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Short Communication

Recovery of astaxanthin from seafood wastewater utilizing fish scales waste

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Abstract

The paper presents basic data on astaxanthin adsorption from fisheries wastewater to fish scales. This process has been proposed to be applicable in fisheries and shrimp waste management [Helgason, Recovery of compounds using a natural adsorbent, Patent WO 01/77230, 2001]. The innovative feature of the method is the application of a solid waste (fish scales) as a natural adsorbent for a carotenoid pigment (astaxanthin) from the seafood industry wastewater. The model investigations were performed with pure synthetic carotenoids to exclude the role of matrix in which astaxanthin is present in the wastewater. Under the experimental conditions used, the maximum loading capacity of astaxanthin onto the scales is 360 mg kg^{-1} dry wt. Studies of the thus formed value added product indicated that drying causes significant loss of astaxanthin activity. Due to the effective filtration characteristics of the studied sorption material, we suggest the scale/astaxanthin sorption process to be suitable for treatment of wastewater from different industries.

Keywords: Astaxanthin recovery; Fish scales as adsorbent; Fisheries waste treatment

1. Introduction

Asthaxanthin (3,3′-dihydroxy-β,β-carotene-4,4′-dione) is a ketocarotenoid, oxidized form of β-carotene being responsible for the pink to red pigmentation of the crustaceans and wild salmonids. It is widely utilized as a coloration agent for the farmed salmonids including the natural or synthetic pigment into the fish diet (Benemann, 1992; Hatlen et al., 1995; Torrissen, 1995). The pigment is also considered in medical and biomedical studies and applications due to its biological function as a vitamin A precursor and its high antioxidative effects, which are stronger than those of β-carotene vitamin E or C (Palozza and Krinsky, 1992; Kobayashi et al., 1997). Gradelet et al. (1997) have demonstrated the preventive effects of astaxanthin against aflatoxin B1 carcinogenicity.

Several procedures for isolating valuable pigments from natural sources have been reported (Spinelli et al., 1974; Choubert and Luquet, 1983; No et al., 1989; Shahidi and Synowiecki, 1991; Johnson, 1992; Shahidi et al., 1992). Extraction methods were reported to get carotenoids out of a shell matrix employing edible oils, hydrochloric acid or organic solvents. They are complementary steps within the chitin extraction procedures from shrimps and shrimp waste. An enzymatic step to improve the extraction of astaxanthin has also been suggested (Cano-Lopez et al., 1987).

The processing of arctic shrimps (a significant source of natural astaxanthin) into food products imposes

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serious environmental problems, especially excessive water use and disposal problems of the coloured wastewater containing organic matter. In this paper we present preliminary experimental results aiming towards an astaxanthin recovery from shrimp waste effluent by the use of fish scales. As an innovative adsorbent we used fish scales, another waste product (Helgason, 2001). In order to verify its sorption efficiency, ctenoid scales from bony fish (Osteichthyes) were investigated. It is a suitable sorption material for at least two reasons: (i) a highly developed structure of the surface offering an adsorptive potential to a variety of suspended material in water; (ii) the availability of the material—bony fish have to be descaled prior to further processing and obtained scales are at the moment considered only as the waste material.

2. Materials and methods

2.1. Fish scales and wastewater

Scales from redfish (*Sebastes marinus*) were collected and washed repeatedly in warm tap water, then dried overnight at 60 °C and stored at room temperature.

Few liters of wastewater from arctic shrimp pooling factory was collected and kept frozen until use in experiments. It is a run-off water from the processing stream, characterized by typical reddish hue, due to a high content of astaxanthin which is bound to shrimp protein residues.

2.2. Astaxanthin solvent extraction and analysis

Duplicate extraction of the studied wastewater was achieved with a petroleum ether (40–60 °C): acetone 1:1 mixture (PEA) in a sample volume (30–100 ml). To avoid poor reproducibility extractions followed by measurements were carried out in sample series of 20–30 using freshly prepared PEA mixture.

Preliminary measurements used in efficiency studies of the process measurements included only determination of free forms of astaxanthin via UV–Vis spectroscopy of centrifuged PEA extracts.

The HPLC measurements involved an isocratic reversed phase analysis (LiChrosorb Merck RP18, *mobile phase* MeCN–CH_2Cl_2–MeOH (0.1 M ammonium formate) 71:7:22 v:v:v) adopted from Yuan and Chen (1998). The system was calibrated using an external astaxanthin standard (Sigma Chemicals). Since majority of astaxanthin occurring in organisms is in the form of fatty acids esters, a saponification procedure taken from literature (Britton et al., 1995) was applied to subsamples of extracts. For this, 10% KOH in ethanol was added to the samples and then kept for 8 h in the dark under anaerobic conditions. The determined content of pigment from hydrolysed extracts was considered as total free and esterified (mono- and diester) astaxanthin.

