Innovative Food Science and Emerging Technologies 47 (2018) 187–194

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

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

High protein ingredients of microalgal origin: Obtainment and functional properties

Aline Massia Pereira, Cristiane Reinaldo Lisboa, Jorge Alberto Vieira Costa*

Laboratory of Biochemical Engineering, College of Chemistry and Food Engineering, Federal University of Rio Grande, Av. Itália km 8, Campus Carreiros, 96203-900 Rio Grande, Brazil

ARTICLE INFO

Keywords:
Protein concentrate
Protein extraction
Protein isolate
Spirulina

ABSTRACT

Protein concentrates and isolates were developed from the microalga *Spirulina* sp. LEB 18 biomass and characterized by functional aspects for food application. Proteins were solubilized at pH 11 and precipitated at pH 4.2 (protein isoelectric point determined by potentiometric titration), with the aid of a high-speed homogenizer. With this procedure, it was possible to obtain a protein concentrate with 83.9 ± 1.7 wt% of protein and a protein isolate with 91.3 ± 1.2 wt%. Protein extraction from this microalga allowed a significant increase in protein solubility and foam stability. Furthermore, proteins from both concentrate and isolate presented higher resistance to thermal denaturation than the original proteins of *Spirulina* sp. LEB 18 biomass. These results show the application potential of the concentrate and the protein isolate from *Spirulina* sp. LEB 18 in foods for protein supplementation, since they have > 80 and 90% protein, respectively.

Industrial relevance text: Using proteins from microalgal sources can serve as a sustainable alternative to meet world food demand. The isoelectric precipitation extraction method can be applied on a large scale, achieving high yields. Protein extracts present potential application in specific foods for protein supplementation. Protein extraction can be allied to other biocomposites extraction, such as carbohydrates and lipids for biofuels production.

1. Introduction

Protein supplements, such as protein concentrates and isolates, have high market demand. They are consumed to improve athletes' performance and practitioners of physical activity and to aid muscle recovery (Agarwal, Beausire, Patel, & Patel, 2015). The consumption of protein-rich supplements by the elderly is also considered important for the purpose of compensating the muscle mass loss and increase the immune system, providing them several health benefits (Bauer et al., 2013; Deutz et al., 2014).

Proteins from animal sources such as whey and vegetable sources like soybeans are the most traditionally used to obtain concentrates and protein isolates, being these used in food supplementation (Brans, Schroën, Sman, & Boom, 2004; Fischer, 2006). However, the obtainment of these protein sources has some inherent disadvantages, as for example soil degradation and depletion of water resources, causing environmental impacts such as loss of biodiversity (Aiking, 2011).

In this context, it should consider that the world population is constantly growing, which means increased demand for proteins. By 2050, the world population is expected to be 9.7 billion people, that is 30% more than the current scenario, which is already twice the number of people that the planet manages to supply sustainably (Nadathur, Wanasundara, & Scanlin, 2017). Efforts to overcome the challenges of sustainable food production to meet the demand for protein, ensuring food and nutritional security involve research into alternative sources of protein.

To be used as protein ingredients, new sources of protein are more accepted by the food industry if they have good technological/functional properties and are available at low cost (Schwenzfeier, Lech, Wierenga, Eppink, & Gruppen, 2013). Microalgae have been reported as new sources of proteins because they have several technological and commercial advantages such as high cell growth rates, low-cost cultivation conditions, and do not compete with food producing areas (Chronakis & Madsen, 2011; Radmer & Parker, 1994).

Microalgae proteins have high nutritional value compared to conventional proteins used in protein supplements considering protein content and essential amino acid content (Indergaard & Minsaas, 1991; Izydorczyk & Biliaderis, 2000).

Abbreviations: GRAS, Generally Recognized As Safe; FDA, Food and Drug Administration; *Sp*PC, *Spirulina* protein concentrate; *Sp*PI, *Spirulina* protein isolate; Td, thermal denaturation temperature

* Corresponding author.
E-mail address: jorgealbertovc@terra.com.br (J.A.V. Costa).

https://doi.org/10.1016/j.ifset.2018.02.015
Received 23 December 2017; Received in revised form 16 February 2018; Accepted 17 February 2018
Available online 21 February 2018
1466-8564/ © 2018 Published by Elsevier Ltd.

The *Spirulina* microalga has a high protein content in its biomass (60 to 70 wt%) and can be used in foods because it has a GRAS (*Generally Recognized As Safe*) certificate granted by FDA (*Food and Drug Administration*) (Belay & Houston, 2002; Fox, 1996). Some foods with added *Spirulina* biomass have been developed (Joshi, Bera, & Panesar, 2014; Marco, Steffolani, Martínez, & León, 2014; Santos, de Freitas, Moreira, Zanfonato, & Costa, 2016), but the addition of protein concentrates or protein isolates from this microalga into foods could provide even greater health benefits. Therefore, the elaboration of such protein concentrates, and protein isolates becomes interesting. The objective of this work was to obtain protein concentrate and protein isolate of *Spirulina* sp. LEB 18, and to evaluate the changes caused by the extraction process in the functional properties, aiming to suggest its use in foods intended for protein supplementation.

2. Material and methods

2.1. Microalgal biomass

Spirulina sp. LEB 18 biomass, cultivated with Zarrouk medium (Zarrouk, 1966) in open raceway type tanks under environmental conditions, was used to perform this study. This microalga, isolated by Morais et al. (2008), was produced in the pilot plant of the Laboratory of Biochemical Engineering, located on the shores of Mangueira Lagoon (33° 30 ′13″S and 53° 08 ′59″W) in the city of Santa Vitória do Palmar, Brazil. The biomass was dried and milled in a ball mill (Model Q298, QUIMIS) to a particle diameter of 0.125 mm (Tyler 115).

