Characterization of Bioactive Compounds Obtained from Starfishes

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Contents

List of Tables

List of Figures

Introduction

Marine organism inferring about 30 phylum, 500 thousand species, which corresponds to about 85 percent of the living organism on the earth (1). Especially, marine organism might have unique self-defensive ability to survive in competition because marine is consist with special ecosystem different from a land. Therefore, a studies for the search of new bioactive compounds from marine organism has been actively doing, and such a possibility is unlimited if variety of marine organisms is considered (2).

In the marine environment of Korea, fishing amount in coastal and offshore has been largely reduced by establishment of exclusive economic zone (EEZ), by fisheries agreement among Japan, China and Korea, by environmental pollution of marine, and by formation of red tide. Therefore, fishing farm has been enlarged by presentation on the keynote of 21th century fisheries policy of government, and followed by change a catching fishery to a feeding fishery and formation of marine ranching. However, starfish, natural enemy of fishing farm, has been gradually increasing as fish farm has been enlarging. It is known that starfish has very strong reproduction-power, namely cutting part of the body as well as the whole body can grow up to new individuals. Starfish gives serious damages to useful shellfish, ark shell, abalone, little clam, scallop etc, inhabiting in shellfish farms of coastal area (3). Generally, the species of starfish grown in Korea sea, which is known to give serious damage to shellfish farm, known as is *Asterias*

amurensis and *Asterina pectinifera* (4), and demage of shellfish farm is very serious situation because a lots of starfishes have already been bred in the marine of Korea (0.5 starfish per $1m^2$) (5).

Therefore, in this study we extracted potential bioactive compounds from two starfishes, *Asterias amurensis* and *Asterina pectinifera*, by stepwise solvent extraction methods after hydrolysis with commercial proteases, Alcalase 0.6L^{TM} and Protamex^{TM}, and then measured bioactivities such as antimicrobial activity, antioxidative activity, ACE inhibitory activity, bile acid binding capacity and antimutagenicity activity *in vitro* of those extracts. Accordingly, if this study is successfully performed, it might be considered that the result of this study could offer the basic data for the further study of availability of bioactive compounds on echinoderms in a future, and could reduce damage of coastal shellfish farm, and also could utilize as a new income of fisherman by efficient usages of unused marine resources.

Research Background

The common five-rayed starfishes, *Asterias amurensis* and *Asterina pectinifera*, are a familiar sight in the coastal area of the Korea. These starfishes are one of the most destructive enemies on culturist by giving serious damages to shellfish farm.

Therefore, Park et al. (3) reported that in order to exterminate effectively starfish, *Asterias amurensis* inhabiting in a village fishing grounds and shellfish farms on coast of Korea, mop and sledge gear were made and their trials in sea for capture efficiency of starfish by each gear and towing distance were carried out by commercial dredger on the coast of Keojedo from April to May in 1995. However the results were not effective. Chang et al. (6) tested killing effects of some chemicals ($Ca(OH)_2$, CaO, $CuSO_4 \cdot 5H_2O$ and NaOH) against starfishes, *Asterias amurensis* and *Asterina pectinifera*, but was not effective because in the concentration of chemicals to kill starfish species, other useful marine organisms such as shellfish, pearl oyster, short necked clam, cockle, abalone etc., were also died.

Meanwhile, Lee et al. (7) reported that in order to use effectively starfish, *Asterias amurensis* was dried, grinded, and mixed with chemical fertilizer. As a result, the experimental crops such as tomato, red pepper, chinese cabbage, etc., used fertilizer mixed with starfish powder, yield higher products than those used chemical fertilizer. Also, the acidity of soil used fertilizer mixed with starfish powder was lower compared to soil using no-fertilizer. Therefore, it is thought that

starfish powder might control the lower of acidity in soil. However, it has not been widely used until now. As result of feeding tests of chicks and rats with a diet containing starfish meal by Champman, O.L. (8), the starfish meal retarded the growth, decreased the egg production, and increased the fatal ratio. These effects are thought to be primarily due to the presence of a thiamine-destructive enzyme. The excess contents of calcium and unbalanced ratio of calcium to phosphorus may be secondary factors affecting on growth (8). Also, as the starfish contains much more calcium than fish, it was necessary to exclude the greater part of the calcium from starfish for the purpose of feed-stuffs preparation rich in protein. Therefore, Hideo et al. (9) attempted to separate the calcium from starfish by digesting it using protease, and made starfish solubles. In the starfish to solubles, protein and vitamine were abundant, and the concentrated starfish solubles with no toxicity were proved by feeding experiment to rats.

Meanwhile, Hideki et al. (10) examined the compositions of the free amino acid (FAA) in four species of starfish, *Asterias amurensis, Solaster paxillatus, Asterina pectinifera,* and *Lysastrosoma anthositcta*. In the FAA compositions of all four starfishes, glycine levels were extremely high, and relatively large amounts of taurine were also found in *Asterina pectinifera*. In addition, new substances, mainly saponins, in an echinoderm have been discovering (2). Komori (11) studied steroidal saponins from *Asterina pectinifera*, and Findlay et al (12) reported that the asterosaponin obtained from *Asterias vulgaris* acts as an antiphlogistic and an analgesic. Andersson et al. (13) also reported that

polyhydroxylated steroidal glycosides (crossasteroside A) obtained from *Porania pulvillus* induce reaction of blood cells and control growth of tumor cell.

Resently, study about bioactive compounds has been actively progressing. In the case of starfish, Seo (2) reported that extracts obtained from *Asterina pectinifera* have antimicrobial and anticancer activities. Cho et al. (14) reported that the starfish *Asterina pectinifera*, one of unused marine resources, exhibits antimicrobial capacities against *B. subtilis* and *S. aureus*. Also, Cho et al. (15~18) separated and identified natural antimicrobial agent, antioxidative agent, anticholesterol agent, and anticoagulant agent from starfish .

Therefore, the objective of this study are 1) to analyze of their components in order to test availability as functional material of starfishes, 2) to increase yields of bioactive materials from the extracts obtained from starfishes using stepwise-extract method after hydrolysis with protease in place of stepwise-extract used in the previous study. Accordingly, this study may contribute exterminate effectively starfishes, which are known to give serious damages to useful shellfish.

Materials and Methods

1. Materials

The materials used in this study were two starfishes such as *Asterias amurensis* and *Asterina pectinifera,* which are known to give serious damages to shellfish farm. These were obtained from coastal region of Jin-jun Mun in Masan of Kyungnam province (The depth of water is within about 15 m), put into an ice chest and transferred to Processing Lab. Dept. of Food & Nutrition, Changwon National University (CNU) within 2 hrs. The materials were taken in the same place in order to keep consistency of this experiment. After transferring to the Lab., these materials were cleaned, washed with streaming water, let on papers for an hour and blended in a blender (Waring blender Co., USA), the blended materials were used immediately in an experiment. The rest raw materials were stored in a freezer (-70℃) until use.

Table 1. Body lengths and weights of starfishes caught in South sea

Species	Body length (cm)	Body weight (g)
Asterias amurensis	22±4	137±10
Asterina pectinifera	9±2	49±5

Mean±S.D (n>10).

2. Methods

1) Extraction of bioactive compounds

The extraction of potentially bioactive compounds followed by a method shown in Fig. 1, and all process of this experiment was performed at 4℃. One kg of raw starfish homogenized was agitated with distilled water (1:3 ratio, w/v) for 5 hrs and centrifuged at 17,500*g* for 10 min. The supernatant as a water fraction was made into powder by vacuum lyophilization (Bondiro, Il Sin Engineeering Co., Korea). In the same way shown above, methanol (1:3 ratio, w/v), acetone (1:3 ratio, w/v), ethylether (1:3 ratio, w/v) and ethyl acetate (1:3 ratio, w/v) were added into the residue in order, and each fraction of methanol, acetone, ethylether and ethyl acetate was obtained. All kinds of the fractions were concentrated using vacuum rotary evaporator (Eyela, N-INW, Tokyo Rikakikai Co., Ltd, Japan), dissolved in DMSO (Dimethyl sulfoxide, Kento, Japan) and stored in a refrigerator (4℃) until use.

On the other hand, the other treatment, which added with protease (Alcalase 0.6L^{TM}, Protamex^{TM}, Novo Nordisk Co., Denmark), was processed. Namely, 1 kg of raw starfish homogenized was agitated with distilled water (1:3 ratio, w/v) and 0.5% of protease (to dried weight of sample) at 50℃ for 5 hrs. The following extraction of organic solvent was processed in the same way applied to Control which no enzyme was added as descirbed above.

