Quinone-mediated non-enzymatic browning in model systems during long-term storage

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ABSTRACT

Non-enzymatic browning induced by polyphenol oxidation is an essential problem during the processing and storage of fruit and vegetable products. Here, the non-enzymatic browning mechanism between catechin (CAT), chlorogenic acid (CQA) and their corresponding quinones was investigated in model systems during the 32-d long-term storage. The results showed that CAT and catechin quinone (CATQ), which contains both A ring with a resorcinol structure and an o-diphenol B ring, are important precursors for browning, while chlorogenic acid quinone (CQAQ)-mediated CAT oxidation (k_{CAT-quinone}) was faster than CAT autoxidation (k_{autoxidation}) and there was no significant difference between CQAQ-mediated CAT oxidation and CATQ-mediated CQA oxidation. These indicate that CQAQ oxidizes CAT to CATQ quickly, and CATQ reacts with CAT subsequently through complex reactions to produce brown pigments in model systems during long-term storage.

1. Introduction

Browning is a strict problem during the processing and storage of fruit and vegetable products, due to high level of polyphenols and high activity of polyphenol oxidase (PPO), and its most distinctive feature is the darkening of color, which is often accompanied by the decrease of flavor and nutritional value, thus lowering consumer preference (Komthong, Katoh, Igura, & Shimoda, 2006; Munoz-Pina et al., 2022; Shen, Zhu, Pan, Cao, Liu, & Li, 2021; Yu et al., 2021). Although phenolic compounds exert health benefits on humans (Cornara, Xiao, Smeriglio, Trombetta, & Burlando, 2020; Sun et al., 2020), they are also responsible for quality deterioration on fruit and vegetables, especially browning. According to the mechanism, browning induced by polyphenol oxidation is divided into enzymatic browning and non-enzymatic browning, respectively (Li, Li, Yan, & Wang, 2021). The former is catalyzed by PPO whereas a wide range of novel processing technologies, such as high-pressure processing, ultrasound and pulsed electric field have been developed to inactivate PPO and hence enzymatic browning is mitigated after processing (Moon, Kwon, Lee, & Kim, 2020; Zhu, Zhang, Mujumdar, & Liu, 2022). However, phenolic compounds are relatively less influenced by processing conditions and remain high chemical reactivity, so non-enzymatic browning is present throughout the processing, storage, transportation and consumption of fruit and vegetable products.

During the non-enzymatic browning process, phenolic compounds are oxidized to quinones catalyzed by oxygen and metal ions, which further self-polymerize or combine with amino acids and proteins, to eventually produce brown macromolecules (Dong, Zhang, Pang, & Qi, 2016; Oliveira, Silva Ferreira, De Freitas, & Silva, 2011). Phenolics are important substrates for browning and catechin (CAT) has been reported to have a high correlation with browning in juices (Benito Martinez-Hernandez, Hazel Alvarez-Hernandez, & Artes-Hernandez, 2019; Derardja et al., 2022; RichardForget & Gauillard, 1997; Song, Yao, Zhai, Du, Chen, & Wei, 2007). Yi, Dong, and Zhu (2014) revealed...
that the concentration of CAT is positively correlated with the degree of browning in the model system. A possible browning mechanism mediated by catechin quinone (CATQ) was proposed that the A ring of CATQ with a resorcinol structure nucleophilically attacks the B ring of CATQ to yield addition product, and then the product undergoes oxidation, intramolecular addition, and isomerization to finally produce a yellow dimer (Guyot, Vercauteren, & Cheynier, 1996; Ma & Waterhouse, 2018). Chlorogenic acid quinone (CQAQ) and its polymerized product with amino acids do not produce intensive browning in the acidic aqueous solution (Murata, Sugita, Sonokawa, Shimamura, & Homma, 2002). Despite of rapid decrease of chlorogenic acid (CQA) during enzymatic browning, browning tends to be more strongly correlated with the initial level of flavan-3-ols (CAT monomers and procyanidins) (Radi et al., 1997). In addition, CQAQ is of great significance to browning and it might be capable of non-enzymatically oxidizing CAT to CATQ, collectively contributing to browning in juices (Amaki, Saito, Taniguchi, Joshiba, & Murata, 2011). This is consistent with our previous research about the interaction between CQAQ and CAT (Liu, et al., 2022). The study on the browning rate of the model system within 30 min indicated that the rate of CQAQ-mediated CAT oxidation (0.1326 mol⋅L⁻¹⋅min⁻¹) was much higher than that of CAT autoxidation (4.25E⁻⁵ mol⋅L⁻¹⋅min⁻¹), and that of CATQ-mediated CQA oxidation (Liu, et al., 2022). These results play an essential role in revealing the browning mechanism. However, it is insufficient to verify the results in the model system within merely 30 min. Real juice is usually stored for 7 to 180 days before consumption (Lee, Yusof, Hamid, & Baharin, 2007; Xu, Wei, Chai, Huang, & Shen, 2021). Whether the same result could be obtained during long-term storage of model systems remain unclear.

