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ORIGINAL ARTICLE

Proximate and Mineral Composition of the Long-Spined Sea Urchin (*Diadema setosum*) Roe

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ABSTRACT - Gonad of sea urchin is considered as food delicacy in many parts of the world due to high nutrition and mineral contents. Several species of sea urchins are available in Malaysia and reported from different habitat, including seagrass beds, coastal breakwater, coral reefs and other ecologically important habitats. The current study was undertaken to evaluate the proximate and mineral composition of long spined sea urchin (*Diadema setosum*) roe collected from breakwater of Terengganu, Malaysia in October 2019, November 2019 and January 2020. The samples were analysed for proximate including protein, lipid, moisture, and ash content of sea urchin roe. The macro, micro minerals and heavy metals were also evaluated including Ca, Fe, Zn, Cu, Co, Se, Mg, Ni, Pb, Al, and Cd. The result suggested that, long spined sea urchin (*Diadema setosum*) roe can be considered as good sources of food due to high percentage of protein (ranged between 36.21±0.44 and 50.14±4.63). The presence of heavy metal such as Ni, Pb, and Cd suggesting the breakwater environments were not good enough and the possible sources of heavy metals contamination in sea water environment must be stopped and eradicated. This study provides important information with regard to the proximate and mineral values of *D. setosum*, as well as the need for a better management of its habitat before *D. setosum* can be widely promoted as delicacy in this region.

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INTRODUCTION

Coastal changes such as erosion poses potential risk to coastal communities across the world [1],[2],[3]. Evidence suggests that the coastline of Terengganu, Malaysia is among one of the most affected areas that experienced severe erosion [4]. Since the expansion of the Sultan Mahmud International Airport (LTASM) runway in 2011 [5], coastal erosion has been exacerbated and imposed a greater problem to the local communities in the nearby region, Kuala Nerus. In order to provide a defense to the coast, several breakwaters have been constructed parallel to the shoreline since 2016 to reduce the impacts of waves and longshore drift [5]. In addition, a better outcome is anticipated from this construction by mean of the enhancement of the biodiversity of coastal marine ecosystems [6]. Generally, studies on the abundance and species diversity of organisms following breakwater construction are focusing on commercial organisms such as fishes [7],[8],[9]. However, such studies have also been carried out on bottom-dwelling organisms such as sea urchin [10][11] considering the commercial value of this invertebrate.

Sea urchin gonad or roe is a well-known nutritious delicacy that provide an economic value in certain parts of the world [12],[13],[14]. Currently, the global captures for sea urchin reached 2,6575 tonnes in

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2016. Japan appeared to the highest-produced country of sea urchin products with 75% of the total imports [15].

In Malaysia, previous studies have highlighted the abundance and distribution of sea urchin inhabit Malaysian waters [16],[17],[18],[19]. Twelve tropical species that have been documented in Malaysia waters, such as *Diadema setosum*, *D. savignyi*, *Echinometra mathaei*, *Astropyga radiata*, *Toxopneustes pileolus*, *Echinothrix calamaris*, *Echinothrix diadema*, *Parasalenia gratiosa*, *Salmacis sphaeroides*, *Pseudoboletia maculata*, *Tripneustes gratilla* and *Salmaciella dussumieri* [19],[20]. The most abundance sea urchin available in Peninsular Malaysia is *D. setosum*. Unlike the ecological status, information on nutritional quality such a protein and trace element composition of *D. setosum* are still scarce. However, the nutrient composition of other species such as *Stomopneustes variolaris* [21],[22], *Paracentrotus lividus* [15] and *Echinometra vanbrunti* [23] have been studied to ensure their suitability for human consumption. From the health point of view, sea urchin roe has high nutritional value as compared to other seafoods which consists of mainly, protein, carbohydrates, vitamins and minerals [24],[25],[26],[27]. In Malaysia, *D. setosum* is still under-consumed, mainly because it is not part of local gastronomic culture except in some local villages in Sabah. In contrast, commercial harvesting of this species is increasing in other countries [28],[29].