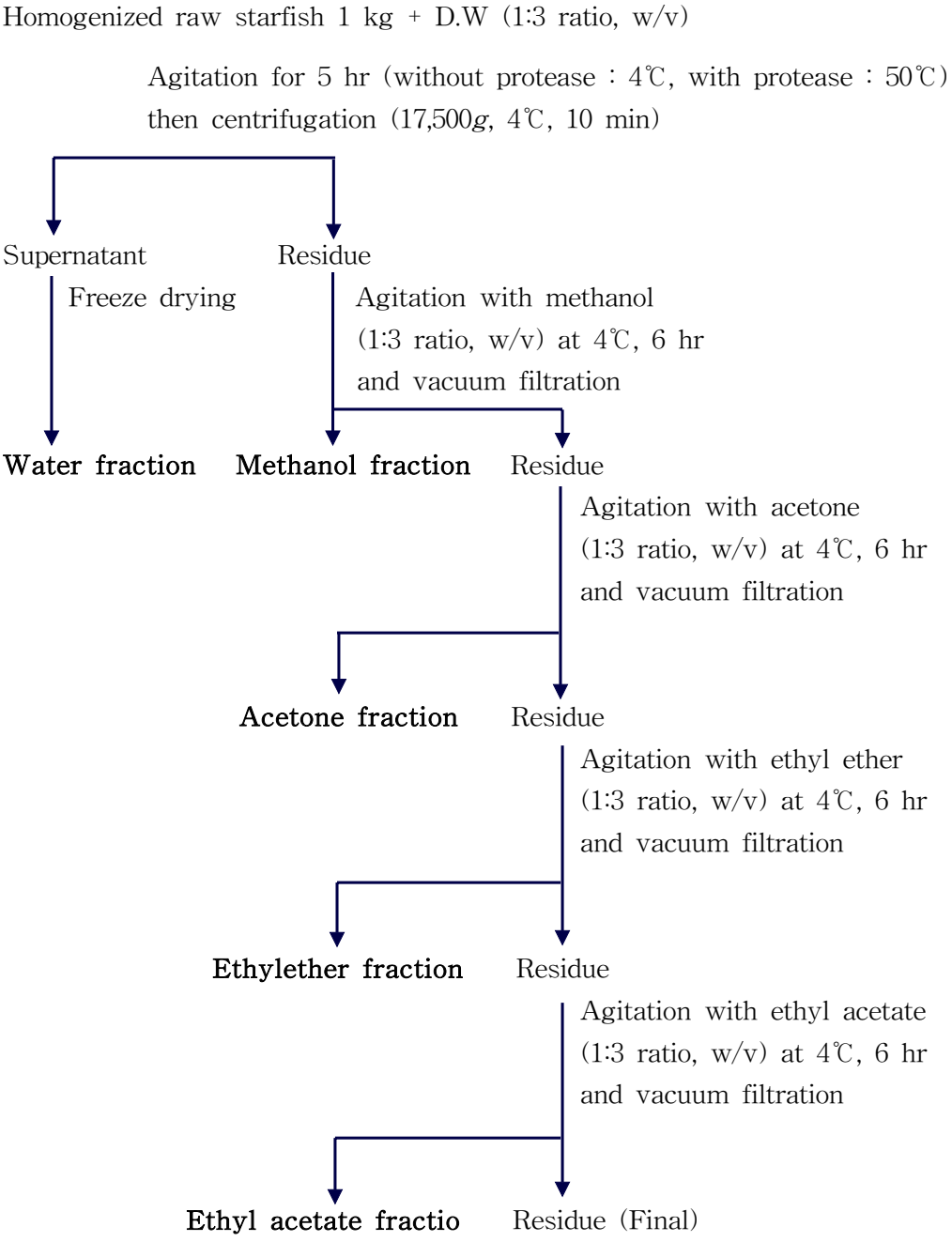

Fig. 1. Procedure for extraction of bioactive compounds from starfishes.

Table 2

2) Analysis of proximate composition and inorganic components

The contents of moisture, crude protein (semi-micro Kjeldahl method), crude lipid (Soxhlet method) and crude ash were determined by A.O.A.C method (19).

The quantitative analysis of mineral and heavy metal was determined, namely 1 g of sample was dissolved in a furnace (Model-101325, Samduc Science and. Co., LTD, Korea) and diluted to 200 mL with 1 N HCl, and the diluted solution was analyzed using ICP (Inductively coupled plasma spectrophotometer, Sequential ICP-AES, Varian, USA).

3) Analysis of carotenoids

(1) Extraction of carotenoids

Two grams of homogenized sample was put into a Erlenmeyer flask (100 mL) with 50 mL of acetone:methanol (1:1, v/v), incubated for 12hrs in the dark and diluted to 100 mL with distilled water after filtration (Whatman No. 2).

(2) Separation of carotenoids

Fifty mL of sample solution prepared was agitated for 30 min after 7.5 g of KOH was added, and put into a separating funnel. It was shaken after adding 50 mL of diethyl ether. 50 mL of 10% NaCl was

added into the solution, and the solution was treated with 150 mL of distilled water for 3 times in order to shift carotenoids to ether layer. The layer of diethyl ether was dehydrated with Na_2SO_4,. The diethyl ether was removed in a vacuum rotary evaporator (Eyela, N-INW, Tokyo Rikakikai Co., Ltd, Japan). The seperated carotenoid was diluted to 10 mL with petroleum ether and measured the absorbance at 445 nm.

Total carotenoids (mg%) was calculated as described follows:

$$\text{Total catotenoids (mg\%)} = \frac{O.D(\lambda max)\times vol\times 10^3}{E^{1\%}_{1cm}(2500)\times \text{weight of sample(g)}}\times 100\times \text{dilution ratio}$$

4) Analysis of total amino acids

Total amino acids were determined according to method of Cha and Cadwallader (21), which was modified from the method of Lee et al. (20). One gram of homogenized sample put into test tube with 5mL of 6N HCl and was decomposed in dry oven for 22-24hr at 115℃. The decomposed solution was filtered with glass wool. After that, the filtered solution was taken into a separating funnel, washed with 50 mL of diethyl ether 2 times to remove lipid and pigment materials and then taken up the lower layer. The lower layer was dehydrated in a vacuum rotary evaporator, dissolved in citric buffer solution (pH 2.2) and finally adjusted to a 25 mL of volume. The final solution was put into test

tube and kept in a refrigerator until analysis. The total amino acids were quantitatively analyzed using an amino acid analyzer (Biochrom20, Pharmacia Biotech, USA).

5) Assay of bioactive compounds

(1) Antimicrobial activity

① Antimicrobial strains and medium

Four kinds of gram-positive bacteria and three kinds of gram-negative bacteria were used to test in this study, which are involved in germ of food poisoning and pathogens, and two kinds of fungi were also used for development of a skin disease (Table 3).

The culture media used for bacteria and fungi were Mueller-Hinton broth and YM broth, respectively. The medium of Mueller-Hinton agar and Potato Dextrose agar was used for bacteria and fungal, respectively. All kinds of medium were purchased from Difco. Co. Ltd.

② Antimicrobial activity test

Antimicrobial and antifungal activity tests were performed using the paper disc method. Mueller-Hinton agar (20 mL) was poured into each petri dishes and dried during all night in a clean bench. 0.1 mL of suspensions of target strain for 6~8 hr were spread on the plates uniformly. Each extract was obtained by filtering through a sterile microfilter (0.45 ㎛ pore size, Millipore). Sterile samples were then

Table 3. Reference strains[1)] used for antibacterial activity experiment

Strain	Strains No.	Medium
Gram (+) bacteria		
Bacillus subtilis	KCTC 1021	Mueller-Hinton agar
Bacillus cereus	KCTC 1012	〃
Staphylococcus aureus	KCTC 1916	〃
Listeria monocytogenes	KCTC 3710	〃
Gram (-) bacteria		
Escherichia coli	KCTC 1924	〃
Salmonella typhimurium	KCTC 2515	〃
Enterobacter aerogenes	KCTC 2190	〃
Mold		
Aspergillus flavus	KCTC 6961	Potato Dextrose agar
Aspergillus niger	KCTC 6982	〃

[1)] Korean Collection of Type Cultures (KAIST)

transferred into the disk (ø 8 mm, Toyo Seisakusho Co.) and inoculated with target strains. The plates were incubated at 37℃ and 25℃ for the bacteria and the fungi, respectively. The diameter of the inhibition zone (mm) was measured after 24 hr and 48 hr for the bacteria and the fungi, respectively.

