Interaction between rice bran albumin and epigallocatechin gallate and their physicochemical analysis

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Abstract Epigallocatechin gallate (EGCG) is sensitive to heat thus its application in food industry is limited. In this work, rice bran albumin protein (RAP) was used as a carrier for EGCG. RAP-EGCG complexes (RAPE) were prepared with the binding number n of 0.0505:1 (EGCG:RAP, w/w) and binding constant K of $(0.74 \pm 0.002) \times 10^4 \text{ M}^{-1}$, which suggests that hydrogen bond/van der Waals forces played important roles in such binding. FTIR analysis demonstrated that EGCG could induce the secondary structure changes of RAP above the ratio of 1.92:1 (EGCG:RAP, w/w). Dynamic light scattering and scanning electron microscope results showed that EGCG could trigger RAP association. Furthermore, the EGCG stability in RAPE was significantly improved than that of free EGCG in 10–60 °C. The antioxidant ability of EGCG in RAPE was partially retained. These findings prove that RAP is a potential carrier for polyphenols and is beneficial for mechanism investigation between protein and polyphenols.

Keywords Tea polyphenols · Rice bran albumin · Carrier · Binding

Introduction

Tea polyphenols is the main active ingredient in green tea, one of whose major ingredients is found to be epigallocatechin-3-gallate (EGCG) (Wang et al. 2012). It has been shown that green tea, especially EGCG, owns many biological properties, such as antioxidant and free radical-scavenging activity (Lorenzo et al., 2013). Meanwhile, its ability to quench oxygen free radicals is more significant than those of Vitamin C and Vitamin E (Yang et al., 2002). However, tea polyphenols are sensitive to heat, metal ion, light, and pH due to the special chemical structure, which limits their commercial application in food, medicine, and health products (Su et al., 2003).

Recently, several materials such as protein, polysaccharides, lipids and other macromolecules all have been used to encapsulate and deliver the bioactive components such as polyphenols in food industry (Pekkinen et al., 2014; Quintanilla-Carvajal et al., 2010; Santos et al., 2013; Schramm et al., 2003; Uchiyama et al., 2011). To realize the effective encapsulation, the water solubility of the materials is an important index. The lipid and the polysaccharide with high molecule weights are usually insoluble in the water, which limits their usages in certain water based systems (Jakobek, 2015). Differently, proteins are a kind of biological macromolecules and they are usually soluble in water due to the amphoteric character which promotes their application in water based foods. In addition, colloidal particles can be formed by the cross-link between each protein molecules; during this process, polyphenols can be effectively encapsulated (Siebert, 1999). More importantly, tea polyphenols can bind to a wide variety of proteins, and molecule bridges such as hydrogen bonds can be formed among protein molecules, phenolic hydroxyls, and aromatic rings (Jöbstl et al., 2006;

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Nagy et al., 2012; Yuksel et al., 2010). Besides, proteins have a complex and porous structure which can trap polyphenols and thus improve their availability while protecting them against oxidation (Quintanilla-Carvajal et al., 2010; Schramm et al., 2003). The ligand-binding proteins can also enhance the bioavailability and bioaccessibility of polyphenols (Ribnicky et al., 2014).

Rice bran is one of the most abundant byproducts in the rice processing industry, and the annual production of rice bran in China is over 10 million tons. There are four kinds of rice bran proteins, namely albumin, globulin, prolamin and glutelin. Albumin is the most abundant and nutritional component among these four kinds of proteins. In addition, rice bran albumin protein (RAP) can be readily absorbed and utilized by the body, and has been found to be of high quality and of importance for food and pharmaceutical applications (Fabian and Ju, 2011; Shoji et al., 2001). Particularly, RAP is hypoallergenic and contains a large quantity of essential amino acids (Shih et al., 1999). Therefore, RAP may act as a suitable ingredient for infant food and diets of children due to its hypoallergenic character (Wang et al., 1999). Nowadays, the extraction and basic properties of RAP have been extensively studied, the RAP hydrolysates are also prepared to be used as nutritional supplements and functional ingredients (Fabian and Ju, 2011). It is noteworthy that RAP is water-soluble and has a porous structure which has the potential for the collection and encapsulation of bioactive compounds. Nevertheless, few studies have referred to the usage of porous structure of RAP as a carrier for the stabilization of bioactive compounds. The encapsulation effect and the interaction mechanism between RAP and tea polyphenol are worthy of investigation.

