Free Amino Acid Pattern in Indian Shrimp (*Metapenaeus Dobsonii*)

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Free amino acids in fish and shell fish are believed to confer characteristic flavors to these sea foods and also serve as an index of microbial spoilage in early stages. The present account describes the quantitative estimation of the free amino acids by ion-exchange chromatography. Glycine, arginine, glutamic acid, threonine, proline, and alanine have previously been reported to figure prominently in the amino acid pool; but the major Indian shrimp species, *Metapenaeus Dobsonii*, is deficient in the last two amino acids. Taurine has been identified for the first time in Indian sea foods.

Free amino acids are believed to confer characteristic flavors to different varieties of fish and shell fish besides serving as an index of spoilage in early stages. Data on the occurrence of free amino acids in Indian fish and other sea foods are lacking except for qualitative studies by Velanker (Velanker and Mahadeva Iyer, 1961) based on unidimensional paper chromatography. Shrimp in particular has engaged our attention in view of its commercial importance and the general belief that glycine and proline are responsible for the sweet flavor in crustacea (Amano and Bito, 1951, De Almeida, 1961; Konosu *et al.*, 1968; Nair and Bose, 1965; Simidu and Masao Hujita, 1954).

In view of the recent work by Hashimoto (1965) regarding the role of several amino acids in fish flavor, it was considered desirable to undertake a quantitative study of the free amino acids of Indian shrimp by ion-exchange chromatography.

EXPERIMENTAL

Fresh shrimp (*Metapenaeus Dobsonii*) transported in ice to the laboratory was employed for sampling and extraction with alcohol (1:4 w./v.) after beheading, shelling, and deveining (Awapara, 1948). The procedure adopted for ion-exchange chromatography was based on that of Moore and Stein (Moore and Stein, 1951; Stein and Moore, 1954). The longer column, employed for the separation of all the amino acids except the basic ones, was operated at 37.5°, 50°, and 75° C by means of a water jacket. Citrate buffers of pH 3.42 and 4.25 were employed for elution (Figure 1). Basic amino acids were separated on the smaller column at room temperature by citrate buffers of pH 5.0 and 6.5 and phosphate buffer at pH 6.81. Arginine appeared in the 120- to 150-ml fraction instead of the 175- to 200-ml fraction as found by Moore and Stein (Figure 2). After adjusting to pH 5.0, an 0.1-ml portion of each effluent fraction was used for the location of the peaks and the rest was employed for paper chromatography and quantitative estimation. Table I compares the findings of the present study with those of other investigators. The authenticity of the peaks due to individual amino acids was checked by two-dimensional paper chromatography employing *n*-butanol–acetic acid–water (4:1:5) and secondary butanol–3% ammonia (3:1) as solvent systems.

RECOVERY EXPERIMENTS

Mixtures of pure amino acids (4.5 mg at pH 2.65 for the 100-cm column and 1.0 mg at pH 4.0 for the 15-cm column) gave 95 to 100% recovery in all cases except hydroxyproline, methionine, and tryptophan where it was around 90%. Arginine again appeared in earlier fractions (120 to 150 ml).

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RESULTS AND DISCUSSION

Since the existing data on the free amino acid pattern in shrimp are based on paper chromatography or microbiological assays (Canien *et al.*, 1951; Velanker and Mahadeva Iyer, 1961), the main objective of this study was to establish the exact nature of the free amino acids present in shrimp so as to serve as a basis for future work on the role of amino acids in shrimp flavor or their significance in quality control. Paper chromatographic studies (Velanker and Mahadeva Iyer, 1961) presented some unusual features in Indian shrimp, such as the absence of glutamic acid and presence of valine and leucine in significant amounts. It was also considered desirable to obtain more information on the glycine content which has been reported as the chief flavor component (Simidu and Masao Hujita, 1954). Data obtained from the present study by ion-exchange chromatography has not revealed the presence of leucine and valine in appreciable quantities. Table I shows that our value for taurine agrees well with those of Simpson (Simpson *et al.*, 1959), while the Japanese workers have not reported its presence. Although the free glycine content in our samples is fairly significant, it is still lower than those quoted for Indian shrimp (Nair and Bose, 1965) and other species (Simpson *et al.*, 1959; Simidu *et al.*, 1952).