(2) Antioxidative activity

Antioxidative activity test was determined by a method of Blios (22). 0.8 mL of 0.4 mM DPPH solution was mixed with 3~4 mL of ethanol. The volume of ethanol was controlled to fix its absorbance within 0.95~0.99 at 525 nm. After that, 0.2 g of sample was put into a test tube with 0.8 mL of 0.0004 M DPPH solution and ethanol controlled as shown above, and it was mixed and incubated for 10 min in the dark. Absorbance of the solution was measured using spectrophotometer (Varian 634S, Australia) at 525 nm.

$$\text{Free radical scavenging activity (\%)} = \left(1 - \frac{\text{O.D of sample}}{\text{O.D of blank}}\right) \times 100$$

(3) Angiotensin-I converting enzyme; ACE Inhibitory Activity

① Preparation of ACE enzyme solution

ACE enzyme solution was made by a method of Cushman and Cheung (23). One g of rabbit lung acetone powder (Sigma Co.) was

Table 4. Procedure for determining of ACE inhibitory activity with TNBS

	Vol (μL)		
	Blank	Control	Sample
Inhibitor	-	-	25
H_2O	25	25	-
ACE solution	50	50	50
0.5 N HCl	400	-	-
12.5 mM Hip-His-Leu	150	150	150
Incubated at 37℃ for 90min			
0.5 N HCl	-	400	400
Kolthoff buffer [1)]	250	250	250
0.1 M TNBS solution	25	25	25
Incubated at 37℃ for 20 min			
Sulfite [2)]	4500	4500	4500
Measured at 416 nm			

[1)] 0.1 M Na_2HPO_4 : 1 N NaOH (1:2 ratio, v/v)

[2)] 4 mM Na_2SO_3 in 0.2 M NaH_2PO_4

mixed with 10 mL of sodium borate buffer (pH 8.5). After mixing for 24 hr at 4℃, the mixture of rabbit lung acetone powder was centrifuged at 17,500*g* for 10 min at 4℃. The supernatant was used as an enzyme solution of ACE, 12.5 mM Hippuryl-Histidyl-Leucine (Sigma Co.) was used as the solution of substrate.

② ACE Inhibitory Activity

ACE inhibitory activity was measured by a method of Toshiro et al. (24) as shown in Table 4. 25 μL of ACE inhibitor and 50 μL of Hip-His-Leu (12.5 mM in borate buffer containing 200 mM NaCl, pH 8.3) were incubated with 50 μL of 25 mU/mL ACE at 37℃ for 90 min. 250 μL of 0.5 N HCl was added as a stop solution of reaction, and the stop solution was then adjusted to the desired pH by adding of 250 μL of Kolthoff buffer (0.1 M Na_2HPO_4 - 1 N NaOH (1:2, v/v)). Then, 25 μL of Trinitrobenzene Sulfonate (TNBS) solution was added there, and it was incubated for 20 min. The solution (4.5 mL of 4.0 mM Na_2SO_3 in 0.2 M NaH_2PO_4) was added, and absorbance of the solution was measured in a spectrophotometer (Varian 634S, Australia) at 416 nm. Inhibitory ratio (%) was calculated as follows :

$$\text{Inhibitory ratio (\%)} = \left(1 - \frac{As-Ab}{Ac-Ab}\right) \times 100$$

Ac, As, and Ab represented the absorbance of control, sample and blank, respectively. The concentration of ACE inhibitor required to inhibit 50% of the ACE activity was defined as IC_{50}.

(4) Bile acid binding capacity *in vitro*

Binding capacity of bile acid was determined by a modified method of Camier et al. (25). An *in vitro* digestion procedure was used. Namely 0.1 g of sample in screw-capped tubes was dissolved with 5 mL of distilled water and 2 mL of 0.1 N HCl and mixed at 37℃ for 1 hr in a shaking water bath. Blank tubes containing reagents only were taken through each step. After this simulated gastric step, samples were adjusted to pH 7.0 with 0.1 N NaOH.

Bile acid solution contained 31.25 μmol/mL in 0.1 M phosphate buffer (pH 7.0). Porcine pancreatin was dissolved in 0.01 M (pH 7.0) phosphate buffer to yield a concentration of 10 mg/mL.

The next step simulated conditions in the small intestine. Each tube was shaken for 1 hr at 37℃ after addition of 4 mL of bile acid solution and 5 mL pancreatin solution. Two mL of 1.33 M phosphoric acid were added in the each tube, and the solution were then quantitatively transferred to 50 mL plastic centrifuge tubes and centrifuged for 10 min at 26,890*g* in a centrifuge (Sorvall RC 28S). Supernatants were taken by Pasteur pipets to a second set of tubes. Five mL of phosphate buffer were added to the centrifuge tubes. The tubes were vortex-mixed and centrifuged as described previously. Supernatants were taken, and mixed with the original supernatants adjusted to pH 7.0 with 1 N NaOH. The 0.1 mL of suprenatant was put into tubes with 0.5 mL of test reagents (nicotinamide adenine dinucleotide (NAD), nitro blue tetrazolium salt (NBT), diaphorase and 3α-hydroxysteroid

dehydrogenase). Sample blanks contained the same reagents as the supernatant but not including the dehydrogenase. Tubes were incubated at 37℃ for 10 min. The reaction was stopped by the addition of 0.5 mL of stopping solution. Absorbance of the solution was measured in a spectrophotometer (Varian 634S, Australia) at 540 nm.

(5) Ames mutagenicity test *in vitro*

① Mutagens

MNNG (N-methyl-N′-nitro-nitrosoguanidine, 0.4 ㎍/plate) was purchased from Aldrich Chemical Co., Milwaukee, WI (USA) and dissolved in distilled water.

② Ames mutagenicity test

Bacterial strains, *Salmonella typhimurium* TA98 and TA100, were used for mutagenicity test and were maintained as described by Marons and Ames (26). The genotypes of the tester stains were checked routinely for their histidine requirement, deep rough (*rfa*) character, UV sensitivity (*uvr* B mutation) and the presence of R factor.

The mutagenicity test (27, 28) was carried out by a modified plate incorporation test (liquid preincubation of the organism with the test compound). For the preincubation, 0.6 mL of phosphate buffer were put into sterilized capped tubes with 0.1 mL of test strain cultured overnight ($1\sim2\times10^9$ cells/mL), 0.1 mL of each sample solution and 0.1 mL of mutagen in an ice bath. After that, the tubes were vortexed and

preincubated at 37℃ for 30 min. Two mL of the top agar supplemented with L-histidine and D-biotin kept at 45℃ were added to each tubes, vortexed for 3 sec and poured on minimal glucose agar plate. The plates were incubated for 48 hr at 37℃ and the number of revertant bacterial colonies were counted. Antimutagenic activity of each sample was expressed as % inhibition compared to the positive control. Toxicity test for the different levels of the solvent fractions obtained from starfishes were also carried out.

Result and Discussion

1. Proximate compositions

Proximate compositions of raw samples and residues of 3 types for starfishes are shown in Table 5. Moisture contents of raw *Asterias amurensis* and *Asterina pectinifera* were 72.35~73.41% range and 64.75~65.37% range, respectively. Ash contents in the raw starfishes of two species were 9.31~13.71% range and 22.37~23.28% range, respectively. No et al. (29) reported that moisture content in the raw starfishes was lower than those of the general fish and shell, whereas content of crude ash was higher than those of the general fish and shell (1~3%) (29). The content of crude protein in raw *Asterias amurensis* and *Asterina pectinifera* was 7.54~12.09% range and 5.98~10.03% range, respectively. In the present study, we identified that the content of crude protein in the starfish species was dependent on the season being captured. In other words, the content of crude protein in the starfish captured on May was much higher than captured on February. The content of crude protein in the starfish increased when the starfish was captured on June and July. It is thought that this time might much coincide with a spawning season. The content of crude protein in this study was similar to those in a previous study (30). The contents of moisture and crude protein in *Asterina pectinifera* were lower than those of *Asterias amurensis,* whereas the content of crude

Table 5

ash in *Asterina pectinifera* was higher than those of *Asterias amurensis*. Accordingly, it is known that component of proximate compositions somewhat differed from starfish species.

