Observations from the Hydrolysis of the Green Sea Urchin (Strongylocentrotus Droebachiensis)

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Abstract

There is a large amount of co-product generated by the sea urchin fisheries around the world, as well as a growing interest in removing large quantities of undersize and low value sea urchins from barren areas in the northern Atlantic and Pacific coasts. The authors believe there is scope to develop a hydrolysate product from this and this study gives preliminary observations on the characteristics of hydrolysate from the sea urchin *Strongylocentrotus droebachiensis*.

The biochemical composition for *S. droebachiensis* were; water 64.1%, protein 3.4%, oil 0.9% and ash 29.8%. Amino acid composition, molecular weight distribution, lipid-class and fatty acid composition are also presented.

The authors suggest a sensory-panel mapping be undertaken on future sea urchin hydrolysates. Possible uses for the hydrolysate are unclear at this stage but the combination of amino acids and the relatively high levels of Glycine, Aspartic acid, and Glutamic acid should be further investigated.

Introduction

Worldwide the supply of sea urchins is approximately 75,000 t [1]. The best described market for sea urchins is in Japan where 80–90% of the total current global supply is consumed. However, there are also domestic markets in many other sea urchins harvesting countries, for example in Chile, New Zealand and the Philippines. In Europe, the market is also traditional and is mainly in the Mediterranean countries, Italy, France and Spain [1]. The roe (also known as the gonad) of the sea urchin is the edible and therefore valuable part of the sea urchin. This can constitute between 0.1–30 % of the total wet weight of sea urchins and this is referred to as the gonad index (GI). Although the GI can range between < 0.1–30%, traditionally the GI is in the order of 10% in wild fisheries and this would equate to 67,500 ton of sea urchin waste product available for processing from the world catch. Currently, this is either sold as cheap fertilizer or as a bait product or simply disposed of as a waste product. There have been some research efforts made to extract useful compounds such as calcium phosphate from this biomass [2]. Mamelona, et al. [3] also analysed the proximate composition and nutrition of the green sea urchins (as well as the Atlantic sea cucumber) and concluded that the co-products from both species presented high potential for valorisation. Most notably in the production of various value-added products such as protein hydrolysates.

In addition to use of sea urchin co-product from wild harvesting there is growing interest in a number of countries (e.g. USA, Canada and Norway) in reducing the high densities of sea urchins (sea urchin barrens) that are causing significant ecological damage and stopping the regeneration of macroalage [4]. Removal of the sea urchins is considered the only option but in order to make this economically viable there must be some financial incentive to remove urchins with low GI levels (less than 10%) that are not economic to fish for the roe product. There is global interest in harvesting and enhancing sea urchins (i.e. to increase the size and quality of the gonad by feeding manufactured feeds over short periods) but these

urchins must be of market size. Often in areas of sea urchin abundance they are either smaller than the required market size, or they have very little gonad (less than 2% are not viable for roe enhancement) [4]. This study looks at other possible uses of sea urchin, waste or co-product, or harvested sea urchins that are too small for roe enhancement or have too little roe to enhance. The process used is enzymatic hydrolysis, a common approach applied when aiming to valorize co-products. One of the reasons this is a common first process is the ease and readiness of scaling, i.e. from laboratory to a commercial scale. The aim of this study was to test if it is possible to produce a hydrolysis product from the sea urchin Strongylocentrotus droebachiensis and to characterize the resulting hydrolysate.

Materials And Methods

Four samples of *S. droebachiensis* (the green sea urchin) were collected by divers in Tromsø, Norway. An estimated 50 individuals were obtained in each sample with an average test diameter of 41.6 mm (± SE 2.6 mm). Immediately after harvesting, the sea urchins were frozen down to -30 °C and kept frozen until further analysis.

Homogenized raw material was hydrolysed with the addition of water and the commercial enzyme Alcalase 2.4L (Novozymes, Denmark) at 0.1% or 1% v/w compared to raw material. Water and raw material were pooled in a reactor 1:3 w/v and heated to 60 °C before the addition of enzyme. The hydrolysis lasted 2hr at 60 °C followed by enzyme deactivation for 20 min at 90 °C. From the reactor, the mix was immediately separated into insoluble shells and liquid and the liquid was separated in a separator to hydrolysate (water and water-soluble proteins) and sediment (insoluble proteins, cell debris etc.) giving three fractions in total. Sediment and shells were not processed further before analyses, but water was removed from the hydrolysate via spray drying at 180 °C inlet temperature and 80 °C outlet temperature.

