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Use of activated carbon for removing phenylalanine from reconstituted skim milk powder hydrolysates

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Abstract

With the aim of preparing dietary supplements for phenylketonurics, the activated carbon was used in this work to remove phenylalanine (Phe) from skim milk powder enzymatic hydrolysates. Six hydrolysates were prepared, using a protease from *Aspergillus oryzae* (AO), isolated or in association with papain (PA). Different conditions were tested for removing Phe, and the best one showed to be the use of a activated carbon:casein ratio of 118 (g) and a temperature of 25 °C, which produced 96–99% of Phe removal. Among the hydrolytic conditions employed, the association of AO with PA (1 h, 1 g of enzyme/100 g of substrate and 4 h, 2 g of enzyme/100 g of substrate, respectively) led to the lowest absolute value for the final Phe concentration (0.060×10^{-4} mg/100 mg of protein).

Keywords: Dietary supplement; Phenylketonuria; Phenylalanine; Skim milk powder; Activated carbon

1. Introduction

Phenylketonuria (PKU) is a metabolic disease associated with the metabolism disorder of phenylalanine (Phe), in which the oxidation of this amino acid is impaired due to the deficiency of the hydroxylase Phe enzyme (Moszczynski & Idziak, 1993). Untreated patients show serious mental retardation and their expectation of life is drastically reduced (Lopez-Bajonero, Lara-Calderon, Galvez-Mariscal, Velasquez-Arellano, & Lopez-Munguia, 1991; Shimamura et al., 1999; Mira & Marquez, 2000).

The nutritional therapy for PKU is based on limitation of protein ingestion, reducing Phe supply to the minimum and promoting the normal growth of patients with other nutrients (Mahan & Stump, 1998; Dutra-De-Oliveira, 1998, Chap. 3).

Considering that the amount of Phe in vegetable or animal proteins is from 3–6g/100 g of protein, the diet for phenylketonurics is generally deficient in proteins in order to attempt the low Phe level required by these patients (Outinen, Tossavainen, Harju, & Linko, 1996; Shimamura et al., 1999). The PKU diet is normally supplemented with a medicinal food containing a mixture of free amino acids or oligopeptides, which provides 50–90% of protein equivalents, 90–100% of vitamins and trace elements and 50–70% of energy (Mira & Marquez, 2000).

Among several protein sources that may be used for preparing dietary supplements for phenylketonurics, isolated casein, the main milk protein, is the choice in most cases (Lopez-Bajonero et al., 1991; Outinen et al., 1996; Shimamura et al., 1999). However, in underdeveloped countries, this protein needs to be imported which represents an important increase in production

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costs. Thus, the use of alternative sources must be investigated. Among them, the skim milk powder may be tested since its cost is lower than casein.

Most of the methods used for Phe removal from protein hydrolysates are based on the principle that a sufficient amount of Phe is liberated by enzymatic hydrolysis, and the free Phe is, then, removed by gel filtration, adsorption by activated carbon or resins (Lopez-Bajonero et al., 1991; Outinen et al., 1996; Shimamura et al., 1999).

The second derivative spectrophotometry (SDS) is an analytical technique of great utility for obtaining qualitative and quantitative data about several compounds (O'haver, 1979; Grant & Bhattacharyya, 1985; Rojas, Ojeda, & Pavon, 1998), such as aromatic amino acids, which absorb specifically between 250 and 300 nm (Ichikawa & Terada, 1977, 1981; Cahill & Padera, 1980; Miclo, Perrin, Driou, Mellet, & Linden, 1995). Concerning the complex spectra of proteins, SDS showed to be a useful tool for separating, identifying and quantifying these amino acids, as well as to reveal important differences among native and denaturated proteins (Ragone, Colonna, Balestrieri, Servillo, & Irace, 1984).

Several authors reported the great reliability of using SDS, between 250 and 270 nm, for quantifying Phe in proteins, since parameters such as pH and the addition of other elements are controlled (Brandts & Kaplan, 1973; O'haver, 1979; Matsushima, Inoue, & Shibata, 1975; Ichikawa & Terada, 1979; Cahill & Padera, 1980; Grant & Bhattacharyya, 1985; Rojas et al., 1998).

Concerning the study of protein hydrolysates, SDS was used by our group for determining the hydrolytic degree of casein hydrolysates, as well as for evaluating the modifications in the protein chain, around aromatic residues, which normally take place when the native structure is broken up. Moreover, we found that the peak intensity, in the second derivative spectra of proteins or peptides is related to the degree of exposure of aromatic amino acids, which becomes higher as the group nears the C- or N-terminal position (Silvestre, Dauphin, & Hamon 1993).

In our group's more recent work, a study of second derivative spectra of aromatic amino acids was carried out in two pH values (7.0 and 13.0), and SDS was used for estimating the degree of Phe exposure in casein hydrolysates prepared by using papain (Barbosa et al., 2002).

The aim of the present work was to test several conditions using activated carbon for removing Phe from protein hydrolysates, in order to prepare dietary supplements for phenylketonurics. The protein source used here was skimed milk powder. SDS was used as a screening method for choosing the best condition for removing Phe by activated carbon, and also for estimating the efficiency of this treatment.

2. Material and methods

2.1. Material

L-pheylalanine, L-tyrosine, L-tryptophan and a protease from *Aspergillus oryzae* (XXIII type) were purchased from Sigma Chemical Co. (St. Louis, MO, EUA). Papain was kindly furnished by BIOBRÁS (Montes Claros, MG, Brazil). The skim milk powder (SMP) was purchased in a supermarket of Belo Horizonte, MG, Brazil.

2.2. Methods

2.2.1. Preparation of skim milk powder (SMP) hydrolysates

Six hydrolysates were prepared from solutions of SMP (0.35 g/100 ml) in 0.01 mol/l phosphate buffer (pH 6.0). Initially, they were pre-heated in a water-bath, at 80 °C for 10 min. Then, the temperature was adjusted to 50 °C, and the enzymes, a protease of *Aspergillus Oryzae* (AO) isolated or in association with papain (PA), were added in such a concentration to attain the desired enzime:substrate ratio (Table 1). The hydrolytic reactions were stopped by lowering the temperature to 10 °C in an ice bath and the hydrolysates were, then, freeze-dried. The other parameters of hydrolysis are listed in Table 1.

2.2.2. Characterization of phenylalanine by SDS

Stock solutions of Phe (6.05×10^{-4} mol/l), Tyr (5.52×10^{-4} mol/l) and Trp (4.90×10^{-4} mol/l) were prepared in a 0.01 mol/l phosphate buffer, pH 6.0. Then, 10 ml of each solution were mixed and successive dilutions of this mixture were made to have Phe concentrations in a range from 0.13 to 1.01×10^{-4} mol/l. Spectra of these diluted solutions were recorded from 250 to 280 nm (CECIL spectrophotometer, CE2041 model, Buck Scientific, England). A software GRAMS-UV (Galactic Industries Corporation,

Table 1
Hydrolytic conditions employed for preparing skim milk powder hydrolysates

Hydrolysates	Hydrolysis time (h)	E:S (g/100 g)	
		AO	PA
H1	AO (5 h)	1	—
H2	AO (1 h) + PA (4 h)	1	2
H3	AO (1 h) + PA (4 h)	10	20
H4	AO (2 h) + PA (8 h)	1	2
H5	AO (5 h) + PA (20 h)	1	2
H6	AO (15 h) + PA (10 h)	1	2

E:S = enzyme:subsgtrate ratio; AO = protease from *Aspergillus oryzae*; PA = papain.