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Incorporating tyramine with transglutaminase weakens gelatin gels – A rheological investigation

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ABSTRACT

Microbial transglutaminase (MTGase) was used to introduce aromatic biogenic amine tyramine (TYR) into porcine skin gelatin (enzymatic amination) and the subsequent rheological properties of gelatin compared to non-treated controls were determined. The impact of MTGase-catalyzed amination conducted at 50 °C was monitored by evaluating gelation and melting temperatures (T_m), storage (G') and loss (G'') modules, as well as gelation rate (K_{gel}) and gel strength (G_n). Treating with MTGase alone without the addition of TYR increased ($p < 0.05$) both gelation and melting temperatures. The gelation temperature and T_m were not different ($p > 0.05$) for the amination treatment group compared to the gelatin control. Overall, incorporating TYR into gelatins with MTGase weakened the gels; this was likely due to the interference of creation of covalent TYR-gelatin bonds that disturbed the normal formation of triple helix networks.

1. Introduction

Gelatin is a denatured fibrous protein made by the partial hydrolysis of collagen, a major component of animal connective tissues. During the production of gelatin, many bonds break randomly and the resulting gelatin represents a broad spectrum of smaller molecular fragments, as well as larger unhydrolyzed main chains (Kaur, Hardman, Melia, Jumel, & Higginbottom, 2002). Commercial gelatins for food consumption are mainly derived from bovine bones and porcine skins due to their low cost and availability (Cho, Gu, & Kim, 2005). Gelatin's unique properties are highly regarded in food manufacturing; as an additive it can improve foods due to its elasticity, consistency and stability (Hashim et al., 2010). The quality of a gelatin depends largely on its rheological properties, which are normally characterized by gel strength, gel elasticity, gelation point and gel melting point (Karim & Bhat, 2009). Gelatin has accessible functional groups which can be coupled with various cross-linkers or targeting-ligands to bear multiple modification opportunities (Elzoghby, Samy, & Elgindy, 2012). Introducing these chemical cross-links, or a combination of cross-linking agents within or between gelatin chains, is a mean of manipulating the characteristics of any given gelatin. This approach has been reported in earlier studies for maltodextrin (Kasapis, Morris, Norton, & Brown, 1993), gellan (Wu, Chiu, Pearce, & Kwei, 2001) and caffeic acid (Mohtar, Perera, & Hemar, 2014). Incorporation of these multifunctional molecules into gelatin chains leads to a three-dimensional covalently cross-linked network with altered rheological properties.

Tyramine (TYR) is one of the most important biogenic amines (BAs) naturally present in a variety of foods and beverages (Spano et al., 2010). As reported in a previous research (Lu, Hrynets, & Betti, 2016), this aromatic and antioxidant BA can be incorporated into pea protein peptides using a GRAS (generally recognized as safe) microbial enzyme - transglutaminase (MTGase). MTGase is a Ca^{2+}-independent enzyme industrially produced from the *Streptoverticillium* species. It catalyzes an acyl transfer reaction between the γ-carboxyamide of peptide- or protein-bound glutamine and the ε-amino group of lysyl residues (Fig. 1 path A). It also catalyzes aminolysis of the γ-carboxamide group of peptide-bound glutamine residues by the variety of primary amines (Han & Damodaran, 1996). A recent report also highlighted the high reactivity of MTGase for the incorporation of the aromatic TYR into protein in model systems (Gundersen, Keillor, & Pelletier, 2014). In this context, we anticipate that MTGase would introduce TYR onto glutamine residues (Fig. 1 path B) of gelatin and alter its functionality. For instance, our previous study (Lu et al., 2016) showed that

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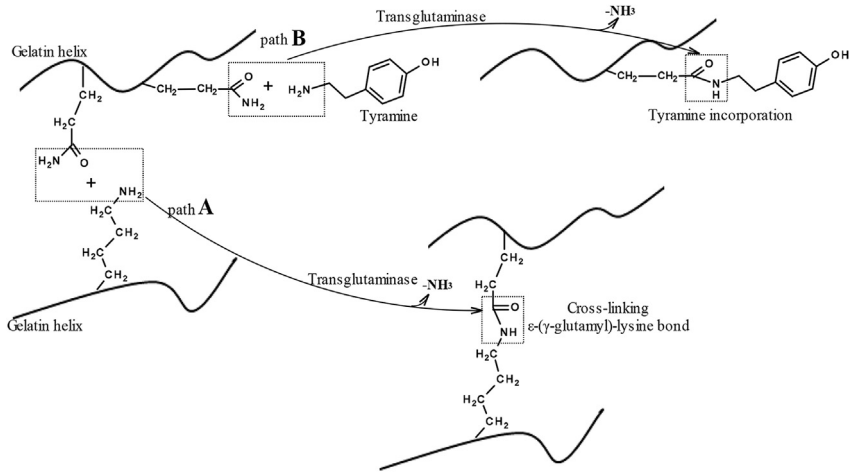

Fig. 1. Schematic representation of two distinct reactions catalyzed by MTGase. Path A shows the formation of an amide bond between glutamine of the gelatin's helix and tyramine (amine incorporation); Path B shows the formation of an amide bond between gelatin's glutamine and lysine side chains (cross-linking).

