Food Chemistry 205 (2016) 229–238

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Immunoreactivity, sensory and physicochemical properties of fermented soy protein isolate

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ARTICLE INFO

Article history:
Received 18 November 2015
Received in revised form 16 February 2016
Accepted 7 March 2016
Available online 8 March 2016

Keywords:
Soybean allergy
Fermentation
Sandwich ELISA and western blot
Principal component analysis (PCA)
Physicochemical properties

ABSTRACT

The effect of induced liquid state fermentation (*Bacillus subtilis*, *Rhizopus oryzae*, *Saccharomyces cerevisiae*, *Lactobacillus helveticus*) on the immunoreactivity, physicochemical and sensory properties of soy protein isolate (SPI) was studied. *L. helveticus* revealed the most abundant reduction in terms of immunoreactivity within soluble protein fractions, up to 100%, which could be measured by *in vitro* sandwich ELISA using mouse monoclonal anti-Glym5 antibodies (mAbs). Almost no binding was found in western blot analysis using mouse monoclonal mAbs and sera from soy sensitive individuals. Fermentation increased water- and oil-binding capacity as well as protein solubility at pH 4.0. Foaming activity was nearly doubled compared to non-fermented SPI. A decreased emulsifying capacity, foaming density, and quantity of soluble proteins at pH 7.0 were observed. Principal component analysis (PCA) confirmed decreased *bitter* and *beany* off-flavors of fermented samples compared to non-fermented SPI. Consequently, fermentation might be a promising method to produce tasty low-allergen food ingredients with good physicochemical properties.

1. Introduction

Food allergy is an abnormal immune response to certain food components. The prevalence of food allergies is rising dramatically, and approximately 220 to 250 million people worldwide suffer from some kind of food allergy (WAO, 2013). Food allergens are typically naturally occurring proteins, with small regions, called epitopes, responsible for the immunoglobulin E (IgE)-mediated allergic response (Taylor & Hefle, 2001).

Soybean (*Glycine max* (L.) Merr.) is an important vegetable protein source for the food industry due to its considerable amount of high quality proteins and nutritional value. However, soybean is among the so-called "big 8" food allergens, which together account for over 90% of all documented food allergies in the U.S. (FDA., 2004; Taylor & Hefle, 2001). The prevalence of soy allergy is not precisely known and it is expected to escalate due to the increasing consumption of soybean products. Currently, eight allergenic proteins (Glym1–Glym8) with molecular masses ranging from 7.5 to 97 kDa have been registered by the International Union of Immunological Societies (IUIS) Allergen Nomenclature Sub-Committee (www.allergen.org). However, the two storage proteins β-conglycinin (Glym5) and glycinin (Glym6) have been related to severe reactions in European allergic populations (Amnuaycheewa & de Mejia, 2010; Holzhauser et al., 2009; Verhoeckx et al., 2015). The estimated allergy threshold is low and small amounts of soy protein, ranging from 0.0013 to 500 mg, may be enough to trigger an immune response (FDA, 2004).

So far, the only way to prevent unintended exposure to a food allergen is complete avoidance of the offending food. Recently, the need to control soy allergy by processing treatments rather than avoidance has pushed the development of new technologies, including genetic modification, thermal and non-thermal treatments (Verhoeckx et al., 2015). Currently, enzymatic hydrolysis using proteases of microbial or plant origin is the most efficient process for disrupting sequential and conformational epitopes, thereby reducing the allergenicity (Wilson, Blaschek, & de Mejia, 2005; Meinlschmidt, Schweiggert-Weisz, Brode, & Eisner, 2016; Meinlschmidt, Sussmann, Schweiggert-Weisz, & Eisner, 2016).

Fermentation is a traditional processing technology in the food industry. Besides the functions of improving the bioavailability of nutrients and reducing pathogenic bacteria in the gastrointestinal tract, a regulation of immunogenicity and possibly reduction of allergenicity by fermentation has been hypothesized (Granato, Branco, Cruz, Faria, & Shah, 2010), but has rarely been investigated. Few studies (Frias, Song, Martinez-Villaluenga, Gonzalez de Mejia, &

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http://dx.doi.org/10.1016/j.foodchem.2016.03.016
0308-8146/

Vidal-Valverde, 2008; Kobayashi, 2005; Song, Frias, Martinez-Villaluenga, Vidal-Valdeverde, & de Mejia, 2008; Song, Pérez, Pettigrew, Martinez-Villaluenga, & de Mejia, 2010; Yamanishi, Huang, Tsuji, Bando, & Ogawa, 1995) have confirmed the degradation of soybean allergens, particularly Glym Bd 30 K, also known as P34, during fermentation by microbial proteolytic enzymes in fermented soybean foods such as soy sauce, miso, tempeh, soybean ingredients and feed-grade soybean meals. It has also been shown that various lactic acid bacteria (LAB) are able to hydrolyze α′- and α-subunits of soybean β-conglycinin (Aguirre, Garro, & de Gioria, 2008). The studies of Frias et al. (2008) and Song et al. (2008) demonstrated that both solid- and liquid state fermentation of cracked soybean seeds, flour, or meals by various mould strains and bacteria, effectively reduced IgE-immunoreactivity by 65–99% as evaluated by an indirect ELISA with human sera. However, these research groups did not consider the effect of fermentation on the sensory and physicochemical properties of the respective soy product as fermentation can often lead to off-flavors or a loss of technofunctionality (Quinn & Beuchat, 1975; Shrestha, Dahal, & Ndungutse, 2013). As sensory and physicochemical properties are equally important parameters for the food industry, the simultaneous investigations are of considerable importance, but not examined to our knowledge up to now. Consequently, the evaluation of residual immunoreactivity of these two proteins is indispensable for the assessment of potential allergenicity of the processed food. However, reports are not available in the literature so far.