Table I. Amounts of Free Amino Acids in Shrimp Muscle

Amino Acids	*Metapenaeus Dobsonii*, mg %	*Penaeus oztecus* (Simpson *et al.* 1959) mg %	*Penaeus Japanicus* (Konosu *et al.* 1968) mg %
Taurine	237.9 ± 2.0	292.1	...
Aspartic acid	35.4 ± 1.19	13.3	Traces
Threonine	52.6 ± 1.05	...	15.0
Serine	60.5 ± 0.90	...	108.0
Sarcocine	29.1 ± 1.00	...	...
Glutamic acid	117.5 ± 1.37	29.2	65.0
Proline	2.20	...	230.0
Glycine	88.2 ± 1.67 200–500[a]	420 450[b]	1250.0
Alanine	15.7 ± 1.2	44.5	58.0
Valine	8.7 ± 0.69	...	19.0
Methionine	4.8 ± 0.63	...	11.0
Isoleucine	8.9 ± 0.45	...	11.0
Leucine	17.5 ± 0.86	...	17.0
Glucosamine	...	...	...
Tyrosine	19.4 ± 0.76	...	1.0
Phenylalanine	7.6 ± 0.88	...	7.0
Tryptophan	18.4 ± 1.94	...	0.9
Histidine	34.9 ± 1.10	...	7.0
			9.3 to 20.6[c]
Lysine	48.2 ± 0.31	...	15.0
Arginine	219.4 ± 2.21	121.8	686.0

[a] Nair and Bose, 1965. [b] Awapara, 1961. [c] Simudu, 1952.

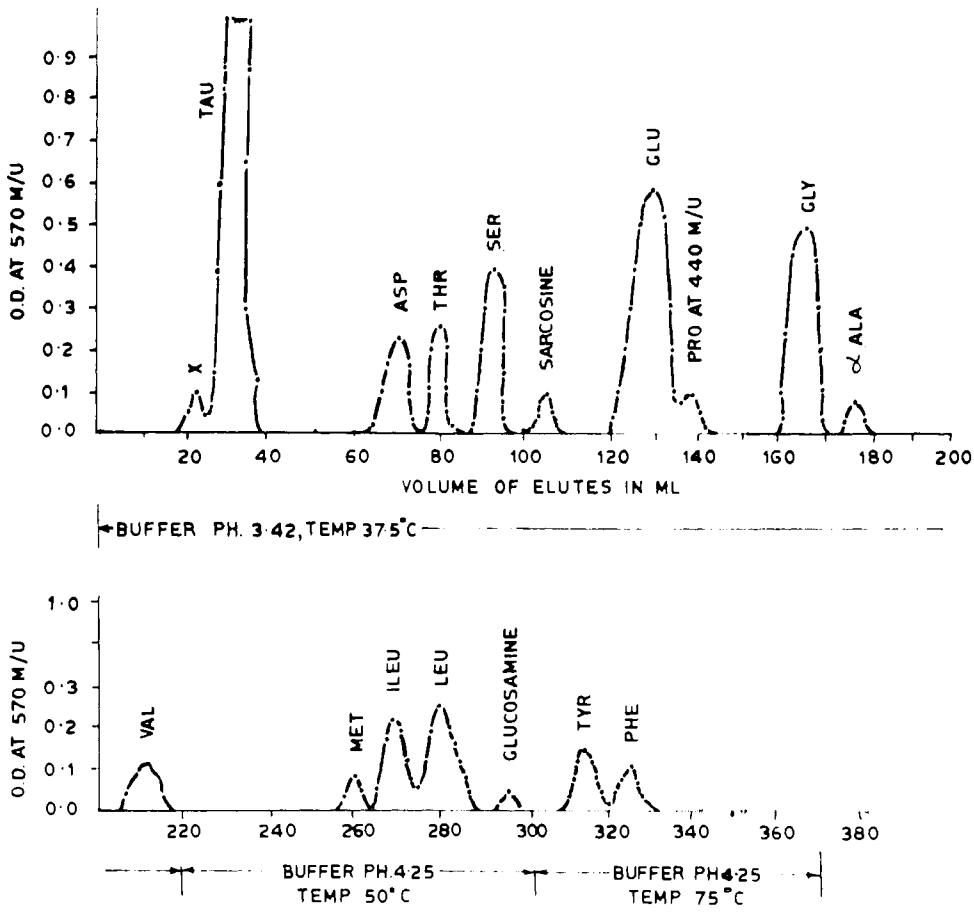

Figure 1. Separation of amino acids from extract of fresh shrimp

The column of Dowex 50-X8, 0.9 × 100 cm. was operated in sodium form with buffers of pH and temperatures as indicated. Effluents were maintained at 4.0 ml. per hour and 1.0-ml. fractions were collected

