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A comparative analysis of lipid and carotenoid composition of the gonads of *Anthocidaris crassispina*, *Diadema setosum* and *Salmacis sphaeroides*

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ABSTRACT

In this study, sea urchins *Anthocidaris crassispina*, *Diadema setosum* and *Salmacis sphaeroides* inhabiting the coastal area of Hong Kong were collected and their gonadal biochemical compositions determined and compared for the first time. The proximate nutritional composition of all species exhibited an order of lipid > protein > carbohydrate. Neutral lipid was the major lipid constituent, accounting for over 80% of the total lipid. The major fatty acids in neutral lipid fraction of the three urchins were C14:0, C16:0, C16:1 (*n*-7), C18:1 (*n*-7) and C20:5 (*n*-3), whereas those in the polar lipid fraction were C16:0, C16:4 (*n*-4), C20:4 (*n*-6) and C20:5 (*n*-3). The dominant carotenoid was echinenone, which accounted for 81.7%, 56.7% and 68.5% of the total carotenoids in *A. crassispina*, *D. setosum* and *S. sphaeroides*, respectively.

1. Introduction

Sea urchin gonads are exploited as a delicacy around the world. This market value varies and is highly dependent on the market demand as well as size and quality of their gonads (Lawrence, 2007). In general, the gonad quality is determined by its taste, colour, texture and firmness, which are greatly influenced by the biochemical composition i.e. lipid, carbohydrate, carotenoid and protein contents (Fernandez, 1997; Lawrence, 2007). Carotenoids, especially echinenone are the major contributor of the bright yellow-orange colour of sea urchin gonads (Shpigel, McBride, Marciano, Ron, & Ben-Amotz, 2005). Carbohydrate and protein contents greatly affect the texture and firmness, whereas protein and lipid contents in general determine the flavour (Siikavuopio, Dale, & Carlehog, 2007).

Sea urchin gonads are rich in valuable bioactive compounds, such as polyunsaturated fatty acids (PUFAs) and β-carotene (Dincer & Cakli, 2007). PUFAs, especially eicosapentaenoic acid (EPA, C20:5 (*n*-3)) and docosahexaenoic acid (DHA, C22:6 (*n*-3)), have significant preventive effects on arrhythmia, cardiovascular diseases and cancer (Pulz & Gross, 2004). β-Carotene and some xanthophylls have strong pro-vitamin A activity and can be used to prevent tumour development and light sensitivity (Britton, Liaaen-Jensen, & Pfander, 2004). The composition of these valuable components, however, varies greatly among different urchin species and is influenced by their natural diet as well as physiological processes i.e. reproductive stage (Fernandez, 1997, Fernandez, 1998; Lawrence, 2007).

In Hong Kong, three common species of sea urchin – green urchin *Salmacis sphaeroides*, long-spined urchin *Diadema setosum* and purple urchin *Anthocidaris crassispina*, inhabit the shallow subtidal waters (Morton & Morton, 1983). *A. crassispina* has been the traditionally exploited species (Chiu, 1986). The other two species of sea urchin have also been found in local seafood restaurants in recent years. Despite the commercial value, their biochemical compositions have not been reported. The aim of the present study, therefore, is to determine the biochemical composition of these three urchin species. The results will not only enhance our understanding of the nutritional values of these three common sea urchins, but also provide essential scientific data for the further exploration of aquaculture of these commercially important species in the water areas of Hong Kong and southern China.

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2. Materials and methods

2.1. Sample collection and preparation

Sea urchins were collected by SCUBA diving during their maturation period, because mature sea urchins have large gonads and thus are rich in nutritional components (Fernandez, 1998). Their natural food, macroalga *Sargassum hemiphyllum*, was also collected. *A. crassispina* and *D. setosum* were collected from the Ninepin Island (22°15′N, 114°21′E), and *S. sphaeroides* was collected from the Shelter Island (22°19′N, 114°17′E). All collected sea urchins were transported to the laboratory in natural seawater. Large sized sea urchins were selected for analysis (*A. crassispina* >40 mm, *D. setosum* >45 mm and *S. sphaeroides* >50 mm). Before dissection, the test diameters were measured using Venier calipers. After dissection, the total wet weight and gonad wet weight were determined accurately. The gonad index was determined as gonad wet weight as percentage of the total wet weight. The gonads from every 15 urchin individuals randomly selected from each species were pooled together and regarded as one group. Three groups of gonad samples from each species were used for analysis. The gonads in each group were freeze-dried, ground to fine powder, mixed well and stored at −20 °C for further analysis.

2.2. Determination of carbohydrate and protein contents

The gonadal carbohydrate content was determined using the phenol–sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). Protein contents were determined using the protein–dye binding method (Bradford, 1976).

