Characterization of sustained-release chitosan film loaded with rutin- β -cyclodextrin complex and glucoamylase

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Abstract Edible chitosan film incorporated with rutin- β -cyclodextrin was developed and characterized. The delivery of rutin was improved via the hydrolyzation function of glucoamylase, and the antioxidant activity of the chitosan film was enhanced by the addition of rutin. Sodium bicarbonate solution at different pHs (pH-adjusting reagent) was employed to afford the mild condition for the incorporated glucoamylase. The enzyme exhibited its hydrolyzation function to improve the release rate of rutin by destabilizing the rutin- β -cyclodextrin complex (RCC) in chitosan film. The optimum pH of glucoamylase was achieved with 5 mL addition amount of 0.5 mol/L sodium bicarbonate solution, and the glucoamylase improved the radical scavenging ratio of chitosan film. The yellowness of chitosan film was enhanced with the addition of RCC solution. The films prepared without water demonstrated

coarse and rough surface, while the water-based films had smoother and even surface as examined by scanning electron microscopy. In contrast, these observations disappeared in the water immersion groups. X-ray diffraction suggested that the hydrolyzation of β -cyclodextrin and the interlinkage between β -cyclodextrin and the chitosan chain exerted a negative function on maintaining the crystal structure of pure chitosan film. Further, the destabilization of RCC complex with the glucoamylase activity was evidenced by the absence of peak associated with β -cyclodextrin as observed from Fourier transform infrared spectra. The enzyme improved the release of rutin and the addition of RCC successfully endowed antioxidant activity to the chitosan film.

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Graphic abstract

Keywords Chitosan film · Rutin · β -Cyclodextrin · Glucoamylase

Introduction

Chitosan, the deacetylated derivative of chitin, is a versatile biopolymer with a number of food applications including environment-friendly packaging, preservation material, edible ingredients and biodegradable substances (Helander et al. 2001). Chitosan possesses wide range inhibition against spectrum for gram-positive and gram-negative bacteria, yeasts and moulds and exhibits biological activities, such as cholesterol reduction, adsorbent of heavy metals and immobilization of angiotensin converting enzyme (Zhou et al. 2013). Researchers focused on the addition of ingredients into chitosan film to enhance its functions with antioxidant activity, scavenging free radicals and other biological activities (Yan et al. 2012). Given the function of chitosan films to provide food quality and safety benefits with its characteristics, they are ideal for the new generation of food packaging (Siripatrawan and Noi-pha 2012). Chitosan possesses excellent film-forming ability and is suitable for the formulation of edible films. Chitosan film is a convenient packaging material, and its different forms, such as composite, modified and sustained-release chitosan films have been extensively investigated (Luo et al. 2015; Pranoto et al. 2005). Chitosan films are used to reduce water vapour, oxygen, lipid and flavour migration between components of multi-component food products, and compositing is one of the useful methods to

append functional properties to chitosan films (Rao et al. 2010). For example, improved mechanical and physical performance is acquired by compositing chitosan films. Reduction in lipid peroxide formation has been observed in ferulic acid-incorporated chitosan films, and strong inhibitory effects on *Escherichia coli* is proved in chitosan-potassium sorbate complex films (Jridi et al. 2014).

Rutin was first detected in *Ruta graveolens*, from which the common name of this pharmaceutically important substance was derived (Kreft et al. 2006). Rutin is a secondary plant metabolite and a highly valued compound with antifungal, antibacterial, anti-inflammatory, anti-cancer, radical scavenging and antioxidant abilities (Dawidowicz et al. 2016). Although rutin is widely present in plants, it is present in negligible amounts in edible plants. The incorporation of rutin into chitosan films enhanced the additional antioxidant function to the films.

