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UV-based protein estimation of prawn species and evaluation of different preservation techniques

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ABSTRACT

Protein is the primary determinant of the nutritional quality of regular dietary foods. Seafood, including prawns, is a vital source of high-quality protein globally. This study evaluates three commercially valuable shrimp species (Acetes indicus, Penaeus monodon, and Fenneropenaeus indicus) for their protein levels by using UV spectrophotometry to measure absorbance at the wavelength of 750 nm. This study also investigates how various methods of preservation (wet, dry, dry - salted and pickled) maintain the amount of protein over time. Protein content was determined through examination of a standard curve based on protein extracts. Significant variation was observed in results, with A. indicus showing highest protein level in its dried form and P. monodon exhibited its maximum amount of protein when fresh. Pickled samples produced very low levels of protein. The quantity of protein was observed more in drying than salting and wet. It was observed least in pickled form The findings of this study indicate that the best way to accurately determine the high protein content is by drying. This finding establishes a clear foundation for future nutritional and processing studies.

INTRODUCTION

Millions of people worldwide, particularly in underdeveloped nations, suffer from malnutrition and protein deficiencies. By providing fish and shellfish items that are accessible to local communities, protein deficiency may be reduced to some degree. It is often recognized that fish and shellfish are extremely nutrient-dense, a vital part of a healthy diet, and a great source of protein and other nutrients that are necessary for human nutrition (4). A key macronutrient for human growth, tissue repair, enzyme production, immune function and general physiological performance of individuals is protein. With a rapidly increasing global population, the availability of affordable high-quality protein sources represents a major public health, nutrition science and food security challenge (1). These attributes are critical for addressing global protein-energy malnutrition (*Wang et al.*, 2021). Aquaculture organisms, such as prawns, represent an environmentally friendly alternative to land-based animal agriculture as they convert feed into body mass more efficiently, have fast growth rates, and have significant environmental impact reductions (FAO, 2022). Therefore, accurate measurement of protein levels in prawns is essential for evaluating nutritional content and creating standards for aquaculture, food processing, and global seafood trading Fish and shellfish are considered nutrient-dense foods providing a rich source of protein (biologically available), as well as all nine essential amino acids.

Therefore, they provide an excellent source of protein and other nutrients needed to address the problem of protein-energy malnutrition in developing nations (4). According to the protein intake guideline, sufficient quality protein is necessary to support metabolic homeostasis and prevent nutrient deficiency diseases. According to U.S. and Canadian dietary reference intakes, the recommended allowance for protein of 0.8 g protein·kg−1·d−1 is “the average daily intake level that is sufficient to meet the nutrient requirement of nearly

all [~98%] healthy individuals" (2) The Institute of Medicine (IOM) also suggests an amount of 0.8 grams per kilogram per day for healthy adults (generally between 40 and 72 grams daily depending on body mass (1). This is the lowest amount needed to satisfy nearly all healthy people's biological requirements (2). Using "athletic" reference body weights of 70 to 90 kg for men and 50 to 70 kg for women, the RDA is equivalent to 56 to72 g/day for men and 40 to 56 g/day for women. The quantity of protein left over after requirements for fat and carbohydrates were satisfied was used to calculate the range of protein intakes advised in the diet. (1)

Depending upon certain circumstances (e.g., physical activity), increased protein intake may be recommended to increase functional results, e.g., greater muscle43 protein synthesis and preservation of metabolism, while 1–1.6 grams per kilogram per day is recommended for greater bioavailability (3). Amino acids (AA) have been linked by bonds of peptides to create protein. Stunting, anaemia, physical weakness, oedema, vascular dysfunction, and weakened immunity are hence the outcomes of protein undernutrition. Dietary intake of 1.0, 1.3, and 1.6 g protein per kg BW per day is advised for those with minimum, moderate, and high physical activity, respectively, in order to satisfy functional goals such as boosting skeletal-muscle protein accretion and physical strength. Healthy individuals can safely consume 2 g of protein per kilogram of body weight per day for an extended period of time. Protein's nutritional benefits are determined by both its amount and quality. Therefore, for human growth, development, and health to be at their best, enough consumption of high-quality proteins from animal products (such as lean meat and milk) is necessary. Twenty distinct amino acids (AA) linked by peptide bonds usually exist in varying concentrations in proteins. One the Greek word "proteios," which means prime or principal, is where the English term "protein" comes from. Since protein is the most basic building block of both human and animal tissues, this word is highly applicable in the field of nutrition. (2). Unless dietary protein is digested by peptidases and proteases in the small intestine lumen to AA, dipeptides, or tripeptides, it lacks nutritional value. They are vital for an organism's survival, growth, development, reproduction, and lactation. (3) Accurate protein quantification is essential for research, quality control, and nutritional labelling.

