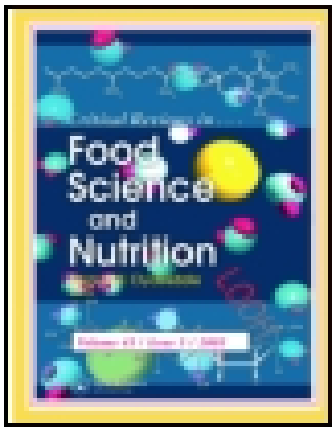

Critical Reviews in Food Science and Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bfsn20

The Interactions between Plant Proteins/enzymes and Other Food Components and Their Effects on Food Quality

Chenyan Lv[a], Guanghua Zhao[ab] & Yong Ning[b]

[a] CAU & ACC Joint-Laboratory of Space Food, College of Food Science and Nutritional Engineering, China Agricultural University, and Key Laboratory of Functional Dairy, Ministry of Education, Beijing 100083, China

[b] School of Laboratory Medicine, Hubei University of Chinese Medicine, NO. 1 Huangjia Lake West Road, Wuhan 430065, China

Accepted author version posted online: 20 Jul 2015.

The interactions between plant proteins/enzymes and other food components and their effects on food quality

Chenyan Lv[1], Guanghua Zhao[1, 2,*], and Yong Ning[2,*]

[1]CAU & ACC Joint-Laboratory of Space Food, College of Food Science and Nutritional Engineering, China Agricultural University, and Key Laboratory of Functional Dairy, Ministry of Education, Beijing 100083, China;

[2]School of Laboratory Medicine, Hubei University of Chinese Medicine, NO. 1 Huangjia Lake West Road, Wuhan 430065, China

*Corresponding author: Guanghua Zhao, Tel: +86-10-62738737, Fax: +86-10-62738737, E-mail: gzhao@cau.edu.cn (G. Z.);

Yong Ning, +86-27-68890259. Fax: +86-27-68890071, E-mail: ningyong128@163.com (Y. N.).

Abstract

Plant proteins are the main sources of dietary protein for human, especially for vegetarians. There are a variety of components with different properties co-existing in foodstuffs, so the

interactions between these components are inevitable to occur, thereby affecting food quality. Among these interactions, the interplay between plant protein/enzyme from fruits and vegetables, cereals and legumes and other molecules plays an important role in food quality, and recently has gained a particular scientific interest. Such interactions not only affect the appearances of fruits and vegetables and the functionality of cereal products, but also affect the nutritive properties of plant foods. Non-covalent forces such as hydrogen bond, hydrophobic interaction, electrostatic interaction, and van der Waals are mainly responsible for these interactions. Future outlooks are highlighted with the aim to suggest a research line to follow for further studies.

Keywords:

Interactions, Food quality, Plant protein, Binding, Enzymes

INTRODUCTION

Proteins in various foodstuffs are the main source of essential amino acids, which are used by cells to build new proteins for different purposes. Human beings use proteins for growth and to build hormones, antibodies and the enzymes that regulate the chemical reactions within the body. Proteins are widely distributed in various foods, such as seafood, meats, dairy, eggs, fruits, vegetables, whole grains, legumes and nuts. These proteins are major components in foods, which are mainly derived from plant and animal sources.

On the other hand, food is a complex system where many other components such as polyphenols (Li et al., 2012; Deng et al., 2009), polysaccharides (Wang et al., 2002), lipids and metal ions (Deng et al., 2009) co-exist with proteins/enzymes and thus their interactions inevitably occur. As we know, food quality encompasses not only sensory properties (appearance, texture, taste and aroma), but also nutritive values, chemical constituents, mechanical properties, and functional properties. The above mentioned interactions between proteins and other food components may affect both the sensory and the nutritive property of foodstuffs. Of these interactions, some are undesirable. For example, the polyphenol oxidases can form dark spots in fruits and vegetables by enzymatic browning (Robinson, 1991). In contrast, there are a few examples in which browning is considered as a desirable process, as it increases the product quality, such as palm dates, prunes, raisins and black tea (Tomás-Barberán and Espin, 2001). Therefore, how to control such interactions appears to be crucial for food quality during food

processing. Prior to controlling the interactions, it is important to understand the mechanism by which proteins interact with other molecules in foods.

Additionally, many proteins have been extensively used in food industry. The interactions between added proteins and small molecules also occur. Soybean proteins are included in a wide variety of formulated foods and developed as a main source of protein for vegetarians nowadays. Interactions between soybean protein and lipids can improve the emulsification property by forming protein-lipid complex. Similarly, ferritin with high bioavailability is being developed as an alternative iron supplement now (Zhao, 2010; Liao, et al., 2014). And polyphenols in plant food has been identified to interact with ferritin through non-covalent bonds, which can improve the digestive stability of ferritin (Li et al., 2012; Wang et al., 2014). This review focuses on a recent progress in the interactions between plant protein and other components in food, and their effects on food quality. In addition, attention is also given to the possible impacts of food processing on these interactions.

PROTEINS IN CEREALS

Cereal grains are grown in greater quantities and provide more food energy worldwide than any other type of crop. So far, cereals have become an important part of the diet of many people. They include maize, sorghum, millets, wheat, rice, barley, oats, teff and quinoa. Proteins from cereals such as gluten protein are usually developed as food additives, due to their important

function in food emulsification or texturing. Therefore, the interactions between cereal proteins and other food components may affect sensory perception and consumer acceptance of cereal food products.

Gluten Protein

Gluten properties are largely responsible for the end-use quality of wheat in many food products. It has been proved that gluten has the main contribution to wheat dough properties with a predominant role determining both dough machinability and textural characteristics of food product. Gluten proteins are composed of two main subfractions. One is glutenin that confers strength and elasticity of dough, and another is gliadin that impairs the viscous properties on the gluten dough (Khatkar et al., 1995). In order to obtain food products with high quality, numerous additives are employed in bakery by reinforcing the role of the gluten or acting as emulsifiers. These components may affect gluten properties by interacting with gluten proteins.

