Protein and polyphenols involved in sediment formation in cloudy litchi juice

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Received: 4 February 2018 / Revised: 8 January 2019 / Accepted: 17 January 2019 / Published online: 6 February 2019
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Abstract Sedimentation is a major issue in juice production. This paper aims to study the mechanisms of precipitate formation during the storage of cloudy litchi juice. The sediment concentration, relative turbidity, and ζ potential were analyzed. The supernatant and sediment were separated to determine the contents of proteins and phenolics. The results showed that the amount of sediment increased during the storage. In addition, the total protein and total phenolic content in the supernatant decreased, whereas the glutelin and total phenolic contents in the sediment increased significantly ($p < 0.05$). Moreover, our results showed that the amounts of procyanidin B₂ and quercetin-3-*O*-rutinose-7-*O*-rhamnoside in the supernatant decreased noticeably. However, these two substances could not be detected in the sediment. In summary, the formation of

sediment from litchi juice is mainly caused by the slow denaturation of proteins and the oxidation of procyanidin B₂ and quercetin-3-*O*-rutinose-7-*O*-rhamnoside.

Keywords Cloudy litchi juice · Sediment formation · Protein · Polyphenols · Glutelin

Introduction

The litchi fruit (*Litchi chinensis* Sonn.) originates from China and is now widely cultivated around the world in areas with a warm climate (Zhang et al., 2013). As a tropical fruit, litchi has bright-red peel, translucent creamy flesh, a unique flavor, and nutritional ingredients (Zhang et al., 2015). Studies have shown that litchi flesh is rich in bioactive compounds such as polyphenols (Brat et al., 2006), which have good antioxidant, anti-radiation, liver protection, and other biological properties (Bhoopat et al., 2011; Khan et al., 2009; Saxena et al., 2011). However, as a result of high temperature and concentrated harvest period, the fruits are highly perishable and the peel is easily browned, which results in preservation difficulties and a decline in quality during storage (Jiang et al., 2004). The processing of litchi fruits into cloudy juice not only solves the problem of litchi preservation but also improves the commercial value.

Compared with clear fruit juice, cloudy juice has a more realistic taste and flavor, which is very popular with consumers and has broad market potential (Grimi et al., 2011). Due to its complexity, the cloudy juice system is extremely unstable during storage and it easily browns, delaminates, and precipitates (Millet et al., 2017). Although these phenomena have no effect on the flavor of the juice, the product can still be rejected by some

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customers. Studies have shown that phenolics and proteins are the main substances that cause the turbidity of clear fruit juice and wine (Esteruelas et al., 2011; Millet et al., 2017). However, few studies have been reported on the sediments of cloudy juice. Phenolic compounds are unstable and usually oxidized during the storage process. Quinone compounds, the oxidation products of phenolics, are highly electrophilic and may lead to the formation of aggregates and a decrease in solubility (Liu et al., 2014; Sun et al., 2013; Vernhet et al., 2014). Studies have also shown that the native phenolic compounds are not involved in turbidity but that their oxidized products aggregate or combine with proteins (Millet et al., 2017). Esteruelas et al. (2011) investigated the sediment of grape wine and found that the slow denaturation of proteins led to aggregation and flocculation, which eventually resulted in sediment. Millet et al. (2017) studied haze composition in three apple-based beverages and found that the main components were polyphenols and proteins. Although the complex mixture of proteins and polyphenols is soluble, it keeps changing until it cannot be dissolved, which eventually results in haze (Siebert, 2006).

Our previous studies showed that the precipitation phenomenon was prone to occur in cloudy litchi juice. We determined the sediment composition and observed that the crude protein content accounted for 23.7% of the dry substances, whereas the total phenolic content accounted for 1.3%. It was suggested that protein–polyphenol interactions were involved in haze and sediment formation in juice and wine (Esteruelas et al., 2011; Millet et al., 2017). The proteins and polyphenols of sediment in litchi juice may come from the soluble proteins and polyphenols. In order to investigate the mechanism of formation of cloudy litchi juice sediment, we have analyzed the changes in proteins and polyphenols in cloudy litchi juice at different storage temperatures.

Materials and methods

Chemical reagents and standards

The standard compounds of Epicatechin, procyanidin B₂, quercetin, rutin, isorhamnetin-3-*O*-rutoside, and kaempferol-3-*O*-rutoside were purchased from Sigma-Aldrich (St. Louis, MO, USA). Mass spectrometry (MS)-grade acetonitrile and acetic acid were supplied by Merck (Darmstadt, Germany). Water for high-performance liquid chromatography (HPLC) and MS was purified using a Milli-Q system (Integral 10, Millipore, Bedford, MA, USA). The Folin–Ciocalteu reagent was purchased from Yuanye Bio-Technology Co., Ltd. (Shanghai, China). All

other chemicals were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Preparation of litchi juice

Ripe red litchi fruits of cv. ‘Huaizhi’ were harvested from an orchard in Guangzhou, China. The pericarp and seed were removed, and the pulp was crushed and filtered through a 74 µm filter cloth. The filtrate was sterilized (95 °C, 1 min), poured into glass bottles under sterile conditions, and stored at 4 °C or 25 °C for 10 weeks.