2. Changes of Amino-N contents in soluble layer of starfish species

1) *Asterias amurensis*

Changes of amino-N contents in soluble layer of *Asterias amurensis* during extraction are shown in Fig. 2. Hydrolysis ratio of *Asterias amurensis* treated with protease was higher than that of control. Especially, it showed that Protamex^{TM} had higher hydrolytic activity than Alcalase 0.6L^{TM}. The contents of amino-N of soluble layers treated with Protamex^{TM} and Alcalase 0.6L^{TM} increased up to 138.01 mg% and 87.80 mg% in 5 hr of reaction time, respectively, and then decreased. However, the content of amino-N slowly increased up to 6 hr in controls (4℃, 50℃), and controls had a low hydrolysis ratio.

2) *Asterina pectinifera*

Changes of amino-N contents in soluble layer of *Asterina pectinifera* during extraction are shown in Fig. 3. Protamex^{TM} also showed the highest hydrolysis ratio in *Asterina pectinifera* as shown in *Asterias amurensis.* The contents of amino-N of soluble layers treated with Protamex^{TM} and Alcalase 0.6L^{TM} increased up to 96.13 mg% and 77.57 mg% in 3 hr of reaction time, respectively, and then decreased slowly.

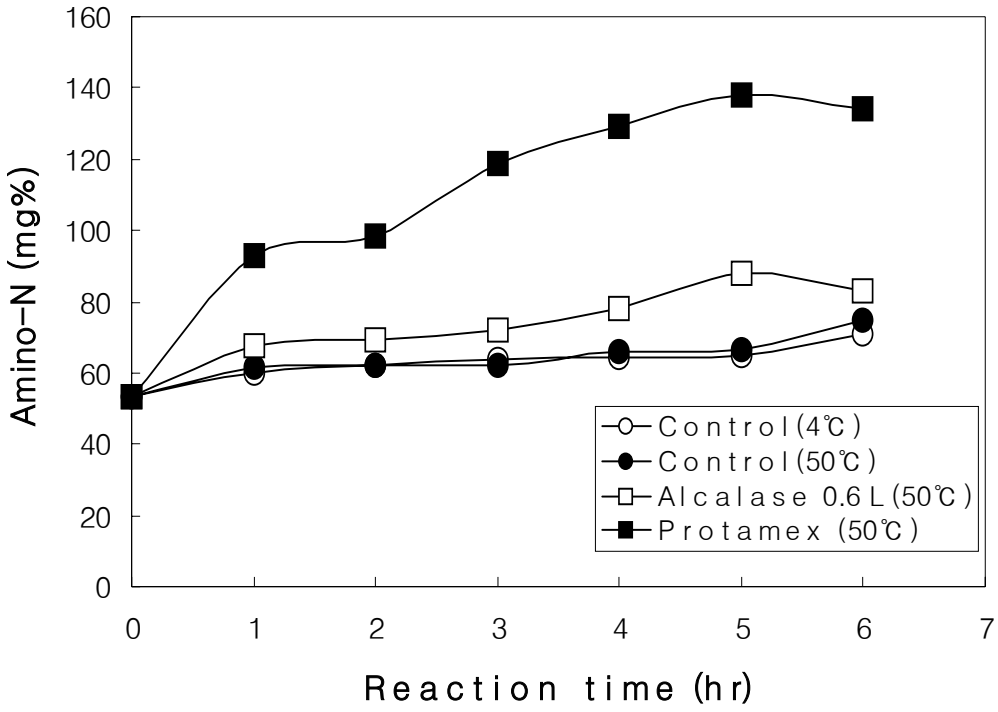

Fig. 2. Changes of contents of Amino-N in soluble layer of *Asterias amurensis* during extraction.

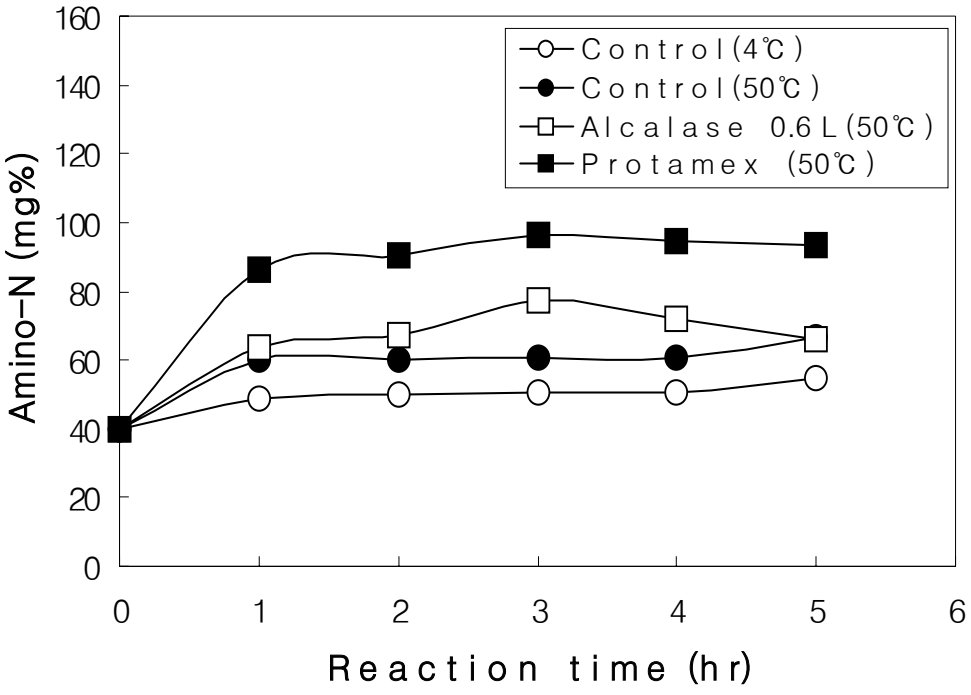

Fig. 3. Changes of contents of Amino-N in soluble layer of *Asterina pectinifera* during extraction.

However, the content of amino-N of soluble layer obtained from control at 50℃ was increased up to 60.20 mg% in 1 hr of reaction time and then increased slowly. It is supposed that because *Asterina pectinifera* might be influenced by autolysis of enzyme on the early stage of reaction.

Accordingly, reaction time of enzyme for the extraction of bioactive compound was fixed as 5 hr by results of amino-N contents on soluble layer of *Asterina pectinifera* and *Asterias amurensis* during extraction.

3. Yields

Yields of fractions obtained from two starfishes by stepwise extractions are shown in Table 6. Total yield in *Asterias amurensis* was 26.19~31.81% range, and yield of water fraction, 15.31~17.71% range, was the highest among several fractions, and methanol fraction (9.27~12.16% range) and acetone fraction (1.44~1.66% range) were followed in order. Yields of ethylether fraction (0.12~0.19% range) and ethyl acetate fraction (0.05~0.09% range) were very low. Total yield in *Asterina pectinifera* was 23.94~29.11% range. Yield of water fraction was also the highest on 18.19~20.78% range, and methanol fraction (5.50~7.93% range), acetone fraction (0.21~0.34% range), ethylether fraction (0.04~0.06% range) and ethyl acetate (0.02~0.04% range) were followed in order. Total yields in *Asterias amurensis* was higher than those in *Asterina pectinifera*, and yield of fractions obtained after hydrolysis with Protamex^{TM} was higher than those of control.

Table 6

In the case of proximate compositions of residues after stepwise extractions (Table 5), the content of crude protein of residue obtained after hydrolysis with Protamex™ was lower than those obtained after hydrolysis with Alcalase 0.6L™ and control. It is thought that yields of extract obtained after hydrolysis with Protamex™ was higher than those obtained after hydrolysis with Alcalase 0.6L™ and control.

4. Contents of inorganic components

The contents of inorganic components of two starfishes were analyzed by ICP and are shown in Tables 7 and 8. The contents of calcium in raw *Asterias amurensis* and *Asterina pectinifera* were 17.93% and 25.80%, respectively. These contents occupied as 37.02% and 39.86% of total crude ash in the two species and was the highest among inorganic components measured in this study. The contents of mineral components measured in this study were similar to those in a previous study (7), in which the contents of calcium in raw *Asterias amurensis* and *Asterina pectinifera* were 16.9% and 20.3%, respectively. The minerals Na, Mg, Si and P were followed in order. Selenium known as antioxidant was contained in the two starfishes with very small amounts. Meanwhile, the contents of heavy metals such as Mn, Cd, Hg and Pb were not detected, whereas the contents of Zn, Cu and Cr were detected with very small amounts.

