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Binding, stability, and antioxidant activity of quercetin with soy protein isolate particles

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ABSTRACT

This work is to study the potential of particles fabricated from soy protein isolate (SPI) as a protective carrier for quercetin. When the concentration of SPI particles increases from 0 to 0.35 g/L, quercetin gives a gradually increased fluorescence intensity and fluorescence anisotropy. The addition of quercetin can highly quench the intrinsic fluorescence of SPI particles. These results are explained in terms of the binding of quercetin to the hydrophobic pockets of SPI particles mainly through the hydrophobic force together with the hydrogen bonding. The small difference in the binding constants at 25 and 40 °C suggests the structural stability of SPI particles. The relative changes in values of Gibbs energy, enthalpy, and entropy indicate that the binding of quercetin with SPI particles is spontaneous and hydrophobic interaction is the major force. Furthermore, SPI particles are superior to native SPI for improving the stability and radical scavenging activity of quercetin.

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1. Introduction

Quercetin (3,5,7,3′,4′-pentahydroxyflavone), one of the major dietary flavonoids, is widely distributed in plants and vegetables (Cook & Samman, 1996). Chemically, quercetin consists of two aromatic rings (A and B) linked by an oxygen containing heterocycle (ring C). The multiple hydroxyl groups in the molecular structure of quercetin may play crucial roles in its widely demonstrated biological and pharmacological activities such as antioxidant, antiinflammatory, antiviral, and anticancer properties (Erlund, 2004; Mendoza & Burd, 2011). However, the use of quercetin as a health promoting agent has been limited by its poor bioavailability, which is caused by its low water solubility and high instability in a neutral and alkaline medium, as well as in the small intestine, colon, liver, and kidney. Therefore, various approaches have been employed to dissolve the problems of quercetin through the delivery systems such as liposomes (Landi-Librandi et al., 2012), micelles (Liu & Guo, 2006), cyclodextrin complexation (Pralhad & Rajendrakumar, 2004), and the formation of phenolipids (Ramadan, 2012).

Many people have proved that quercetin can bind with animal proteins, including human serum albumin (Sengupta & Sengupta, 2002), bovine serum albumin (Fang et al., 2011), collagen (Yang et al., 2009), and β-casein (Mehranfar, Bordbar, & Parastar, 2013), which may promote its solubility, stability, and bioavailability. The binding mechanism between quercetin and these animal proteins has been investigated; however, plant proteins, which are inexpensive, less allergenic, more biocompatible and biodegradable compared to animal derived proteins, have not received much attention for the encapsulation of bioactive flavonoids, like quercetin. Soy protein isolates (SPIs) are highly refined and concentrated plant protein fractions, produced from soybean. SPI is mostly constituted of two globular protein fractions: the hexameric glycinin, with a molecular mass of approximately $3–3.8 \times 10^5$, and the trimeric β-conglycinin, with a molecular mass of approximately $1.8–2 \times 10^5$ (Keerati-u-rai, Miriani, Iametti, Bonomi, & Corredig, 2012). Owing to its high nutritional value and many useful functional properties such as emulsification, solubility, and film-forming capacity, SPI has been used as wall material for the encapsulation of flavor compounds (Charve & Reineccius, 2009), fish oil (Gan, Cheng, & Easa, 2008), and paprika oleoresin (Rascón, Beristain, García, & Salgado, 2011).

Recently, considerable attention has been paid to particles constructed from proteins being used as delivery systems, which have the advantages of easily manipulated size and surface characteristics, controlled release, and controlled particle degradation (Jahanshahi & Babaei, 2008). SPI particles can be prepared by different methods including adding desolvant or crossing-linking agents (Teng, Luo, & Wang, 2012), heat induced aggregation (Jones & McClements, 2011), alkaline hydrolysis (Jong & Peterson, 2008), and cold-gelation (Zhang, Liang, Tian, Chen, & Subirade,

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2012). Generally, soy protein particles have a core–shell structure with basic polypeptides and β subunits interacting together forming the hydrophobic core, and acidic polypeptides, α′ and α subunits, located on the outside the core, forming the hydrophilic shell (Chen, Lin, Sun, & Zhao, 2014). Several compounds, like vitamins have been reported to be successfully encapsulated by SPI particles (Teng, Luo, & Wang, 2013).

