanthin was found to vary from 14 to 39 mg kg^{-1} in the samples of wet shrimp shells. In relation to its use as a raw material for chitin production, no clear seasonal variation was found in the intrinsic viscosities/molecular weights of the chitin extracted from the shrimp shells using an optimized extraction procedure for chitin extraction, suggesting that chitin producers can rely on shrimp shell waste as a stable raw material for chitin production independent of season.

Keywords: Shrimp shell; Seasonal variation; Chitin; Intrinsic viscosity

1. Introduction

The global annual production of shell waste from crustacean harvest calculated on a dry basis is estimated to 1.44 million metric tons (Knorr, 1991). The stock of the deep-water shrimp *Pandalus borealis* in the Barents Sea and in the Spitzbergen area was estimated to 161 000 metric tonnes in 1994, and the annual harvest of shrimps in Norway is estimated to about 60 000 tonnes, although a decreasing trend was seen in 2005 and 2006. The northern shrimp is harvested by industrial trawlers operating in the Barents Sea, and the shrimps are machine peeled in large factories in northern Norway where the meat is separated from the shells. The meat recovery is about 25% (w/w) while almost 40% is solid waste (Gildberg & Stenberg, 2001). Previously published results have demonstrated that shrimp shell proteins are well balanced in their amino acid composition and, as such, they may serve as an excellent component of starter feed for animals and for aquaculture industries (Shahidi & Synowiecki, 1992). So far the shell fraction has not been extensively utilized, although for the last 10–15 years a limited fraction have either been processed to shrimp meal as a feed additive, or used as raw material for production of chitin.

Crustacean shells constitute the traditional and current commercial source of chitin (Kim & Rajapakse, 2005). Chitin is one of the most abundant natural biopolymers (Roberts, 1992), and is a linear homopolymer of β(1-4)-linked 2-acetamido-2-deoxy-D-glucose. Chitin is mainly used as a raw material to produce chitin-derived products, such as chitosans, chito-oligosaccharides and glucosamine.

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An increasing number of useful products derived from chitin continue to attract commercial development. Currently the major driving force in the market is the increasing sale of glucosamine as a dietary supplement (Sandford, 2002).

The three main components of crustacean shells together with chitin are minerals (mainly calcium carbonate) and proteins. These three components exist closely associated and account for about 90% of the dry weight of the shell (Ferrer et al., 1996). Chemical isolation of chitin from crustacean shells involves demineralisation and deproteination which may cause depolymerisation and de-*N*-acetylation of the chitin. Optimization of the chitin extraction in order to minimize the degradation of chitin, while at the same time bringing the impurity levels down to a satisfactory level for specific applications is therefore important. Percot, Viton, and Domard (2003) reported an optimized extraction procedure of biological grade chitin with high molecular weight and a high degree of acetylation.

In order to grow, the shrimps must regularly change their exoskeletons (molting). Female *P. borealis* spawn once a year and extrusion of eggs starts in late summer to early autumn and the ovigerous period lasts until spring. Shortly after the time of egg hatching the shrimp undergo a molt. Molting records indicate 2–4 additional molts before a new egg laying can take place. It is assumed that no molting takes place when the shrimp carries the eggs (Shumway, Perkins, Schick, & Stickney, 1985). The molting process involves production of both chitinases and enzymes involved in chitin biosynthesis, suggesting that the chitin quality may vary with season. However, to our knowledge, no systematic study of the annual variation in the chemical composition or chitin quality of shrimp shells has been reported, and a possible seasonal variation in e.g., the chitin content and quality would be of importance to the chitin producers. In relation to the use as a raw material for chitin production, we have investigated the seasonal variations in chitin quality and chemical composition of monthly samples of shrimp shells harvested in the Barents Sea.

