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Effect of ultrasound pre-treatment on formation of transglutaminase-catalysed soy protein hydrogel as a riboflavin vehicle for functional foods

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ABSTRACT

High intensity ultrasound (HIU) treated soy protein isolate (SPI) and non-HIU-treated SPI were cross-linked by transglutaminase to form hydrogels. SDS-PAGE showed that HIU increased the amount of high molecular weight aggregates, likely due to the formation of ε-(γ-glutamyl) lysine bonds. Moreover, HIU pretreatment increased the hydrophobic nature of transglutaminase gels as demonstrated by FT-Raman. HIU changed the 3D-network structure of transglutaminase induced SPI gel with riboflavin (TSGR). Furthermore, 40 min HIU increased gel yield, riboflavin encapsulation efficiency and gel strength of TSGR. HIU decreased swelling and protein erosion of TSGR in simulated gastrointestinal fluids. It also resulted in reduced riboflavin release rate and altered the release mechanism in simulated gastrointestinal fluids both in the absence and presence of digestive enzymes. In conclusion, HIU may facilitate covalent cross-linking, increase hydrophobicity and change the 3D network of TSGR, leading to differences in hydrogel stability, as well as riboflavin encapsulation and release profiles.

1. Introduction

Hydrogels are three dimensional polymeric networks that can absorb and retain large amounts of water (Guo, Zhang, & Yang, 2012; Hennink & Van Nostrum, 2012). Due to the hydrophilic and soft tissue biocompatibility, different hydrogels have been synthesised as novel materials in biomedical applications, such as drug and bioactive compound delivery and tissue engineering (Hennink & Van Nostrum, 2012; Hoare & Kohane, 2008;

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Teixeira, Feijen, van Blitterswijk, Dijkstra, & Karperien, 2012; Yin, Su, Qi, & He, 2012). Recently, more and more works have focused on using natural-polymer-based biomaterials that can be considered as nontoxic (Song & Zhang, 2008) and have advantages of biodegradability, biocompatibility and wide availability (Chen, Remondetto, Rouabhia, & Subirade, 2008). Food proteins are natural biopolymers, which are generally recognised as safe. Moreover, globular proteins, such as whey, soy and egg white protein, show excellent gelation properties (Chen, Remondetto, & Subirade, 2006) that may be useful for the formation of globular protein hydrogels as bioactive compound/nutraceutical delivery systems.

Soy protein isolate (SPI), an important byproduct of the soybean oil industry (Song, Tang, Wang, & Wang, 2011), is used extensively in food manufacturing as it is an abundant, high nutritional quality, inexpensive and renewable natural material (Chen et al., 2008; Maltais, Remondetto, & Subirade, 2009). Furthermore, SPI has excellent gelation property (Kinsella, 1979). However, the traditional SPI gelation is often achieved by heat treatment (Gu, Campbell, & Euston, 2009; Tang, Chen, & Foegeding, 2011), which limits its application as a carrier of heat-sensitive compounds (Chen et al., 2006). Thus formation of cold-induced SPI hydrogel (Maltais, Remondetto, Gonzalez, & Subirade, 2005; Maltais et al., 2009; Tang, Luo, Liu, & Chen, 2013) may be a more appropriate approach for the formation of SPI hydrogel as a controlled release vehicle for this purpose.

Transglutaminase (TGase; EC 2.3.2.13) is considered as an excellent cold protein gel coagulant for food or drug industry because of its low price and safety, with GRAS (generally recognised as safe) status. TGase catalyses an acyl transfer reaction between the γ-carboxamide group of a peptide-bound glutaminyl residue and a variety of primary amines. When the amine-containing substrate is the ε-amino group of a peptide-bound lysyl residue, peptide chains can be covalently connected through ε-(γ-glutamyl) lysine isopeptide bonds (Fuchsbauer et al., 1996; Hernàndez-Balada, Taylor, Phillips, Marmer, & Brown, 2009; Yokoyama, Nio, & Kikuchi, 2004). Thus TGase is widely used in food processing industry to improve the gelation property of food proteins (Yokoyama et al., 2004). For example, a majority of the subunits of β-conglycinin and the acidic subunits of glycinin SPI could be polymerised by TGase (Tang, Wu, Yu, Li, Chen, & Yang, 2006b), and a cold gel could be formed from soy milk using TGase as coagulant (Tang, Li, Wang, & Yang, 2007). Moreover, Song et al. (Song & Zhang, 2008) pointed out the possibility of using TGase-induced cold soy protein gel as a carrier for the controlled release of 5-aminosalicylic acid. These findings indicate the potential to develop TGase cross-linked SPI gel as a bioactive compound/nutraceutical carrier.

