Inhibition mechanism of thermally induced furfural in simplified sugarcane juice model system by polyphenols

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ABSTRACT

The inhibitory mechanisms of 5 polyphenols including vanillin (V), chlorogenic acid (CA), hydroxybenzoic acid (HA), 3, 4-dihydroxybenzoic acid (P) and gallic acid (GA) on furfural (FF) in sugarcane juice model systems were investigated in this study. The inhibition rates of all these 5 polyphenols on the FF could reach over 90 %. The contents of Fru and 3-DG in polyphenol system were lower than those in Fru/Ser. The adducts formed from polyphenols and Fru were found in all systems and exhibited a trend of increasing first and then decreasing. The results of correlation analysis indicated CA, HA and P exerted negative effects on of FF. Polyphenols probably inhibited FF generation by 2 ways: competitive binding with the precursors and preventing the conversion of intermediates to FF by scavenging free radicals. These results provided basis for improving the quality and safety of sugarcane juice and the application of polyphenol in food industry.

1. Introduction

Sugarcane is the most widely cultivated sugar crop in the world, which is abundant in various amino acids, minerals and bioactive substances (Rodrigues et al., 2021; Wang et al., 2024; Wang et al., 2025). Besides the traditional sugar products, sugarcane juice has become a primary pathway for the extension of sugarcane industry chain (Wang et al., 2020). Thermal sterilizations are essential for ensuring product safety and prolonging shelf life of sugarcane juice. However, the deterioration of color and flavor in sugarcane juice after thermal treatment has become a constraint for its industrial development. It has been demonstrated in our previous study that the formation of furfural (FF) during thermal processing is closely related to the quality deterioration of sugarcane juice (Wang, Deng, et al., 2019). FF is a kind of furan aldehyde with a roasted almond aroma and a bitter taste (Liu et al., 2021). In addition, the accumulation of FF and its derivatives might had adverse effects on human bodies (Rong et al., 2022). Consequently, inhibiting the formation of FF during thermal treatment is an effective way to prevent quality deterioration and ensure the safety of sugarcane juice product.

Polyphenols have been considered as natural scavengers of harmful Maillard reaction products due to their antioxidant properties and ability to capture carbonyl compounds (Han et al., 2024). Mildner-Szkudlarz et al. (2019) found that gallic acid can inhibit acrylamide in bread models by up to 73 %. According to Zhu et al. (2020a), trans-resveratrol, apigenin, kaempferol, and nordihydroguaiaretic acid could inhibit the Maillard reaction in milk through the formation of adducts with acetaldehyde. As reported in previous study (Huang et al., 2023), the formation of FF is closely related to caramelization and Maillard reaction. It could be speculated that the formation of FF could also be blocked by adding polyphenols. However, there are few studies specifically focusing on the inhibition of FF and the related mechanisms of natural products.

It has been confirmed that polyphenols could prevent the Maillard reaction by scavenging free radicals, chelating metal ions, binding precursors and capturing carbonyl compounds (Guerra & Yaylayan, 2014; Spagnuolo et al., 2021). The identification of the adducts by Zheng et al. (2024) indicated that catechin inhibited FF formation by competitive reacting with the precursors and trapping the critical intermediates during thermal treatment. The aromatic ring of phenols, influenced by hydroxyl groups, was highly susceptible to electrophilic aromatic substitution reactions (Song et al., 2021; Zhu et al., 2020b). Quinones formed upon oxidation of epicatechin can undergo nucleophilic addition with the nucleophiles lysine or glycine during thermal process (Guerra & Yaylayan, 2014). Besides, the intermediates products of Maillard reaction such as carbonyl compounds, usually have partially positively

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charged carbon atom on their carbonyl groups, making them prone to be attacked by nucleophiles. Therefore, nucleophilic phenols could react with carbonyl compounds to inhibit the formation of FF. Therefore, we hypothesized that polyphenols could also actively contribute to the elimination of FF in sugarcane juice. However, due to the complex of caramelization and Maillard reactions, the variety of components in sugarcane juice and the difference of structures and properties of polyphenols, it remains unclear whether all polyphenols possess the same inhibitory mechanism. Thus, this study was designed to evaluate inhibitory effects of 5 kinds of polyphenols including vanillin (V), chlorogenic acid (CA), hydroxybenzoic acid (HA), 3, 4-dihydroxybenzoic acid (P) and gallic acid (GA) on FF in sugarcane juice model systems containing fructose (Fru) and serine (Ser). The changes of precursors, intermediate, adducts and free radicals were also investigated to explore the inhibitory mechanism of these 5 polyphenols. The results of this study will provide a theoretical basis for the optimization of sugarcane juice process and create a paradigm for future studies on the inhibition of off-flavor and harmful substance during thermal treatment of fruit and vegetable juice.