The ability of the body to digest and absorb that protein can vary depending on the amino acid composition and digestibility of vegetable protein sources. That is why animal proteins (e.g., those from shellfish, such as prawns) often have a higher biological value than do plant proteins. Crustaceans, specifically prawns, have recently gained popularity as highly effective sources of quality protein, micronutrients, and bioactive compounds that benefit human health. Prawns are edible crustaceans. They are found in marine and fresh water habitat. They are obtained naturally or can be cultured. Small aquatic crustaceans belonging to the decapod order that have ten legs and an exoskeleton are referred to as prawns. Some of these species are edible. The structure of prawns provides additional benefits due to the fact that they are easy to digest, contain a balanced amino acid profile, and are relatively low in calories. Prawns, a popular seafood choice, are highly consumed and recognized as an excellent source of protein in a nutritious diet. When compared to other aquaculture species, prawns are an agriculturally and economically important source of protein, with their relatively low-fat content and broad family/community acceptance as food. Although the protein content of prawns is generally high and because of this high biological value, prawns can be an excellent source of protein for individuals living in areas where the diet is predominantly comprised of plant foods and where the risk for protein deficiency is the highest.

Prawns are one of the most significant groups of crustaceans in the global aquaculture and fisheries industry and also widely distributed and inhabit many different types of marine and freshwater ecosystems, and are among the most important species globally in aquaculture and fisheries, however despite their widespread consumption, the quantity of protein found in prawn species can be considerably different depending upon the environmental conditions that they live in and how they have been handled after harvest (5)

Black Tiger Prawn (Penaeus Monodon) and Indian White Prawn (Fenneropenaeus Indicus) are among the most commercially valuable and highly utilized species within India and throughout much of the tropical areas of the world. the black tiger prawn Penaeus monodon, commonly known as big tiger shrimp, exists in the wild in the Indian Ocean and in the Pacific Ocean Black tiger shrimp account for 56% of worldwide shrimp output. The black tiger shrimp has a delicate and sweet taste, and the cooked flesh is firm and juicy. (Pascoal et al., 2010). P. monodon are so significant to the international markets that they constitute around 80% of the total farmed shrimp output globally (Rosenberry, 2001). Fenneropenaeus indicus, also known as"Indian white shrimp," is another commercially important shrimp species that is farmed in large facilities in Southeast Asia and widely cultured in India, the Middle East, and Eastern Africa (Rosenberry, 2001).

Fenneropenaeus indicus is the scientific name of the white prawn found in India - this species falls under the

penaeid family and belongs to the genus Fenneropenaeus. This prawn has a very high commercial value throughout the Indo-West Pacific and is found in all coastal and marine waters off the coast of India. This marine species resides in a sandy or muddy environment on the bottom of the ocean; its adults generally occupy depths up to 90 Metres (300 Feet). Youth, however, often inhabit the more shallow waters at the maximum depth of the river mouth (Estuary). Therefore all juveniles live in both coastal and Estuarine waters while the adult lives mainly in oceanic habitats. (21)

The Indo-West Pacific region is home to the small sergestid shrimp, Acetes indicus, aka jawla. In particular, shrimp live in shallow estuarine and coastal waters, mainly along the Indian subcontinent (i.e., India, Sri Lanka, Bangladesh, etc.) and South East Asia *(SeaLifeBase, 2023*). As such, the shrimp form dense swarms and support local, traditional fisheries, and are often processed into different dried and fermented shrimp products in these coastal areas *(Omori, 1975*). Previous research described a very complicated life cycle for the species that includes multiple naupliar, protozoeal, mysis, and post-larval developmental stages, as observed from the populations along the Bombay coastline (Pillai, 1973). Recent phylogenetic research has revealed that A. indicus is not an individual species but a species complex that is present across multiple genetically distinct lineages in South and South East Asia, thereby resulting in an intensively revised taxonomy and distribution of the species *(Hanamura et al., 2024*).

