Effects of tea polyphenols on physicochemical and antioxidative properties of whey protein coating

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Abstract Effects of tea polyphenols (TP) incorporation on physicochemical and antioxidative properties of whey protein isolate (WPI) coating were studied. Two WPI coating solutions were prepared by heating WPI solutions (pH 8, 90 °C) for 30 min and then TP was incorporated. TP addition could increase the negative zeta potential of 5% solution. The surface hydrophobicity index of both solutions was increased and intrinsic fluorescence intensity decreased greatly after addition of TP. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azinobis (2 ethylbenzothiazoline-6-sulfonate) (ABTS) radical scavenging capacities of both solutions increased with increasing TP. Compared with apple pieces coated with whey protein only, those with TP containing whey protein coatings showed lower browning index and slight changes in weight loss during

24 h storage. Data indicated that TP could influence the physicochemical properties and improve the antioxidant activity of WPI coating solutions and can be used to retard the enzymatic browning of fruit during storage.

Keywords Tea polyphenol · Whey protein · Physicochemical property · Antioxidant activity · Fresh-cut apple

Introduction

Whey protein isolate (WPI), the by-product during cheese-making process, is commonly used as protein-based coating. WPI coating is transparent, odorless and tasteless with desirable barrier properties to oxygen and lipids (Kadam et al., 2013). WPI edible coating has been used on peanuts, salmon, fruits, or cereals where they offered good aroma, fat, humidity and oxygen barriers (Markus et al., 2012). Natural antioxidants are favored for incorporation into whey protein coatings to further extend the shelf life of foods (Akcan et al., 2017). Natural antioxidants such as α -tocopherol and plant extracts are recently tested antioxidants to improve the oxygen-barrier property of WPI coating (Agudelo-Cuartas et al., 2020; Alp Erbay et al., 2017; Wang et al., 2020).

Tea is a daily beverage and crude medicine in China for thousands of years. Tea polyphenols (TP), hydrophilic antioxidants, are the most abundant active antioxidant compounds in tea leaf and are mainly responsible for the health benefits of tea consumption (Yan et al., 2020a, b). TP have been reported to act as antioxidants by donation of hydrogen atoms, acceptance of free radicals, interruption of chain oxidation reactions or by chelating metals (Giménez et al., 2013). When TP were incorporated into protein

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based coating solutions, the two compounds may interact by hydrophobic interaction and hydrogen bonding between the carbonyl group of protein-peptide bond and the phenolic hydroxyl group of polyphenols (Thongkaew et al., 2014; de Moraes et al., 2020), thus, the properties of each other may be changed. Interactions between polyphenols and whey protein decreased the antioxidant and antimicrobial performance of Argentinean green tea (Jakobek, 2015; Kanakis et al., 2011; Staszewski et al., 2011). Incorporation of TP into gelatin film lowered peroxide value of fish oil and increased the radical-scavenging activity (Bao et al., 2010). Green tea extract improved the antioxidant power of agar and agar-gelatin films (Giménez et al., 2013).

Incorporation of tea polyphenols (TP) into whey protein-based coating may be a good choice for making antioxidant edible coating. The objectives of this study are to investigate the impacts of TP (0.1–0.5%, w/v) on physicochemical and antioxidative properties of whey protein coating solutions at two concentrations (5% or 10%, w/v), and then effects of whey protein coating with or without TP against enzymatic browning and water loss in fresh-cut apples were also studied.