Inclusion, used for numerous purposes, such as a flavour carrier in food, incorporates health-promoting ingredients in food and provides protection for active compounds against thermal oxidation and destruction of acids and bases (Karathanos et al. 2007). This approach is also used for improving water solubility, release rate and bioavailability of insoluble or poorly soluble ingredients (Zhu et al. 2007). β -Cyclodextrins is cyclic glucose oligomers having seven glucose units linked by α -1, 4-glucosidic bonds, it was formed by the enzymatic modification of starch and have the ability to form inclusion complexes with hydrophobic compounds. Recently, β -cyclodextrin is widely used to prepare inclusion products in food and pharmaceutical science. The inclusion of β -cyclodextrin

with the guest molecules can change physical and chemical properties of the guest molecules, such as increasing aqueous solubility and stability. Much interest has been concentrated on preparing inclusion complexes of β -cyclodextrin with various small molecules (Bhandari et al. 1998). Due to the α -1, 4-glucosidic bonds of this compound, it can be hydrolyzed by the glucoamylase that was used to destabilize the RCC to improving the release of rutin.

The solubility of chitosan and release rate of incorporated ingredients is critical factors in chitosan film application. This work developed a chitosan solution with moderate pH to protect the glucoamylase activity and embed glucoamylase into films to facilitate the release of rutin by hydrolyzing the RCC. The obtained chitosan film exhibited antioxidant activity with the addition of complex of β -cyclodextrin and rutin.

Material and methods

Materials

Medium-molecular-weight chitosan (CAS, 9012-76-4, deacetylation degree > 90%, Bide Biotechnology Ltd., Shanghai, China), rutin (CAS, 207671-50-9, Puyihua Biotechnology Ltd., Beijing, China), β -cyclodextrin (CAS, 68168-23-0, Zhongtai Food Ltd., Henan, China) and glucoamylase (CAS, 9032-08-0, 10^5 U/g, optimum pH area of 4.5–6.5, Aobo Kang Xing Biotechnology Ltd., Beijing, China) were used in this work.

Film formation

Preparation of rutin- β -cyclodextrin complex (RCC)

Saturated rutin solution (dissolved in ethanol at 25 °C) was slowly sprayed by the injector into the β -cyclodextrin aqueous solution (1%) with 15 min stirring condition, finally the solution of RCC was obtained by filtration.

Film-forming solution

Solution (100 mL) containing 1 g chitosan, 1 mL glycerol and 0.6 mL acetic acid was prepared, and 30 mL of this solution was mixed with certain volumes of 0.5 mol/L sodium bicarbonate, RCC solution and 1% glucoamylase solution, therefore the film-forming solution was obtained with the vacuum degassing. The films were prepared following the procedures described by Clara Pastor with slight modification (Pastor et al. 2013): the film-forming solution was casted into a plastic plate (9 cm diameter)

and dried at 40 °C for 48 h, and then the films were incubated at 50% relative humidity for 24 h.

Optimization of film-forming solution

PH change of film-forming solution during drying

Film-forming solution with certain volume (1, 3, 5, 7 and 9 mL) of sodium bicarbonate (0.5 mol/L), 4 mL of glucoamylase solution (1%) and 3 mL of RCC solution was dried at 40 °C. PH of the solution was measured following the evaporation of solution at its residual volumes of 100, 70, 40 and 10 mL. There were triplicate parallel trails for each sample.

Determination of RCC solution addition and glucoamylase solution addition

Films were prepared by certain volume of RCC solution (0, 1, 2, 3, 4 and 5 mL), 4 mL of glucoamylase solution (1%) and 30 mL of film-forming solution to determine the optimum addition amount of RCC, and then it was submerged in 30 mL water for 1 h. After taking out the film, they were immersed in to 30 mL water again, and the solution was taken and replaced with the same volume of distilled water every day. The solution at the first and the third day were used to evaluate antioxidant activity by ABTS radical scavenging assay. Certain volume of glucoamylase (0, 1, 2, 3, 4 and 5 mL) solution and 4 mL of RCC solution were added into the film to determine the optimum addition amount of glucoamylase. There were triplicate parallel trails for each sample.

