Innovative Food Science and Emerging Technologies 30 (2015) 79–88

Contents lists available at ScienceDirect

Innovative Food Science and Emerging Technologies

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

Effect of thermal and electric field treatment on the conformation of Ara h 6 peanut protein allergen

Sai Kranthi Vanga *, Ashutosh Singh, Vijaya Raghavan

Department of Bioresource Engineering, Faculty of Agriculture and Environmental Studies, McGill University, Sainte-Anne-de-Bellevue, QC H9X 3V9, Canada

ARTICLE INFO

Article history:
Received 13 August 2014
Received in revised form 31 December 2014
Accepted 2 March 2015
Available online 19 March 2015

Keywords:
Peanut allergen
Root mean square deviation
Ara h 6
Radius of gyration
Molecular dynamic simulation

ABSTRACT

The study uses molecular dynamic simulation to evaluate the effect of static and oscillating electric field (2450 MHz) of intensity 0.05 V/nm at different temperatures 300 K, 380 K and 425 K on structural conformation of Ara h 6 peanut protein allergen. The conformational changes in the protein were studied with respect to root mean square deviation, radius of gyration, dipole moment and solvent accessible surface area. The increase in temperature and application of external electric fields, both static and oscillating fields had significant effect on the conformation of Ara h 6, specifically the helical secondary structures. It was observed that the root mean square deviation increased with a rise in temperature and application of external electric fields had no significant effect on it at any given temperatures. This study also demonstrated that exposure to external stresses including thermal and electric fields induces conformational changes in the protein structure, which may impact its physico-chemical properties.

Industrial relevance:

- The work was performed to understand the influence of food processing on protein and the changes in their structure using molecular modeling concept.
- Molecular Dynamics Simulations have been applied to visualize the folding and unfolding of the protein structure depending on the amount of stress applied on the system.
- This work can help in modification and optimization of process parameters (like temperature and time) to enhance the protein functional properties and digestibility in the end product.
- Increasing digestibility would automatically result in higher nutritional absorption in the body, decreased immunoreactivity and overall better nutritional quality.

1. Introduction

Food allergy is defined as an immune response towards a particular protein or a group of proteins present in a food. Although, any food may cause an immune response, peanuts, tree nuts, milk, soybean, wheat, egg, fish and shellfish are responsible for most of the food allergies around the world (Scott H. Sicherer & Sampson, 2006). It has been estimated that approximately 3% of the adult population and 3–8% of the infants are allergic to one or more foods and this number is growing (Rona et al., 2007; Venter et al., 2008; Zuidmeer et al., 2008). Several researchers have suggested that genetic predisposition (Arshad, Stevens, & Hide, 1993; Sampson, 2004) could also be another major issue. The inter-exchange between various cultures confers dissipation of various forms of cuisines increasing the diversity in food available for consumption. This exposure to numerous forms of food has been indicated as one of the reasons for an increase in population that suffer from food allergies (Helm & Burks, 2000b). Moreover, hygiene hypothesis, which states that excessive cleanliness around an individual interrupts with the normal development of immune system because of lack of external environmental triggers may also lead to an increase in susceptibility to allergens related to environment and food (Guarner et al., 2006; Helm & Burks, 2000a; Yazdanbakhsh, Kremsner, & van Ree, 2002).

In North America, peanuts are one of the major sources of food allergy. Sicherer, et al. (Scott H. Sicherer, Muñoz-Furlong, Godbold, &

Abbreviations: IgE, Immunoglobulin E; SASA, Solvent Accessible Surface Area; RMSD, Root Mean Square Deviation; SR, Systemic Allergic Reaction; OAS, Oral Allergy Syndrome; NMR, Nuclear Magnetic Resonance Imaging; MD, Molecular Dynamics; FTIR, Fourier Transformation Infrared Spectroscopy; PDB, Protein Data Base; PME, Particle Mesh Ewald; NVT, Ensemble in which number of atoms, volume and temperature are constant; NPT, Ensemble in which number of atoms, pressure and temperature are constant; PPI, Peanut Protein Isolate.