How to measure the protein content of prawns accurately is important for all aspects of nutrition labelling of prawns, and as such, is critical for food processors, dietitians, and quality assurance personnel in the seafood industry. A variety of preservation techniques exist, such as drying, salting, freezing and pickling, that allow prolonged storage, but each of these preservation methods may alter the protein composition (amount and/or type) of the prawn via methods of protein analysis (6). An understanding of how these preservation techniques affect the protein content of the product is critical for consumers to know that preserved seafoods have a reliable nutrition source. However, the use of post-harvest preservation methods to ensure the nutritional quality of seafood is critical to success. The biochemical breakdown of seafood begins almost immediately after harvest due to naturally occurring enzymes within seafood, microorganisms, and oxidizing agents, all of which can adversely impact the ability to measure and identify protein (*Ahmed et al.*, 2023). preservation techniques impart increased shelf stability, some of the changes made will be to the physiological form and solubility of proteins, including denaturation of protein structures or the loss of nitrogen during processing. A common laboratory-based method is the colorimetric assay using the Lowry method; this method uses the chromogenic properties of the protein to determine the amount of peptide bonds formed due to amino acid interactions. Another method is the ultraviolet (UV) spectrophotometry method, which allows for a more rapid means of protein measurement without the need to add a reagent; however, UV spectrophotometry measures the absorbance of the sample at specific wavelengths that correspond to certain aromatic amino acids. Each of the above-mentioned sample analysis techniques has unique advantages and disadvantages with respect to the sensitivity of the method to the sample, preparation time required to prepare the samples and method susceptibility to changes that may occur during preservation. (*Mahesha & Yuvaraja's College, Mysore, n.d.-b*)

The evaluation of protein content within marine food is based on principles of biochemistry such as chemical means and selection of methodologies. Biochemical methods for evaluating protein content have varying specificity, sensitivity and potential for interference from other substances. This variation should be taken into account when determining the protein content of a given marine food. Recent studies indicate that the mostconsistent method used to establish protein content in any one type sample should be determined by conducting tests using multiple methods (*Kulawik et al*., 2020).

The findings of prior investigations suggest analytical approaches will give different protein determinations and hence merit corroborative investigations (4). Redundant preservation processes may denature proteins or cause loss of nutrients or even affect concentration of proteins because of moisture removal. Thus, it is essential to evaluate a good method to systematically check for varying preservation options to determine the best method for the analysis of prawns.

The objective of this study was to determine the total protein content of prawns treated by three different methods of preservation: drying, salting, and pickling; then compare three commonly used estimation techniques. These were aimed at establishing the best practice for estimating total protein based on how each method reacts to biochemical changes caused by the preservation of prawns. The research will also provide a basis for nutritionally standardising protein profiles across prawn species, allowing for a more efficient and practical application of resources within fisheries science.

METHODOLOGY

1. Acquisition of Samples

Acetes Indicus, Penaeus Monodon, and Fenneropenaeus Indicus were obtained from local markets like Malad Fish Market and Versova Fish Market. To avoid contamination due to microbial activity, all samples were brought to the Laboratory in ice-packed containers. All samples were processed within 24 hrs after collection to maintain the biochemical integrity.

2. Preservation Methodology

There are four treatment groups for preserving.

2.1 Fresh (Wet): Fresh samples were washed, cleaned, and used directly after collection for protein extraction without any preservation treatment.

2.2 Dried Prawns: Samples were allowed to dry in the sun for two to three days until they reached a constant weight. These samples were kept in airtight sterile containers.