ABTS radical scavenging assay

The ABTS radical scavenging assay was performed following the describe of Xican Li with slight modification (Li et al. 2012). The stock solution was diluted by PBS (pH 7.4) after settled in the dark for 24 h. The absorbance of the diluted solution was 1.00 ± 0.05 at 734 nm (PBS was the blanking solution). Leaching solution (0.5 mL) was mixed with the above-mentioned solution (ABTS reaction solution) and reacted for 6 min, and then the same volume of PBS (0.5 mL) was used for the blank. The absorbance of the ABTS reaction solution (A_0) and mixed solution (A) was employed to calculate the radical-scavenging activity (RSA) of leaching solution by the following the equation (Li et al. 2012):

$$RSA = (A_0 - A) / A_0 * 100\%. \quad (1)$$

Color properties

Colourimetry (CM-3600A, Konica Minolta, Japan) was employed to determine the color properties of the films. Total colour difference (ΔE) was calculated as follows (Yang et al. 2016).

$$\Delta E = \sqrt{(L^*)^2 + (a^*)^2 + (b^*)^2}. \quad (2)$$

Scanning electron microscopy (SEM)

Chitosan film (ChF), chitosan film containing glucoamylase and RCC (ChGRF), chitosan film containing RCC (ChRF), chitosan film with 5 days of water immersion (ChFW), chitosan film containing glucoamylase and RCC with 5 days of water immersion (ChGRFW) and chitosan film containing RCC with 5 days of water immersion (ChRFW) were detected by SEM.

The microstructure of the surfaces was intuitive using a scanning electron microscope (SEM) (FEI Sirion 200, Hong Kong, China). Before observing, the samples were placed on a metal stub with a double-sided adhesive tape and then coated under vacuum with gold in an argon atmosphere. All films were examined by an accelerating voltage of 5 kV.

X-ray diffraction (XRD) detection

Chitosan powered (ChP), glucoamylase powered (GP), β -cyclodextrin powder (CP), rutin powder (RP), powder of β -cyclodextrin and rutin (CRP), RCC powder (RP), powder of chitosan and RCC (ChRP), powder of chitosan, glucoamylase and RCC (ChGRP), ChF, ChGRF, ChRF, ChFW, ChGRFW and ChRFW were prepared in this trial.

Samples were analysed via XRD (D8-ADVANCE, Bruker AXS, Germany) equipped with a copper tube operated at 35 kV and Cu radiation of 0.154 nm wavelength. Diffractograms were obtained by scanning from 3° to 50° at a rate of 0.5°/min with the step size of 0.02 (Shujun et al. 2006).

Fourier transform infrared (FTIR) spectroscopy

FT-IR was employed to determine the interaction of ingredients in film formation and water immersion. A Nicolet 5700 spectrophotometer (Thermo Nicolet 5700, USA) was used to record the spectra of the samples. The powdered samples were mixed separately with analytical grade KBr and then pressed into discs. The spectra of the samples were recorded in the region of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} (Zarski et al. 2016).

Thermogravimetry (TG) analyses

The same samples in SEM were used to investigate thermodynamic property via TG, derivative thermogravimetry (DTG) and differential thermal analysis (DTA) by a thermogravimetric analyzer (Mettler Toledo, Germany). TG of films was heated from 40 to 600 °C with a heating rate of 10 °C/min in nitrogen atmosphere.

Results and discussion

Optimisation of film-forming solution constituent

Enzyme activity was critical in this edible film preparation. The film-forming solutions made by 1, 3, 5, 7 and 9 mL of sodium bicarbonate solution were evaporated at 40 °C, and the pH levels of the solutions were measured at the residual volumes of 70, 40 and 10 mL (Fig. 1a). This trial simulated the pH variation in film drying process to ensure that the pH variation was suitable to maintain the glucoamylase activity. Five curves with different volumes of sodium bicarbonate solution possessed the same rising trend with a similar slope. Given that the optimal pH range of the enzyme is 4.5–6.5, the original pH of the pre-preparation solution should exceed 4.5, that is 5 mL of sodium bicarbonate solution was added into film-forming solution.

Color property, an important quality factor, is essential to define the appearance of the coated product (Pereda et al. 2012). L_* represents lightness from complete opaque or blackness (value of 0) to complete transparency or whiteness (value of 100), a_* indicates redness (positive value) and greenness (negative value), and b_* reflects yellowness (positive value) and blueness (negative value). The total color difference (ΔE), obtained by $(a_*^2 + b_*^2)^{1/2}$, refers to the purity or saturation of color (Gonçalves et al. 2007). Values of L_* , a_* and ΔE decreased gradually following the increase in addition amount of RCC solution, and no significant change was observed in each colour component ($p > 0.05$). The addition of RCC solution significantly enhanced the yellowness of film, but more RCC had no contribution to the increase in b_* (Fig. 1b). This result suggested that the interlinkage between RCC, β -cyclodextrin or un-embedded rutin was responsible for the color change, and the addition of 1 mL of solution was sufficient to saturate the interlinkage.