In this work, the RAP and EGCG were used as materials to prepare the rice bran albumin protein-EGCG complexes (RAP-EGCG). The stoichiometry and binding constant were obtained by fluorescence spectrometry. The structural characteristics of RAP and RAP-EGCG were compared by using Fourier transform infrared (FTIR) spectroscopy and scanning electron microscope (SEM). Dynamic Light Scattering (DLS) was used to explore the size of protein influenced by EGCG. Furthermore, the stability of EGCG induced by RAP was also studied. This work provides a new approach to fabricate protein–polyphenols co-assemblies and is also beneficial for the mechanism investigation between protein and polyphenols.

Materials and methods

Materials

Rice bran was purchased from Qinghe Oil Co. Ltd (Jiangxi, China), its particle size is 60 meshes and the content of fat is less than 2%. Epigallocatechin-3-gallate (EGCG) was obtained from Solarbio Biotechnology Co. (Beijing, China), and its purity is higher than 98%. All other reagents used were of analytical grade.

The extraction of RAP

The defatted rice bran (100 g) was extracted with distilled water of 1.5 L for 4 h at room temperature by the use of a magnetic stirring apparatus, which was followed by centrifugation at 6000 r/min for 15 min to obtain the albumin fraction (supernatant). The albumin was obtained by adjusting the pH of the extracts to 3.3 (isoelectric point), and the ensuing solution was placed in the dark for 1 h to produce the precipitates. The precipitated proteins were obtained by centrifugation at 6000 r/min for 15 min and were redistilled in distilled water. Then the pH was neutralized before freeze-drying.

Protein gel electrophoresis

Electrophoresis of proteins under denaturing conditions was done in 15% SDS-polyacrylamide gel. The 3% protein samples (5 μ L) were mixed with the (10 μ L) sample buffer, which consisted of 25% glycerol, 12.5% 0.5 M Tris-HCl, pH 6.8, 2% (w/v) SDS, 1% (w/v) bromophenol blue and 5% β -mercaptoethanol. After the solution was boiled for 10 min, electrophoresis was conducted at 15 mA for 2 h. Gels were stained with Coomassie Brilliant Blue R-250. Protein concentration was determined according to the Lowry method by the use of bovine serum albumin as a standard.

Preparation of RAPE

The RAP-EGCG complexes (RAPE) were prepared by mixing the RAP (1 mg/mL, 5.0 mL, pH 7.0) with different amounts of EGCG (a mass ratio of 0, 0.64:1, 1.28:1, 1.92:1, 2.56:1, 3.20:1, EGCG: RAP, w/w) and stirring for 30 min, and then the complexes were stood at 25 °C for 2 h and were frozen-dried to powder before use.

Fluorescence spectrometry measurements

Fluorescence quenching titration experiments were performed by using the Cary Eclipse fluorescence

spectrophotometer (Varian Incorporation, USA), and the quartz cuvettes is 1 cm path length. All experiments were carried out in 20 mM PBS buffer (pH 7.0) at 25 °C temperature. In the fluorescence titration experiment, the concentration of RAP was 0.8 mg/mL in 20 mM PBS, pH 7.0, at 25 °C. The titrations were conducted by adding EGCG (22.0 mg/mL) of 0.82–17.18 μ L increments to RAP (1 mg/mL) of 1.5 mL to make different mass concentration ratios (0, 0.015:1, 0.030:1, 0.045:1, 0.060:1, 0.075:1, 0.105:1, 0.135:1, 0.150:1, 0.165:1, 0.180:1, 0.240:1, 0.255:1, 0.300:1, EGCG:RAP, w/w), which was followed by fluorescence intensity detection. The excitation wavelength was 290 nm, and the emission wavelength was 300–500 nm, a slit width of 5 and 10 nm were set for excitation and emission, respectively. To obtain the stoichiometry and binding constant, the data were fitted to Eq. (1) for the binding of EGCG to n independent binding sites on the protein according to previously described methods (Bou-Abdallah et al., 2008; Lv et al., 2013).