The biochemical parameters that were mapped – water, oil, protein and fat, were based on the methods ISO 6496 (water), Bligh and Dyer (fat), NS-EN ISO 5983-2 (protein) and ISO 5984 (ash). Briefly, water-content was measured gravimetrically after incubation at 105 °C in a drying cabinet for 48 h, ash-content was also measured gravimetrically after incubation at 550 °C in a muffle furnace for 24 h, protein-content was estimated using kjeldahl nitrogen determination with a conversion factor (nitrogen to protein) of 6.25. Molecular weight distribution was performed on an Agilent 1200 series HPLC-system with a superdex peptide 10/300 column, 0.5 ml/min flow with 30/70 ACN/H2O and 0.1% Trifluoroacetic acid, free amino acids and total amino acids quantifications were performed on the dried water-soluble hydrolysates with reverse phase chromatography, derivatization and fluorescent detection. Lipid class and fatty acid composition analysis were performed on the raw material and sediment according to AOCS C1 1b-89 by either titration or methyl esterification and detected with a capillary GC-FID. All analyses were performed at the Nofima Biolab facilities in Bergen, Norway.

Results from the four samples were averaged and standard deviation calculated.

Results And Discussion

In this paper, we demonstrate the biochemical parameters of *S. droebachiensis*, hydrolysate, sediment and shells; the amino acid composition and molecular weight distribution of the hydrolysate, and fatty acid- and lipid compositions of *S. droebachiensis* and sediment after hydrolysis. Additionally, two enzyme doses are compared in their recovery rates of protein, oil and ash. This was performed to contribute to the discussion in what commercial use the sea urchins may have as an invasive species necessitating removal.

General observation of the hydrolysate includes the following. After spray drying it had a red colour and the relative amounts of the constituents were water > > protein > ash > oil. Due to spray-drying which removes much of the water, the water content is not reflected well in the tables. A coarse taste test reflected a product that was very different from the hydrolysate of any other product previously tested with a citrus-like taste.

Biochemical composition

Water, oil, protein and ash were measured in all relevant fractions; whole animal, dried hydrolysate, sediment and shell fraction, in order to be able to measure the distribution of each in the different fractions obtained (Table 1). Since two different enzyme-concentrations were applied, an increase in protein recovery could also be detected when increasing the enzyme-concentration from 0.1 % to 1 % (Table 2). The moisture content, i.e. water, is substantial in all fractions (the hydrolysate contained 98–99 % water before drying, Table 1) and typically accounts for up to 70 % of the raw material. The hydrolysate and shells contain more ash than the sediment fraction. The shell fraction is expected to contain high levels of ash as shells typically contain much minerals whereas the hydrolysate is somewhat high.

The biochemical composition of two sea urchin species (Echinometra lucunter and Lytechinus variegatus) was reported by Diniz, et al. [5] and it appears the lipid-content (oil) is much larger in these species at 8% than in *S. droebachiensis* where the lipid content has been approximately 1%.

Haider, et al. [6] studied the biochemical composition of two species of sea cucumber and the results shown are similar to what is presented here.

A study on the composition and amino acid profile of co-products from sea urchin processing plants [3] investigated the urchin digestive tract and non-commercial gonads for proteins, amino acids and fatty acids. The protein contents observed in the digestive tract (processing by-products) were higher than what is presented here for the whole animal (5.3 vs. 3.4%). Gonads displayed a higher protein content. The ash-content varies enormously between the two experiments; where Mamelona, et al. [3] reports ash-contents of 1.6%, the results of this experiment indicates ash-levels of 29.8%. This may be due to the calcareous shell-fraction which is also included in the whole-urchin analyses and presumably excluded

from the digestive tract. This would need to be considered in the use of co-product from the sea urchin fishing industry and/or use of whole small sea urchins to produce hydrolysate.

The moisture and ash content in the gonads of sea urchins has previously been shown to vary with changing diets [7–9].

Table 1
Biochemical composition of all the different fractions: whole animal, dried hydrolysates (0.1 and 1% enzyme), hydrolysis sediment and shell fraction.