Determination of sediment concentration

At the point of storage, six bottles of juice were removed. After centrifugation at 4200×*g* for 15 min, the sediment was collected and freeze dried. Then, the lyophilized powder was weighed and the concentration of sediment was expressed in mg/L.

Determination of relative turbidity

Cloudy litchi juice was centrifuged at 4200×*g* for 15 min at room temperature. Then, the supernatant was separated, and its absorbance was measured at 660 nm in a spectrophotometer (UV1800, Shimadzu, Kyoto, Japan). The relative turbidity ($\tau_{\text{super}}/\tau_{\text{total}}$) was expressed as the percentage of the absorbance value of supernatant after centrifugation to the absorbance value of the juice sample before centrifugation (Schultz et al., 2014).

Determination of ζ potential

The ζ potential is the potential located at the electrical double layer of charged particles, which reflects the stability of a suspension. The ζ potential was measured by using a Zetasizer Nano instrument (ZS90, Malvern, Worcestershire, UK) with a 1 cm polystyrene colorimetric cell and a pair of 0.45 cm² platinum electrodes at 0.4 cm distance. The determination was carried out at 25 °C. The results were calculated according to the Smoluchowski equation, $\zeta = \varepsilon\mu/\eta$, in which ε is the dielectric constant of the solution, μ is the electrophoretic mobility of the particles in the presence of an electric field, and η is the viscosity of the solution (Schultz et al., 2014).

Analysis of protein content

Determination of total protein content in the supernatant

The soluble total protein in the supernatant was determined by using the Coomassie Brilliant Blue method (Bradford, 1976; Grintzalis et al., 2015), which has sensitivity limit of 1 µg/mL. Bovine serum albumin (BSA) was used as the standard, and quantitative analysis was performed by the external standard method.

Determination of total protein content in the sediment

The Osborne fractionation method was used to extract the proteins from the sediment (Horax et al., 2010; Osborne, 1924). Briefly, 0.5 g of sediment was mixed with 10 mL of water. After the mixture had been magnetically stirred for 2 h and centrifuged at 10,000×g for 25 min at 4 °C, the supernatant (albumin) was collected. The residue was mixed with 10 mL of 1 mol/L NaCl solution. After the mixture had been magnetically stirred for 2 h and centrifuged at 10,000×g for 25 min at 4 °C, the supernatant (globulin) was collected. The residue was then mixed with 10 mL of 70% ethanol. After the mixture had been magnetically stirred for 2 h and centrifuged at 10,000×g for 25 min at 4 °C, the supernatant (gliadin) was collected. Finally, 10 mL of 0.1 mol/L NaOH solution was added to the remaining residue. After the mixture had been magnetically stirred for 2 h and centrifuged at 10,000×g for 25 min at 4 °C, the supernatant (glutelin) was collected. The protein content of each fraction was determined by the Coomassie Brilliant Blue method. The result was expressed as mg/L litchi juice.

Extraction of free phenolics and bound phenolics

The extraction of free phenolics was performed according to the method of Sun et al. (2002) with some modification. Briefly, 30 mL of supernatant was added to 60 mL of 80% ethanol. The sample was sonicated at 30 °C for 10 min and then centrifuged at 4500×g for 10 min; the supernatant was collected. The sediment was then extracted twice with 80% ethanol. The extracts were combined and concentrated with a rotary evaporator. The concentrate was placed into a Sep-Pak C18 cartridge (Waters, Milford, MA, USA) and eluted sequentially with water and methanol. The methanol eluent was collected. It was concentrated under vacuum, freeze dried, and then dissolved in 2 mL of 80% methanol. The extraction of bound phenolics was carried out according to the method of Tang et al. (2016). Sediment

(0.1 g) was mixed with 10 mL of 2 mol/L HCl. The mixture was incubated at 85 °C for 1 h and then adjusted to pH 2.0 with 10 mol/L NaOH. After that, the extract was centrifuged at 6000×g for 5 min. The supernatant was extracted six times with ethyl acetate/diethyl ether (1:1, v/v). The combined extracts were then concentrated under vacuum, freeze dried, and dissolved in 2 mL of 80% methanol.

Determination of total phenolic content

The total phenolic content was determined according to the Folin–Ciocalteu method as described previously (Zou et al., 2017). Specifically, 1 mL of an appropriately diluted sample was mixed with 2.0 mL of Folin–Ciocalteu solution. The mixture was left to stand for 5 min. Then, 2.0 mL of 10% (m/v) Na₂CO₃ solution was added, and the solution was allowed to stand at 25 °C in the dark for 1 h. The absorbance was measured at 760 nm. Gallic acid was used as the standard, and the total phenolic content of the sample was expressed as gallic acid equivalents (GAE).