Therefore, a long-term storage experiment was carried out in this paper where the role of CATQ, CAT and CQA in browning were further investigated. To study browning mechanism, model systems are usually employed. Aka, Courtis, Louarme, Nicolas, and Billaud (2013) used buffers in combination with phenolic compounds as model systems to study enzymatic browning. Phosphate buffer solutions comprised of phenolic substrates and enzymes, have been widely used to construct a model system to explore the phenolic-dominated browning mechanism (Dong, Zhang, Wang, & Qi, 2016; Liu, et al., 2022; Yi, Dong, & Zhu, 2014; Yi, Dong, Zhu, & Zhao, 2015). In our previous study, the model system was also developed to mimic the real juice (Liu et al., 2022). In this manuscript, two model systems were modified based on these researches. One system includes the target quinones and phenols and the other includes the PPO and phenols. These two model systems could reveal the browning system in two different ways, leading to more confident results. Moreover, the three browning pathways, CQAQ-mediated CAT oxidation, CAT autoxidation, as well as CATQ-mediated CQA oxidation were compared, respectively, to uncover the non-enzymatic browning mechanism in long-term stored model systems.

2. Materials and methods

2.1. Reagents and chemicals

Catechin, chlorogenic acid, ferrous chloride, copper chloride, Amberlyst A-26(OH) Ion exchange resin, periodic acid, tetrahydrofuran, ether, anhydrous formic acid and anhydrous methanol were purchased from Shanghai Macleans Biochemical Co., Ltd (ShangHai, China). Disodium hydrogen phosphate anhydrous and citric acid monohydrate were purchased from Sinopharm Chemical Reagent Co., Ltd. Benzenesulfinic acid (BSA) was purchased from Shanghai Bi De Pharmaceutical Technology Co., Ltd (ShangHai, China). Water was purified using a Milli-Q system (Millipore, Billerica, MA, USA). All chemicals were of analytical grade or of the highest available purity.

2.2. Sample preparation

Na₂HPO₄-Citrate buffer was prepared by mixing 0.2 mol/L Na₂HPO₄ solution and 0.1 mol/L citric acid solution to reach the pH of 3.7. Ferrous chloride and copper chloride were added to the buffer system till the final concentration of Fe²⁺ and Cu²⁺ achieved 20 mg/L and 5 mg/L, respectively.

2.2.1. CQA and CAT oxidation catalyzed by PPO

CQA solution (8 mM), CAT solution (8 mM) and PPO solution (530 U/mL) were prepared, respectively. 0.5 mL phenolic compound (CQA or CAT) was added to 19.5 mL PPO solution (530 U/mL). Then 0.5 mL reaction solution was sucked out to mix with 0.5 mL BSA (0.4 mM) to quench quinone at 0 s, 10 s, 20 s, 30 s, 60 s, 2 min, 4 min, 8 min, 16 min, 32 min, 64 min, 128 min, respectively.

2.2.2. Long-term storage experiment in quinone oxidation model systems

Using the method of Ma and Waterhouse (2018) with some modifications, CATQ and CQAQ were prepared by dissolving CAT and CQA in 8 mL anhydrous methanol (20 mM). The solution was degassed under nitrogen for 10 min using a magnetic stirrer with 400 rpm. Then, 250 mg activated periodate resin was added to the solution for 7 min under the same condition. The quinone solution was decanted from the resin and used within 30 min.

