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Effects of gamma radiation on microbial, physicochemical, and structural properties of whey protein model system

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ABSTRACT

Gamma radiation has been used in food processing for many years, though it has certain effects on food components. Whey protein solutions (10%/30%, wt/vol) were treated with gamma radiation at various dosages (10–25 kGy) and evaluated for microbial changes in the solutions and physicochemical and structural changes of whey proteins. Whey protein solutions after gamma radiation showed substantially lower populations of all viable microorganisms than those of controls. The 10% whey protein solution treated at radiation of 20 or 25 kGy remained sterile for up to 4 wk at room temperature. Gamma radiation increased viscosity and turbidity and decreased soluble nitrogen of whey protein solutions compared to nonradiated control samples regardless of radiation dosage. Nonreducing sodium dodecyl sulfate-PAGE suggested that whey proteins under gamma radiation treatment formed aggregates with high molecular weights. Reducing sodium dodecyl sulfate-PAGE showed that disulfide bonds played a role in gamma radiation-induced whey protein cross-linking. Scanning and transmission electron microscopy micrographs exhibited large aggregates of whey proteins after gamma radiation treatment. Results suggested that gamma radiation could be applied to whey protein solution for purposes of reducing microbial counts and cross-linking protein molecules.
Key words: gamma radiation, whey protein, physicochemical property, structural property

INTRODUCTION

Nonthermal processing technologies, such as ultrasonic (Foegeding et al., 2002; Shen et al., 2017), high-pressure (Camp et al., 1997; Keim and Hinrichs, 2004), enzymatic (Ju et al., 1997; Ju and Kilara, 1998), and gamma radiation (Chawla et al., 2009), have generated much attention in food industry. In particular, the use of gamma radiation has drawn special attention due to the dual effects for both inhibiting microorganisms and altering structure of molecules by targeted processes. Gamma radiation has been proven to be an effective and safe method for sterilization of certain products. A wide variety of perishable food products (meat, fruits, and so on) are submitted to gamma radiation for preservation to control food-borne pathogens and reduce microbial load and insect infestation, thereby extending shelf life (Oliveira et al., 2007; Kasera et al., 2012). Gamma radiation can penetrate the products and eliminate microorganisms present in crevices and creases effectively (Prakash et al., 2010). Low-dosage gamma radiation has been accepted as neither presenting any toxicological hazard nor introducing any special nutritional or microbiological problems, thus being safe for human consumption (Tsiotsias et al., 2002). The Joint FAO/IAEA/WHO Expert Committee concluded in 1980 "that the gamma radiation of any food commodity up to an overall average dosage of 10 kGy presents no toxicological hazard" (FAO/IAEA/WHO, 1980).

Gamma radiation may induce changes in the molecular characteristics of components in foods at certain dosages. Foods or an ingredient have been treated by gamma radiation to ensure a change for facilitated processing purposes (Sabato and Lacroix, 2002; Cieśla et al., 2004). Gamma radiation generates direct or indirect effects to foods in a dry or aqueous state, respectively. For solid foods, molecules absorb radiation energy directly and result in changes in the structure of food components. Aqueous solutions in water first exposed to gamma radiation generate hydroxyl radicals and hydrated electrons, which can in turn react with molecules to form covalent bonds (Oliveira et al., 2007). Radiation treatment is known to affect protein molecules (Silva et al., 2006); it can affect proteins by causing conformational changes (Mcparland, 2010) and

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promoting reactions such as oxidation of AA, cleavage of peptide, and formation of disulfide bonds by association of aromatic and heterocyclic residues (Abu et al., 2006). Gamma radiation has been employed to improve water vapor permeability and chemical stability of protein-based films (Ouattara et al., 2002), mechanical resistance of milk protein gels (Cieśla et al., 2004), viscosity of whey dispersions (Atlay and Alfred, 2004), oil absorption, and emulsion capacities of cowpea protein isolates (Abu et al., 2006). In addition, gamma radiation was shown to have reduced the allergenicity of different allergenic foods by structurally altering the human IgE-binding epitopes in food allergens (Lee et al., 2001).

