IUFoST 2006
DOI: 10.1051/IUFoST:20060479

**Transglutaminase Polymerization of Peanut Proteins**

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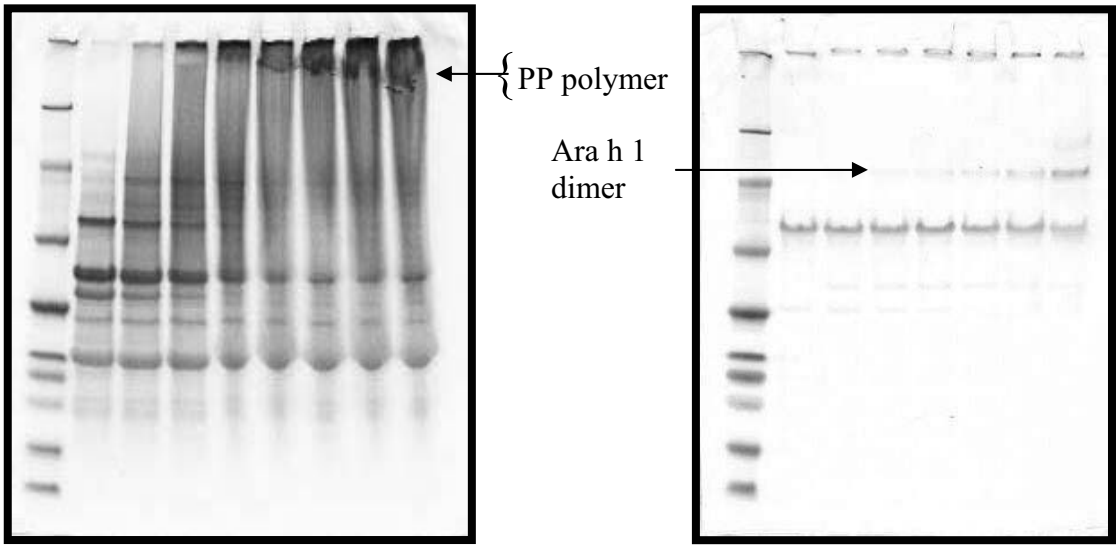
**INTRODUCTION:** Transglutaminase (TGase) [protein-glutamine:amine γ-glutamyl-transferase, EC 2.3.2.13] promotes protein cross-linking reactions through an acyl transferase mechanism (1) involving protein-bound glutaminyl residues and primary amines, including the ε-amino group of lysine residues in soy, myosin, gluten, oat globulin, casein and whey (2,3,4). Herein, we present a first report of microbial TGase catalysis of protein fractions prepared from peanut, *Arachis hypogaea L.*, and the effects of polymerization on experimental parameters listed below.

**OBJECTIVES:** Characterize the effects of TGase covalent cross-linking reactions on (*i*) SDS-PAGE banding patterns, (*ii*) the degree of peanut protein (PP) polymerization, and (*iii*) IgE reactivity with TGase modified PP and glycoprotein conjugates.

**METHODS:** SDS-PAGE electrophoresis, *O*-Phthaldialdehye (OPA) analyses, and enzyme linked immunosorbent (ELISA) assays were accomplished according to standard procedures.

**RESULTS:** (1) TGase polymerization of lightly roasted peanut flour dispersions, containing 12% fat (LR-12), resulted in a wide distribution of higher molecular weight species (Fig.1A) while cross-linking of purified Ara h1 resulted in distinct dimer formation (Fig. 1B).

**Figure 1: TGase Polymerization of Peanut Protein Dispersions**




**(A) LR-12 (+) TGase** **(B) Purified Ara h1 (+) TGase**

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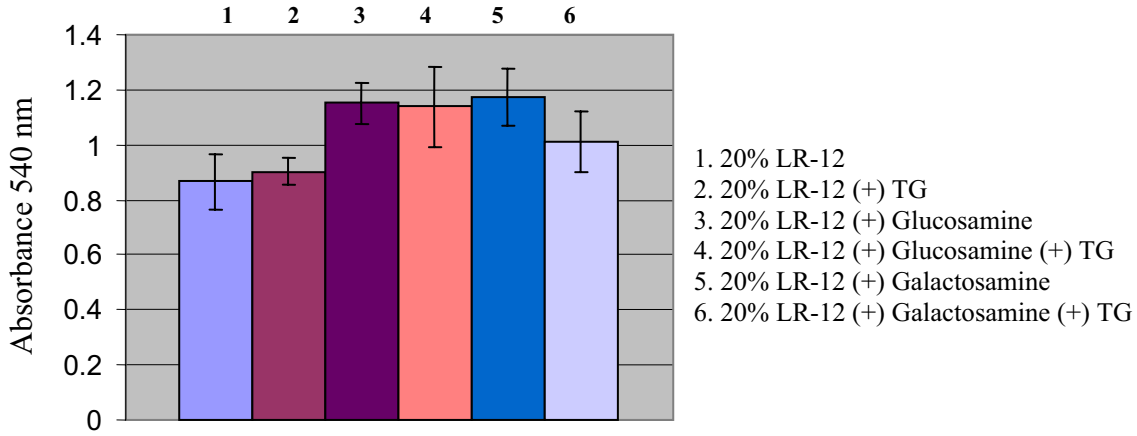
(2): OPA assays revealed ~30% coupling after incubation of LR-12 PP dispersions with TGase for ~ 4 h at 37°C.

**Table 1: Quantitative Analysis of TGase Cross-linking: (LR-12)**
(OPA assay)

| PP Sample | Incubation Time | $A_{340nm}$ |
|---|---|---|
| LR-12 (-) TGase | 0 h | .84 ± 0.04 |
| LR-12 (+) TGase | 2 h | .68 ± 0.05 |
| LR-12 (+) TGase | 4 h | .58 ± 0.03 |

(3) IgE responses, an indicator of potential allergic reactions, were measured for LR-12 PP and glycoPP-conjugates, created by TGase linkage of monosaccharide amino sugars with LR-12 protein substrates. These results showed slightly elevated IgE binding to all polymers as compared to LR-12 (control).

**Figure 2: ELISA Results**




**CONCLUSIONS:** The polymerization of PP was catalyzed by microbial TGase, and lightly roasted PP samples were cross-linked ~ 30%. TGase modification did not diminish IgE binding responses.

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**Keywords:** Peanut proteins, Transglutaminase, and Allergenicity