The contents of calcium in water fraction for control and *Asterias amurensis* pre-treated with Alcalase 0.6L™ and Protamex™ were

Table 7

Table 8

0.92%, 0.94%, and 0.93 %, respectively, and in the case of *Asterina pectinifera* calcium contents were 3.29%, 3.45% and 3.43%, respectively. Water fraction was only extracted with relatively low amounts and most of calcium was existed residues. In addition, the contents of calcium in starfishes pre-treated with proteases were similar to control. Therefore, this is thought that the extraction of calcium was not affected by protease.

5. Carotenoids

Although more than 600 carotenoids have been characterized in nature, only about 10% of carotenoids have potential vitamin A activity (31). Carotenoids, mainly used as food colourants, are characterized by its strong reactive conjugated double bonds related to oxidation by heat, light, acid and metal ions (32). There have been continuous interests in the study of dietary carotenoids due to the nutraceutical studies. It has suggested that diets rich in carotenoid-containing foods may reduce the risk of certain types of chronic diseases such as cancer (33), cardiovascular disease (34~36), macular degeneration (37) and cataracts (38, 39).

The contents of carotenoids in starfishes are shown in Table 9. The contents of carotenoids in *Asterias amurensis* and *Asterina pectinifera* were 0.62 mg% and 5.88 mg%, respectively. In the case of *Asterina pectinifera,* the contents of carotenoids were higher than those in muscle of had-shelled mussel (0.4 mg% in male and 2.7 mg% in

female) and muscle in blue mussel (1.1 mg% in male and 3.2 mg% in female) (40). Therefore, carotenoids in starfishes are expected to be used in the various fields as food colourants, health foods, medical supplies and feeds.

Table 9. Total carotenoid amounts in the starfishes

(Unit : mg%)

	Asterias amurensis	*Asterina pectinifera*
Total carotenoids	0.62	5.88

6. Total amino acid

The contents of total amino acid in starfishes are shown in Table 10. The contents of total amino acid in *Asterias amurensis* and *Asterina pectinifera* were 10,786.95 mg% and 8,511.33 mg%, respectively. In the case of *Asterias amurensis,* proline was 3,528.21 mg%, which was 32.71% of total contents of amino acids, and proline was the highest in amounts among the amino acids identified in this study. The content of glutamic acid, cystathionine, β-alanine, L-arginine and L-lysine was 2,552.45 mg% (23.65%), 934.82 mg% (8.37%), 766.83 mg% (7.11%), 542.15 mg% (5.03%) and 494.96 mg% (4.59%), respectively. On the other hand, in the case of *Asterina*

Table 10

pectinifera, glycine was 1,025.11 mg%, which corresponds to 12.04% of total contents of amino acids. Glycine was the highest in amounts among the amino acids identified in this study. Glutamic acid (11.64%), L-arginine (6.71%), L-threonine (6.35%), aspartic acid (5.97%) and L-leuicine (5.67%) were followed in order. In this study, it suggests that compositions of amino acids somewhat differed from species to species of starfish.

7. Assay of bioactive compounds

1) Antimicrobial activity

The antimicrobial activities of each fraction obtained from *Asterias amurensis* and *Asterina pectinifera* are shown in Table 11 and Figs. 4 to 9. The results are shown that two starfishes exhibited an extremely strong antimicrobial activity against all of the tested microorganisms. The antimicrobial activity of the fraction obtained from *Asterina pectinifera* was stronger than that of *Asterias amurensis* (Fig. 4 to 9), and the antimicrobial activity of the fraction obtained from starfishes pre-treated with protease was stronger than that of control. In the case of solvents of extract, methanol fraction had the strongest antimicrobial activity, and acetone fraction, ethylether fraction, ethyl acetate fraction were followed in order. However, water fraction did not show any antimicrobial activity.

MIC (Minimum inhibitory concentration) of methanol fraction

Fig. 4

Fig. 5-1, 5-2

Fig. 6

Fig. 7

Fig. 8-1, 8-2

Fig. 9-1, 9-2

Table 11

obtained after hydrolysis with Protamex^{TM} against *Bacillus subtilis* in *Asterias amurensis* and *Asterias amurensis* were 250 ppm, respectively. These results were similar or stronger than those in a previous study (41-44), in which MIC of para-oxy ethyl benzoate (41), ethanol extracts of *Brassica juncea* (42), ethanol extracts obtained from steamed green tea (43) and protamine (44) were 1,000 ppm, 10,000 ppm, 250 ppm and 200 ppm, respectively. In the other hand, para-oxy ethyl benzoate and protamine were known as a synthetic food preservative and a natural antimicrobial agents. In the case of *Bacillus cereus* and *Staphylococcus aureus,* MIC of methanol fraction obtained after hydrolysis with Protamex^{TM} in *Asterias amurensis* and *Asterias amurensis* were also 250 ppm, respectivey. MIC against *Listeria monocytogenes (*500 ppm and 250 ppm) were almost similar to those in a previous study (45-47) where MIC of linoleic acid (45), extracts of *Rheum palmatum L.* (46) and hexane fraction of *Ouercus mongolica* leaf (47) were 200 ppm, 500 ppm and 250 ppm, respectively. In the case of gram negative bacteria, all of the three bacteria, *Enterobacter aerogenes, Escherichia coli* and *Salmonella typhimurium*, showed the same inhibitory effects with MIC of 500 ppm, respectively.

Meanwhile, Cho et al. (15) reported that MICs of ethylether extract obtained from *Asterina pectinifera* of the South sea were 379, 304 and 506, and MICs of ethyl acetate extract were 300, 260 and 500 μg/disk against *Bacillus subtilis, Staphylococcus aureus* and *Escherichia coli*, respectively. Therefore, methanol fraction obtained after hydrolysis with Protamex^{TM} in our study showed the stronger inhibition potency than

those of Cho et al. (15).

Extracts obtained from two starfishes exhibited an extremely strong antimicrobial and antifungal activity against gram positive and gram negative bacteria and fungi. Especially, the antimicrobial activity was stronger against gram positive bacteria than that of gram negative bacteria. This might be thought because of differences of characters among bacteria, which the cell membrane of gram positive bacteria was easily attacked by antimicrobial materials because peptidoglycan was exposed, whereas the cell envelope of gram negative bacteria contains significant amounts of lipopolysaccharide and lipoprotein in association with the thin peptidoglycan layer. The layer of lipid material outside the peptidoglycan is called the outer membrane, therefore, because the layer suppresses an inflow of hydrophobic material or hydrophilic material of large molecular, resistance for antimicrobial material of gram negative bacteria was stronger than those of gram positive bacteria (48).

In the case of fungi, MIC of methanol fraction obtained after hydrolysis with Protamex^{TM} against *Aspergillus flavus* and *Aspergillus niger* were 500 ppm, respectively. However, water fraction did not show any antimicrobial activity. Accordingly, it is supposed that antimicrobial compounds obtained from two starfishes might not be watersoluble but be liposoluble.

2) Antioxidative activity

The antioxidative activity of each fraction obtained from two starfishes was tested using the method of α,α-diphenyl-β-picrylhydrazyl (DPPH) radical scavenging activity. The result are shown in Table 12, and the values represented as 50 percent electron donating ability (EDA_{50}). The antioxidative activities (EDA_{50}) of extracts obtained from *Asterina pectinifera* were stronger than those of *Asterias amurensis,* and methanol fractions in the both species have shown the strongest activity among the solvent fractions. Especially, the activities of methanol fractions obtained after hydrolysis with Protamex^{TM} were somewhat more effective than those of control. Namely, the EDA_{50} of methanol fractions obtained from *Asterias amurensis* and *Asterina pectinifera* pre-treated with Protamex^{TM} were 1.67 and 0.68 mg/mL respectively, but the EDA_{50} of methanol fractions of control were 1.91 and 0.72 mg/mL respectively. The EDA_{50} of methanol fraction (0.68 mg/mL) obtained from *Asterina pectinifera* pre-treated with Protamex^{TM} was similar or stronger than those of Cho et al. (49), which the EDA_{50} of L-ascorbic acid and *Yulmu* (young radish) were 0.65 and 0.72 mg/mL, respectively. This is supposed that antioxidative activity of starfishes might undergo influences of carotenoids. Nakio (50) reported that the carotenoids protect cell and tissue from reactive oxygen as well as the singlet oxygen quenching ability in vital reaction. Also, it was reported that demand of carotenoids increased as aging-prevention agent, anticancer agent and nutrition-enrichment agent after

Table 12

approvement antioxidative activity of carotenoids in FDA (U.S.A)(51).