The construction of breakwater in Kuala Nerus district created a new habitat with different characteristics and functions. These new habitats have in turn, increased the population of sea urchin [10]. The emergence of *D. setosum* in the breakwater areas offer a new opportunity to be explored in terms of seafood product. Whilst the suitability of *D. setosum* for human consumption is still unknown, a study on the nutrient compositions of this species is imperative before in can be commercialized.

The aim of the present study, therefore, is to determine the proximate and element composition of *D. setosum* collected around the breakwater in Kuala Nerus coasts. Findings from this study will provide an important baseline information on the proximate value of *D. setosum*. In addition, due to its characteristics such as sedentary, tolerant to pollution and grazing-feeder [30] the present understanding the proximate values of *D. setosum* will provide more insights on the safety of seafood products.

MATERIALS AND METHODOLOGY

Collection and preparation of specimens

The sea urchins were collected around Tok Jembal breakwater area located in Kuala Nerus district, Terengganu, Malaysia (Figure 1) in October 2019, November 2019 and January 2020. Three individuals of sea urchins were collected by hand from the same locality at the same low tides in each month, making altogether 9 individuals of this organism collected. All specimens were transported to the laboratory in 10 L bucket and processed within 4 h of collection. Sea urchin roe, the important part for human consumption was used for all analyses. The shells were opened, and gonads removed. The weight of the roe was recorded using an analytical balance with precision of up to 0.001 g (And, HR-250AZ). Individual roe was divided into four pieces for proximate (moisture and ash, lipid and protein) and trace elements analysis.

Proximate analysis

Moisture content analysis

Moisture content was calculated based on the percentage of weight loss after drying to a constant weight at 60°C for a minimum 48 h in a heat oven (Memmert, UFB500). To determine the ash content, the dry samples were weighed and transferred to a muffle furnace (Carbolite, ELF1123) at 600°C for 24 h and the ash content was calculated as a percentage value [31].

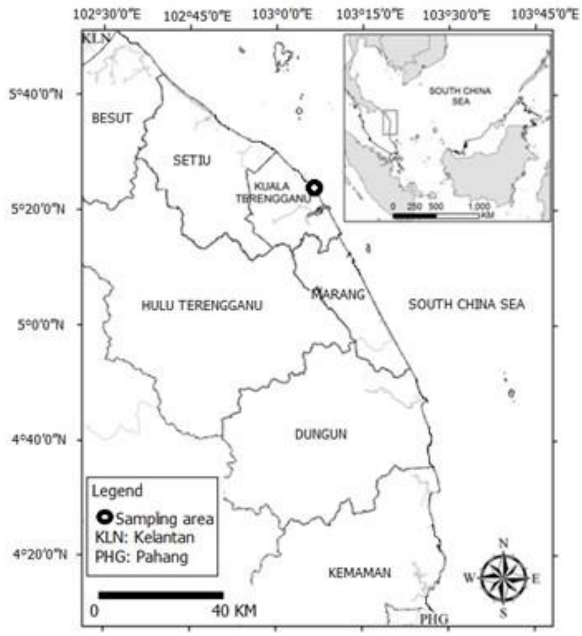

Figure 1. Map of the sample collection in Tok Jembal breakwater.

Protein analysis

Composite samples of urchin used to analyze protein content by taking 0.5 g of homogenized samples were taken. The protein content was determined according to the [31]. The process started by, taking the hydrolyzed sample with 5 ml concentrated sulphuric acid (h_2so_4) and adding one tablet of kjedahl in the micro kjedahl tube at 420°c for 1 h. After cooling, h2o was added to the hydrolysates before neutralization and titration. The amount of total nitrogen in the raw material was multiplied with a traditional conversion factor of 6.25 to determine the e total protein content. Protein content was expressed as a % of the dry sample weight.