$$I = I_0 - \left(\frac{I_0 - I_\infty}{2n[P]_0} \right) \times \left[1/K + [EGCG]_0 + n[P]_0 \right] - \sqrt{\left(1/K + [EGCG]_0 + n[P]_0 \right)^2 - 4n[P]_0[EGCG]_0} \quad (1)$$

where n is the binding site number and K is the apparent binding constant, $[P]_0$ and $[EGCG]_0$ are the RAP and EGCG concentrations, and I_0 and I_∞ are the relative fluorescence intensities of the protein in the absence and presence of EGCG when the binding sites are fully saturated, respectively.

Fourier transform infrared (FTIR) spectroscopy measurements

The infrared spectra of samples were measured with the potassium bromide pellet method by the use of a Spectrum Nicolet iS50 Fourier transform spectrophotometer (Thermo Fisher). Frozen-dried samples RAPE (a mass ratio of 0, 0.64:1, 1.28:1, 1.92:1, 2.56:1, 3.20:1, EGCG:RAP, w/w) were mixed with potassium bromide (KBr) in a ratio of 1:150 (w/w) and homogenized in an agate grinder. The FTIR scanning conditions were as follows: a spectral range of 400–4000 cm^{-1} , 16 scans and a resolution of 4 cm^{-1} . KBr was used as a reference. Spectrum acquisition of each sample was repeated for three times under the same condition. FTIR spectra were smoothed out and their baselines were corrected automatically by using Thermo Scientific OMNIC software.

Scanning electron microscope (SEM) analysis

The structural changes of RAP and RAPE under different conditions were analyzed by using field scanning electron microscopy (SEM, Hitachi SU-1510, Japan). The samples were fixed with conductive adhesive on aluminum support and sputter-coated with gold; and then they were observed at an accelerating voltage of 20 kV. The average cross section area of the pores was calculated by using image-processing tools of the ImageJ software.

Dynamic light scattering (DLS) experiments

The dynamic light-scattering measurements were performed at 25 °C by using a Viscotek model 802 dynamic light-scattering instrument (Viscotek Europe Ltd.) as previously reported (Li et al., 2009). The hydrodynamic radius R_H was calculated with the regularization histogram method by using the spheres model, from which an apparent molecule mass was estimated according to a standard curve calibrated from known globular protein. OmniSIZE 2.0 software was used to calculate the size distribution of aggregated protein from the addition of EGCG.

The determination of the retention ratio of EGCG

The de-stabilizing of EGCG is usually characterized at the low temperature ranges (≤ 100 °C) and the high temperature ranges (> 100 °C) (Wang et al., 2008). In this work, we chose to carry out the stability experiment at a lower temperature range of 4–80 °C to simulate the storage process (4–30 °C), incubation process (40–60 °C), pasteurization process (70–90 °C), and boiling process (100 °C), respectively. The samples were analyzed by measuring the absorbance at 274 nm through HPLC according to the previous report (Yang et al., 2017). EGCG standard curve was made out to calculate the retention of EGCG in different heated free EGCG and that of RAP system. Briefly, to evaluate the stability of EGCG in RAPE exposed to thermal processing, RAPE solution of 10.0 mL was poured into a water bath (Model 181 DK-8D, Tianjin Honour Instrument Co., Tianjin, China) at 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 °C in a dark tube for 12 h, respectively. At each point in time, 0.3 mL of each above solution was extracted and adjusted to pH 2.4 by the addition of HCl (1 M) to separate the EGCG from RAP, which resulted in the release of the EGCG. Then the solution was transferred to an Amicon Ultra-3K centrifugal filter device (Pall Corp.). What follows is the centrifugation at 5000 rpm for 20 min, free EGCG which penetrated the Ultracel membrane was determined by HPLC (Xue et al.,

