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The effects of pulsed ultraviolet light, cold atmospheric pressure plasma, and gamma-irradiation on the immunoreactivity of soy protein isolate

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ABSTRACT

This study investigates the effect of nonthermal processing technologies on soy immunoreactivity. Soy protein isolate was treated with pulsed ultraviolet (PUV) light, direct and remote cold atmospheric pressure plasma (CAPP), and gamma-irradiation (3–100 kGy). Sample weight, surface temperature, hydrogen peroxide content, and pH value have been measured. SDS-PAGE analysis revealed reduced protein intensity bands corresponding to major soy allergens β-conglycinin (Gly m5) and glycinin (Gly m6). Sandwich ELISA using specific mouse monoclonal anti-Gly m5 antibodies (mAbs) confirmed a loss of soy immunoreactivity following PUV light, direct CAPP, and gamma-irradiation with increasing dose and time. The maximum reduction in immunoreactivity (91–100%) in the soluble protein fraction was achieved by direct CAPP as well as PUV light and gamma-irradiation treatment. A decreased immunoreactivity up to 89% was observed for samples treated with remote CAPP. These innovative technologies might have great potential for industrial application due to their effectiveness in reduction of soy immunoreactivity.

1. Introduction

The ubiquitous presence of allergens in human food supply coupled with an increased awareness of food allergies has intensified increasing effort for developing allergy mitigation methods. Food allergy results from an adverse immunoglobulin E (IgE)-mediated reaction of the immune system towards dietary antigens, commonly proteins. The antigenic determinant of allergenic proteins is called epitope, which can be classified into linear and conformational epitopes (Taylor & Hefle, 2001). Although nearly any food is capable of causing an allergic reaction, it was found that nearly 90% of all allergic reactions in the U.S. are triggered by eight main protein sources, which compromise milk, eggs, fish, crustacean/shellfish, tree nuts, peanuts, wheat, and soy. These foods are called the "big 8", which were defined as "major food allergens" by the Food Allergen Labelling and consumer Protection Act of 2004 (FALCPA). Allergy to soy is one of the most common food allergies, especially among infants. Up to now, eight allergenic soy proteins (Gly m1–Gly m8) have been registered by the International Union of Immunological (IUIS) Societies Allergen Nomenclature Sub-Committee (www.allergen.org), but only the two storage proteins β-conglycinin (Gly m5) and glycinin (Gly m6) have been identified as related to severe allergic reactions (Holzhauser et al., 2009). Although the prevalence of soy allergy is not precisely known, it is expected to escalate due to the increasing consumption of soy-containing food products (FAO, 1995).

Total avoidance of soy-containing foods and prompt treatment to allergic shocks with medicine i.e. epinephrine are still likely to be the only way to avoid severe outcome. Consequently, a great demand for methods, which reduce food allergens without affecting the nutritional value, is becoming a popular topic of various research activities.

Science and industry are searching for thermal and nonthermal technologies to control soy allergy by modifying epitopes (Shriver & Yang, 2011). The technological approach hitherto has mainly been focused on thermal technologies, which commonly retain the ability of soy to elicit an immune response. More recently, nonthermal food processing technologies have emerged in the food industry due to their negligible effects on food properties (Huang, Hsu, Yang, & Wang, 2014; Shriver & Yang, 2011). Different studies have shown that nonthermal technologies such as high pressure processing (HPP) hold a great promise for the development of food ingredients with reduced allergenicity (Tammineedi, Choudhary, Perez-Alvarado, & Watson, 2013; Yang et al., 2010).

