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Determination of Biogenic Amines in Different Shrimp Species for Export

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Abstract

This study is part of the project on the "Quality Assurance of Different Shrimp Species for Export". Local shrimp samples were collected from Ministry of Livestock and Fisheries and various private enterprises. Contents of biogenic amines were determined by using benzoyl chloride derivatization method with HPLC (reverse phase high performance liquid chromatography). Based on the biogenic amines, quality index of shrimps were correlated with freshness index so that the grade of shrimp samples can be classified as excellent, good, and acceptable. All sizes of shrimps such as extra large, large, medium were found to excceptable respectively.

Keywords: biogenic amines, shrimp species, derivatization method (HPLC)

Introduction

Naturally occurring biogenic amines are widely present in all living cells and a variety of food stuffs. Some amines such as putrescine, histamine, cadaverine, spermidine and spermine have been implicated in growth processes. Biogenie amines are biologically active compounds synthesized from amino acids. These biogenic amines are useful to determine as quality indices for the decomposition of shrimp for export. Many chromatogrphic methods especially HPLC, have been developed

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and widely used in the determination of the concentration of biogenic amines.

Among them, amine derivatization with fluorescine agents can give specific and sensitive method for the determination of biogenic amines. However, the reaction products have a short life and the method requires the preparation of amines before derivatization.

The use of tosyl, dansyl or benzoyl chloride is preferred as they can derivatize most of the naturally occurring amines, and form more stable reaction products. The naturally occurring polyamines (putrescine, spermidine and spermine) are low molecular weight organic cations present in all living cells.

Stimulation of polyamines synthesis is usually included in the rate of DNA, RNA and protein synthesis. The application of benzoyl derivatives is advantageous. The biogenic amine content is useful in estimating freshness or degree of spoilage of fish and fishery products.

In this paper, a modification of a simple and rapid derivatization procedure of different biogenic amines with benzoyl chloride and their determination by reversed phase HPLC is described.

The present paper describes how to draw the quality index in frozen shrimp species for export.

Materials and Methods

Collection of samples

Local export shrimp samples were collected from the Ministry of Livestock and Fisheries and various export enterprises. In exporting ,it is necessary to process before exporting. Processing was performed as follows.

The contents of polyamines were measured depending on the sizes of shrimp species.

Reagents

Standard biogenic amines including putrescine hydrochloride, cadaverine dihydrochloride, histamine dihydrochloride ,spermidine trihydrochloride, spermine tetrahydrochloride were obtained from Sigma (St. louis, Mc. U.S.A)Methanol.(HPLC grade was obtained from Merck (Germany).

Standard biogenic amines solution

Putrescine dihydrochloride (10mg), 10mg of cadaverine dihydrochloride, 10mg of histamine dihydrochloride ,10mg of spermidine trihydrochloride, 10mg of spermine tetra hydrochloride were dissolved in 10 ml of doubly distilled water . The final concentration of each amine was 1 mM.

Determination of biogenic amines by using benzoyl chloride derivatization method

To 2 ml of mixed amine solution containing 0.1 mg of each amine standard, 1 ml of 2 M sodium hydroxide was added., followed by 10 μl of benzoyl chloride, the solution was mixed by using a vortex mixer and was allowed to stand at 20, 30 , 40 and 60 ^{o}C for 20, 40, and 60 min. The benzoylation was stopped by adding 2 ml of saturated NaCl solution, and the solution was extracted with 3 ml of diethyl ether. After centrifugation, the organic layer was transferred into a tube and evaporated to dryness in a stream of nitrogen. The residue was dissolved in 1 ml of methanol and 20 μl aliquot were injected for HPLC analysis(D-Hwang 1997 and P.Malle 1996).

Determination of the calibration curve of standard amine

To 2 ml of mixed standard amine solution containing 0-0.5 mg of each amine, 1 ml of 2 M sodium hydroxide was added , followed by 10µl of benzoyl chloride. The reaction temperature and time were set at 30°C for 40 min, respectively. After benzoylation, the standard sample was extracted and determined by HPLC analysis as described in section 2.4.

