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A new process for advanced utilisation of shrimp waste

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Abstract

A high quality chitosan for application in cosmetics can be produced from the processing waste of Northern shrimp (*Pandalus borealis*). A major fraction of the shrimp waste is protein tissues, which are normally wasted during conventional chitosan preparation. The present work shows that the shrimp waste proteins can be hydrolysed by a commercially available protease (Alcalase) and recovered as a protein hydrolysate with a high content of essential amino acids, before the scales are processed to chitosan. The Alcalase treatment had no adverse effect on either yield or quality of the chitosan. By this new method the total recovery of Kjeldahl nitrogen was 68.5% as compared with only 12.8% by the conventional method. In addition, a concentrate of astaxanthin was recovered in the sediment after centrifugation of the crude protein hydrolysate. This concentrate may be a valuable supplement in the feed to salmonid fishes.

Keywords: Shrimp waste; Chitosan; Protein hydrolysate; Astaxanthin

1. Introduction

In Norwegian fisheries the annual catch of Northern shrimp is about 40 000 tons. In commercial processing, the meat recovery is about 25% (w/w), whereas almost 40% (w/w) is solid waste containing 25–30% dry matter. About 30% of this dry matter are tissue proteins, while minerals and chitin are the other major fractions [1,2]. Recently, a commercial production of chitosan from shrimp waste has been established in Norway. Chitosan is produced according to a patented process yielding a high quality product suitable for application in various cosmetic and hair conditioning products [3,4]. During processing of the shrimp waste to chitosan, both minerals and tissue proteins are chemically extracted and drained off, and only about 10% of the raw material dry matter is recovered as chitosan. This means that for each kg chitosan produced, about 3-kg protein is wasted. It has been shown earlier that tissue proteins in shrimp waste can be hydrolysed and recovered by treatment with Alcalase, a commercially available protease preparation, and that the hydrolysate obtained contained bio-active peptides, which may be valuable as pharmacological tools or as a growth stimulating agents in animal feed [5,6]. Earlier a method for enzymic removal of proteins from demineralised shrimp waste was presented [7]. This method, however, is poorly designed for commercial recovery of protein since the hydrolysate obtained is very diluted, and it is not compatible with the recovery of astaxanthin.

The main objectives of the present work were to demonstrate the recovery of amino acid, nitrogen and astaxanthin by Alcalase pre-treatment of shrimp waste before it is further processed to chitosan, and to reveal if the enzymic pre-treatment has any influence on either yield or quality of the chitosan.

2. Materials and methods

2.1. Materials

Northern shrimp (*Pandalus borealis*) waste (heads and scales) from a processing factory was provided by BioHenk AS. The waste was packed in plastic bags and stored at −20°C before use. Alcalase (2.4 l FG) was

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provided by Novo Nordisk A/S. All reagents used were of analytical grade.

2.2. Chemical analyses

Dry weight was determined after drying for 2 days at 105°C, and ash after heating to 550°C for 20h. Total N and crude protein were determined by the Kjeldahl procedure. Lipid content was determined by Soxhlet extraction with petroleum benzene. Astaxanthin was determined according to the method of Skrede and Storebakken [8]. Amino acids were determined after hydrolysis (6 N HCl, 24 h, 110°C) under nitrogen pressure. Phenyl-thiocarbamoyl derivatives were prepared according to Waters Associates [9]. The derivatives were separated by high-pressure liquid chromatography (HPLC) reverse-phase chromatography using a Supelco LC-18-DB column and recorded by UV absorption at 254 nm.

2.3. Protein hydrolysis

Shrimp waste (2.00 kg) was thawed overnight at about 10°C and mixed with 2.00 l warm (80°C) distilled water (Fig. 1). After equilibration of the temperature to 40°C, Alcalase (20 ml) was added and the sample was hydrolysed for 2 h in an open stirred reactor ($\phi = 26$ cm) before the enzyme was inactivated by heating ($>$ 90°C, 20 min). Solid tissues and crude hydrolysate were separated by manual pressing of the sample in a sausage press (Vogt Ideal) with a nylon net screen (1.5 mm) mounted above the outlet. The press cake (904 g) was packed in a plastic bag and stored at -20°C until further processing to chitosan. The crude hydrolysate was cooled to 4°C before suspended solids were separated by centrifugation (40 min, 10 000 $\times$ g).

