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Enzymatic hydrolysates from food wastewater as a source of peptones for lactic acid bacteria productions

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Abstract

A diverse group of peptones obtained by enzymatic hydrolysis of wastewater from the industrial processing of octopus showed their effectiveness to promote the growth of lactic acid bacteria (LAB) and the production of bacteriocins. The highest nisin formation by *Lactococcus lactis* was reached using peptones from papain hydrolysis for 24 h (enzyme concentration: 1.25 mg papain/g protein). On the other hand, the highest pediocin production by *Pediococcus acidilactici* was obtained with peptones derived from 4 h of pepsin digestion (enzyme concentration: 3.75 mg papain/g protein). Thus, these marine peptones are promising alternatives to currently available and expensive commercial medium as well as a possible solution to valorise this problematic wastewater.

Keywords: Marine peptones; Lactic acid bacteria production; Nisin and pediocin production; Lactic acid production; Waste medium; Logistic equation; Waste valorisation

1. Introduction

One of the most important environmental problems which are characteristic of coastline areas is the large volume of waste generated by fishing, aquaculture or foodstuff processing industries. Usually, these by-products are dumped into the sea without a previous treatment of depuration neither evidently nor by valorisation. Among these food products, the cooked cephalopod (particularly octopus) has higher commercial value and larger production of wastewater in the Galician coasts (NW Spain). These massive spills with high protein concentration generate a negative environmental impact on the *Galician Rías*, marine ecosystems of great ecological wealth and very sensitive to the contamination.

A possibility to eliminate this problem and to valorise those rich nitrogen and phosphorus organic sources (proteins, peptides and amino acids), is their use as nutrients in the formulation of optimum media for the micro-organisms culture. To check their validity, the lactic acid bacteria (LAB) may be the most appropriate group of micro-organisms.

From the viewpoint of their industrial importance, LAB are classified as one of the greatest and most important microbial groups due to their significant role in food fermentation and preservation, as a natural microflora or as an inoculum added under controlled process conditions [1–3]. Among the molecules produced by these micro-organisms which present antimicrobial activity are lactic and acetic acid, ethanol, diacetyl, 2,3-butanediol and bacteriocins [4–6]. Bacteriocins produced by LAB, are peptides with antimicrobial activity and have great importance to the food industry, as they are innocuous, sensitive to digestive proteases of vertebrates, and do not change the organoleptic properties of the food [7,8].

LAB and, specifically, bacteriocins productions are very fastidious due to the need for rich growth media containing nutrients such as carbohydrates, nucleic acids, minerals, vitamins and, mainly, amino acids, proteins or protein hydrolysates [9–14]. For example, the standard laboratory media (MRS, TGE, APT) solve the problem of protein sources, incorporating products such as bactopeptone, tryptone, meat extract or yeast extract (sometimes all of these) on formulations which reach expensive costs. Also, these media are of bovine or porcine origin that disable their utility to generate *kosher* and *halal* products [11,15] and to avoid the bovine spongiform encephalopathy risk (BSE) [16].

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Hence the use of low-cost proteins or protein fractions will bring about a reduction in large-scale production costs [10–13,17,18]. Furthermore, whether food waste is used to obtain these nutritional sources (e.g. waste generated by industries which process foodstuffs of marine origin), a complete productive cycle is closed: the recycling and valorisation of pollutant waste and the obtaining of a product of high added value, used for control and preservation of foodstuffs (LAB and bacteriocins).

Bearing this in mind, this work examined the suitability of media formulated with enzymatic hydrolysates of wastewater from the industrial processing of octopus, for the culture of LAB and the production of bacteriocins. The micro-organisms assayed were *Lactococcus lactis* and *Pediococcus acidilactici* producing of nisin and pediocin, respectively. The experimental approach consisted of kinetic studies of the protein fractions, using as criteria for comparison among the cultures, the parameters values obtained of the numeric adjustment of the experimental data to the proposed mathematical models.

2. Materials and methods

2.1. Preparation of marine peptone from octopus

The wastewaters from the industrial processing of octopus (*Octopus vulgaris*) were prepared in the laboratory, in a similar way to their commercial formulation, carrying out the following steps: the octopus was frozen immediately after capture and maintained at −50 °C until its use, subsequently they were thawed in brine for 24 h (brine solution: 20 g l^{-1} of NaCl; brine-to-octopus ratio: 500 ml per kg (dry basis)) and thermally processed in two separate stages at 90 °C/10 min in an autoclave. On average, the industrial processing of octopus generates approximately 0.75 l of wastewater with high protein content per kilo (db) of material processed.