Materials and methods

Materials

Whey protein isolate (WPI, 93.14% protein on dry weight basis) was purchased from Fonterra Co-operative Group (Auckland, New Zealand). The protein was composed of 69.2% (w/w) β -lactoglobulin, 14.2% α -lactalbumin, 3.3% bovine serum albumin, and 2.1% immunoglobulin G. Other components are as follows (w/w): 0.36% fat, 1.6% ash, 0.7% lactose monohydrate and 0.07% calcium. Green tea polyphenols powder was purchased from Kemai Biotech. Co., Ltd (Changchun, China). The composition of the tea polyphenols powder was provided by the manufacturer: it contains 98.35% polyphenols (determined by UV) and 50.84% epigallocatechin gallate (EGCG) (determined by HPLC) and 0.65% caffeine (determined by HPLC). Apples (Golden Delicious) were purchased from local market (Changchun, China). Reagents used for antioxidative properties analysis and 8-anilino-1-naphthalene sulfonic acid (ANS) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Other chemicals were purchased from Beijing Chemical works (Beijing, China). Deionized water was obtained using a Milli-Q deionization reversed osmosis system (Millipore Corp., Bedford, MA, USA).

Preparation of WPI coating solutions containing TP

Whey protein stock solution (20%, w/v) was prepared by slowly dissolving WPI powder in deionized water at room temperature and then stirred (700 rpm) for 2 h. The stock solution was stored at 4 °C overnight for complete hydration. Whey protein stock solution was returned to ambient temperature and then diluted to either 5% or 10% (w/v) using deionized water. The solutions were adjusted to pH 8 with 2 M sodium hydroxide. Both solutions contained in beakers were coated with aluminum foil and then heated at 90 °C for 30 min in a water bath. The coating solutions were obtained by cooling heated whey protein solutions in mixed water–ice quickly to room temperature (25 ± 1 °C). A series of TP containing solutions were prepared by adding TP powder slowly into coating solutions at levels from 0.1% to 0.5% (w/v) and then all samples were mixed for at least 1 h for the interaction between TP and protein. Samples are prepared in triplicates for two batches.

Particle size and zeta potential measurement

Particle size and zeta potential of all samples diluted to 1% (w/v) were determined by a Zetasizer (Nano-ZS, Malvern Instruments, Malvern, Worcestershire, WR, UK). Samples were filtrated using a 0.22 μ m pore-size filter membrane and ultrasound treated before determination. Z-average diameter (D_h) and polydispersity index (PDI) were recorded with a solvent viscosity of 0.8872 mPa.s and solvent refractive index of 1.33. The measurement angle was set at 173° backscatter with a run time of 10 s for each measurement. Data analysis was performed according to Stokes–Einstein equation. Zeta potential of all samples was recorded based on the technique of Laser Doppler Electrophoresis and calculated based on Henry equation.

Surface hydrophobicity and intrinsic fluorescence spectra determination

The surface hydrophobicity of all samples was determined using 8-anilino-1-naphthalene sulfonic acid (ANS) probe method by a Spectrofluorometer (RF-5301PC, Shimadzu, Kyoto, Japan). All samples were diluted to 0.005–0.025% concentrations and determined for fluorescence intensity at emission wavelength of 485 nm and excitation wavelength of 390 nm. Twenty millilitres of ANS probe (8 mM) were added into 4 mL samples and then kept in dark for 15 min before determination. Index of the protein surface hydrophobicity (H_0) was obtained as the slope of the ANS fluorescence intensity against protein concentration calculated by linear regression.

All samples were diluted to 0.1% and then measured for intrinsic fluorescence spectra with a Spectrofluorometer

(RF-5301 PC, Shimadzu, Kyoto, Japan). The emission spectra were measured in the range of 300 to 450 nm at the constant excitation wavelength of 280 nm.

Antioxidative properties analysis

Samples diluted to the concentrations of 0.01%, 0.025%, 0.05% and 0.1% were performed for ABTS radical scavenging ability analysis. Stock solution containing ABTS (7 mM) and potassium persulfate (2.45 mM) were prepared and stored in dark at 4 °C for 12–16 h. The stock solution was then diluted with distilled water until the absorbance achieved 0.7 ± 0.02 at 734 nm. Subsequently, ABTS solution (100 μ L) was mixed with 50 μ L sample and kept resting for 1 h in the dark at room temperature. The absorbance of ABTS, blank and ABTS/samples were recorded at 734 nm.