This work aims to investigate the binding behaviors of quercetin with SPI particles and evaluate their potential to act as a protective carrier for a typical flavonoid quercetin. The fluorescence emission spectra and fluorescence anisotropy of quercetin were monitored upon adding different concentrations of SPI particles. The quenching of intrinsic fluorescence of SPI particles induced by quercetin was used to determine the thermodynamic parameters involved in their binding. Moreover, compared to native SPI, the stability and the radical scavenging activity of quercetin with SPI particles have been investigated.

2. Materials and methods

2.1. Materials

Quercetin was purchased from Alfa Aesar. Defatted soy flour was purchased from the Yuwang group. 2,2′-Azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) was obtained from Sigma–Aldrich Chemical Company. All other chemical reagents used were of analytical grade, and water was double distilled.

2.2. Methods

2.2.1. Preparation of SPI particles

SPI powder was first prepared from defatted soy flour by alkaline extraction followed by acid precipitation (Teng et al., 2012). The protein content of the SPI powder was 91%, as determined by the Bradford method using bovine serum albumin as a standard (Bradford, 1976). SPI particles were then prepared from SPI powder according to a cold-gelation method (Zhang et al., 2012). The pH of the SPI solution (10 g/L in double distilled water) was raised to pH 12 using 1 mol dm^{-3} NaOH. One part of the alkaline treated SPI solution was heated to 85 °C for 30 min in a tightly closed tube to induce SPI unfolding and disrupt large particles. The heated protein solution was cooled to 25 °C, diluted to 6 g/L and its pH adjusted to 7.4 with 1 mol dm^{-3} HCl. Finally, calcium chloride was added into the protein solution to induce the formation of SPI particles, and the final concentration of calcium chloride was 2×10^{-3} mol dm^{-3}. For comparison, the remaining SPI solution was also adjusted to pH 7.4 as the sample of native SPI. Quercetin is poorly soluble in water so it was dissolved in ethanol at 1 g/L as stock. A small quantity of quercetin stock solution was added to the solutions of native SPI and SPI particles at room temperature. The quercetin concentrations were 50 μM in all samples, except for the determination of the binding constant and radical scavenging assay.

2.2.2. Particle size and zeta potential measurements

The particle size and zeta potential of SPI particles were determined using a Malvern Zetasizer Nano ZS (Malvern Instruments, Malvern, U.K.) at 25 °C (Zhang et al., 2012). While the particle size was controlled *in situ* by dynamic light scattering, the zeta potential was determined by the electrophoretic mobility according to the Smoluchowski equation. During the measurements, the sample was diluted 10-times, and each value was measured at least three times.

2.2.3. Steady-state fluorescence measurements

Steady-state fluorescence measurements for the binding of quercetin with SPI particles, was carried out on a Hitachi F-4500 FL spectrophotometer. The fluorescence emission spectra of 50 μM quercetin with different concentrations of SPI particles, were taken at 25 °C between 500 and 625 nm with an excitation wavelength at 350 nm (Sengupta & Sengupta, 2002).

The intrinsic fluorescence of 0.5 g/L SPI particles were recorded between 300 and 450 nm, at an excitation wavelength of 280 nm (Keerati-u-rai et al., 2012). By varying quercetin concentration from 0 to 3.5 mg/L, the binding constants of quercetin with SPI particles (at 25 and 40 °C) could be calculated from the maximum fluorescence of SPI particles, according to the double logarithm equation (Barik, Priyadarsini, & Mohan, 2003).

2.2.4. Fluorescence polarization technique

The fluorescence anisotropy (r) of quercetin in the presence of different concentrations of SPI particles was measured by an Edinburgh FLS900 spectrofluorophotometer (Edinburgh Instruments Ltd, Livingston, UK) at 25 °C. Quercetin was excited at 350 nm, and the emission spectra were scanned between 500 and 600 nm. The fluorescence intensities were obtained at angle settings of 0–0°, 0–90°, 90–0°, and 90–90° at 25 °C. The value of r was obtained using Eq. (1) (Shinitzky & Barenholz, 1978):

$$r = (I_{//} - G \times I_{\perp})/(I_{//} + 2G \times I_{\perp}) \quad (1)$$

where $I_{//}$ and $I_{\perp}$ are the fluorescence intensities of the emitted light, polarized parallel and perpendicular to the exciting light, respectively, and G is the grating correction factor, which is the ratio of sensitivities of the instrument for vertically and horizontally polarized light.