The major non-starch polysaccharides of wheat flour are pentosans. Pentosans originate from the endosperm, cell wall and bran of wheat grain (Wang et al., 2002). The abilities to immobilize water and to form viscous solutions or gels by covalent cross-linking are important attributes that also can have direct functional implications in gluten formation and properties. Water extractable pentosans (WEP) interfere with gluten formation in both direct and indirect ways. On the one hand, WEP can compete with gluten for water during the first stage of dough formation, resulting in a

delay in the development time of gluten (Labat et al., 2002). This corresponds to the indirect effect of WEP on gluten formation. On the other hand, WEP are able to directly cross-link with gluten, consequently affecting the extensibility of dough and gluten (Wang et al., 2002). Additionally, the ferulic acid in WEP is proposed to involve the interaction of pentosans with gluten (Wang et al., 2004). Similarly, hydroxypropylmethylcellulose (HPMC) has been shown to interact with gluten protein as well. The presence of HPMC did not modify the viscoelastic behavior of gluten dough during cooling at 25 °C. However, the presence of HPMC increased the solubility of gluten proteins in sodium dodecyl sulphate, this is possibly because HPMC interferes with the protein association and its further aggregation during heating by occupying the space of the proteins in the gluten network (Rosell and Foegeding, 2007).

The effects of hydrocolloids on the functional properties of wheat bread have been investigated recently. κ-Carrageenan, sodium alginate, xanthan gum, pectin and some cellulose derivatives can affect the dough rheology, bread volume, crumb texture and the shelf life during storage to different extents, by forming hydrophilic complexes with gluten proteins (Mandala and Sotirakoglou, 2005; Qiu et al., 2015). In detail, pectin and λ-carrageenan strengthened wheat dough and sodium alginate augmented the extensibility of dough. The formed weak complexes are mainly due to either attraction between local dipoles of carbohydrate residues of the polysaccharide and charged groups of the protein or the formation of unstable Shiff bases between the aldehyde groups of the polysaccharide and ε-amino groups of the proteins (Rosell and

Foegeding, 2007). Likewise, emulsifiers widely used in bakery product such as sodium stearoyl lactylate (SSL) can induce protein folding including an increase in α-helix conformation and a decrease in β-sheet, turns and random coil (Figure 1). The conformation change may be resulted from the low burial of tryptophan residues to a more hydrophobic environment and the low percentage area of the C–H stretching band for GS0.25 (Gluten + 0.25% SSL) (Gómez et al., 2013).

Additionally, bread making starts by adding water (and other ingredients) to flour and applying kinetic energy (by mixing), thereby forming extensible dough that contains a developed gluten network. During mixing, dough entraps air. Wheat flour free lipids become bound or trapped within the gluten fraction and can align at the interface of the gas cells (Pareyt et al., 2011). Research on wheat flour lipids demonstrated that lipids played a significant role during dough mixing, fermentation and proofing, baking and bread storage (Chin et al., 2010). Meanwhile, bread loaf volume is sensitive to the composition, extractability and overall content of wheat flour lipids. In consequence, the food quality, especially the bakery products, can be significantly changed by other components in wheat flour, and such changes can help improve the properties of gluten protein, especially emulsification property, during food processing.

Sorghum Protein

Sorghum (*Sorghum bicolor* L. Moench) is an important food cereal in many parts of Africa,

Asia and the semi-arid tropics worldwide, which acts as a principal source of protein, vitamins and minerals for millions of the poorest people living in these regions. Porridges appear to be the most common types of food prepared from sorghum by cooking with boiling water. Protein fractionation studies have shown that prolamine and glutelin are the principal protein fractions (Duodu et al., 2003). However, the nutritive value of sorghum grain is relatively low due to its resistance to protease digestion. The factors affecting wet cooked sorghum protein digestibility may be categorized into two main groups: endogenous factors (disulphide and non-disulphide crosslinking, kafirin hydrophobicity and changes in protein secondary structure) and exogenous factors (grain organizational structure, polyphenols, phytic acid, starch and non-starch polysaccharides) (Duodu et al., 2003). Previous studies showed that cooking sorghum in the presence of β-mercaptoethanol increased protein digestibility by reducing the covalent crosslinking (disulfide bond) with other amino acids in the same or another protein molecule (Hamaker et al., 1987).

One important characteristic of sorghum is an abundance of tannins. It has been established that proteins generally interact with tannins by means of hydrogen bonding, hydrophobic interaction, electrostatic attraction and covalent bonding associated with oxidation (Butler et al., 1984). Such interaction may lead to precipitation because of the large size of the tannins, rendering most of the proteins insoluble. As expected, more than tannins (2%-4%) in sorghum were found to strongly bind with sorghum proteins which have a loose, open structure, and be rich in proline

(Butler et al., 1984). However, the anti-nutritional effects of sorghum tannin may be alleviated by treating the grain with dilute aqueous ammonia, strong alkalies and formaldehyde or by dehulling (McGrath et al., 1982). Moreover, the production of tannin-free sorghum by genetically modification can also improve the nutritional quality of sorghum products.

Similarly, phytic acid with high concentration naturally occurs in the germ of sorghum. Phytic acid is highly charged with six phosphate groups, and also forms insoluble complexes with proteins (Ryden and Selvendran, 1993) by interactions. This leads to reduced protein digestibility, which was attributed to the possible formation of a phytate–protein complex and the protein is less susceptible to enzymatic attack (Duodu et al., 2003). However, there was no significant correlation between the percentage improvements in protein digestibility and dietary total phytic acid concentration. Many questions remain unanswered regarding the effect of phytate on sorghum proteins in particular.