2. Materials and methods

2.1. Chemicals

The standards of Fru, V, CA, GA, P, HA, 3-deoxyglucosone (3-DG) and N-(1-Deoxy-D-fructos- 1-yl)-L-serine (FS) were all purchased from Macklin (Shanghai, China). Amino acid mixture standard solutions were purchased from Wako Pure Chemical Industries (Osaka, Japan). EDTA, *o*-phenylenedi- amine(OPD), sodium hydroxide and sodium dihydrogen, L-ascorbic acid, ferric chloride, deoxyribose, hydrogen peroxide, trichloroacetic acid, hexobarbital, resorcinol, luminol, dimethylphenylene orange, ferrous sulfate, and acetonitrile, formic acid, methanol xylenol orange were in analytical grade and bought from Macklin (Shanghai, China). Methanol, formic acid and acetonitrile were of high-performance liquid chromatography (HPLC) grade and bought from Thermo Fisher Scientific Co., Ltd. (Shanghai, China).

2.2. Preparation of sugarcane juice

The sugarcanes with green rind collected in Datian County, Fujian Province, China were used as the raw material of sugarcane juice. The juice was extracted using an automatic machine (YF-T80, Xiuling Kitchen Ware Co., Ltd., Guangdong, China) and filtered through a 0.07 mm mesh. Five kinds of polyphenols (0.012 mmol/100 mL) including V, CA, P, HA and GA were diluted to 100 mL with sugarcane juice individually. Fresh juice without polyphenols was set as the control group. Then, all samples with the volume of 10 mL were subjected to oil bath heating at 120 °C for 20 min and removed to ice water bath immediately. Each sample was prepared in triplicate.

2.3. Preparation of model systems

As reported in our previous study, the critical precursors of FF in sugarcane juice was Fru and Ser (Huang et al., 2023). Thus, the control group was established using a phosphate-buffered solution (pH 5.4) with Fru at a concentration of 15 mmol/100 mL and serine (Ser) at 5 mmol/100 mL based on the actual composition of sugarcane juice. Each of the polyphenols (0.12 mmol/100 mL) was added in to the control group to form the experimental system of V/Fru/Ser, CA/Fru/Ser, HA/Fru/Ser, P/Fru/Ser, and GA/Fru/Ser, respectively. The solution of each model system (10 mL) was transferred into a headspace vial and sealed. Subsequently, samples were subjected to heating in oil bath at 120 °C for 30 min. Samples were collected at intervals of 5 min and cooled in an ice water bath immediately for further analysis. Each sample was prepared in triplicate.

2.4. Determination of furfural by GC–MS

The detection of FF was conducted as the method reported by Wang, Wang, et al. (2019) with a recovery rate of 86.63 % - 110.38 % and a quantification limit of 1×10^{-7} mmol/mL. The heated sample in headspace vial was equilibrated at 50 °C for 40 min. Afterward, a SPME extraction fiber (50/30 m , DVB/CAR/PDMS, Supelco, Bellefonte, PA, USA) was inserted into the headspace vial and adsorbed for 40 min. Subsequently, the desorption of the fiber was carried out in the injection port of GC–MS (7890 A GC and a 5975C MS, Agilent Technology Co., Ltd., Santa Clara, CA, USA) at 280 °C for 5 min. A fused silica capillary column (DB-WAX, 60 m × 320 μm, 0.25 μm, Agilent Technology Co., Ltd., Shanghai, China) was used for FF detection. The temperature program started at 50 °C for 5 min, then rose to 150 °C at the speed of 5 °C/min, followed by an increase to 240 °C at the rate of 10 °C/min and held for 2 min. A constant helium flow rate was maintained at 1 mL/min. The MS was operated in EI mode and associated parameters were set as: ionization energy, 70 eV; ion source temperature: 230 °C; quadrupole temperature, 150 °C; emission current: 34.6 μA; mass scan range: 50 to 500 amu; solvent delay: 3 min. Quantitative analysis of FF were performed by establishing a standard curve.