Qualitative and quantitative analysis of polyphenols

The identification of polyphenols was performed by HPLC (LC-20AT, Shimadzu, Kyoto, Japan) coupled to electrospray ionization–tandem mass spectrometry (5600, SCIEX, Concord, Ontario, Canada) (HPLC–ESI–MS/MS). The column was a WondaSil C18 (250 × 4.6 mm, 5 µm), and the mobile phase consisted of 0.4% acetic acid solution (A) and acetonitrile (B). Gradient elution was applied as follows: 0–40 min for 5% B to 25% B, 40–45 min for 25% B to 35% B, and 45–50 min for 35% B to 50% B. The column temperature was 30 °C, flow rate was 1 mL/min, and detection wavelength was 200–700 nm (Zhang et al., 2013). ESI was used as the ion source with negative-ion mode. The capillary voltage was 4.5 kV, atomizer pressure was 1.5 bar, and mass spectrometry (*m/z*) range was 100–1000. The polyphenol content was determined by HPLC (LC-20AT, Shimadzu). Epicatechin, procyanidin B₂, rutin, and isorhamnetin-3-*O*-rutanose were quantified by using the corresponding standards. Due to the lack of commercial standards, quercetin-3-*O*-rutinose-7-*O*-rhamnoside, kaempferol-3-*O*-rutinose-7-*O*-rhamnoside, and isorhamnetin-3-*O*-rutanose-7-*O*-rhamnoside were quantitated by using quercetin, kaempferol-3-*O*-rutanose, and isorhamnetin-3-*O*-rutanose, respectively.

Data analysis

The results were expressed as mean ± standard deviation, and the data were analyzed by using Tukey's multiple

range of ANOVA with SPSS 18.0 (Chicago, IL, USA). The statistical significance was defined at $p < 0.05$.

Results and discussion

Changes in stability of cloudy litchi juice during storage

As shown in Fig. 1A, the sediment concentration of cloudy litchi juice during the storage process increased gradually. In the first 8 weeks, the sediment from the litchi juice stored at 25 °C was significantly ($p < 0.05$) higher than that stored at 4 °C. After 9 weeks of storage, the sediment concentrations of the two samples were similar, and the value increased to 7900 mg/L. In general, the higher the temperature, the faster the sedimentation occurred in the juice. In addition, during the storage process, some of the soluble compounds transformed into insoluble compounds. Our results are consistent with the results of Kolniak-Ostek et al. (2014), who reported that the content of insoluble substances in cloudy apple juice kept increasing during the first 4 months of storage.

Relative turbidity is one of the important indicators for characterizing the cloud stability of fruit juice (Schultz et al., 2014). It was reported that relative turbidity was sensitive to the extent of flocculation within the cloud (Schultz et al., 2014). Our results showed that the relative turbidity increased significantly ($p < 0.05$), indicating that the supernatant of cloudy litchi juice became more stable as the storage time increased. This is attributed to haze formation during the storage. Our results are consistent with previous reports of cloudy apple juice (Oszmiański et al., 2009).

The ζ potential can be used to characterize the charge properties of the particle surfaces in the solution, which is also an important indicator of the juice system (Genovese

and Lozano, 2001). The greater the absolute value of the ζ potential, the stronger the surface charge and the lower the tendency for aggregation between particles (Genovese and Lozano, 2001; Schultz et al., 2014). In general, particles with the absolute value of ζ potential greater than 30 mV are considered stable (Genovese and Lozano, 2001). It has been reported that the ζ potential of apple juice is -19 to -20 mV (Genovese and Lozano, 2001) and the potential of orange juice is -23 mV (Croak and Corredig, 2006). Our study showed that the initial value of the ζ potential of litchi juice was only -2.4 mV, which indicated that the surface charge of the particles in the system was small and the repulsion between the particles was weak. Therefore, the aggregation would occur easily in litchi juice. After 10 weeks of storage at 4 °C and 25 °C, the ζ potential of cloudy litchi juice decreased to -11.2 mA and -10.9 mA, respectively (Fig. 1C). This is consistent with the results of Mollov et al. (2006), who reported that the surface charge on the surface of the particles in apple juice increased after refrigeration. These changes may be due to adsorption of free hydroxyl groups by macromolecules such as proteins in the juice.

Changes in protein content during the storage of cloudy litchi juice

The stability of juice does not correlate well with the protein concentration, because individual proteins have different properties (Esteruelas et al., 2009). The research of Hsu and Heatherbell (1987) indicated that unstable proteins usually possess lower isoelectric points (4.1–5.8) and smaller relative molecular masses (13–30 kDa). We first analyzed the changes in the total protein content in the supernatant of cloudy litchi juice during storage at 4 °C and 25 °C (Fig. 2). Within the first 4 weeks of storage at 4 °C, the protein content decreased rapidly, with a decreasing amplitude of 39.7%; this was followed by a

Fig. 1 Changes in the sediment concentration (A) and the ζ potential and relative turbidity (B) of cloudy litchi juice

Fig. 2 Changes in the content of protein fractions in cloudy litchi juice. **(A)** Protein content in the supernatant, **(B)** albumin content in the sediment, **(C)** globulin content in the sediment, **(D)** gliadin content in the sediment, **(E)** glutelin content in the sediment

slow declining trend. At 25 °C, the protein content in the supernatant decreased significantly ($p < 0.05$) in the first 3 weeks (45.2%), and then decreased slowly. Compared with that at 4 °C, the decreasing amplitude of protein was greater at 25 °C. The above results show that the changes in the protein content in the supernatant are negatively correlated with the sediment concentration. Thus, it can be

speculated that the proteins in the supernatant are involved in the formation of the sediments.

