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Protein extractionfrom muscle tissue of Scomberomorouscommerson(Spanish Mackerel) using 2D Electrophoresis and SDS PAGE

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Abstract

2-D electrophoresis and SDS-PAGE are being pioneer in protein identification and evaluation, proteomics profiling of this species via 2DE and SDS- PAGE could help in identify various protein components along with its metabolic function.Spanish Mackerel (*Scomberomoruscommerson*) being economically important species and consumed worldwide, proper nutritional profile is still in research. Proper protein profiling of this species could lead to discovery of many nutritional facts. Present study focused on protein extraction of this species using 2DE and SDS-PAGE and obtained approximate molecular weight lying between 37 and 145 kDa in 13cm IPG strips.

Keywords: 2D electrophoresis, Proteomics, SDS-PAGE, Spanish Mackerel

1.Introduction

Proteins being the functional compound for maintaining proper metabolism of human is considered to be the highly required nutrition. It plays major role in all cellular metabolic activities like translation process and even a minor deficiency of protein could lead to major metabolic diseases. Proteomics is the study which deals with characterization, identification and analysis of protein and encompasses protein expression profiling, post translational modifications, Proteome mining, structural and functional proteomics and protein- protein interaction as its application to biology.Therefore, this field deals with research on signal transduction, tracing and curing disease pathway especially on drug designing (Graves and Havstead, 2002). As per proteomic data there has been significant work carried on virus, bacteria, archaea kingdoms compared to other kingdom and the reason could be due to complexity of genes and protein structure produced by higher order organisms especially on vertebrates. Lacunae of proteomic research on commercially available fish and crustacean species especially in India still exist. In India,*Scomberomoruscommerson*commonly called as Spanish Mackerelis considered to be the highly commercial fish species and research work on this species is almost is still an unattained task. *Scomberomoruscommerson*belonging to family Scombridae (WoRMS, 2021) is economically and commercially very important species and also has high rank for its quality, taste and nutritive value worldwide (FAO, 1994). There has been considerable increase and demand for consumption of this fish in India in regular basis (Food and beverage news, 2016). Fish is considered to be the cheapest and healthier option for proper protein intake. Lysine is the dominant amino acid present in fish meal, and abundant quantity of essential amino acids (G.Lozanno and A. Hardisson, 2003; A.Arino*et al.,* 2013). Proteomics research on this fish species could help to identify and validate important and indigenous protein

components using the 2-D electrophoresis and SDS-PAGE method. 2-D gel-based proteomics has proved to be the most appropriate methodologyas it has the ability to separate high resolution protein even in intact stage with its collection of post translational modifications (Rabilloud and Lelong,2011) and visualization of protein spots made superior in this method (Magdeldin*et al.,* 2014). SDS- PAGE helps in separation of protein sample according to its molecular weight (Chevalier, 2010) and also one of the most reliable method in proteomics research. 2-DE method has been employed in various proteomics approaches for several species including *L. innocua*, *L. monocytogenes*, *S. aureus*, *O. catharinensis*etc. (Calvo *et al.,* 2005; Pocsfalvi*et al.,* 2008; Dias *et al.,* 2010).The main advantage of using 2-DE and SDS PAGE are they are useful in proper visualization of protein spots and these visualized spots can then be computed using various methods one such example is preferring Euclidian distances for making similarity matrix for all spots and this method is followed in species *Arabidopsis thaliana* for hierarchical clustering of protein spots (Chevalier,F*et al.*,2004). 2-D Electrophoresis and SDS-PAGE are employed for protein separation process based on pI value and on molecular weight of protein sample obtained from *S. commerson*. LC-MS could be used to identify the protein components. The aim of this study is to procure different protein profile from muscle of *Scomberomoruscommerson* using2-D gel electrophoresis and SDS- PAGE.

2. Materials and Methods:

2.1 Specimen for the experiment:

Fresh adult specimen of *Scomberomoruscommerson*(Family: Scombridae) were collected from the fish landing center. And it was preserved in dry ice for eliminating rigor mortis condition.