2.5. Determination of fructose by HPLC

According to the method proposed by Wang, Cui, et al. (2022), the content of Fru was determined using a HPLC system (Waters 2695, Waters Corp. Shanghai, China) equipped with a RID detector (Waters 2414, Waters Corp. Shanghai, China). Separation was performed using a Waters Sugar-Pak chromatography column (6.5 mm × 300 mm, 10 μm, Waters Corp. Shanghai, Chin) with a column temperature of 65 °C. The mobile phase was ultrapure water with the flow rate of 0.2 mL/min. The injection volume was 10 μL. Quantitative analysis of Fru was conducted by establishing a standard curve.

2.6. Determination of serine

The content of Ser was determined using an amino acid analyzer (L-8900, Hitachi Co., Ltd., Tokyo, Japan) following the method proposed by Wang, Wu, et al. (2022). Sample of 20 μL was injected directly into the equipment. Quantitative analysis was conducted by comparing the chromatographic peak areas with the calibration curves.

2.7. Determination of polyphenols by HPLC

Based on the protocol reported by dos Lima et al. (2015), the determination of polyphenols compounds was carried out using a HPLC system (Waters 2695, Waters Corp. Shanghai, China) and a SunFire C18 analytical column (250 mm × 4.6 nm, 5 μm, Waters Corp. Shanghai, China). The mobile phase consisted of 0.1 % phosphoric acid aqueous solution (A) and methanol (B) with a flow rate of 1.2 mL/min. The elution program was set as follow: 73 % A at 0 min, 50 % A at 10 min, 0 % A at 22 min and 73 % A at 26 min and held for 4 min. The column temperature was 30 °C, the injection volume was 10 μL and the quantification wavelength was 280 nm. Each polyphenol was identified by comparing the retention times with those of the standards. The contents of polyphenols were also calculated based on the standard curves.

2.8. Identification of adducts

The determination of 3-DG was conducted according to the method of our previous study using OPD for the derivatization of 3-DG (Huang et al., 2023). Accurately weighed 6 g of OPD and dissolved in phosphate buffer (pH 5.4) to a final volume of 100 mL. Mixed 2 mL of the sample with an equal volume of OPD solution in a 10 mL centrifuge tube. The mixture was heated at 60 °C in a water bath for 30 min and stirred in the dark at 50 rpm for 30 min. The samples were filtered through a 0.22 μm
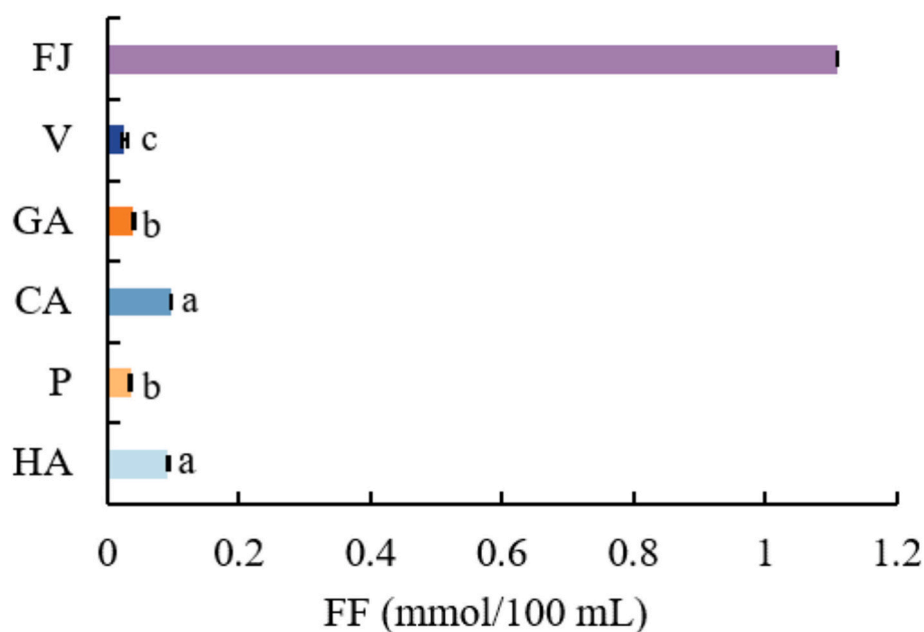
Fig. 1. FF in thermal treated sugarcane juice with polyphenols.
This graph illustrated the levels of FF in sugarcane juice with the addition of different polyphenols after heated for 20 min.
Note: FJ, fresh juice; GA, gallic acid; P, 3, 4 p-hydroxybenzoate acid; HA, p-hydroxybenzoate acid; CA, chlorogenic acid; V, vanillin; FF, furfural. The standard error bars were denoted in black. Different lowercase letters indicate a significant difference ($P < 0.05$) among treatments as determined by Fisher's LSD.