Sonication technology is attracting much attention of food protein researchers (Jambrak, Lelas, Mason, Krešić, & Badanjak, 2009). Low frequency high intensity ultrasound (HIU) (16–100 kHz, power in the range 10–1000 W cm^{-2}) can be used to alter food properties (Soria & Villamiel, 2010), and several studies have reported that HIU can change the physicochemical properties of soy proteins (Arzeni et al., 2012; Hu, Cheung, Pan, & Li-Chan, 2015; Hu, Wu, et al., 2013; Jambrak et al., 2009; Tang, Wang, Yang, & Li, 2009). The surface hydrophobicity, protein solubility, and emulsifying properties increased after HIU, while the particle size decreased (Hu, Li-Chan, Wan, Tian, & Pan, 2013). Furthermore, our recent studies found that HIU of SPI increased the gelation property of heated gels formed by glucono delta lactone and $CaSO_4$ (Hu, Fan, et al., 2013; Hu, Li-Chan, et al., 2013). These reported effects of HIU on SPI suggest the possibility of using HIU to change the gelation properties and microstructures of SPI cold gels induced by TGase. Moreover, the microstructure of cold gels can influence their properties as carriers (Maltais et al., 2009; Song & Zhang, 2008). In other words, HIU may influence the microstructure of SPI–TGase cold gel, resulting in the regulation of its release of encapsulated bioactive compounds or nutraceuticals such as riboflavin. Riboflavin (vitamin B_2), a water-soluble vitamin, is involved in numerous metabolic reactions (Yoshimatsu et al., 2014). Riboflavin deficiency may lead to impaired handling of iron and night blindness. Moreover, riboflavin is important in protecting against cardiovascular diseases and cancers (Powers, 2003). However, humans are not able to synthesise riboflavin and thus must obtain it as a nutrient via intestinal absorption (Yoshimatsu et al., 2014). People who consume little milk or meat products have the risk of having poor riboflavin status. Previous studies have reported that the absorption of riboflavin could be enhanced if riboflavin is retained in the gastrointestinal system for a longer period of time (Kagan et al., 2006; Levy & Jusko, 1966). Therefore, creating a riboflavin vehicle that can lead to longer retention in the gastrointestinal system would be beneficial for supplementation of riboflavin. Therefore, riboflavin was chosen as a model bioactive compound/nutraceutical compound in this study, with the objective of investigating HIU-treated SPI–TGase cold gel as a riboflavin carrier.

2. Materials and methods

2.1. *Materials*

Soy protein isolate (SPI, >90% protein d.b., as determined by the Kjeldahl method) was donated by Yuwang Company (Shandong, China). Transglutaminase (Activa TI; 100 units of enzyme activity per gram of powdered preparation) was a gift from Ajinomoto North America, Inc. (Itasca, IL, USA). Riboflavin (≥98%) and pancreatin (8 × USP) were products from Sigma–Aldrich (St. Louis, MO), and pepsin from porcine stomach mucosa (5 × USP) was obtained from Fisher Scientific. Other chemicals used in this study were of analytical grade.

2.2. *Preparation of high intensity ultrasound treated soy protein isolate*

SPI dispersions (10%, w/v) were prepared by adding distilled water into protein powder and then gently stirring for 2 h. A sonication processor model JY92-2D (Ning Bo Scientz Biotechnology Co. Ltd, Ningbo, Zhejiang, China) with a 0.636 cm diameter titanium probe used for high intensity ultrasound treatment of 100 mL of SPI dispersions in 125 mL flat bottom conical flasks immersed in an ice-water bath. Samples were treated at 20 kHz at 400 W for 0, 20 or 40 min (pulse duration of on-time 5 sand off-time 1 s). The temperatures of ultrasonic treated SPI suspensions did not exceed 20 °C. After ultrasound treatments, all samples were freeze-dried and then