Sunflower Protein

Sunflower (*Helianthus annuus* L.) is one of the larger sources of vegetable oil and protein of good nutritional quality. Sunflower flours and protein concentrates have potential food uses because of their high protein content, white color, bland flavor, and absence of anti-nutritive factors (Robertson and Morrison, 1977). Sunflower seeds also contain significant quantities of phenolic compounds which remain in the flour after oil extraction. Phenolic compounds naturally

present in sunflower seeds, mainly are chlorogenic and caffeic acids. To improve the quality of sunflower protein meal, attempts have been made to remove these polyphenolic substances to obtain colorless sunflower isolates from sunflower meal, for instance, acidic butanol extraction (Prasad, 1988). The acidic butanol extraction can remove 90% of the phenolics from sunflower meal, resulting in a great reduction in the color and flavor changing of sunflower meal. While it is possible to reduce the content of these compounds in protein products by modifying the extraction procedure (Salgado et al., 2011), it is impossible to eliminate them completely due to their strong interaction with proteins (Salgado et al., 2011). Under neutral and alkaline conditions, sunflower proteins develop dark green and brown colors because of bonding with oxidation products of polyphenolic compounds, especially chlorogenic acid (Prasad, 1988). Nevertheless, their final color tone was more dependent on the conditions used in the preparation process than on the amount of phenolic compounds in the product. It has been reported that the hydrogen bond between hydroxyl groups of phenolic compounds and the peptide bond in proteins is unusually strong. In aqueous solutions, such strong interaction by the hydrogen bond greatly favors the formation of complexes between phenols and proteins. On the other hand, the interaction between sunflower protein and phenolic compounds conferred the antioxidant properties on the sunflower protein films. Consequently, these specific protein films in packaging are of potential usefulness for preserving oxidation-sensitive products (Salgado et al., 2012).

PROTEINS IN LEGUMES

Legumes are widely recognized as important sources of food and feed proteins. In many regions of the world, legume seeds are the unique supply of protein in the diet. Proteins are a major component of legume seeds. Their nutritional and functional properties dramatically affect the overall quality of the seeds and their technological performances. Soybean is the most important member in a family of legumes in the world. In developed countries, proteins from soybean seeds can now be regarded as versatile functional ingredients or biologically active components more than as essential nutrients. Moreover, other components in soybean seeds such as flavones, polyphenols and other food additives may interact with soybean proteins, affecting their functional properties.

Soybean Protein Isolate

Soybean protein isolates (SPI) due to their desirable functional properties, high nutritional value and associated health effects, have been employed in a wide variety of formulated foods and developed as a main source of protein for vegetarians, especially for Asian populations. The major protein components of soybean are glycinin and β-conglycinin, which are used as emulsifiers due to the surface active properties of their constitutive proteins in bakery goods, chocolate, instant products (milk powder), margarines and mayonnaise (Rydhag and Wilton, 1981).

However, other molecules such as lipids and isoflavones coexist with soybean protein in

soybean products, thereby affecting the properties of soybean protein by interactions during food processing. It has been reported that interactions between lecithin and soy protein can enhance the emulsification activity of soybean protein by forming the protein-lipid complex (Beckwith, 1984), which may be attributed to that the components such as proteins and phospholipids possess charges or have the capability to be ionized in food emulsions. Meanwhile, pH has been identified as an important factor in emulsifying activity of soy protein and lecithin (Figure 2). And different behaviors displayed by soy protein isolates are due to different protein structure and pH values. In other words, the presence of lecithin can enhance the initial characteristics of emulsions and diminish the creaming rate in both systems (Comas et al., 2006).

Isoflavones have been postulated as responsible for at least part of the beneficial health effects of soybean consumption. The affinity between soybean proteins and isoflavones depended on their diverse polarity and hydrophobicity as well as their abilities to form hydrogen bonds, which may affect the emulsification activity of soybean protein. Furthermore, it has been reported that enthalpic interactions (such as hydrogen bonding) between genistin and the proteins would appear to come into play at pH 3.5 or 4.5 or 5.6, with the resulting affinities being weaker with β-conglycinin than with glycinin. Meanwhile, malonylgenistin would likewise undergo an enthalpic interaction with proteins at pH 4.5 and 5.6, whereas at pH 3.5 hydrophobic bonds are favored (Speroni et al., 2010). These results help us to select the optimal conditions during food processing to get food products with good taste and appearance. Nevertheless, the effect of

isoflavones on the structures and properties of soybean protein remains to be further determined.

Ferritin

Ferritin is abundant in legume seeds. Ferritin as an iron storage protein has been extensively studied recently (Zhao, 2010; Harrison and Arosio, 1996). From the standpoint of nutrition, biofortification of staple food with iron caged within phytoferritin from legumes is believed to be an effective strategy to combat with iron deficiency anemia which affects ~2 billion people in the world. However, there are many other components co-existing with ferritin in foodstuffs, and thus their interactions could occur, resulting in a change in the property of ferritin. It has been identified that the reductants in foodstuff such as anthocyanins, phenolic acids and ascorbic acids can induce iron release from ferritin cavity (Deng et al., 2009), without influencing the primary/secondary structure of ferritin. The iron release rate partially depends on the structures and chelating activities of reductants. For example, the order of iron release from soybean seed ferritin (SSF) is as follows: delphinidin > cyaniding > petunidin > malvidin > delphinidin-3-*O*-glucoside > petunidin-3-*O*-glucoside. More interestingly, the pigments can inhibit ferritin degradation during iron release to different extents (Deng et al., 2009). Moreover, it has been reported that tannic acid and epigallocatechin gallate (EGCG) can induce ferritin association (Li et al., 2012; Wang et al., 2014) (Figure 3). The hydrogen bond and hydrophobic interaction may be two main factors responsible for the interaction between them. It was also found that ferritin association induced by

these small molecules can further improve the digestive stability of ferritin in *vitro*, but the evidence *in vivo* has been lacking.

