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Extraction and characterization of protein from Irish brown seaweed *Ascophyllum nodosum*

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ABSTRACT

This study investigates traditional and non-conventional methods of extraction of protein from Irish brown seaweed *A. nodosum*. Acid, alkali, combined acid-alkali with and without ultrasound pretreatment were investigated for extraction of protein from *A. nodosum*. Molecular weight of protein was determined using high performance size exclusion chromatography and amino acid profiling was carried out using an amino acid analyzer. Combination of first acid and then alkali extraction was found to be the most efficient method of extraction among all methods investigated (59% of recovery); followed by single step of alkali extraction assisted with ultrasound (68.4 μm) which was able to extract 57% of total protein. Alkaline extraction was shown to yield the best protein/algae liquefaction ratio (1.28). This can be attributed to the release of polysaccharide complexes first by acid and then solubilization of proteins by alkali solvent. The molecular weight of extracted protein was found to be relatively low, in the range of 2–4 kDa average MW. The alkali method of extraction was found to be optimum for extraction of amino acids from *A. nodosum*.

1. Introduction

Traditionally, seaweed has been exploited as a source of food in many Asian countries, whereas elsewhere it has been widely used as source of biochemicals for food, pharma and cosmetic applications. Seaweeds are an important source of biologically active compounds including polysaccharides, carotenoids, phycobilins, fatty acids, vitamins, sterols, tocopherol and phycocyanins among others (Kadam & Prabhasankar, 2010). The popularity of seaweeds is attributed to their high polysaccharide content which is used for stabilizing and thickening applications in the food industry. Seaweeds are also increasingly exploited as a source of proteins. However, to date, extraction and fractionation of macroalgae proteins, peptides and amino acids has mainly been performed at laboratory scale. One of the main disadvantages of using seaweed as a protein source is the large variability on protein content in the raw material, which can vary from 3 to 47% (Harnedy & FitzGerald, 2013), depending on species, geographical location and season of growth (Fleurence, 1999). Generally, due to a lower photosynthetic activity which results in carbohydrates being produced and stored at a lower rate, relative protein content is higher in winter. Also the protein content of brown seaweeds is lower compared to green or red seaweeds. The protein content of *A. nodosum*, employed in the present study, generally varies from 3 to 15% of dry weight (Fleurence, 1999) and contains acidic amino acids, ranging from 18 to 44% of protein content (Harnedy & FitzGerald, 2011). *Ascophyllum nodosum* belongs to the class Phaeophyceae and is mainly confined to the north Atlantic basin where it dominates the rocky intertidal zones and can be easily harvested (Guiry, 2013). It is widely employed as supplement in food and agricultural applications (Fan et al., 2011). In general, protein from seaweed is extracted by means of aqueous, acid and alkaline extraction or enzymatic hydrolysis from dried seaweed powders; the supernatant rich in proteins is collected after centrifugation and the proteins are recovered by ultrafiltration, precipitation using ammonium sulfate or chromatographic techniques (Galland-Irmouli et al., 1999). Enzymatic extraction involves the use of enzymes such as proteases, cellulases, amylases, glucanases or endoproteases (Kadam, Álvarez, Tiwari, & O'Donnell, 2015), which degrade the seaweed matrix and release the proteins. Chemical hydrolysis or subcritical water hydrolysis have been also investigated (Kadam, Álvarez, et al., 2015). These conventional methods of protein extraction are time consuming and require large amount of solvents and the efficiency of extraction is limited. One of the most important factors influencing isolation and extraction of seaweed proteins is the complex seaweed matrix which supposes a physical barrier (Harnedy & FitzGerald, 2013). Proteins of seaweed species are bound to other non-protein components such as polysaccharides and polyphenols (Wijesinghe & Jeon, 2012); which are believed to be the main components that obstruct seaweed protein extraction (Conde, Balboa, Parada, & Falqué, 2013). Because of the above-mentioned reasons, non-conventional extraction techniques are currently being investigated and developed to improve the extraction yield while minimizing the time and resources required. Microwave

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assisted extraction (MAE) (Chemat & Cravotto, 2012), supercritical fluid extraction (SFE) (Liang & Fan, 2013; Sereewatthanawut et al., 2008), ultrahigh pressure extraction (UPE), pressurized fluid extraction (PFE) (Nobre et al., 2006), pressurized liquid extraction (PLE) and ultrasound assisted extraction (UAE) can enhance the extraction rate and yield. All these techniques improve the mass transfer rate, increasing the interaction between solvent and solute, helping to reduce extraction challenges caused by complex seaweed matrices.

In this study ultrasound was investigated as a tool for enhancing extraction from *A. nodosum*. When ultrasound is applied, rarefactions and compression occurs. If the pressure is higher than the tensile strength of the liquid vapor bubbles are formed. Such bubbles, under high ultrasound fields, collapse generating a cavitation effect (Vilkhu, Mawson, Simons, & Bates, 2008). This process of cavitation leads to peeling, erosion, particle breakdown and degradation of the solid-liquid surfaces, which facilitates the release of the target compound. Besides, an enhanced mass transfer can be observed (Vilkhu, Manasseh, Mawson, & Ashokkumar, 2011). UAE has been used for extraction of protein from various sources including microalgae (Parniakov, Apicella, et al., 2015); wheat germ (Zhu, Sun, & Zhou, 2009), defatted rice bran (Chittapalo & Noomhorm, 2009), brewers spent grain (TaNg et al., 2010), rapeseed (Dong et al., 2011), sorghum (Bean, Ioerger, Park, & Singh, 2006) and perilla seed (Zhu & Fu, 2012). However, to date UAE has not been reported for the extraction of protein from *A. nodosum*. The objective of this study was to investigate the effect of ultrasound assisted acid/alkali extraction of protein from *A. nodosum* on yield and amino acid degradation.

