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ScienceDirect
Bioresource Technology 99 (2008) 7723–7729
BIORESOURCE TECHNOLOGY

Utilisation of *Chlorella vulgaris* cell biomass for the production of enzymatic protein hydrolysates

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Received 19 July 2005; received in revised form 28 January 2008; accepted 29 January 2008
Available online 24 March 2008

Abstract

Studies on enzymatic hydrolysis of cell proteins in green microalgae *Chlorella vulgaris* 87/1 are described. Different proteases can be used for production of hydrolysates from ethanol extracted algae. The influence of reaction parameters on hydrolysis of extracted biomass with pancreatin was considered, and the composition of hydrolysates (Cv-PH) was investigated in relation to the starting materials. Significant changes in the degree of hydrolysis were observed only during the first 2 h and it remained constant throughout the process. An enzyme-substrate ratio of 30–45 units/g algae, an algae concentration of 10–15% and pH values of 7.5–8.0 could be recommended. Differences in the chromatographic patterns of Cv-PH and a hot-extract from *Chlorella* biomass were observed. Adequate amounts of essential amino acids (44.7%) in relation to the reference pattern of FAO for human nutrition were found, except for sulfur amino acids. Cv-PH could be considered as a potential ingredient in the food industry.

Keywords: Green microalgae; *Chlorella*; Enzymatic hydrolysis; Protein hydrolysate; Food industry

1. Introduction

Since microalgae provide an efficient mean of converting solar energy into biomass (Hall and Rao, 1994), biotechnology of microalgae has gained importance in recent years due to the development of new production and environmental technologies (Pulz et al., 2000). Because their growth requires unexpensive substrates, microalgae can be used as economical and effective biocatalysts to obtain high added-value compounds and during productive processes, the algal biomass formed may be used as a food source such as proteins (Olaizola, 2003; Shimizu and Li, 2006).

The unicellular green algae (*Chlorophyta*, *Chlorophyceae*) are suitable for protein products sold as health foods and food supplements (Iwamoto, 2003; Merchant et al., 2002; Oh-Hama and Miyachi, 1988; Pulz and Gross, 2004). However, intact green algae *Chlorella* and *Scenedesmus* have a low protein digestibility due to their strong wall (Shelef and Soeder, 1980). The enzymatic hydrolysis of cell proteins has been described as a promising method to improving algae protein digestibility, which makes the product useable in human nutrition (Stoilov et al., 1995; Tchorbanov and Bozhkova, 1988).

Progress in hydrolysis techniques has led to the production of hydrolysates of many food proteins, using proteolytic enzymes such as pancreatic proteases, bacterial proteases and pepsin (Guadix et al., 2000; Kislukhina, 2002). Because enzymatic protein hydrolysates appear to be more effective than either intact protein or free amino acids, they have been widely used in specific formulations with clinical applications (Frokjaer, 1994; Vioque et al., 2004).

The sources most commonly used in nutritional products are casein and whey proteins and soybean proteins

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doi:10.1016/j.biortech.2008.01.080

(Clemente et al., 1999). In this sense, green microalgae biomass would represent in tropical countries an innovative proteinaceous bioresource for developing enzymatic protein hydrolysates suitable for pharmacological nutrition (Morris et al., 2007). An extended knowledge of the protein quality and functional properties in microalgae hydrolysates would be useful in understanding their use as potential additives for food and dietary items. This paper reports the results of a study on the enzymatic hydrolysis of *Chlorella vulgaris* biomass with pancreatin and also, the biochemical analysis of the obtained hydrolysates.

2. Methods

2.1. Microorganism, cultivation conditions and biomass processing

Algae samples were obtained by autotrophic outdoor cultivation of *C. vulgaris* 87/1 in open circulating cascade systems of 500 m^2 from September to December. This strain was isolated from Chalons dam in Santiago de Cuba and is deposited at the culture collection of the Solar Energy Research Center (CIES). The growth medium contained (g L^{-1}): NH_4NO_3 (1.2), $MgSO_4 \cdot 7H_2O$ (1.0) and a food grade NPK (8:12:12) fertilizer formula (0.9). The algal suspension was bubbled with 1% CO_2. The algae were harvested by continuous-flow centrifugation (separator Alfa Laval, Sweden) up to 10% dry matter in the slurry and then submitted to disruption in a DynoMill KDL apparatus with a disruption time of 3 min. The dark-green algae slurry was spray dried in a Niro Atomizer drier (input 200–210 °C, output 80–90 °C). The powder thus obtained (moisture content 7%) was preserved in plastic boxes for further use. Dry algae samples of 500 g were extracted with ethanol (2L) at 45 °C for 3 h via gentle agitation.