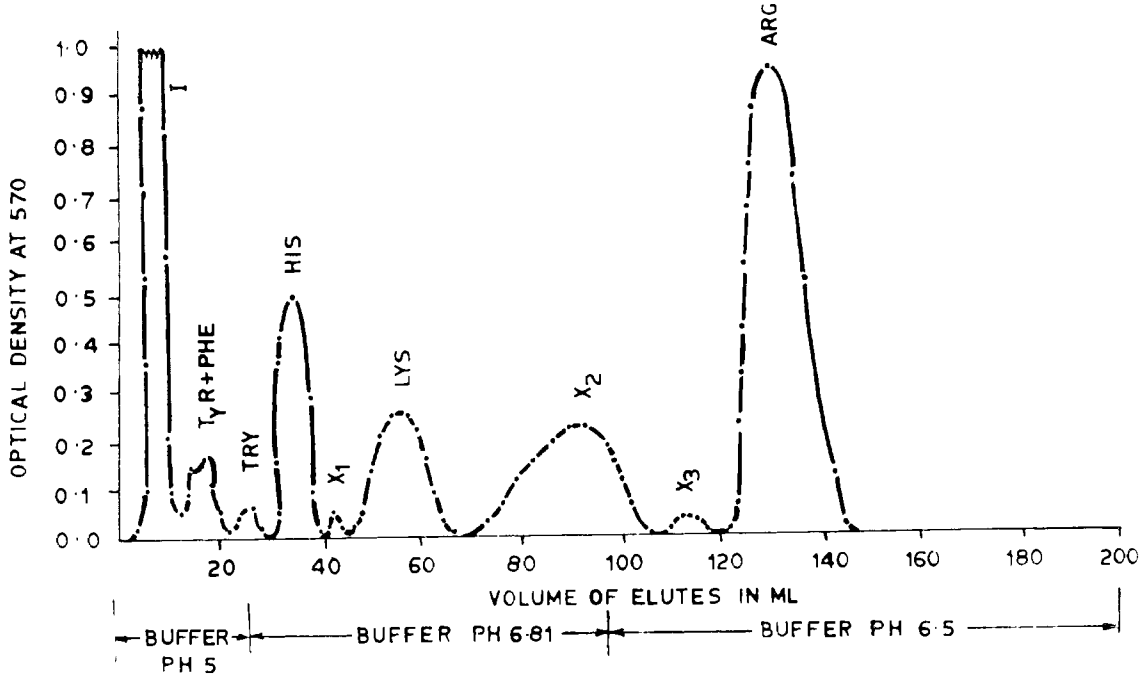

Figure 2. Separation of basic amino acids from extract of fresh shrimp on a column of Dowex 50-X8, 0.9 × 15 cm.

The column was operated at room temperature with the buffers of pH as indicated. The large peak 1 comprises all the amino acids emerging before tyrosine in Figure 1. Flow rate was 4.0 ml. per hour and 1.0-ml. fractions were collected

Japanese work (Konosu *et al.*, 1968) indicates a still higher value, while proline and alanine which have been reported by other workers to occur invariably in shrimp form likewise minor constituents in Indian shrimp. Our material, although rich in aspartic acid, contains less serine than the Japanese variety. *Metapenaeus Dobsonii* is also richer in tyrosine and phenylalanine than the Japanese shrimp. Arginine, glycine, and glutamic acid, however, are quite prominent as reported by other workers, the latter being higher in our sample. We are also able to confirm the presence of taurine, which in fact forms the major constituent. It may be mentioned that Kittredge (James S. Kittredge *et al.*, 1961) has reported its presence in addition to glycine, arginine, and tyrosine-*o*-sulfate, the last being absent.

Regarding taurine, this is the first instance that it has been detected in Indian fish or shell fish. Threonine and lysine are among the other significant free amino acids present in Indian shrimp. Among the noteworthy features of the free amino acids of Indian shrimp is absence of branched chain and aromatic amino acids in significant quantities, a feature common to marine organisms according to Simpson (Simpson *et al.*, 1959). Taurine constitutes the only sulfur amino acid, ignoring traces of methionine. On the other hand the histidine level is slightly higher than the reported values (34.4 mg % as against 7.0% and 9.3 to 20.6 mg %). Glucosamine was only present in traces.

Although the seasonal variation may form a partial explanation, our experience suggests that the glycine content of Indian shrimp appears to be lower than values reported by Nair and Bose (1965). It is proposed to study this aspect in greater detail because of its technological significance. It is proposed to identify the four unknown peaks, X_1, X_2, X_3, and X_4 (Figures 1 and 2) which do not correspond to any other known constituents of shrimp. This accounts for the fact that free amino acids described above after quantitative separation forms only 41.4% of the alpha-amino nitrogen by the Pope and Stevens method.

Taste panel observations at this laboratory reveal that glycine contributes to the sweet flavor of shrimp, while leucine, glutamic acid, and proline confer a desirable flavor. Arginine was also found to affect the flavor to an appreciable extent. On the other hand, taurine was found to give an unusual after taste.

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