All experiments were undertaken in preventing from light conditions and, when possible, under N_2 conditions to avoid possible *cis*/*trans* isomerisation and oxidation of the astaxanthin.

2.3. Sorption experiments

In different sorption experiments from dry scales packed without pressure in 10–70 cm long PE columns (i.d. 5 cm), supported with a reservoir filled with wastewater (Fig. 1). Flow, amount of pigment and eluent composition were varied and then optimized. One litre of wastewater was passed at the initial flow of 3 ml min^{-1} through 4 g of scales, yielding in an optimal penetration and thus adsorption of astaxanthin onto the scales. The flow however was not continues through the process being gradually inhibited by scales saturated with wastewater. This process, however, took about 5 h. The experiment was carried out preventing the system from light and at room temperature. The following five samples were taken and analysed after saponification by HPLC: (i) wastewater (with floating suspension), (ii) the first 25 cm of suspension on top of scales, containing the largest fragments of solid shrimp waste, (iii) the first 10 cm of scales with sorbed pigment, (iv) the next 10 cm of

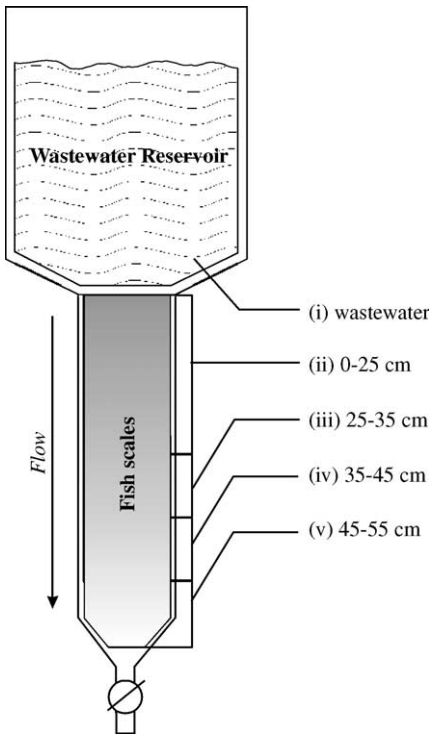

Fig. 1. Experimental set-up with indicated sampling ports.

the same of scales and (v) rest of the scale material (10 cm).

3. Results and discussion

3.1. Astaxanthin in the scale pigment product

The results of the astaxanthin determination in each fraction are given in Table 1. Additionally, the samples from fractions (ii) to (v) were taken for astaxanthin determination after drying (40 °C, 12 h).

The results show that astaxanthin is bound onto the scales, were 88–95% of the total sorbed pigment was found to be bound in its esterified form. The maximum loading capacity of astaxanthin onto the scales (362 mg kg^{-1} dry wt.) was found in fraction (iii).

3.2. Effects of different particle size on adsorption of pigments from the wastewater

In order to define the influence of the size of pigment containing matter in the wastewater the following 3 samples were prepared: (i) filtrates of wastewater from the paper filter, (ii) filtrates from 0.45 μm membrane vacuum filtration and (iii) supernatant from centrifugation at 4000*g* for 10 min. The concentration of astaxanthin in all the filtrates was low compared to that in unprocessed wastewater. In the supernatant, about half of the wastewater content was found. This shows that most of astaxanthin fraction is located in suspension, which can be separated by paper filter but some pigment is located in a fraction small enough not to be separated by centrifugation. From the preliminary structure-activity relationship considerations it seems probable that the carotenoid is a part of the lipid component of the wastewater.

3.3. Sorption capacity and efficiency of the fish scales

During preliminary experiments it was observed that the pink coloured suspension is spreading quite uniformly within a column packed with scales, gradually loosing its intensity towards the bottom. This shows that retention of the pigment onto scales is not only due to the mechanical filtration of the wastewater suspension at the top of scale column but also occurs in the deeper layers of the scales. The capacity of not milled, mildly compressed scales was estimated to be in the range of 80–120 ml g^{-1} (wastewater/scales). When this ratio is reached, the flow of wastewater through the scales is no longer constant but continuously slowed down due to the large particles and fragments at the top of the column. Thus a pre-step including milling, pigment dissolution and/or pre-filtration prior to scales-sorption has been identified. The sorption efficiency (defined as the percentage amount of astaxanthin remaining onto scales) of uncompressed scales is much lower. Here the breakthrough of pigment containing suspension is already observed after 14 ml g^{-1}. Fig. 2 shows the result of an experiment to determine the loading capacity of a scale column. The sorption efficiency (% of retention) is rather constant for the first 180 ml of wastewater and then starts to fluctuate. Then only 70–80% of the pigment remained on the scales. The final breakthrough of astaxanthin (also indicated by the slow down of the flow) was observed at 290 ml (83 ml g^{-1} of scales).

