African Journal of Biotechnology Vol. 10(62), pp. 13610-13616, 12 October, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2056
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Chemical constituent analysis of the crown-of-thorns starfish *Acanthaster planci* and potential utilization value of the starfish as feed ingredient for animals

Peng Luo, Chao-qun Hu*, Jian-jun Xia, Chun-hua Ren and Xiao Jiang

Key Laboratory of Marine Bio-resources Sustainable Utilization, CAS, South China Sea Institute of Oceanology, Guangzhou 510301, China.

Accepted 7 September, 2011

The crown-of-thorns starfish *Acanthaster planci* is a major management issue on coral reefs and the exploring of effective control methods to the starfish is an interesting goal. In this study, the chemical constituent of the starfish were analyzed and the toxicity of the starfish was tested when it was used as mice diet. The results showed that protein content of the starfish was 19.8 to 22.0% of dry weight and the amino acid composition was similar to that of fish meal. Though the starfish had little fatty acids (<1%), the fatty acids contained rich variety and unsaturated fatty acids on average accounted for more than 60% of total fatty acids. In addition, per gram (dry weight) of the starfish contained 65.4 to 97.4 µg astaxanthin, which was higher than that of shrimps. The starfish used as the feed for mice did not have negative influence on the growth and the health of the mice. Based on these results, we consider that the crown-of-thorns starfish *A. planci* has the potential to be an ingredient for animal feeds, thus reducing the usage of fish meal, fish oil and carotenoids. Hence, a method for resource utilization and control of *A. planci* was suggested.

Key words: Chemical constituents, *Acanthaster planci*, astaxanthin, resource utilization, feed ingredient.

INTRODUCTION

The crown-of-thorns starfish *Acanthaster planci* inhabiting tropical and subtropical marine waters in Indo-Pacific area is regarded as the major predators to corals (Souter et al., 2000; Pratchett et al., 2009), and every outbreak of *A. planci* causes large-scale destruction of coral reefs (Sebens et al., 1994; Sweatman et al., 2008). Thus, *A. planci* has become a major management issue on coral reefs (Sweatman et al., 2008) that play an important role in marine ecosystem (Souter et al., 2000). Besides its damage to coral reefs, *A. planci* is notorious for its venom in the spines on body surface (Shiomi et al., 1985; Karasudani et al., 1996). Consequently, many fundamental researches on *A. planci* have been done, including distribution characters (Marsh and Tsuda, 1973; Yokochi et al., 1991), toxicity and components of venom (Shiomi et al., 1988, 1990, 1994; Ota et al., 2006), population genetics (Nishida and Lucas, 1988; Nina et al., 2009), feeding behavior (Hanscomb et al., 1976; Teruya et al., 2001), control strategies (Kenchington and Kelleher 1992; http://www.gbrmpa.gov.au/ _data/assets/ pdf_file/0009/4203/ws018_paper_16.pdf), and etc. Among these researches, the control strategies to *A. planci* are interesting and key targets.

In order to control the outbreak of *A. planci* and decrease its damage to coral reefs, some measures including manual capture, cutting up, underwater fences, and manual injection of chemical reagents have been adopted (Brian, 1995). However, these measures are either expensive and laborious or harmful to the survival of other marine organisms. Therefore, it is almost impossible that these measures can been widely used in effective control of the amount of *A. planci*. As a result, many researchers attempted to look for biologically active substances in *A. planci* and thus turned *A. planci* into valuable medical raw materials. Some active substances

*Corresponding author. E-mail: cqhu@scsio.ac.cn. Tel: +086-020-89023216. Fax: +086-020-89023218.

Table 1. Proximate composition, Ca, P, and astaxanthin content of starfish, *Acanthaster planci*.