Quinone oxidation model systems were constructed, system 1 (CQA+CAT), system 2 (CQAQ+CQA), system 3 (CATQ+CAT), system 4 (CATQ+CAT), system 5 (CQAQ+CQA), system 6 (CQAQ+CQA), system 7 (CATQ+CATQ), system 8 (CQA+CQA), system 9 (CAT+CAT), system 10 (CQA+CQA). Na₂HPO₄-Citrate buffer (100 mL) was sucked into the headspace bottle, and oxygen was introduced into the solution for 2 h to achieve oxygen saturation. Then 1 mL prepared quinone or phenolic solution (20 mM) was added to 100 mL Na₂HPO₄-Citrate buffer, followed by the addition of 1 mL another quinone or phenolic solution (20 mM). The 10 model systems were stored at room temperature. Then 0.2 mL reaction solution was sucked out to mix with 0.2 mL BSA (0.8 mM) to quench quinone at 1, 2, 3, 4, 5, 6, 7, 8, 11, 14, 17, 20, 23, 26, 29, 32 d.

2.2.3. Long-term storage experiment in PPO oxidation model systems

Ten PPO oxidation model systems were constructed, system 1 ([PPO-CQA] + CQA), system 2 ([PPO-CQA] + [PPO-CQA]), system 3 (CQA+CQA), system 4 ([PPO-CAT] + CAT), system 5: [PPO-CAT] + [PPO-CAT], system 6 (CAT + CAT), system 7 ([PPO-CQA] + CAT), system 8 ([PPO-CAT] + CQA), system 9 ([PPO-CQA] + [PPO-CAT]), system 10 (CQA + CAT). System 1, 4, 7 and 8 were prepared as follows: 0.5 mL phenolic compounds (CQA or CAT) (40 mM) were added to 100 mL Na₂HPO₄-Citrate buffer, followed by the addition of 1 mL PPO solution (2900 U/mL). Then oxygen was introduced into the systems for 2 h to achieve oxygen saturation. Later, the reaction systems were placed in a constant temperature water bath at 95 °C for 3 min to inactivate the enzymes and then in an ice-water bath for 5 min. Another phenolic compound (CQA or CAT) (0.5 mL, 40 mM) was added to the systems. System 2, 5 and 9 proceeded the same steps as the former three systems, while the inactivation of PPO was conducted after the addition of the two phenol solutions. System 3, 6 and 10 were not added with PPO and they just undergo oxygen saturation and the addition of two phenolic compounds. The 10 model systems were stored at room temperature. Then 0.2 mL reaction solution was sucked out to mix with 0.2 mL BSA (0.8 mM) to quench quinone at 1, 2, 3, 4, 5, 6, 7, 8, 11, 14, 17, 20, 23, 26, 29, 32 d, respectively.

2.3. Analytical procedure

2.3.1. Phenolic and quinone-BSA analysis

Owing to the instability and high reactivity of quinones, BSA was used to capture quinones to form the stable products. Hence, quinones were determined as quinone-BSA adducts. UHPLC-MS/MS was used to analyze polyphenols and quinone-BSA products. Samples were filtered with 0.22 μm Nylon membrane filter, of which 3 μL were injected into a
Waters ACQUITY UPLC System coupled with a Waters Xevo TQ-S and a reversed-phase C18 column (2.1 × 100 mm, 1.7 μm particles, Waters). The mobile phases were composed of (A) 0.1 % formic acid in water and (B) 0.1 % formic acid in methanol. The flow rate was 0.3 mL/min, and the column temperature was 30 °C. The gradient was as follows: 0 min, 10 % B; 4 min, 40 %; 6 min, 60 % B; 7 min, 100 % B; 8 min, 10 % B; 9 min, 10 % B. Samples in the automatic sampler tray were held at 10 °C.

The analysis was carried out in negative ion mode with an ESI interface. The source parameters were as follows: capillary voltage, 1.57 KV; cone gas flow, 700 L·Hr⁻¹; desolvation temperature, 550 °C. Multiple reaction monitor (MRM) was used, where the parent ion and daughter ion were \( m/z = 289 \) and \( 203 \) for CAT, \( m/z = 353 \) and \( 191 \) for CQA, \( m/z = 428.9 \) and \( 125.06 \) for CATQ-BSA, \( m/z = 492.9 \) and \( 301.05 \) for CQAQ-BSA.

### 2.3.2. Browning analysis

Browning was determined as the absorbance of the supernatant by UV–vis spectrophotometer at 405 nm. An aliquot of 200 μL sample was sucked to determine the absorbance. \( \text{Na}_2\text{HPO}_4\)-citrate buffer was used as a blank control.

