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Isolation and analysis of proteins in shrimp waste

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Abstract

The objective of this study is to measure and quantify the proteins present in shrimp waste. Which is estimated by lowrys protein assay after the extraction of the proteins from the shrimp waste using MLB lysis buffer. The correlation of the amino acids present in it are found by using thin layer chromatography technique, then the peptides present in the proteins are separated out by sodium dodecyl sulphate-polyacrylamide gel electrophoresis and native polyacrylamide gel electrophoresis.

Keywords: Protein isolation; Shrimp waste; Thin-layer chromatography; Protein analysis.

Introduction

Shrimp processing is one of the major food industries in India. Shrimp processing waste is the single largest industrial fish waste in the country (Grant, 2007). So this waste can be analysed as this contains a considerable amount of proteins, the proteins mainly present in these wastes are glycoprotein as these proteins are linked with a complex polysaccharide chitin (Laemmli, 1970; Hempelmann *et al*., 1984). Therefore, the analysis and purification of these proteins may help in the usage of these proteins in many fields of application.

Methods and materials

Sample collection

The shrimp wastes such as the head and the shell of the shrimp were collected from the local fish market located in kelambakkam (Chennai). These wastes were wrapped in aluminium foil carefully and kept in a sterile ice-box which are then examined in laboratory.

Protein extraction

The samples are then washed with sterile distilled water for 2 times and then washed with phosphate buffer. After the washing procedure, the samples are homogenized using a normal homogenizer along with MLB (magnesium lysis buffer). Then the sample is kept at 4°C for 10 minutes and then in orbital shaker under cool conditions for 20 minutes. Now the samples were carefully transferred to the Eppendrof tubes and centrifuged at 10,000rpm for 10 minutes then the supernatant is carefully pipetted out to a clean Eppendorf tube and stored at -4°C in a deep freezer.

Protein estimation

The protein sample extracted from the shrimp waste are now quantified using Lowry's protein assay to measure the amount of peptide concentration present in it using BSA (Bovine serum albumin) as standards.

Thin layer chromatography

Now the proteins samples are spotted on thin layer of activated silica-G along with 6 standard aminoacids glycine, serine, leucine, tryptophan, phenylalanine and cysteine with a space of 1.5cm, then placed in the scintillation chamber containing solvent system of butanol, acetic acid and water in the ratio 8:2:2. Now the mobile phase (solvent) is allowed to develop until it reaches approximately 2cm below the top of the stationary phase. The TLC plate is taken out and kept out for solvent evaporation then ninhydrin reagent is sprayed on the TLC plate and kept in hot air oven for 5-10 minutes for colour development (Jonathan *et al*., 2007).

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Table 1. Protein estimation (lowry's method)

	Blank	Std 1	Std 2	Std 3	Std 4	Std 5	Test 1 Dil^{-10}	Test 2
OD(660nm)	-	0.538	0.989	1.484	1.558	1.951	0.429	0.851
Protein concentration	-	200	400	600	800	1000	-	-

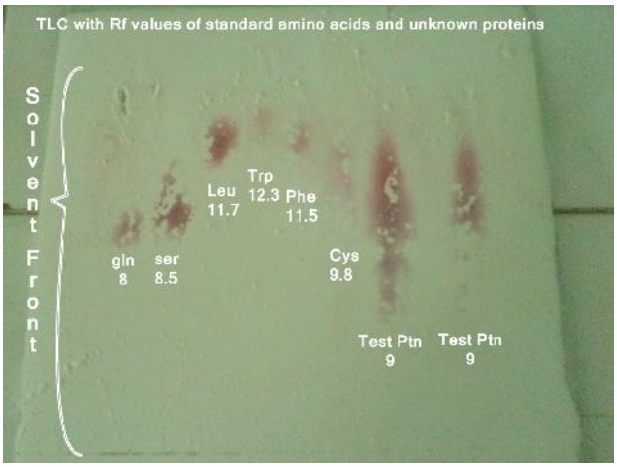

Fig.1. Thin layer chromatography

SDS-page

Here the proteins are separated based on their molecular size, weight, therefore 15% polyacrylamide gel is prepared, and the protein sample is prepared by mixing the protein sample with Laemmli sample buffer in the ratio of 2:1 and heating the sample in a water bath at 100^{o}c for 3 minutes (Raymond and Weintraub, 1959; Rüchel *et al*., 1978). Then these samples are loaded on to the polyacrylamide gels along with a molecular weight protein ladder (100Kb) in a separate well and the gel is run in vertical slab gel electrophoresis apparatus at 50V for 1hr and 100V for 4hrs till the tracking dye (bromophenol blue) reaches the bottom of the separating gel. Now the gel is taken out and rapid coomassie brilliant blue is done for the detection of the proteins.

Native page

Same procedure is followed as previous, but non-denaturing conditions are carried out to maintain the native state of proteins, therefore majorly sodium dodecyl sulphate is excluded and heating is ignored. Hence, directly the protein sample in the ratio of 2:1 with sample buffer is added to the 15% prepared polyacrlamide gel directly. The gel is run in vertical slab gel electrophoresis apparatus for 5hrs at 50V to maintain the native state of the proteins. After 5hrs rapid coomassie blue staining is done for the detection of proteins.

Results and Discussion

Protein estimation (Lowry's protein assay)

The protein concentration exceeds the final concentration of the standards so 1in 10 dilution is followed to calculate the protein concentration using spectrophotometer (Table 1), the protein (test) 0.2ml corresponds to 0.43 O.D, this corresponds to 150ug. Therefore, 1ml corresponds to 750ug; the protein concentration was calculated to be 7500ug/ml. The total concentration of protein was found to be 7.5mg/ml.

Thin layer chromatography

The amino acids correlation was studied in this thin layer chromatography by calculating the Rf values of the amino acids with the unknown protein sample (Fig.1). The unknown protein was correlated to 2 amino acids mainly serine and cysteine (Fig.2 and 3).

Fig. 2. SDS-page

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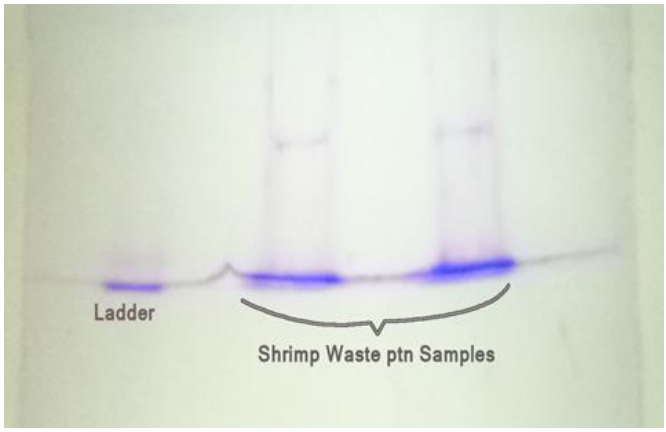

Fig. 3. Native page

Conclusion

The presence of high number of serine and cysteine reveals that the protein is highly stable as these amino acids form the basis of disulphide bonds in the protein. The proteins were separated out as peptides in the SDS-PAGE along with the molecular weight marker. The protein was separated out as native protein just separating 2 peptides from the protein sample.

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