Besides HPP, high-energy, short-wavelength electromagnetic gamma(γ)-irradiation turned out to be an effective preservation method, extending the shelf-life of perishable foods (Kasera et al., 2012). Although the utilization of gamma irradiation is limited to a few food applications depending on the individual national legislation, the ability of irradiation to reduce the allergenicity of quite different

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allergenic food products, including almond, cashew nut, walnut proteins, ovalbumin, bovine serum albumin, milk proteins (beta-lactoglobulin) as well as shrimp tropomyosin and legume proteins (kidney bean, peanut, black gram) has been reported (Byun, Lee, Yook, Jo, & Kim, 2002; Kasera et al., 2012; Seo et al., 2007; Su, Venkatachalam, Teuber, Roux, & Sathe, 2004). It has been assumed that irradiation structurally alters IgE-binding epitopes by generating primary free radicals, reacting with proteins, which results in protein fragmentation, polymerization (dimerization), and aggregation (Kuan, Bhat, Patras, & Karim, 2013). In contrast, Moriyama et al. (2013) found that gamma-irradiation of soybeans applying a dose rate between 2.5 and 30 kGy resulted in apparent band profiles of major soy allergens Gly m5, Gly m Bd 30 K, Gly mTI, and Gly m4, while protein band intensities were not significantly changed by irradiation. ELISA analyses using allergen-specific antibodies (Gly m5, Gly m Bd 30 K, Gly mTI, and Gly m4) suggested no significant changes in the allergen contents, except for a decrease in Gly mTI. Chemical changes of proteins that are caused by gamma-irradiation are commonly fragmentation (depolymerization), inter-protein cross-linking (aggregation), including the formation of disulfide bonds, hydrophobic interactions that could lead to protein aggregation, and oxidation by oxygen radicals that are generated in the radiolysis of water (Davies & Delsignore, 1987; Lee & Song, 2002). Irradiation emits electrons and generates radicals from the breakdown of the cobalt-60 isotope. Proteins can be converted into higher molecular weight aggregates due to the generation of inter-protein cross-linking reactions, hydrophobic and electrostatic interactions as well as formation of disulfide bonds due to the water-radiolysis (Davies & Delsignore, 1987). Gamma-irradiation as a physical mean of decontamination by photon-induced changes at the molecular level. Ionizing radiation with low dose rates up to 1 kGy is usually applied to control food-borne pathogens as well as to reduce the microbial load and insect infestation, thereby extending the shelf-life of perishable products for commercial purposes (FDA, 1997). As side effect, this technology may change antigenicity of food proteins by the destruction or modification of epitopes. According to Vaz et al. (2013) the types of modification that food proteins might undergo during irradiation, including protein unfolding and aggregation, could be not observed at a low-dose range. Therefore, the potential of gamma-irradiation to affect soy Gly m5 immunoreactivity after a low dose compared to a high dose of radiation was investigated in this study.

More recently, the use of pulsed ultraviolet (PUV) light treatment, a high-peak power technology, has attracted considerable attention as an alternative food preservation method that consists of intense flashes of broad-spectrum of light containing wavelength from near-infrared to ultraviolet (Koutchma, Forney, & Moraru, 2009). Previous studies have shown that PUV has the ability to reduce the level of allergenicity in peanut products (Chung, Yang, & Krishnamurthy, 2008; Yang, Mwakatage, Goodrich-Schneider, Krishnamurthy, & Rababah, 2012) as well as soybean (Yang et al., 2010), shrimp (Shriver, 2011), almond (Li et al., 2013), and wheat extracts (Nooji, 2011). The efficiency to reduce immunoreactivity has been attributed to photothermal, photochemical, and photophysical reactions, contributing to a change in protein structure and reduction in IgE-binding ability (Krishnamurthy, Demirci, Krishnamurthy, Irudayarj, & Yang, 2009; Yang et al., 2010). PUV is commonly regarded as nonthermal if the time of exposure is limited to some seconds where the temperature rise is insignificant (Krishnamurthy et al., 2009). However, recent studies confirmed that prolonged exposure with PUV light can induce significant photothermal effects by the infrared portion of PUV light spectra (Krishnamurthy et al., 2009; Yang et al., 2010). Consequently, a considerable temperature rise, moisture loss, and simultaneously sample weight loss could occur due to increased energy absorbance (Chung et al., 2008; Li et al., 2013; Nooji, 2011; Yang et al., 2010).