2.2.5. Measurement of quercetin loading efficiency

Immediately after preparing SPI particles encapsulated with quercetin, the product was transferred into an Amicon Ultra-3000 centrifugal filter device (Millipore Corp., Billerica, MA, USA), and centrifuged at 10,000 rpm for 2 h in order to separate the unencapsulated quercetin (in the tube) from the encapsulated form (in the filter) (Shpigelman, Israeli, & Livney, 2010). The mass of free quercetin in the tube was analyzed according to a quercetin standard curve, through the measurement of maximum absorbance at 365 nm by UV–vis spectroscopy (Shimadzu, UV-1800). The quercetin loading efficiency in SPI particles was calculated to be about 87%.

2.2.6. Evaluation of quercetin stability

In order to investigate the stability of quercetin, the absorbance of free quercetin, quercetin with native SPI and quercetin with SPI particles was measured at 365 nm, at different time intervals, at 25 °C on a UV–vis spectrophotometer (Shimadzu UV-1800) (Fang et al., 2011).

2.2.7. Antioxidant activity of quercetin

The ABTS radical cation decolorization test was used in the assessment of antioxidant activity for quercetin (Ozgen, Reese, Tulio, Scheerens, & Miller, 2006). Briefly, $ABTS^{\cdot+}$ solution was prepared by mixing 7.4 mM ABTS with 2.6 mM potassium persulfate and kept in the dark at least for 12 h. The obtained $ABTS^{\cdot+}$ solution was diluted to an absorbance of 0.7 ± 0.02 at 734 nm, and mixed with free quercetin, quercetin with native SPI, and quercetin with SPI particles, respectively. The absorbance of each sample was measured at 734 nm by a Shimadzu UV-1800 spectrophotometer at 25 °C.

2.2.8. Statistical analysis

Generally, analyses were repeated at least three times and evaluated by their means and standard deviations. The averaged data obtained from the binding studies, were used in the calculations for the binding parameters. The best fit values for binding parameters were achieved by applying a nonlinear least squares regression using the software Microcal Origin 6.0 (Microcal Software Inc., Northampton, United States).

3. Results and discussion

3.1. Properties of SPI particles

We selected a cold-gelation method to prepare SPI particles as it does not require the use of organic solvents, and the obtained SPI particles have the advantages of a uniform size distribution, the ability to adjust size easily and good stability (Zhang et al., 2012). During the preparation process the treatment of the protein solution at an alkaline pH improved the protein solubility, owing to stronger repulsions along polypeptide chains (Kristinsson & Hultin, 2004). Thermal treatment of the SPI solution at 85 °C led to denaturation and a reorganization of protein secondary and tertiary structures, exposing more hidden hydrophobic amino acids, and the uncoiling of polypeptide chains (Wang, Li, Jiang, Qi, & Zhou, 2014). Finally, the addition of calcium chloride induced the formation of SPI particles by serving as a bridge between the negatively charged carboxylic groups on neighboring protein molecules (Zhang et al., 2012). The mean particle diameter of SPI particles was determined to be 220 ± 3 nm, and a zeta potential of about −20.3 ± 0.1 mV. The obtained SPI particles often exhibit a good stability due to their small particle size and high negative charge.

