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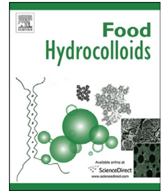

Gelation behaviors of denaturated pea albumin and globulin fractions during transglutaminase treatment

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The behavior of pea albumins (Alb) and globulins (Glob) in a denatured state toward microbial transglutaminase (MTGase) treatment was studied by SDS-PAGE analysis, free amine group determination, dynamic rheology and confocal laser scanning microscopy. The denaturation of pea proteins by chemical reduction with dithiothreitol (DTT) or by thermal treatment at 80 °C enhanced the enzymatic crosslinking degree of both protein isolates with greater crosslinking for the Glob fraction. The chemical denaturation affected preferentially the participation in crosslinking reaction of legumin acid subunits (40 kDa) for Glob sample and albumin polypeptides PA2 (26 kDa) for Alb sample, whereas the heat treatment led to complete polymerization of 55, 35 and 30 kDa vicilin polypeptides for pea globulins as shown by SDS-PAGE analysis. Up to 10 wt% concentration, the Alb fraction was not able to form MTGase crosslinked gels whatever the initial native or denaturated state. Compared to the native state, chemical and thermal denaturation of Glob fraction before enzymatic treatment led to the formation of weaker and stronger viscoelastic gels respectively. These contradictory results indicated that the enzymatic crosslinking reaction is highly related to polypeptides composition and conformation of proteins and the use of denaturation as a strategy to enhance gel forming properties by transglutaminase treatment has to be used with caution in the case of plant proteins.

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1. Introduction

Transglutaminase (TGase; E.C.2.3.2.13) is an enzyme that catalyzes acyl-transfer reactions through the transfer of γ-carboxamide groups (acyl donor) of glutamine (Gln) residues to free amino groups (acyl acceptor). In proteins, the ε-amino groups of lysine (Lys) residues act as acyl acceptors and lead to the formation of intra- and inter-molecular ε-(γ-glutamyl)-lysine (G-L) cross-links (Djoullah et al., 2015b). Transglutaminase of microbial origin (MTGase) is the more used source in the food industry as it is available in commercial preparations (Kieliszek & Misiewicz, 2014). As a consequence of TGase-induced crosslinking, the functional and textural properties of food proteins, such as thermal stability, water retention, solubility, interfacial and gelling properties, can be improved to open a wide range of applications (Gaspar & de Góes-Favoni, 2015). The generation of G-L isopeptide bonds has a predominant role in the gel formation where stable protein network is required. Highly elastic and irreversible gels can be obtained by the use of Tgase with different protein substrates, even at relatively low concentrations (Motoki & Kumazawa, 2000). The most traditional applications concern reconstituted meat or fish foodstuffs, dairy gels, bread or baked pastry products and soy-derived products such as tofu, where the crosslinking of proteins makes it possible to favorably influence the texture of the final products (Kieliszek & Misiewicz, 2014).

The main condition for a protein to be a good substrate for TGase is to have enough accessible Gln and Lys residues (De Jong & Koppelman, 2002). This depends, on the one hand, on the primary structure of the protein which defines quantitatively the composition of the protein in these two amino acids and on the other hand on the conformation of the protein (ternary and quaternary structures) which represents a limiting factor to the accessibility of TGase to these two reactive groups. Another important factor influencing this enzymatic reaction is the neighborhood distance between the two residues (Jaros, Partschefeld, Henle, & Rohm, 2006). Indeed, if the two residues belong to the same protein molecule, the transglutaminase can form

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intramolecular G-L bond if the Gln and the Lys are sufficiently close to each other. In the case of intermolecular bonds, i.e. binding between two distinct proteins, steric hindrance or repulsive interactions between proteins may limit the proximity of the two residues thus preventing the enzymatic reaction.

Among protein sources, casein, myosin and soybean glycinin were evaluated as excellent candidates for MTGase because of their richness in Gln and Lys residues (De Jong & Koppelman, 2002; De Jong, Wijngaards, Boumans, Koppelman, & Hessing, 2001; Jaros et al., 2006). Their Gln and Lys residues, in contrast to those of β-lactoglobulin or bovine serum albumin, appeared to be easily accessible for MTGase and sufficiently close to each other for enzymatic catalysis. It is possible to improve the susceptibility of proteins to the enzymatic treatment. The principle is based on the destabilization of the protein in order to unfold the structure thus allowing a better exposure of the Gln and Lys residues to the TGase to catalyze the reaction. Several strategies have been exploited in the literature to improve crosslinking efficiency: e.g. pH variation, use of reducing agents, enzymatic hydrolysis by protease or thermal denaturation.

In the case of whey proteins, variation in pH may affect the structure of the protein and make it partially unfolded and thus more susceptible to enzymatic treatment (Eissa, Bisram, & Khan, 2004). The reducing electrophoretic profile of the whey proteins at pH 8 treated with MTGase showed that the migration bands were less intense than those treated at pH 6 and 7, in particular for β-lactoglobulin; this accounted for the formation of a greater number of high molecular weight aggregates by covalent bonds initiated by MTGase.

In its native state, the folded structure of β-lactoglobulin is stabilized by two disulfide bridges, which limits the crosslinking between protein molecules by MTGase (Eissa & Khan, 2006). It has thus been shown that in the presence of a reducing chemical agent such as dithiothreitol (DTT) (10 mM) whose role is to break the protein-stabilizing disulfide bridges, the enzymatic reaction was markedly improved compared to the native sample (De Jong & Koppelman, 2002). However, since DTT is not allowed in the food industry, other reducing agents have been tested. Sodium sulphite and cysteine improved enzymatic cross-linking of β-lactoglobulin by MTGase, but to a lesser degree than DTT.