Samples diluted to the concentrations of 0.1%, 0.25%, 0.5% and 1% were performed for DPPH radical scavenging ability analysis. DPPH solution (0.2 mM) was prepared by dissolving the powder in ethanol slowly. A portion of 150 μ L sample was mixed with equivalent volume of DPPH solution under agitating for 10 s, and then kept for 30 min in the dark at room temperature. The absorbance of DPPH, blank and DPPH/samples solution were read at 517 nm.

For calculating the radical scavenging activity (AA) of DPPH and ABTS, the following equation was used:

$$AA(\%) = 100 - (A_S - A_B)/A_d \times 100 \quad (1)$$

where A_B , A_S and A_d represent the absorbance of the blank, DPPH/ABTS with samples and DPPH/ABTS solutions, respectively.

Coating apple preparation

Coating solution was mixed with same weight of glycerol and then filtered through two layers of muslin cloth to remove any coagulation. The coating solution with glycerol was used for coating apple slices (approximate 4 cm $\times$ 3 cm $\times$ 1.5 cm). Apple slices was first immersed in 30 mL sodium hypochlorite-citric acid solution (100 mg L^{-1} , pH $\approx$ 6) for 2 min to avoid initial browning. After draining, apple pieces were immersed in coating solutions for 5 min and then drained for 10 min. Coated apple slices were stored at 20 °C for 1 day and measured for the color and weight at 0, 1, 6, 17 and 24 h.

Color change measurement

Color of all samples was measured using a Konica Minolta Colorimeter (CM-2300d, Tokyo, Japan). Each measurement was taken at three locations. The lightness L^* (0 = black, 100 = white) and chromaticity coordinates a^*

and b^* , which indicate red/green and yellow/blue direction, respectively, were measured. Samples were covered with a piece of cloth and measured in dark. The parameters were set as light source of D65, observation angle of 2°, and determination mode of SCE. Color change of coated apple piece models during 1 day was evaluated using browning index (BI) (Al-Asmar et al., 2020). Browning index (BI) was calculated using the following equation using measured values of L^* , a^* , and b^* :

$$BI = \frac{(x - 0.31)}{0.172} \times 100 \quad (2)$$

where x is the chromaticity coordinate calculated from L^* , a^* , b^* tristimulus values according to the following formula: $x = L^*/(L^* + a^* + b^*)$

Weight loss calculation

Weight change of all coated apple samples was evaluated using percentage weight loss. Percentage weight loss was expressed as the percentage of weight loss to the initial weight (0 h). Weight loss was calculated by subtracting weight of apple slices at different time point from that of 0 h.

Data analysis

Data obtained from analysis were expressed as mean $\pm$ standard deviation (S.D.). The significant differences of data between samples and control were calculated using Version SPSS 21 (SPSS Inc. Chicago, IL, USA). The significance level was set at $p < 0.05$. Data were checked for homogeneity by Levene is test. When the data were homogeneous, one-way analysis of variance (ANOVA) and then a least squared differences (LSD) model was used. All figures were drawn by origin 2018b (OriginLab Corporation, Northampton, NC, USA).

Results and discussion

Effects of TP on particle size and zeta potential of WPI coating solution

Dynamic light scattering (DLS) technique has been used to determine the size of protein–polyphenol aggregates over a wide range of conditions (Thongkaew et al., 2014). Effects of TP on the particle size of coating solutions were studied using DLS. All samples showed polydispersity index (PDI) values in the range of 0.2–0.5 (data not shown here), indicating the monodisperse systems with relatively narrow size distribution (Osvaldo et al., 2015). Effects of TP on the Z-average diameter (D_h) of coating solutions are shown in

Fig. 1 Effects of tea polyphenols on average diameter (A) and zeta potential (B) of coating solutions (CS). Note Different lowercase letters denote significant difference between samples with different TP level at $p < 0.05$