2.2. Chemical analysis

Total nitrogen, total fibre and ash were analyzed according to AOAC (1995) approved methods. Crude protein content was calculated using a conversion factor of 6.25. Total soluble sugars were estimated colorimetrically by the phenol–sulfuric acid method using a standard curve of glucose (Dubois et al., 1956), and total lipids were measured according to Kochert (1978). Pigments were determined in methanolic extracts according to Wellburn equations (1994). Nucleic acids (RNA + DNA) were measured as described by Rut (1973). *In vitro* protein digestibility (IVPD) was estimated measuring the pH of the protein suspension immediately after 10 min of digestion with a multienzyme solution of trypsin–chymotrypsin–peptidase. It was found that the pH of a protein suspension thus measured was highly correlated with the *in vivo* apparent digestibility of rats (Hsu et al., 1977). The enzymes, trypsin (porcine pancreatic trypsin type IX), chymotrypsin (bovine pancreatic chymotrypsin type II) and peptidase (porcine intestinal peptidase grade III) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

The amino acid profile was determined by reverse phase-HPLC after 24 h hydrolysis at 110 °C with 6 mol/L HCl and further derivatisation with phenylisothiocyanate. The tryptophan content was analyzed by derivative spectrophotometry (Fletouris et al., 1993). The amino acid scores (essential amino acid content in test protein hydrolysate/content of same amino acid in reference protein) were calculated. The lowest value corresponding to the limiting amino acid was designated as the chemical score. The FAO amino acid requirement pattern for the 2–5 year old child was used as the reference protein for comparison. This pattern is used, because it is the most demanding of any age group other than infants (FAO/WHO/UNU, 1985).

In protein hydrolysates, free amino acids content was estimated by the colorimetric ninhydrin reaction (Kalant, 1956) and soluble hydrolysed protein was assessed by the Biuret's method according to Lu et al. (2005).

All the chemicals used were of analytical grade.

2.3. Enzymatic hydrolysis

The influence of the nature of proteolytic enzymes on the hydrolysis of *Chlorella* cell proteins in ethanol extracted biomass was studied using the following proteases: pancreatin, pepsin and papain (MERCK), trypsin (Léciva, Praha), bromelain (UNICA, Cuba) and a crude extract obtained by submerged culture of *Bacillus subtilis* CEBI Bs-06-4. The specific activities and optimum pH for these proteases are showed in Table 1. One proteolytic unit was expressed as the amount of enzyme necessary to catalyze at an initial rate the release of 1 μmol tyrosine from a 2% denatured casein solution at 37 °C and the optimum pH for each enzyme within 1 min (Anson, 1938). The hydrolysis was carried out for 4 h at 20 U/g, algae concentration of 10%, temperature of 37 °C and the pH considered as optimum. The enzyme reaction was stopped by heat treatment at 85 °C for 15 min. The slurry thus obtained was centrifuged at 3000*g* for 10 min and the supernatants were used further for analysis.

Table 1
Specific activity (U/mg of protein) and optimum pH of proteases used on the enzymatic hydrolysis of cell proteins of *Chlorella vulgaris* 87/1

Proteolytic enzymes	Specific activity (U/mg of protein)[a]	Optimum pH
Pancreatin	0.470	7.5
Pepsin	3.280	2.0
Trypsin	0.070	7.5
Papain	0.130	7.0
Bromelain	1.197	7.0
Crude of *Bacillus subtilis*	0.030	8.5

[a] One proteolytic unit was expressed as the amount of enzyme necessary to catalyze at an initial rate the release of 1 μmol tyrosine from a 2% denatured casein solution at 37 °C and the optimum pH for each enzyme within 1 min.