2. Materials and methods

2.1. Raw materials and preparation

Deep-water shrimp (*P. borealis*) was harvested by seagoing trawlers in the Barents Sea. On board the trawler the shrimps were frozen within two hours after catch. The trawlers typically harvest shrimps for several weeks before they return to the mainland. All samples included in this work were received from a local shrimp peeling factory in Troms county (Lenvik Fiskeindustri). The size ratio of the shrimps was 230–260 shrimps kg^{-1}, which means that males and transitionals (intersexual phase between males and females) dominates. At the factory the frozen blocks of raw shrimps were thawed, steam-boiled and shelled using pilling machines. Representative shell samples were taken out, packed in plastic bags and stored at −20 °C before and during transport to the lab. Prior to the chemical investigations the shells were thawed slowly (over night) in a cold room, rinsed several times with water and either dried at 100 °C. Alternatively extraction of chitin was performed from non-dried shells. The dried shells were then milled in a hammer mill through a 1-mm sieve.

2.2. Dry weight and content of ash (minerals)

The dry weight was determined after drying for 24 h at 105 °C. Ash content was determined by heating to 530 °C for 20 h. The precision of the ash content was estimated to ±2% (standard deviation of the mean ($n = 5$)). All measurements were done in triplicate.

2.3. Protein

The content of total nitrogen in dried and milled shells was determined ($n = 5$) using a Carlo Erba Elemental Analyzer Model 1104 with a Hewlett–Packard 3373B Integrator (Kirsten, 1979). In order to estimate the percentage of proteins the non-protein nitrogen fraction (nitrogen from chitin) must be withdrawn from the total nitrogen value as shown in the following equation:

$$P_{\%} = (N_{total} - N_{chitin}) \times 6.25 \quad (1)$$

The nitrogen content in chitin was calculated from the gravimetric analysis using the theoretical percentage of nitrogen in fully acetylated chitin (6.896). The value 6.25 corresponds to the theoretical percentage of nitrogen in proteins. The purity of the chitin fraction and thus the validity of the factor 6.896 (theoretical N content in chitin) was checked both by elemental analysis and by the traditional Kjeldahl method. Both methods were tested with commercial samples of chitin and monomer/dimer fractions of GlcNAc and were found to be in accordance with theoretical calculations.

2.4. Extraction of chitin

2.4.1. Demineralisation

Cold 0.25 M HCl (300 ml) was added to 50.0 g thawed shrimp shells (not dried). This extraction was allowed to proceed for 5 min on ice. The suspension was then filtered and additional 300 ml of cold 0.25 M HCl was added to the pellet. The supernatant was kept for later analysis. After 35 min of cold extraction the suspension was filtered again. The supernatant was combined with the first one and the pellet was washed with water (300 ml). The suspension was then filtered and the water from the washing procedure was added to the acid supernatants. The exact total volume of this acid extract was noted. The extract was kept in a cold room until the content of calcium carbonate was determined by an Atomic Absorption Spectrophotometer (Perkin–Elmer, model 290 B).

2.4.2. Deproteination

The final pellet from the demineralisation step was extracted with NaOH (1 M, 100 ml) at 95 °C for 2 h. The suspension was then cooled to room temperature, filtered and the pellet was extracted again under the same conditions (1 M NaOH, 100 ml, 95 °C) for 2 h. The same filtering procedure was then repeated one more time and the final alkaline extraction was allowed to proceed for 1 h. Then the extract was cooled to room temperature, filtered and washed with water until neutrality was achieved. The pellet was finally washed with ethanol (96%) and dried at 80 °C. The content of chitin was determined gravimetrically.

2.5. Minerals

The acid extracts from the demineralisation step were analysed for 10 selected minerals (Ca, Na, K, Mg, Sr, Ba, Cu, Ni, Co, Fe) by atomic absorption (Varian, model 400). The precision of the methods (given as 95% confidence limits) are ±2% for Ca, Na, K, Sr and Mg, ±5% for Fe and Cu, and ±20% for Ba and Ni. For Co the values are just around the detection limit of the method.

2.6. Total lipids

Total lipids were determined according to Bligh and Dyer (1959) with the modifications described by Hardy and Keay (1972).