Fig. 1(A). Whey protein solution heated at pH 8 where far away from the isoelectric point of β -lactoglobulin tends to form linear filamentous aggregates which do not scatter dynamic light strongly and usually has nano-meter scale (Santipanichwong et al., 2008). In a whole, 10% coating solution showed diameter of 60 nm, which was significantly higher than that of 5% solution (about 45 nm) ($p < 0.05$). Whey protein aggregation can be influenced by protein concentration and higher concentration may result in higher particle size. Similar results were reported by our previous study (Zhang et al., 2019). Incorporation of TP did not change the average diameter of whey protein in both 5% and 10% solution significantly ($p > 0.05$), indicating the relative stable complexes. Similar results were reported for rice glutelin–procyanidin dimer interactions by Dai et al. (2019).

Zeta potential is an indicator for stability of protein solution by surface charge density. Zeta potential of all coating solutions were measured and the results are shown in Fig. 1(B). WPI coating solution alone at 5% concentration displayed a zeta potential value of -16.24 mV which was significantly decreased to -22.10 – -57 mV by TP addition ($p < 0.05$). At pH 8, the protonated phenolic groups of polyphenols could be deprotonated and the generated oxygen center imparted a high negative charge density which further decreased the zeta potential value of the complexes (Mariana et al., 2011). Previous study also showed that reaction with phenolic compounds caused change in the net charge of Cinnamomum camphora seed kernel protein molecules (Yan et al., 2020a, b). However, no significant changes were observed between samples with TP level higher than 0.3% ($p > 0.05$). Similar results were reported by Mariana et al. (2011). The stability of whey protein solution was enhanced by TP incorporation. System with zeta potential lower than -10 mV (usually between -25 and -30 mV) is stable due to the high-energy barrier between particles (Xiao et al., 2017).

Whey protein coating solution alone at 10% concentration had a zeta potential value of about -62.28 mV, which is significantly higher than that of 5% coating solution ($p < 0.05$), indicating that more negatively charged amino acid residues are exposed. Variation of TP did not cause significant changes on the negative charge of 10% coating solution ($p > 0.05$). The possible reason may be that the change of TP caused was not high enough to produce a significant difference.

Effects of TP on surface hydrophobicity of WPI coating solution

Whey protein can interact with TP via both hydrophobic interactions and hydrogen bonds (Jakobek, 2015). Hydrophobic interactions happen between hydrophobic regions of the protein amino acids (benzene and aliphatic side chains) and the aromatic nuclei of the polyphenols. Hydrogen bonds can be formed between the hydroxyl groups of polyphenols and carbonyl groups of peptide chain (Tugba et al., 2013). Interactions between whey protein and TP may cause change in the conformation of protein and surface hydrophobicity may be a good indicator. Thus, effects of TP on surface hydrophobicity of coating solutions are measured and the results are shown in Fig. 2.

Whey protein coating solution alone at 5% concentration showed a surface hydrophobicity index (H_0) of 259.62 ± 3.40 and addition of 0.1% TP significantly enhanced the value to 342.94 ± 4.50 ($p < 0.05$). Further increase of TP from 0.2% to 0.5% did not cause significant differences between samples ($p > 0.05$). Compared with

Fig. 2 Effects of tea polyphenols on surface hydrophobicity (H_0) of coating solutions (CS). Note Different lowercase letters denote significant difference between samples with different TP level at $p < 0.05$

5% coating solution alone, 10% coating solution without TP displayed a significantly higher surface hydrophobicity index at 324.70 ± 3.50 ($p > 0.05$). No significant surface hydrophobicity index was observed for 10% coating solutions prepared with 0, 0.1, 0.2 and 0.3% TP ($p > 0.05$). While at TP levels of 0.3% and 0.4%, the surface hydrophobicity index of 10% coating solution was significantly increased ($p < 0.05$). In contrast, previous studies showed that polyphenols could induce aggregation of whey protein and decrease the surface hydrophobicity (Cao and Xiong, 2017; Feng et al., 2017). The different results may be due to the different conformation of whey protein which was native and polymerized whey protein in previous and our study, respectively.