The objective of this research was to evaluate the degradation of the major soybean allergens Glym5 and Glym6 (SDS–PAGE) and the reduction of Glym5 immunoreactivity (sandwich ELISA and western blot) by induced liquid state fermentation of SPI using *Bacillus* (*B.*) *subtilis*, *Rhizopus* (*R.*) *oryzae*, *Saccharomyces* (*S.*) *cerevisiae*, and *Lactobacillus* (*L.*) *helveticus*. The sensory (smell, taste, and mouthfeeling) and physicochemical properties (protein solubility, emulsifying, foaming, oil- and water-binding capacity) have been analyzed as well.

2. Material and methods

2.1. Raw materials and chemicals

Untoasted soybeans (*G. max* (L.) Merr.) were purchased from Naturkost Ernst Weber (Munich, Germany). *L. helveticus* DSM 20075, *B. subtilis* DSM 10, and *R. oryzae* DSM 2200 were purchased from the Germany Resource Center (DSMZ, Braunschweig, Germany), while *S. cerevisiae* TMW 3.210 was obtained from the collection of the chair 'Lehrstuhl für Technische Mikrobiologie' in Weihenstephan (TMW, Freising, Germany).

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

2.2. Preparation of soy protein isolate (SPI)

SPI was prepared from untoasted soybean seeds using the technique as previously described in Meinlschmidt, Sussmann, et al. (2016).

2.3. Fermentation of SPI

2.3.1. Strains, media, growth conditions, and preparation of inocula

All strains were cultivated overnight under their respective optimal growth conditions (see Table 1).

For solid media, 15 g L^{-1} agar was added to the respective broth. After cell enumeration, a calculated aliquot of the preculture was centrifuged for 10 min at 9000 × *g*, the pelleted cells were washed twice with Ringer solution (Merck KGaA, Darmstadt, Germany) and were resuspended in 1 ml sterile distilled water, which was used as inocula.

2.3.2. Experimental design of induced liquid state SPI fermentation

Liquid state fermentations were performed in SPI that was suspended in sterile deionized water (5% w/w, pH 6.7). Prior to inoculation, the SPI dispersion was pasteurized at 85 °C for 10 min in order to reduce the endogenous microbiota of the raw materials. After cooling down, the dispersions were supplemented with 2% (w/v) glucose (Sigma–Aldrich Inc., St. Louis, USA) to favor the growth of the inoculated strains. Two independent replicates of the SPI dispersion were inoculated with strains from precultures to obtain a concentration of either 1×10^8 colony forming units (CFU) per ml of bacteria/yeast or 10% (v/v) mould.

Fermentations were carried out under strain-specific growth conditions (Table 1) for 24 and 48 h in conical flasks without agitation. Fermentation was stopped by heat treatment at 90 °C for 20 min to terminate residual proteolytic activity. All samples were neutralized (pH 7.0) with 1 M NaOH, kept at −50 °C and lyophilized (BETA 1–8, Martin Christ Gefriertrocknungsanlagen GmbH, Germany). The pH value of the samples was measured initially and after fermentation using a disinfected pH electrode (WTW, Weilheim, Germany) calibrated at pH 7.0 and 4.0. All fermentations were performed in duplicate.

2.4. Microbiological analysis

2.4.1. Determination of the total viable counts by measuring the colony forming units (CFU)

Directly after inoculation and at time intervals of 24 h and 48 h, the fermentation process was monitored by determining the total viable counts. Aliquots (100 µl) of fermented SPI were in triplicate serially diluted 1:10 (v/v) with Ringer solution (Merck, Germany) and 100 µl of certain dilutions was spread homogenously with a Drigalski spatula in duplicate on the surface of pre-dried plate-count agar plates (Merck KGaA, Darmstadt, Germany). In addition, fermented samples were plated on strain specific agar to quantify and favor the growth of the respective strain. Plate-count agar plates were incubated aerobically at 30 °C for 48 ± 2 h, while all other plates were incubated under strain specific growth conditions (see Table 1). The colonies were counted and expressed as colony forming units per milliliter (CFU ml^{-1}).

2.4.2. Matrix assisted laser desorption ionization-time of flight-mass spectrometry (MALDI-TOF-MS)

MALDI-TOF-MS was performed to verify that the inoculated strains were dominant in the samples during fermentation. Fresh

Table 1
List of employed strains including abbreviations, and growth/culture conditions.

Strain	No.	Abbreviation	Type	Growth/culture conditions
Bacillus subtilis	DSM 10	*B. subtilis*	Bacteria	30 °C, aerob, nutrient
Lactobacillus helveticus	DSM 20075	*L. helveticus*	Lactic acid bacteria	37 °C, anaerob, MRS
Rhizopus oryzae	DSM 2200	*R. oryzae*	Mold	30 °C, aerob, potato dextrose
Saccharomyces cerevisiae	TMW 3.210	*S. cerevisiae*	Yeast	30 °C, anaerob, potato dextrose

DSM = Deutsche Sammlung von Mikroorganismen; TMW = Technische Universität Weihenstephan; MRS = DeMan, Rogosa and Sharpe