Separation of amines by HPLC

Amines were determined by using a Hitachi liquid chromatograph (Hitachi, Tokyo, Japan) consisting of a Model L 6200 pump, a Rheodyne Model 7125 syringe loadng sample injector, a model L- 400 UV –visible detector, set at 254nm, and a Model D- 2500 chromato integrator. A Lichrospher 100 RP- 18 reversed phase column (5 µm, 125 x 2.5 mm I.D .Merck) was used for the separation. The gradient elution program was set at 0.8 ml/min, starting with a methanol- water mixture (50:50 , v/v) for 0.5 min. The program proceeded linearly to methanol- water 85 :15, v/v, with a flow rate of 0.8 ml/ min over 6.5 min.

Recovery of biogenic amines from frozen shrimp samples for export

After processing for export, frozen shrimp samples were homogenized in a Waring blender for 2 min. Each ground sample (5 g) was transferred to a Sorvall centrifuge tube, mixed with 100, 200 , 300, 400, 500 µl of 1 mg/ml of each standard amine and separately homogenized with 10 ml of 20% of $HClO_4$ for 2 min. The homogenized solution was centrifuged (10 min, 40°C). The filtrate was transferred into a centrifuge tube and each extract (2 ml) was derivatized with benzoyl chloride using the same procedure as standard biogenic amines measurement. The recovery of amines in frozen shrimp was determined (Hwang 1997 and M.Valle 1996).

Results and discussion

The chromatographic profile of these biogenic amines by the gradient system was developed as shown figure 2. All these biogenic amines were well separated in a total duration of 30 min with good peak resolution and sharpness. Benzoylation of biogenic amines at 37°C gave the best peak and maximum peak areas. The calibration curve for each amine was linear within the range of 0.02- 4 μg. The contents of biogenic amines in *P.monodon* (Black tiger) are described in Table 1,2 ,3 and 4 and Figures 1and 2. It is interesting to note that contents of biogenic amines have some correlation with the size of shrimp samples. In the smaller size of shrimp samples, more decomposition was found and in the larger size of shrimp samples, less decomposition was found. So, the smaller the shrimp, the larger the spoilage becomes.

$$\text{Quality Index} = \frac{\text{histamine(ppm)+putrescine(ppm)+cadaverine(ppm)}}{\text{1+spermidine+spermine}}$$

Mietz and karmas (1971) proposed a chemical quality index based on biogenic amines (H.H. Huss,1995). It is very interesting to find that the value of quality index is smaller as the value of freshness index becomes larger.

Quality Index is inversely proportional to freshness index. Freshness index is related to Quality Index (Pierre Malle and Michel Valle 1996). This present study showed that the value of Quality Index (%) was calculated by plotting correlation between quality index and freshness index. It is very interesting to find that contents of putrescine, cadaverine and histamine diamines increased in smaller size of shrimp species and decreased in greater size of shrimp species. On the other hand, contents of spermidine and spermine decreased in smaller size of shrimp species as in Figures 3, 4, 5, 6 and 7. Contents of spermine and spermidine increased in larger size of shrimp species and they are rarely

found in smaller size shrimp species. Spermidine and spermine were found in promoting growth potential in animal tissue. It is known that putrescine, spermidine and spermine are essential cell growth and cell division.

Conclusion

In this study, it was found that spermidine and spermine show the greatest growth in promoting potential in shrimp. These polyamines play an important role in promoting cell growth and cell division. Therefore, biogenic amines should not always be considered as potential toxicants. The contribution of this work is that this study serves as a potential application for biogenic amines in shrimp species for export. The content of polyamines present in shrimp samples for export are within the acceptable range fixed by the U.S Food and Drug Administration (FDA). European commission and Australian Food Safety Community set the limited at 100mg/kg. for some biogenic amines for e.g (histamine). But, some biogenic amines have not set until now. In this study, a rapid, sensitive and reproducible HPLC procedure was developed for the determination of these biogenic amines in frozen shrimp samples using benzoylation with benzoyl chloride.