Effects of TP on intrinsic fluorescence of WPI coating solution

Due to the sensitivity of intrinsic fluorescence to the microenvironment changes around proteins, fluorescence analysis is often used to provide information about proteins and small molecules interactions (Zhang and Zhong, 2013). Whey protein contains tryptophan (Trp), tyrosine (Tyr) which can emit fluorescence when excited at a wavelength of 280 nm (Li and Muriel, 2012; Shuang et al., 2018). All samples were determined for the intrinsic fluorescence intensity at excitation wavelength of 280 nm and the spectra are shown in Fig. 3. The intrinsic fluorescence intensity from whey protein in both coating solutions was appreciably decreased when TP was increased from 0.1 to 0.5%, indicating the interactions between whey protein and TP. Similar fluorescence quenching effect was reported for

Fig. 3 Effects of tea polyphenols on intrinsic fluorescence intensity of coating solutions (CS)

epigallocatechin and β -lactoglobulin (Wu et al., 2011). This phenomenon may be explained by the formation of non-fluorescent protein/TP complexes where the microenvironment of Trp and Tyr residues in the whey protein are altered (Dai et al., 2019). In addition, lysine, histidine and cystine residues have the ability to quench the fluorescence of Trp residue, whey protein-TP interaction induced conformation change in the protein may make these residues closer to Trp residue and quench the intensity (Chen et al., 2019). Coating solution at 5% concentration had a maximum peak position ($\lambda_{\max}$) of 343 nm while addition of TP induced no shift or a small shift of 1–4 nm to longer wavelength (Fig. 3A), indicating that TP addition did not influence the polar environment of Trp greatly. Whey protein in 10% coating solution only exhibited a fluorescence emission maximum ($\lambda_{\max}$) at 342 nm while TP induced red shift of $\lambda_{\max}$ up to 356 nm (Fig. 3B),

indicating that the micro-environment of Trp and Tyr residues was altered by interaction with TP (Li and Muriel, 2012).

Effects of TP on ABTS and DPPH scavenging ability of WPI coating solution

ABTS assay is a spectrophotometric method widely used for the assessment of antioxidant activity of various substances (Li et al., 2008). Effects of TP on the ABTS scavenging ability of WPI coating solutions are determined and the results are shown in Fig. 4(A), (B). The ABTS radical scavenging activity of all samples increased with an increase in concentration from 0.01 to 0.1%, which was concentration-dependent. Whey protein and tea polyphenols both have good ABTS radical scavenging activity (Zhang et al., 2019). As expected, for both coating solutions, more potent ABTS radical scavenging activity at all tested concentrations was observed by addition of TP. Figure 4(A) shows the influence of TP on ABTS radical

scavenging activity of 5% coating solution. At 0.1% tested concentration, 5% coating solution alone, samples with 0.1% to 0.5% TP could scavenge 37.10%, 41.66%, 48.43%, 51.04%, 56.44% and 66.79%, respectively. Similar trends are observed for 10% coating solution (Fig. 4B). The highest antiradical activity of 74.83% was found for 10% coating solution with 0.5% TP at test concentration of 0.1%.

DPPH radical scavenging activity assay was used to test the substances of quenching reactive species by hydrogen donation. Whey protein had DPPH scavenging ability due to containing antioxidative amino acids such as cysteine, tryptophan, tyrosine and phenylalanine (Gao et al., 2018). TP have also been reported to act as antioxidants by donation of hydrogen atoms (Giménez et al., 2013). Samples diluted to 0.1%, 0.25%, 0.5% and 1% were determined for DPPH scavenging ability and the results are shown in Fig. 4(C), (D). Coating solutions showed different DPPH scavenging capacity depending on the protein concentration. Coating solutions at 5% concentration had DPPH

Fig. 4 Effects of tea polyphenols on ABTS (A and B) and DPPH (C and D) scavenging activity of coating solutions (CS)

scavenging activity in the range of 5.59–22.82% while that for 10% solutions showed almost double values which increased from 12.81 to 30.78%. The reason may be due to whey protein aggregation and thus the occupancy of antioxidative amino acids were affected by protein concentration. Addition of TP substantially improved the DPPH-scavenging activity of both solutions. Similar results were reported for gelatin film (Bao et al., 2010).

