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Improvement of functional and antimicrobial properties of brewery byproduct hydrolysed enzymatically

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ABSTRACT

The aim of this study is to investigate the characteristics of brewer's spent grain (BSG) hydrolysates. Hydrolysis was performed using a *Bacillus cereus* sp. extracellular peptidase. The proteins were hydrolysed for 2, 8 and 24 h, achieving degree of hydrolysis ranging from 2.65 to 21.80%. During enzymatic hydrolysis, average peptide chain length decreased rapidly and the soluble forms increased. Solubility of the hydrolysates showed a good correlation with turbidity. The water/oil holding capacity, the emulsifying properties and the foaming expansion were analysed, and improved functional properties were found respect to the control. For the studied hydrolysates concentrations no gel formation were obtained. However, BSG hydrolysates exhibited also desirable rheological properties making their good candidate for many food formulations. Thermal characterization revealed the energy recovery by the enzymatic hydrolysis process. The understanding of hydrolysates antimicrobial properties may lead to utilize their as potent natural antimicrobial against *Escherichia coli* O157:H7. In the light of the results, hydrolysates made from BSG can be converted into a high value protein food ingredient.

1. Introduction

Protein-rich byproducts from agro industrial industry have limited uses due to their dark colour, susceptibility to oxidation and off-flavour. As a sequence, they are discarded or processed into low marked-value products. The application of enzyme technology to recover food protein may produce a broad spectrum of food ingredients or industrial products. Recent advances in biotechnology have also demonstrated the capacity of enzymes to produce novel food products, modified foodstuffs and improved waste management.

Technological advancement in the past two decades in the commercial protein production has focused on the use of plant protein sources instead of animal sources (Henry & Kettlewell, 1996). Barley (*Hordeum vulgare* L.) is an extensive grown cereal and is used mainly for the brewing industry. In recent years, increased incorporation of barley into the human diet is recommended (Newman, Lewis, Newman, Boik, & Ramage, 1989) due to nutraceutical properties. Brewer's spent grain (BSG), high-volume by-product from the brewing industry, primarily contains proteins, barley cell wall carbohydrates, and lignin (Treimo et al., 2009). However, to date its use has mainly been limited to animal feeding. To increase the potential application of such insoluble proteins, they can be hydrolysed.

With regard to BSG utilization, our research group has already optimized the conditions for fermentation using *Bacillus cereus* spp., a bacteria isolated from fermented cabbage (Pérez Borla, Davidovich, & Roura, 2010). The fermentation is an eco-friendly method for the recovery of biomolecules from BSG.

In recent years, a significant growth of interest in functional protein hydrolysates can be observed. Protein modifications by enzymatic or chemical means can usually improve certain functional properties. Particular attention has been paid to enzymatic hydrolysis of protein. Enzymatic modification using specific proteases has several advantages over acid or alkali hydrolysis; it is mild and does not destroy amino acids (Desslie & Cheryen, 1988). Protein hydrolysis has also been applied to improve the functional, organoleptic and nutritional value of foodstuff (Navarrete del Toro & García-Carreño, 2002). Functional protein hydrolysates can be

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used in food systems as additives, as food texture enhancers or as pharmaceutical ingredients. Some of these properties are water/oil binding, emulsification, foam formation, viscosity and gelation. Many studies have demonstrated that the enzymatic hydrolysis of various substrates improved its functional properties, including solubility, emulsifying and foaming characteristics (Puski, 1975; Were, Hettiarachchy, & Kalapathly, 1997). Functional properties of proteins are those physicochemical properties of proteins which affect behaviour of proteins in food systems during preparation, processing, storage, and consumption, and contribute to the quality and sensory attributes of food systems (Kinsella, 1976).

These properties are affected by intrinsic protein factors such as molecular structure and size, and other many environmental factors. The importance of these properties varies with the type of food products in which the hydrolysate is used (Yu, Ahmedna, & Goktepe, 2007). Is scare the information about the antimicrobial activity of hydrolysates from processing by-products against food pathogens. Protein hydrolysates are widely used as nutritional supplements, functional ingredients and flavours enhancers in food, coffee whiteners, cosmetics, personal care products and confectionery and in the fortification of food products. The latter is critically needed in developing countries where protein deficiencies remain a mayor health problem, especially among children (Yu et al., 2007). The hydrolysis process generates smaller peptides with improved nutritional characteristics compared to the original protein (Kalili, Mohamed, Taha, & Karisson, 2003).

The hydrolysed protein rich liquor is expected to show beneficial bio-functionalities like antibacterial properties. Protein hydrolysates from different sources, such as soy (Joo, Yi, Lee, Lee, & Oh, 2004), whey (Shinam, Radha, Prakash, & Kaul, 2007), meat and fish proteins (Jang, Jo, Kang, & Lee, 2008) and defatted peanut kernels (Huang, Shyu, Wang, & Hsu, 2010), have been found to possess antimicrobial activity. The operational conditions employed in the processing of protein isolates, the type of protease (Celus, Brijs, & Delcour, 2007) and the degree of hydrolysis (DH) also affect the antimicrobial activity.