2.2. Sample preparation

Samples for the experiment were prepared by grinding the fish muscle tissue in presence of Liquidnitrogen (-196°C) and solubilized in optimized 2D lysis buffer containing7M Urea, 2% CHAPS, 50mM DTT and 10mM PMSF at pH 7.5. Sonication was performed to acquire complete lysis of muscle tissue. The samples were then further centrifuged at 12,000 rpm for 15 min at 4°c. Supernatant was aliquoted and protein estimation was performed by Bradford assay using BSA as standard.

2.3. Gel analysis

For preparative gels, the homogenates were adjusted to 150 µg of total protein along with rehydration buffer containing 7M Urea, 2% CHAPS, 50mM DTT and 10mM PMSF and loaded on 13cm IPG strips of pH 3–10 and pH 4-7 (GE Healthcare, Uppsala, Sweden) were employed in the first dimension. Isoelectric focusing carried after this stage. Proteins were focused for a total voltage of 50,000 Vhs at constant temperature (20^{o}C) under linear voltage ramp after an active IPG rehydration at 30 V in a IPG Phor III (GE Healthcare, Uppsala, Sweden) apparatus with following IEF conditions 500 V step-n-hold for 1 h, 1000 V gradient for 1 h, 5000 V gradient for

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3 h and 5000 V step-n-hold for 7 h. Following Isoelectric focusing, each IPG strip was placed in the equilibration buffer containing 2% DTT first followed by incubation in another buffer in which the DTT was replaced by 2.5% iodoacetamide. Second dimension was carried out in 12.5%SDS-PAGE gels at 10°C in an SE600 (GE Healthcare, Uppsala, Sweden) at 1 W/gel for 1 hr and 13 W/ gel for 3 hr.Protein spots were visualized in colloidal blue stain and the gel was scanned using image scanner (Rocher *et al.*, 2015).

3. Results

Gel image was processed to determine molecular weight and Isoelectric value of protein spots. SDS-PAGE gel image was visualized (Fig 2). From the gel images proper bands are formed approximately between 45 and 50 kDa in SDS-PAGE. 2-DE gel image of seer fish muscle tissue at pH of 3-7 were visualized along with molecular weight (Fig 3.1 and 3.2). After visualizing the 2DE gel image multiple distinct protein spots were distributed and concentrated between 37 and 145 kDa molecular weight on 13 cm IPG strips. And this represents the presence of some essential as well as non-essential amino acids especially glycine, methionine, proline etc.,

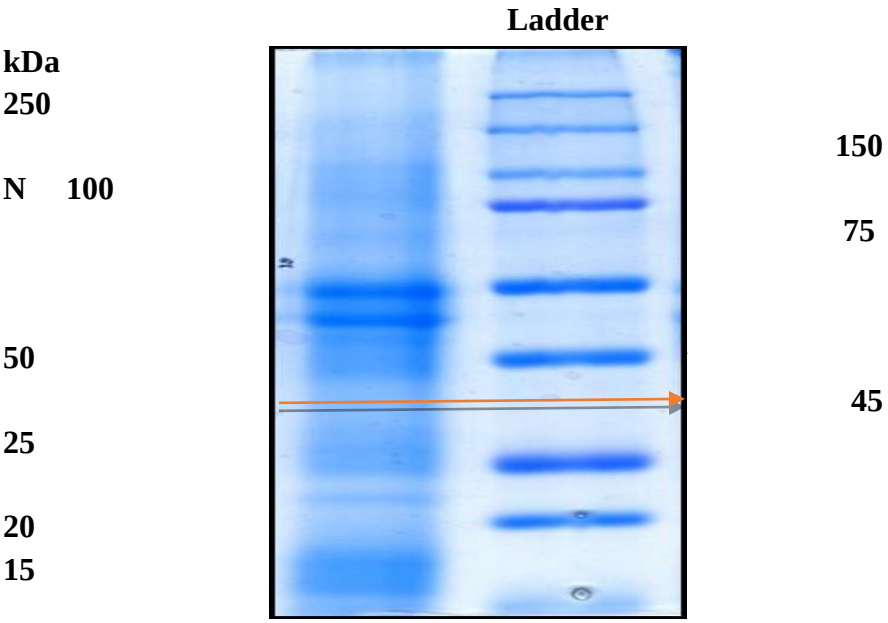
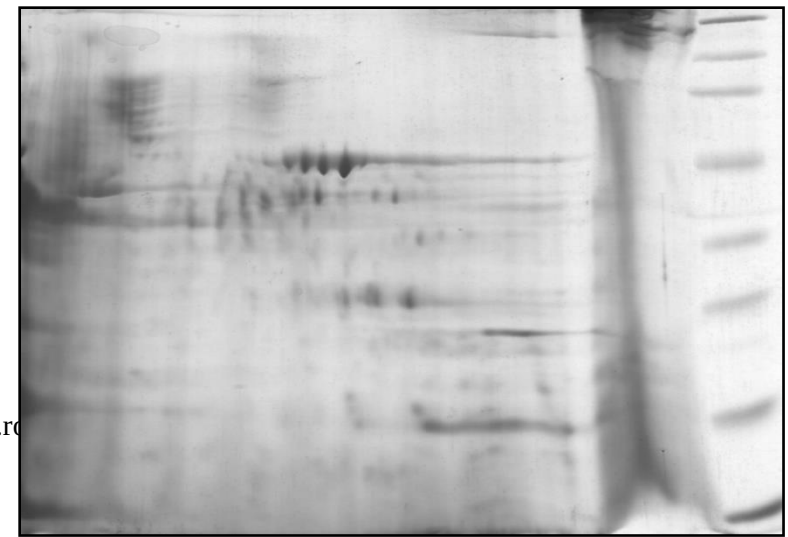