### 2.3.3. Statistical analysis

Statistical calculations were performed with GraphPad Prism 9. Pairwise comparisons were conducted by T-test, and global \( P \) values were obtained by ANOVA. The level of significance was <0.05 (\( P < 0.05 \)) if not stated otherwise. Statistics were carried out on experimental replicates.

### 3. Results and discussion

#### 3.1. Long-term storage experiment in quinone oxidation model systems

### 3.1.1. Browning analysis

Fig. 1A showed the color changes of 10 different quinone oxidation model systems during 32 days of storage, meanwhile the browning was monitored by the absorbance at 405 nm (Fig. 1B). According to the storage time, the browning of 10 systems was analyzed in 3 different stages. Overall, no significant change in browning was observed in system 2 (CQAQ + CQA), system 6 (CQAQ + CQAQ), and system 8 (CQA + CQA), while the browning of other seven systems exhibited an upward trend with time going on. The browning of system 1 (CQAQ), system 4 (CQAQ + CAT), system 6 (CQAQ + CAT), system 5 (CATQ + CQAQ) and system 7 (CATQ + CATQ) showed a similar trend. In the initial stage (0–8 d, IS), the browning of these systems was in a steady state without significant change. In the second stage (8–23 d, SS), the browning increased rapidly, followed by a slow upward trend during the third stage (23–32 d, TS). Different from the former five systems, the browning of system 9 (CAT + CAT) and system 10 (CAT + CQA) increased slowly during the second stage whereas rise swiftly in the last stage.

A longitudinal comparison of the browning between each system was conducted. According to browning, the ranking order of the 10 quinone oxidation systems was as follows: system 7 (CATQ + CATQ) > system 4 (CATQ + CAT) > system 5 (CATQ + CQAQ) > system 3 (CATQ + CQA) ≈ system 1 (CQAQ + CAT) > system 6 (CQAQ + CQAQ) > system 2 (CQAQ + CQA) > system 8 (CQA + CQAQ). System 7 (CATQ + CATQ) had the highest degree of browning. System 4 (CATQ + CAT) and system 5 (CATQ + CQAQ) had similar absorbance in the first stage while the browning of system 4, which contained a higher level of CATQ, exceeded that of system 5 during the second stage. Due to the highest browning level of system 7 (CATQ + CATQ) and ranking order of CATQ + CAT > CATQ + CQAQ > CATQ + CQA, it was speculated that CATQ is an important precursor in the non-enzymatic browning, followed by CAT. This is consistent with the findings of Derardji et al. (2022), which revealed that CAT, epicatechin and B-type proanthocyanidins have the greatest effect on browning, and CATQ is an important browning precursor. CATQ contains A ring with naphthol properties, which can undergo head-to-tail polymerization reaction, resulting in self-polymerization of CATQ and the product has a certain amount of conjugated double bonds and provides visible absorbance, contributing to browning (Guyot, Vercauteren, & Cheynier, 1996). Lopez-Serrano and Barcelo (2002) also reported that brown pigments were detected as oligomeric compounds resulting from CAT oxidation. Despite of the absence of quinones in system 9 (CAT + CAT) and system 10 (CAT + CQA), the substantial increment in absorbance indicated their great potential for browning. In addition, the browning of system 9 (CAT + CAT) was higher than that of system 10 (CAT + CQA), which further verified the crucial role of CAT on browning. CAT has the B ring with ortho-diphenol and a resorcinol structure on the A ring. It was speculated that CAT was slowly oxidized to quinone by oxygen, which could explain the stable absorbance during the initial stage, and then the large amount of CATQ generated promoted its polymerization to arise sharp increment of browning during the third stage. The system containing CAT or CATQ had a higher degree of browning. In the mechanism of CATQ-induced browning, both the A and B rings are involved in the polymerization reaction. Therefore, it is inferred that both the A and B rings play a crucial role in browning.

When CATQ or CAT were absent in the quinone oxidation model systems, the browning of system 2 (CQAQ + CQA), system 6 (CQAQ + CQAQ) and system 8 (CQA + CQAQ) was low and had no remarkable difference during the whole storage. Hence, the system which merely contained CQA or CQAQ, was not prone to browning. Murata, Sugiura,
Sonokawa, Shimamura, and Homma (2002) also reported that self-polymerization of CQAQ or polymerization products with amino acids did not result in intensive browning. The minor effect of CQA on browning could be attributed to that CQA only contains an o-diphenol B ring on its structure. The research by Radi et al. (1997) indicated that although CQA is the best substrate for PPO, flavan-3-ol which contains both A ring and B ring rather than CQA makes great contributions to brown pigments. Oszmianski and Lee (1990) found that copolymers of oxidation products of CAT and CQA were less brown compared to the oxidation products of CAT. Moreover, areas under the browning curve of three systems system 3 (CATQ + CQA), system 8 (CQA + CQA) and system 10 (CAT + CQA) were significantly different (Fig. 1C). These results revealed that A ring and B ring collectively contribute to browning. Phenolic compounds without A ring, such as CQA, probably have no marked influence on browning.