Component	Group 1	Group 2	Group 3	Group 4	Mean ± SD
Moisture (%)	69.1	68.2	67.7	69.1	68.5 ± 0.7
Ash (%)	23.3	22.2	20.5	20.5	21.6 ± 1.4
Protein (% dry weight)	21.0	22.0	21.1	19.8	21.0 ± 0.9
Fat (%)	<1.0	<1.0	<1.0	<1.0	-
Ca (% dry weight)	26.7	22.3	23.0	25.4	24.4 ± 2.1
P (mg/g dry weight)	1.4	1.9	1.8	1.6	1.7 ± 0.2
Astaxanthin (mg/kg dry weight)	65.4	97.4	68.9	82.4	78.5 ± 14.6

*The starfish *Acanthaster planci* were distributed into four groups according to their weight. Every group includes 8 individuals. Group 1: 107.2 ± 8.4g/ individual; group 2: 178.6 ± 13.7g/ individual; group 3: 240.9 ± 17.4 g/ individual; group 4: 368.1 ± 20.3 g/ individual.

such as glycosides (Fleminga et al., 1976; Minalea et al. 1985), gangliosides (Smirnova et al., 1990) and anti-coagulant peptide (Koyama et al., 1998) have been isolated from *A. planci*, but due to the cost of production and the long process from the finding of new active substances to the approval of drug production until now there is no extensive application of *A. planci* or other starfish to medicine industries. Consequently, looking for new low-cost approaches for control and resource utilization of *A. planci* is an important and interesting target. Besides the application of *A. planci* to the extraction of biologically active substances, using *A. planci* as feed materials may be a new way for resource utilization worthy of exploration, although there has been no report relevant to the collection of starfishes as feed materials.

In the present study, we analyzed chemical constituent of the crown-of-thorns starfish *A. planci* in the sight of animal nutriology and tested its toxicity as the diet to mice in three different feeding ways. Based on these experiments, we evaluated potential utilization value of *A. planci* as feed component for animals.

MATERIALS AND METHODS

Preparation of the crown-of-thorns starfish *Acanthaster planci*

The crown-of-thorns starfish *A. planci* were captured in Yongle islands in South China Sea and stored at -20°C for the following experiments. For chemical constituent analysis, the starfish were sorted into four groups (groups 1 to 4, every group includes eight starfish) according to their weight (Table 1). And the starfish in every group were homogenized and used as the samples for the analysis of chemical constitutes.

Proximate chemical composition

Moisture content was determined by drying the samples of minced starfish in an oven at 105°C for 5 h. Ash content was determined by heating the dried samples for 3 h at 550°C according to Association of Official Analytical Chemists (AOAC) method 938.08. Protein content was calculated by converting the nitrogen content, determined by AOAC method 940.25. Total lipid content was determined in a 20 g sample of minced starfish through acid hydrolysis extraction reported in AOAC method 948.15.

Inorganic analysis

Freeze-dried minced starfish (approximately 2.0 g/sample) was treated with 20 ml of concentrated nitric acid in a Kjeldahl tube. The mixture was stood overnight and then digested carefully on a hotplate. After cooling, 10 ml of perchloric acid was added into each tube and the tubes were heated again followed by evaporation to near dryness. Then, the mixture was transferred with 50 ml of 1.3 M Hydrochloride. Calcium (Ca) contents were determined by atomic absorption spectrometry on a Perkin-Elmer 3100 AAS (Norwalk, CT) (Dong et al., 2005). Ammonium molybdate was added for color to the digested solution and then Phosphorus (P) content was determined by a colorimetric method with a Shimadzu UV-300 spectrophotometer (Kyoto, Japan) (Dong et al., 2005).

Analysis of amino acid composition

The freeze-dried minced starfish were treated with 6 N Hydrochloride at 110°C for 22 h. Amino acids in hydrolysate were analyzed directly after pre-column derivatization with o-phthaldialdehyde (OPA). The amino acid composition was analyzed using Hewlett-Packard (HP) (Palo Alto, CA, USA) high performance liquid chromatography (HPLC) 1050 system with a Hypersil ODS column from Phenomenex (125×4 mm ID, 5μm particle size and 80Å pore size, Torrance, CA, USA). Other operations for the determination of amino acid composition were performed according to the reported method (Dong et al., 2005).

Analysis of fatty acids

The freeze-dried minced starfish were used for lipid extracted with a chloroform-methanol (2:1, v/v) solution. Other preparation for the analysis of fatty acid composition was carried out as previously described (Kim et al., 2010). Fatty acid composition was analyzed with gas chromatograph (Agilent 6820, USA) equipped with a flame-ionization detector according to the protocol by Kim et al. (2010).