Subsequently, we extracted the proteins in the sediment by the Osborne fractionation method (Horax et al., 2010; Osborne, 1924). According to Osborne classification, proteins are divided into 4 classes based on solubility. Albumin is soluble in water, globulin is soluble in salt solution, gliadin is soluble in 70–90% alcohol, and glutelin is

soluble in alkaline solution (Horax et al., 2010; Osborne, 1924). The changes in the contents of different protein fractions are shown in Fig. 2. Under storage at 4 °C and 25 °C, the content of glutelin was much higher than those of the other three proteins, among which the content of globulin was the lowest. Under storage conditions of 4 °C, albumin did not change significantly ($p > 0.05$), whereas the other three proteins showed an increasing trend. Glutelin showed the greatest change, and its content increased from 32.2 to 70.0 mg/L. Under storage at 25 °C, the change trends of albumin, gliadin, and glutelin in the sediment were the same as those at 4 °C, and globulin showed a decrement in the first 5 weeks followed by an increase after 6 weeks. Compared with 4 °C, the contents of albumin and globulin at 25 °C were slightly lower, whereas the contents of gliadin and glutelin were slightly higher.

The above results indicate that the supernatant mainly contained albumin. With prolongation of the storage time, the solubility of albumin gradually reduced, which resulted in the formation of sediment. At present, the changes in protein contents of different fractions during fruit juice storage have not been reported. Some literature has suggested that the role of proteins in the formation of turbidity in grape wine has two stages (Van Sluyter et al., 2015). In the first stage, the folded proteins are unfolded, whereas in the second stage, the unfolded proteins aggregated and the haze was formed (Van Sluyter et al., 2015). During the storage of cloudy litchi juice, the folded albumin was unfolded, and it subsequently aggregated and precipitated from the juice system, to form the sediment. Temperature has a great impact on protein unfolding: the higher the temperature, the easier the unfolding (Van Sluyter et al., 2015). In addition, proteins in litchi juice may combine with other substances, such as polyphenols and polysaccharides, and thus form the sediment. It was reported that glutamate was the main amino acid of cloudy proteins in beer (Siebert, 2009) and apple-based beverages (Millet et al., 2017). However, the amino acid composition of different protein fractions remains to be studied further.

Changes in phenolic compounds during the storage of cloudy litchi juice

The changes in the total phenolic content in the supernatant are shown in Fig. 3A. In the first week, the total phenolic contents decreased sharply, with decreasing amplitudes of 41.1% and 49.7% at 4 °C and 25 °C, respectively. After 10 weeks, the total phenolic contents decreased by 85.1 mg/L and 45.0 mg/L, respectively. In contrast, the total phenolic contents in the sediment increased first and then decreased. Ten weeks later, the contents in the sediment increased by 2.75 mg/L and 1.61 mg/L, respectively;

these values were much lower than the decreased amounts of the total phenolic compounds in the supernatant. The above results indicate that polyphenols may be involved in the sediment formation of cloudy litchi juice. As the determination of the total phenolic content by the Folin–Ciocalteu method is based on a redox reaction, the reducing sugars, amino acids, proteins, and vitamin C in cloudy litchi juice influence the results (Millet et al., 2017). In addition, the chromogenic degrees are not the same for different phenolic compounds and the reduction capacity of oxidized polyphenols is weakened, which leads to the lower result values (Millet et al., 2017). This eventually results in the decreasing amount of total phenolic content in the supernatant being much higher than the increasing amount in the sediment.

In order to investigate which phenolic compounds play major roles in the formation of the sediment, the structures of the polyphenols in cloudy litchi juice were analyzed by HPLC–ESI–MS/MS. As shown in Table 1, 7 phenolic compounds were identified, 4 of which were epicatechin, procyanidin B₂, quercetin-3-*O*-rutinose-7-*O*-rhamnoside, and rutin, shown as peak 1, peak 2, peak 3, and peak 6, respectively. Peak 4 shows a molecular ion $[M]^-$ at m/z 739, that has a fragment at m/z 593 that was obtained by loss of a rhamnose moiety $[M-146]^-$. The fragment at m/z 285, which matches a kaempferol monomeric ion, was obtained by loss of rhamnose and rutinose moieties $[M-146-146-162]^-$. Thus, this compound can be putatively identified as kaempferol-3-*O*-rutinose-7-*O*-rhamnoside. Peak 5 shows a molecular ion at m/z 769 and a fragment at m/z 623, corresponding to the loss of a rhamnose moiety $[M-146]^-$. The fragment at m/z 315, corresponding to isorhamnetin, was obtained from the loss of rhamnose and rutinose moieties $[M-146-146-162]^-$. Thus, this compound can be putatively identified as isorhamnetin-3-*O*-rutinose-7-*O*-rhamnoside. The compound with a molecular ion at m/z 623 and a fragment at m/z 315 was putatively identified as isorhamnetin-3-*O*-rutanoside.

