Food Research International 99 (2017) 971–978

Contents lists available at ScienceDirect

Food Research International

journal homepage: www.elsevier.com/locate/foodres

Assessment of the functional properties of protein extracted from the brown seaweed *Himanthalia elongata* (Linnaeus) S. F. Gray

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ARTICLE INFO

Article history:
Received 9 May 2016
Received in revised form 18 June 2016
Accepted 28 June 2016
Available online 29 June 2016

Keywords:
Brown seaweed *Himanthalia elongata*
Functional properties
Amino acid profile
Foaming properties
Emulsifying properties

ABSTRACT

A protein extract from the brown seaweed *Himanthalia elongata* (Linnaeus) S. F. Gray was prepared and its functional properties, colour and amino acid composition were assessed for its potential future use by the food industry. The total content of amino acids was determined as 54.02 ± 0.46 g amino acids/kg dry weight, with high levels of the essential amino acids lysine and methionine. SDS-PAGE showed 5 protein bands with molecular weights of 71.6, 53.7, 43.3, 36.4 and 27.1 kDa. The water holding capacity and oil holding capacity were determined as 10.27 ± 0.09 g H_2O/g and 8.1 ± 0.07 g oil/g respectively. Foaming activity and stability were higher at alkaline pH values. The emulsifying capacity and stability of the extract varied depending on the pH and oil used. These results demonstrate the potential use of *Himanthalia elongata* protein extract in the food industry.

1. Introduction

The world's population is expected to reach 9.1 billion people within the next 40 years and it is predicted that food production will need to double in the next four decades. In this scenario protein supply will be critical for both human food and animal feed uses (Aiking, 2014).

At present, animal protein production for human consumption is inefficient, and on average the production of 1 kg of animal protein requires 6 kg of plant protein (Aiking, 2014). In terms of food sustainability, utilisation of less animal protein could be beneficial in terms of preventing the effects of climate change (Aiking, 2014). Plant proteins are cheaper to produce than animal proteins but lack essential amino acids. For example, lysine and tryptophan are deficient in cereals and methionine in legume crops (Ufaz & Galili, 2008). It is necessary therefore to find economically viable alternatives to both animal and terrestrial plant protein sources (Suresh Kumar, Ganesan, Selvaraj, & Subba Rao, 2014).

Protein contributes to the technofunctional properties of food products and can act as emulsifying agents, texture modifiers in addition to assisting with fat and water absorption and the whipping properties of foods (Ogunwolu, Henshaw, Mock, Santros, & Awonorin, 2009). These features all contribute to the taste, texture and consumer acceptance of food products (Ogunwolu et al., 2009). The functional properties of a protein concentrate depend on its physicochemical characteristics which include molecular weight, amino acid composition, net charge and surface hydrophobicity. The physicochemical characteristics of a protein extract often depend on the extraction conditions employed. For example, cowpea and pigeon pea protein isolates displayed differences in hydrophobicity, colour and enthalpies depending on the extraction technique (micellation technique versus isoelectric point precipitation) and the conditions employed (pH) (Mwasaru, Muhammad, Bakar, & Man, 1999).

Recently, demand for seaweed for human consumption has increased due to consumer demands for new and healthy "natural foodstuffs" produced in a sustainable manner. Seaweeds are known to be rich in minerals and certain vitamins, but they also can be a rich source of protein. The protein composition of seaweed and the primary sequences of the protein amino acids are different from those of land proteins and may be better suited for human consumption compared to other vegetable protein sources (Joel Fleurence, 1999). Most seaweeds also contain all the essential amino acids and brown macroalgae were reported to contain higher levels of the acidic amino acids aspartic and glutamic acid than red and green macroalgae (Joel Fleurence, 1999). *Himanthalia elongata* belongs to the brown macroalgae or Phaeophyta. It has a history of safe use and acceptability in cooking and was previously used to add a beefy or nutty-like flavour to dishes (Rhatigan, 2009). Indeed, previously, beef patty formulations produced using the seaweed *H. elongata* (40% inclusion) were rated the highest in terms

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http://dx.doi.org/10.1016/j.foodres.2016.06.023
0963-9969/

of overall acceptability due to improvement in texture and mouth-feel without losing its sensory quality (Cox & Abu-Ghannam, 2013). However, only a few studies have been carried out detailing the functional properties of seaweed protein extracts (Kandasamy, Karuppiah, & Rao, 2012; Suresh Kumar et al., 2014).

The aim of the present study was to investigate the functional properties of protein extracts generated using food grade chemicals from the brown seaweed *H. elongata* commonly known as sea spaghetti. The solubility, water activity, water and oil holding capacity, emulsifying and foaming properties of the extracted protein were assessed. In addition the amino acid composition, colour and pH of the *H. elongata* protein extract were studied to assess the potential of this seaweed protein for use in the food industry.

2. Materials and method

2.1. Materials

Himanthalia elongata (Linnaeus) S. F. Gray provided by Porto-Muiños, Galicia, Spain was hand-harvested and collected at Muros, A Coruña, Galicia, Spain on the 20th of May 2013. Samples were freeze-dried, milled and vacuum preserved until further analysis. To avoid physicochemical modifications of the protein all reagents used during these experiments were food grade chemicals. Sunflower and olive oils from Musgrave House Ltd. (Cork, Ireland) and rapeseed, peanut and walnut oils from Lakeshore Foods Ltd. (Drogheda, Ireland) were purchased for use in this study.