3.2. Binding characteristics of quercetin with SPI particles

The steady-state fluorescence spectra of quercetin in different concentrations of SPI particles are shown in Fig. 1. Free quercetin emits a relatively weak fluorescence with an emission peak at about 520 nm, owing to its proton transfer tautomer fluorescence band. After the addition of SPI particles, there is a marked increase in the fluorescence intensity of quercetin with a clear red-shift in the fluorescence maximum, which is in good agreement with previous reports on the binding of quercetin to animal proteins (Fang et al., 2011; Mehranfar et al., 2013; Sengupta & Sengupta, 2002). On one hand, the change in fluorescence intensity of quercetin suggests that quercetin can transfer from the polar aqueous solution, to nonpolar hydrophobic pockets in the SPI particles, where 3-hydroxyflavone readily yields an excited state with a strong fluorescence (Fang et al., 2011). Furthermore, the hydrophobic pockets of the SPI particles could shield the excited species of quercetin from quenching and nonradioactive decay (Mehranfar et al., 2013). Wang et al. previously found that the formation of SPI particles may induce a self-reassembly from a β-sheet structure to an α-helix and β-turn structure (Wang et al., 2014), leading to the exposure of more hydrophobic groups on the protein. The hydrophobic binding of the aryl groups in quercetin to the hydrophobic pockets of protein was previously identified by Rawel et al. by the addition of urea to destroy the noncovalent interactions, leading to a decrease in fluorescence intensity of quercetin in the presence of BSA (Rawel, Meidtner, & Kroll, 2005). On the other hand, the red-shift in the fluorescence maximum of quercetin in the presence of SPI particles may indicate the formation of intermolecular hydrogen bonds between the 5,7-hydroxy in ring A of quercetin and the sulfhydryl groups or the hydroxyl groups of the protein (Fang et al., 2011).

Fluorescence polarization measurements can provide information on the anisotropy (r) of quercetin, which can give evidence for the binding location of quercetin with SPI particles. The higher r value reveals a greater restriction of the rotation of the quercetin molecule. Fig. 2 gives the r values of quercetin in different concentrations of SPI particles. The r value of free quercetin is 0.19, which is close to the reported r value of quercetin in water (Sengupta & Sengupta, 2003). After the concentration of SPI particles increases from 0.05 to 0.35 g/L, the r value further increases from 0.28 to 0.33. The change of r value is consistent with the increased fluorescence intensity of quercetin, with increasing the concentrations of SPI particles. The higher r values of quercetin with SPI particles relative to that in water supports the above discussion that quercetin is bound in the hydrophobic pockets of the protein. The hydrophobic pockets buried inside the SPI particles could not only give a smaller polarity, but also a stronger, more rigid microenvironment for quercetin (Barik et al., 2003; Zhang et al., 2012). Thus, quercetin experiences a restricted motion and, therefore, an increase in anisotropy occurs.

The change of protein intrinsic fluorescence can be employed to understand the binding characteristics of quercetin with SPI particles. Fig. 3 depicts the spectra of steady-state fluorescence of SPI particles as a function of quercetin concentration. Without addition of quercetin, SPI particles exhibit a characteristic fluorescence maximum at 340 nm, which results from the intrinsic fluorescence of tryptophan residues (Keerati-u-rai et al., 2012). When quercetin is added, the fluorescence intensity of SPI particles decreases gradually. This is because tryptophan residues are generally considered to be located in the hydrophobic pockets of globular proteins. The hydrophobic interactions of quercetin with the hydrophobic groups near tryptophan residues may result in the fluorescence

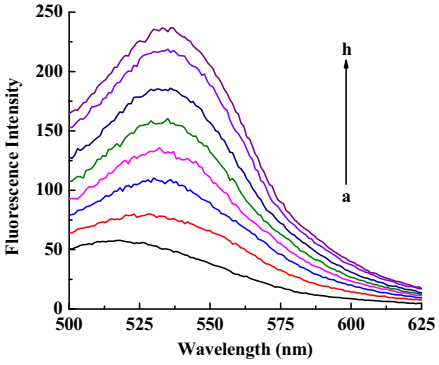

Fig. 1. Fluorescence spectra of 50 μM quercetin in the presence of SPI particles at different concentrations: (a) 0, (b) 0.05, (c) 0.1, (d) 0.15, (e) 0.2, (f) 0.25, (g) 0.3, (h) 0.35 g/L.

