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Enzyme assisted degradation of potential soy protein allergens with special emphasis on the technofunctionality and the avoidance of a bitter taste formation

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ABSTRACT

Soy protein hydrolysates are often rejected by consumers due to their unpleasant bitter taste. In the present study, soy protein isolate (SPI) was hydrolyzed using five proteases and combinations thereof to prevent bitterness. Hydrolysis was performed as one- and two-step process and the extent of hydrolysis was evaluated by SDS-PAGE and degree of hydrolysis analyses. Enzymatic hydrolysis performed as both, one- and two-step process, increased the degree of hydrolysis from initially 2.1% up to 30%. Most peptides generated were < 20 kDa and major soybean allergens (glycinin, β-conglycinin) were effectively degraded as shown by SDS-PAGE and liquid chromatography mass spectrometry (LC-MS/MS) analyses. The bitterness of SPI hydrolysates was evaluated by a sensory panel on a 10-cm continuous scale, ranging from 0 (no perception) to 10 (strong perception). Bitterness of the resulting hydrolysates was remarkably reduced to a minimum of 1.3 compared to unhydrolyzed SPI with a bitterness intensity of 2.8. Hydrolysates showed increased protein solubility at both pH 4.0 and 7.0, emulsifying capacity up to 810 mL g^{-1} and foaming activity up to 2706%. The study shows that enzyme combinations are an effective approach to produce hypoallergenic soy hydrolysates that combine low bitterness and superior techno-functional properties.

1. Introduction

Soybean (*Glycine max* (L.) Merr.) is the most important valuable crop that provides the largest source of vegetable protein to human diet. Particularly, soy protein isolates are often favoured as functional and nutritional ingredients due to the high protein content (ca. 90%).

However, consumption of soy-containing food products can cause severe and even fatal allergic reactions such as anaphylactic shock. The Food and Agriculture Organization of the United Nations (FAO) listed soybean as one of the eight priority allergens ("big 8") which comprises those foods that cause 90% of all Immunoglobulin E (IgE)-mediated allergies (FDA, 2004). At least 16 IgE-binding soy proteins have been characterized, but only the two storage proteins Gly m5 (β-conglycinin) and Gly m6 (glycinin) have been suggested to be the major fractions containing allergens (Amnuaycheewa & de Mejia, 2010; FARRP, 2015; Holzhauser et al., 2009).

In recent years, the need to control soybean allergy by methods other than avoidance has spurred the development of new technologies, including genetic modifications as well as thermal and non-thermal treatments (Shriver & Yang, 2011; Verhoeckx et al., 2015). An effective approach to reduce the allergenicity of soybean is the enzymatic hydrolysis, which has already been proven in numerous studies using selective proteases from animal, plant or microbial origin (Wilson, Blaschek, & de Mejia, 2005; Yamanishi et al., 1996). Generally, enzymatic hydrolysis is widely applied to upgrade the functional features, including solubility, foaming and emulsifying properties (Ortiz & Wagner, 2002; Tsumura et al., 2005).

However, protein hydrolysates are accompanied by the formation of bitter peptides, which impedes their utilization as food ingredients. The bitterness of hydrolysates is due to the release of low molecular weight peptides composed of mainly hydrophobic amino acid residues (Ishibashi et al., 1988; Ney, 1979; Seo, Lee, &

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Baek, 2008). Thus, the prevention, reduction, or elimination of bitter taste is essential to make the hydrolysates acceptable to consumers.

Effective debittering methods, however, have not yet been successfully developed. Numerous options have been investigated in the debittering of food protein hydrolysates including selective separation or adsorption of bitter peptides such as treatment with activated carbon, extraction with alcohol, isoelectric precipitation, and chromatography (Saha & Hayashi, 2001). The bitterness in hydrolysates was also masked via the addition of various agents such as polyphosphates, specific amino acids and through the admixture with intact proteins (FitzGerald & O'Cuinn, 2006; Sujith & Hymavathi, 2010; Sun, 2011). Debittering via transpeptidation of specific amino acids by T-plastein reaction in addition to cross-linking using microbial transglutaminase as well as the modification of taste signaling have also been conducted (Sun, 2011).

Much effort has been made in peptidase-mediated debittering of protein hydrolysates that means an enzymatic hydrolysis of bitter peptides with, particularly exo-peptidases such as amino-peptidases, alkaline/neutral proteases and carboxypeptidases. A reduction in bitterness has been observed in proteinase hydrolysis of food proteins during concomitant or subsequent incubation with exopeptidase-rich enzyme preparations which cleave adjacent to hydrophobic amino acid residues (Chae, In, & Kim, 1998; FitzGerald & O'Cuinn, 2006).

Although many studies have been applied in either the reduction of the allergenicity or the debittering of hydrolysates, literature on an effective enzyme combination for sufficiently reducing both allergenicity and bitterness, while improving the techno-functional properties, is not available.