2.2. Centesimal composition

Determination of total protein, ash and moisture content was performed according to methods described by AOAC (2000). The nitrogen conversion factor into proteins used to determine the amount of protein (Kjeldahl) was 5.95 according to López et al. (2010) for cyanobacteria. The lipid content was determined according to the method of Folch, Lees, and Stanley (1957). Carbohydrates were determined by the method of Dubois, Gilles, Hamilton, Rebers, and Smith (1956).

2.3. Isoelectric point of Spirulina sp. LEB 18 proteins

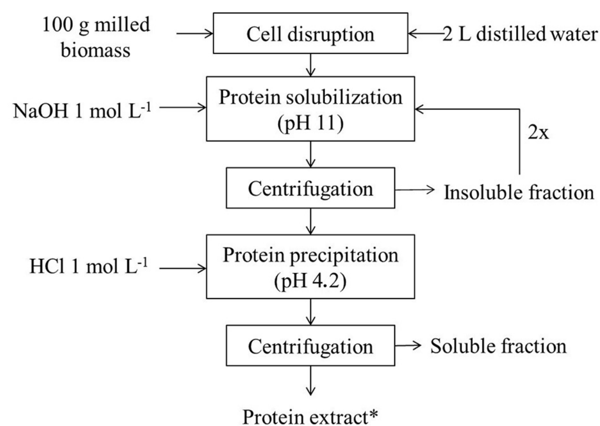
The isoelectric point of proteins was determined by potentiometric titration (Harris, 2010) using 0.1 mol L^{-1} HCl. The microalga extract was performed using 0.5 g biomass in 10 mL of distilled water, submitted to cell disruption in a 60 Hz ultrasonic probe, for 10 min in cycles of 59 s (59 s on/59 s off).

2.4. Chemical extraction of Spirulina sp. LEB 18 proteins

The process of chemical extraction by pH exchange was employed, according to the method of Furtado (2013) with modifications, knowing the isoelectric point of these microalgae proteins (Fig. 1). The microalga biomass was homogenized with distilled water in a high-speed ultra-turrax homogenizer (Ika, T25, Germany), at 9000 rpm, in a 1:20 (w/v) proportion, using 1 mol L^{-1} NaOH to adjust to pH 11 (Lisboa, Pereira, & Costa, 2016), separating soluble proteins and the insoluble fraction after centrifugation (15,200 × g for 30 min) (Hitachi, CR22GIII, Japan).

Insoluble fraction coming from the first centrifugation was then resuspended and submitted to alkaline solubilization twice. Solubilized proteins were precipitated at pH 4,2 using 1 mol L^{-1} HCl for 15 min in ultra-turrax homogenizer. The precipitated proteins were separated by centrifugation, frozen at −70 °C for 24 h in ultrafreezer (New Brunswick Scientific, U535–86 Innova®, EUA), freeze-dried (Labconco, 7753040, EUA) for 48 h and stored at room temperature, thereby obtaining the protein extract.

The protein content of the obtained protein extract was determined



*Protein extract denominated *Spirulina* protein concentrate (≥ 80 % protein), used to obtain *Spirulina* protein isolate (≥ 90 % protein) by repeating the extraction process.

Fig. 1. Flowchart of protein extraction by isoelectric precipitation.

by micro-Kjeldahl (AOAC, 2000). The extract with a protein content higher than 80 wt% was denominated *Spirulina* protein concentrate (*Sp*PC). For protein extract obtainment with > 90 wt% of protein, the protein extraction was performed over again, utilizing the protein concentrate obtained in the first extraction as the raw material. The obtained extract was called as *Spirulina* protein isolate (*Sp*PI).

2.5. Protein extraction process efficiency

The weight yield of the process (η) was calculated considering the initial dry weight of *Spirulina* sp. LEB 18 and the weight of lyophilized protein extract (*Sp*PC or *Sp*PI), as shown in Eq. (1). The protein extraction yield (PEY) was calculated knowing the initial and final protein content obtained, as shown in Eq. (2).

$$\eta(\%) = \frac{W_{protein\ extract}}{W_{biomass}} \times 100 \quad (1)$$

$$PEY(\%) = \frac{W_{protein\ extract} \times P_{protein\ extract}}{W_{biomass} \times P_{biomass}} \times 100 \quad (2)$$

wherein: $w_{protein\ extract}$ = final weight of protein extract (g); $w_{biomass}$ = initial weight of biomass (g); $P_{protein\ extract}$ = protein concentration of the protein extract (%); $P_{biomass}$ = protein concentration of initial biomass (%).

2.6. Determination of protein molecular weight

The approximate molecular weight of proteins from the microalga biomass, *Sp*PC and *Sp*PI was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) in continuous buffer system: tris buffer 1.5 mol L^{-1} and SDS 10% (w/v) based on Laemmli (1970) method. Samples (1 mg mL^{-1}) were diluted at 1:1 with sample buffer and boiled for 5 min. These sample solutions (10 μL) were loaded into 5% stacking gel and separated on 12.5% separating gel. The electrophoretic running was performed at 150 V for 2 h. The molecular weight marker *Precision plus protein standard* (Dual color 161-0374, Bio-Rad Laboratories, California, EUA), ranging from 10 to 250 kDa was also loaded (7 μL) to estimate proteins' molecular weight. The gel staining was performed overnight with a solution containing 40% methanol, 10% acetic acid, containing 0.1% Coomassie Blue G-250 dye. The gel was de-stained with a 20% methanol and 5% acetic acid solution.

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