Another molecule which can induce iron release from ferritin is a reduced form of nicotinamide-adenine dinucleotide (NADH). This compound is also widely distributed in foodstuffs. However, the mechanism of iron release from ferritin induced by NADH is different from those molecules listed above. NADH cannot contact the iron core directly due to the larger size of NADH (1.5 nm) than the size of ferritin channels (Masuda et al., 2010). Instead, NADH molecules bind on the surface of ferritin shell close to the 4-fold channel of pea seed ferritin (PSF), which is 1.58 nm from the tryptophan residues calculated by fluorescence resonance energy transfer (Lv et al., 2013) (Figure 4). The interaction between them has been ascribed to van der Waals interactions or hydrogen bonds, as suggested by isothermal titration calorimetry (ITC) measurement (Chaikuad et al., 2005). Furthermore, since plastid DNA coexists with ferritin in the amyloplast of legume seeds, their interactions have been also investigated recently. Results demonstrated that the presence of DNA can enhance the rate of protein association during iron uptake by ferritin (Yang et al., 2014). On the other hand, soybean seed ferritin exhibited a marked DNA-protective function against oxidative damage at a low loading of Fe^{2+} (≤ 48 Fe^{2+}/shell) (Liao et al., 2012) (Figure 4). Thus, the interactions between ferritin and other molecules significantly affect the stability and iron content of ferritin.

Importantly, the existence of dietary factors such as phytic acid, polyphenols and calcium

may affect the ferritin iron absorption by human. At cell levels, it has been suggested that tannic acid increased Fe uptake from intact ferritin, possibly by interfering with ferritin or ferritin mineral core assembly due to its amphoteric properties and releasing Fe for absorption. However, other dietary factors like phytic acid, ascorbic acid and calcium have no effect on the ferritin iron absorption (Kalgaonkar and Lönnerdal, 2008). Therefore, the interaction between dietary factors and ferritin could improve the stability and iron bioavailability of ferritin, especially for the tannins, but research *in vivo* needs to be elucidated.

PROTEINS/ENZYMES IN FRUITS AND VEGETABLES

One prominent feature for fruits and vegetables is that proteins occurring in them are mainly composed of enzymes. Although these enzymes are usually much lower in content as compared to carbohydrate, they play a key role in fruits and vegetables quality including appearance, aroma, flavor, hand-feel, mouth-feel and chewing sounds. Consumers integrate all of those sensory into a final judgment of the acceptability of fruit or vegetable.

Fruits and vegetables usually have a very short post-harvest life, due to their relatively high metabolic activity and high sensitivity to fungal attack. Furthermore, during handling, storage, and marketing, they are highly susceptible to physical damage leading to disruption of its cellular structure and consequently a speedup of softening and browning phenomena (Chisari et al., 2007), and some of such damages are induced by interactions between enzymes widely distributed in

fruits and vegetables with small molecules (oxygen, metal ions and pigments etc.). Phenolic compounds play an important role in food visual appearance. Anthocyanin pigments are responsible for most of the blue, purple, red and intermediate hues of plant-derived foods. Therefore, the interaction between phenolic compounds and plant polyphenol oxidases is considered to have significant impact on plant food quality. Elucidating such interactions seems to be very important, and fortunately great progress has been made in this area as bellow.

Polyphenol Oxidases

Browning of damaged tissues of fresh fruits and vegetables mainly occurs from the oxidation of phenolic compounds and contributes significantly to a quality loss. A large body of work reports the characterization of oxidative enzymes from various fruits and vegetables, such as apples, grapes, pears, eggplants, and strawberries (Carbonaro and Mattera, 2001). The primary enzymes responsible for the browning reaction are polyphenol oxidases (PPOs). The PPOs usually have a dinuclear copper center which catalyzes to insert oxygen in a position *ortho*- to an existing hydroxyl group in an aromatic ring (Virador et al., 2010) (Figure 5). The structure of the active site of the enzyme is highly conserved, in which copper is bound by six or seven histidine residues and a single cysteine residue. (Mayer, 2006), and the presence of the seventh histidine unit binding Cu contributes to the high enzyme activity (Hernandez-Romero et al., 2006).

In plants, the PPOs are predominantly located in the chloroplast thylakoid membranes, and

are thereby physically separated from its natural substrate phenolic compounds, which occur in the vacuoles. However, upon any cell-damaging treatment, the enzyme and substrates come into contact, leading to rapid oxidation of phenols (Chazarra et al., 2001). Chlorogenic acid, caffeic acid, epicatechin, and catechin are all polyphenols commonly found in fruits and vegetables, which can act as substrates for PPOs and be oxidized to quinones by oxygen in the presence of PPOs. In turn, such quinones are very reactive and can react with each other and other cellular components to generate a black or dark-brown pigment called melanin. This causes dark spots to form in the plant tissue, frequently leading to a decrease in the quality of fruits or vegetables, especially for fresh-cut ones. It has been proved that pH is crucial for the oxidation of polyphenols by PPO, not only due to the optimal condition for PPO activity but also to the ionization state of enzymes (Kazandjian and Klibanov, 1985). In addition, many inhibitors of PPO have been described, which belong to very diverse chemical structures. For example, the inhibition of glucose and fructose showed that the increasing concentrations of sugar caused a progressive inactivation of both enzymes, and such inhibition was much more evident in strawberry polyphenol oxidase than in others (Chisari et al., 2007). Salicylic acid as another inhibitor has been proved to competitively inhibits the activity of PPO by forming hydrogen bond with amino acids in PPO (Zhou et al., 2015). Differently, the development of browning is desirable for improvement of the product quality of plant foods (Tomás-Barberán and Espin, 2001). For example, a black color due to enzymatic browning is considered a criterion of quality in certain dried products such

as black tea, coffee, and prune skins.