It can be concluded that the importance of quality indices rely on biogenic amines for quality control for consumption. Assurance of seafood safety depends on numerous factors and consideration. It can also be reported that QIM method is beneficial to be used in our country .This work was proposed new information in seafood production and on seafood spoilage. Quality index and freshness Index for fish were found in the literature. This new study was proposed to be used as the quality index for fish and shrimp.

It is observed that bigger shrimp is preferable for export and local consumption because smaller shrimp can be contaminated (D.F.Hwang 1997).

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Table 1 Contents of Biogenic Amines in *P. monodon* (B. tiger), (Small Size) (mean) (S.D) for Export by using benzoyl chloride derivatization method with HPLC

Sample	Put (ppm)	Cad (ppm)	His (ppm)	Spd (ppm)	Spm (ppm)	QI	FI
1	9.05±0.2	5.01±0.10	4.90±0.00	10.28±0.0	0.146±0.10	165%	0.6
2	9.08±0.1	5.12±0.10	4.93±0.10	10.31±0.10	0.150±0.10	166%	0.6
3	9.10±0.1	5.21±0.20	3.19±0.10	10.93±0.10	0.148±0.20	144%	0.7
4	9.54±0.1	4.67±0.20	4.52±0.20	9.98±0.20	0.152±0.20	168%	0.7
5	9.75±0.2	5.01±0.30	4.77±0.20	9.70±0.20	0.155±0.40	180%	0.75
6	9.99±0.3	5.45±0.30	5.01±0.30	9.46±0.10	0.140±0.50	210%	0.65
7	9.69±0.3	5.87±0.40	5.23±0.30	9.57±0.20	0.152±0.50	193%	0.85
8	10.02±0.2	4.97±0.40	5.08±0.40	9.44±0.20	0.142±0.40	184%	0.75
9	10.23±0.1	4.99±0.40	5.25±0.50	10.01±0.30	0.149±0.30	183%	0.75
10	10.37±0.2	5.04±0.30	5.55±0.30	10.44±0.30	0.143±0.40	180%	0.7

Table 2 Contents of Biogenic Amines in *P. monodon* (B. tiger), (Medium Size) (mean) (S.D) for Export by using benzoyl chloride derivatization method with HPLC

Sample	Put (ppm)	Cad (ppm)	His (ppm)	Spd (ppm)	Spm (ppm)	QI	FI
1	7.10±0.10	3.91±0.10	3.10±0.20	16.05±0.10	2.01±0.10	74%	1.2
2	7.21±0.10	3.37±0.20	3.72±0.30	15.29±0.20	2.41±0.20	76%	1.4
3	7.19±0.20	3.10±0.20	3.59±0.30	15.56±0.20	2.55±0.30	77%	1.1
4	7.40±0.20	3.81±0.20	3.52±0.30	16.18±0.20	2.25±0.20	75%	1.4
5	7.95±0.30	3.20±0.20	3.25±0.40	15.73±0.30	2.08±0.20	76%	1.2
6	7.79±0.30	3.49±0.20	3.02±0.40	15.73±0.30	2.28±0.20	75%	1.4
7	8.01±0.30	3.51±0.20	3.44±0.40	15.80±0.30	2.80±0.20	76%	1.1
8	7.54±0.30	3.77±0.30	3.26±0.30	15.37±0.40	2.35±0.20	78%	1.1
9	7.94±0.40	3.55±0.30	3.40±0.30	15.40±0.40	2.77±0.30	77%	1.1
10	7.34±0.40	3.88±0.30	3.22±0.30	15.68±0.40	2.57±0.30	75%	1.3

Table 3 Contents of Biogenic Amines in *P. monodon* (B. tiger), (Large Size) (mean) (S.D) for Export by using benzoyl chloride derivatization method with HPLC