As shown in Table 2, quercetin-3-*O*-rutinose-7-*O*-rhamnoside is the main phenolic compound in cloudy litchi juice, accounting for 55.3% of the total phenolics determined by HPLC. This phenolic compound decreased by 15.8% after the first week at 4 °C and then remained stable, whereas, it decreased gradually during the first 4 weeks at 25 °C. The highest decline was observed for procyanidin B₂, which decreased by 53.5% and 54.4% at 4 °C and 25 °C, respectively. Relatively, kaempferol-3-*O*-rutinose-7-*O*-rhamnoside and isorhamnetin-3-*O*-rutinose-7-*O*-rhamnoside were more stable. Therefore, it can be speculated that, among the polyphenols, procyanidin B₂ and quercetin-3-*O*-rutinose-7-*O*-rhamnoside are the main phenolic compounds that cause the sediment of cloudy litchi juice. Numerous studies have shown that the stability of

Fig. 3 Changes in the total phenolic content in cloudy litchi juice. (A) Total phenolic content in the supernatant, (B) Total phenolic content in the sediment

Table 1 Identification of phenolic compounds in cloudy litchi juice

Peak	Retention time	UV/vis (nm)	[M-H] ⁻ <i>m/z</i>	MS/MS <i>m/z</i>	Identification
1	27.6	288	289.07	245.08, 205.05, 179.03	(-)-Epicatechin
2	28.9	279	577.13	425.11, 407.12, 289.15	Procyanidin B ₂
3	40.5	255, 355	755.20	609.10, 489.12, 300.01	Quercetin-3- <i>O</i> -rutinose-7- <i>O</i> -rhamnoside
4	44.1	265, 348	739.20	593.31, 285.12, 255.08	Kaempferol-3- <i>O</i> -rutinose-7- <i>O</i> -rhamnoside
5	44.3	265, 348	769.21	623.21, 315.17, 300.17	Isorhamnetin-3- <i>O</i> -rutanoside-7- <i>O</i> -rhamnoside
6	45.3	278	609.14	343.00, 301.02	Rutin
7	47.1	279	623.15	315.11, 300.27	Isorhamnetin-3- <i>O</i> -rutinoside

polyphenols is related to the number of hydroxyl groups, degree of polymerization, and molecular weight (Esteruelas et al., 2011; Millet et al., 2017; Siebert, 2009). More galloyl groups, hydroxyl groups, and a higher degree of polymerization make polyphenols combine with proteins more easily (Zhu et al., 2018). In our study, the decreased amount of procyanidin B₂ was markedly higher than that of (+)-catechin, which is consistent with the previous reports (Millet et al., 2017). During the storage of litchi juice, polyphenols may combine with proteins to form polymers and precipitate.

According to the work of Millet et al. (2017), procyanidin accounted for 59–96% of the total phenolics in hazes of apple-based beverages. In order to verify the direct participation of procyanidin B₂ and quercetin-3-*O*-rutinose-7-*O*-rhamnoside in the formation of the sediment, the phenolic compounds in the sediment were analyzed. Unfortunately, regardless of whether acid degradation or phloroglucinol degradation was used, neither procyanidin B₂ nor quercetin-rutinose-rhamnoside could be detected in the sediment, and neither could the monomer compounds (+)-catechin and quercetin. These results suggest that

procyanidin B₂ and quercetin-3-*O*-rutinose-7-*O*-rhamnoside may be oxidized during storage, and thus, they cannot be detected by HPLC–DAD–MS. The study of Millet et al. (2017) also indicated that oxidized polyphenols were difficult to detect by HPLC–DAD–MS. In the following work, we will study the changes of these two polyphenols in a juice model system.