2014). Retention ratio of EGCG after treatment was calculated according to Eq. (2) as following,

$$\text{Retention ratio}(\%) = \frac{\text{Residual EGCG}}{\text{Encapsulated EGCG}} \times 100 \quad (2)$$

DPPH radical-scavenging activity

The antioxidant activities of the EGCG before and after RAP binding were determined according to the method of DPPH radical-scavenging capacity. Briefly, 1 mL of RAPE (protein concentrations were set as 0.01 mg/mL, 0.02 mg/mL, and 0.03 mg/mL, respectively) were added to 3 mL of DPPH (0.04 mg/mL) dissolved in deionized water. The free EGCG and RAP (with the same EGCG or protein concentrations to RAPE) were used as control samples for detection. Absorbance at 517 nm was determined after 1 h incubation at room temperature in the dark. All experiments were carried out in triplicate, and the antioxidant activity was calculated as followings:

$$\text{DPPH radical-scavenging ratio}(\%) = (1 - A_e/A_o) \times 100 \quad (3)$$

where A_o is the absorbance without sample and A_e is the absorbance with sample.

Statistical analysis

All data analysis was performed by using Origin 8.5 software (Micro Cal Inc.). The results were analyzed by using the Statistical Analysis System (SAS 9.0) package software for the analysis of variance, Duncan's test. The significance was established at $P \leq 0.05$. All experiments were carried out in triplicate.

Results and discussion

Preparation of RAP

After calculation of the protein content in RAP sample by the Lowry method, a content of 0.712 g protein per 1 g RAP sample was obtained with a purity of 71.2% (w/w). SDS-PAGE was performed to analyze the purity of RAP. It was found that the molecular weight of the RAP subunit was approximate 14.4 kDa (Fig. 1), which was consistent with the previous reports (Chanput et al., 2009; Hamada, 1997) and indicative of a successful preparation. However, some reported molecular weights of rice bran albumin are mainly in the range of 10–100 kDa (Adebisi et al., 2009; Tang et al., 2003). The reason behind the distinction of

Fig. 1 SDS-PAGE analysis of RAP

molecular weights may be due to differences of the materials and extraction method. It should be noted that the protein band was not.

Fluorescence and FTIR spectra analysis

The intrinsic fluorescence from tyrosine (Tyr) and tryptophan (Trp) is a very useful tool for studying the interaction and binding between polyphenol and protein. As is well-known, Trp has intrinsic fluorescence residues when exciting at 290 nm, and the intrinsic fluorescence emission from Trp residue is sensitive to changes of the surrounding microenvironment (Ware, 1962). The fluorescence spectra of RAP in the absence and presence of different concentrations of EGCG in 20 mM PBS buffer (pH 7.0) was shown in Fig. 2(A). Results showed that RAP exhibited a strong fluorescence peak at 360 nm at $\lambda_{ex} = 290$ nm, which indicated that most of the observed fluorescence was attributed to the Trp residue (Wang et al., 2015), whereas EGCG had no fluorescence under the present experimental conditions. Meanwhile, a significant fluorescence quenching of RAP was observed with the increase of EGCG concentration, which demonstrated that there was a strong interaction between EGCG and RAP. In addition, to establish the binding stoichiometry, the fluorescence data were fitted to Eq. (1) to calculate the binding of EGCG to n independent binding sites on the protein and the binding constant K (Bou-Abdallah et al., 2008; Lv et al., 2013). Fluorescence titration revealed that approximately $n = 0.0505$ of the EGCG (mass ratio) was attached to a RAP molecule and the binding constant K of EGCG to RAP was $(0.74 \pm 0.002) \times 10^4 \text{ M}^{-1}$ [Fig. 2(B)], which indicates that the Hydrogen bond and van der Waals forces plays important roles in the interaction between EGCG molecules and RAP (Frazier et al., 2006; Lv et al., 2013). This finding is consistent with previous reports that the polyphenols can interact with proteins by non-covalent forces including hydrogen bonding, hydrophobic bonding,