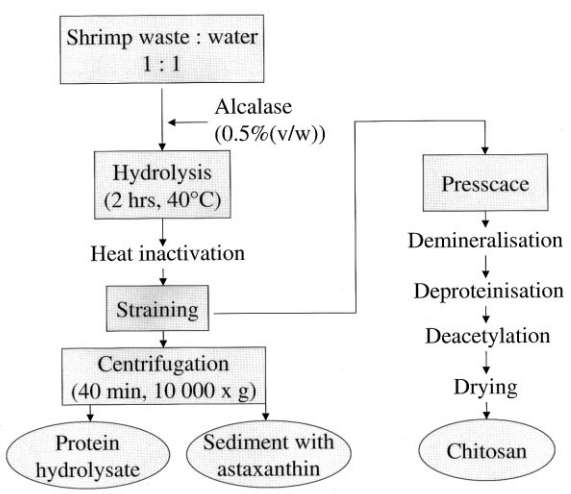

Fig. 1. Flow chart of the new process for utilisation of shrimp wastes.

2.4. Preparation of chitosan

Chitosan was prepared from 2.00 kg shrimp waste (A) and the presscake obtained after Alcalase treatment of 2.00 kg shrimp waste (0.90 kg presscake + 1.10 l water) (B) by a method developed by Wachter et al. [3]. Demineralisation was obtained by adding 4.00 l tap water and 0.50 l HCl (37%) to A or B and stirring the mixtures (three blade stirrer; $\Phi = 22$ cm, 150 rpm) for 12 h at 17–18°C in a plastic reactor ($\Phi = 32$ cm). After draining on a stainless steel screen ($\Phi = 0.16$ cm) the scales were washed three times with 5 l cold tap water. Water was then added to a total amount of 5 kg before 0.55 l of a 50% (w/w) NaOH solution was mixed in. Residual proteins were solubilized by stirring the mixtures at about 80°C for 2 h in a stainless steel reactor and drained off on the steel screen. The scales were washed five times with 5 l cold tap water. After draining overnight at 4°C, the scales were mixed with 4.00 l tap water and 0.25 l HCl (37%) and stirred for 1.5 h at room temperature (about 21°C) in a plastic reactor before they were drained and washed on the screen three times with 5 l cold tap water. Then water was added to yield a total amount of 1.00 kg before 1.00 kg solid NaOH was mixed in during heating and stirring in a glass reactor ($\Phi = 16$ cm). Complete deacetylation of the chitin was obtained by stirring the mixtures for 4 h at about 80°C. The chitosan preparations were washed on the screen with cold tap water until the pH of the washing water was lowered to 7.3 before it was frozen and dried in a freeze-dryer (Leybold-Heraeus, DT2).

2.5. Viscosity measurements

Chitosan (4 g) was solubilized in 400 ml glycolic acid (0.4% (w/w)) and the temperature equilibrated to 20°C. Viscosity was measured with a Brookfield DV-1 + viscosimeter.

3. Results and discussion

The shrimp waste contained 26.5% dry matter of which 74% was organic material (mainly tissue proteins and chitin) and 26% minerals. Only 0.4% of the dry matter was extractable lipids. About 60% of the extractable lipids were recovered in the sediment fraction after centrifugation of the crude protein hydrolysate, and on a dry matter basis the sediment contained 1500 ppm astaxanthin. This is ten times higher than the normal astaxanthin content in high quality shrimp meal (Guro Eilertsen, personal communication) and corresponds to a astaxanthin recovery of about 40% [10]. After centrifugation, the hydrolysate had a dry matter content of 4.9% (w/w) of which 86% were crude protein and 10% minerals.

Table 1
Amino acid composition of shrimp waste hydrolysate compared with the amino acid composition of Atlantic cod muscle protein and Bacto-Tryptone; a high quality nitrogen source for cultivation of micro-organisms [a]

Amino acid	Shrimp waste hydrolysate	Code muscle protein [b]	Bacto-Tryptone [c]
Essential			
Histidine	3.12 ± 0.00	3.1	2.5
Isoleucine	5.77 ± 0.03	4.8	4.4
Leucine	8.86 ± 0.09	8.4	8.3
Lysine	8.31 ± 0.32	9.5	7.2
Methionine	3.30 ± 0.04	3.1	2.4
Phenylalanine	5.55 ± 0.09	4.0	4.6
Threonine	6.04 ± 0.02	4.6	4.5
Tryptophan	nd	1.2	nd
Valine	6.72 ± 0.04	5.3	6.7
Non-essential			
Tyrosine	5.08 ± 0.06	3.5	2.9
Aspartic acid	2.84 ± 0.26	10.6	7.7
Glutamic acid	8.92 ± 0.57	15.5	24.2
Glycine	6.76 ± 0.01	6.3	2.3
Serine	6.29 ± 0.04	4.2	6.1
Arginine	8.90 ± 0.21	6.2	3.4
Alanine	7.58 ± 0.01	5.0	3.5
Proline	5.67 ± 0.00	3.7	9.3
Cystine	0.34 ± 0.02	1.1	0.0

[a] The values given are amounts of amino acids in g/100 g.
[b] Calculated from data given by Shahidi et al. [11].
[c] From Clausen et al. [12].