Peroxidases

Peroxidase (POD) occurring in almost all vegetables is another oxido-reductase enzyme which plays a crucial role in enzymatic browning as well since diphenols may function as reducing substrates in its reaction (Robinson, 1991). Peroxidases usually consist of a family of isozymes which contain identical heme groups but differ in the precise composition of the glycoprotein. Peroxidases normally increase in activity and number during ripening and peroxidase can combine with hydrogen peroxide and produce an activated complex that can react with a wide range of donor molecules (Reed, 1975), causing undesirable changes in food materials including off-flavor, aroma, and color. The involvements of POD in browning are reported by different research groups. So far, a number of peroxidases from different fruits and vegetables have been identified using SDS-PAGE followed by specific activity staining recently (Préstamo and Manzano, 1993). Similarly, recent studies have showed that cantaloupe melon POD activity appears to be consistent with that of ascorbate peroxidase (Lamikanra and Watson, 2000) and that POD activity in minimally processed cantaloupe melons could be the result of a preservative response to increased oxidative stress in the cut fruit (Lamikanra and Watson, 2001).

To further understand the interactions between peroxidases and substrates, the crystal structure of horseradish peroxidase isozyme has been solved and the key residues (Phe residues,

142, 68 and 179) involved in direct interactions with aromatic donor molecules have been identified (Gajhede et al., 1997). In addition, ascorbic acid and other natural antioxidants have been shown to inhibit the peroxidase activities. All antioxidants used are able to terminate the oxidation by preventing the formation of free radicals (Hemeda and Klein, 1990; Lamikanra and Watson, 2001). It has been reported that temperature preconditioning treatment can suppress the increase in peroxidase activity in squash during subsequent storage at 5 °C (Wang, 1995).

Importantly, peroxidase is considered as the most heat-stable enzyme in plants, and there is an empirical relationship between residual peroxidase activity and the development of off-flavors and off-odors in foods. Thermal inactivation kinetic studies in POD (in the range of 70 to 100 ºC) exhibited biphasic curves, providing evidence for the presence of isoenzymes with different thermal stabilities (Morales-Blancas et al., 2002). Therefore, inadequate thermal processing can cause reactivation of peroxidase and a loss of food quality. The detailed mechanism behind the thermal inactivation remains unclear by this time. In addition, a pH of 2.4 at 25 °C with low chloride concentrations causes total detachment of the heme. Once the heme-protein interaction is disturbed, there is a loss of protein stability. It was concluded that lipid oxidative activity of peroxidase aggregates was due to either increased heme exposure with a change in temperature or pH or an increased number of active sites induced by heme migration (Burnette, 1977). A better understanding of interactions between peroxidase and substrates should enable the production of improved human food products with improved flavor and overall quality, resulting in longer

storage periods.

Phenylalanine Ammonia-lyase

Phenylalanme ammoma-lyase (PAL, EC 4.3.1.5) was firstly discovered in barley seedlings by Koukol and Conn (Koukol and Conn, 1961), and it has become the most studied enzyme concerned with secondary metabolism in plants. PAL activity in plants increases in response to several kinds of stress including wounding (Ke and Saltveit, 1989), exposure to ethylene, low temperature and fungal infection. PAL as a key enzyme in phenolic synthesis catalyzes the first reaction in the biosynthesis of plant phenylpropanoid products (Figure 6). The synthesized phenolic compounds can further be oxidized by polyphenol oxidase (PPO) producing brown polymers that contribute to tissue browning (Ke and Saltveit, 1989). In detail, PAL catalyzes the non-oxidative deamination of *L*-phenylalanine to form *trans*-cinnamic acid and a free ammonium ion which can induce the biosynthesis of a large range of phenylpropanoid-derived secondary products, such as flavonoids and isoflavonoids, coumarins, lignins, wound-protective hydroxycinnamic acid esters and other phenolic compounds (Jones, 1984). High activity of PAL is associated with accumulation of anthocyanins and other phenolic compounds in fruit tissue of several species.

One of the major causes for quality loss in minimally processed lettuce (*Lactuca sativa L.*) is the browning of the cut pieces induced by PAL activity (López-Gálvez et al., 1996). In addition, it

has been reported that strawberry fruits have a developmental-dependent expression of PAL activity and accumulation of phenolic substances derived from the phenylpropanoid pathway (Cheng and Breen, 1991). Importantly, chitosan and chitin treatments can lead to increase of the PAL activity, which has been demonstrated to be one of the earliest responses of plants to the onset of infection by pathogens and are often considered as indicators of resistance (Khan et al., 2003). Chilling damage has also been shown to induce an increase in PAL activity (Lafuente et al., 2003). Accordingly, many distinctive developmental features of flesh fruits, such as a loss of astringency and appearance of characteristic color at ripening, are related to PAL activity and changes in the synthesis and accumulation of phenolic compounds.

Pectinases

Food texture is a major determinant of consumer acceptance and preference for fruits and vegetables (Van Buggenhout et al., 2009). Pectic substances account for about one-third of the dry substance of the primary cell walls of fruits and vegetables. Previous studies on fruits and vegetables suggested that the interactions between pectic substances and the pectinases coexisting in fruits and vegetables might affect their quality (Kashyap et al., 2001). Pectinase is a complex macromolecule and plays an important role in the mechanical properties of plant tissue and in (pre-) processed plant-based foods. They catalyze numerous pectin conversion reactions strongly degrading the pectic substances, thereby impacting on the quality of fruits, vegetables, and the

related intermediate and end products (Duvetter et al., 2009).

Generally, pectinesterases known as pectinmethyl hydrolase are involved in changes in the pectic substances of fruits and vegetables during ripening, storing and processing by catalyzing deesterification of the methoxyl group of pectin. Meanwhile, depolymerizing enzymes can catalyze hydrolysis or cleaving of α-1, 4-glycosidic linkages between galacturonic monomers in pectic substances. In addition, protopectinases, protopectin-solubilizing enzymes, which liberate water-soluble and highly polymerized pectin from protopectin, have been reported to react with the polygalacturonic acid region of protopectin and with the polysaccharide chains that may connect the polygalacturonic acid chain and cell wall constituents (Alkorta et al., 1998).