Quantification of astaxanthin in *A. planci*

The concentration of astaxanthin in the body of *A. planci* was determined by the method of Bjerkeng et al. (1997). The carotenoids were extracted from accurately weighed homogenized samples (4 g/sample) using a 1:1:3 mixture of distilled water,

methanol (containing 500 mM butylated hydroxytoluene) and chloroform. An isocratic HPLC system (Waters, USA) with the chromatographic column (Nova Pak C18, Waters, USA) was used to determine the carotenoid concentrations. External standards of astaxanthin (Sigma, USA) with known concentrations were prepared to establish a response line and the concentration was calculated using peak areas from the chromatograms. Mobile phases A, B and C were distilled water, acetone and methanol, respectively.

Toxicity test of *A. planci* as the food for mice

Though it has been confirmed that *A. planci* has venom (Shiomi et al., 1985; Karasudani et al., 1996), the toxicity of the starfish as food for animals is unknown. To evaluate the possibility of the starfish as the feed component, we analyzed the toxicity of the starfish as the food for mice. The starfish were homogenized with distilled water in equal weight and the homogenate was centrifugated at 500 g to remove the big particles. The supernatant was used as test sample A. The supernatant was also boiled and used as test sample B. In addition, the homogenate of the starfish was dried at 55°C and used as test sample C. The toxicity test was carried out using 36 male and female mice weighing 18 to 22 g each. The mice were randomly distributed into one control group (D) and three treated groups (A, B and C), containing 9 animals per group. In group A, the animals were fed with 1 ml of sample A twice in four days through gavage method. In group B, the animals were fed with 1 ml of sample B twice in four days through gavage method. In group C, the animals were starved for 24 h and fed with dried sample C through cafeteria feeding in four days. After 4-day trial period, all the animals were fed with normal diet for 10 days, and any death or changes in general behavior and other physiological activities were observed and recorded (Shah et al., 1997). After 14 days, the mice in different groups were weighed.

RESULTS AND DISCUSSION

Moisture, ash, Ca and P content

The results of moisture, ash, Ca and P content of the starfish *A. planci*, are shown in Table 1. Moisture content of the starfish detected ranged from 67.7 to 69.1% by weight, which is similar to those of other starfish (Raymond et al., 2007) and some fishes (El-Masry AND Wold, 2008; Keba et al., 2009). The ash content of the starfish reached 20.5 to 23.3% of weight, which may be due to plenty sclerites or spines in or on their bodies. Likewise, high level of Ca content was observed (22.3 to 26.7% of dry weight) in the inorganic analysis, coupled with a relative high level of P content (1.7 mg g^{-1} dry weight), as Ca and P are main inorganic elements in skeleton-like structure.

Protein content and amino-acid composition

The total protein content of the starfish calculated by total nitrogen was found to be 19.8 to 22.0% of dry weight and the four starfish groups with different weight ranges did not show obvious difference on the total protein content. This value of the total protein is within the range of 18 to 27% protein content in fish (http://www.weightlossforall.com/protein-fish.htm). A total of 18 amino acids were detected in *A. planci* and the contents of amino acids are shown in Table 2. The comparison of amino acid composition of the starfish in different groups showed that glycine (2.38 to 3.82 g/100 g dry weight) and glutamate (1.75 to 2.20 g/100 g dry weight) were the most abundant essential amino acid in the starfish, while taurine (0.07 to 0.09 g/100 g dry weight) was the lowest amino acid. Relative values of various amino acids of the starfish were compared with those of fish meal (http://www.svn.vefir.net/srmjol/content/view/24) and it was found that the starfish had similar profile of amino acid composition with that of fish meal (various amino acids in the starfish had similar relative value with the corresponding constituents in fish meal), with the exception that glycine level (18.8% on average) was approximately 3 times as much as that of fish meal (6.3%) (Figure 1). According to the report by National Research Council (NRC. 1993), essential amino acids requirements of *Nile tilapia* include tryptophan, lysine, histidine, arginine, threonine, valine, methionine, isoleucine, leucine, phenylalanine and tyrosine. Supposing the essential amino acids requirements of *N. tilapia* were set as a standard, the starfish contained all the essential amino acids required by the fish, *N. tilapia*. Total protein content and amino acid profile of the starfish therefore suggested that it has the potential to be the substitute for fish meal as the partial protein source.