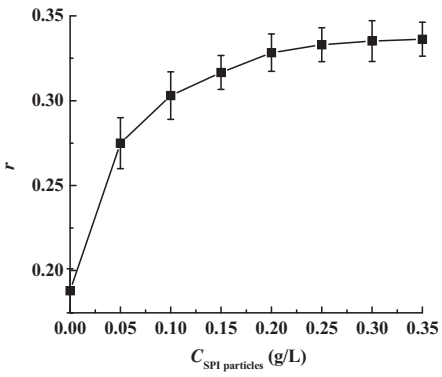

Fig. 2. The variation of fluorescence anisotropy r of quercetin with increasing concentration of SPI particles.

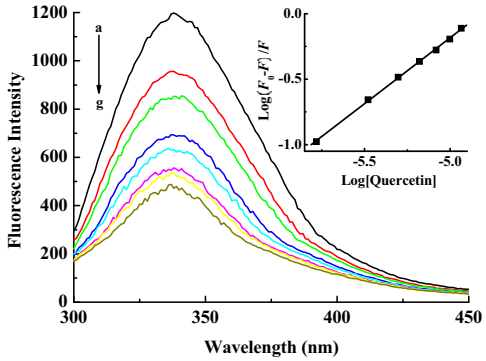

Fig. 3. The variation of fluorescence spectra of SPI particles as a function of quercetin concentration at 25 °C. Spectra (a–g) correspond to 0, 0.5, 1, 1.5, 2, 2.5, 3, and 3.5 mg/L quercetin, respectively. (Inset) Linear plot of $\log\frac{F_0-F}{F}$ vs log [Quercetin] for the determination of binding constant of quercetin with SPI particles at 25 °C.

quenching of SPI particles. This is in agreement with previous findings, suggesting that the aryl groups of the polyphenols hydrophobically bind to the hydrophobic pockets of proteins (Kawamoto, Mizutani, & Nakatsubo, 1997). Meanwhile, the decrease in the fluorescence of SPI particles here corresponds to the increase in fluorescence due to quercetin shown in Fig. 1, which indicates a possible energy transfer between quercetin and the tryptophan residues in SPI particles.

The fluorescence quenching of tryptophan residues in proteins with polyphenolic compounds is generally initiated by static quenching, from the formation of ground-state complexes between the protein and the quencher (Shpigelman et al., 2010). It is assumed that there are '*n*' same and independent binding sites in SPI particles where quercetin binds according to the following model (2):

$$P + nL \xrightarrow{k_b} PL_n \tag{2}$$

where '*P*' denotes SPI particles, '*L*' is quercetin and 'PL_n' is the complexes of quercetin with SPI particles. For this static quenching, in which small molecules bind independently to a set of equivalent sites on a macromolecule, the equilibrium between the free and bound molecules is given by the double logarithm Eq. (3) (Barik et al., 2003):

$$\log\frac{(F_0-F)}{F} = \log K_b + n\log[\text{Quercetin}] \tag{3}$$

where F_0 and F are the fluorescence intensities of SPI particles at 340 nm in the absence and in the presence of different concentrations of quercetin, respectively; [Quercetin] is quercetin concentration, K_b is the binding constant and n is the number of binding sites. From the linear fitting plot of the double logarithm curve of $\log(F_0-F)/F$ vs log [Quercetin] in the inset of Fig. 3, the value of K_b for the binding of quercetin with SPI particles is 7.1×10^4 dm^3 mol^{-1} at 25 °C. To study the influence of temperature on the binding of quercetin with SPI particles, a K_b value at 40 °C was also calculated and found to be 8.5×10^4 dm^3 mol^{-1}. These two values are within the limits of the reported data for the binding of quercetin with proteins (Fang et al., 2011). Native protein generally exhibits a much greater binding affinity for small hydrophobic compounds at enhanced temperatures, due to the heat induced partial unfolding of the protein. However, little difference of K_b values at the two investigated temperatures may indicate the structural stability of SPI particles, which do not allow the protein to undergo a significantly increased partial unfolding at higher temperatures.

