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Characterization of oil-in-water emulsions stabilized by tyrosinase-crosslinked soy glycinin

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ABSTRACT

The effect of crosslinked soy glycinin with tyrosinase from *Bacillus megaterium* (TyrBm) on o/w emulsion properties was studied. The ability of TyrBm to crosslink soy glycinin was evaluated in the presence or absence of three phenolic mediators. It was observed that crosslinking of glycinin is facilitated by a phenolic mediator and is negligible in its absence. Subsequently, the glycinin-stabilized emulsions were evaluated in two systems: (i) homogenization after crosslinking in the presence of a mediator, caffeic acid, and (ii) homogenization prior to crosslinking in the absence of caffeic acid. Emulsions were prepared using a high-pressure homogenizer and their particle size, creaming resistance, viscosity and microstructure were measured. Results indicate that the method of emulsion preparation affected the emulsion physical stability, thus, the first system led to a decrease in emulsion stability against creaming while the second system resulted with improved properties after the enzymatic treatment; Crosslinking after homogenization eliminated the need for a phenolic mediator and led to a lower creaming velocity and higher viscosity. In addition, fluorescence microscopy observations demonstrated that the crosslinking reaction of TyrBm after homogenization led to the formation of cold-set gel-like structures of small droplets linked by covalent bonds.

1. Introduction

Soy proteins are widely used in various food products, due to their high nutritional value and their ability to improve texture as emulsifying and gelation agents (Friedman & Brandon, 2001; Hughes, Ryan, Mukherjea, & Schasteen, 2011; Liu, 1997; Mujoo, Trinh, & Ng, 2003; Renkema, Lakemond, de Jongh, Gruppen, & van Vliet, 2000; Zhang, Wu, Lan, & Yang, 2013). They are correlated with many health benefits, such as reduced LDL cholesterol uptake and prevention of diabetes (Friedman & Brandon, 2001). They were also approved by the US Food and Drug Administration (FDA) as a health claim in foods stating that soy proteins reduce the risk of coronary heart disease (Zhang et al., 2003). Glycinin and β-conglycinin, which account for 65–85% of the total seed protein, are found to be the most important soy proteins and have been utilized and modified for various purposes, such as for food functionalities (Liu, 1997; Mujoo et al., 2003). Glycinin is an oligomeric protein with a molecular mass of ~350 kDa. The individual glycinin monomer consists of an acidic polypeptide subunit (AS) with a size of ~38 kDa and a basic polypeptide subunit (BS) with a size of ~20 kDa, linked by a single disulfide bridge. At least six acidic polypeptides (A1a, A1b, A2-A4 and A5) and five basic polypeptides (B1a, B1b and B2–B4) have been isolated (Mujoo et al., 2003). When compared to other soy fractions, glycinin showed a lower emulsifying ability than soy protein isolate or than the other major protein, β-conglycinin. This is probably due to its high molecular mass and globular structure that limits its ability to adsorb at the oil droplet surface (Keerati-u-rai & Corredig, 2010; Kinsella, 1979; Wagner & Guéguen, 1999). Better control of the final soy product can be obtained by understanding the influence of each protein alone on the emulsion properties. Glycinin has been investigated for its emulsifying properties and different methods have been used to improve its emulsification ability and emulsion stability, such as: heat and pH treatment, high pressure and chemical crosslinking with formaldehyde (Keerati-u-rai & Corredig, 2009; Tang, Chen, & Foegeding, 2011; Yasir, Sutton, Newberry, Andrews, & Gerrard, 2007). A different approach for altering protein structure and improving its properties is enzymatic crosslinking. The great advantage of enzymatic modifications over chemical

Abbreviations: TyrBm, tyrosinase from *Bacillus megaterium*; SPB, sodium phosphate buffer; AP%, percentage of adsorbed proteins.

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modifications is the mild conditions of enzymatic reactions, the ability to control the reaction through slight changes of temperature and pH, and finally, the higher specificity of enzymatic reactions, which allow the avoidance of possible side products. To date, enzymatic crosslinking has been investigated in some detail using the enzyme transglutaminase that catalyzes acyl transfer reactions, resulting in formation of ε-(γ-glutaminyl) lysine interactions (Bao et al., 2011; Castro-Briones et al., 2009; Hu, Zhao, Sun, Zhao, & Ren, 2011; Min & Green, 2008; Muguruma et al., 2003; Siu, Ma, Mock, & Mine, 2002). Transglutaminase treatment of soy proteins-stabilized emulsions led to an increase in droplet size and improved emulsion stability against creaming (Hu, Xu, Fan, Cheng, & Li, 2011; Yang, Liu, & Tang, 2013).