Fig. 2 (A) Fluorescence intensity of RAP in PBS (pH 7.0) treated with different mass concentrations of EGCG at 25 °C. Conditions: [RAP] = 0.8 mg/ml; [EGCG]: 0, 0.015, 0.030, 0.045, 0.060, 0.075, 0.105, 0.135, 0.150, 0.165, 0.180, 0.240, 0.255, 0.300 mg/ml.

and van der Waals forces (Bourvellec and Renard, 2012; Li et al., 2015).

To further investigate the interaction between RAP and EGCG, FTIR analysis was applied to detect the FTIR spectra changes of both native and EGCG-binding state of RAP. FTIR is a very useful technique for studying the structure in protein and the spectra changes of the amide regions which could suggest the conformational changes in the secondary structure of proteins (Metrick and MacDonald, 2015). The amide I and II regions between the wavenumbers 1600–1700 and 1480–1575 cm^{-1} can respectively give information on the secondary structure of protein (Murayama and Tomida, 2004; Yang et al., 2015). The transmittance spectra of RAP in the absence and

$\lambda_{\text{Ex}} = 290 \text{ nm}$, λ_{Em} was from 300 to 500 nm, slits for excitation and emission of 5 and 10 nm, respectively. (B) The fitting equation of fluorescence intensity according to Eq. (1)

presence of different concentrations of EGCG in 20 mM PBS buffer (pH 7.0) were depicted in Fig. 3. With the increases of EGCG concentration, the wavenumbers of the amide I and II bands shifted from 1650 and 1550 to 1625 and 1525 cm^{-1} , respectively, which indicated that the interaction between EGCG and RAP could result in the secondary structure changes of RAP (Dembereldorj et al., 2012). Meanwhile, the shift would happen until the mass ratio was more than 1.92 (mass ratio). These results suggested that the EGCG dose was an important parameter that significantly influences the secondary structure of RAP. Previous studies have shown that polyphenol binding to the back bond of the protein molecule could lead to the unfolding the protein chain, and this binding may affect the secondary and tertiary structure of protein molecules (Bandyopadhyay et al., 2012; Maiti et al., 2006). Therefore, associated with fluorescence result, EGCG molecules could affect the folding of the protein chain, thus changing the secondary structure of protein through the Hydrogen bond and van der Waals forces.

RAP association induced by EGCG

SEM analysis was used to study the morphology of RAP induced by EGCG. Loosened porous structure of RAP without EGCG can be seen in the bright-field pictures shown in Fig. 4(A). After RAP was exposed to EGCG, the pores of protein decreased and the aggregation increased significantly [Fig. 4(B)], which indicated that EGCG was able to facilitate RAP association. The fluorescence and FTIR results obtained in this work suggested that the

Fig. 3 Representative FTIR transmittance spectra of RAP in pH 7.0 treated with different mass concentrations ratio of EGCG (0, 0.64:1, 1.28:1, 1.92:1, 2.56:1, 3.20:1, EGCG: RAP, w/w) at 25 °C

Fig. 4 SEM images of (A) single RAP and (B) RAPE upon treatment with EGCG (3.2:1, EGCG: RAP, w/w), all the scale bar is 10 μm

EGCG molecules could induce the association of protein by hydrogen bond and hydrophobic interactions, which is consistent with the previous work (Wang et al., 2014).