Lipid content

Lipids were extracted using a Soxtec apparatus, as described by [32]. Samples were heated with 50 ml petroleum ether at 90°C for 1 hour. The extracted lipid was removed from the apparatus at 100°C in the oven. The lipid content was expressed as a % of the dry sample weight.

Analysis of mineral composition

For mineral analysis, 0.5 g samples of urchin-hins roe were dissolved in HNO_3 and subjected to acid digestion procedure US EPA Method, 1994 [33]. Mineral concentrations were analysed by inductively coupled plasma mass spectrometry (ICP-MS).

Statistical analysis

One-way ANOVA (analysis of variance) was performed using the SAS 9.4 software for Windows (version 9.4; SAS Institute Inc., 2012, Cary, NC, USA) [34], while tukey-test was performed to determine the mean comparison and significance level among different sampling time. A multivariate analysis by mean of Principal component analysis (PCA) was performed on mineral compositions of sea urchin roe. This ordination was measured usingthe Euclidean distance matrix (dissimilarity) to determine the similarity between months. All the multivariate analysis was performed using PRIMER v6 [35].

RESULTS AND DISCUSSION

The ash content was found significantly ($p < 0.0001$) higher in October (10.89±0.18%), followed by December (5.72±0.14%), and January (5.37±0.25%) (Figure 2). In contrast, no significant difference was observed in moisture content during sampling periods. Meanwhile, the lipid content was significantly ($p = 0.0007$) higher in October (26.18±1.24%), followed by January (20.87±1.02%), and December (20.17±0.80%) (Figure 2). The protein content of sea urchin roe was found significantly ($p = 0.0016$) higher in January (50.14±4.63 %), followed by October (38.60±0.61%), and December (36.21±0.44%) (Figure 2).

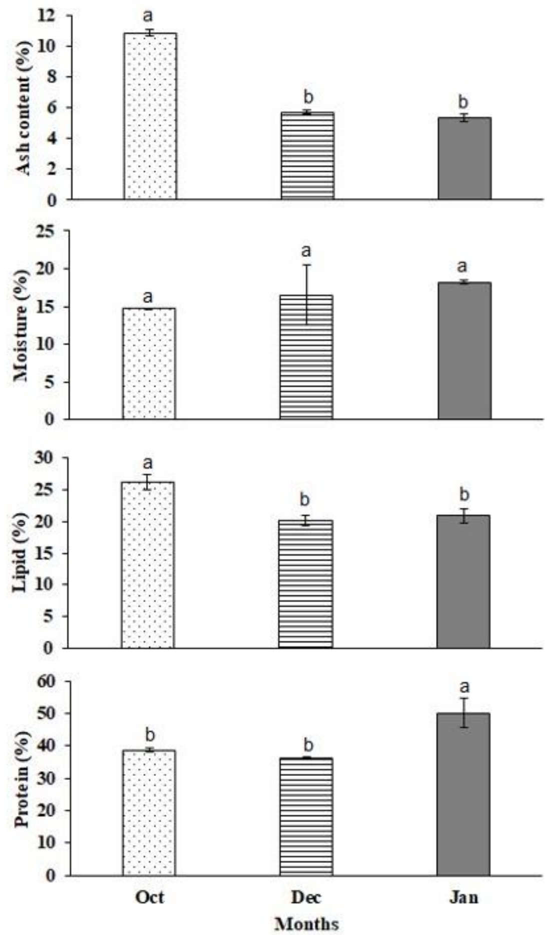

Figure 2. Proximate composition of sea urchin roe for three different months. Data expressed in mean ± standard error