Sample	Put (ppm)	Cad (ppm)	His (ppm)	Spd (ppm)	Spm (ppm)	QI	FI
1	2.50±0.1	1.93±0.2	1.20±0.1	17.83±0.2	2.90±0.1	25%	2.0
2	1.99±0.1	2.01±0.2	0.31±0.2	17.68±0.2	2.70±0.1	20%	2.1
3	1.85±0.1	1.95±0.2	0.56±0.2	18.05±0.1	2.50±0.1	20%	2.2
4	1.55±0.2	2.97±0.1	0.37±0.2	18.10±0.1	1.92±0.2	25%	2.4
5	3.01±0.1	4.44±0.1	0.99±0.2	18.77±0.1	1.93±0.2	38%	2.8
6	2.21±0.1	1.37±0.2	1.07±0.1	18.03±0.1	1.76±0.2	22%	1.6
7	1.59±0.2	2.05±0.2	0.37±0.2	17.96±0.2	1.78±0.2	20%	1.8
8	2.29±0.1	3.51±0.1	0.88±0.2	18.00±0.1	1.65±0.2	32%	1.4
9	2.09±0.1	3.40±0.1	0.91±0.2	18.01±0.1	1.95±0.1	30%	2.3
10	2.31±0.1	4.71±0.1	1.08±0.1	18.10±0.1	1.55±0.2	38%	2.2

Table 4 Contents of Biogenic Amines in *P. monodon* (B. tiger), (Extra Size) (mean) (S.D) for Export by using benzoyl chloride derivatization method with HPLC

Sample	Put (ppm)	Cad (ppm)	His (ppm)	Spd (ppm)	Spm (ppm)	QI	FI
1	1.88	1.02	0.90	25.24	6.01	12%	2.6
2	1.92	0.59	0.57	22.10	6.50	10%	2.7
3	1.99	0.91	1.02	22.02	6.90	13%	2.5
4	1.05	0.95	0.55	21.50	6.70	8.73%	2.7
5	1.51	0.82	0.74	18.95	6.92	12%	2.6
6	1.71	0.95	0.86	20.19	6.37	11.42%	2.6
7	1.65	0.91	0.76	22.01	6.27	11.3%	2.6
8	1.57	0.80	0.32	21.02	6.47	9.44%	2.7
9	1.42	0.77	0.44	20.73	6.59	9.2%	2.7
10	1.63	0.87	0.81	20.79	6.92	1.52%	2.6

Figure 1 Separation of Benzoylated polyamines by HPLC. Operation: column (250x4.6mm) reverse phase column mobile phase, methanol water (65:35); column tempemratuure, ambient; flow rate, 1ml/min. Peaks identification: 1=Putrescine, 2=Cadaverine, 3=diaminohexane (internal standard), 4= Histamine, 5 = Spermidine, 6= Spermine, 7 = Tyramine