* Corresponding author.
E-mail addresses: sai.vanga@mail.mcgill.ca (S.K. Vanga), ashutosh.singh@mail.mcgill.ca (A. Singh), vijaya.raghavan@mcgill.ca (V. Raghavan).

http://dx.doi.org/10.1016/j.ifset.2015.03.003
1466-8564/

Sampson, 2010) estimated that about 1% of the population in United States of America is sensitive to peanuts. They also suggested that over the years there has been an overwhelming increase from 0.6% in 1999 to 2.1% in 2008 in the younger population (<18 years) who are affected by sensitivity towards peanuts (Scott H. Sicherer et al., 2010). Within Peanuts, there are about 13 protein allergens recognized by The International Union of Immunological Societies Nomenclature subcommittee, (*Arachis hypogaea* — Ara h) Ara h 1 to Ara h 13, of which Ara h 1, 2 and 3 are major peanut allergens (Breiteneder & Radauer, 2004; Burks et al., 1992; Sampson, 2004; Schmitt, Cheng, Maleki, & Burks, 2004; Scott H. Sicherer & Sampson, 2007). These allergens of peanuts cause immunoglobulin E (IgE) - mediated immune responses. The portion of the allergen, which is recognized by IgE is called an epitope. These epitopes consist of linear contiguous stretch of amino acids or three dimensional structural motif that crosslink with IgE triggering an allergenic response in a sensitized individual (Sathe & Sharma, 2009).

The allergenicity of a specific compound can be defined as its ability to introduce allergic response and it may vary with varying environmental conditions. In food, proteins are found within a complex matrix where its interaction with other food components such as carbohydrates may alter its functionality and make it allergic (S. H. Sicherer & Sampson, 2010; Clare Mills, Sancho, Rigby, Jenkins, & Mackie, 2009). Processing of food also impacts proteins and alters their digestibility and conformation, which alters their ability to induce allergenic responses (S. H. Sicherer & Sampson, 2010). In recent years several studies have revealed that food processing can also play an alternative role in moderating the allergic response of food proteins (Clare Mills et al., 2009; Cucu, De Meulenaer, Bridts, Devreese, & Ebo, 2012; Thomas et al., 2007). In 2012, Cucu et al. (Cucu et al., 2012) studied the impact of thermal processing and glycation on the basophil activation by hazelnut proteins. They reported that thermal processing of hazelnut protein in the presence or absence of wheat protein had no significant effect on the stimulatory activity of basophil for patients with systemic allergic reaction (SR) or oral allergy syndrome (OAS). They also observed that incubation of hazelnut protein with glucose completely suppressed the stimulatory activity of basophil in OAS patients. They concluded that SR patients were more susceptible to allergic reaction for both processed and unprocessed hazelnut protein as compared to OAS patients. In another study Yang et al.(Yang, Mwakatage, Goodrich-Schneider, Krishnamurthy, & Rababah, 2012) applied pulsed ultraviolet light on raw and roasted peanuts and peanut buttery slurry to reduce peanut allergenicity. Their SDS-PAGE and ELISA analysis revealed that with increase in treatment time the allergenicity of peanut protein reduced compared to control. Hence it can be assumed that by introducing novel food processing techniques allergenicity of food proteins can be altered and reduced to a significant level.

It is well known that during processing food components undergo several chemical and physical changes; hence protein subjected to external stresses such as thermal, chemical and electrical stresses may lead to conformational changes to the molecular structure (Singh, Orsat, & Raghavan, 2013b). The functional properties of proteins depend on their structure and any change in it may alter the functionality (Singh, Munshi, & Raghavan, 2013a). In recent years several studies have been conducted to understand the impact of processing on protein conformation and its relation to the functional properties (Clare Mills et al., 2009; Davis, Smales, & James, 2001; Mondoulet et al., 2005; Singh, Orsat, & Raghavan, 2012; Singh et al., 2013a, 2013b). Several techniques including Fourier Transformation Infrared Spectroscopy (FTIR), Nuclear Magnetic Resonance Imaging (NMR), X-ray diffraction can be used to study the conformational changes in protein during processing, but these techniques are expensive and can only relate the structure of proteins before and after processing. To overcome these shortcomings Singh et al. (Singh et al., 2013b) applied molecular dynamic (MD) simulation technique to gain insight into protein dynamics under the influence of external static electric field. MD simulation technique is widely used to study the structural and dynamic properties of biomolecules and has been applied in the field of pharmacology and molecular biology for development of accurate and novel drug systems.