These octopus waters (12 l) were centrifuged at 7500 × *g* for 15 min at 20 °C and divided into 24 aliquots of 500 ml each one, necessary to obtain the peptones corresponding to three types of enzymes, in two concentration levels and four incubation times. Papain (PAP; Sigma: 3.2 U/mg solid), trypsin (TR; Fluka: ∼9000 U/mg) and pepsin (PEP; Merck: 700 FIP-U/g) were used as enzymes at the following levels and initial pH values:

(a) PAP: 1.25 mg enzyme/g protein aliquot, 12.50 mg enzyme/g protein aliquot, pH 6.4.
(b) TR: 1.25 mg enzyme/g protein aliquot, 3.75 mg enzyme/g protein aliquot, pH 6.4.
(c) PEP: 1.25 mg enzyme/g protein aliquot, 3.75 mg enzyme/g protein aliquot, pH 2.5.

Hydrolyses were carried out maintaining at 20 °C with orbital shaking at 100 rpm for 0, 4, 10 or 24 h and, subsequently, the proteolytic process was ended by heating to 101 °C for 10 min. After each incubation period, the hydrolysates were treated in a decanting centrifuge at 7500 × *g* for 15 min, obtaining the corresponding sediment and supernatants. The supernatants (or marine peptones) were typified determining the levels of total nitrogen, soluble protein and total sugars, and then stored at −20 °C until the time of their use in the formulation of culture media. The basic composition of peptones with the hydrolysis degree corresponding to initial time was (in g l^{-1}): 20.3 of protein (Lowry), 0.22 of reducing sugars, 3.7 of total nitrogen and 1.67 of total sugars.

2.2. Microbiological methods

The micro-organisms used were *L. lactis* ssp. *lactis* (abbreviated key Lc HD1) [8,19] and *P. acidilactici* NRRL B-5627 (Pc 1.02) [9,12]. *Leuconostoc mesenteroides* ssp. *lysis* (kindly donated by Dr. Ray, University of Wyoming, Laramie, USA) and *Carnobacterium piscicola* CECT 4020 (Spanish Collection Type Culture) were employed as indicators for the nisin and pediocin bioassays, respectively. Stock cultures were stored at −75 °C MRS medium with 25% glycerol [20]. Inocula (1%, v/v) consisted of cellular suspensions from 12 h (Lc HD1) and 24 h (Pc 1.02) aged in MRS, adjusted to an OD (700 nm) of 0.900.

Table 1
Composition of culture media used in microbiological assays (g l^{-1})

	MP media[a]	MRS medium
Glucose	20.00	20.00
Yeast extract	4.00	4.00
Sodium acetate	5.00	5.00
Ammonium citrate	2.00	2.00
K_2HPO_4	2.00	2.00
$MgSO_4$	0.20	0.20
$MnSO_4$	0.05	0.05
Tween 80	–	1.00
Meat extract	–	8.00
Bactopeptone	–	10.00
Marine peptone protein (Lowry)	10.00	–

[a] Marine peptone from octopus defined in text.

2.3. Culture media

The composition of the media is shown in Table 1. For the purpose of comparison a MRS commercial medium (Pronadisa, Hispanlab S.A., Spain) was used. In all cases, initial pH was adjusted to 7.0 with NaOH 5 M and solutions sterilised at 101 °C for 1 h. Micro-organisms were grown in 300 ml Erlenmeyer flasks with 200 ml of medium at 30 °C, with 200 rpm orbital shaking. The cultures were carried out in duplicate. At pre-established times, each culture was divided into two aliquots. The first was centrifuged at 4000 × *g* for 15 min, and the sediment washed twice and resuspended in distilled water to the adequate dilution for measuring the optical density (OD) at 700 nm. The dry weight can then be estimated from a previous calibration curve. The supernatant was used for the measure of reducing sugars [21], proteins, glucose, lactic acid and acetic acid. The second aliquot was used for the extraction and quantification of bacteriocin (nisin and pediocin). All assays were carried out in triplicate.

2.4. Analytical methods

The proteins were determined by the method of Lowry et al. [22]. In the case of the hydrolysates, total nitrogen (method of Havilah et al. [23], applied to digests obtained by the classic procedure of Kjeldahl), and total sugars (Dubois et al. [24], according to the application of Strickland and Parsons [25]) were also determined. In the case of the supernatants, glucose, lactic acid and acetic acid were quantified by HPLC analysis (refractive-index detector), using an ION-300 column (Transgenomic, USA) with 6 mM sulphuric acid as a mobile phase (flow = 0.4 ml min^{-1}), at 65 °C. Methods for the extraction and quantification of bacteriocin were described in detail by Cabo et al. [20] and Murado et al. [26,27], using *L. mesenteroides* ssp. *lysis* (for nisin) and *C. piscicola* CECT 4020 (for pediocin) as indicators.

2.5. Mathematical models

The mathematical models used to describe and to typify kinetically the sigmoid growth of micro-organisms and the corresponding LAB productions were the following ones [28] (see symbol notation Table 2 for the definition of the parameters and their units):

$$X = \frac{K}{1 + \exp[2 + (4 \cdot v_{mx}/K)(\lambda_x - t)]} \quad (1)$$

$$L = \frac{L_m}{1 + \exp[2 + (4 \cdot v_{ml}/L_m)(\lambda_l - t)]} \quad (2)$$