Whey is an abundant by-product of cheesemaking. Whey proteins, enriched protein fractions from whey, have various functional properties, including emulsifying, gelation, thickening, and water-holding capacities (Zhang et al., 2015). Whey proteins can be designed for enhanced functional properties by altering the protein structure through physical, chemical, or other means (Foegeding et al., 2002). The effects of gamma radiation on whey proteins were mostly limited to the edible film mechanical properties and allergenicity reduction. Furthermore, the effect of gamma radiation on microbiological properties of perishable food products such as meat and fruits has been studied with low dosages of gamma radiation. No information is available on the effects of gamma radiation on microbiological, physicochemical, and structural properties of whey protein solution. In the current study, the effects of gamma radiation on whey protein (10 and 30% solutions) at high dosages (10–25 kGy) were investigated for changes in microbiological and physicochemical properties as well as protein-protein interaction and microstructure.

MATERIALS AND METHODS

Materials

Whey protein isolate (**WPI**; 92% protein, wt/wt) was purchased from Fonterra Co-operative Group (Auckland, New Zealand). The protein content was verified by Kjeldahl method (Standardization Administration of the People's Republic of China. 2016). Other components were (wt/wt) 0.36% fat, 1.6% ash, 0.7% lactose monohydrate, and 0.07% calcium. Aerobe, yeast, and mold count plates were 3M Petrifilm (3M, St. Paul, MN). Prefilled buffer (9 mL) was purchased from Fisher Scientific Inc. (Pittsburgh, PA). All other chemicals used were of reagent grade and purchased from Sigma-Aldrich Co. LLC (St. Louis, MO). Deionized water (resistivity was above 18.2 MΩ) was obtained using a Milli-Q deionization reversed osmosis system (Millipore Corp., Bedford, MA).

Gamma Radiation Treatment

Whey protein solutions (10 and 30%, wt/vol) were prepared by dissolving WPI powder slowly into deionized water at room temperature with the help of magnetic stirring (500 rpm) for 2 h before storage at 4°C overnight to allow complete hydration. When calculating the amount of whey protein solutions to be added, a correction for the protein content of the powder was taken into account. The solutions remained at the original pH of approximately 6.8. Gamma radiation treatment of WPI samples (each in 50-mL centrifuge tubes) were performed with a ^{60}Co irradiator (Gammacell 220, Nordion, Ottawa, Canada) at average dosages of 10 to 25 kGy at ambient temperature with presence of oxygen. The Gammacell 220 operates on a 220 V, 60 Hz, and 15 A supply. Samples were then left at room temperature for up to 4 wk and withdrawn periodically for microbial and physiochemical analysis. Untreated whey protein solutions stored under the same condition were used as controls.

Microbial Analysis

Each sample (1 mL) was diluted to gradient concentration by 10^{-1}, 10^{-2}, and 10^{-3} with 9 mL of prefilled sterile buffer. Each dilution was applied on aerobe, yeast, and mold counts according to the manufacturer's instructions (3M Petrifilm; https://multimedia.3m.com/mws/media/236194O/petrifilm-aerobic-interpretation-guide.pdf). The top of film was lifted to expose the surface and 1 mL of each dilution (10^{-1}, 10^{-2}, and 10^{-3}) from each sample was added inside a circular foam barrier on aerobe, yeast and mold count Petrifilms. After drying for 5 min, aerobe count Petrifilms were put into an incubator at 37°C for 48 h, whereas yeast and mold count Petrifilms were incubated at 21°C for at least 48 h. The readable plate count was between 25 and 250 cfu and the final data were expressed as log cfu/mL, which was the logarithm to base 10 of the microorganism's population value per milliliter. Triplicates for each sample for 3 trials were tested.

Viscosity Measurement

Viscosity of whey protein solutions before and after gamma radiation at various dosages was determined at room temperature (23 ± 1°C) using a Brookfield Viscometer (Model DV-I Prime, Brookfield Engineering Labs Inc., Stoughton, MA). Samples were tested