In food industry, pectinases are extensively used in increasing the yield of fruit and vegetable juices, controlling cloud stability in juices, enzymatic peeling of fruits, controlling the rheological properties of purees and pastes, engineering the texture of fruits and vegetables, manufacture of wine, extraction of pigments and food colorings, and so on. Commercial pectic enzymes are used in apple juice manufacturing to depectinize pressed juices in order to remove turbidity and prevent cloud-forming. Like other enzymes in fruits and vegetables, the pH and temperature may affect pectinase activity significantly (Ceci and Lozano, 1998). Therefore, controlling of interactions between pectinases and pectic substances during food storage and processing is of prime importance since desirable or deleterious reactions can be tailored (accelerated or inhibited) meeting specific quality targets.

EFFECTS OF FOOD PROCESSING ON THE INTERACTIONS BETWEEN PROTEINS/ENZYMES AND OTHER FOOD COMPONENTS

Food processing has been known to affect content, activity and bioavailability of food components, and it also plays an important role in the interactions between plant protein and other food components. The traditional food processing methods include heat treatment, fermentation and germination. Heat treatment has been usually used for inactivation of enzymes in plant food such as fruits and vegetables (Morales-Blancas et al., 2002). Phytic acid of soy meal (SM) could influence protein and important mineral digestion of monogastric animals. Recent studies have demonstrated that two-stage temperature protocol achieves better phytic acid degradation during *A. oryzae* solid state fermentation. As a consequence, the fermented SM has lower antinutritional factors (phytic acid and oligosaccharides) and higher nutritional value for animal feed (Chen et al., 2014). As an interesting alternative to traditional food processing and preservation methods, high-pressure processing has a potential for food preservation because it can inactivate microorganisms and enzymes responsible for shortening the life of a product. In addition to lengthening the shelf-life of food products, high hydrostatic pressure can modify functional properties of components such as proteins, which in turn can lead to the development of new products (Hendrickx et al., 1998). Moreover, ultrasound has attracted considerable interest in food science and technology due to its promising effects in food processing and preservation. As one of

the advanced food technologies it can be applied to develop gentle but targeted processes to improve the quality and safety of processed foods and offers the potential for improving existing processes as well as for developing new process options (Knorr et al., 2004).

High hydrostatic pressure (HHP) has been showed to improve protein solubility and dispersion stability of mineral-added soybean protein isolate. In detail, HHP-denatured soybean proteins may coexist with different minerals at different pH values in the form of soluble species (Manassero et al., 2015). Thermally- and HHP- denatured calcium-added soybean proteins exhibited very different solubility values. HHP may lead to dissociation of calcium from binding sites of soybean proteins, whereas thermal treatment cannot. Similarly, high intensity ultrasonic (HUS) pre-treatment can affect the properties of soybean protein. It has been shown that the surface hydrophobicity and free sulfhydryl (SH) content of soybean protein isolate can increase with HUS-treatment time (Hu et al., 2013), which will further affect the interaction forces between proteins and small molecules.

As we all know, enzymes are responsible for the quality of fruits and vegetables. In order to improve the quality of fruits and vegetables, enzymes have been inactivated by many methods to preventing the interactions between enzymes and their substrates. As for thermal technologies, in addition to traditional heating, there are several methods such as ohmic heating, which can raise the temperature in a very short time to a critical level (Jaeger et al., 2010). Although traditional heat treatments can ensure safety and extend the shelf life of juices, undesirable brown color

development as a result of the Maillard reaction between amino and carbonyl compounds. In contrast, high pressure (HP) processing (at low and moderate temperatures) has a limited effect on pigments (chlorophyll, carotenoids, and anthocyanins) responsible for the color and flavor of fruits and vegetables (Oey et al., 2008). However, due to cell disruption, HP processing facilitates the occurrence of enzymatic and non-enzymatic reactions related to the texture of fruits and vegetables. This is because substrates, ions and enzymes which are located in different compartments in the cells can be liberated and interact with each other during HP treatment (Oey et al., 2008). Moreover, pressure can enhance the action of pectinmethylesterase (PME), and lower the polygalacturonase (PG) activity (occurring mostly at moderate temperature). Pectinases from different sources (Van den Broeck, et al., 2000; Ly Nguyen et al., 2002) exhibits differences in their pressure and temperature stability. Consequently, different pressure and temperature combinations can be used to activate or inactivate some specific pectinases during processing to create textures, which cannot be formed by thermal processing.

In order to avoid detrimental changes in sensory and nutritive properties, pulsed electric field (PEF) pasteurization of fruit juices is a promising preservation method (Jaeger et al., 2010). It has been reported that the PEF processed juices had a lower 5-hydroxymethylfurfural concentration than those treated by heat, a fact that can be attributed to the reduced thermal load to which the product is exposed during PEF preservation. Thus, it seems that a combination of the non-thermal and thermal technologies could improve the food quality better during food processing.

CONCLUSIONS AND PERSPECTIVES

The interactions between proteins and other molecules in different food systems have been extensively investigated recently. The crucial enzymes (PPO, POD or PAL) in fruits and vegetables responsible for enzymatic browning should be inactivated or activated according to the need of customer. The interactions between plant proteins and other molecules such as polysaccharides, lipids and metal ions occur constantly during food processing. These studies mainly focus on the interactions *in vitro*, and their effects on protein structures and functions. Non-covalent forces such as hydrogen bond, hydrophobic interaction, electrostatic interaction, and van der Waals are responsible for these interactions. Meanwhile, the pH value of an aqueous solution is also crucial to the interactions due to the electrical charge of all the molecules involved. All of these interactions can further affect the appearance, nutrition and texture of food. What's more, food processing technologies can significantly affect the interactions between plant protein and other molecules. Therefore, during food processing, it is of upmost importance to select the optimal conditions to control these interactions for different applications. It is likely that other unidentified conditions related to the interaction co-exist, which also make important contribution to this process.

However, so far, there have been some questions which remain to be answered. Firstly, better evaluation systems should be established to assess the effect of interactions on food quality.

Secondly, the mechanism of interactions between plant protein and other molecules needs more detailed information to better control such interactions. Thirdly, most of interactions studied so far focus mainly on bi-molecule system. However, the interactions among a real food system are much more complicated than those in the bi-molecule system. Finally, the bioavailability of the entire foodstuff, which is a major concern during food intake, has not yet been elucidated.