	Ash			Oil			Protein			Moisture		
S. droebachiensis	29.8 %	±	6.1 %	0.9 %	±	0.2 %	3.4 %	±	0.3 %	64.1 %	±	4.9 %
0.1% enzyme	33.9 %	±	4.8 %	8.2 %	±	2.0 %	38.8 %	±	0.9 %	5.8 %	±	1.8 %
1% enzyme	31.9 %	±	1.4 %	5.0 %	±	0.4 %	47.4 %	±	1.7 %	3.3 %	±	0.2 %
Sediment	12.7 %	±	3.1 %	6.1 %	±	1.0 %	7.7 %	±	0.6 %	68.0 %	±	3.8 %
Shells	56.0 %	±	2.7 %	0.5 %	±	0.1 %	1.3 %	±	0.1 %	38.0 %	±	2.8 %

Table 2
Difference in recovery of the different fractions in the hydrolysate when increasing enzyme concentration 10-fold.

	Recovery 0.1% enzyme	Recovery 1% enzyme	Recovery increase
Moisture	92.0 %	86.9 %	-5.5 %
Oil	45.7 %	89.9 %	96.8 %
Ash	61.3 %	72.5 %	18.2 %
Protein	37.0 %	91.3 %	146.5 %

Recovery

Enzyme efficacy can manifest itself in the recovery of protein in the aqueous hydrolysate compared to the raw material. Recovery in general is calculated based on a recording of all biochemical parameters (i.e. water, ash, oil and protein) in each step of the hydrolysis. Knowing each fraction's relative contribution to the whole and its content will allow for a tracking of parameters throughout the process. Protein and oil are commonly the two factors that are followed most closely in a commercial perspective due to their role in human consumption and are also the two parameters most commonly affected by change in enzyme

concentration or enzyme type. Recovery parameters were affected by the amount of enzyme used. Oil and protein recovery increased substantially, ash somewhat and water recovery remained at a similar level displaying a slight decrease (Table 2) when enzyme dosage was increased.

Amino Acid Analysis

The amino acid analyses of all four hydrolysate samples were similar. An average of all samples is displayed in Table 3.

Similar amino acids as found in the sea urchin dominate the sea cucumbers: Glycine, Aspartic acid, and Glutamic acid [10]. Sea cucumber is also high in Alanine and Arginine which appear to be lower in *S. droebachiensis*. Glutamic acid, Glycine and Alanine are all known to promote sweet and umami flavor in sea urchin roe [11] and two of the three (Glutamic acid and Glycine) are clearly above average distribution both in the total and free form samples from the current study. In free form, Leucine and Glycine dominate whereas Glycine, Glutamic acid and Aspartic acid dominate in total.

The authors suggest a sensory-panel mapping analysis should be undertaken on future sea urchin hydrolysates. Possible uses for the hydrolysate are unclear without further testing but the unusual combination of amino acids and the relatively high levels of Glycine, Aspartic acid, and Glutamic acid should be further investigated in terms of product placement.

Table 3
Distribution of amino acids both total (bound + free) and free (in solution) in the hydrolysate.

	Total	Free
Amino acid	**g/100 g hydrolysate**	**g/100 g hydrolysate**
Aspartic acid	3.25 (± 0.27)	0.12 (± 0.08)
Glutamic acid	4.95 (± 0.44)	0.41 (± 0.07)
Hydroksyproline	0.36 (± 0.08)	0.02 (< 0.01)
Serine	1.85 (± 0.23)	0.29 (± 0.10)
Glycine	7.98 (± 0.97)	5.05 (± 0.90)
Histidine	0.71 (± 0.05)	0.13 (± 0.04)
Arginine	2.33 (± 0.30)	0.64 (± 0.16)
Threonine	1.73 (± 0.19)	0.30 (± 0.11)
Alanine	1.98 (± 0.24)	0.64 (± 0.14)
Proline	1.40 (± 0.27)	0.23 (± 0.16)
Tyrosine	1.14 (± 0.12)	0.56 (± 0.09)
Valine	1.70 (± 0.14)	0.46 (± 0.09)
Methionine	0.92 (± 0.06)	0.47 (± 0.08)
Isoleucine	1.43 (± 0.11)	0.41 (± 0.10)
Leucine	2.33 (± 0.18)	1.25 (± 0.17)
Phenylalanine	1.30 (± 0.07)	0.75 (± 0.08)
Lysine	2.18 (± 0.29)	0.61 (± 0.07)

Lipid class and fatty acid analysis

The sediment and raw material were subjected to lipid class and fatty acid analyses (Tables 4 and 5). In both samples the lipid class triacylglycerol is most abundant followed by free fatty acids and cholesterol. In total neutral lipids account for 50.15 and polar lipids 1.15 g/100 g extracted fat. Of the typically marine fatty acids (EPA, DPA and DHA), the EPA is most abundant. Other fatty acids of notable amounts compared to the mean are 14:0, 16:0, 18:1 and 20:4 n-6. These analyses were only performed on two samples.