2.7. Astaxanthin

Extraction (50 g shrimp shells, 200 ml acetone, N_2 atmosphere, −18 °C, 24 h) and quantitative determination (spectrophotometric) of astaxanthin were performed according to Schiedt, Liaaen-Jensen, and Pfander (1995, chap. 5). Quantification and separation of astaxanthin, astaxanthin monoester and astaxanthin diester forms were performed by HPLC according to Egeland, Johnsen, Eikrem, Throndsen, and Liaaen-Jensen (1995).

2.8. Intrinsic viscosity of chitin

The chitin was dissolved in alkali as described by Sannan and co-workers (Sannan, Kurita, & Iwakura, 1976) and the intrinsic viscosity of the chitin and its respective molecular weights were determined as previously described (Einbu, Naess, Elgsaeter, & Vårum, 2004).

3. Results and discussion

3.1. Chemical composition of shrimp shell

The average dry matter content of the samples of shrimp shells was 22 ± 2%, with no significant seasonal variation. Fig. 1 shows the content of the three main components, i.e., minerals (ash), protein and chitin given as percentage of the dry weight of shrimp shells harvested at different times of the year.

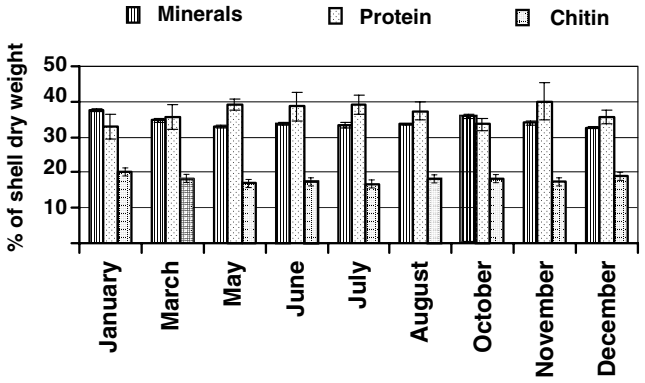

Fig. 1. Content of minerals (ash), total proteins, and chitin in industrially produced shrimp shells (year 2000) given as percentage of dry weight.

The protein content was found to vary between 33% and 40% of the dry weight (Fig. 1), with no obvious seasonal variation. The lowest protein concentrations were detected in the samples from January and October (33% and 34%, respectively), which also had the highest ash contents. Re-calculations on an ash-free basis did not alter the relative relationships between the samples (results not shown). The accuracy of the protein determination is not unambiguous since the results arise as the difference between the total nitrogen in dried shrimp shells determined by a Carbon–Nitrogen-analyser (accuracy of this method is ±4–10% of the mean) and the nitrogen content in the isolated chitin. A one-way ANOVA was used to test whether the seasonal difference in the protein content was significant, predicting a probability of 0.87 for an insignificant seasonal variation in our data.

The chitin content of the shrimp shells was found to vary from 17% to 20% of the dry weight. The accuracy of the method was found by triplicate analysis of the samples, the variation was ±6% of the mean. A one-way ANOVA was used to test whether the seasonal difference in the chitin content was significant, predicting a probability of 0.62 for an insignificant seasonal variation in our data. The chitin content was determined gravimetrically and the purity of the chitin samples was verified by comparing the nitrogen content in the isolated chitin (determined from the Carbon–Nitrogen-analyser) with the theoretical nitrogen content in a pure chitin sample. These results showed no significant impurities in the chitin samples ⩽2% variation between theoretical and estimated values (Roberts, 1992).

The content of minerals (ash) varied between 32% and 38% of the dry weight. A one-way ANOVA was used to test whether the seasonal difference in the ash content was significant, predicting a probability of 0.75 for an insignificant seasonal variation in our data. The other ions contributing to the difference between the ash content and the $CaCO_3$ content were quantified, and the results are shown in Table 1.

