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Optimal extraction and hydrolysis of *Chlorella pyrenoidosa* proteins

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HIGHLIGHTS

- For the first time two new methods were applied to whole protein extraction in microalgal cells.
- Two new methods: ionic liquid (IL) and low-temperature high-pressure cell breakage (LTHPCB).
- The newly developed LTHPCB can facilitate the development of Chlorella industry.
- The extracted proteins were hydrolyzed with three enzymes (papain, trypsin and alcalase).
- The obtained protein hydrolysates should be useful in nutritional supplement and medical foods.

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ABSTRACT

In this study, for the first time, the applications of two new methods, ionic liquid and low-temperature high-pressure cell breakage methods, to the extraction of whole proteins in *Chlorella pyrenoidosa* cells were explored. Meanwhile, the comparison with three traditional methods was also made. The results indicated that the extraction rate for ionic liquid is only at moderate level, but the new low-temperature high-pressure cell breakage method can obviously increase the protein extraction rate up to 2- to 15-fold. Subsequently, the hydrolysis of the extracted proteins was conducted with three enzymes (papain, trypsin and alcalase). The data presented that the degree of hydrolysis for each enzyme under the optimal conditions is in the order of: alcalase (18.31%) > papain (14.33%) > trypsin (8.47%), demonstrating the potential of *C. pyrenoidosa* protein hydrolysates obtained here in nutritional supplement and medical foods.

1. Introduction

Enzymatic protein hydrolysates appear to be more effective than either intact protein or free amino acids in clinical applications for nutrition and pathologies with reduced absorptive capacity and food allergies caused by intact protein epitopes (Clemente et al., 1999). There is great interest on the production of hydrolysates of many food proteins using proteolytic enzymes. The mostly commonly used protein sources in specific nutritional formulations are casein, whey proteins and soybean proteins (Potier, 2008).

Recently, microalgae proteins have been proposed as an alternative protein source due to their abundant content and amino acid profile (Garcia et al., 2012). The unicellular green algae *Chlorella* are suitable for protein products sold as health foods and food supplements (Morris et al., 2008). However, intact green algae *Chlorella* have a low protein digestibility due to their rigid cell wall. To access them various methods like alkali cell dissolution, organic solvent extraction, freeze–thawing or ultrasonication have been applied (Schwenzfeier et al., 2011). In particular, ionic liquids, which are ionic and salt-like materials that are liquid below 100 °C, have recently gained wide attention in separation and extraction of bioactive materials from plants (Tang et al., 2012), including proteins (Cheng et al., 2008; Ge et al., 2010). In this study, two new methods, ionic liquid and low-temperature high-pressure cell breakage methods, were developed to extract whole proteins from *Chlorella pyrenoidosa* cells. To our knowledge, there is no report on the applications of such two methods to the protein extraction of *Chlorella* sp. Subsequently, their extraction efficiencies were compared with other methods. Then, the extracted proteins were subjected to hydrolysis with three enzymes (papain, trypsin and alcalase), and their optimal process conditions were determined.

2. Methods

2.1. Materials and chemicals

C. pyrenoidosa powder (48.2% of total protein contents) was from Yuejian Bioengineering Co. Ltd., China. Bio-Rad Protein Assay

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