Haider, et al. [6] presented similar distribution of polyunsaturated and monounsaturated fatty acids, in addition to the ratios between the n-3 and n-6.

Table 4
Lipid classes in raw material and sediment. Average of two samples, all amounts are g/100 g extracted fat (Following lipid classes had readings of zero: Diacylglycerol, Monoacylglycerol, Cholesterol esters, Phosphatidylinositol, Phosphatidylserin, Phosphatidylcholin and Lyso-Phosphatidylcholin).

Lipid class/fatty acid	Raw material		Sediment	
Triacylglycerol	32.5	± 5.5	25	± 3
Free fatty acids	10.45	± 1.55	7.7	± 1.2
Cholesterol	6.55	± 0.35	6.9	± 0.5
Phosphatidyletanolamin	1.15	± 0.05	2.9	± 1.6
Total polar lipids	1.15	± 0.05	14.35	± 2.55
Total neutral lipids	50.15	± 7.55	40.4	± 3.3
Total sum lipids	51.25	± 7.45	54.65	± 5.85

Table 5
Fatty acids in raw material and sediment. Average of two samples, all amounts are g/100 g extracted fat.

Fatty acid	Raw material		Sediment	
14:0	5.25	± 0.25	4.3	± 0.1
16:0	7.35	± 0.15	6	± 0.1
16:1 n-7	1.8	0	1.45	± 0.05
16:2 n-4	0.1	0	0.1	0
16:3 n-4	0.2	0	0.2	0
18:0	1.45	± 0.05	1.25	± 0.05
18:1 (n-9)+(n-7)+(n-5)	5.6	± 1.1	3.5	± 0.1
18:2 n-6	1.6	± 0.1	1.05	± 0.15
18:3 n-3	1.15	± 0.15	0.8	± 0.2
18:3 n-6	0.2	0	0.15	± 0.05
18:4 n-3	4.45	± 0.05	5.05	± 0.65
20:0	0.3	± 0.1	0.25	± 0.05
20:1 (n-9)+(n-7)	4	± 0.4	3.7	± 0.6
20:2 n-6	1.15	± 0.25	1	± 0.2
20:3 n-3	1.05	± 0.35	0.95	± 0.35
20:3 n-6	0.35	± 0.05	0.25	± 0.05
20:4 n-3	0.65	± 0.35	0.5	± 0.2
20:4 n-6	5.4	± 0.1	5.6	0
20:5 n-3 EPA	8.05	± 2.05	8.25	± 2.15
21:5 n-3	0.1	0	0.1	0
22:0	0.1	0	0	0
22:1 (n-11)+(n-9)+(n-7)	1.8	± 0.3	1.45	± 0.35
22:4 n-6	0.1	0	0.1	0
22:5 n-3 DPA	0.2	0	0.15	± 0.05
22:6 n-3 DHA	0.75	± 0.25	0.6	± 0.2

Fatty acid	Raw material		Sediment	
24:1 n-9	0.15	± 0.05	0.1	0
Sum saturated fatty acids	14.45	± 0.55	11.8	± 0.1
Sum monoenoic fatty acids	13.35	± 1.85	10.2	± 0.9
Sum total-PUFA fatty acids	25.5	± 3	24.8	± 3.9
Sum PUFA (n-3) fatty acids	16.4	± 2.7	16.35	± 3.45
Sum PUFA (n-6) fatty acids	8.8	± 0.3	8.15	± 0.45
Sum identified fatty acids	53.3	± 1.7	46.8	± 3.1
Sum unidentified fatty acids	18.95	± 1.75	17	± 0.9

Size exclusion chromatography

To give a general view of the size distribution of the peptides in the hydrolysate a size exclusion chromatography with a gel filtration column was performed on all hydrolysates. The results were quite similar on all and are presented as mean and standard deviation (Fig. 1). The distribution is based on a standard curve made from known compounds. It appears that most of the sample consists of the smallest range of proteinaceous compounds– from single amino acids to tripeptides.