Lipid and fatty-acid analysis

Total lipid content was determined with 20 g sample (wet weight) in each group. The method failed to give the exact lipid content, as 20 g sample did not produce detectable lipid extract with the method based on weighing, thus only the proximate lipid level (<1%) could be acquired. In another aspect, the result reflected that the starfish has less lipid content than usual fish (http://www.eatatease.com/fat-content-fish-shellfish.html). Composition of fatty acids of the starfish is shown in Table 3. Though *A. planci* had low lipid content, it had a good profile of fatty acid composition, which was manifested by the results that unsaturated fatty acids reached at 59.84 to 68.36% of total fatty acid and polyunsaturated fatty acids accounted for half of the unsaturated fatty acids. Also, polyunsaturated fatty acids contain timnodonic acid (EPA) (C20:5 ω-6, 1.31 to 2.73%) and docosahexaenoic acid (DHA) (C22:6 ω-3, 0.89-1.71%), which have important physiological functions to humans and animals. Some unsaturated fatty acids had relatively high content, such as C20:1 ω-9 (26.95 to 33.44%) and C20:4 ω-6 (13.29 to 17.70%). Thus, though lipid content was not rich in *A. planci* and using the starfish as part feedstuff can decrease the

Table 2. Amino acid contents of starfish, *Acanthaster planci* (g/100g dry weight).

Amino acid	Group 1	Group 2	Group 3	Group 4	Fish meal	Mean ± SD
Taurine	0.08	0.09	0.07	0.07	0.6	0.08 ± 0.01
Tryptophan	0.09	0.12	0.10	0.09	0.7	0.10 ± 0.01
Aspartate	1.49	2.10	1.82	1.58	6.8	1.75 ± 0.27
Glutamate	1.75	2.46	2.20	1.95	9.8	2.09 ± 0.31
Serine	0.67	0.94	0.82	0.71	2.9	0.79 ± 0.12
Glycine	2.38	3.82	3.21	2.69	4.1	3.03 ± 0.63
Threonine	0.63	0.97	0.82	0.71	2.9	0.79 ± 0.15
Histidine	0.17	0.27	0.24	0.22	1.4	0.23 ± 0.04
Alanine	0.89	1.29	1.10	1.02	4.4	1.08 ± 0.17
Arginine	0.86	1.42	1.13	1.02	4.0	1.11 ± 0.24
Tyrosine	0.41	0.52	0.47	0.46	2.6	0.47 ± 0.05
Valine	0.57	0.87	0.75	0.62	3.7	0.70 ± 0.14
Methionine	0.17	0.28	0.25	0.21	2.1	0.23 ± 0.05
Phenylalanine	0.38	0.58	0.50	0.43	2.7	0.47 ± 0.09
Isoleucine	0.51	0.71	0.66	0.56	3.0	0.61 ± 0.09
Leucine	0.79	1.13	1.04	0.87	5.4	0.96 ± 0.16
Lysine	0.60	0.97	0.88	0.71	5.4	0.79 ± 0.17
Proline	0.41	1.13	0.97	0.93	2.5	0.86 ± 0.31

*Amino acid contents of fish meal were acquired from online (http://www.svn.vefir.net/srmjol/content/view/24)