The zinc (Zn) content of sea urchin roe was significantly ($p = 0.0048$) higher in December (57.27±5.85 µg/Kg), followed by October (39.83±5.84 µg/Kg), and January (30.53±6.60 µg/Kg). The cobalt (Co) content was significantly ($p = 0.0037$) higher in October (0.13±0.01 µg/Kg), followed by December (0.09±0.02 µg/Kg), and January (0.06±0.00 µg/Kg). The selenium content of sea urchin roe was also temporally significantly different ($p = 0.0027$) with the highest recorded in December (1.98±0.22 µg/Kg), followed by October (1.38±0.22 µg/Kg) and January (0.69±0.33 µg/Kg). The highest manganese content was recorded in October with 11.37±2.47 µg/Kg, which was significantly higher ($p = 0.0027$) than in January (4.72±0.84 µg/Kg) and December (3.86±1.19 µg/Kg). October also recorded the highest cadmium (Cd) content with 0.23±0.05 µg/Kg. This was significantly higher ($p = 0.0018$) than in December (0.13±0.03 µg/Kg), and January (0.05±0.01 µg/Kg) (Table 1). Meanwhile, no significant difference between months was recorded for calcium, iron, copper, nickel, lead, and aluminum concentration. The differencein mineral composition between months is graphically presented in the Principal Component Analysis (PCA) (Figure 3).

Table 1. Mineral composition of the sea urchin roe in three different months

Elements (µg/Kg)	October	December	January	*p*-value
Ca	538.00±185.07	784.00±758.72	401.00±136.96	0.6091
Fe	158.67±36.25	114.63±42.11	112.37±15.48	0.2377
Zn	39.83±5.84	57.27±5.85	30.53±6.60	0.0048*
Cu	184.57±157.23	55.00±77.20	15.59±12.14	0.1833
Co	0.13±0.01	0.09±0.02	0.06±0.00	0.0037*
Se	1.38±0.22	1.98±0.22	0.69±0.33	0.0027*
Mn	11.37±2.47	3.86±1.19	4.72±0.84	0.0027*
Ni	1.25±0.25	0.82±0.06	0.90±0.30	0.1108
Pb	0.33±0.08	0.29±0.14	0.18±0.06	0.2367
Al	48.37±7.83	43.47±13.50	87.53±91.38	0.5735
Cd	0.23±0.05	0.13±0.03	0.05±0.01	0.0018*

*Indicate significant difference $p < 0.05$; all data presented based on fresh weight. Values are mean value and ± SD: n, the number of samples.

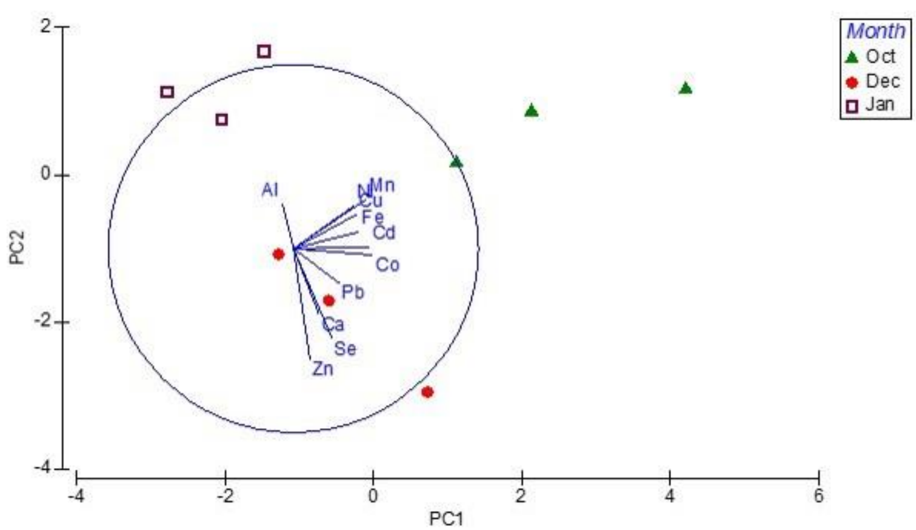

Figure 3. A PCA ordination plot of the difference between months according to the trace element composition. The difference is based on the Euclidean distance