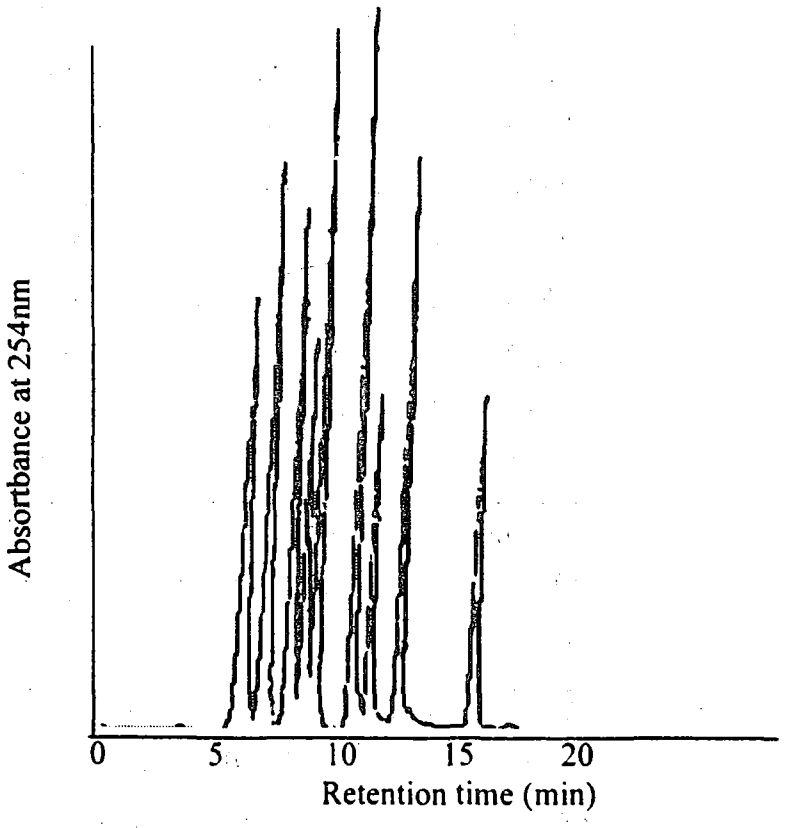


Figure 2 Comparison of biogenic amines from P.monodon (Black Tiger) (Upper) and standard biogenic amines Chromatogram (Lower) with HPLC. Peaks identification: 1= Putrescine, 2 = Cadaverine, 3 = diaminohexane (internal standard), 4 = Histamine, 5 =Spermidine, 6= Spermine