3.1.2. CQAQ-mediated CAT oxidation vS CAT autoxidation

In order to compare CQAQ-mediated CAT oxidation and CAT autoxidation in the long-term storage, the changes of CAT, CATQ, CQA and CQAQ in system 1 (CQAQ + CAT) and system 10 (CQA + CAT) were monitored, which represent the CQA-mediated CAT oxidation and CAT autoxidation, respectively. Fig. 1B revealed that the browning of system 1 (CQAQ + CAT) was higher than that of system 10 (CQA + CAT) starting from 8 d. Here, the rate constant of browning was calculated after 8 d, where the browning rate of system 1 (CQAQ + CAT) was significantly higher than that of system 10 (CQA + CAT) (Fig. 2A). CAT and CQA in system 10 (CQAQ + CAT) showed no marked change during the 32-d storage (Fig. 2B–C), which indicated that autoxidation of both CQA and CAT was weak. A substantial increment of CQAQ was observed (Fig. 2E), corresponding to the increased browning during the third stage. For system 1 (CQAQ + CAT), CAT and CQA decreased remarkably and were almost empty after 32-d storage (Fig. 2B–C), meanwhile CATQ surged rapidly (Fig. 2D), illustrating that the oxidation of CAT mediated by CQAQ was thorough. After a period of surge, CATQ quickly dropped back to its original level. It is possible that CQAQ converts CAT to CATQ via electron transfer and itself is reduced to its origin phenol, as presumed that CQAQ may function as a self-perpetuating oxidizer in CAT oxidation (Liu et al., 2022). The browning, browning rates and concentration changes of CQA, CAT, CATQ and CQAQ in two systems 1 (CQAQ + CAT) and system 10 (CQA + CAT) revealed that CQAQ-mediated CAT oxidation is faster than CAT autoxidation in the long-term storage. This is consistent with the previous finding that CQAQ-mediated CAT oxidation is stronger than CAT autoxidation in the short-term model system (Liu et al., 2022).

3.1.3. CQAQ-mediated CAT oxidation vS CATQ-mediated CQA oxidation

In 3.1.1, no significant difference in browning was found between system 3 (CATQ + CQA) and system 1 (CQAQ + CAT). In both of the two systems, CQA and CAT gradually declined throughout the 32-d storage (Fig. 3B–C), and CATQ and CQAQ increased sharply in the third stage.
The change of these phenolic compounds and quinones showed a similar trend in system 3 (CATQ + CQA) and system 1 (CQAQ + CAT). Their browning rates and phenolic-quinone concentrations also had no marked difference (Fig. 3A-E).

Fig. 3. CQAQ-mediated CAT oxidation vs CATQ-mediated CQA oxidation in the system of [CQAQ + CAT] and [CATQ + CQA]. (A) Rate constants ($k_{A405}$). The concentration of (B) CAT and (C) CQA. The peak area of (D) CATQ and (E) CQAQ. All data are presented as mean ± SD (n = 3). The different marks represented statistically significant differences according to the independent samples T-test (none significant difference, ns; $P < 0.05$, *; $P < 0.01$, **).

Fig. 4. CQA and CAT oxidation catalyzed by PPO within 128 min. (A) The concentration of CQA and CAT within 128 min. (B) The peak area of CQAQ and CATQ within 128 min. (C) The peak area of CQAQ and CATQ within 8 min. All data are presented as mean ± SD (n = 3).
3.2. CQA and CAT oxidation catalyzed by PPO