2.3 Dried-Salted: samples were mixed with common salt (NaCl) in a 1:4 (Prawns: Salt) ratio. These samples were left to dry in the sun. store in airtight, sealed containers. so they are not contaminated.

2.4 Preparing prawn pickle:

1. Clean the Prawns: Wash, peel, and remove the central vein of the prawns.
2. Blanching: Boil prawns for 10 minutes in 1% brine containing 0.1% citric acid.
3. Drain & Weigh: Drain the prawns and weigh them.
4. Prepare Ingredients (for 100g blanched prawn)

Oil: 25g, Mustard: 1.5g, Garlic: 10g, Green chillies: 5g, Ginger: 7.5g, Chilli powder: 5g, Turmeric & Pepper: 0.3g each, Cinnamon, Clove, Asafoetida: 0.1g each, Salt: 7.5g, Vinegar: 30g, Potassium sorbate: 01g

5. Frying:

Heat oil to 180–190ºC.

6. Add mustard, garlic, chillies, and ginger. Fry until light brown.
7. Add chilli powder, turmeric, and pepper. Stir and take off the flame.
8. Mixing:

Add cinnamon, clove, asafoetida, salt, and prawn. Mix well.

9. Cool slightly, then add vinegar and sorbate.
10. pH Check: Make sure pH is less than 4 (add more vinegar if required).
11. Packing: Cool, weigh, and pack in bottles. Seal tightly. (9)

3.Methods used for protein estimation:

1. Lowry's Method: A biological method for measuring the total amount of protein in a solution is the Lowry protein assay. A colour change in the sample solution proportionate to the protein content indicates the total protein concentration, which may subsequently be determined using colorimetric methods.(7)

2. Standard Preparation: Dissolve 10 mg of BSA in 1 mL of distilled water.

Tube	Desired Conc. (mg/ml)	Volume of Stock (10 mg/	Add Buffer (up to 1 m total)
1(Blank)	0.0	0	1.0
2	1	100 µL	900 µL
3	2	200 µL	800 µL
4	3	300 µL	700 µL
5	4	400 µL	600 µL
6	5	500 µL	500 µL

Table 1: Standard Preparation

UV Spectrophotometer-

UV spectrophotometry, or UV-V is spectroscopy, whose fundamental idea is used to measure the intensity of

visible or ultraviolet light passing through a liquid sample contained within a cuvette. It compares this measured light intensity to that of the original light beam prior to entering the sample. (*METTLER TOLEDO, 2021*)

RESULTS:

Processing Form	*A.Indicus* (whole)	*P. monodon* (meat)	*F.indicus* (meat)	Comparative result Per 1 g
Wet (Fresh/Raw)	150 – 200 mg	190 – 240 mg	180 – 230 mg	Low conc.
Dried	480 – 640 mg	400 – 500 mg	450 – 530 mg	Highest conc.
Dried - salted	300 – 450 mg	250 – 400 mg	280 – 420 mg	Moderate Conc.
Pickle	50 – 150 mg	50 – 150 mg	50 – 150 mg	Lowest conc.