Meanwhile, it is thought that antioxidative activity might be influenced by peptides increased after enzyme hydrolysis. Recently, studies for antioxidant effects of protein hydrolysates have been reported (52, 53). Especially, Yeum et al. (52) reported that the antioxidant activity of protein hydrolysates was influenced by existence of specific amino acids in peptides or side chain of their terminal, and the antioxidative activity was dependent on the peptide type produced by various enzymes.

3) ACE inhibitory activity

Angiotensin I-converting enzyme (ACE) is physiologically important in the regulation of blood pressure; it catalyzes the conversion of the inactive angiotensin I to a potent vasoconstrictor, angiotensin II, by cleavage of a dipeptide from the c-terminal of angiotensin I, and inactivates the vasodilator bradykinin. Thus, the specific inhibition of ACE could be of great value for prevention of hypertension (24).

ACE inhibitory activities for two starfishes are shown in Table 13. ACE inhibitory activity (IC_{50}) of methanol fraction showed 21.7, 18.3 and 16.4 μg/mL for *Asterias amurensis* (control), *Asterias amurensis* pre-treated with Alcalase 0.6L^{TM}, and with Protamex^{TM}, respectively, Especially, ACE inhibitory activity (IC_{50}) of methanol fraction obtained after hydrolysis with Protamex^{TM} was lower than those of the others. It was positively insured that ACE inhibitory activity of methanol fraction

Table 13

obtained after hydrolysis with Protamex^{TM} is predominant. Meanwhile, ethyl acetate fraction also showed weak ACE inhibitory activities, and ACE inhibitory activities of water fraction and acetone fraction were appeared to be completely inactive. In the case of *Asterina pectinifera*, ACE inhibitory activity (IC_{50}) of methanol fraction showed 31.8, 28.9 and 18.7 μg/mL for control, *Asterina pectinifera* pre-treated with Alcalase 0.6L^{TM}, and with Protamex^{TM}, respectively, and methanol fraction obtained after hydrolysis with Protamex^{TM} is also predominant. Lee (54) reported that IC_{50} of water fraction and boiling water fraction obtained from *Laminaria japonica* and *Agarum cribrosum* were 10.4 ~ 15.5 μg/mL and 13.3 ~ 24.0 μg/mL range, respectively, and it was known that these have strong antihypertensive effect. Accordingly, because ACE inhibitory activity (IC_{50}) of methanol fraction obtained from starfishes pre-treated with Protamex^{TM} was similar to those of water fraction and boiling water fraction obtained from *Laminaria japonica* and *Agarum cribrosum*, it was apparent that methanol fraction obtained from starfishes pre-treated with Protamex^{TM} showed higher ACE inhibitory activity.

Generally, it has been reported that ACE inhibitory activity is influenced by hydrolysates of proteins, and especially peptides having low molecular weight such as dipeptide and tripeptide possess higher ACE inhibitory activity (55, 56). Yeum et al. (56) reported that ACE inhibitory activity of hydrolysates of protein is influenced by the type of peptide not by the amounts of protein and peptide, which is presumed by the complex effects of length or structure of the low

molecular peptides and composition and sequence of amino acids etc obtained by hydrolysates.

4) Bile acid binding capacity (*in vitro*)

Binding capacity of bile acid for starfishes are shown in Table 14. In the case of *Asterias amurensis*, among the several extracts, methanol fraction had the strongest bile acid binding capacity. Especially, methanol fraction obtaind after hydrolysis Protamex^{TM} had the strongest capacity. Namely, binding capacity of bile acid of methanol fraction showed 24.01, 34.18 and 36.72 mM/g for control, for *Asterias amurensis* pre-treated with Alcalase 0.6L^{TM} and with Protamex^{TM}, respectively. Acetone fraction was followed in order, and even though low capacity, water fraction and ethyl acetate fraction of *Asterias amurensis* pre-treated with Protamex^{TM} showed the binding capacity of 17.37 and 4.23 mM/g, respectively. However, other extracts including ethylether fraction were appeared to be completely inactive for bile acid binding capacity. In the case of *Asterina pectinifera,* binding capacity of bile acid of methanol fraction for control, for *Asterina pectinifera* pre-treated with Alcalase 0.6L^{TM} and with Protamex^{TM} showed 61.86, 78.25 and 114.12 mM/g, respectively. Methanol fraction had also the strongest bile acid binding capacity among the solvent fractions. Especially, methanol fraction of obtained after hydrolysis with Protamex^{TM} had the strongest among those of the others. Ethyl acetate fraction, acetone fraction, ethylether fraction and water fraction was

Table 14

followed in order.

From the result shown above, it was suggested that binding capacity of bile acid of fractions obtained from *Asterina pectinifera* pre-treated with Protamex^{TM} was stronger over about 2 times than control. Cho et al. (17) reported that binding capacity of bile acid of acetone fraction obtained from *Asterias amurensis* and *Asterina pectinifera* of the South sea were 809.3 and 234.7 μM/g, respectively. Therefore, in this study, it is thought that methanol fraction obtained from *Asterina pectinifera* pre-treated with Protamex^{TM} was stronger than those of Cho et al. (17).

5) Antimutagenic effect (*in vitro*)

Toxicity test for the solvent fractions obtained from starfishes are shown in Tables 15 and 16. The solvent fractions obtained from starfishes for toxicity test in this study did not show any toxicity to the tester strain. The antimutagenic effects of solvent fractions obtained from two starfishes were examined using the Ames test with *Salmonella typhimurium* TA98 and TA100 for the mutagenicity induced by MNNG (Tables 17 and 18). Inhibiton ratio in each fractions of *Asterias amurensis* was weak against *Salmonella typhimurium* TA98. Meanwhile, in the case of *Asterina pectinifera,* methanol fraction among the solvent extracts showed the strongest inhibition ratio on MNNG, and inhibition ratio of methanol fraction obtained after hydrolysis Protamex^{TM} (98%) was higher than that in control (96%). Byoun et al.

Table 15

Table 16

Table 17

Table 18

(57) reported that water extracts (50 mg) of the garlic showed inhibition ratio of 97.9% on 2-aminofluorene against *Salmonella typhimurium* TA98. Ha et al. (58) reported that several carotenoids isolated from the tunicates and shellfishes showed inhibition ratio of 46~87% on 2-amino-3-methylimidazol [4.5-f] quinoline (IQ) against *Salmonella typhimurium* TA98. In addition, Lee et al.(59) reported that mugwort extract (250 μg) showed inhibition ratio of 23% on 2-nitrofluorene against *Salmonella typhimurium* TA98. These reports were similar with result of this study. The next effective ones were acetone fractions, namely acetone fractions showed 94, 95 and 95% for control, *Asterina pectinifera* pre-treated with Alcalase 0.6L™, and with Protamex™, respectively. Ethylether fractions, ethyl acetate fractions and water fractions exhibited very weak inhibition ratio.

Each solvent fraction of *Asterias amurensis* showed weak inhibition of base pair exchange mutation agent Salmonella typhimurium TA100 (Table 18). Meanwhile, in case of *Asterina pectinifera*, acetone fractions showed the highest inhibition ratio as from 83% to 89% range. Water fractions and methanol fractions were not observed. Lim (60) reported that 5% of *doenjang, chungkukjang* and persimmon leave extracts showed inhibition ratio of 60, 52 and 77% on MNNG mutagenicity against *Salmonella typhimurium* TA100, respectively. Therefore, it was also suggested that acetone fraction obtained from *Asterina pectinifera* have strong inhibition ratio compared to report by Lim.

Meanwhile, Han et al. (61) reported that a new component, 5 alpha-cholest-7-en-3beta-ol, isolated from butanol fraction of starfish,

Asterina pectinifera, possesses potent antigenotoxic activity against the mutagen, MNNG, with *Salmonella. typhmurium* TA1538. Axuine et al. (62) reported that β-carotene, 8´-apo-β-carotene-methylester, 8´-apo-β-carotenal and canthaxanthin also inhibit a mutation for the mutagenicity induced by MNNG against *Salmonella. typhimurium* TA100.

