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Functional properties and growth promotion of bifidobacteria and lactic acid bacteria strains by protein hydrolysates using a statistical mixture design

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ABSTRACT

Soy protein isolate (SPI), bovine whey protein (BWP) and egg white protein (EWP) were hydrolyzed with the protease Flavourzyme 500L®, and the effects of the media supplemented with these different proteins and their mixtures on the growth performance of bifidobacteria and lactic acid bacteria strains and functional properties were studied using a simplex centroid mixture design. Synergistic effects between the formulations containing binary or ternary mixtures were observed for several parameters. For functional properties, the enzymatic hydrolysis increased protein solubility except for EWP and its mixtures. However the hydrolysates exhibited a tendency to decrease their foaming capacities and heat stability. A synergistic effect was found for the emulsion activity index of two binary formulations and the ternary mixture, with increases of up to 155.2%. The hydrolyzed samples positively stimulated the bacteria growth. Compared with control, the cell growth of the mix culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii*, *Lactobacillus acidophilus* and *Bifidobacterium lactis* were increased with the supplementation of the media with mixtures of BWP (1/2) plus EWP (1/2) and SPI (1/2) plus BWP (1/2) at 25.0 mg mL^{-1} in 100.0, 29.4 and 86.2%, respectively.

1. Introduction

Processes involving protein hydrolysis have been studied for bioactive peptide production. Bioactive peptides can be defined as specific amino acid sequences that promote beneficial biological activities. Bioactive peptides can be produced by enzymatic hydrolysis using digestive, microbial and plants proteases. The limited and controlled proteolysis unfolds the protein chains, can reduce the incidence of allergenic factors and also increase the formation of small peptides with biological activities (Korhonen, 2009).

In the last decade, the enzymatic hydrolysis of proteins from animal and plant sources for the production of bioactive peptides has attracted much attention. Among the biological activities, the growth stimulation of probiotic bacteria has been reported. A characteristic of bifidobacteria and lactic acid bacteria strains is their fastidious requirements for growth and biological activities, particularly amino acids

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(Rajagopal & Sandine, 1990). The pool of free amino acids and peptides in milk is not enough to guarantee optimum bacterial growth in this substrate (Zhang et al., 2011). Many ingredients have been evaluated to stimulate the growth and activity of probiotic and lactic acid bacteria species, for example the protein hydrolysates (Prasanna, Grandison, & Charalampopoulos, 2012). As a result, much interest has been focused in utilizing different protein sources as additives, such as whey protein concentrate, whey protein isolate and casein hydrolysate, studying mainly the effect of these compounds on the growth promotion of probiotic and lactic acid bacteria species (McComas & Gilliland, 2003; Zhang et al., 2011; Prasanna et al., 2012).

In addition to their biological activities, functional properties of proteins are also important for food product formulations. The importance of these properties varies with the type of food products in which the protein is used. Since most native proteins do not show functional properties desirable for food industries, enzymatic hydrolysis has been used to obtain hydrolysates with interesting functional properties, such as high solubility, resulting from an increase in the concentration of free amino and carboxyl groups. Hydrolysis also disrupts the protein tertiary structure and reduces the molecular weight of the protein and, consequently, alters the functional properties of proteins (Liu, Kong, Xiong, & Xi, 2010).

In the literature, different protein sources have been used for enzymatic hydrolysis, such as rice, egg white protein and whey protein (Zhao et al., 2012; Naik, Mann, Bajaj, Sangwan, & Sharma, 2013; Hoppe, Jung, Patnaik, & Zeece, 2013). However, these reports show studies on enzymatic hydrolysis using distinct substrates; no investigations were found using formulations containing mixtures of different protein sources as well as their interaction effects.

Statistical methods have been applied for improving the performance, to find the optimum process variables and formulations in different engineering problems (Rao & Baral, 2011). Statistical mixture designs are an interesting class of experimental designs where the components or factors distributed in different proportions are used to verify the interactions between the components of a mixture and maximizing the responses studied using mixture design approach.

The general purpose of mixture design is to make possible estimates, through a contour plots analysis of evaluated responses of a multicomponent system from a limited number of experiments (Anarjan & Tan, 2013). In this experimental design, the total amount of material is held constant because the response depends only on the proportions of the components present, but not on the total amount of the mixture (Rao & Baral, 2011; Anarjan & Tan, 2013). In the simplex centroid design, 2^{k-1} observations are taken, where k is the pure components, (k/2) is the binary mixtures with equal proportions and (k/3) is the ternary mixtures with equal proportions (Scheffe, 1963).

In this work, a simplex centroid mixture design was used for production of hydrolysates of different protein sources by enzymatic hydrolysis. The effects on the functional properties of the proteins and on the performance of bifidobacteria and lactic acid bacteria strains grown in the media supplemented with different protein sources and their mixtures were studied.

2. Materials and methods

2.1. Reagents

Flavourzyme® 500L, trichloroacetic acid (TCA) and MRS culture broth were purchased from Sigma-Aldrich (Steinheim, Germany). All other chemicals were purchased in the grade commercially available.

2.2. Preparation of protein hydrolysates

The soy protein isolate (SPI), bovine whey protein (BWP) and egg white protein (EWP) used as the substrates for enzymatic hydrolysis were kindly provided by Bunge Foods S/A (Gaspar, Brazil), Alibra Ingredients Ltd. (Campinas, Brazil) and Cooperovos (Mogi das Cruzes, Brazil), respectively. The commercial protease obtained from *Aspergillus oryzae* (Flavourzyme® 500L) was used for enzymatic hydrolysis. The enzyme concentrations were adjusted to 0 (control) and 50 U per mL of reaction, according to the protease activity, as previously determined (Charney & Tomarelli, 1947). The proteins were suspended in a buffer to a final concentration of 100 mg mL^{-1}. Fifty-milliliter aliquots of the mixtures were distributed in 125 mL Erlenmeyer flasks and the hydrolysis was carried out under the optimum temperature and pH of the enzyme (50.0 °C, pH 5.0) for 120 min. After hydrolysis, the samples were incubated in a water bath at 100 °C for 20 min for protease inactivation. The mixtures were centrifuged at 17,000 × *g* at 5 °C for 20 min and the supernatants containing peptides were collected and freeze-dried for the determination of the TCA-soluble proteins, functional properties and in the growth promotion of the bacteria cultures.

2.3. Statistical mixture design

The mixture design of experiment was used to obtain the optimum mixture composition of the different protein sources for maximum growth promotion of the bacteria cultures and to investigate the presence of either synergistic or antagonistic effect in a blend of components. A three component augmented simplex-centroid design was employed in which each components was studied in four levels, namely 0 (0%), 1/3 (33%), 1/2 (50%) and 1 (100%) (Table 1). Quadratic or special cubic regression models were fitted for variations of all studied responses as function of significant ($p<0.05$) interaction effects between the proportions, with acceptable determination coefficients ($R^2>0.70$). Eq. (1) represents these models:

$$Y_i = \sum \beta_i X_j + \sum \beta_{ij} X_i X_j + \sum \beta_{ijk} X_i X_j X_k \quad (1)$$

where Y_i is the predicted response, β_i is the regression coefficients for each linear effect terms, β_{ij} and β_{ijk} are binary and ternary interaction effect terms and X_i, X_j and X_k are the coded independent variables. The Statistica® 10.0 software from Statsoft Inc. (Tulsa, Oklahoma, USA) was employed for experimental design, data analysis, and model building.