Table 2: Results of protein concentrations

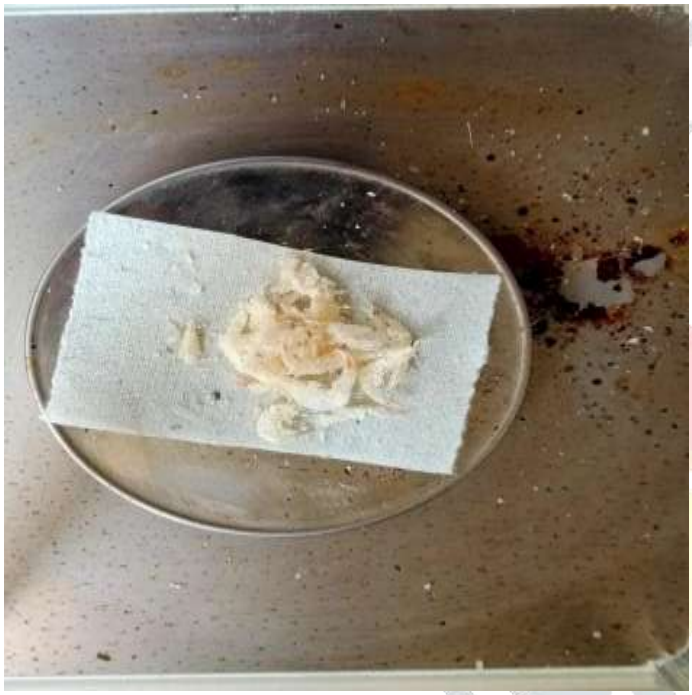

Fig.1: *A. indicus* sampling

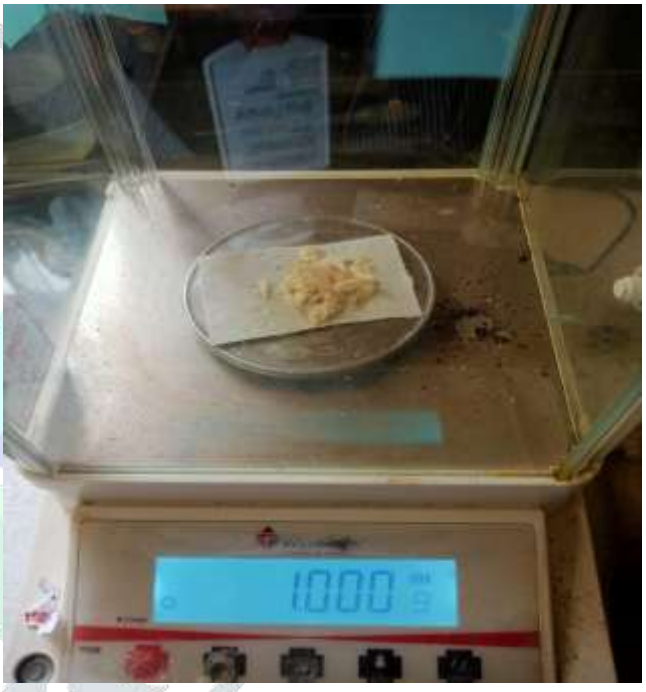

Fig. 2: Weighing samples

Fig.3 Dried – salted samples

Fig.4 Dried samples

Protein Concentration Based on Processed Form:

The concentration of protein found in three crustaceans (*A. indicus*-whole*; P. monodon*-meat*; F. indicus*-meat) varied significantly based on their processed form. The variation was from the lowest concentration of 50 mg/g (pickled) to the highest concentration of 640 mg/g (dried) of protein. A summary of these protein concentration ranges can be seen in Table 2.

Fresh Samples: Among the wet or unprocessed samples, they were all organic protein with protein concentrations between 150 mg/g and 240 mg/g; *A. indicus* had the lowest value (150 mg/g and 200 mg/g) as it had a larger amount of water per dry food ratio before receiving heat treatment.

Dried Samples: All the dried samples had the highest protein content, with the highest level of protein being found in *A. indicus* (Whole) at between 480 mg/g and 640 mg/g, which was found to be greater than that of the meat-only samples of *P. monodon* and *F. indicus* (dried).

Dry-Salted Samples: Of the three tested samples, the Dry-Salted ones were in between the Dried Forms and at the lower range of Wet Forms. The range of the Dry-Salted samples was between 250 mg/g and 450 mg/g, which is well below that of the Dry Form but above that of Wet and Pickled Forms.

Pickle Samples: The Pickled form exhibited the lowest and narrowest range of concentration, with a range of about 50 -150. When examining Pickled form, the protein contents for three different fish species, there was little to no difference, indicating that the original raw material had little or no effect on the end protein composition.

DISSCUSION:

This study demonstrated that dried crustaceans had higher levels of protein than other types of fish. The lower levels of protein in dry-salted or pickled crustaceans were supported by other research in which researchers found that drying crustaceans increased their overall protein content and decreased their moisture content. One recent example includes murrel fish, where drying processes resulted in protein concentrations in fish powder reaching double that of raw murrel fish (~20.7g/100g vs. ~42g/100g dry) (*Raut et al., 2025*). Several different drying techniques were evaluated for shrimp meat, and sun-dried shrimp produced higher protein levels than either raw or processed shrimp.