Dynamic light scattering (DLS) was used to further investigate RAP association in solution. All samples were allowed to stand for 10 min at room temperature prior to DLS measurements at pH 7.0. Specifically, one population with size distribution of 19.9 nm was evident in the scattered light intensity distribution curve of the RAP sample (Fig. 5). After the addition of EGCG to RAP, the size distribution of the sample was markedly altered towards larger aggregates. Particularly, after adding EGCG to RAP at a ratio of 2.0: 1 (w/w), the size populations were distributed in about 52, 114 and 451 nm, respectively (Fig. 5). These results demonstrated that the cross-linking between RAP and EGCG led to the aggregation of RAP, which is in accord with the SEM results (Fig. 4). Previous studies

showed that EGCG had a high affinity for proline-rich proteins and polymers, and the interaction between salivary proteins and polyphenols even resulted in insoluble aggregates by hydrogen bonding and hydrophobic interactions (de Freitas and Mateus, 2001; Poncet-Legrand et al., 2007). Regarding RAP is rich in proline residues (Adebiyi et al., 2009), we inferred that EGCG could likewise induce protein association by hydrogen bond and van der Waals forces between the phenolic hydroxyl of EGCG and the amide carbonyl of the peptide backbone in RAP.

Effect of RAP on the stability of EGCG

Phenolic compounds such as EGCG are generally not chemically stable when exposed to light, heat and oxidation, and this leads to low bioavailability and short biological half-life (Dube et al., 2010; Liazid et al., 2007; Su et al., 2003; Xue et al., 2014). Proteins have a complex and porous structure which is potential for encapsulating or binding with polyphenols. On the other hand, polyphenols can interact with proteins by non-covalent forces which may stabilize the polyphenols and meanwhile retain the natural polyphenol structure (Shpigelman et al., 2010). This raised an interesting question whether such RAP could protect EGCG against high temperature treatment. To confirm this idea, the thermal stability of the EGCG incorporated with RAP was evaluated. Wang et al. has reported that the degradation and epimerization of EGCG could occur in thermal processes (Wang et al., 2008). Below 44 $^{\circ}\text{C}$, the degradation of EGCG was more profound; above 44 $^{\circ}\text{C}$, the epimerization from gallic catechin gallate to EGCG is faster than degradation. Komatsu et al. (1993) found that 82 $^{\circ}\text{C}$ was a turning point in the degradation kinetics of tea catechins. Based on these findings, we chose to carry out the experiment at a lower

Fig. 5 Relative scattered intensity distribution curves of RAP upon treatment with different mass concentrations ratio of EGCG (0, 0.5:1, 1.0:1, 2.0:1, EGCG: RAP, w/w)

temperature range of 4–100 °C to simulate the storage process (4–30 °C), incubation process (40–60 °C), pasteurization process (70–90 °C), and boiling process (100 °C), respectively. We anticipate some useful information for further relevant investigation at relatively low temperature ranges. As shown in Fig. 6. Retention ratios during storage at 4, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100 °C in vitro were investigated. Free EGCG with the same treatment was used as control samples. Results indicated that, at low temperatures (4 and 10 °C), the EGCG in RAPE and free EGCG samples could be remained with similar retention ratios of 94.9% and 92.8%, respectively, with no significant differences ($P > 0.05$). However, as temperatures increased below 50 °C (10 to 50 °C), the retention ratios of EGCG in RAPE decreased to 83.1, 76.1, 66.3, 61.5, and 47.8% (mean values), which were significantly higher than those of 76.2, 62.8, 56.7, 51.1, 40.6% (mean values) in free EGCG samples ($P < 0.05$), respectively. The retention ratio of EGCG in free EGCG sample (19.1%) even remained only half of that in FECs (39.5%) after 60 °C treatment for 12 h. Higher temperature treatments (70–100 °C), however, exhibited no significant differences of retention ratios between free EGCG and RAPE groups ($P > 0.05$).