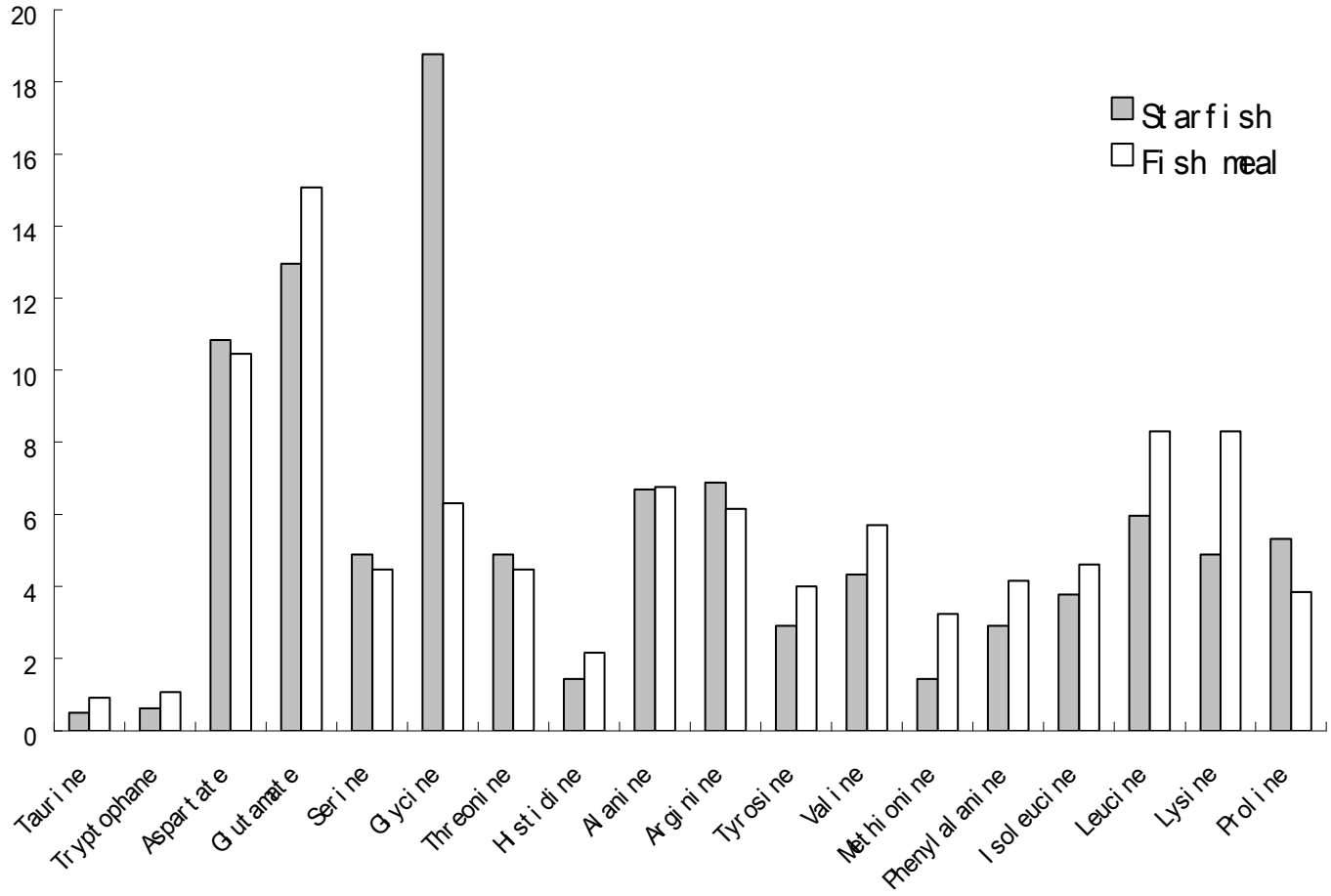

Figure 1. Comparison of amino acid composition between starfish *Acanthaster planci* and fish meal.

Table 3. Fatty acid composition of starfish, *Acanthaster planci.*

Fatty acid	Group 1	Group 2	Group 3	Group 4	Mean ± SD
Saturated					
C10:0	0.08	0.06	0.05	0.05	0.06 ± 0.01
C12:0	0.21	0.11	0.10	0.13	0.14 ± 0.05
C13:0	0.06	0.02	0.02	0.06	0.04 ± 0.02
C14:0	1.65	0.59	0.87	0.89	1.00 ± 0.45
C15:0	1.94	1.15	0.88	1.72	1.42 ± 0.49
C16:0	8.29	7.76	10.73	9.46	9.06 ± 1.32
C17:0	0.86	1.52	0.79	1.23	1.10 ± 0.34
C18:0	10.62	15.07	15.43	12.38	13.38 ± 2.29
C19:0	2.47	2.70	2.82	2.50	2.62 ± 0.17
C20:0	0.95	1.21	1.7	0.92	1.20 ± 0.36
C21:0	2.00	0.92	1.31	1.16	1.35 ± 0.46
C22:0	0.45	1.42	0.99	0.67	0.88 ± 0.42
C23:0	0.3	0.95	0.62	0.76	0.66 ± 0.27
C24:0	1.65	3.36	3.8	1.02	2.46 ± 1.33
Subtotal	31.53	36.84	40.11	32.95	35.36 ± 3.88
Monounsaturated					
C16:1ω-7	3.10	3.32	2.21	1.01	2.41 ± 1.05
C18:1ω-9	4.71	3.95	3.62	4.28	4.14 ± 0.47
C20:1ω-9	26.95	23.87	27.11	33.44	27.84 ± 4.2
C22:1ω-9	0.91	0.53	0.47	0.50	0.60 ± 0.21
Subtotal	35.67	31.67	33.41	39.23	35.00 ± 3.26
Polyunsaturated					
C18:2 ω-6	2.00	1.94	0.88	0.44	1.32 ± 0.78
C18:3 ω-3	0.98	1.15	1.59	0.61	1.08 ± 0.41
C20:2ω-6	4.39	3.28	3.41	4.04	3.78 ± 0.52
C20:3ω-6	0.40	0.60	0.26	0.46	0.43 ± 0.14
C20:4ω-6	17.69	17.70	13.29	15.49	16.04 ± 2.11
C20:5ω-6	1.37	1.31	2.73	1.54	1.74 ± 0.67
C22:3ω-3	3.68	3.79	2.73	3.93	3.53 ± 0.54
C22:5ω-5	0.47	0.28	0.42	0.42	0.40 ± 0.08
C22:6ω-3	1.71	1.44	1.18	0.89	1.31 ± 0.35
Subtotal	32.69	31.49	26.49	27.82	29.62 ± 2.94

