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Nutritional ingredients from spent brewer's yeast obtained by hydrolysis and selective membrane filtration integrated in a pilot process

Manuela Amorim [a], Joana O. Pereira [a], David Gomes [b], Carlos Dias Pereira [b], Hélder Pinheiro [c], Manuela Pintado [a, *]

[a] Universidade Católica Portuguesa, CBQF – Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Arquiteto Lobão Vital, Apartado 2511, 4202-401 Porto, Portugal
[b] Politécnico de Coimbra, Escola Superior Agrária, Bencanta, 3045-601, Coimbra, Portugal
[c] Departamento de Farmacologia e Terapêutica, Faculdade de Medicina da Universidade do Porto, Al. Prof. Hernâni Monteiro, 4200-319, Porto, Portugal

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ABSTRACT

Spent brewer's yeast is a natural surplus from brewing industry. In order to up-grade this by-product, isolation of compounds has been tentatively assessed. The main objective of this work focuses on the use of ultrafiltration and nanofiltration pilot system for recovering cell compounds. Initially, yeast was autolyzed and ultrafiltered with a 10 kDa cut-off, and the two fractions obtained were hydrolyzed with Cynara cardunculus extract and nanofiltered with 3 kDa cut-off. Four fractions with different molecular weights were obtained, with protein and sugar contents ranging between 30–69% and 20–48%, respectively. Sodium and potassium were the major minerals present, whereas glutamine, glutamic acid and alanine, the most representative free amino acids. Peptide profile showed peptides with hydrophilic and hydrophobic characteristics, usually associated with biological activities, including antihypertensive and antioxidant. Thus, based on their compositions, all fractions show technological and biological potential, and can be used as nutritional ingredients in food and feed.

1. Introduction

Beer manufacture involves production of several residues and by-products e.g., spent grains, hops and yeasts (Mussatto, 2009). Brewer's yeast is the second largest by-product originated by the food industry, and most of this is sold as animal feed at low price or has to be disposed as waste. Therefore it merits considerable attention, not only for the large amounts produced, but also due to its valuable nutritional composition. Residual brewing yeast is predominantly composed by proteins (35–60% dry basis) that include all the essential amino acids and have high biological value (referring to the amount of essential amino acids in its structure) (Chae et al., 2001) thus being an excellent source of high-quality protein, comparable in value with soy protein (Otero et al., 2000). The second highest compound is carbohydrates that represent 35–45% of dry basis. In addition, this by-product has other substances biologically important, such as, minerals (5–7.5% of which, Ca, P, K, Mg, Fe, among others) lipids, B vitamins and enzymes (dos Santos Mathias et al., 2014).

Yeasts have been traditionally used in fermentation processes, as food flavoring and enrichment ingredients as yeast extract and autolysate (Pacheco et al., 1997), and are generally recognized as safe - GRAS (Briggs et al., 2004). Due to this, for inactivated yeast derivatives, new applications have been explored as nutritive complements and ingredients for formulations in food industry (Chae et al., 2001; Dikit et al., 2010).

This increasing interest in food by-products led to the development of novel bioprocessing technologies for isolation of bioactive substances to be used as functional foods and nutraceuticals. Improvement of these functional ingredients, involves certain biotransformation processes through enzyme-mediated hydrolysis in combination with membrane techniques for fractionation of peptides hydrolysate. Porous membrane filtration is employed as a physical barrier in order to separate particles with different characteristics, based on size and shape, using pressures and membranes specifically designed for the process, with different pore

* Corresponding author.
E-mail address: mpintado@porto.ucp.pt (M. Pintado).

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diameters (Malik et al., 2013). Although, it exist different methods of membrane filtration - reverse osmosis, nanofiltration, ultrafiltration and microfiltration, in ascending order relative to the diameter of the pores, they are all intended for separation or concentration of substances (Pouliot et al., 2014).

Several studies relate the application of this technology to the production of food ingredients and nutraceuticals. Dairy by-products, lead the utilization of this technology in the production of purified and hydrolyzed proteins, lactose and salts (Lieske and Konrad, 1996; Saxena et al., 2009; Tavares et al., 2012), but other food industries including canned fish, meat, cereals, among others have been using this technology (Samaranayaka and Li-Chan, 2011). Thereby, membrane-based process can provide the required product quality, purity, yield and throughput with low cost and environmental sustainability. All these assumptions lead to the main objective of this work, the use of selective membranes, in pilot system, to transform the spent brewer's yeast in hydrolyzed fractions with different molecular weights and with improved chemical and nutritional richness.

2. Material and methods

2.1. Spent brewer's yeast

Spent brewer's yeast was obtained as a byproduct of beer production and it was kindly provided by UNICER, Porto, Portugal. Each batch of spent brewer's yeast processed in pilot process was initially submitted to autolysis performed at 70 °C for 4 h in a 100 L double-walled, steam-supplied vat heated with burning gas with control for stirring rate and temperature.