Fig 2 SDS-PAGE image showing the band molecular weight lying between 45 and 50kDa



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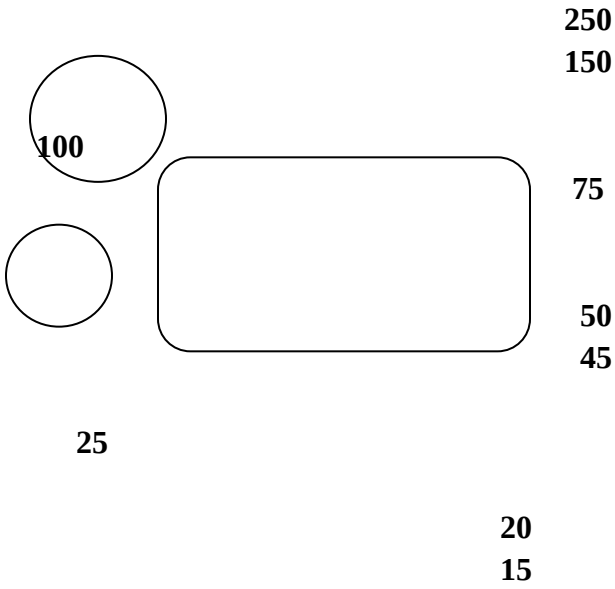