The PCA shows that all samples were different in terms of trace elements composition. This is evidenced by the clear cluster of samples according to months. However, the influence of each element on contributing to the difference is somewhat low, judging from the short vector lines, except for Zn and Se. Samples in October are separated from December largely on PC_2, in the direction of increasing Mn, Ni, Cu, Fe, Cd and Pb, while decreasing levels of Zn, Se, Ca, and Pb, where these elements were recorded higher in December to October. The difference in C2 is also shown between samples in December and January. December recorded a higher content of Zn, Se, Ca and Pb while the only element with higher proportion recorded in January was Al. This element (Al) also determined the difference between October and January (as seen on PC1). The distribution of samples based on trace elements composition was also influenced by the weight of the sea urchins (Figure 4). Samples that were influenced by Zn, Se, Ca and Palso corresponded to the higher weight.

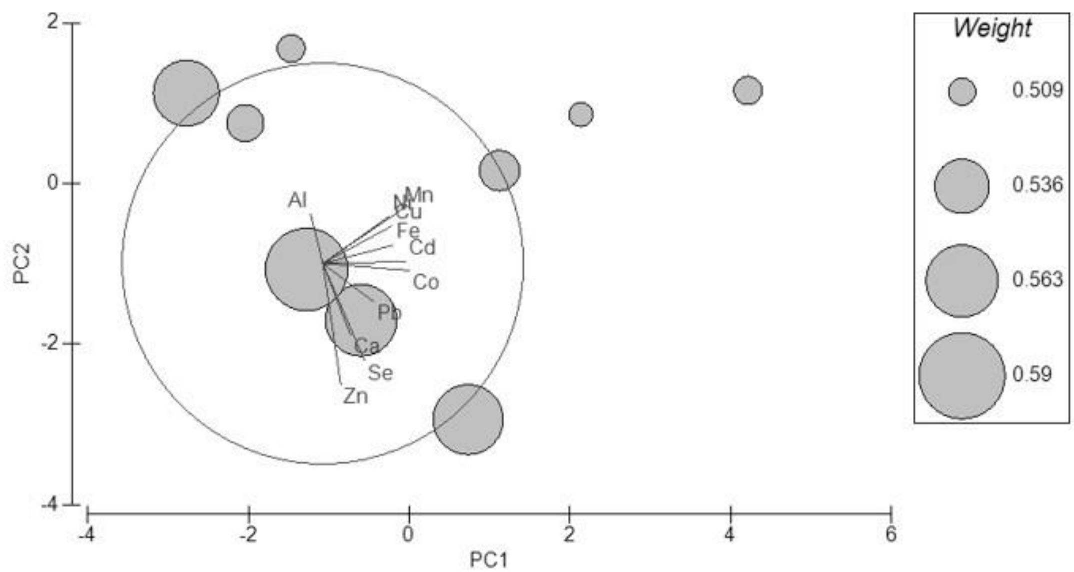

Figure 4. A PCA ordination plot of the trace elements composition with super-imposed samples weight of each sample

DISCUSSION

Evaluation of the proximate analysis of the sea urchin roe indicates that this species has high protein, comparable to other commercial sea urchins from other parts of the world. This study also provides the trace element composition available in the sea urchin roe. The main outcomes of this study are consistent with h previous study on sea urchin showing high protein [21],[36], with low lipid content [25],[37],[38] and, rich in essential elements such as Fe and Zn [39],[40].

The ash content of *D. setosum* ranged between 5.3% and 10.9% in the current study was also in accordance to the previous studies on the sea urchin roe. [41], found 8% of ash in gonadal parts of *D. setosum* collected in the northeast Kenya. However, a lower range was recorded in the offshore waters on off Nagasaki ranging from 1.8 to 2.6 % [42]. Study on other species of sea urchin by [21] reported the ash content of *Stomopneustes variolaris* might reach up to 3.76% ± 0.25% in Indian waters. The moisture content of *D. setosum* roe ranged between 14.7 and 18.2% in the present study was consistent with the study of Kaneko36 where the ash content varied between 63.9 and 76.4%. Meanwhile different species *Stomopneustes variolaris* contained 69.3% [25] and 77.5% ± 0.80% of moisture in gonad [21]. Nevertheless, the food quality available in the environment could influence the proximate composition of the urchins [43].