Accordingly, as the results of antimutagenic effects of two starfishes, methanol and acetone fractions obtained from *Asterina pectinifera* showed strong inhibition capacities for the mutagenicity induced by MNNG in *Salmonella. typhimurium* TA98. Meanwhile, in the case of *Salmonella typhimurium* TA100, acetone fraction obtained from *Asterina pectinifera* showed strong inhibition ratio. The inhibition ratio was stronger in the fraction obtained after hydrolysis with Protamex^{TM} than in the fraction of control.

In order to use effectively starfishes for human being life, isolation and structural elucidation of functional compounds, and the establishment of safety of functional compounds should be further needed in the next experiment.

Conclusion

Materials used in this study were two starfishes, *Asterias amurensis* and *Asterina pectinifera,* which are known to giving serious damages to shellfish farm on coast of Korea. To study the possibility of availability as functional materials, proximate composition, inorganic components, carotenoids pigments and total amino acids of the two starfishes were analyzed. Antimicrobial activity, antioxidative activity, ACE inhibitory activity, bile acid binding capacity and antimutagenicity activity in starfishes after hydrolysis with two kinds of proteases, Alcalase 0.6L^{TM} and Protamex^{TM}, to increase yield and bioactive materials, were tested.

1. In the case of proximate composition of two starfishes caught from the South sea, moisture content was lower than those of the general fish and shell, whereas crude ash prepared from two starfishes was rather higher than those of fish and shellfish. The contents of moisture and crude protein in *Asterina pectinifera* were lower than those of *Asterias amurensis,* whereas content of crude ash was higher than those of *Asterias amurensis*. Content of crude protein increased in the starfishes caught on May, June, and July compared to those caught on February

2. Content of calcium in raw *Asterias amurensis* and *Asterina*

pectinifera was 17.93% and 25.80%, respectively. These contents occupied as 37.02% and 39.86% of total crude ash in the two species and was the highest among inorganic components measured in this study. Na, Mg, Si and P were followed in order. Selenium, which was known as antioxidant, was contained in the two starfishes with very small amounts. Water fraction was extracted relatively with low amounts, and most of calcium existed in their residues.

3. The content of carotenoid pigments in *Asterias amurensis* and *Asterina pectinifera* was 0.62 and 5.88 mg%, respectively.

4. The contents of total amino acid in *Asterias amurensis* and *Asterina pectinifera.* were 10,786.95 and 8,511.33 mg%, respectively. In the case of *Asterias amurensis,* proline (32.7%) accounted for the most part in contents among the amino acids identified in this study, and glutamic acid (23.7%), cystathionine (8.4%), β-alanine (7.1%) were followed in order. In the case of *Asterina pectinifera,* glycine (12.0%) accounted for the most, and glutamic acid (11.6%), arginine (6.7%), threonine (6.4%) were followed in order.

5. In the case of antimicrobial activity, antioxidative activity, ACE inhibitory activity and bile acid binding capacity, methanol fractions in starfishes pre-treated with Protamex^{TM} showed stronger than those of control, and methanol fraction obtained from *Asterina pectinifera* were stronger than those of *Asterias amurensis* in all activities except for

ACE inhibitory activity

6. The results of antimutagenic effects of two starfishes using the Ames test with *Salmonella typhimurium* TA98 and TA100 for the mutagenicity induced by MNNG are shown as follows: methanol and acetone fractions obtained from *Asterina pectinifera* showed higher inhibition capacity for the mutagenicity induced by MNNG against *Salmonella typhimurium* TA98 than other fractions. In contrast, acetone fractions showed higher inhibition capacity for the mutagenicity against *Salmonella typhimurium* TA100. The effect of extracts in *Asterina pectinifera* pre-treated with Protamex^{TM} was stronger than that of control. Whereas, each fractions obtained from *Asterias amurensis* showed weak inhibition potency.

Therefore, in order to use effectively starfishes for human being life, isolation and structural elucidation of functional compounds, and the establishment of safety of functional compounds should be further needed in the next experiment.

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초 록

불가사리로부터 생리기능성 물질의 탐색

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해양생물은 육상과 다르게 특이한 생태계를 이루는 환경 때문에 적자생존의 경쟁 속에서 살아남기 위해 특이한 자기방어능력을 소유하고 있을 가능성이 매우 높다. 따라서, 이러한 가능성에 초점을 맞추어 해양생물로부터 새로운 생리활성물질을 개발하려는 시도가 활발히 진행되고 있는 상태이고 해양생물의 다양함을 고려한다면 그 가능성은 무한하다고 볼 수 있다.

특히 이중에서 극피동물에 속하는 불가사리는 서식환경에서의 생존능력이 뛰어나 우리나라 연안해역의 패류 양식장의 피조개, 전복, 바지락, 가리비 등 고급 패류를 무차별 포식함으로써 연안 패류자원의 황폐화 및 양식어업인에게 경제적으로 막대한 피해를 주어 심각한 해양환경 문제로 야기되고 있다. 현재까지 알려진 우리나라 패류 양식장에 피해를 주는 불가사리류는 아므르 불가사리 (*Asterias amurensis)*와 별 불가사리 (*Asterina pectinifera*) 2종이다.

따라서 본 연구에서는 이들 2종의 불가사리로부터 기능성 소재로서의 활용 가능성을 검토할 목적으로 불가사리의 화학적 성분의 분석과 동시에 단백질 분해효소를 이용하여 생리기능성 물질의 효과를 극대화시킬 수 있는 방법을 시도하였다. 그리고 얻어진 용매추출부에서의 생리기능적 특성을 탐색하여 다음과 같은 결과를 얻었다.

불가사리의 일반성분 조성은 일반 어패류에 비하여 수분함량이 적고, 회분 함량이 높았다. 아므르 불가사리의 경우 전체 고형물 함량에 대한 회분의 함량이 약 50%, 별 불가사리의 경우에는 60% 이상을 차지하고 있었

으며, 다음으로 단백질 함량이 많았다. 각 채취시기별로 보면 2월에 비해 5월, 6월, 7월달에 채취한 불가사리에서 증가하는 경향을 보였다. 그리고, 무기질을 분석한 결과, 아므르 불가사리와 별 불가사리에서 각각 Ca 함량이 17.93 g%과 25.80 g%로 전체 회분의 37.02% 및 39.86%를 차지하였고, 다음으로 Na, Mg, Si 및 P등의 순이었으며, 항산화 작용을 하는 것으로 알려진 Se도 미량 함유되어 있었다.

구성 아미노산은 아므르 불가사리의 경우 총 10,786 mg%였고, 별 불가사리에서는 8,511 mg%였다. 조성은 아므르 불가사리의 경우 proline (32.7%), glutamic acid (23.7%), cystathionine (8.4%), β-alanine (7.1%)순이었고, 별 불가사리에서는 glycine (12.0%), glutamic acid (11.6%), arginine (6.7%), threonine (6.4%)순이었다. 총 카로티노이드 함량은 아므르 불가사리의 경우 0.62 mg%, 별 불가사리의 경우는 5.88 mg%였다. 따라서 아미노산, 무기질 및 카로티노이드 색소등과 같은 유용성분은 안정성만 확립된다면 소재로서의 가능성이 충분히 있었다.

식품부패, 병원성 및 식중독 유발균류 7종 (*B. subtilis, B. cereus, Staphy. aureus, L. monocytogenes, E. coli, Sal. typhimurium, E. aerogenes)*과 피부염과 관련된 진균류 2종 (*Asp.. flavus*와 *Asp. niger)*을 대상으로 항균력실험을 한 결과, 아므르 불가사리보다 별 불가사리에서의 추출물의 효과가 더 우수하였고, 효소를 처리하지 않은 대조구에 비해 효소를 처리한 추출구에서 상대적으로 효과가 좋았다. 특히 단백질분해효소에서는 Protamex^{TM}로 처리한 경우가 모든 추출구에서 더 우수하였다. 추출 용매별로 살펴보면, Protamex^{TM}로 처리한 별 불가사리의 methanol 추출물은 500 μg/disk의 낮은 농도에서도 실험 균주 9종 모두에 대하여 항균력이 가장 우수하였고, 다음으로 acetone, ethylether 및 ethyl acetate 순이었으며, 물 추출구에서는 활성이 나타나지 않았다.