Tyrosinase from the soil bacteria *Bacillus megaterium* (TyrBm) was isolated and characterized in our lab and was investigated for its monophenolase and diphenolase activities (Goldfeder, Egozy, Shuster Ben-Yosef, Adir, & Fishman, 2012; Sendovski, Kanteev, Shuster Ben-Yosef, Adir, & Fishman, 2010; Shuster & Fishman, 2009). TyrBm is a "type-3-copper" enzyme, which contains two copper ions in its active site that are necessary for its activity. It is the enzyme responsible for the production of melanin in organisms and its native substrate is L-tyrosine. Tyrosinases from different origins have been investigated for their protein crosslinking ability on milk and wheat proteins, but not on soy glycinin (Heijnis, Wierenga, van Berkel, & Gruppen, 2010; Selinheimo, Autio, Kruus, & Buchert, 2007; Thalmann & Lötzbeyer, 2002). Studies demonstrated that the crosslinking ability of tyrosinase is limited by the structure of the substrate protein and the exposure of its tyrosine residues and in most cases, required a low molecular weight phenolic agent as a mediator (Fig. 1) (Heck, Faccio, Richter, & Thöny-Meyer, 2013; Thalmann & Lötzbeyer, 2002).

In this study, we used TyrBm for crosslinking soy glycinin in order to improve its emulsion properties. The crosslinking reaction was tested both in the presence and in the absence of a phenolic agent. In order to overcome the need for a phenolic reagent, the crosslinking reaction in the absence of a reagent was carried out after the homogenization. We assumed that during homogenization, some of the hidden tyrosine residues become more exposed to the tyrosinase active site. By introducing covalent bonds within the soy protein network, we have improved emulsion stability against creaming and delayed phase separation.

2. Experimental

2.1. Materials

The commercial soy glycinin standard (98% purity), sodium bisulfite, caffeic acid, chlorogenic acid, coumaric acid and Nile red were obtained from Sigma Chemical Co. (Rehovot, Israel). Refined corn oil was purchased from the local supermarket (Haifa, Israel).

2.2. Methods

2.2.1. Purification of TyrBm

TyrBm was isolated in our laboratory from soil samples, and the gene encoding for the tyrosinase was cloned into *Escherichia coli* BL21, expressed and purified as previously described, and the enzyme activity was determined on L-tyrosine, and L-dopa (Shuster & Fishman, 2009). In this work, the enzyme activity on caffeic acid was determined as well.

2.2.2. Isolation of soy protein fractions

Glycinin-rich fraction was isolated from defatted soybean flakes, kindly provided by Shemen Industries Ltd. (Haifa, Israel). The isolation was done according to the method of Nagano et al. (Nagano, Hirotsuka, Mori, Kohyama, & Nishinari, 1992), with slight

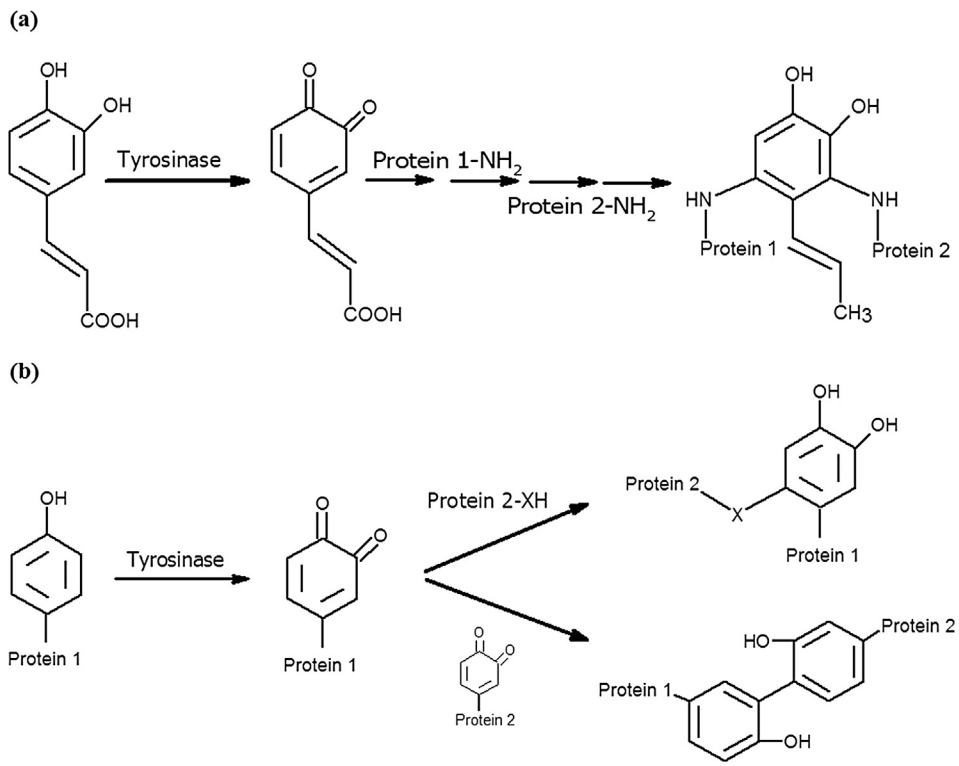

Fig. 1. Reaction scheme of a tyrosinase generated crosslinking of proteins (adapted from (Heck et al., 2013)); (a) in the presence of caffeic acid as a low molecular weight phenolic mediator; (b) in the absence of a low molecular weight phenolic reagent.

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