The radical scavenging ratio of films with different volumes of RCC solution was detected at the first and the third day of water immersion (Fig. 1c). The radical scavenging ratio at third day of water immersion showed the

Fig. 1 Optimisation of film-forming solution constituent. **a** Changes in pH of film-forming solution during drying. The numbers (1, 3, 5, 7 and 9) were the addition volume of sodium bicarbonate solution, **b** color properties of film with different volumes of RCC solution (groups with same symbol had significant difference with $p > 0.05$), **c** radical scavenging ratio of films with different volumes of RCC

solution at 1 and 3 days of water immersion (groups with same symbols had significant difference with $p > 0.05$), **d** radical scavenging ratio of films with different volumes of glucoamylase solution at 1 and 3 days of water immersion (groups with same symbol had significant difference with $p > 0.05$) (color figure online)

optimum volume of RCC solution addition with the turning point at 3 mL addition, and the tendency of straight line was observed in the radical scavenging ratio at the first day of water immersion. The turning point at 3 mL addition of RCC solution in the radical scavenging ratio at the third day of water immersion illustrated that the addition of RCC solution should be more than 3 mL.

The enzyme was not the critical factor that controlled the release of rutin, and there was no significant difference between the radical scavenging ratios of five samples at the first water immersion day. The radical scavenging ratio at the third day showed a gradual increase, and films with 4 and 5 mL of enzyme solution presented the higher radical scavenging ratio than the films without enzyme addition at the third day of immersion ($p > 0.05$).

Morphology of films

SEM was employed to observe the morphology of the films. The black spot formed by glycerol permeation exhibited an almost round shape, and the smooth surface was observed in ChF (arrowheads in column ChF of Fig. 2). Large spots were found in ChGRF, while spots were barely observed in films without water immersion. This morphology suggested that the hydrolyzation of RCC and β -cyclodextrin afforded more possibility for the permeation of glycerol during film drying (arrowheads in column ChGRF of Fig. 2 and arrowheads in column ChRF of Fig. 2). A coarse surface with granular protrusion that possibly induced by the addition of RCC and β -cyclodextrin was observed in the chitosan- β -cyclodextrin composite film, and the hydrolysis of glucoamylase also induced less

Fig. 2 Morphologies of films observed using SEM (Bars in first, second, third and fourth rows are 300, 100, 50 and 20 μm , respectively). *ChF* chitosan film, *ChGRF* chitosan film containing glucoamylase and RCC, *ChRF* chitosan film containing RCC, *ChFW*

chitosan film with 5 days of water immersion, *ChGRFW* chitosan film containing glucoamylase and RCC with 5 days of water immersion, *ChRFW* chitosan film containing RCC with 5 days of water immersion

granular protrusions in *ChGRF* rather than in *ChRF* (arrows in the *ChGRF* and *ChRF* columns of Fig. 2).

Water immersion leading to the disappearance of glycerol pots and dissolution of granular protrusions endowed a tight and accordant surface to the water immersion group (columns *ChFW*, *ChGRFW* and *ChRFW* in Fig. 2). Scratches formed in the film preparation process and disappeared in water immersion were probably induced by the dissolution of RCC and β -cyclodextrin or the hydrolysis of the enzyme (arrowheads in column *ChGRFW* of Fig. 2). However, few granular protrusions were found in the surface of *ChRFW* (arrowheads in *ChRFW* of Fig. 2).