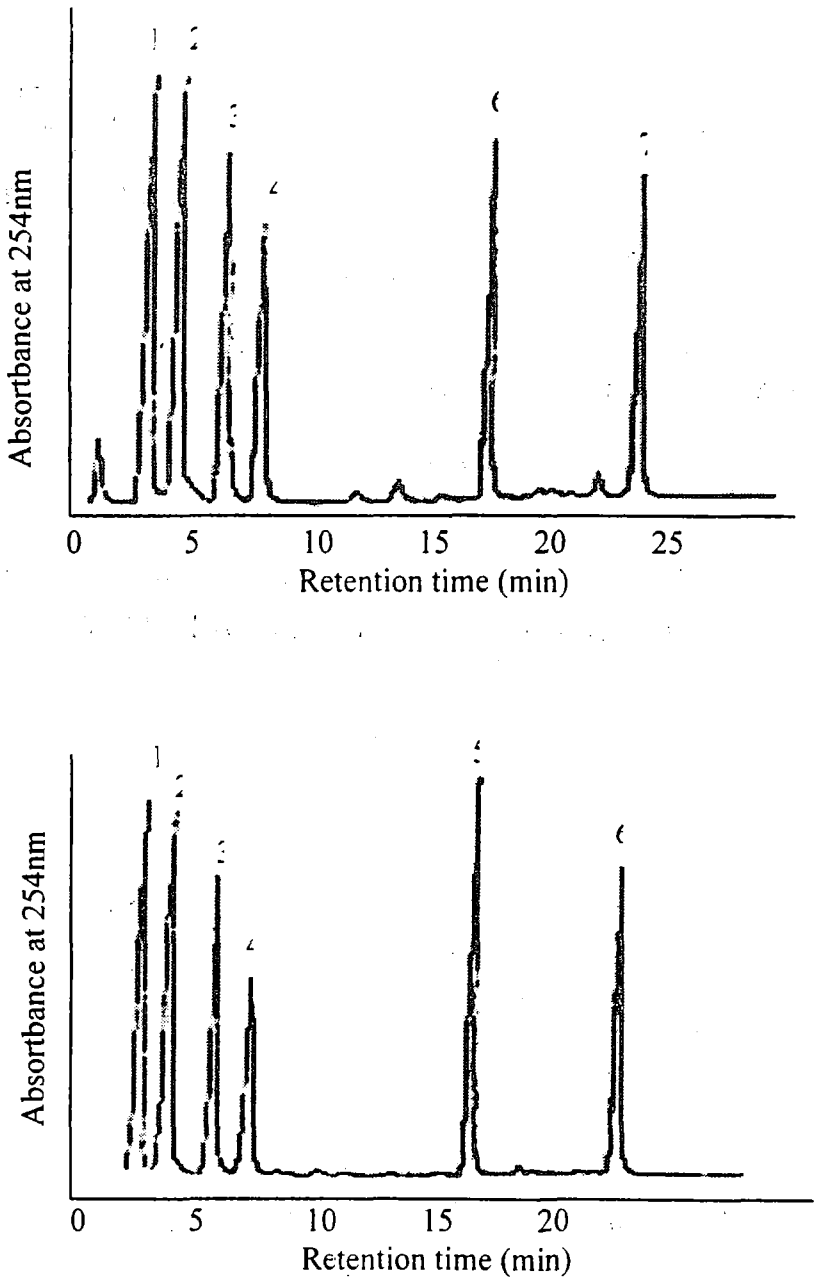


Figure 3 Content of spermine in different shrimp species for export by using benzoyl chloride derivatization with HPLC

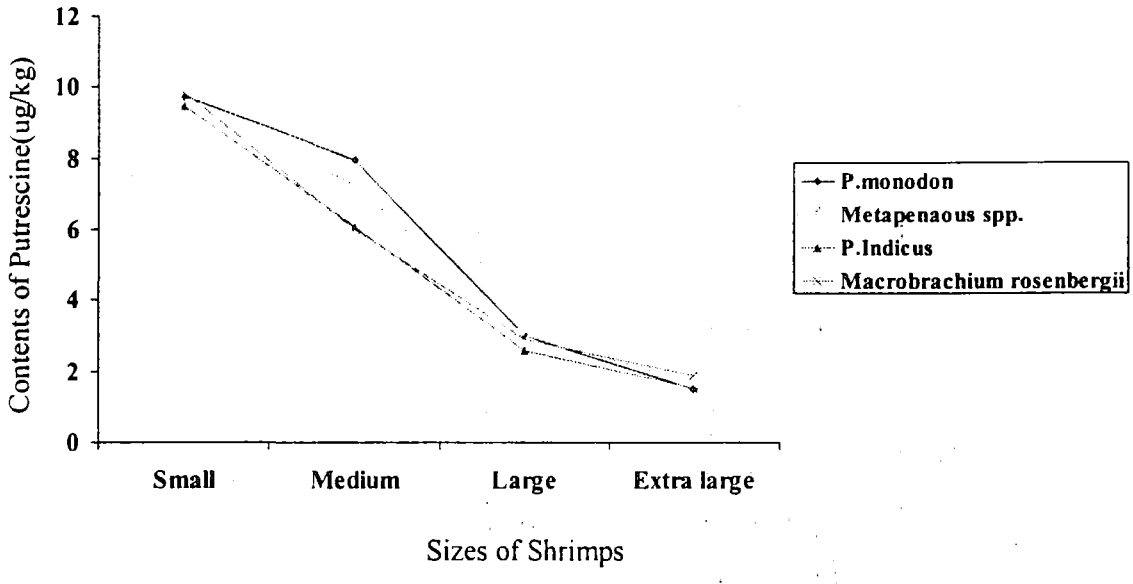


Figure 4 Content of spermidine in different shrimp species for export by using benzoyl chloride derivatization with HPLC