*Values are percentages of total fatty acids.

usage amount of fish oil in feeds since fish oil is just added in a small amount.

Astaxanthin content

Astaxanthin is a kind of carotenoids that has attracted considerable interest in recent years because of the potent antioxidant activity and possible role in delaying or preventing degenerative diseases (Terao et al., 1989; Palozza and Krinsky, 1992). Astaxanthin has important appli-cations in human and animal food industries, specifically in nutraceutical, pharmaceutical and cosmetic industries (Khanafari et al., 2007: Park et al., 2010). In this study, we also analyzed astaxanthin content in *A. planci* and the result indicated that per gram (dry weight) of the starfish contained 65.4 to 97.4 µg astaxanthin, and this content did not show correlation with the weight of the starfish (or presumable age). The average value of astaxanthin content was 2.2 times to that of astaxanthin

Table 4. Mice weight before and after the feeding trial.

Group	Before experiment	After experiment
Treated group 1	18.9 ± 0.8	24.5 ± 1.3
Treated group 2	18.9 ± 1.2	24.4 ± 1.6
Treated group 3	19.1 ± 1.1	23.8 ± 1.4
Control group	19.0 ± 1.2	24.1 ± 1.2

in Litopenaeus vannamei (Latscha et al., 1989), 1.5 times to that of astaxanthin in *Penaeus monodon* (Latscha et al., 1989) and 2.6 times to that of astaxanthin in *Marsupenaeus japonicus* (Latscha et al., 1989). Shrimp wastes are one main sources of extracted astaxanthin that can be used as fish feed (Lee et al., 1999; Handayani et al., 2008). Compared with shrimps, *A. planci* has higher astaxanthin content and therefore has the potential to be the new sources of astaxanthin.

The toxicity to mice

To test whether *A. planci* is toxic to animals when it is used as feed, the toxicity of the starfish to mice was analyzed. The results indicate that all the mice in different groups fed with the starfish in different ways did not show any toxicant symptoms, and their behavior and vitality were the same as the mice in the control group. After a 4-day trial period, all the trial mice fed with the starfish can normally eat the diet without apocleisis. After the whole trial period the mice were weighed. The weights of mice before and after experiment are listed in Table 4. The weight of the mice in three treated groups did not have obvious difference with those in control groups. Even the weights of the mice in two treated groups (1 and 2) were slightly higher than those of the mice from the control group. It manifested that the starfish used as the feed for mice did not have negative influence on the growth and the health of the mice. Though it has been confirmed by toxicity tests that the starfish has venom secreted by the spines (Shiomi et al., 1988, 1998; Karasudani et al., 1997), all the toxicity tests were performed using crude extract (Shiomi et al., 1985, 1994) or purified toxin (Shiomi et al., 1988, 1998; Karasudani et al., 1996) through intraperitoneal injection or intravenous injection. It still remains unknown whether the toxin can act on digestive system of animals when the animals are fed with the starfish. Our preliminary result suggested that the toxin could not have influence on the health of the mice according to the present dosage. Certainly, the toxicity test using the mice in this study is only a preliminary and promising result, long-term feeding observation or using other experimental animals may be adopted in future works. Furthermore, if the starfish is to be used as the feedstuff, some measured should be adopted as well to inactivate the toxin in the feed processing.