XRD detection

No peaks were found at 2θ of 20 in rutin, β -cyclodextrin, RCC and mixture of β -cyclodextrin and rutin, but the chitosan film and powder presented peaks at around the same position (Fig. 3a, b). This result suggested that the main peaks in all films located at about 2θ of 20 were inherited from the peak of chitosan powder or chitosan film (chitosan in Fig. 3b). Jagged peaks in the 2θ of 8–12.88 in *ChGRF* of Fig. 3c, d were caused by RCC, β -cyclodextrin, glucoamylase, rutin and their mixture (Fig. 3a). The complex showed peaks at $2\theta = 10.58$, which was covered by chitosan and glucoamylase in the film and powder mixture

(Fig. 3a). Interlinkage between β -cyclodextrin and chitosan chain was destroyed by hydrolysis, which induced the aggregation of β -cyclodextrin resulting in the appearance of peak at 2θ of 10.7 in the water immersion groups (Fig. 3d). Furthermore, the peaks at 2θ of 22.54 in the water immersion groups were also induced by the hydrolyzation of aggregation of β -cyclodextrin. The chitosan film presented a higher peak at about $2\theta = 20$ than *ChRF* and *ChGRF* illustrating that the addition of RCC and glucoamylase decreased the crystal structure of the chitosan film (chitosan of Fig. 3c). Peaks at 2θ of 27.96 and 28.3 in *ChGRF* and *ChGRFW* were supposedly induced by glucoamylase, nevertheless, water immersion decreased the content of glucoamylase in films (the low peak appeared at $2\theta = 28.3$ in *ChGRFW*). RCC with two noticeable peaks at 2θ of 31.92 and 34.8 showed no peaks at the same position after mixing with chitosan, and they shifted to 2θ of 32.1 and 33.94 in *ChGRF* (Fig. 3a, b).

ChGRF showed a higher peak than *ChRF* in Fig. 3c, however, a reverse result was observed in the water immersion groups (Fig. 3d). Interlinkage between β -cyclodextrin and the chitosan chain showed a negative effect in maintaining the crystal structure of chitosan film. Therefore, the rearrangement of ingredients in the film induced by the hydrolysis of enzyme during film drying led to a tight crystal structure of chitosan film. Glucoamylase induced β -cyclodextrin destabilization that resulted in the

Fig. 3 XRD detection of the combination of ingredients and films. *RP* rutin powder, *RCCP* RCC powder, *GP* glucoamylase powder, *ChP* chitosan powder; *CP* β -cyclodextrin powder, *CRP* powder of β -cyclodextrin and rutin, *ChRP* powder of chitosan and RCC, *ChGRP* powder of chitosan, glucoamylase and RCC, *ChF* chitosan film,

ChGRF chitosan film containing glucoamylase and RCC, *ChRF* chitosan film containing RCC, *ChFW* chitosan film with 5 days of water immersion, *ChGRFW* chitosan film containing glucoamylase and RCC with 5 days of water immersion, *ChRFW* chitosan film containing RCC with 5 days of water immersion

release of rutin and the breakage of the interlinkage between β -cyclodextrin and chitosan. Therefore, the typical peaks of chitosan at 2θ of 20.38 was distinct (Fig. 3d). Even the typical peaks of chitosan at ChFW and ChRFW presented similar height, the crystal structure destroyed by the complex showed a wide peak in ChRFW (Fig. 3d).

FT-IR spectra analysis

Peaks in the range of 700–850 cm^{-1} indicating the benzene ring of rutin, seven-membered ring of β -cyclodextrin and the RCC were reduced by the addition of chitosan and glucoamylase (Fig. 4a, b) (Anjali et al. 2005), moreover, reduced peaks were also identified in the chitosan film (Fig. 4c, d). The film with water immersion presented a smoother line in the same range compared with the groups without water immersion. In particular, release of ingredients from ChGRFW in water immersion was induced by the hydrolyzation of β -cyclodextrin and resulting in the release of rutin and the smooth line (Fig. 4d). Peaks

identified at around 856 and 940 cm^{-1} in I, M, β -cyclodextrin and ChRP shifted to 850 and 930 cm^{-1} , respectively, and peaks identified at around 856 cm^{-1} were identified as the typical peaks of β -cyclodextrin (probably the cyclic structure). These above-mentioned typical peaks were eliminated by the addition of glucoamylase (Fig. 4b), which was proven by the hydrolysis and dissolution of β -cyclodextrin in the water immersion groups (Fig. 4d). The peak of β -D configuration that should be attributed to chitosan at about 898 cm^{-1} was concealed by the addition of enzyme and RCC (Fig. 4c and ChGRP of Fig. 4b), and the appearance of this peak in the water immersion groups was observed. The function of glucoamylase induced a relatively high peak at 898 cm^{-1} because of its hydrolysis function (Fig. 4d).