2.2. Protein extraction and determination

Crude protein was extracted in triplicate according to the method of Galland-Irmouli et al. (1999). Briefly, 10 g of freeze-dried seaweed were suspended in 1 L of ultrapure water and ultra-sonicated for 1 h using a Bransonic® 3510EMT (Branson Ultrasonic SA, Switzerland). This sample was left to stir overnight on a magnetic stirrer plate (IKA RCT basic safety control, Germany) at 4 °C. After 24 h, the solution was centrifuged at 10,000 × *g* for 1 h and the supernatant decanted. The pellet fraction was re-suspended in 0.5 L of ultrapure water and subjected to a second extraction procedure as described above. Supernatants from both days were pooled together, and saturated to 80% with ammonium sulphate for 1 h at 4 °C followed by centrifugation at 20,000 × *g* for 1 h to precipitate the protein. The protein precipitates were diluted in a minimum volume of water and were subsequently dialyzed using Thermo Scientific™ SnakeSkin™ 3.5 kDa molecular weight cut off (MWCO) tubing (Fisher Scientific, New Hampshire State, USA) against ultrapure water at 4 °C overnight. Conductivity values were obtained for the water in dialysis tanks following incubation using a conductivity meter (Wissenschaftlich Technische Werkstätten, Germany). Dialyzed protein extracts were freeze-dried in an industrial scale freeze-drier FD 80 model (Cuddon Engineering, New Zealand), vacuum sealed and stored at −20 °C until further analysis. The protein yield of this process was calculated as g protein extract/g seaweed on dry weight (DW). Nitrogen was analyzed using a Leco Protein Analyser (Leco FP 628, Leco Corporation, USA). A factor of 6.25 was used to compute the protein value for the seaweed protein extract.

2.3. Total and free amino acid composition

For total and free amino acid composition analysis, *H. elongata* protein concentrate was hydrolysed in 6 M HCl at 110 °C for 23 h following the method of Hill (1965). Samples were then deproteinized by mixing equal volumes of 24% (w/v) tri-chloroacetic acid (TCA) and sample, these were allowed to stand for 10 min before centrifuging at 14,400 × *g* (Microcentaur, MSE, UK) for 10 min. Supernatants were removed and diluted with 0.2 M sodium citrate buffer, pH 2.2 to give approximately 250 nmol of each amino acid residue. Samples were then diluted 1 in 2 with the internal standard norleucine, to give a final concentration of 125 nm/mL. Amino acids were quantified using a Jeol JLC-500/V amino acid analyzer (Jeol (UK) Ltd., Garden city, Herts, UK) fitted with a Jeol Na^+ high performance cation exchange column.

2.4. Polyacrylamide gel electrophoresis

Tris-Tricine-SDS-PAGE was performed using a Mini-PROTEAN® electrophoresis unit (Bio-Rad laboratories, USA) using Mini-PROTEAN® 10–20% Tris-Tricine Precast Gels (Bio-Rad laboratories, USA). Protein separation was performed according to the manufacturer's recommendations. Briefly, samples were diluted 1:1 in with loading buffer containing 200 mM Tris-HCl pH 6.8, 2% SDS, 40% glycerol, 0.04% Coomassie Blue G-250 and 350 mM DTT. Samples were heated at 55 °C for 10 min and loaded in the precast gels in the electrophoresis unit in the presence of running buffer containing 100 mM Tris-base, 100 mM Tricine and 0.1% SDS. Running conditions were 30–35 mA for 3 h and 15–20 mA for 2 h. The Precision Plus Protein™ Dual Xtra Prestained Protein Standard (Bio-Rad laboratories, UK) was used as a molecular mass marker (250–2 kDa). After migration, protein bands were detected by Coomassie staining. Proteins were fixed in 40% methanol and 10% acetic acid for 40 min at low speed in an orbital shaker (VWR, USA). The gel was washed three times in ultrapure water for 5 min and then stained in Coomassie G-250 staining solution (Bio-Rad laboratories, USA) for 60 min in an orbital shaker. The gels were left overnight in ultrapure water with shaking and images were captured using a GS-800 densitometer (Bio-Rad laboratories, USA). All images were analyzed by Quantity One® software version 4.5.2 (Bio-Rad Laboratories, USA).

2.5. Colour evaluation

The colour of the extracted protein powder generated from *H. elongata* was measured using an UltraScan PRO Spectrophotometer (HunterLab, Germany) with illuminant D_{65}, diffuse 8° observer angle and included automated specular component. White and black standards (HunterLab, Germany) were used during the calibration procedure previous all measurements. Readings are reported in the CIE L*, a*, and b* system, being L* (lightness), a* (redness/greenness), and b* (yellowness/blueness). The chroma (C*) and hue (h°) values were calculated using the following equations:

$$C* = \sqrt{a*^2 + b*^2}$$

$$h^\circ = \tan^{-1}\left(\frac{b*}{a*}\right) \times \frac{180}{\pi}$$

To compare the colours of this study with other colours we used the ΔE*ab that represents the distance between any two colours in CIELAB space defined by its three orthogonal coordinates L*, a* and b*. ΔE*ab was calculated as follows

$$\Delta E*ab = \sqrt{(L*_1 - L*_2)^2 + (a*_1 - a*_2)^2 + (b*_1 - b*_2)^2}$$

L^*_1, a^*_1 and b^*_1 are the colour attributes of one colour sample and L^*_2, a^*_2 and b^*_2 the colour attributes of another colour sample to compare. A result of $\Delta E^*ab < 2$ is generally considered to be perceptually equivalent.

2.6. Determination of pH

Freeze-dried *H. elongata* protein was resuspended in distilled water at 1% w/v and the pH measured using a pH meter Orion, model 420A (Thermo Orion, Cambridgeshire, UK).