In this study, the conformational changes in Ara h 6 peanut protein allergen under the influence of external stresses including thermal and static/oscillating electric field have been evaluated. Ara h 6 is selected as the model protein for this study because it is an important allergen in peanuts and is approximately 59% homologues with the Ara h 2, which is regarded as a major peanut allergen. Several researchers have reported that Ara h 2 and Ara h 6 allergens are harder to digest in humans compared to the Ara h 1 or Ara h 3 (Koppelman, Hefle, Taylor, & De Jong, 2010; Koppelman et al., 2005). Moreover, recent studies have also suggested that allergic reactivity to various isoforms of Ara h 6 can be observed in the growing allergic population (Bernard et al., 2007; Flinterman et al., 2007; Peeters et al., 2007). The isoforms are the same protein but with a slight disparity in the sequence i.e., a very similar duplicate. In 2007, Bernard et al., (Bernard et al., 2007) found an isoform of Ara h 6 allergen and its derivative compound from peanut protein that were as reactive or more reactive than that of Ara h 2. All the aforementioned findings suggested that studying the behavior of Ara h 6 under the influence of external stresses might provide an insight on how various food processing techniques might affect their allergenic properties of peanut proteins.

Hence, in this study emphasis has been given to the quantification of conformational change using root mean square deviation, radius of gyration, dipole moment and its effect on surface hydrophobicity and hydrophilicity of Ara h 6.

2. Materials and methods

MD simulation on Ara h 6 was performed using a classical MD algorithm as implemented in Groningen machine for chemical simulations (GROMACS) software package, version 4.5.5 from the Stockholm Center for Biomembrane Research, Stockholm, Sweden (Hess, Kutzner, Van Der Spoel, & Lindahl, 2008). Peanut allergen Ara h 6 consists of 127 amino acid residues, where more than 40% of the secondary structure consists mainly of helices (6 helices) and one 3/10 helix. Ara h 6 starting configuration with the Protein Data Bank (PDB) (Berman et al., 2000) accession code 1W2Q (Lehmann et al., 2006) was used for this study. All atom CHARMM27 force field was used to describe the potential energy and provide functions and parameters for every type of atom in the system (Astrakas, Gousias, & Tzaphlidou, 2012). The protein configuration was enclosed in a periodic cubic water box of dimensions 10.215 X 10.215 X 10.215 (nm) containing 34838 water molecules to satisfy the minimum image convention. The water model selected for this study was TIP3P and three sodium ions were added to neutralize the system. The neutral solvated protein system was first energy minimized with converging criterion of maximum force value of 10 kJ/nm/mol using steepest descent for 20000 steps and equilibrated to constant volume (NVT) and temperature (NPT) for 200 ps.

During the MD simulation the temperature was maintained using Berendsen thermostat and pressure was set at 1 bar. The constant temperature and pressure coupling was 0.1 ps and 2 ps respectively and to limit the short-range non-bonded interactions, van der Waals interaction and long-range electrostatic interactions, a cut-off of 1 nm was used. PME algorithm was used with grid spacing of 0.16 nm and the time step during the simulation was 2 fs. A total of nine MD simulations were run to evaluate the effect of temperature 300 K (27 °C), 380 K (107 °C) and 425 K (151 °C) static electric field of intensity 0.05 V/nm and oscillating electric field of intensity 0.05 V/nm and frequency of 2450 MHz. All the electric fields were applied at the *x* axis of the equilibrated solvated protein system.