2. Materials and methods

2.1. Seaweed samples

Brown seaweed *A. nodosum* was harvested at Silver Strand beach, Co. Galway, Ireland in June 2014. Harvested seaweed was washed to remove impurities, chopped and hot air oven dried at 40 °C for 12 h. Dried seaweed was powdered using a hammer mill (Retsch SM100, GmbH, Germany) and sieved through a 0.5 mm mesh. Samples were stored at 4 °C prior to extraction studies.

2.2. Extraction of protein

All chemicals (NaOH, HCl, molecular weight markers for HPSEC, trichloroacetic acid, phosphate salts and ultrapure water) employed in this work were supplied by Sigma Aldrich (Ireland, Vale Rd, Arklow, Co. Wicklow). All chemicals were grade reagent ACS.

Protein extraction from *A. nodosum* was carried out using a modification of the method of Harnedy and FitzGerald (2013) (Fig. 1). Dried seaweed samples of 2 g were dissolved in 40 ml of distilled water and incubated at 4 °C for 16 h. The solution was centrifuged at 9000 rpm for 20 min at 4 °C (Sigma 2-16PK, United Kingdom). After centrifugation, the pellets obtained were treated with acid (HCl) and alkali (NaOH) at concentrations of 0.1, 0.2, 0.3, and 0.4 M. A solid to solvent ratio of 1:15 was used and solutions were stirred for 1 h at 4 °C and then centrifuged at 9000 rpm for 20 min at 4 °C. The resultant pellets were dried at 40 °C for 18 h and analyzed for protein content and total liquefaction. Protein content was measured in the supernatant obtained in first extraction step combined with the supernatant from the second extraction step.

The second type of extraction investigated was sequential extraction where first 0.1 M HCl was added to the pellet obtained after rehydration step, followed by centrifugation and the pellet obtained from the first extraction was subsequently treated with 0.1 M NaOH. Supernatants obtained from both acid and alkali assisted extractions were mixed and dried for protein yield estimation. In a similar manner, extraction was also carried out by reversing the order of solvent addition, namely first adding 0.1 M NaOH solvent followed by 0.1 M HCl addition. A solid to solvent ratio of 1:15 was used for both extractions.

In the third type of extraction, a ultrasound pretreatment was carried out. Pellet from rehydration was suspended in either 0.1 M HCl or 0.1 M NaOH buffer using the same solvent to solids ratio of 1:15; then

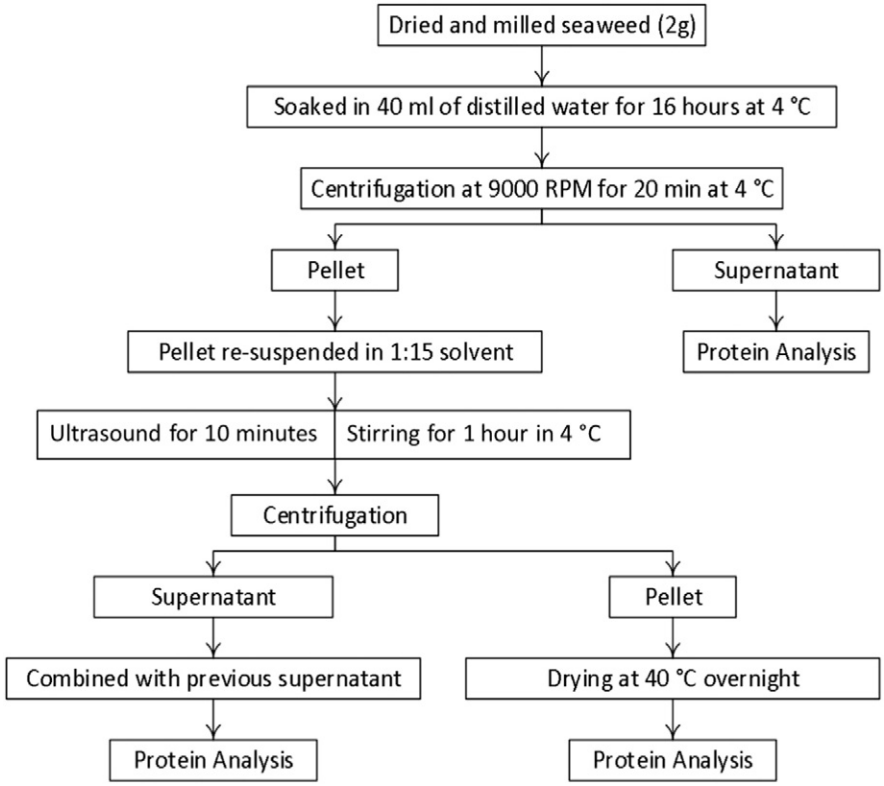

Fig. 1. Flow-chart for extraction of protein.