Application of cold atmospheric pressure plasma (CAPP) has gained considerable attention as an alternative microbial inactivation technology due to its germicidal effects (Ehlbeck et al., 2011). Although the effect of CAPP on protein structure is not well studied so far, it has been assumed that CAPP might promote reactions in liquids by injecting reactive oxygen radicals, altering the epitope structure. Recent studies showed that CAPP might be an effective method to reduce immunoreactivity of wheat and shrimp proteins (Nooji, 2011; Shriver, 2011). However, to the best of our knowledge, reports on the effect of CAPP on the residual soy immunoreactivity are not available in the literature up to now.

Currently, little is known about how gamma-irradiation, PUV light, and CAPP treatment may alter food allergens, and hence there is a need to investigate the relationship between food protein allergenicity and the effect of food irradiation. Further, the effect of these technologies on soy Gly m5 immunoreactivity has not been investigated so far. As Gly m5 is one of the most abundant proteins in soy and sensitization against this protein is highly indicative for severe allergic reactions (Holzhauser et al., 2009), the evaluation of its residual immunoreactivity is indispensable for the assessment of potential allergenicity of modified foods. Therefore, this study aimed to investigate the effect of PUV light, gamma-irradiation as well as direct and remote CAPP on soy immunoreactivity. The degradation of major soy allergens Gly m5 and Gly m6 and residual immunoreactivity have been evaluated by SDS-PAGE analysis and sandwich ELISA using mouse monoclonal anti-Gly m5 antibodies (mAbs). In addition, sample weight, surface temperature, hydrogen peroxide content and pH value have been measured.

2. Materials and methods

2.1. Raw materials and chemicals

Untoasted soybeans (*Glycine max* (L.) Merr.) were purchased from Naturkost Ernst Weber (Munich, 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 isolates (SPI)

SPI was prepared from soybeans using the technique as previously described in Meinlschmidt, Sussmann, Schweiggert-Weisz, and Eisner (2016b). Briefly, soybeans were de-hulled, flaked, and defatted with *n*-hexane. SPI was prepared by acidic pre-extraction (pH 4.5, 1:8 w/v flakes to water ratio, 1 h) of soybean flakes. After stirring for 1 h at room temperature, the suspension was separated using a decanter (3250 ×*g*, 60 min). Subsequently, alkaline protein-extraction (pH 8.0, 1:8 w/v, 1 h) of flakes residue was performed and the suspension was separated (3250 ×*g*, 60 min). The supernatant was adjusted to pH 4.5 for protein precipitation, followed by centrifugation (5600 ×*g*, 130 min). The obtained SPI was neutralized, pasteurized (70 °C, 10 min) and spray-dried.

2.3. Nonthermal food processing technologies

2.3.1. Pulsed ultraviolet (PUV) light treatment

SPI dispersions (5 mg mL^{-1}, 10 mL each in an aluminum dish with a diameter of 7 cm) were treated in a pulsed light chamber (Claranor, Avignon, France), which was equipped with a three Xenon tubes reflector. The lamp is connected to a capacitor and emits a broad spectrum intense light flash of 200 to 1100 nm. The applied voltages ranged between 1.5 and 2.8 kV, which corresponded to a total available energy of 0.27 and 0.98 J cm^{-2} s^{-1} at a distance of 10 and 8 cm from the central axis of the pulsed UV lamp system, respectively. The total energy input was determined with a Solo2 Power and Energy Meter (Gentec, Quebec City, Canada). Three pulses per second with a width of 300 μs were produced. Treatment duration was set to 1, 2, 4 and 6 min (three replicates each). Sample weights, pH value using pH-indicator strips (pH 2.0–9.0; Merck, Darmstadt, Germany), and surface temperature using a