As shown in Table 1, Ca, Na, Mg and Sr were found to be the main ions. Relatively high concentrations of strontium were found in all the samples. The ratio (weight) between calcium and strontium in the shrimp shells was slightly higher as compared to the same ratio in seawater ($Ca/Sr_{seawater} = 53$ and $Ca/Sr_{shrimp\ shells} = 67$), indicating that calcium as compared to strontium is not selectively incorporated into the crustacean shells. Radionucleotides originating from nuclear weapons fallout, the Chernobyl accident (1986) and discharges from European reprocessing facilities (Sellafield, La Hague and Dounreay) contributes to contamination of the Barents Sea area, and since ^{90}Sr behaves like an analogue for calcium this ion will probably be incorporated and found in shrimp shells. The level of radionucleotides in marine animals and plants is, however, very low. In a previous work, discards of Newfoundland shrimp (*P. borealis*) were analysed (method not given) and the levels of Ca, Mg and Sr were found to be in the same range as those found in this work (Shahidi & Synowiecki, 1992).

Our results show low contents of lipids from 0.3% to 0.5% of the dry weight, with no significant seasonal variation in the lipid content of the shrimp shell. Lipid content of whole shrimps (*P. borealis*) has been reported to increase from April to September (Hopkins, Sargent, & Nilssen, 1993).

The content of astaxanthin was found to vary from 14 to 39 mg kg^{-1} in our samples of wet shrimp shells. The relative distribution of astaxanthin forms (free, mono- and diester, in % of total astaxanthin) in the shells was determined and is given in Table 2. The relative amount of astaxanthin forms suggests a possible conversion from mono- and diester forms to free astaxanthin during storage. Samples stored for longer time had generally a higher content of free astaxanthin. The remaining astaxanthin was found to exist as mono and diesters forms. This may explain the variations observed in Table 2, as the samples were stored (at −20 °C in the dark) from 10 to 22 months prior to analysis. A significant loss in astaxanthin contents has been demonstrated during storage (0–12 months) of atmospheric air-packed shrimp samples (Bak, Andersen, Andersen, & Bertelsen, 1999) and the results presented herein should be judged accordingly.

3.2. Extraction and characterisation of chitin

Chitin was extracted from the different samples of shrimp shell in order to investigate seasonal variations in relation to the quality of the shrimp shells as a raw material for chitin production. In order to minimise the depolymerisation of the chitin and the de-*N*-acetylation during the extraction procedure, initial extraction experiments were performed where the conditions for demineralisation and deproteinisation were varied. The method described by Hackman (1954) using cold 2 M HCl for 48 h and 1 M NaOH for 24 h at 100 °C was found to result in a chitin with significantly lower (~20%) intrinsic viscosities than chitin obtained from the method described in Section 2. This is in accordance with our previous results on treatment of chitin with 3 M HCl for 24 h (Einbu et al., 2004), showing significant depolymerisation of the chitin during such acid treatment. Also, from our recent results on de-*N*-acetylation of chitin in 1 M NaOH at 95 °C (Einbu & Vårum, submitted for publication), it was found that the deproteinisation method described by Hackman could be expected to significantly lower the degree of *N*-acetylation of the chitin sample. In addition, control experiments were performed with drying and milling of the shrimp shells prior to chitin extraction, showing that the drying and milling procedure will result in a small but significant reduction in the intrinsic viscosities of the chitin. Thus, in order to minimize depolymerisation and de-*N*-acetylation of the chitin samples during the extraction procedure, we

Table 2
Distribution of astaxanthin in dried shrimp shells (year 2000)

	Free astaxanthin (% of total)	Monoester (% of total)	Diester (% of total)
January	19	25	56
March	11	20	70
May	8	23	69
June	11	28	62
July	9	24	67
August	7	28	65
October	6	25	69
November	6	24	71
December	6	23	71

Table 1
Composition of selected ions in dried shrimp shells expressed as mg kg^{-1} dried shrimp shell (year 2000)