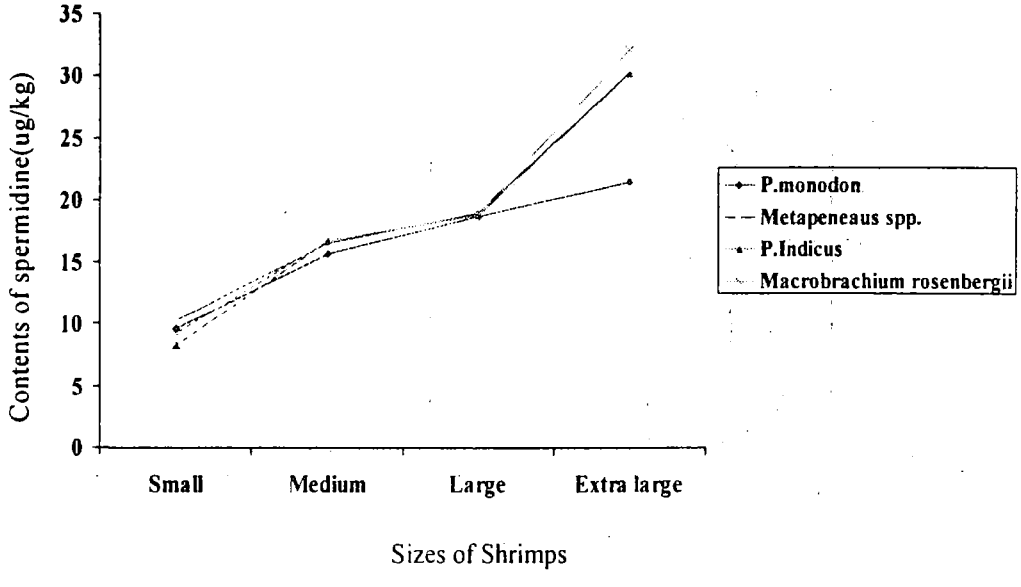


Figure 5 Content of spermidine in different shrimp species for export by using benzoyl chloride derivatization with HPLC

