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Comparison of Extraction Assays and Quantification of Protein from *Ulva anandii* (Cholorophycota)

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ABSTRACT

Seaweeds contain many macronutrients including protein, therefore they can be utilized to fulfil the protein requirements of human beings. This research focused on extracting total protein in green seaweed *Ulva anandii* from the crude extracts, by using the trichloroacetic acid (TCA) and acetone precipitation methods, and the estimation of crude extract (water-soluble proteins), and those obtained from the two above-mentioned methods. The results indicate that the water-soluble proteins had the highest quantity (949.75µg/mL) followed by the TCA precipitation method (831µg/mL), while the acetone precipitation method had the least concentration of total protein (100 µg/mL). The study concludes that treatment with organic solvents lowers the quantity of protein extracted from *U. anandii*.

Keywords: *Ulva anandii*, Protein estimation, Trichloroacetic acid, Green seaweed

1. INTRODUCTION

Proteins are one of the major macronutrients required by human beings as they are involved in most of the biological functions (Rosni, *et al.*, 2015). But animal sources are rather expensive, and due to the increased demand for veggie proteins, plant sources can become scarce over time (O'Connor, *et al*., 2020; Thiviya, *et al.*, 2022). Moreover, the human population is expected to reach up to 2100 billion people by the year 2100 (Reynolds, *et al.*, 2022), therefore it is necessary to find safe alternatives for fulfilling protein requirements. Seaweeds have emerged as a promising protein reservoir due to their rich protein content (Subramoni & Abraham, 2023), offering a viable alternative to conventional sources (Samarathunga, *et al.*, 2023). Notably, members of Chlorophycota exhibit moderate protein levels (Fleurence, et al., 2018), with *Ulva* spp., a member of Chlorophycota, boasting protein content of up to 44% (Holdt, & Kraan, 2011). Generally, many types of proteins are present in seaweeds including phycolectins, phycoerythrins, glycoproteins, and mycosporine-like amino acids (Wijesekara, *et al*., 2017). However, methodologies for seaweed protein extraction vary across studies and lack standardization (Angell, *et al.*, 2016) mainly due to the complex cell wall structure as the primary reason (Pliego-Cortés, *et al.*, 2020). Hence, this study investigates the impact of extraction protocols on total protein estimation.

2. MATERIALS AND MATHODS

2.1 Materials and Reagents

Green seaweed Ulva anandii was collected from the intertidal zone of Paradise Point, Karachi, during May and June 2022. The samples were brought to the laboratory, where they were washed thoroughly with tap water to remove epiphytes and other debris, and then shade-dried. The completely dried samples were ground into powder form and then stored in polythene bags until further use.

The analytical reagent (C) for protein estimation through Lowry's method was prepared by adding 100 mL of Lowry's reagent A (2% Na_2CO_3 mixed with 0.1N NaOH) with 2 mL of Lowry's reagent B (0.5% $CuSO_4$ mixed with 2.37% Sodium Potassium Tartrate Solution). Fresh Folin Ciocalteu reagent (2N), was prepared by diluting with an equal volume of distilled water (1:1 v/v).

2.2 Extraction

The proteins were extracted by modifying the multiple procedures described by Barbarino, & Lourenço, (2005). Briefly, the total proteins were extracted by dissolving 2.5g of dried seaweed powder in 125 mL of deionized water and incubated for 16 hours in a shaking water bath. The resultant was centrifuged at 6000 rpm for 2 hours. The supernatant was separated using 3 mL and 10 mL syringes, the pellet was again incubated with deionized water and centrifuged. The supernatant collected after both cycles was combined, while the pellet was discarded. The combined supernatants were filtered through a Millipore 0.22µm pore size filter paper. Part of this supernatant (considered as crude extract or water-soluble proteins) was tested for total proteins. For the remaining crude xtract, two distinct protocols were further tested, the trichloroacetic acid (TCA), and the acetone precipitation methods.

2.3 TCA (Trichloroacetic acid) Method

The TCA method of Berges, *et al.*, (1993), as explained by Barbarino, & Lourenço, (2005), was used with some modifications. 2.4 mL of crude extract was mixed with 6 mL of 25% TCA and incubated for 30 minutes at 4°C, after which it was centrifuged at 6000 rpm for 2 hours. The supernatant was carefully removed, while the pellet was washed with 10% TCA. Then add 6 mL of 10% TCA, incubate at 4°C for 30 minutes, and centrifuge at 6000 rpm for 2 hours. Again the supernatant was removed, and the pellet was resuspended in 12 mL of 5% TCA at 4°C for 30 minutes, then centrifuged at 6000 rpm for 2 hours. The supernatant was rigorously discarded, and the pellet of precipitated protein was dried by turning down the tube on tissue paper. The 100 µL of deionized water was added to the pellet and mixed. Finally, protein suspension was stored at -20°C till further use.

2.4 Acetone Precipitation Method

The modified method of Nejadi, et al., (2014) was used for the protein extraction. A mixture of crude extract of *U. anandii* and pre-chilled acetone (100%) was prepared in (1:4 v/v) ratio and kept at -20 °C for 24 hours. After that, the solution was centrifuged for an hour at 6000 rpm. The supernatant was decanted and the pellet was dried by placing the inverted tubes on tissue paper. The pellet was kept in the freezer at -20 °C for further use.