Conclusion

This work aims at targeting some of the challenges connected to the usage of sea urchins not well fitted for the established gonad markets. In this respect biochemical data, amino acid composition, lipid and fatty-acid composition in addition to size exclusion chromatography have been presented.

Declarations

Author contributions

PJ and RGS wrote the paper

PJ and RGS analysed the data

PJ collected samples

RGS performed experiments

Additional information

The authors declare no competing interests.

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Figures

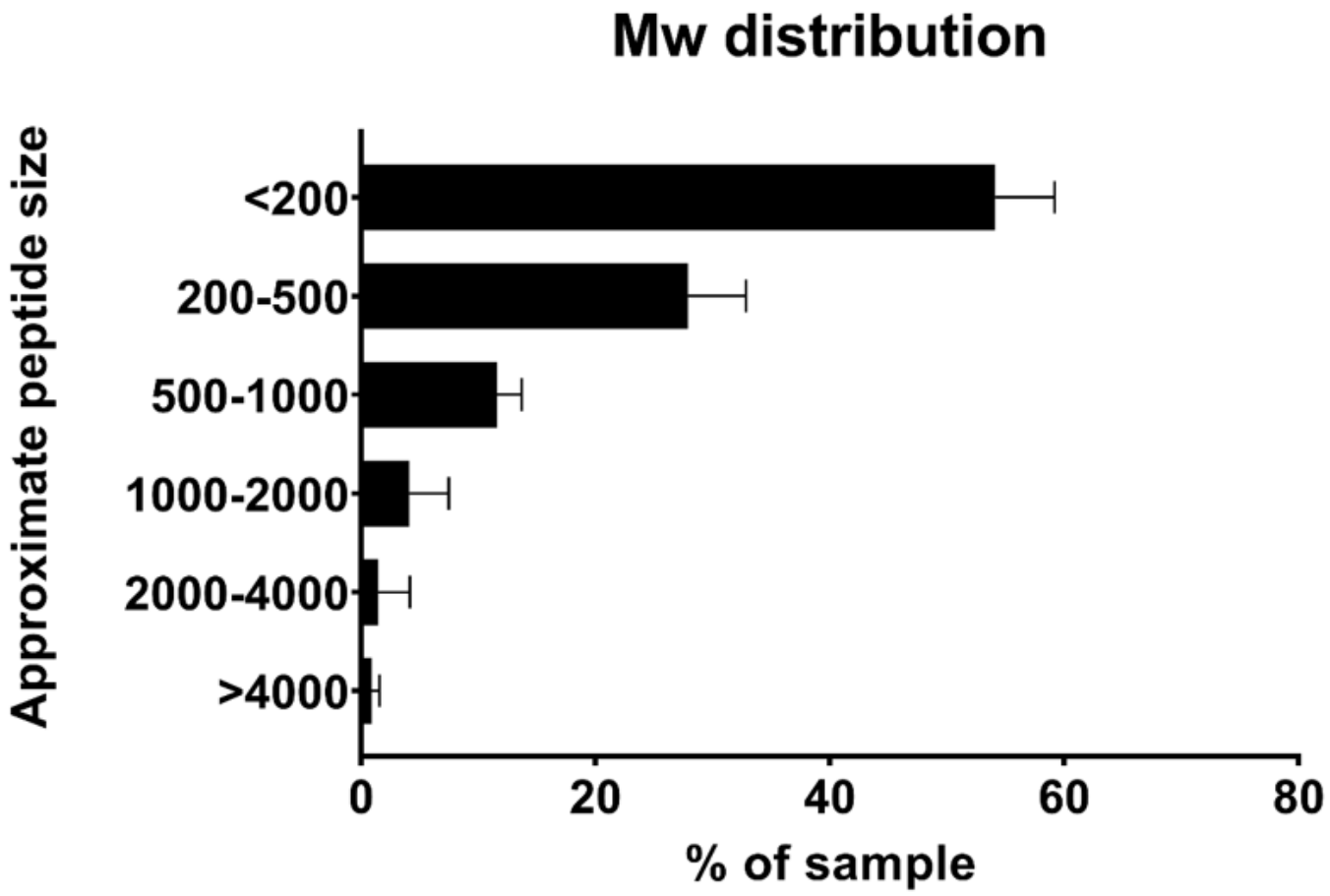

Figure 1

Mw distribution of the peptides in the hydrolysate. The approximate peptide sizes indicate that ~50% of the peptides contain no more than 2-3 amino acids. Average of four hydrolysates.