Ross et al. have characterized the nature and magnitude of the thermodynamic parameters associated with various individual kinds of molecular interactions, that play an important role in the drug–protein binding process (Ross & Subramanian, 1981). The thermodynamic parameters for the binding of quercetin with SPI particles, i.e., the relative changes in value in Gibbs energy, enthalpy, and entropy (ΔG, ΔH, and ΔS) can be calculated from the following equations:

$$\ln(K_{40\,^\circ C}/K_{25\,^\circ C}) = (1/T_{25\,^\circ C} - 1/T_{40\,^\circ C})\Delta H/R \tag{4}$$

$$\Delta G = -RT\ln K_b \tag{5}$$

$$\Delta G = \Delta H - T\Delta S \tag{6}$$

The calculated values of ΔG are −27.7 and −29.5 kJ mol^{-1} at 25 and 40 °C respectively, which reveals the spontaneity of the binding of quercetin with SPI particles. The values of ΔH and ΔS are 9.3 kJ mol^{-1} and 124 J K^{-1} mol^{-1}, respectively. The positive value of ΔH is possibly dictated by two endothermic factors (Li, Chen, Wang, & Lu, 2013): (1) quercetin molecules partly destroy the hydrophobic hydration structure when they approach SPI particles, which is an endothermic process, and (2) when quercetin molecules insert into the hydrophobic pockets of SPI particles, the original iceberg structure surrounding the quercetin molecules is destroyed, which also causes an endothermic effect. Meanwhile, the positive value of ΔS may be attributed to the release of combined water molecules to buffer medium, including those from the molecule pocket, hydration layer on the surface of the SPI particles and iceberg structure surrounding the hydrophobic parts of quercetin. On the other hand, the much smaller value of ΔH compared to the interaction energy of the chemical bond (>100 kJ/mol) suggests that the interaction force between quercetin and SPI particles is a weak intermolecular force. Although hydrogen binding is involved, the positive values for both ΔH and ΔS mean that a hydrophobic interaction is the major binding force between quercetin and SPI particles, similar to the interaction study in the binding of α-tocopherol with human serum albumin (Li et al., 2013).

3.3. Stability of quercetin with SPI particles

The changes in the relative intensity of the characteristic maximum absorption of quercetin at 365 nm as a function of time, are used to evaluate the stability of quercetin in the samples of free quercetin, quercetin with native SPI, and quercetin with SPI particles. As shown in Fig. 4, about 90% of free quercetin is decomposed rapidly after 300 min of incubation, whereas quercetin combined with native SPI is degraded about 60%, and less than 10% quercetin is degraded when quercetin is mixed with SPI particles. Zenkevich et al. investigated the oxidative degradation of quercetin in water and in water–ethanol solutions, in a moderately-basic media at

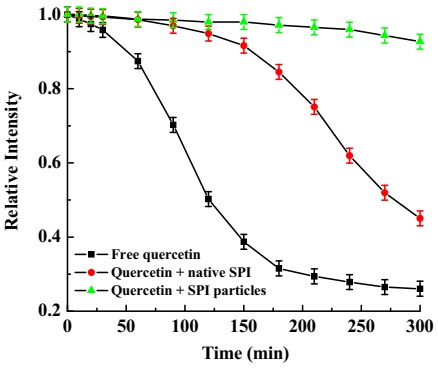

Fig. 4. Relative intensity of the maximum absorption of quercetin in different samples as a function of time.

ambient temperature without any radical initiators or irradiation of the reaction media (Zenkevich et al., 2007). Through direct head-space analysis of the gas phase of acidified quercetin solutions, they proposed that the rapid degradation of free quercetin is mainly ascribed to the oxidative decarboxylation of ring C in quercetin. When quercetin binds to the hydrophobic pockets of native SPI, the somewhat enhanced stability of quercetin indicates that the active groups in ring C are effectively protected from the aqueous medium. The higher suppression yield of SPI particles compared to native SPI may be ascribed to more available hydrophobic pockets. Meanwhile, Fang et al. previously compared the binding behaviors of quercetin with three kinds of protein particles (bovine serum albumin, lysozyme and myoglobin) and found, besides the main hydrophobic interaction occurring in the tryptophan residues, hydrogen bonding also contributes to the highest binding capacity of quercetin with BSA particles (Fang et al., 2011). Therefore, the formation of hydrogen bonds between quercetin and SPI particles cannot be neglected, due to the high stabilization effect of SPI particles.