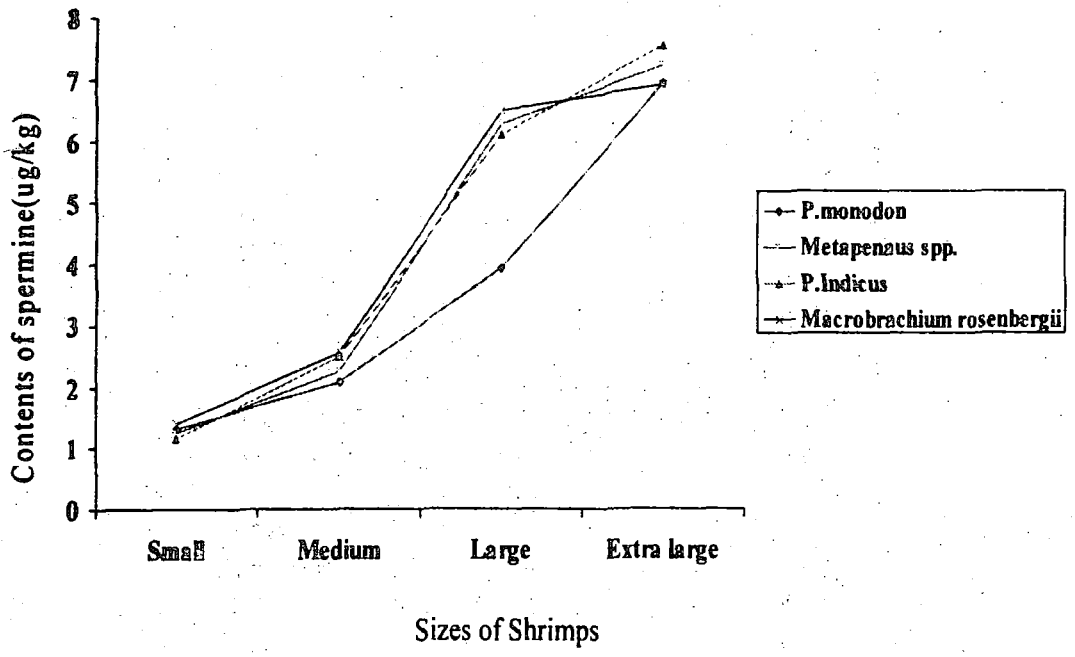
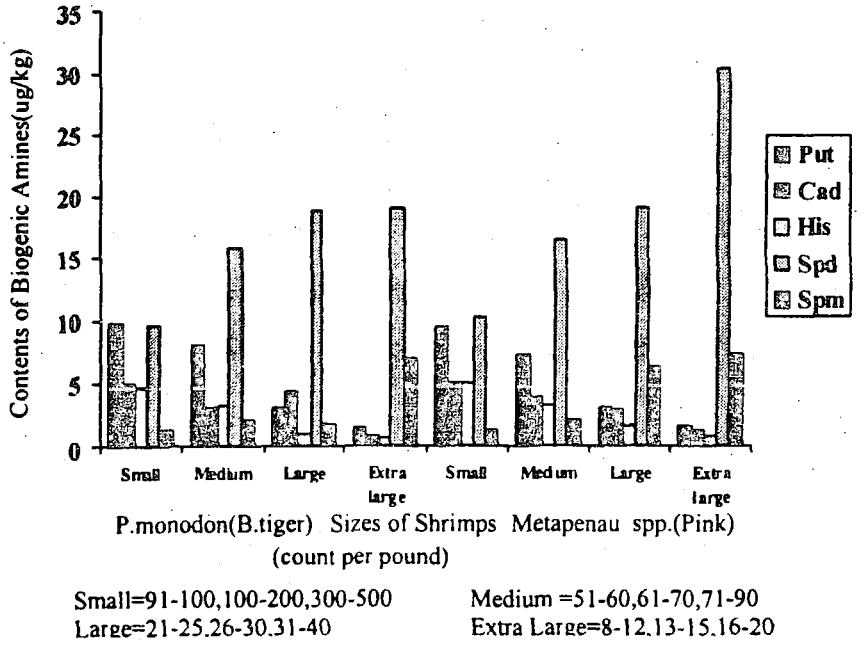
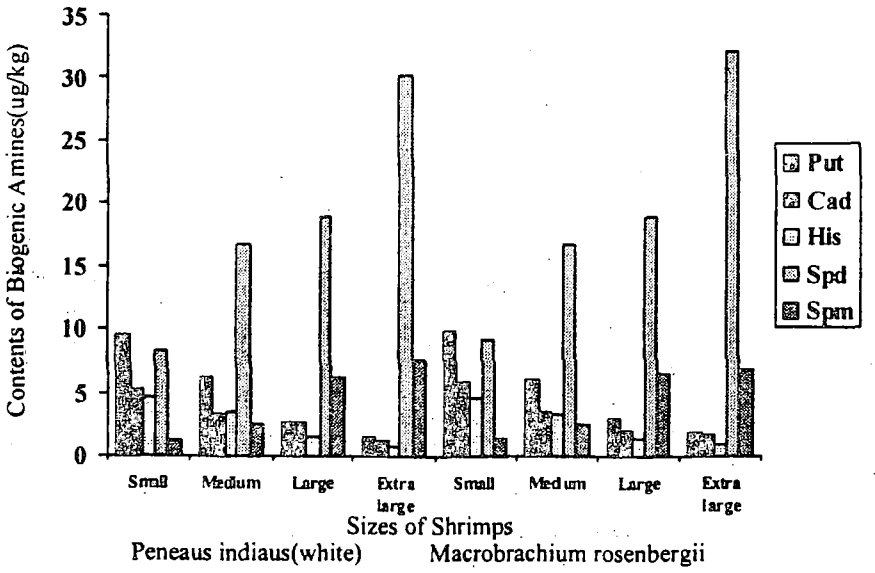


Figure 6 Contents of Biogenic Amines in Different Shrimp Species for Export by using benzoyl chloride derivatization with HPLC



Small=91-100,100-200,300-500 Medium =51-60,61-70,71-90
Large=21-25.26-30.31-40 Extra Large=8-12.13-15.16-20

Figure 7 Contents of Biogenic Amines in Different Shrimp Species for Export by using benzoyl chloride derivatization with HPLC



Small=91-100,100-200,300-500 Medium=51-60,61-70,71-90
Large=21-25,26-30,31-40 Extra Large=8-12,13-15,16-20 (count per pound)