In this paper, changes in sediment amount, ζ potential, proteins, and polyphenols in cloudy litchi juice during storage were investigated. The results showed that the sediment amount of cloudy litchi juice increased gradually, whereas the total protein content in the supernatant decreased. The glutelin content in the sediment was much higher than that of other protein components and showed an increasing trend. The total content of phenolics in the supernatant decreased. In addition, the greatest declines were observed in the contents of procyanidin B₂ and quercetin-3-*O*-rutinose-7-*O*-rhamnoside. However, an increase in the total phenol content in the sediment was observed. Taken together, it was speculated that, during the storage process, the denaturation of soluble proteins slowly occurred in the cloudy litchi juice with a gradual

Table 2 Changes in content of individual phenolic compounds in cloudy litchi juice

Week	(-)-Epicatechin	Procyanidin B ₂	Quercetin-3- <i>O</i> -rutinose-7- <i>O</i> -rhamnoside	Kaempferol-3- <i>O</i> -rutinose-7- <i>O</i> -rhamnoside	Isorhamnetin-3- <i>O</i> -rutanoside-7- <i>O</i> -rhamnoside	Rutin	Isorhamnetin-3- <i>O</i> -rutoside
4 °C							
0	4.45 ± 0.03 ^{a(1)}	6.47 ± 0.01 ^a	55.63 ± 0.57 ^a	14.96 ± 0.17 ^a	8.49 ± 0.22 ^a	3.65 ± 0.11 ^a	6.96 ± 0.10 ^a
1	3.54 ± 0.00 ^c	6.29 ± 0.05 ^{ab}	45.43 ± 0.40 ^b	14.61 ± 0.29 ^{ab}	7.74 ± 0.15 ^b	2.64 ± 0.01 ^{cd}	4.45 ± 0.12 ^{bc}
2	3.48 ± 0.00 ^{cd}	5.85 ± 0.06 ^c	42.28 ± 0.41 ^d	14.25 ± 0.09 ^{bc}	7.35 ± 0.11 ^{bc}	2.69 ± 0.01 ^{cd}	4.26 ± 0.08 ^{bcd}
3	3.53 ± 0.00 ^c	6.08 ± 0.00 ^{bc}	43.01 ± 0.18 ^{cd}	13.91 ± 0.17 ^{cde}	6.86 ± 0.13 ^d	2.32 ± 0.08 ^e	4.05 ± 0.08 ^{ef}
4	3.65 ± 0.03 ^b	5.59 ± 0.07 ^d	42.95 ± 0.06 ^{cd}	13.95 ± 0.17 ^{cd}	6.90 ± 0.16 ^d	2.52 ± 0.07 ^{de}	3.97 ± 0.09 ^f
5	3.42 ± 0.00 ^d	5.44 ± 0.15 ^d	42.23 ± 0.51 ^d	14.07 ± 0.17 ^c	6.94 ± 0.05 ^d	2.97 ± 0.07 ^b	4.15 ± 0.09 ^{def}
6	3.30 ± 0.00 ^e	4.76 ± 0.10 ^e	42.81 ± 0.11 ^{cd}	14.01 ± 0.18 ^{cd}	6.85 ± 0.16 ^d	2.77 ± 0.08 ^{bc}	4.36 ± 0.03 ^{bcd}
7	3.13 ± 0.05 ^{fg}	4.25 ± 0.09 ^f	43.92 ± 0.80 ^c	13.93 ± 0.08 ^{cd}	6.95 ± 0.07 ^{cd}	2.92 ± 0.06 ^b	4.47 ± 0.04 ^b
8	3.18 ± 0.01 ^f	3.83 ± 0.09 ^g	43.97 ± 0.13 ^c	13.54 ± 0.10 ^{de}	6.84 ± 0.08 ^d	2.54 ± 0.08 ^d	4.30 ± 0.04 ^{bcd}
9	3.34 ± 0.03 ^e	3.23 ± 0.05 ^h	43.74 ± 0.04 ^c	13.98 ± 0.06 ^{cd}	6.79 ± 0.18 ^d	2.68 ± 0.06 ^{cd}	4.07 ± 0.04 ^{ef}
10	3.10 ± 0.00 ^g	3.00 ± 0.07 ⁱ	41.01 ± 0.34 ^e	13.42 ± 0.27 ^e	6.84 ± 0.09 ^f	2.60 ± 0.05 ^{cd}	4.25 ± 0.05 ^{cde}
25 °C							
0	4.45 ± 0.03 ^a	6.47 ± 0.01 ^a	55.63 ± 0.57 ^a	14.96 ± 0.17 ^a	8.49 ± 0.22 ^a	3.65 ± 0.11 ^a	6.96 ± 0.10 ^a
1	3.53 ± 0.01 ^b	4.71 ± 0.04 ^b	45.29 ± 0.41 ^b	14.03 ± 0.18 ^b	7.42 ± 0.18 ^b	2.54 ± 0.07 ^{bc}	4.67 ± 0.08 ^b
2	3.46 ± 0.05 ^b	3.30 ± 0.03 ^c	43.08 ± 0.33 ^c	13.69 ± 0.17 ^b	7.13 ± 0.13 ^{bc}	2.31 ± 0.10 ^{bc}	4.26 ± 0.11 ^c
3	3.20 ± 0.03 ^c	2.98 ± 0.01 ^d	43.29 ± 0.20 ^c	13.51 ± 0.17 ^{bc}	6.90 ± 0.10 ^c	2.44 ± 0.11 ^{bc}	4.24 ± 0.06 ^c
4	2.75 ± 0.02 ^{de}	2.40 ± 0.04 ^f	40.38 ± 0.52 ^d	12.72 ± 0.20 ^d	6.96 ± 0.14 ^c	2.32 ± 0.05 ^{bc}	4.11 ± 0.13 ^c
5	2.83 ± 0.05 ^d	2.38 ± 0.35 ^f	40.82 ± 0.49 ^d	12.64 ± 0.08 ^{de}	6.76 ± 0.13 ^c	2.46 ± 0.11 ^{bc}	4.03 ± 0.07 ^c
6	2.65 ± 0.01 ^e	2.88 ± 0.15 ^d	38.57 ± 0.53 ^e	12.91 ± 0.15 ^{cd}	6.21 ± 0.25 ^d	2.56 ± 0.11 ^{bc}	4.24 ± 0.06 ^c
7	2.34 ± 0.02 ^f	2.58 ± 0.06 ^e	36.50 ± 0.09 ^g	12.50 ± 0.09 ^{def}	5.89 ± 0.11 ^{de}	2.27 ± 0.07 ^c	4.01 ± 0.09 ^c
8	2.44 ± 0.01 ^f	3.26 ± 0.05 ^c	37.45 ± 0.53 ^{efg}	12.40 ± 0.20 ^{def}	5.23 ± 0.07 ^f	2.57 ± 0.06 ^{bc}	4.18 ± 0.09 ^c
9	2.23 ± 0.08 ^g	2.83 ± 0.00 ^d	38.41 ± 0.11 ^{ef}	11.90 ± 0.11 ^f	5.56 ± 0.09 ^{ef}	2.42 ± 0.11 ^{bc}	4.31 ± 0.08 ^c
10	2.20 ± 0.01 ^g	2.95 ± 0.03 ^d	37.22 ± 0.58 ^{fg}	12.05 ± 0.50 ^{ef}	5.33 ± 0.14 ^f	2.46 ± 0.12 ^b	4.28 ± 0.19 ^c