Conclusion

Based on chemical constituent analysis of the starfish A. *planci* and the toxicity test to mice, we found that protein content of the starfish is within the range of protein content in fish and the protein from the starfish have a good profile of amino acid composition. Though the starfish has little fatty acids, the fatty acids contain rich variety and unsaturated fatty acids account for more than 60% of total fatty acids. *A. planci* has higher astaxanthin content than most common shrimps and they do not produce toxicity to mice when used as the feed. In summary, the crown-of-thorns starfish *A. planci* has the potential to be an ingredient for animal feeds, which can reduce the usage of fish meal, fish oil, and carotenoids. Therefore, a method for resource utilization of *A. planci* is suggested in the present study, hoping that it can provide a new strategy to control the amount of *A. planci*.

ACKNOWLEDGEMENTS

This work was supported by the National Key Technology R&D Program (2009BAB44B02), the Natural Science Fund of China (No.31070106) and the Frontier Key Program for Youngsters in SCSIO (SQ200801).

REFERENCES

Bjerkeng B, Følling M, Lagocki S, Storebakken T, Olli JJ, Alsted A (1997). Bioavailability of all-E-astaxanthin and Z-isomers in rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 157: 63-82.

Brian L (1995). Controlling crown-of thorns starfish. (http://www.reef.crc.org.au/publications/explore/feat45.html).

Dong YL, Sheng GY, Fu JM, Wen KW (2005). Chemical characterization and anti-anaemia activity of fish protein hydrolysate from *Saurida elongate*. J. Sci. Food Agric. 85: 2033-2039.

El-Masry G, Wold JP (2008). High-speed assessment of fat and water content distribution in fish fillets using online imaging spectroscopy. J. Agric. Food Chem. 56 (17): 7672-7677.

Fleminga WJR, Wyllie SG, Howdena MEH (1976). Isolation and partial characterization of steroid glycosides from the starfish *Acanthaster planci*. Comp. Biochem. Physiol. (Part B: Comp. Biochem.) 53(3): 267-272.

Handayani AD, Sutrisno, Indraswati N, Ismadji S (2008). Extraction of astaxanthin from giant tiger (*Panaeus monodon*) shrimp waste using palm oil: studies of extraction kinetics and thermodynamic. Bioresour. Technol. 99: 4414-4419.

Hanscomb NJ, Bennett JP, Harper G (1976). Biochemical stimuli for feeding in *Acanthaster planci* (L.). J. Exp. Mar. Biol. Ecol. 22(2): 193-197.