2.3. Lipid and fatty acid analyses

Total lipids were extracted from 200 mg of lyophilized samples according to the modified Folch procedure, and subsequently separated into neutral lipids and polar lipids using solid-phase extraction (Chen, Jiang, & Chen, 2008). Fatty acids were transmethylated with sulphuric acid in methanol. The fatty acid methyl esters were detected using a HP-6890 gas chromatography (Hewlett Packard Company, Wilmington, Delaware, USA) equipped with a HP7673 injector, a flame-ionization detector and a HP-INNOWAX™ capillary column (HP 19091N-133, 30 m × 0.25 mm × 0.25 μm) (Chen et al., 2008). The inlet and detector temperatures were set at 250 °C and 270 °C, respectively. The temperature was programmed from 170 °C to 230 °C with an increment of 1 °C/min. High purity nitrogen gas was used as carrier gas. The fatty acid methyl esters were identified by comparison of their retention times with those of the authentic standards (Sigma). Hexacosanoic acid (C23:0) was used as internal standard.

2.4. Carotenoid analyses

The gonadal carotenoids were extracted with 3 mL acetone. The extraction procedure was repeated several times until the samples turned white. The extracted carotenoids were resuspended in 1 mL acetone and stored at −20 °C prior to HPLC analysis (Haug, Guillou, Connan, Goulard, & Diouris, 2003). The carotenoid profiles were analyzed using a Waters 2695 Separation Modules HPLC (Waters, Milford, Massachusetts, USA) equipped with a photodiode array detector (Waters 2996) and a reverse phase column (Waters Spherisorb 5 μm ODS2, 4.6 × 250 mm) (Huang, Liu, Li, & Chen, 2008). The mobile phase consisted of solvent A (acetonitrile–methanol–0.1 M Tris–HCl (84:2:14, v/v), pH 8.0) and solvent B (methanol–ethyl acetate (68:32, v/v)) at a gradient of 0–100% solvent B for 0–15 min, 100% solvent B for 15–25 min and 100–0% solvent B for 25–28 min. The flow rate was 1.2 mL/min. The sample injection volume was 40 μL. Chromatographic peaks were identified by comparing the retention times and spectra against those of authentic standards. Quantification of individual carotenoids was carried out using calibration graphs obtained from its authentic standard (Sigma Company, Milwaukee, WI, USA).

2.5. Calculation of energetic values

The energetic values of the gonads were calculated by multiplying the level of each organic constituent by its energy equivalent (1 g carbohydrate = 17.15 kJ, 1 g protein = 23.64 kJ, and 1 g lipid = 39.54 kJ) and by summarizing the energetic values of all the constituents (Brody, 1945).

2.6. Statistical analysis

All data were expressed as mean ± standard deviation (SD) of triplicate. Differences in each nutritional parameter among sea urchin species were detected using one-way analysis of variance (ANOVA), followed by multiple comparisons using the least significant difference (LSD) at $p < 0.05$ by the Statistical Analysis System (SAS Institute, Inc., Cary, NC, USA).

3. Results and discussion

3.1. Gonad index

The gonad index, total wet mass and test diameter were significantly different among the three species (Table 1). The gonad index was highest in *D. setosum* (11.9%) and lowest in *S. sphaeroides* (4.5%). The gonad index of *D. setosum* in this study was higher than that of the same species collected from Kubbar island reef of Kuwait (1.5–5.6%) (Alsaffar & Lone, 2000). *A. crassispina* has a wide geographical distribution from coastal areas of Japan to southern China. In this study, the mean wet weight and gonad index of *A. crassispina* (46 mm test diameter) was 50.3 g and 7.6%, respectively. The weights of *A. crassispina* (38.8–60.5 g) were similar with the similar sized individuals (40.9–48.4 mm test diameter) of the same species collected from Wakasa bay in Japan, but were lower (90.0–128.6 g) than the larger individuals collected from the same area (55.6–64.6 mm test diameter) (Yatsuya & Nakahara, 2004). The differences might be due to the variations in environmental conditions such as habitats, water temperature and diet (Cook, Bell, Black, & Kelly, 2000).

3.2. Proximate biochemical composition

The biochemical composition of all three species shows a descending order: lipid > protein > carbohydrate (Fig. 1). Lipid was the most abundant component, ranging from 135.8 mg/g dry gonad in *S. sphaeroides* to 300.6 mg/g dry gonad in *A. crassispina*. Over 80% of their total lipid was neutral lipid. In the gonads of other sea urchins i.e. *Strongylocentrotus droebachiensis*, *Psammechinus miliaris* and *Paracentrotus lividus*, lipid was also the dominant

Table 1
Size, weight and gonad index of three species of sea urchin.

Urchin species	Average diameter (mm)	Average weight (g)	Gonad index (%)
Anthocidaris crassispina	46.1 ± 2.3[c]	50.3 ± 7.7[c]	7.6 ± 2.0[b]
Diadema setosum	66.1 ± 4.0[a]	142.9 ± 23.9[a]	11.9 ± 2.5[a]
Salmacis sphaeroides	56.8 ± 3.8[b]	83.4 ± 18.1[b]	4.5 ± 1.8[c]

Data marked with different lowercase letters in the same groups were significantly different ($p < 0.05$).