2.2. Development of pilot process

Spent brewer's yeast autolysate (stored and transported at refrigerated temperature) was submitted to a sequence of selective filtration processes (Fig. 1), combined with enzymatic hydrolysis to obtain different peptide extracts, using pilot-scale equipment (Proquiga, Spain). As described in Fig. 1, 100 L of spent brewer's yeast autolysate was factionated in an ultrafiltration batch system at 45–50 °C, with an organic membrane Hydranautics model (Dairy 10k 3838-30) with 7.4 m^2 filtration area and 10 kDa cut-off. Thereafter, protein retentate (PR) (molecular weight (MW) > 10 kDa) and protein permeate (PP) (MW < 10 kDa) were submitted to hydrolysis at optimal conditions previously determined (data not shown): 4% (v/v) of *Cynara cardunculus* extract (Formulab, Maia, Portugal), for 4 h at 55 °C and pH adjusted to 5.2 with lactic acid (Sigma – Aldrich, St. Louis MO, USA). After hydrolysis, each hydrolyzed fraction (protein retentate and permeate) was then nanofiltred at 45–55 °C using an organic membrane PTI Advanced Filtration (model NF 3838/30-FF), area 6, 9 m^2 with 3 kDa cut-off.

Four fractions were obtained (i) Protein Retentate Hydrolyzed >3 kDa (PRH >3 kDa); (ii) Protein Retentate Hydrolyzed <3 kDa (PRH <3 kDa); (iii) Protein Permeate Hydrolyzed >3 kDa (PPH >3 kDa) and (iv) Protein Permeate Hydrolyzed <3 kDa (PPH <3 kDa). Each of these fractions was concentrated by reverse osmosis (concentration rate approximately 40 times), frozen at −80 °C and then freeze dried and stored under vacuum at ambient temperature, protected from light.

2.3. Nutritional composition

Protein content was determined according AOAC procedures (Nx5.8) (Horwitz et al., 2010) using a Kjeltec system 1002 distilling unit (Tecator; Höganäs, Sweden).

Total sugars were determined by colorimetric method as described by Dubois et al. (1956), using glucose (Sigma – Aldrich, St. Louis MO, USA) as standard. Ashes were determined by heating for 5 h in a muffle at 525 °C (AOAC, 1995).

Mineral concentration was carried out by optical emission spectrometer Model Optima 7000 DV ™ ICP-OES (Dual View, PerkinElmer Life and Analytical Sciences, Shelton, CT, USA) with radial configuration as described by Chatelain et al. (2014). Free amino acids content of each fraction was performed by pre-column derivatization with orthophthalaldehyde (OPA) methodology. Isoindole-type fluorescent derivatives were formed in an alkaline solution (borate buffer pH 10.4) from OPA, 2-sulfanylethanol and the primary amine group of the amino acid. The derivatives were

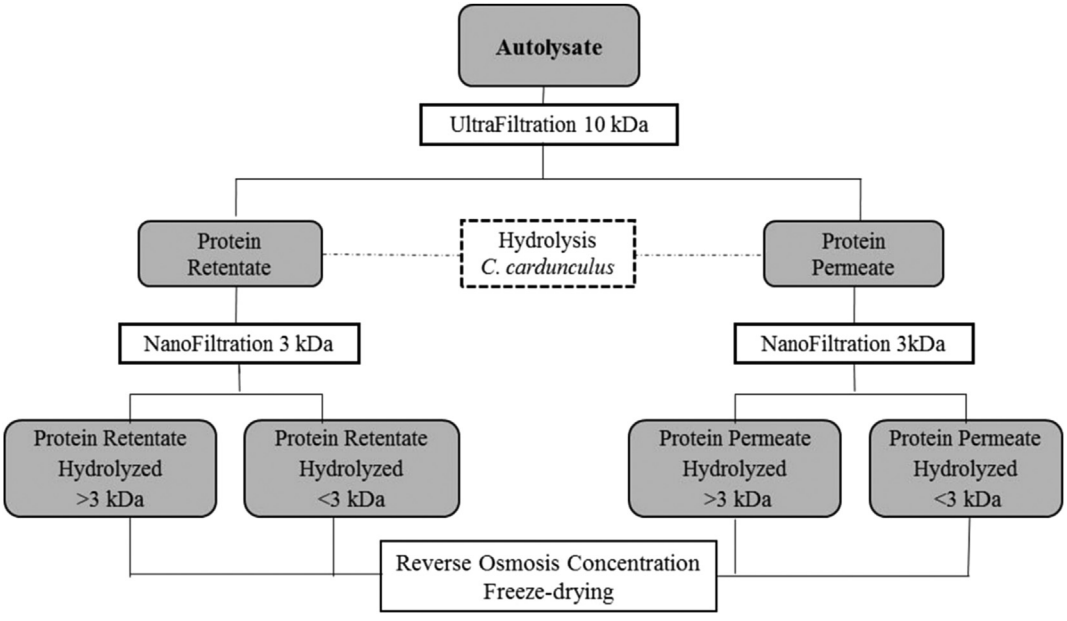

Fig. 1. Diagram of pilot scale for filtration of spent brewer's yeast autolysate.