	Na	K	Mg	Sr	Ba	Cu	Ni	Co	Fe
January	2270	310	5670	1840	24.8	10.3	10.0	<0.12	30.7
March	2230	370	5830	1800	17.3	13.7	3.0	<0.12	35.4
May	1530	240	5120	1660	18.5	16.6	4.3	<0.13	43.1
June	1740	280	5590	1840	20.7	14.8	2.6	<0.12	48.7
July	1690	280	5150	1590	19.3	18.8	2.7	<0.14	26.9
August	1940	290	5660	1860	23.0	14.0	2.8	<0.12	35.8
October	1460	260	5770	1780	20.2	14.4	0.8	<0.13	31.1
November	1530	250	5600	1730	19.0	16.2	0.8	<0.13	43.6
December	1680	270	5500	1650	21.3	14.1	1.8	<0.13	59.3

Table 3
Intrinsic viscosities of chitin samples from shrimp shells together with the calculated molecular weights

Time of shrimp harvest	Intrinsic viscosity (ml/g)	Molecular weight* (kg/mol)
January	1170	960
March	1160	950
May	1150	940
June	1180	970
July	1250	1060
August	1190	990
October	1250	1060
November	1160	950
December	1200	1000

* Molecular weights determined by the MHS-equation determined by Einbu et al. (2004).

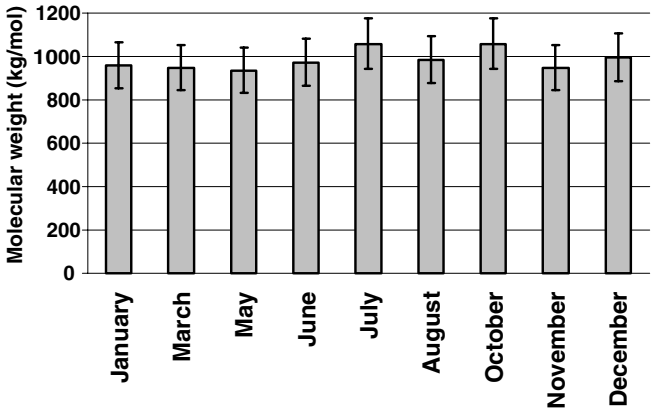

Fig. 2. Molecular weights of chitin extracted from shrimp shells (year 2000).

used cold 0.25 M HCl in the demineralisation process and subsequently in the deproteinisation process 1 M NaOH at 95 °C (see Section 2 for details). The intrinsic viscosities of the resulting chitin were determined and the intrinsic viscosities of the chitin from the different shrimp shell samples are shown in Table 3 together with their estimated molecular weights, as determined from the MHS-equation using $K = 0.1$ and $a = 0.68$ (Einbu et al., 2004).

The molecular weights of the samples varied between 940 and 1060 kDa, with a standard deviation of 4%. The standard deviation of the molecular weights were previously determined to ±11% (Einbu et al., 2004).

Fig. 2 shows the determined molecular weights of the chitin from different shrimp shell samples harvested monthly (year 2000). It is unlikely that the molecular weights determined reflects the "native" molecular weights of the chitin as it occurs in the shrimp shell matrix. However, the presented data in Fig. 2 was obtained by a mild and optimized extraction procedure, and as such they should represent chitin chain lengths that are relevant to chitin producers. Both the conditions for extraction and the solubilisation procedure will probably to some extent influence the chain length of the chitin in solution. With this limitation, the variation in the molecular weight in Fig. 2 is within the experimental error and from this, no significant seasonal variation can be seen in the molecular weight of the chitin.

4. Conclusion

In relation to its use as a raw material for chitin production, no clear seasonal variations were found in the chemical composition of shrimp shells from *P. borealis*. From our optimized and mild procedure for chitin extraction, no significant seasonal variations were found in the molecular weight of the chitin extracted from the shrimp shells. This indicates that the chitin producers can rely on the shrimp industry as a stable source of raw material without large seasonal variations, provided that the shells are adequately stored after processing.

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