The present study was conducted to investigate (1) the effectiveness of different enzyme combinations on the degradation of the major soybean allergens (glycinin, β-conglycinin), (2) their effects on the *bitter* taste, and (3) the techno-functional properties of the resulting hydrolysates. Protein degradation was evaluated by determining the degree of hydrolysis as well as electrophoresis (SDS-PAGE) and mass spectrometry (LC-MS/MS) analyses. A specific emphasis has been given to the debittering effect of enzyme combinations. The effects on the techno-functional properties (protein solubility, emulsifying capacity, foaming activity, density, and stability) were also studied.

2. Materials and methods

2.1. Raw materials and chemicals

Soybeans (*G. max* (L.) Merr.) were purchased from Naturkost Ernst Weber (Munich, Germany). Alcalase® 2.4 L FG (2.4 AU-A/g, endoprotease from *Bacillus licheniformis*), Flavourzyme® 1000 L (1000 LAPU/g, endo- and exoprotease from *Aspergillus oryzae*), and Neutrase® 0.8 L (0.8 AU-N/g, endoprotease from *Bacillus amyloliquefaciens*) were kindly provided by Novozymes A/S (Bagsvaerd, Denmark). Papain (cysteine-protease from *papaya latex*) (≥10 units/mg, E.C. 3.4.22.2, Sigma no P4762) was purchased from Sigma–Aldrich Inc. (St. Louis, U.S.A) and Corolase® 7089 (850 UHb/g, endopeptidase from *Bacillus subtilis*) was kindly provided by AB Enzymes GmbH (Darmstadt, Germany).

All chemicals used were of analytical grade and obtained from Th. Geyer GmbH & Co. KG (Renningen, Germany) if not stated separately.

2.2. Preparation of soy protein isolates (SPI)

Soy protein isolate (SPI) was prepared from soybean seeds (*G. max.* (L.) Merr.) as previously described by Meinlschmidt, Sussmann, Schweiggert-Weisz, and Eisner (2016).

2.3. Enzymatic hydrolysis of SPI

For the experiments, five different commercially available food-grade enzymes were used. These protease preparations are commonly used in the food industry for the production of protein hydrolysates. Reaction conditions (50 °C and pH 8.0) were chosen according to producers' application sheet. The dosage of 0.5%, 0.05% and 1% for endo- and exoproteases has been applied according to unpublished preliminary experiments.

Enzymatic hydrolysis was performed with various enzyme combinations (Table 1) in thermostatically controlled reactors. Therefore, SPI was dispersed in deionized water at a protein concentration of 5% (w/w) and adjusted to 50 °C and pH 8.0 with 1 M NaOH prior to enzyme addition. A total volume of 1000 mL was used for the proteolysis reaction. Hydrolysis was performed either as one-step or two-step process and 10 enzyme combinations were carried out using various endo- and exoproteases (Table 1). The enzyme concentrations used were 500 LAP-U/g for Flavourzyme, 0.6 AU-A/g for Alcalase, 212.5 UHb/g for Corolase 7089, 0.2 AU-N/g for Neutrase and ≥250 units/g for Papain, which correspond to an enzyme to substrate ratio (E/S) of 1% (v/w), 0.5% (v/w), 0.5% (v/w), 0.5% (v/w) and 0.05% (w/w) of the total protein, respectively.

For the one-step process, enzymes were simultaneously added to the vessel and SPI was digested for 4 h, while aliquots were taken after 30, 120, and 240 min. During the two-step process, an endo-protease was applied in the first hydrolysis stage for pre-digestion (see Table 1). After 1 h incubation, Flavourzyme (1% v/w) was added for subsequent debittering by additional hydrolysis for another 2 h and aliquots were taken after 30, 120, and 240 min. During hydrolysis, the mixture was stirred, while the pH-value and temperature were maintained constantly.

Hydrolysis was stopped by heat treatment at 90 °C for 20 min. Control dispersions (no enzyme addition) were prepared under the same incubation conditions and heat inactivation treatment. All samples were frozen at −50 °C and lyophilized (BETA 1–8, Martin Christ Gefriertrocknungsanlagen GmbH, Germany). Experiments were performed in duplicate.

2.4. Determination of protein degradation due to enzymatic hydrolysis

2.4.1. Degree of hydrolysis (DH) using the o-phthaldialdehyde (OPA) method

The DH of all samples was calculated in triplicate (n = 3) by determining the free α-amino groups with *o*-phthaldialdehyde (OPA) using serine as standard according to Nielsen, Petersen, and Dambmann (2001).

2.4.2. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The molecular weight distribution of the samples was determined according to Laemmli (1970) using SDS-PAGE under reducing conditions. The sample preparation and gel running conditions were chosen as described elsewhere (Meinlschmidt et al., 2016). Protein visualization was performed by Criterion Stain-Free Gel Doc™ EZ Imager (Bio-Rad).

2.5. Protein identification by liquid chromatography tandem mass spectrometry (LC-MS/MS)

2.5.1. Sample preparation for LC-MS/MS analysis

Protein separation was performed using SDS polyacrylamide gel electrophoresis (SDS-PAGE). The soy protein isolate was mixed with