Fig.3.1. 2D gel- pI determination with IPG strip of 3-11cms

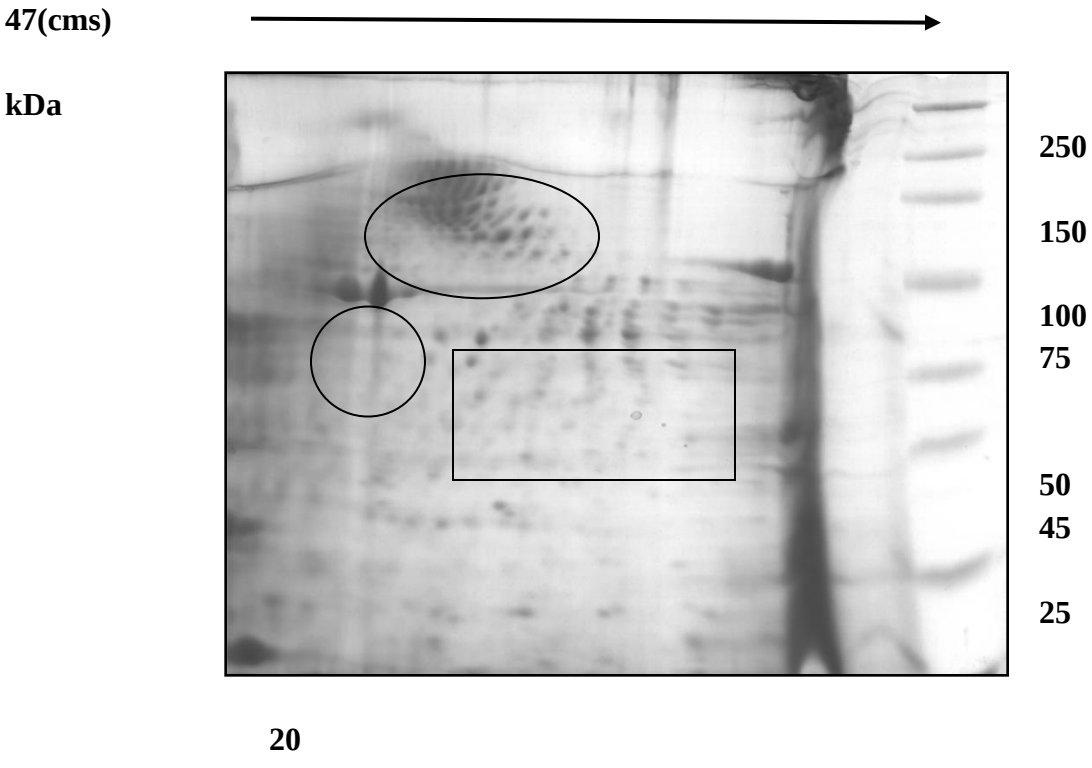

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Fig.3.2. 2D gel- pI determination with IPG strip of 4-7cms

4. Discussion

SDS PAGE is extensively used in proteomics study for separation of protein based on size and molecular weight. Profiling protein compounds via SDS PAGE for microorganisms proved to possess high diagnostic value compared to other type of protein profiling method (Bilal Aslam *et al.,* 2017). Consumption rate of Seer fish is comparatively very high worldwide compared to other commercially important species. This proteomic analysis in *Scombemoruscommerson*is a pioneer and new work(Fig 5) and approximate results has been figured out. Thousands of protein spots have been visualized in the gel along with their molecular weight. This experiment clearly explained the presence of multiple proteins in muscle of this species as the protein spots are densely located and distributed through out gel. Further work on this species along with identification and validation using LC-MS method could reveal the exact protein concentration and could bring light in the study of this commercially important species. KEGG pathway could provide exact protein function in a metabolic pathway which is further useful in proceeding advance research. Being economically important and readily available species,this work on proteomics answers the puzzle on proper protein intake via food. For exact quantification of amino acids present in the sample, various algorithms could be used to determine pk_a value. But out of all algorithms available, SVM and Co factor methods observed to provide accurate value for large sets instead of iterative method which could be used for small sets of values (Audain*et al.,* 2015).

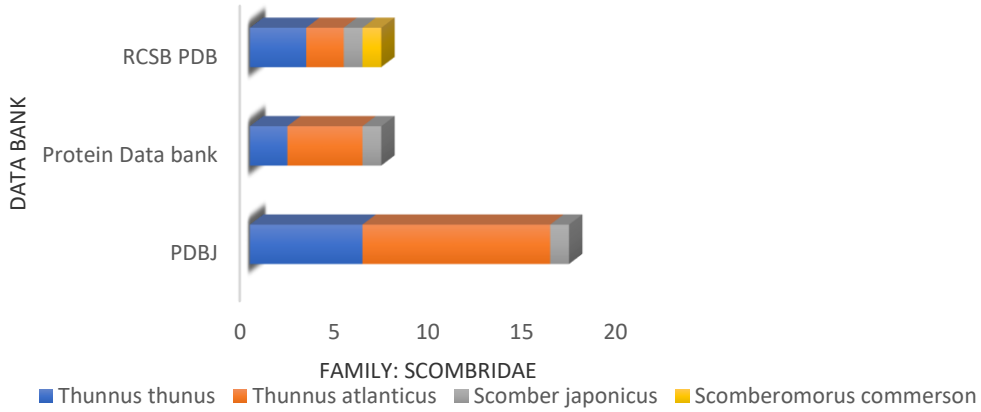

Fig 5. Graph depicting the number of works carried out and submitted in data bank on family Scombridae

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