CQA and CAT oxidation catalyzed by PPO were conducted to detect the concentrations of CQA, CAT, CQAQ and CATQ, respectively, within 2 h. Fig. 4A showed that PPO oxidized CQA and CAT rapidly within 10 s, while residual CAT was much more than CQA, and generated CQAQ was much more than CATQ (Fig. 4B-C). CQAQ and CATQ all exerted a trend of rising firstly and then falling. Because quinones are highly reactive and once produced, they will further react with other compounds to form stable products. The results indicated that PPO is more susceptible to oxidize CQA compared to CAT, consistent with the previous finding that CQA is a powerful substrate for PPO in apples (Amaki, Saito, Taniguchi, Joshiba, & Murata, 2011). Janovitz-Klapp, Richard, and Nicolas (1989) demonstrated that CQA is a better substrate for apple PPO than CAT at pH 4. It was also found that CQA is the most efficient for coffee leaves PPO and lettuce PPO (Altunkaya & Goekmen, 2008; Melo, Shimizu, & Mazzaferra, 2006). Therefore, PPO can preferentially catalyze the oxidation of CQA to produce large amounts of CQAQ. Although CQA and CQAQ themselves have no significant effect on browning as shown in 3.1, CQAQ is of great significance to the oxidation of CATQ (Amaki, Saito, Taniguchi, Joshiba, & Murata, 2011; Liu, et al., 2022), which readily oxidize CAT to CATQ to induce browning in fruit and vegetable products. So, these results illustrated the reliability of the proposed browning pathway mediated by CQAQ.

3.3. Long-term storage experiment in PPO oxidation model systems

3.3.1. Browning analysis

In order to approximate a more realistic browning situation, PPO oxidation model systems were established. The color changes and the absorbance at 405 nm of 10 different PPO oxidation model systems during 32 days of storage were shown in Fig. 5A and Fig. 5B, respectively. The browning of system 4 ([PPO-CAT] + CAT), system 5 ([PPO-CAT] + [PPO-CAT]), system 6 (CAT + CAT), system 7 ([PPO-CQA] + CAT), system 8 ([PPO-CAT] + CQA), system 9 ([PPO-CAT] + [PPO-CQA]), and system 10 (CQA + CATQ) showed a similar change trend. Their browning had no significant change in the first stage, but proceeded a slow increase in the second stage and a substantial rise in the third stage, respectively. The browning of system 1 ([PPO-CQA] + CQA), system 2 ([PPO-CQA] + [PPO-CQA]), and system 3 (CQA + CQAQ) had no obvious change. The horizontal change trend is similar to that in quinone oxidation model systems, as discussed in 3.1.1.

The ranking order of browning in the longitudinal direction was that system 5 ([PPO-CAT] + [PPO-CAT]) > system 4 ([PPO-CAT] + CAT) > system 6 (CAT + CAT) > system 7 ([PPO-CQA] + CAT) > system 9 ([PPO-CAT] + [PPO-CQA]) > system 8 ([PPO-CAT] + CQA) > system 1 ([PPO-CQA] + CQA) > system 10 (CQA + CATQ) > system 3 (CQA + CQAQ). Actually, PPO is capable of oxidizing CAT, CQA to produce corresponding quinones. Thus the system ([PPO-CAT] + CAT) corresponds to the system (CAT + CAT) and the difference is the approach by which phenolic compounds are oxidized. Generally, the browning ranking of each system was also similar to that of quinone oxidation system in 3.1.1. The systems containing CATQ had higher browning degree, further shoring up that CATQ is a critical precursor for browning. The browning of system 2 ([PPO-CQA] + [PPO-CQA]), system 1 ([PPO-CQA] + CQA) and system 3 (CQA + CQAQ) remained lower than that of the system containing CAT or CATQ, supporting the view that CQA without the structure of A ring is not prone to cause browning in fruit and vegetables. Consequently, the change rule of browning in 10 PPO oxidation model systems and their ranking order of absorbance at 405 nm were similar to that in quinone oxidation model systems. The crucial role of CAT and CATQ in browning was further demonstrated.

3.3.2. CQAQ-mediated CAT oxidation vs CAT autodissociation

Fig. 5B showed no significant difference between system 7 ([PPO-CQA] + CAT) and system 10 (CQA + CAT) in browning, and the concentrations of CQA, CAT, CQAQ and CATQ were similar in the two systems (Fig. 5C-F). These results contradicted the conclusion obtained in the quinone oxidation system. The difference of quinone oxidation systems and PPO oxidation systems is the approach of phenolic compounds oxidation. It is possible that CQA oxidation catalyzed by PPO is weaker than that catalyzed by activated peroxidate resin, thus resulting in less CQAQ generated than expected.