Another means used to improve the susceptibility of proteins to enzymatic treatment is the use of chelating agents such as ethylenediaminetetraacetic acid (EDTA). The latter was used to improve the crosslinking of α-lactalbumin by eliminating the calcium (calcium bridge) stabilizing the structure of this protein (Jaros et al., 2006).

Partial hydrolysis of proteins by proteolytic enzymes caused an increase in the degree of crosslinking as shown for soy proteins (Walsh, Cleary, McCarthy, Murphy, & FitzGerald, 2003). The partial cleavage of the amino acids following proteolysis makes it possible to alter the compact structure of the proteins and thus to better expose the Gln and Lys residues to the MTGase.

Finally, an alternative to the chemical modification of structured proteins is thermal denaturation. Indeed, heat treatment destabilizes the structure of β-lactoglobulin and thus makes the Gln and Lys residues more accessible to MTGase (De Jong & Koppelman, 2002).

To our knowledge a few studies reported the improvement of TGase-induced crosslinking by the application of chemical or thermal pretreatments on plant proteins in relation to their gelling properties. The gelation induced by the enzymatic treatment of soy proteins is generally carried out on freshly extracted proteins without major denaturation or alternatively on commercial isolates whose denaturation state is not controlled (Dube, Schäfer, Neidhart, & Carle, 2007; Gaspar & de Góes-Favoni, 2015). This is also true for pea proteins which are interesting alternative candidates to soybean proteins for such application Recent studies have reported the effect of transglutaminase on pea protein gel formation from native and/or commercial pea protein isolates (Schafer, Zacherl, Engel, Neidhart, & Carle, 2007; Shand, Ya, Pietrasik, & Wanasundara, 2008; Sun & Arntfield, 2011). In these studies the link between pea globulin denaturation and gel forming properties upon transglutaminase treatment had never been established. So in the present paper, we propose to elucidate this relation by taking into account the effect of enzymatic crosslinking on constitutive polypeptides of pea proteins after their chemical reduction or heat denaturation.

Pea (*Pisum sativum* L.) proteins representing 20–30% of total dry pea seeds, are mainly composed of 50–60% globulins and 15–25% albumins (Boye, Zare, & Pletch, 2010). Pea globulins (Glob) are salt-extractible proteins and are composed of the two major groups of legumin-type (11S) and vicilin-type (7S) families. Legumin has been described as a hexameric protein of between 360 and 400 kDa. Each monomer consists of a 40 kDa acidic subunit and a 20 kDa basic subunit linked by a disulfide bond (Croy, Gatehouse, Evans, & Boulter, 1980a). Vicilin is a trimer of approximately 160–200 kDa. Vicilins consist of ~50 kDa primarily subunit and different polypeptidic subunits of 30–35 and 13–20 kDa resulting from post-transductional processing (Croy, Gatehouse, Evans, & Boulter, 1980b). A third major storage protein, convicilin (7S), has a subunit of ~71 kDa and a molecular weight of 210–290 kDa in its trimeric native form (Croy, Gatehouse, Tyler, & Boulter, 1980). The albumin fractions (Alb; 2S) are water-soluble proteins including two major components richer in sulfur amino acids and lysine than pea Glob (Croy, Hoque, Gatehouse, & Boulter, 1984). The most abundant component is the albumin protein (PA2), a homodimer of two polypeptides of approximately 25–26 kDa linked with non specific interactions. The second component is the low molecular weight albumin protein (PA1) containing two polypeptides of approximately 4 and 6 kDa. Lipoxygenases, glycosidases, protease inhibitors, and lectins present in less quantity also belong to the albumin fraction (Bérot, Le Goff, Foucault, & Quillien, 2007).

In a previous paper we evaluated the influence of the operating conditions on the cross-linking yield by the MTGase of the Alb and Glob fractions of pea protein in the native state (Djoullah, Djemaoune, Husson, & Saurel, 2015a). Alb fractions had never been considered in previous studies concerning enzymatic crosslinking of pea protein. It was determined that a temperature of 40 °C, pH 7, activity of 20 MTGase units/g protein, 20 mM NaCl ionic strength and 2 h incubation time constituted parameters offering a good compromise between high crosslinking level and stability of enzymatic activity. Compared to the behavior of soy protein (Gan, Cheng, & Easa, 2009), it was concluded that native albumin and pea globulin are classified as poor and medium substrates respectively for MTGase, despite their amino acid composition rich in lysine and glutamine. It was concluded that the structure and conformation of the proteins play a decisive role and their denaturation would allow a better accessibility of the enzyme to the lysine and glutamine residues, thus improving the yield of crosslinking.

In this new work, our objective was to understand the effect of chemical (reducing treatment by DTT) and physical (heat treatment) denaturation of the Alb and Glob fractions of pea proteins on the enzymatic reaction by MTGase involving the different polypeptides constituting these fractions. This enables us to establish as novel findings a link between enzymatic crosslinking effect at the molecular level and gel forming properties of denaturated pea proteins. For this reason, the resulting rheological and structural properties of the formed protein networks were also investigated by comparison to the native fractions previously studied.

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