The absorption bands at around 1031 and 1081 cm^{-1} were characteristics of C–O–C and C–O (Subramanian and Vijayakumar 2012), in addition, they shifted to 1033 and 1076 cm^{-1} in the water immersion groups, respectively (Fig. 4d). The C–O group was eliminated in the film

Fig. 4 FT-IR spectra of ingredients and films. *RP* rutin powder, *RCCP* RCC powder, *GP* glucoamylase powder, *ChP* chitosan powder, *CP* β -cyclodextrin powder, *CRP* powder of β -cyclodextrin and rutin, *ChRP* powder of chitosan and RCC, *ChGRP* powder of chitosan, glucoamylase and RCC, *ChF* chitosan film, *ChGRF*

chitosan film containing glucoamylase and RCC, *ChRF* chitosan film containing RCC, *ChFW* chitosan film with 5 days of water immersion, *ChGRFW* chitosan film containing glucoamylase and RCC with 5 days of water immersion, *ChRFW* chitosan film containing RCC with 5 days of water immersion

without water immersion by hydrogen bonds between chitosan, β -cyclodextrin and rutin. Water immersion induced the breakage of hydrogen bonds, the production of monosaccharide and polysaccharide resulting in strong C–O vibration.

Vibrations at around 1080 (glucose ring), 1155 (glucose ring), 1380 ($-\text{CH}_2$) and 1410 cm^{-1} ($-\text{CH}_2$) were attributed to the glucose ring, and they did not change in the film (Deladino et al. 2008; Harish Prashanth et al. 2002). The vibration at 1650 cm^{-1} in Fig. 4a was formed by the formation of RCC and disappeared in ChIP and ChGIP. The C=C bending of benzene ring, which represented rutin (Fig. 4a), also disappeared after incorporated by the β -cyclodextrin. The vibrations at 1602 ($-\text{NH}_2$) and 1654 cm^{-1} ($-\text{NH}$) in Fig. 4b shifted to 1563 and 1658 cm^{-1} respectively, which was attributed to the formation of hydrogen bonds between chitosan and β -cyclodextrin (Fig. 4c).

Meanwhile they were transferred to 1574 and 1647 cm^{-1} , respectively, in the water immersion groups (Fig. 4d) (Mun et al. 2008; Subramanian and Vijayakumar 2012). ChIGFW possessed a sharp peak at 1574 cm^{-1} and decreased absorption at 1647 cm^{-1} showing that the hydrogen bonds between β -cyclodextrin were destroyed, therefore much more free $-\text{NH}_2$ was produced by enzyme hydrolysis.

The bands at 2925 cm^{-1} in Fig. 4a and 2924 cm^{-1} in Fig. 4b were ascribed to the vibration of methylene in chitosan and methylene in β -cyclodextrin (Fig. 4b) (Li et al. 2011). These bands were located at 2885 and 2973 cm^{-1} in films with water immersion and at 2854 and 2924 cm^{-1} in films without water immersion. ChRFW showed a sharper peak at 2924 cm^{-1} compared with ChGRFW and ChFW specifying that the destabilization of β -cyclodextrin decreased the peak.

TG and DTG analyses

Troughs at around 80–90 °C identified in film without water immersion were mainly induced by water evaporation (Fig. 5a, c), while groups with water immersion presented low evaporation temperature at 70 °C (Fig. 5b, d). Following water evaporation, hydrogen bonds between chitosan to water decreased, which increased interlinkage between chitosan to chitosan, improved mechanical property of the film. The water in the film interlinked with the chitosan chain, therefore this portion of water could not escape easily and the water evaporation observed at a high temperature (circles in Fig. 5c, d).

Expanded width of the water immersion line was attributed to the penetration of water molecules, which resulted in the increased of the binding between water and chitosan chain. The facilitated evaporation induced by the alleviated binding force of hydrogen bond led to low water evaporation temperature of the water immersion groups (Fig. 5d). A small amount of weight loss was detected after water immersion owing to the same weakened binding force between water and chitosan (Table 1).