ACKNOWLEDGEMENTS

The work was supported by the National Natural Science Foundation of China (31471693).

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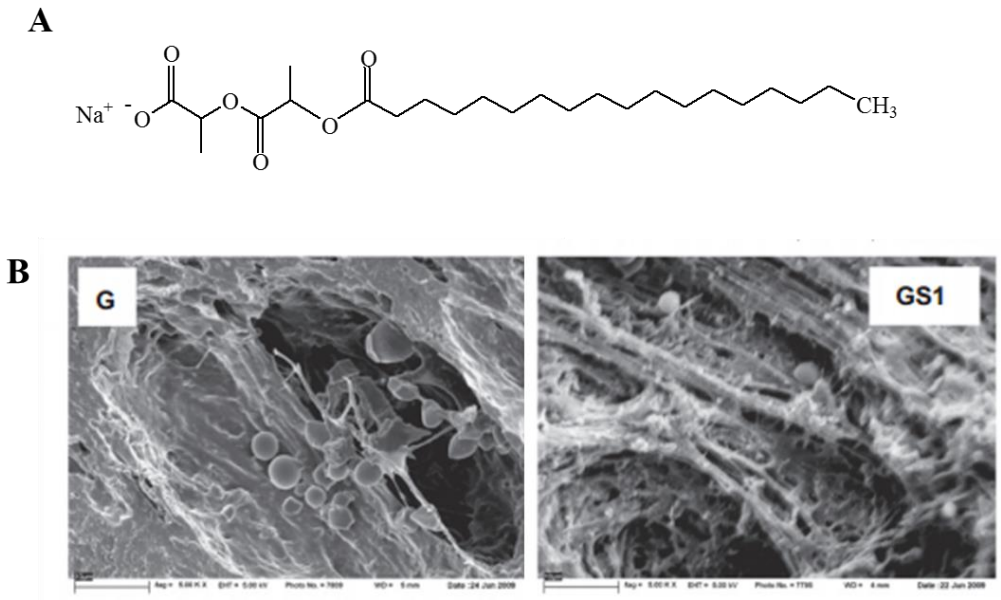

Figure 1 (A) Structure of sodium stearoyl lactylate (SSL); (B) Scanning electron microscopy of gluten prepared with emulsifier (1.0%). Native gluten (G), gluten-SSL (GS1). Magnification: 5000× (Gómez et al., 2013).

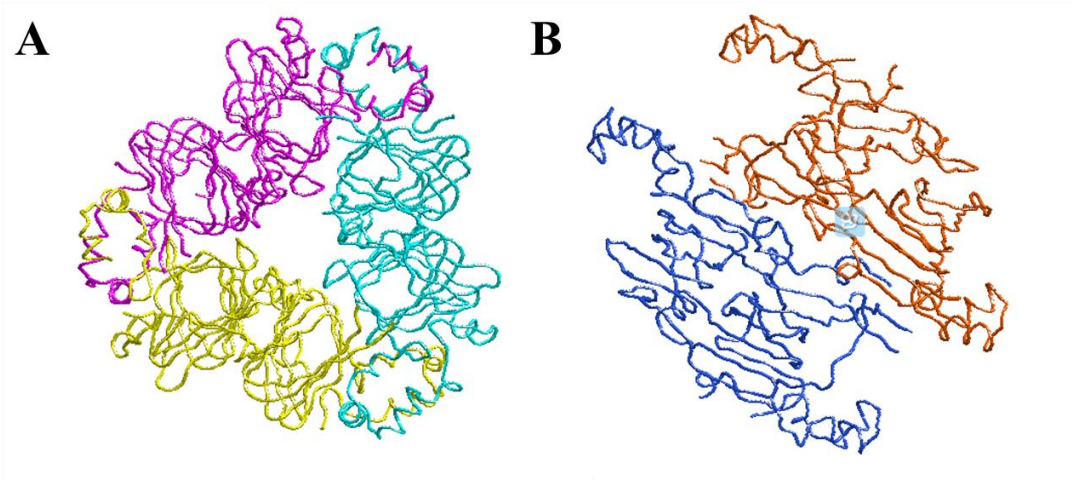

Figure 2 Crystal structures of glycinin (A) and β-conglycinin (B).

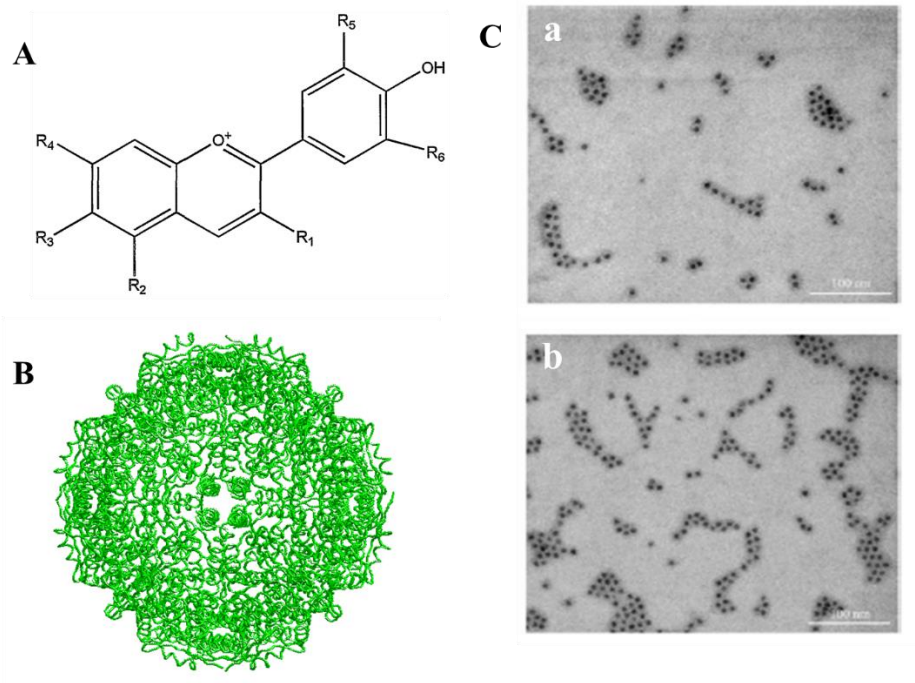

Figure 3 (A) Chemical structure of anthocyanin; (B) Crystal structure of phytoferritin; (C) The SSF aggregation was initiated by mixing SSF with different concentrations of tannic acid (6.8–6.8 μg/mL). Transmission electron micrographs of holoSSF in the absence (a) and presence (b) of tannic acid (Li et al., 2012).