Effects of whey protein coating with or without TP on color change of fresh-cut apples

Whey protein coatings against enzymatic browning in apples were investigated. Glycerol, as the plasticizer, can be added before or after heating. However, study showed that presence of glycerol in heated whey protein may resulted in the increase of denaturation temperature (Chanasattru et al., 2009). Thus, glycerol was added after heating. Enzymatic browning is a major problem reducing shelf-life of fresh-cut fruit and vegetable due to the reaction of phenolic compounds with atmospheric oxygen diffusing into the tissue (Perez-Gago et al., 2004). Study showed that whey protein coatings can delay fruit and vegetable browning by acting as oxygen barriers (Tien et al., 2001). Effects of TP on enzymatic browning of whey protein coated fresh-cut apple piece were studied. Browning index (BI) of all samples were calculated from L^* , a^* and b^* values (Fig. S1). Changes in BI during 1 day for apple slice models with different coatings are shown in Fig. 5(A), (B).

All samples showed a slight change and even decrease in BI values during 1 day of storage at room temperature. Similar results were reported by Azevedo et al. (2018). The decreased index may be attributed to increase in a^* and b^* values, indicating more red and yellow color. Compared with samples coated with 5% whey protein, samples coated with 10% whey protein showed a relatively lower BI values, indicating a more effective anti-browning agent. This may be due to the more compact whey protein network formed at higher protein concentration (Zhang et al., 2019). Addition of TP reduced the BI values of fresh-cut apples coated with both coating solutions to some extent, maybe due to the antioxidative properties of TP, which retarded the diffusion of atmospheric oxygen into the tissue and enzymatic reactions of phenolic compounds with released endogenous polyphenol oxidase (Yan et al., 2020a, b).

Effects of whey protein coating with or without TP on percentage weight loss of fresh-cut apples

Weight loss occurs during fruit and vegetable storage due to their respiratory process, the transference of humidity and some processes of oxidation (Pajak et al., 2016).

Fig. 5 Effects of whey protein coating with or without TP on color change of fresh-cut apples

Coating provides a semi-permeable barrier against gases and moisture, thereby reducing respiration, water loss and oxidation reaction rate. Weight loss is an important index in the evaluation of protein-based coating. Coating at higher protein concentration led to slightly lower weight loss rate of apple slices during storage. Apples coated with 5% whey protein solutions showed percentage weight loss of 4.23%, 12.48%, 35.66% and 49.66% at timepoints of 1st, 3rd, 17th and 25th h while those for samples treated with 10% solution were 3.96%, 10.16%, 31.43% and 47.76%, respectively. The main driving forces for whey protein-based coating are disulfide bonds and hydrogen bonds. Therefore, interactions between whey protein and TP via hydrophobic force and hydrogen bond may influence the coating effect of whey protein. However, it seemed that addition of TP did not present significant differences on weight loss of apple pieces (Fig. 6C, D), although it delayed the browning rate. Similar results were reported by previous study that water vapor permeability

Fig. 6 Effects of whey protein coating with or without TP on percentage weight loss of fresh-cut apples

and water resistance of agar film was not affected by addition of green tea extract (Giménez et al., 2013).

In conclusion, tea polyphenols may interact with whey protein and change its physicochemical and antioxidative properties depending on tea polyphenols level and whey protein concentration. Tea polyphenol incorporated whey protein-based coating showed better protecting effect for fresh-cut apple slices during storage. Tea polyphenols addition may be a good choice to make whey protein-based antioxidative coating.

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