Table 1 Thermal degradation temperature and the weight loss of samples

	Td	Δw	Td	Δw
ChF	83.8	12.73%	232.8	52.24%
ChGRF	90.3	13.62%	237.8	54.05%
ChRF	84.3	9.89%	230.3	50.25%
ChFW	72.9	11.99%	291.3	36.31%
ChGRFW	79.7	10.68%	297.3	40.49%
ChRFW	71.3	9.71%	257.8	42.22%

ChF chitosan film, *ChGRF* chitosan film containing glucoamylase and RCC, *ChRF* chitosan film containing RCC, *ChFW* chitosan film with 5 days of water immersion, *ChGRFW* chitosan film containing glucoamylase and RCC with 5 days of water immersion, *ChRFW* chitosan film containing RCC with 5 days of water immersion

Thermal degradation temperatures (Td) and the weight loss (Δw)

β -Cyclodextrin was hydrolyzed by glucoamylase and the hydrolyzate was released into water, which increased the exposure of binding sites in ChGRFW. The binding sites exhibited similar water binding capacity as shown by the flat peak of ChGRFW (stable water evaporation rate), by

Fig. 5 TG and DTG analyses of six films. *ChF* chitosan film, *ChGRF* chitosan film containing glucoamylase and RCC, *ChRF* chitosan film containing RCC, *ChFW* chitosan film with 5 days of water

immersion, *ChGRFW* chitosan film containing glucoamylase and RCC with 5 days of water immersion, *ChRFW* chitosan film containing RCC with 5 days of water immersion

contrast, the bottom of the troughs (curve of ChGRFW in Fig. 5d) was difficult to be identified. Hydrolysis function of glucoamylase decreased the binding force between water and chitosan, subsequently; more water was lost in ChGRFW compared with ChRFW (Table 1). β -Cyclodextrin and its hydrolyzate also presented strong capacity to combine with water in films during film drying, which reinforced the water combining capacity of film and improved the evaporation temperature of ChGRF (Fig. 5a, c). The water combination capacity of ChGRF was reinforced and more water was maintained in film drying process, which brought about the more obvious water evaporation in TG detection process of ChGRF than other groups (Table 1). ChRFW that underwent the dissolution of β -cyclodextrin in water immersion showed a low evaporation temperature.

The decomposition temperature of chitosan films of 230 °C in the non-water immersion groups (rectangle in Fig. 5c) and 290 °C in the water immersion groups (rectangle in Fig. 5d) were ascribed to a complex process that included dehydration of the saccharide rings and the depolymerisation and decomposition of the acetylated and deacetylated units of polymers (Kweon et al. 2001). The space between groups in water immersion samples was enlarged by hydrolysis and dissolution, which resulted in the difficult reaction of two groups and the improvement in decomposition temperature. The large space generated by hydrolysis in water immersion in ChGRFW maintained the highest decomposition temperature. The amalgamation and decomposition (interacting with chitosan) of small-molecule compounds, such as β -cyclodextrin and their hydrolyzate, were possibly responsible for the high weight loss (Table 1). The weight loss at 600–640 °C was induced by the thorough decomposition of the main chain of the film, but no weight loss was observed in the non-water immersion groups. Less space and tight interlinkage of those samples improved the thermostability of films. ChGRFW lost most of the small-molecule compounds by hydrolysis because of the water dissolution.

Conclusion

More than 5 mL of sodium bicarbonate solution (2.5 mmol/L) could improve the pH to fit the optimum pH of enzyme in film drying process. The loop of β -cyclodextrin was broken by the hydrolyzation of glucoamylase resulting in the release of rutin from RCC in film drying and water immersion process. Decomposition of RCC also facilitated the penetration of glycerol and, which induced the generation of large black spots on the ChGRF. The coarse surface with granular protrusion in ChGRF and ChRF was probably formed by the addition of RCC,

however, water immersion was responsible for the disappearance of glycerol pots and the dissolution of granular protrusions. The characteristic peak of chitosan located at 2θ of 20 was decreased by the addition of RCC and glucoamylase, which suggested that the tight structure of chitosan film was weakened and the release ratio of rutin was promoted. The results also illustrated that the glucoamylase facilitated the decomposition of RCC and the rutin release. The addition of glucoamylase promoted the release of rutin from the chitosan-RCC complex film, and the incorporated RCC was successfully appended the antioxidant ability to the film.

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