3.4. ABTS scavenging activity of quercetin with SPI particles

The ABTS radical cation decolorization test is used to assess the radical scavenging ability of quercetin. When an $ABTS^{\cdot+}$ radical encounters a proton-donating substance, such as an antioxidant, the radical is scavenged and the absorbance of $ABTS^{\cdot+}$ is reduced (Lee & Yoon, 2008). Fig. 5 outlines the maximum absorbance values at 734 nm of $ABTS^{\cdot+}$ alone and in the presence of pure quercetin, native SPI, quercetin with native SPI, SPI particles, and quercetin with SPI particles. It is observed that the presence of pure quercetin can reduce the absorption of $ABTS^{\cdot+}$ by about 57%. The high ABTS antioxidant activity of quercetin is mainly contributed by 3′ and 4′ hydroxyl groups in ring B, which could scavenge the $ABTS^{\cdot+}$ radical by donating H^+ ions (Heijnen, Haenen, van Acker, van der Vijgh, & Bast, 2001; Lee & Yoon, 2008). Native SPI and SPI particles are observed to reduce the absorption of $ABTS^{\cdot+}$ by about 29% and 39%, respectively. Beermann et al. previously explained that the presence of small peptides and C-terminal aromatic tyrosine residues may lead to the mild radical scavenging abilities demonstrated by native SPI (Beermann, Euler, Herzberg, & Stahl, 2009). Because of the structure reorganization and exposure of active groups for H^+ donation, SPI particles show a higher ABTS antioxidant activity than native SPI. However, it is noted that the ABTS antioxidant activities for quercetin with native SPI or SPI particles are lower than the total antioxidant activities from free quercetin and native SPI or SPI particles. The masking effect on the antioxidant capacity of protein with polyphenol could be attributed to the formation of intermolecular hydrogen bonds that prevents them from donating H^+ ions to the radical. Nevertheless, when combined with native SPI or SPI particles, the ABTS antioxidant ability of quercetin is even better than free quercetin. The strongest antioxidant of quercetin with SPI particles suggests that this kind of plant protein particles can provide a better microenvironment for the 3′ and 4′ hydroxyl groups in ring B of quercetin, which are not participating in the formation of hydrogen bonds with the protein, and easily donate H^+ to reduce $ABTS^{\cdot+}$ into its nonradical form.

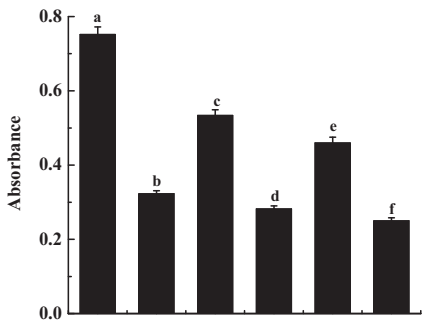

Fig. 5. $ABTS^{\cdot+}$ absorption at 734 nm in different samples. (a) $ABTS^{\cdot+}$ alone; (b) $ABTS^{\cdot+}$ with quercetin; (c) $ABTS^{\cdot+}$ with native SPI; (d) $ABTS^{\cdot+}$ with quercetin and native SPI; (e) $ABTS^{\cdot+}$ with SPI particles; (f) $ABTS^{\cdot+}$ with quercetin and SPI particles.

4. Conclusions

In summary, fluorescence measurements reveal that quercetin can bind to the hydrophobic pockets of SPI particles through hydrophobic interactions and hydrogen bonding. The hydrophobic binding of the aryl groups of quercetin to the hydrophobic pockets of SPI particles results in an increase of fluorescence intensity and anisotropy of quercetin. The observed clear red-shift in fluorescence maximum of quercetin in the presence of SPI particles, indicates the formation of hydrogen bonds between quercetin and SPI particles. The thermodynamic parameters suggest the spontaneous binding nature and the major role of hydrophobic interactions. SPI particles exhibit greater abilities for improving the stability and radical scavenging activity of quercetin compared to native SPI. The present work reveals that particles constructed from plant proteins could be used as a promising candidate for encapsulating and delivering hydrophobic bioactive compounds in functional foods.

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