TYR was incorporated into pea peptides with subsequent positive effects on the antioxidant capacity of the hydrolysates. The action of MTGase has been demonstrated to be largely responsible for cross-linking gelatins (Crescenzi, Francescangeli, & Taglienti, 2002; Dickinson & Yamamoto, 1996; Fuchsbauer et al., 1996; Lim, Mine, & Tung, 1999), but, to the best of our knowledge, no information is available on the MTGase-catalyzed amination of gelatin, and its subsequent specific effects on rheological properties. The advantage of using this approach to modify the functional properties of gelatin was due to the high specificity of an enzymatic reaction, the possibility of working at mild conditions and the utilization of a known GRAS enzyme.

It is hypothesized that incorporation of TYR into the gelatin molecule would affect the gel network formation during the cooling phase. The TYR conjugation would increase surface hydrophobicity of the gelatin and thus may induce the faster formation of hydrogen bonding of gelatin chains (inter-molecular hydrogen bonds) to speed up gel formation. This faster reaction would be desirable in gelatin applications in both the food and pharmaceutical industries. On the other hand, the incorporation of TYR could decrease inter-molecular hydrogen bonds due to steric hindrance and protein folding. In this case the formation of intra-hydrogen bonds would be dominant over the inter-molecular ones, and as a consequence, the gelatin chains would fold in such a manner to reduce the interactions between the gelatin chains thus negatively affecting the rheological properties. The overall aim of this study was to evaluate and compare the viscoelastic properties of aqueous MTGase-treated gelatin dispersions in the presence or absence of TYR. It was important to understand the impact of incorporating TYR on rheological characteristics of gelatin.

2. Materials and methods

2.1. Materials

Commercial gelatin extracted from porcine skin (type A) was from Sigma-Aldrich (St. Louis, MO, USA). Gelatin prepared by acid treatment (type A) and not by the base treatment (type B) was chosen, because treatment with base hydrolyzes the amide groups of glutamine residues and thus suppresses enzymatic reactions (Crescenzi et al., 2002). Tyramine hydrochloride (TYR, ≥ 98.0%), *N*-Carbobenzoxy (CBZ)-L-glutaminyl-glycine (CBZ-Gln-Gly), L-glutamic acid γ-monohydroxamate and hydroxylamine were purchased from Sigma-Aldrich. MTGase (Activa-TI; 99% maltodextrin and 1% MTGase) was from Ajinomoto Food Ingredients (Eddyville, IA). All other chemicals and reagents were from Sigma-Aldrich or Fisher Scientific (Fair Lawn, NJ, USA) and were of analytical grade.

2.2. Experimental design

Porcine skin gelatin (G) solutions was used at final concentrations of 5 and 6.67 g/100 mL and were subjected to TYR amination through MTGase catalysis reactions conducted at 50 °C for 4 h. The latter concentration of gelatin is typical for determining the "Bloom" strength measurement. Two concentrations of TYR (0.5 and 1 g/100 g) were added to the gelatin solutions in the presence or absence of MTGase. Thus, for each of the two gelatin concentrations 6 treatments were produced: a control gelatin without MTGase and TYR (G), a treatment of gelatin and MTGase (G + MTGase), a treatment of gelatin, MTGase and addition of 0.5 g/100 g TYR (G + MTGase + 0.5 g/100 g TYR) and its respective control (G + 0.5 g/100 g TYR), a treatment of gelatin, MTGase + 1 g/100 g TYR (G + MTGase + 1 g/100 g TYR) and its respective control (G + 1 g/100 g TYR). The whole experiment was repeated three independent times using gelatin from different batches. For each trial two tubes per treatments (including controls) were incubated at 50 °C for 4 h.

2.3. Determination of enzyme activity

MTGase activity was measured using CBZ-Gln-Gly and hydroxylamine as substrates following the method of Folk (1970) with some modifications. The final 0.23 mL reaction mixture contained 174 mmo/L Tris buffer, 31 mmol/L CBZ-Gln-Gly, 87 mmol/L hydroxylamine, 8.7 mmol/L glutathione (reduced form), 4 mmol/L calcium chloride and 0.06 unit transglutaminase. The reaction mixture was incubated at 37 °C and 50 °C for 10 min and terminated by adding 500 μl of ferric chloride/trichloroacetic acid reagent (0.7 g/100 mL). After centrifugation for 5 min at 1000×*g*, the absorbance of the resulting supernatant was recorded at 525 nm (V-530, Jasco Corporation, Tokyo, Japan). L-glutamic acid-γ-monohydroxamic acid was used as a standard for calibration. One unit of enzyme activity was equivalent to a change in absorbance of 0.29/min at 525 nm, corresponding to the formation of 1 μmol of hydroxamate/min from CBZ-Gln-Gly and hydroxylamine at pH 6.0. The enzyme MTGase activity was expressed as the initial reaction