Protein plays a vital role as it serves as an energy supply, particularly during reproductive activity [26]. The protein content of the *D. setosum* (ranged between 36.2% and 50.1%) in the present study was on the higher end of the range recorded in previously studied sea urchins [36] found 187.5 mg/g of protein in *D. setosum* from Hong Kong coast, whereas [42] reported the gonadal protein of the *D. setosum* might be ranged between 14.1 and 17.0% from Nagasaki, Japan coast. The other species of sea urchin, *Stomopneustes variolaris* recorded an average of 12.1% of the protein content from the Indian water [21]. Protein is the main constituent of the sea urchin roe which possibly can be as an alternative's sources instead of other seafood supplements.

After protein, lipid was the next dominant organic component of the urchins. Lipid content of *D. setosum* ranged between 20.2% and 26.2% in the present study. This supported by previous study on the lipid content of *D. setosum* collected from central Vietnam was found to be 25.0% [44]. On the other hand, previous research observed a lower lipid content on the urchins available in Nagasaki and Kenya [42] and Kenya [41]. Overall, *D. setosum* in the present study recorded a comparable content of lipid to the other sea urchin species such as *Paracentrotus lividus, S. variolaris and Evechinus chloroticus* [21],[25],[37],[38]. However, by comparison the lipid content in the present study was lower than protein, which was in contrast with study by [36] and [40] where the lipid was consistently higher in composition compared to the protein.

In general, mineral compositions of *D. setosum* in the present study were within the recommended daily intake [45] (Table 2), except for Zn which recorded a much higher range. The possible explanation is that the Zn content is highly fluctuate especially among samples taken at different time or environmental conditions [39],[40]. Most of minerals contained in *D. setosum* in the present study were also comparable to previous studies [39][25][40]. In addition, the findings from the present study were also consistent with previous studies on other sea urchin species such as *P. lividus, S. variolaris and E. chloroticus* [15],[21],[30],[46].

As for the multivariate perspective on mineral compositions, temporal variation seemed to affect the mineral compositions, and this is conformed to the findings in the previous study [40]. The variation was fairly apparent for Al where this mineral was highly corresponded to January while other minerals were more associated to October and December.

This contrast composition might be due to the uptake of aluminium in aquatic organisms essentially increases when the water hardness decreasing, such as in the condition low dissolve minerals in the water [47],[48]. The aluminium composition in the present study was generally higher when the other minerals were lower, and vice versa when the other minerals were higher. Similarly, [49] also found out that in the presence of zinc and copper, the aluminum content in flagfish *Jordanella flowidae* was generally low.

Table 2. Recommended Daily Intake (RDI) and Tolerable Upper Intake (TUI) of nutrients based on Malaysian standard [45]

Elements	Recommended Daily Intake	Tolerable Upper Intake
Calcium (Ca)	1000 mg	2500 mg
Magnesium (Mg)	320 mg	350 mg
Iron (Fe)	1.3 – 2.94 mg	4.7 – 5.5 mg
Zinc (Zn)	4.3 – 6.2 mg	35 mg
Copper (Cu)	900 µg	1000 µg
Manganese (Mn)	1.8 – 2.3 mg	11 mg
Selenium (Se)	24 µg	400 µg
Cadmium (Cd)	1 µg	NA
Lead (Pb)	3.57 µg	NA

CONCLUSION

This study revealed the high proximate and mineral compositions of sea urchin *D. setosum* roe, which can be considered as one the major nutritional sources. This study also found the presence of heavy metals in the *D. setosum* roe, which suggest that environment is moderately polluted and *D. setosum* can be used as the bio-indicator for marine habitats. To better understand the environmental variation and its effects on the mineral uptake by organisms, it is recommended that this type of study be expanded for a longer spatial scale across different monsoon seasons.

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