그리고 항산화능 실험에서는 Protamex^{TM}로 처리한 불가사리류의 methanol 추출구에서 전자공여능 (EDA_{50})이 각각 1.67, 0.68 mg/mL으로 가장 우수함을 알 수 있었다. 그리고 항고혈압성을 ACE 저해활성으로 측

정한 결과에서도, methanol 추출구가 각각 IC_{50} 16.4, 18.7 μg/mL으로 기존의 용매순차추출법보다 우수함을 알 수 있었다.

항콜레스테롤성 효과는 bile acid binding capacity로 측정하였는데, Protamex^{TM}로 처리한 불가사리류의 methanol 추출구에서 각각 36.72, 114.12 mM/g으로 우수한 효과를 나타내었다. 한편, 불가사리에서 얻어진 추출물의 항돌연변이효과를 측정하고자 *Sal. typhimurium* TA98 및 100균주에 직접 돌연변이원 MNNG로 돌연변이를 유도하여 실험한 항돌연변이성은 별 불가사리의 methanol과 acetone 추출물이 *Sal. typhimurium* TA98에서 억제효과가 나타났으며, *Sal. typhimurium* TA100에서는 별 불가사리의 acetone 추출물만이 돌연변이 억제효과가 있었다. 그러나 아므르 불가사리에서는 효과가 나타나지 않았다.

따라서 불가사리를 Protamex^{TM}로 50°C에서 5시간동안 가수분해한 다음 얻어진 별 불가사리의 methanol추출물은 생리기능적 효과가 전반적으로 우수함을 알 수 있었고, 아므르 불가사리에서도 Protamex^{TM}처리후의 methanol추출물은 항돌연변이성을 효과를 제외하고는 생리기능적효과가 우수함을 알 수 있었으나 별 불가사리보다는 낮았다.

감사의 글

돌이켜 생각해 보면 참으로 귀한 시간들이었습니다. 그 동안 너무나 많은 분들이 도와 주셨고, 그 분들을 통해 비록 작지만, 저에겐 너무나 소중한 이 결실을 얻게 되어 모든 분들께 깊은 감사를 드립니다.

먼저, 오로지 연구의 길만을 걸어오시고, 과학도로서 과학도가 가져야 할 자세를 늘 강조하시면서 진정한 스승의 상을 실천으로 보여주시는 지도교수님이신 존경하는 차용준 교수님, 제 논문 심사위원장님이신 김양하 교수님, 심사위원이신 보건 생화학과 문자영 교수님, 그리고, 대학 1학년 때부터 너무나 많은 도움을 주신 윤현숙 교수님, 김 창순 교수님, 이경혜 교수님, 문혜경 교수님께 진심으로 머리 숙여 감사드립니다. 또한, 제 논문을 위해 많은 도움과 격려를 이끼지 않으신 유영재 교수님, 조용권 교수님, 경상대학교 김진수 교수님, 오광수 교수님, 정보영 교수님, 그리고 주동식 박사님께도 진심으로 감사드립니다.

이 논문을 시작하기 위해 처음으로 불가사리를 잡으러 가던 날, 유난히도 추웠던 2001년 2월 달에 저희 신랑과 신랑친구인 김인식씨. 얕은 바닷물에서 주워 담을 생각으로 아무 준비없이 수건만 달랑들고 갔었는데, 웬걸... 깊은 바닷 속에서 희미하게 비치는 불가사리들.... 하는 수 없이 팬티바람으로 바닷물에 들어가 1봉지씩 잡으면서, 손, 발이 조개껍질에 베여 피나고, 온 몸이 벌겋게 되어 동사할 뻔 했던 그 날. 정말 그날을 평생 잊을 수가 없습니다. 그리고, 이 날을 계기 삼아 매 실험할 때마다 스쿠버 장비를 챙겨들고 불가사리를 잡으러 간 저희신랑과 후배 임문섭씨와 명환선배. 이들이 없었으면, 과연 이 실험을 해 낼 수 있었을까? 의문스럽습니다. 이들에게 진심으로 감사드립니다.

그리고, 기나긴 밤을 같이 밤샘하면서 많은 도움을 준 우리 실험실 부원들, 영원한 우상 우리 훈이선배, 장생 도라지 우진선배, 항상 차분한 소정이, 실장 은정이, 이쁜 영미, 야무진 조은이와 은경이, 파래 미영이, 참한 우리 지영이, 딸기 혜진이, 귀여운 민숙이와 현주와 설희, 영양학 실험실의 은실

이 언니, 임신했다고 매일 칼슘우유 사다준 고마운 우리 권증이, 현진이, 식품화학실험실의 김래영씨, 재희, 유경이 언니, 현주와 생화학실험실의 양정래 선생님, 현정이에게 진심으로 감사의 마음을 전하고 싶습니다. 또한 오랜 시간동안 많은 격려를 아끼지 않으신 김현진 이사님, 이현화 교수님, 나진희 선생님, 지금 영국에서 열심히 공부 중인 우리 변정순 언니, 나의 고마운 친구들인 영하, 미정이, 현화, 현주, 소희와 지영이, 예진이, 상희, 정민이, 그리고 예쁜 우리 현희와 항상 친구들의 우정이 뭔지 배우게 해 주는 임식씨, 정규씨를 비롯한 신랑 동아줄 친구들과 고향친구인 광재씨에게도 감사드립니다.

마지막으로, 일찍 들어가면 새벽 2시고, 밤샘도 밥먹듯이 하면서 청소, 빨래는 물론 제때 제대로 식사도 못 챙겨줬는데도 불구하고, 항상 열심히 하는 내가 자랑스럽다며 격려와 사랑을 아낌없이 보내준 사랑하는 저희 신랑, 이민호씨, 진심으로 감사합니다. 그리고, 유난히 자식들에게 아낌없는 사랑을 주시고, 며느리도 자식이라며 하고싶은 공부 해야된다며 힘든 사과농사지으시면서 지금까지 학비며, 생활비 다 대주시고, 잘 챙겨먹고 다니는지 걱정으로 일주일이 멀다하시며 반찬과 먹거리를 해다 주시며, 집안 대소사에 실험 때문에 바빠서 못 가봐도 당연히 이해해 주신 저희 시아버님, 시어머님과 재치와 유머가 넘치고 친구같은 예비 의사 우리 도련님.
저희 신랑을 갓난아기 때부터 어린 시절까지 사랑으로 키워주시고, 지금까지도 뵈면 용돈을 챙겨주시는 사랑이 넘치시는 할머님.
항상 자기 며느리처럼 너무나 예뻐해 주시고, 가면 언제든지 예술 같은 음식솜씨로 우리를 기쁘게 해주시는 큰 이모부님, 이모님.
만나면 언제나 포옹으로 시작하며, 너무나 예뻐해 주시는 이 시대 최고로 멋진 남자 외삼촌이신 양진수님과 천사 같은 외숙모님.
멀리 서울에서도 아낌없는 사랑을 보내주시는 막내 이모부님, 이모님과 창원 고모부님, 고모님.
너무나 큰 사랑을 주신 이 모든 분들께 진심으로 감사드립니다.
그리고, 지금까지 항상 정신적인 기둥이 되어주신 너무나 사랑하는 친정식구들, 할아버지, 아버지, 어머니, 오빠와 동생 현수에게도 진심으로 감사드리며, 이 작은 결실을 바칩니다.
또한, 바빠서 많이 신경 써 주지 못했지만, 10달 동안 뱃속에서 너무나 건

강하게 잘 커 준, 곧 태어날 사랑하는 우리 예쁜 아기에게도 감사하는 마음을 전합니다.

끝으로 지금까지 저에게 많은 도움과 사랑을 주신 모든 분들께 다시 한 번 더 진심으로 감사의 마음을 올리며, 항상 건강하시고 앞으로 좋은 일만 있으시길 진심으로 바랍니다.

응 시 부 문		연락처	
	국립 창원대학교 CHANGWON NATIONAL UNIVERSITY	휴대폰	

이 력 서

사 진 3cm×4cm	성 명	한 글		주민등록 번 호	
		한 문		생년월일	(만 세)
주 소					
호 적 관 계	호주성명			호주와의 관 계	

년	월	일	학 력 및 경 력 사 항

년	월	일	자 격 · 특 기 · 상 벌 사 항	시 행 청

위 사항은 사실과 틀림없음.

년 월 일

㊞

自 己 紹 介 書

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자연과학대학　　식품영양학과　　성 명 :

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