In the light of the above facts, a characterization of the hydrolysates resulting from enzymatic hydrolysis of BSG protein was done. Firstly, protein hydrolysates, with variable DH, were prepared from pre-treated BSG by a *B. cereus* spp. extracellular protease. After that, the hydrolysates were characterized by their functional properties (such as solubility, clarity, foaming, emulsification and gelation) to determine the potential application in various food products.

Additionally, other physicochemical properties as the thermal characterization, reological behaviour and colour of the hydrolysis product were investigated.

Finally, this study determined the antimicrobial activity of the protein hydrolysate against *Escherichia coli* and *Listeria monocytogenes*. The understanding of antimicrobial properties may lead to utilize it as a potent natural antimicrobial. In future works, we will emphasize the search of antioxidant capacity of these hydrolysates.

2. Materials and methods

2.1. Raw materials

Brewer's spent grain (BSG) [~32.5 g protein/100 g on a dry basis (db)] was purchased from Antares S.A. (Mar del Plata, Argentina). The BSG was pre-treated according to the procedure describing in Kotlar, Belagardi, and Roura (2011) in order to standardize the hydrolysis substrate (HS). Enzymatic hydrolysis was produced in our lab following the procedure described in Section 2.1.

2.2. Preparation of protein hydrolysate

2.2.1. Enzymatic hydrolysis

The enzyme source was obtained from a *B. cereus* strain previously isolated by Pérez Borla et al. (2010).

Thirty six gram of HS was added to 100 mL Minimal Salt Medium (MSM) which contained: K_2HPO_4 (0.1 g/100 mL), $MgSO_4 \cdot H_2O$ (0.02 g/100 mL), $CaCl_2$ (0.01 g/100 mL) and Na_2CO_3 (0.1 g/100 mL), and then was autoclaved at 121 °C for 15 min (Kotlar, Ponce, Sansevero, & Roura, 2010), to inactivate microorganisms initially present in the HS. Sodium carbonate was sterilized separately and added to the rest of the medium after cooling to room temperature. 5 mL of the enzyme source (crude enzyme) were added to 100 mL of the above mentioned medium in 150 mL Erlenmeyer, and incubated at 32 °C on an orbital shaker (TS-1000, Zhejiang, China) for 24 h. To evaluate the effect of the incubation medium alone over this timeframe, a control sample was prepared in a similar manner, but without enzyme (control).

Hydrolysate samples were collected at 0, 2, 8 and 24 h of incubation period (HP_0, HP_1, HP_2 and HP_3, respectively), and enzymatic activity stopped by heating in a water bath at 95 °C for 5 min.

Then, each sample was centrifuged at 2000 × *g* at 4 °C for 10 min using a Labnet 7M centrifuge (Labnet International, Inc., New York, USA) and the supernatant was collected.

Hydrolysates were freeze-dried using a freeze dryer (Karaltay Scientific Instruments Co., Ltd., Beijing, China) to yield a powered hydrolysate. Lyophilizes were stored at 4 °C until analysed.

2.2.2. Determination of degree of hydrolysis (DH)

The DH of BSG hydrolysates was measured by the o-phthaldialdehyde (OPA) method (Nielsen, Petersen, & Dambmann, 2001). The OPA reagent was prepared by combining 7.620 g disodium tetraborate, 200 mg sodium dodecyl sulphate (SDS), 160 mg OPA 97% (in 4.0 mL 95% ethanol), and 176 mg dithiothreitol (DTT) (Sigma, cod. D0632) and adding deionized water to a final volume of 200 mL. A L-serine (Merck, cod. Art. 7769) concentration range (0–0.2 mg/mL) was used as the standard curve. Total hydrolysate was diluted (1:80) in SDS (1 g/100 mL). OPA reagent (3.0 mL) was added to all samples (400 μL), and the absorbance (340 nm) was measured after 20 min. The increase in amino groups between control sample and hydrolysates was attributed to proteolysis, and the degree of hydrolysis (DH) was calculated by the following equation:

$$DH\_(\%) = \frac{h}{h_{tot}} * 100 \tag{1}$$

Where *h* represents hydrolysis equivalents [mequivalents (mequi)/g protein]; and h_{tot} is the total theoretical number of peptide bonds per unit weight present in BSG protein, for hordeins h_{tot} = 7.52 mequi/g protein (Bamdad, Wu, & Chen, 2011). The degree of hydrolysis expressed for each sample was the mean of three determinations.

The expression for *h* in the OPA method is:

$$\text{serine\_}NH_2 = \frac{\left(OD_{sample\_} - OD_{blank}\right)}{\left(OD_{standard} - OD_{blank}\right)} * 0.9516 \text{ mequi} / L * 0.1 * 100 / X * P \tag{2}$$

Where serine_NH_2 is the mequi serine NH_2/g protein; *X* are the grams of sample; *P* is the protein concentration in the sample; 0.1 is the sample volume in litre (L), *h* is then: