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Short communication

Release behavior of quercetin from chitosan-fish gelatin edible films influenced by electron beam irradiation

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ABSTRACT

This work dealt with the study of the release kinetics of quercetin incorporated into chitosan-gelatin edible films after electron beam irradiation. The aim was to determine the influence of irradiation dose (at 40 and 60 kGy) on the retention of quercetin in the films, their release in a hydroalcoholic medium (30% ethanol v/v) at 25 °C. Irradiation induced a reduction of the release rate for quercetin, revealing that cross-linking probably occurred during irradiation. Indeed, the content (%) of quercetin remaining in the film after the release increased from 23.4 ± 5.7% for non-irradiated sample to 33.6 ± 2.1% after a 60 kGy irradiation dose. But the effective diffusion coefficient of quercetin was not significantly modified by the irradiation process. However, it was noticed a significant increase of the lag-time (time required before the release starts) by ten times. Thus, the irradiation influenced the retention by creating new interactions or linkages between biopolymers and the quercetin, which finally led to an entrapment of a significant amount of the antioxidant. As expected, the electron beam irradiation allowed modulating the retention and then the release of the antioxidant encapsulated in the chitosan-gelatin matrices.

1. Introduction

Edible films and coatings offer the opportunity to effectively control mass transfer among different components within a food system or between the food and its surrounding environment (Debeaufort, Voilley, & Guilbert, 2002; Hernandez-Izquierdo & Krochta, 2008). Moreover, a modern trend for developing active edible films and coatings is to combine different biological polymeric materials and to incorporate various functional ingredients, such as nutritional supplements, antimicrobial or antioxidant agents (Cheng, Wang, & Weng, 2015). The most frequently used materials for edible films and coatings are polysaccharides (such as starch, cellulose derivatives, alginate, pectin and chitosan), proteins (such as gelatin, zein, gluten, milk casein, whey and soy proteins) and lipophilic materials (such as glycerides, beeswax and shellac). These materials can be used either individually or in combination to produce films and coatings (Nesterenko, Alric, Silvestre, & Durrieu, 2013). Active packaging can be achieved when functional ingredients are incorporated.

Chitosan is a natural polymer obtained by the deacetylation of chitin, which is a fish industry by-product. It is among the most investigated polysaccharides for active edible films and coatings development due to its inherent antimicrobial, antifungal properties and good film forming ability (Fernández-Pan, Maté, Gardrat, & Coma, 2015). Gelatin is another widely used bio-based material obtained by the controlled hydrolysis of the insoluble fibrous collagen present in the bones and skin generated by fish processing wastes. Its excellent film forming ability is well-known (Hoque, Benjakul, & Prodpran, 2010). Gelatin and chitosan based films used for coating or packaging could maintain the quality of foods during storage, due to their good barrier to oxygen, light and prevention of dehydration and lipid oxidation (Jongjareonrak, Benjakul, Visessanguan, & Tanaka, 2006; Park & Zhao, 2004). In order to improve the food-protective capacity of chitosan and/or gelatin films, various active substances including synthetic antimicrobial and antioxidant agents or natural plant extracts have been added into the film for increasing the food shelf life (Wu et al., 2015). In acidic environment (pH < pKa) the amino groups of chitosan are protonated and their positive charges can interact with polyanions such as gelatin, at a pH lower than its isoelectric point, forming a polyelectrolyte complex. Due to these characteristics, chitosan and gelatin have been widely used for

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the production of edible films (Benbettaïeb, Karbowiak, Brachais, & Debeaufort, 2015a).

The incorporation of antioxidants in these biodegradable edible polymers is an interesting alternative to food preservation, since oxidation is one of the major problems affecting food quality as well as biopolymer film ageing (Martins, Cerqueira, & Vicente, 2012). The use of natural, non-toxic antioxidants such as ferulic acid or α-tocopherol is sought in order to be consistent with the consumer health (Benbettaïeb, Karbowiak, Assifaoui, Debeaufort, & Chambin, 2015; Fabra, Hambleton, Talens, Debeaufort, & Chiralt, 2011). Very few studies have established the effects of polymer structure, in particular chitosan-gelatin films, on the retention and release properties of the antioxidant compounds (Papadokostaki, Amarantos, & Petropoulos, 1998). Besides, irradiation have been shown as a promising technique to induce cross-linking between polymers and to improve the physical and functional properties of edible coatings (Benbettaïeb, Karbowiak, Brachais, & Debeaufort, 2015b; Vachon et al., 2000). However, the effects of irradiation treatment on the release mechanism of active compounds from edible films are not well-known. Lacroix et al. (2002) showed that gamma-irradiation was efficient enough for inducing cross-links in calcium caseinate edible films and could thus be envisaged for the immobilization of enzymes or active compounds. Gamma irradiation of caseinate also contributed to control the release. However, these works neither explained the mechanism involved in the release rate delay nor the impact of irradiation on the diffusivity of active molecules into the simulant media. The objectives of this study were to investigate the effects of electron beam irradiation on the release behaviour of quercetin in hydroethanolic medium.

2. Materials and methods

2.1. Materials and reagents

Commercial grade chitosan (CS) (France Chitine, ref 652, molecular weight of 165 kDa, low viscosity, deacetylation degree of 85%, France) and commercial grade fish gelatin (G) (Rousselot 200 FG 8, Bloom degree = 180, viscosity = 4 mPa s at 45 °C and pH = 5.4) were used as film-forming matrix. Anhydrous glycerol (GLY) (Fluka Chemical, 98% purity, Germany) was used as a plasticizer. Glacial acetic acid (Sigma, 99.85% purity) helped to improve the solubility of chitosan. Quercetin (minimum purity 99%) having a molecular weight of 302 g mol^{-1}, a melting point of 316 °C, a logP value of 1.47 and a solubility in water of 0.06 g L^{-1} (data from Chemspider) was purchased from Sigma Aldrich and used as a model of natural antioxidant molecule.

2.2. Film making

A 2% (w/v) chitosan solution was prepared by dispersing the chitosan powder in 1% (v/v) aqueous acetic acid. The solution was homogenized at 1200 rpm with an Ultra Turrax (RW16 basic- IKA-WERKE, Germany). Then, glycerol (10% w/w polymer dry matter) was added to the solution under stirring. A 6% (w/v) fish gelatin solution (pH = 4.9 ± 0.2) was prepared in distilled water under continuous stirring and heated at 70 °C for 30 min. Glycerol (10% w/w polymer dry matter) was added to this film forming solution after dissolution of gelatin. Gelatin and chitosan film forming solutions (CS and G) were then mixed in equivalent weight proportion 50%CS–50%G (dry matter), and stirred for 30 min, while adjusting pH between 5.2 and 6. This condition aimed at obtaining a polyelectrolyte complex between chitosan and gelatin, that can only occur at a pH above the isoelectric point of gelatin (pI = 4.5–5.2) to be negatively charged, and below the pKa of the amino group of chitosan (pH = 6.2–6.5) to be positively charged, and to prevent any phase separation. Quercetin was finally added to this film forming solution at a concentration of 50 mg per gram of dry matter. The aqueous dispersion was homogenized at 1200 rpm using an Ultra Turrax until complete dissolution. The film forming solution containing the antioxidant was then poured into plastic Petri dishes (13.5 cm diameter). They were then dried in a ventilated climatic chamber (KBF 240 Binder, ODIL, France) at 25 °C and 45% relative humidity (RH) for 18–24 h. After drying, films were peeled off from the surface and stored up to equilibrium in a ventilated climatic chamber (KBF 240 Binder, ODIL, France) at 50% RH and 25 °C before all measurements.

2.3. Radiation treatment

Radiation processing was carried out at the AERIAL pilot plant (Innovation Park, Illkirch, Strasbourg, France), using a linear electron accelerator at a temperature of 20 ± 0.5 °C. Dried films (65–80 μm thickness) were irradiated with electron beam carrying 2.2 MeV energy and 0.3 kGy/s dose rate. The doses used for this study were 40 and 60 kGy. One batch (with and without antioxidant) was also preserved as the non-irradiated reference. Dosimetry was performed using alanine pellet dosimeters calibrated according the international standard ISO 51607(2004E).

2.4. Release of quercetin in water/ethanol medium

The release of the quercetin was carried out in triplicate using a rotating paddle dissolution apparatus (AT7 Smart type II, Sotax, Basel). 600 mg of film was immersed in 1 L of a 30% ethanol solution (v/v) under stirring (50 rpm) at 25 ± 1 °C. A 3 mL sample was periodically withdrawn and assayed for quercetin release up to equilibrium. The amount of antioxidant in the release medium was determined by UV–vis spectrophotometery (Biochrom Libra S22) at 376 nm. A series of standard solutions (1, 2, 4, 5, 10, 25 and 50 mg/L) was used for calibration, according to the Beer–Lambert's law. The initial concentration of quercetin in the film during preparation was used to calculate the percentage of retention in the film at the end of the release kinetics by comparison with the amount released at equilibrium.

The effective diffusion coefficient of quercetin in the film (D) was also determined from the release kinetics assuming a Fickian mechanism, considering the transient state of the transfer.

The experimental method chosen corresponded to the case of diffusion from a stirred solution of limited volume. As the solution was constantly stirred, we assumed no boundary layer and an always uniform concentration in the solution. The initial concentration of antioxidant in the solution was equal to zero. The concentration of antioxidant in the film was assumed to be uniformly distributed within the film at time zero. We also considered a unidirectional diffusion of the antioxidant in the film, and a diffusivity which neither depended on the concentration nor on the time. Under those conditions, the following analytical solution to Fick second law was thus used (Crank, 1975):

$$\frac{C_t}{C_\infty} = 1 - \sum_{n=1}^{\infty} \frac{2\alpha(1+\alpha)}{1+\alpha+\alpha^2 q_n^2} \exp\left(-\frac{Dq_n^2 t}{l^2}\right) \quad (1)$$

where C_t is the concentration of the antioxidant determined in the dissolution medium over time, C_∞ is the maximum concentration of the antioxidant determined in the dissolution medium when equilibrium is achieved, $\alpha = V_s/(K \times V_f)$ with V_s the volume of solution (m^3), V_f the volume of film (m^3) and K the partition coefficient. q_n are the non-zero positive roots of $\tan(q_n) = -\alpha q_n$ using n values between 1 and 6. D is the effective diffusion coefficient