The results are expressed in mg/L of juice

⁽¹⁾Different alphabets in the same column at the same temperature indicate significant differences ($p < 0.05$)

transformation into insoluble glutelin, which leads to the formation of sediment. In addition, the oxidation and aggregation of procyanidin B₂ and quercetin-3-*O*-rutinose-7-*O*-rhamnoside may also be involved in the formation of the sediment. The properties and structures of different protein fractions, as well as the structures of the phenolic compounds in the sediment, need to be further studied.

Acknowledgements This work was supported by the National Natural Science Foundation of China (No. 31501541), the Natural Science Foundation of Guangdong Province (No. 2015A030312001), and the Science and Technology Program of Guangdong Province (No. 2017B020207005), China.

Compliance with ethical standards

Conflict of interest The authors declare that they no conflict of interest.

References

- Bhoopat L, Srichairatanakool S, Kanjanapothi D, Taesotikul T, Thananchai H, Bhoopat T. Hepatoprotective effects of lychee (*Litchi chinensis* Sonn.): a combination of antioxidant and anti-apoptotic activities. *J. Ethnopharmacol.* 136: 55–66 (2011)
- Bradford MM. A rapid method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248–254 (1976)
- Brat P, Georgé S, Bellamy A, Du Chaffaut L, Scalbert A, Mennen L, Arnault N, Amiot MJ. Daily polyphenol intake in France from fruit and vegetables. *J. Nutr.* 136: 2368–2373 (2006)
- Croak S, Corredig M. The role of pectin in orange juice stabilization: effect of pectin methylesterase and pectinase activity on the size of cloud particles. *Food Hydrocolloid* 20: 961–965 (2006)
- Esteruelas M, Kontoudakis N, Gil M, Fort MF, Canals JM, Zamora F. Phenolic compounds present in natural haze protein of Sauvignon white wine. *Food Res. Int.* 44: 77–83 (2011)
- Esteruelas M, Poinaut P, Sieczkowski N, Manteau S, Fort MF, Canals JM, Zamora F. Characterization of natural haze protein in sauvignon white wine. *Food Chem.* 113: 28–35 (2009)