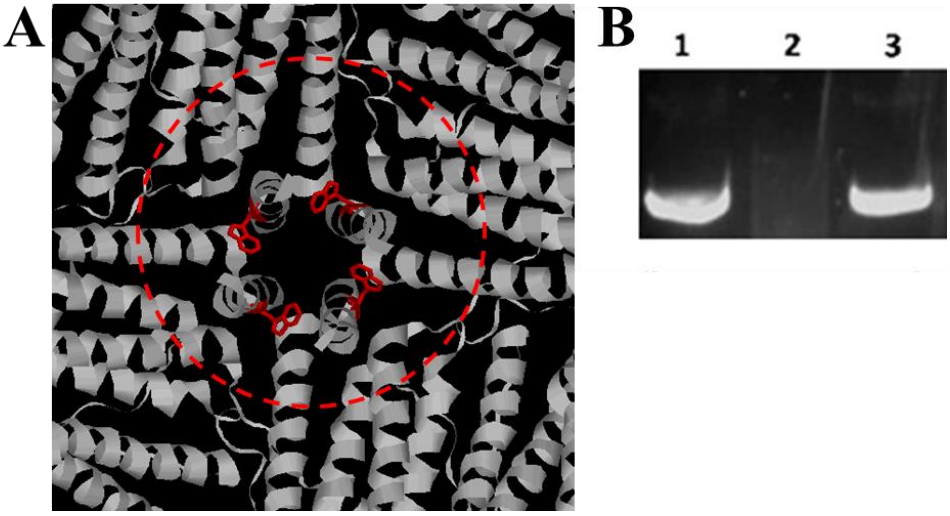

Figure 4 (A) Crystal structure of 4-fold channel of phytoferritin. The tryptophan residues were labeled with red color; the red circle indicated the putative location of NADH binding sites. (B) The DNA protective role of soybean seed ferritin upon aerobic addition of 48 Fe^{2+}/protein to apoferritin *in vitro*. Lane 1, plasmid DNA; lane 2, plasmid DNA+48 μM $FeSO_4$; lane 3, plasmid DNA+1 μM apoSSF+48 μM $FeSO_4$ (Liao et al., 2012).

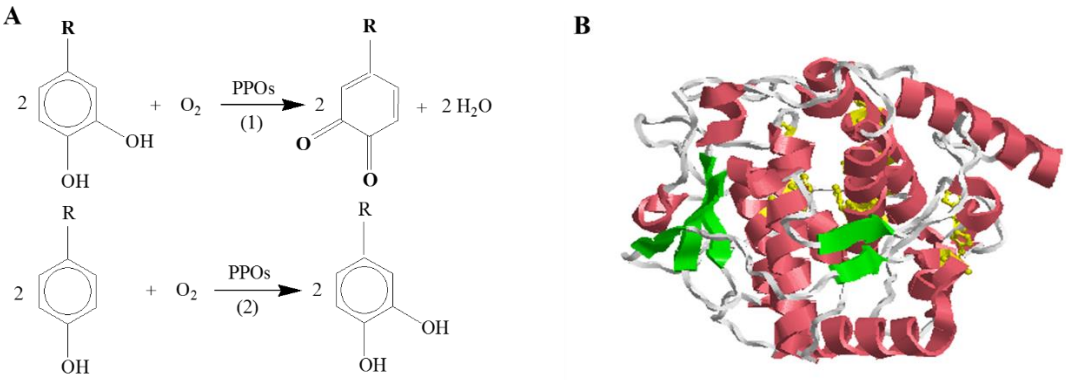

Figure 5 (A) The pathways for polyphenol oxidation by polyphenol oxidases (PPO); (B) Crystal structure of polyphenol oxidases (Virador et al., 2004). The histidine residues were labeled with yellow color.

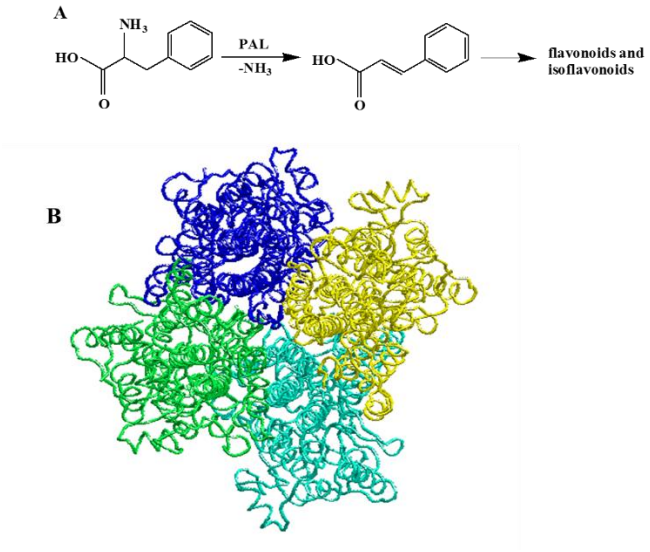

Figure 6 (A) The pathway for phenolic synthesis catalyzed by phenylalanme ammoma-lyase (PAL); (B) The crystal structure of phenylalanme ammoma-lyase (Calabrese et al., 2004).