Table 1 shows the amino acid composition of the hydrolysate compared with the amino acid composition of cod muscle protein and Bacto-Tryptone; a high quality microbial peptone. Generally, the shrimp waste hydrolysate has a high content of essential amino acids, indicating a high nutritional value both used for food, feed or as a nitrogen source in growth media for micro-organisms. In addition to containing growth, stimulating peptides and being a potential nutritious product, it has also been proved that shrimp waste hydrolysates contain compounds that stimulates non-specific immune response reactions in salmon marchrophages (unpublished results). Hence, supplementing salmon feed with shrimp waste hydrolysate may improve both the growth and the disease resistance of the fish.

Fig. 2 shows the recovery of dry matter and Kjeldahl nitrogen in the new combined utilisation process as compared with the conventional process where only the chitosan is recovered. In the conventional process, only 12.8% of the nitrogen and 8.8% of the total dry matter were recovered, whereas the corresponding values for the new method were 68.5 and 33.2%, respectively. This is mainly due to the high recovery of proteinaceous materials in the enzyme hydrolysate. In a standard chitosan production procedure, this material is all waste and represents a potential pollution problem instead of contributing to value addition.

Table 2 shows weight recovery and some chemical and physical characteristics of the chitosan obtained with the two methods. The total weight recovery of chitosan is similar with the two methods, although marginally lower with the new combined process. There were no significant differences in the content of either dry matter ash or Kjeldahl-N. The viscosity of chitosan solutions made from the press cake after enzyme hydrolysis was significantly higher than the viscosity of solutions made from chitosan prepared by the conventional procedure. The reason for this is unknown. One possible explanation is that chitinolytic enzymes active at acid conditions are degraded and removed during the initial Alcalase treatment [13].

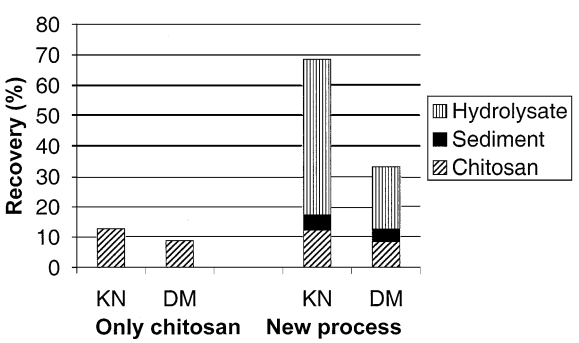

Fig. 2. Recovery of Kjeldahl nitrogen (KN) and dry matter (DM) in conventional chitosan production compared with the new process where the shrimp waste is subjected to hydrolysis by Alcalase, before chitosan is produced.

Table 2
Recovery, chemical composition and viscosity of chitosan made directly from shrimp waste and from the press cake of shrimp waste after enzymic protein hydrolysis

	Waste	Press cake
Weight recovery (%)	2.37	2.32
Dry matter (%(w/w))	98.75 ± 0.03	98.73 ± 0.17
Ash (%(w/w))	0.070 ± 0.007	0.064 ± 0.007
Kjeldahl-N (%(w/w))	8.07 ± 0.10	7.92 ± 0.05
Viscosity of 1% solution (mPas)	327 ± 5	700 ± 64

It is likely that initial deproteinisation of shrimp waste with Alcalase may simplify subsequent chitosan production, but this remains to be investigated.

4. Conclusion

Shrimp waste can be subjected to protein hydrolysis by Alcalase to obtain a high value protein hydrolysate yielding more than half of the Kjeldahl-N of the raw material. A large fraction of the astaxanthin in the shrimp waste can be recovered in the sediment after centrifugation of the crude protein hydrolysate. The Alcalase treatment does not influence negatively either on the recovery or the quality of the chitosan produced from the shrimp scales. By employing the new method, it is possible to recover about 70% of the total amino-N in valuable products as compared with less than 15% in the conventional chitosan production process.

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