3.3.3. CQAQ-mediated CAT oxidation vs CATQ-mediated CQA oxidation

As shown in Fig. 5B, there was no significant difference in the browning of system 7 ([PPO-CQA] + CAT) and system 8 ([PPO-CAT] + CQA), which represent CATQ-mediated CQA oxidation and CQAQ-mediated CAT oxidation, respectively. The rate constant of browning showed the same trend as that in quinone oxidation model systems that there was no significant difference in the browning of system 7 ([PPO-CQA] + CAT) and system 8 ([PPO-CAT] + CQA) (Fig. 5G). The concentrations of CAT, CQA, CATQ and CATQ showed similar change trends in two systems, and they also had no remarkable difference (Fig. 5H-K). These results demonstrated that there was no remarkable difference between system 7 ([PPO-CQA] + CAT) and system 8 ([PPO-CAT] + CQA), consistent with the result in 3.1.3.

Since the oxidizing capacity of CQAQ was comparable to that of CATQ (Liu, et al., 2022), it is inferred that CQAQ-mediated CAT oxidation and CATQ-mediated CQA oxidation to result in browning are all present and possibly exhibit similar oxidative intensity. Our previous research showed a significant difference of k between CQAQ-mediated CAT oxidation (g) (k = 0.1326 mol·L⁻¹·min⁻¹) and CATQ-mediated CQA oxidation (h) (k = 0.01739 mol·L⁻¹·min⁻¹) (Liu, et al., 2022). However, our result showed little differences in the concentrations of CAT, CATQ, CQA and CATQ in the two oxidation systems. It was possible that the redox reaction rate between CQAQ and flavanols is fast (Nikolantonaki & Waterhouse, 2012), which results in the prompt pathway of the CQAQ-mediated oxidation of CAT, leading to tremendous collections of CATQ and CQA quickly in the system 1 (CQA + CAT) within 1 day. And thus, system 1 (CQA + CAT) and system 3 (CATQ + CQA) were similar after 1 day and no marked difference was observed between the two systems during the long-term storage.

4. Conclusion

Consequently, this study further demonstrated the crucial role of CATQ and CAT containing both an o-diphenol B ring and A ring with resorcinnol in browning of model systems during long-term storage. Moreover, CQAQ might oxidize CAT to generate CATQ quickly within one day, and CATQ reacts with CAT subsequently through complex reactions to produce brown pigments during long-term storage. In future, this mechanism will be validated in the real apple juice, including the sugars, soluble and insoluble fibers effects. Additionally, a new mitigation strategy is inspired that A and B rings of phenolic compounds might be modified structurally via the addition of suitable exogenous substances to quench browning precursors and block the browning pathway.

CRediT authorship contribution statement

Jingting Su: Formal analysis, Writing – original draft, Writing – review & editing. Yaqian Geng: Formal analysis, Writing – original draft, Writing – review & editing. Jinbo Yao: Investigation, Writing – review & editing. Yuan Huang: Investigation, Writing – review & editing. Junfu Ji: Supervision, Conceptualization, Writing – review & editing. Fang Chen: Supervision, Conceptualization, Writing – review & editing. Xiaosong Hu: Supervision, Conceptualization, Writing – review & editing. Lingjun Ma: Funding acquisition, Project administration, Supervision, Conceptualization, Writing – review & editing.
Fig. 5. Changes of ten PPO oxidation model systems during 32 days' storage. (A) Color and (B) absorbance at 405 nm of ten PPO oxidation model systems. The concentration of (C) CAT and (D) CQA and the peak area of (E) CATQ and (F) CQAQ in the system of [PPO-CQA]+[PPO-CAT] and [PPO-CAT]+[PPO-CQA]. (G) Rate constants ($k_{A405}$) in the system of [PPO-CQA]+[PPO-CAT] and [PPO-CAT]+[PPO-CQA]. The concentration of (H) CAT and (I) CQA and the peak area of (J) CATQ and (K) CQAQ in the system of [PPO-CQA]+[PPO-CAT] and [PPO-CAT]+[PPO-CQA]. All data were presented as mean ± SD (n = 3). The different marks represented statistically significant differences according to the independent samples T-test (none significant difference, ns; P < 0.05, *; P < 0.01, **).
Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Lingjun Ma reports financial support was provided by National Natural Science Foundation of China. Lingjun Ma reports financial support was provided by Young Elite Scientists Sponsorship Program. The remaining authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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