- Genovese DB, Lozano JE. The effect of hydrocolloids on the stability and viscosity of cloudy apple juices. *Food Hydrocolloid* 15: 1–7 (2001)
- Grimi N, Mamouni F, Lebovka N, Vorobiev E, Vaxelaire J. Impact of apple processing modes on extracted juice quality: pressing assisted by pulsed electric fields. *J. Food Eng.* 103: 52–61 (2011)
- Grintzalis K, Georgiou CD, Schneider YJ. An accurate and sensitive Coomassie Brilliant Blue G-250-based assay for protein determination. *Anal. Biochem.* 480: 28–30 (2015)
- Horax R, Hettiarachchy N, Over K, Chen P, Gbur E. Extraction, fractionation and characterization of bitter melon seed proteins. *J. Agric. Food Chem.* 58: 1892–1897 (2010)
- Hsu JC, Heatherbell DA. Heat-unstable proteins in wine. I. Characterization and removal by bentonite fining and heat treatment. *Am. J. Enol. Vitic.* 38: 11–16 (1987)
- Jiang YM, Duan XW, Joyce D, Zhang ZQ, Li JR. Advances in understanding enzymatic browning of harvested litchi fruit. *Food Chem.* 88: 443–446 (2004)
- Khan IU, Asghar MN, Iqbal S, Bokhari TH, Khan ZD. Radical scavenging and antioxidant potential of aqueous and organic extracts of aerial parts of *Litchi chinensis* Sonn. *Asian J. Chem.* 21: 5073–5084 (2009)
- Kolniak-Ostek J, Oszmiański J, Rutkowski KP, Wojdyło A. Effect of 1-methylcyclopropene postharvest treatment apple and storage on the cloudy juices properties. *LWT Food Sci. Technol.* 59: 1166–1174 (2014)
- Liu XR, Xie RP, Fan YW, Hu JN, Luo T, Li HY, Deng ZY. Polymerization of proanthocyanidins catalyzed by polyphenol oxidase from lotus seedpod. *Eur. Food Res. Technol.* 238: 727–739 (2014)
- Millet M, Poupard P, Le QJ, Bauduin R, Guyot S. Haze in apple-based beverages: detailed polyphenol, polysaccharide, protein, and mineral compositions. *J. Agric. Food Chem.* 65: 6404–6414 (2017)
- Mollov P, Mihalev K, Buleva M, Petkanchin I. Cloud stability of apple juices in relation to their particle charge properties studied by electro-optics. *Food Res. Int.* 39: 519–524 (2006)
- Osborne TB. The vegetable proteins. *J. Soc. Chem. Ind.* 43: 440 (1924)
- Oszmiański J, Wojdyło A, Kolniak J. Effect of enzymatic mash treatment and storage on phenolic composition, antioxidant activity, and turbidity of cloudy apple juice. *J. Agric. Food Chem.* 57: 7078–7085 (2009)
- Saxena S, Hajare SN, More V, Kumar S, Wadhawan S, Mishra BB, Parte MN, Gautam S, Sharma A. Antioxidant and radioprotective properties of commercially grown litchi (*Litchi chinensis*) from India. *Food Chem.* 126: 39–45 (2011)
- Schultz AK, Anthon GE, Dungan SR, Barrett DM. Effect of acidification on carrot (*Daucus carota*) juice cloud stability. *J. Agric. Food Chem.* 62: 11528–11535 (2014)
- Siebert KJ. Haze formation in beverages. *LWT Food Sci. Technol.* 39: 987–994 (2006)
- Siebert KJ. Haze in beverages. *Adv. Food Nutr. Res.* 57: 53–86 (2009)
- Sun J, Chu YF, Wu X, Liu RH. Antioxidant and antiproliferative activities of common fruits. *J. Agric. Food Chem.* 50: 7449–7454 (2002)
- Sun X, Bai R, Zhang Y, Wang Q, Fan X, Yuan J, Cui L, Wang P. Laccase-catalyzed oxidative polymerization of phenolic compounds. *Appl. Biochem. Biotech.* 171: 1673–1680 (2013)
- Tang Y, Zhang B, Li X, Chen PX, Zhang H, Liu R, Tsao R. Bound phenolics of quinoa seeds released by acid, alkaline and enzymatic treatments and their antioxidant and α -glucosidase and pancreatic lipase inhibitory effects. *J. Agric. Food Chem.* 64: 1712–1719 (2016)
- Van Sluyter SC, McRae JM, Falconer RJ, Smith PA, Bacic A, Waters EJ, Marangon M. Wine protein haze: mechanisms of formation and advances in prevention. *J. Agric. Food Chem.* 63: 4020–4030 (2015)
- Vernhet A, Carrillo S, Poncetlegrand C. Condensed tannin changes induced by autoxidation: effect of the initial degree of polymerization and concentration. *J. Agric. Food Chem.* 62: 7833–7842 (2014)
- Zhang Z, Huber DJ, Qu H, Yun Z, Wang H, Huang Z, Huang H, Jiang Y. Enzymatic browning and antioxidant activities in harvested litchi fruit as influenced by apple polyphenols. *Food Chem.* 171: 191–199 (2015)
- Zhang R, Zeng Q, Deng Y, Zhang M, Wei Z, Zhang Y, Tang X. Phenolic profiles and antioxidant activity of litchi pulp of different cultivars cultivated in Southern China. *Food Chem.* 136: 1169–1176 (2013)
- Zhu W, Jia Y, Peng J, Li CM. Inhibitory effect of persimmon tannin on pancreatic lipase and the underlying mechanism in vitro. *J. Agric. Food Chem.* 66: 6013–6021 (2018)
- Zou B, Wu J, Yu Y, Xiao G, Xu Y. Evolution of the antioxidant capacity and phenolic contents of persimmon during fermentation. *Food Sci. Biotechnol.* 26: 563–571 (2017)

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