Further studies showed that slowing down the outflow of wastewater passing through the scales improves percent of astaxanthin retention onto scales even to the range of 90–97% elongating however the time of the process. Apart for prolongation of the flow, physical interference should be applied such as mechanical mixing during the process. This should increase and improve the contact of pigment containing particles with the scales. Stirring by aeration should not be considered due to the possible oxidation of the astaxanthin.

3.4. Sorption and desorption of synthetic astaxanthin in the free form

Binding capacity of pure astaxanthin (standard solution SIGMA Chemicals) onto scales was examined. Because of the poor solubility of caretonoids in water

Table 1
Astaxanthin concentrations in the fractions (ii)–(v) (mg kg^{-1} dry wt.) and wastewater (mg l^{-1} wet wt.) from the sorption experiment before and after drying in 40 °C for 12 h

Sample	Astaxanthin concentration before drying (% of total pigment)			Astaxanthin concentration after drying (% of total pigment)		
	Free	Esters	Total	Free	Esters	Total (loss)
(i) Wastewater	0.2 (24)	0.5 (76)	0.7			
(ii) 0–25 cm	11.9 (12)	93.2 (88)	105.1	0.6 (20)	2.3 (80)	2.9 (97)
(iii) 25–35 cm	18.4 (5)	343.7 (95)	362.1	2.0 (3)	55.7 (97)	57.7 (84)
(iv) 35–45 cm	10.0 (8)	114.8 (92)	124.8	1.5 (10)	14.2 (90)	15.7 (87)
(v) 45–55 cm	4.3 (10)	38.4 (90)	42.7	1.2 (8)	12.2 (92)	13.3 (69)

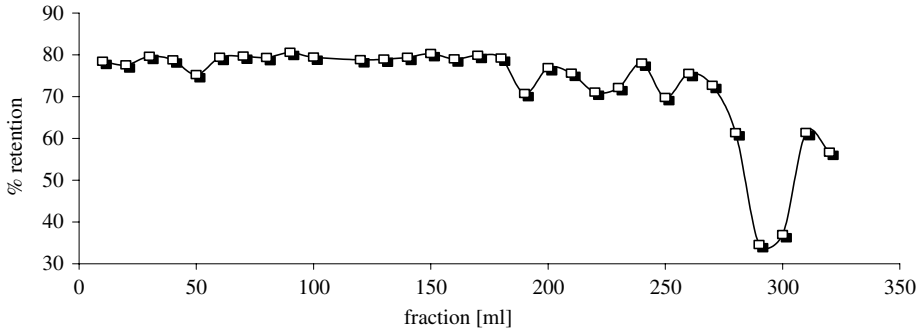

Fig. 2. Sorption capacity of astaxanthin from wastewater on compressed not milled scales (column length: 10 cm; scales: 3.5 g (dry wt.); 52 ml g^{-1} (volume); fractions: 10 ml each.

different amounts of acetone was added. To understand the influence of acetone on the sorption–desorption processes in simultaneous runs acetone was also added to the wastewater at comparable concentration.

It was found that adding lower than 0.5% of acetone together with a small amount of NaCl (minimum 0.01 mol l^{-1}) the sorption of pure astaxanthin occurred with a comparable efficiency as found for wastewater pigment.

4. Conclusions

We could demonstrate that astaxanthin itself and adsorbed to wastewater suspension and particles can be adsorbed onto fish scales. This opens up a chance to use this process for a new technology: (i) the extraction of pigment containing fishery wastewater yielding in a new value-added product—astaxanthin bound scales, (ii) it seems possible for to re-use the processing water, (iii) scale adsorption process technology would minimize the scale waste of fishery industry. More detailed studies are underway in our laboratory to understand the molecular mechanisms of the sorption phenomena, to analyze the scale matrix, to optimize the sorption process and to upscale it towards a technology for wastewater treatment and re-cycling.

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