2.4.1 Estimation*:* The investigation of the total protein of crude extract, TCA and acetone treated samples was carried out by Lowry's method (Lowry, *et al*., 1951). Briefly, concentrations of 0.05, 0.1, 0.2, 0.4, 0.6, 0.8, and 1 mL were made from a stock solution of BSA (1 mg/mL) for plotting the standard curve. The final volume of each concentration was 1 mL. Similarly, 1 mL of the water-soluble protein solution (crude extract), the acetone-treated protein precipitates, and the TCA-treated protein precipitates were taken in separate test tubes. From each concentration of BSA and samples to be tested, 0.2 mL of solution was pipetted out in separate test tubes. In each tube, 2 mL of reagent C was mixed, and the tubes were incubated at room temperature. After 10 minutes, 0.2 mL of Folin Ciocalteu reagent was added to each test tube. After 20 minutes of incubation, the optical density (O.D) was taken at 750 nm on a spectrophotometer. The values of the BSA standard were used to create a standard curve, after which the values of the samples tested were estimated through the standard graph.

3. RESULTS & DISCUSSION

Seaweeds are utilized throughout the world as human food due to their high nutritional value, as seaweeds are a rich source of vitamins, minerals, carbohydrates, lipids, and proteins. Green seaweeds, in particular, are known to possess a moderate amount of proteins. However, the extraction of these proteins varies from species to species and the protocol used for extraction. Therefore, this research focused on estimating the protein contents of green seaweed *U. anandii* collected from the Karachi coast. The proteins were extracted through three different methods: using deionized water (used through the crude extract), acetone-precipitated proteins, and TCA-precipitated proteins. It was observed during this study that the crude extract i.e. the water-soluble extract had the highest concentration of proteins followed by the TCA precipitate protocol, while the least concentration was observed in the acetone-precipitation protocol. The concentration of crude extract (water soluble) was recorded as 949.75µg/mL. The proteins extracted in this study after TCA protein precipitation were 831µg/mL. The acetone-precipitated protein's concentration was low i.e. 100µg/mL the results revealed the mean value of protein concentrations (Figure 1).

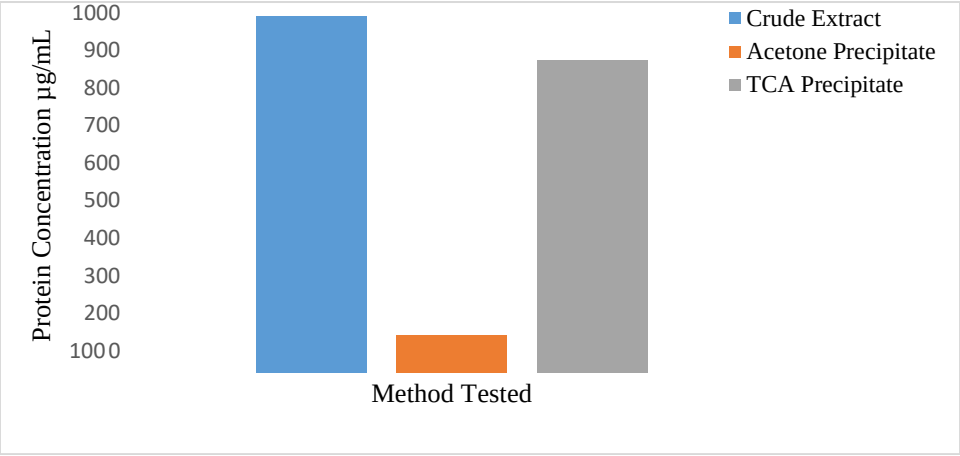

Figure 1. Amount of proteins extracted after each protocol

The seaweeds have been gaining attention over the years for their protein concentrations, with recent advancements focused on the nutritional quality of these proteins (Juul, *et al*., 2022). But the extraction of proteins from seaweeds is considered a complicated task due to the presence of many compounds including phenolic compounds and tannins, as well as the resistant nature of algal cell walls (Field, *et al.,* 2017). Usually, the proteins from seaweeds are extracted through either enzyme hydrolysis, or by using aqueous, alkaline, or acidic solvent (Kadam, *et al.,* 2017). This study was carried out to estimate the protein concentrations in crude extracts (water-soluble proteins), acetone-precipitated proteins, and TCA-precipitated proteins in green seaweed *Ulva indica*. The protein samples were estimated by Lowry's Method. The study of Wijesekara, *et al*., (2017) has reported that the concentration of proteins in *Ulva* sp. varies according to the extraction process and also according to the season of collection. Previously, a study conducted by Amano & Noda, (1992), has also shown that water-soluble fraction had a higher protein content in seaweeds *Ulva pertusa* and *U. fasciata*, while Harrysson, *et al.,* (2018) also concluded that the traditional method of water extraction results in high protein yield in *U. lactuca*. Niemi, *et al*., (2023) compared protein estimation using different methods and concluded that crude extract had higher protein concentration while using the TCA precipitation method slightly reduced the protein concentration. Berges, *et al*., (1993) also observed that precipitating proteins through the TCA method results in lower protein content According to Crowell, *et al.,* (2013), many reports indicated protein loss in the acetone-precipitation method. Nejadi *et al*., (2014) have suggested that the amount of proteins extracted is inversely proportional to the complexity of the procedure tested, i.e., the more complex the procedure tested, the lower the amount of protein extracted. Moreover, a study conducted by Wijesekara, *et al.,* (2017) revealed that usually use of organic solvents increases protein extraction but in the case of *Ulva* sp., the organic solvents decrease the amount of extracted protein concentrations.

4. CONCLUSION

It was observed that green seaweed *U. anandii* revealed the highest concentration of water-soluble proteins present in the crude extract as compared to acetone-precipitated and TCA-precipitated proteins. It is further recommended that more *Ulva* species should be tested with more replicates and the proteins extracted from each species should be analyzed for their structures.

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