Karasudani I, Koyama T, Nakandakari S, Aniya Y (1996). Purification of anticoagulant factor from the spine venom of the crown-of-thorns starfish, *Acanthaster planci*. Toxicon. 34(8): 871-879.
Keba N, Yucel S, Baki B (2009). Comparative analysis of nutritive composition, fatty acids, amino acids and vitamin content of wild and cultured gilthead seabream (*Sparus aurata* L. 1758). J. Anim. Vet. Adv. 8(3): 541-544.
Kenchington R, Kelleher G (1992). Crown-of-thorns starfish management conundrums. Coral Reefs 11(2): 53-56.
Khanafari A, Saberi A, Azar M, Vosooghi G, Jamili S, Sabbaghzadeh B (2007). Extraction of astxanthin esters from shrimp waste by chemical and microbial methods. Iran J. Environ. Health Sci. Eng. 4(2): 93-98.
Kim JS, Kim KS, Kim MJ (2010). Analysis of food components of freeze-dried Alaska Pollack (Hwangtae). Afr. J. Biotechnol. 9(24): 3629-3633.
Koyama T, Noguchi K, Aniya Y, Sakanashi M (1998). Analysis for sites of anticoagulant action of plancinin, a new anticoagulant peptide isolated from the starfish *Acanthaster planci*, in the blood coagulation cascade. Gen. Pharmacol. 31(2): 277-282.
Latscha T (1989). The role of astaxanthin in shrimp pigmentation. Adv. Trop. Aquat. 9: 319-325.
Lee SH, Rob SK, Park KH, Yoon KR (1999). Effective extraction of astaxanthin pigment from shrimp using proteolytic enzymes. Biotechnol. Bioprocess Eng. 4: 199-204.
Marsh JA, Tsuda RI (1973). Population levels of *Acanthaster planci* in the Mariana and Caroline Islands, 1969-1972. Atoll. Res. Bull. 170: 1-16.
Minalea L, Riccioa R, Grecoa OS, Menouc JL (1985). Starfish saponins—XVI. composition of the steroidal glycoside sulphates from the starfish *Luidia maculate*. Comp. Biochem. Physiol. (Part B: Comp. Biochem.) 80(1): 113-118.
National Research Council (1993). Nutrient requirements of fish. National Academy Press, Washington DC, USA.
Nina Y, Satoshi N, Masami H, Ken O, Karin G, Kazuo N (2009). Gene flow of *Acanthaster planci* (L.) in relation to ocean currents revealed by microsatellite analysis. Mol. Ecol. 18(8): 1574-1590.
Nishida M, Lucas JS (1988). Genetic differences between geographic populations of the crown-of-thorns starfish throughout the Pacific region. Mar. Biol. 98(3): 359-368.
Ota E, Nagai H, Nagashima Y, Shiomi K (2006). Molecular cloning of two toxic phospholipases A2 from the crown-of-thorns starfish *Acanthaster planci* venom. Comp. Biochem. Physiol. (Part B: Biochem.Mol. Biol.) 143: 54-60.
Ota E, Nagashima Y, Shiomi K, Sakurai T, Kojima C, Walkes MP, Himeno S (2006). Caspase-independent apoptosis induced in rat liver cells by plancitoxin I, the major lethal factor from the crown-of-thorns starfish *Acanthaster planci* venom. Toxicon. 48(8): 1002-1010.
Palozza P, Krinsky NI (1992). Astaxanthin and canthaxanthin are potent antioxidants in a membrane model. Arch. Biochem. Biophys. 297: 291-295.
Park JS, Chyun JH, Kim YK, Line LL, Chew BP (2010). Astaxanthin decreased oxidative stress and inflammation and enhanced immune response in humans. Nutr. Metab. 7: 18-27.
Pratchett MS, Schenk TJ, Baine M, Syms C, Baird AH (2009). Selective coral mortality associated with outbreaks of *Acanthaster planci* L. in Bootless Bay, Papua New Guinea. Mar. Environ. Res. 67: 230-236.
Raymonda JF, Himmelman JH, Guderleya HE (2007). Biochemical content, energy composition and reproductive effort in the broadcasting sea star *Asterias vulgaris* over the spawning period. J. Exp. Mar. Biol. Ecol. 341(1): 32-44.
Sebens KP (1994). Biodiversity of coral reefs: what are we losing and why? Integr. Comp. Biol. 34(1): 115-133.
Shah AM, Garg SK, Garg KM (1997). Subacute toxicity studies on pendimethalin in rats. Indian J. Pharm. 29: 322-324.
Shiomi KA, Itoh K, Yamanaka H, Kikuchi T (1985). Biological activity of crude venom from the crown-of-thorns starfish *Acanthaster planci*. Bull. Jpn. Soc. Sci. Fish. 51(7): 1151-1154.
Shiomi KA, Kazama A, Shimakura K, Nagashima Y (1998). Purification and properties of phospholipases A2 from the crown-of-thorns starfish (*Acanthaster planci*) venom. Toxicon. 36(4): 589-599.
Shiomi KA, Yamamoto S, Yamanaka H, Kikuchi T, Konno K (1990). References and further reading may be available for this article. Toxicon. 28(5): 469-475.
Shiomi KA, Yamamoto S, Yamanaka H, Kikuchi T (1988). Purification and characterization of a lethal factor in venom from the crown-of-thorns starfish (*Acanthaster planci*). Toxicon. 26(11): 1077-1083.
Smirnova GP (1990). Gangliosides with the sialic acid residue in the inner part of the oligosaccharide chain and with the terminal galactofuranose residue from the starfish *Acanthaster planci*. Russ. J. Bioorg. Chem. 16(6): 830-838.
Souter DW, Lindén O (2000). The health and future of coral reef systems. Ocean Coast. Manage. 43: 657-688.
Sweatman H (2008). No-take reserves protect coral reefs from predatory starfish. Curr. Biol. 18(14): 598-599.