What's the reason why the stability of EGCG in RAPE was obviously higher than that of free EGCG in 10–60 °C temperature ranges? It has been reported that human serum albumin could improve the stability of EGCG in neutral and slightly alkaline pH conditions via hydrogen bonds interactions (Li et al., 2015). The pore structures of protein could encapsulate EGCG through hydrogen bonds interaction and thus prevent the oxidation and degradation of EGCG, behind which the space steric of human serum

albumin is an important reason. In this work, EGCG could bind with RAP and lead to the association of RAP through the Hydrogen bond and van der Waals forces. We inferred that the protein might be unfolded during EGCG-RAP interactions, which resulted in the proteins association and thus exhibited a space steric effect on EGCG molecules. The typical porous structure of RAP may also have a certain shelter effect on EGCG, which making it resistant against thermal treatment. Regarding the bioavailability of the EGCG, previous report has shown that the phenolic compounds have no specific receptors on the surface of small intestinal epithelial cells, which results in a poor small intestinal absorption and low bioavailability due to passive diffusion (Li et al., 2015). Differently, to encapsulate or bind with polyphenols by using nanoparticle delivery system is a promising method to enhance its absorption and bioavailability of polyphenols (Puligundla et al., 2017). The polyphenol-nanoparticle complex can be absorbed through a different pathway such as the endocytosis and macropinocytosis. Thus, after encapsulation of EGCG in the RAP, the absorption and the bioavailability of EGCG may be improved. Further investigation should be carried out to make it clear.

Antioxidant property of RAPE

EGCG is a kind of polyphenol that owns strong antioxidant and free radical-scavenging activities (Lorenzo et al., 2013). To investigate whether the RAPE retains antioxidant ability will be helpful for further application of EGCG based on RAP carrier. Figure 7 showed the antioxidant activities of free EGCG, RAP, and RAPE, as measured by the DPPH radical-scavenging assay. It was found that the radical-scavenging abilities of the RAP were significantly lower than those of free EGCG and RAPE in the

Fig. 6 Retention ratio of EGCG in free EGCG sample and RAP-EGCG complex (EGCG/RAP = 1.6:1, W/W) at different temperatures. * $P < 0.05$

Fig. 7 DPPH radical-scavenging ability of RAPE, RAP, and free EGCG. Each column represents the mean of DPPH radical-scavenging ratio and standard deviation. * $P < 0.05$

concentration ranges of 0.01–0.03 mg/mL ($P < 0.05$). After binding of EGCG with RAP, the DPPH radical-scavenging ability of RAPE was remarkably lower than that of free EGCG in the concentrations of 0.01–0.03 mg/mL. For example, RAPE with a higher concentration of 0.02 mg/mL only retained about 76.4% of the DPPH radical-scavenging ability of free EGCG in a same EGCG dose (Fig. 7). It has been found that the DPPH radical-scavenging ability closely associates with the hydrogen-donating ability of the sample (Yang et al., 2008). After EGCG loading with the RAP, the obvious decrease of the DPPH radical-scavenging ability in RAPE suggested a possible change in the hydrogen-donating capacity of EGCG due to the RAP–EGCG interaction. We inferred that this change might attribute to the formation of the hydrogen bonds between hydrogen atoms in the hydroxyl groups of EGCG and the electro-negative atoms of RAP (Yang et al., 2008). Consequently, the hydrogen bonds may weaken the hydrogen-donating capacity of EGCG, which results in a decrease of DPPH radical-scavenging ability of RAPE as compared to that of free EGCG.

The present work demonstrates the potential use of rice bran albumin protein to stabilize the tea polyphenol-EGCG. The EGCG could interact with RAP with binding site number n of 0.0505:1 (EGCG: RAP, w/w) by hydrogen bond and van der Waals forces. FTIR analysis demonstrated that EGCG could induce the change in the secondary structure of RAP above the ratio of 1.92:1 (EGCG: RAP, w/w) at 25 °C. Furthermore, the stability of EGCG is significantly improved after being incorporated with rice bran albumin protein. The antioxidant property of RAPE is also partially retained. These findings are beneficial for the utilization of RAP as a novel carrier for the improvement of polyphenol stability, as well as the application of EGCG in food industry.

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Compliance with ethical standards

Conflict of interest The authors declare no competing financial interest.

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