Related to our findings of lower protein concentrations in dry-salted samples when compared with fully dried samples, the work of *Mebratu et al*. 2024 found that applying a brine (salt) treatment to fish before drying reduced the total protein content by approximately 6% compared with untreated fish (58.8 g/100 g dry matter to 55.4 g/100 g dry matter) regardless of the temperature at which they were dried. In addition, studies that compare muscle properties have demonstrated that salting/brining with very high salt concentrations causes denaturation of myofibrillar proteins such as myosin and actin; decreased solubility for myofibrilar proteins when in contact with brine; and decreased WHC, which may reduce the amount of extractable/measurable protein present in the final product (*Martínez- Álvarez et al. 2006, Thorarinsdottir et al. 2002*).

Additionally, the effects of high ionic strength on muscle fibre contraction and dehydration ("myofibrillar"), and consequent denaturation of proteins present in the salted seafood has been studied (*Ünlüsayın et al., 2016*): the contraction and dehydration of myofibrils occurs when the salt concentration is ~20% because of the high ionic strength. The low protein concentration found in uncooked muscle from the dry-salted muscle samples would also correlate with the potential aggregation (forced) of part of muscle protein into the dry- salted muscle samples.

Limited published research exists on the impact on protein content after the pickling process involving crustaceans. However, studies of salted/brined fish indicate that soluble muscle protein (myofibrillar proteins) may be lost during this process by the osmotic leaching of the brine or through denaturation/aggregation induced by salt addition (*Mebratu et al., 2024; Martínez-Álvarez et al., 2006; Ünlüsayın & al., 2016*). Thus, it would not be surprising for these factors to reduce the quantity of protein measured in crustaceans that were subjected to pickling, given that they experienced the same losses experienced with salted/brined fish.

The current study supports what has previously been shown in other studies that drying (which concentrates) and salt (due to denaturation, dehydration, aggregation, and leaching) both reduce protein content, though sometimes they do so more than species differences do. Since there are substantial differences in how fish are processed (e.g., dried versus pickled), it is likely that some (but not necessarily all) of these differences provide more nutritional (protein) value than respective species do. Because dried products have been shown to yield higher protein values than salted/pickled ones, the results also have implications for how nutritional evaluations and food-processing recommendations are made. For instance, consumers, nutritionists, and the aquaculture/processing industries should be mindful that simply using "dried" as a protein source could result in an overestimation of the amount of protein available, while salt-preserved/pickled seafood may have low or unquantified amounts of protein available as usable protein.

CONCLUSION:

As per the work conducted we can assert that, preservation type strongly influences the resulting amount of protein found in prawns as compared to the inherent variations between the three species (*Acetes indicus, Penaeus monodon, and Fenneropenaeus indicus*). The use of ultraviolet (UV) spectrophotometry indicated that the method of preservation for maximising the concentration of protein was drying. The high protein content of the dried whole A. indicus was primarily due to the combination of protein preservation due to drying methods and the incorporation of the exoskeleton into the dried product.

The dried-salted product contained moderately high protein content, but showed a decrease in protein content compared to the dried product because in salted seafood, where the salt concentration reaches about 20%, high ionic strength causes dehydration of protein (22). Pickled samples from all three species had much lower protein content than dried and dried-salted products due to the dilution of their protein content caused by using oil, salt and acetic acid as their preservatives.

Overall, the relative ranking for the holding on to protein content is: Dried>dried salted> Wet> Pickled. This ranking clearly illustrates how much the way in which prawns are preserved affects their nutritional value. It provides valuable information to consumers, nutritionists, and the seafood industry by highlighting that the method of preservation selected has a direct effect on how concentrated the protein is in the prawn. Dried prawns are the best option when considering protein in diets, while packed prawns should be used primarily as flavour-enhancing agents and not as a source of concentrated protein. This study aims to develop a rapid and precise spectrophotometric method for measuring protein content in prawns. The results of this research should serve as a resource for all those who work in food technology and seafood industries or for researchers involved in quality control, product development, and nutritional analysis.

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