filter for HPLC detection. Sample (10 μL) was injected into a HPLC system (Waters 2695, Waters Corp. Shanghai, China) coupled with a PDA UV detector (Waters 2889, Waters Corp. Shanghai, China). A SunFire C18 column was used to separate the sample at 40 °C. The detection wavelength was 320 nm. The mobile phase consisted of 0.1 % phosphoric acid aqueous solution (A) and methanol (B) with a flow rate of 1 mL/min. The elution gradient was set as: 70 % A at 0 min, 40 % A at 10 min, 70 % A at 12 min and 70 % A at 15 min. Quantitative analysis of 3-DG was conducted by establishing a standard curve.

As the protocol reported in our previous study (Huang et al., 2023), the peak area of the characteristic fragment detected by UPLC-QTOF-MS/MS (Waters YEA1172, Waters Co. Stamford Avenue, UK) was employed to calculate the content of FS based on the standard curve. The detection was conducted in positive ESI mode with an ACQUITY C18 column (1.7 μm, 2.1 × 100 mm Waters Co., Ltd., Shanghai, China). Acetonitrile was used as solvent A and 0.1 % formic acid in water (*v*/v) was used as solvent B at a rate of 0.3 mL/min. The elution gradient was set as: 20 % A at 0 min, 80 % A at 10 min, 80 % A at 12 min and 20 % A at 15 min. The injection volume was 2 μL, the column temperature was 45 °C, the capillary voltage was 2.5 kV, the detector voltage was 1.8 kV and the drying gas flow was 600 L/h.

2.9. Determination of hydroxyl radical (·OH) scavenging activity

The hydroxyl radical (·OH) scavenging rate in model system was determined following the method described by Gao et al. (2019). Briefly, 0.4 mL of phosphate buffer, 1.04 mmol/L EDTA solution, 1 mmol/L $FeCl_3$, 2 mmol/L ascorbic acid, 0.10 mL of the sample, 0.1 mL of 60 mmol/L deoxyribose and 0.1 mL of 10 mmol/L H_2O_2 were mixed in a test tube. The mixture was incubated at 37 °C for 1 h. The reaction was terminated by the addition of 1 mL of 20 % trichloroacetic acid. Then, 1 mL of 0.8 % thiobarbituric acid was added and heated in a boiling water bath for 15 min. After cooling immediately in ice water, the absorbance was measured at a wavelength of 532 nm by an ultraviolet spectro-photometer (UV-1100B, Shanghai MAPADA Instruments Co. Ltd., Shanghai, China). The ·OH scavenging rate was calculated as:

$$OH \text{ scavenging rate} = \frac{A_0 - (A_1 - A_2)}{A_0} \times 100\%$$

where, A_0 was the absorbance of the control group (Fru/Ser), A_1 was the absorbance of model system and A_2 was the absorbance of blank.

2.10. Determination of superoxide anion ($O_2^{\cdot-}$) scavenging activity

The determination of superoxide anion ($O_2^{\cdot-}$) scavenging activity was conducted by a pyrogallol-luminescence-phosphate buffer system (pH 10.2). Firstly, 10 μL of the sample and 20 μL of 1 mmol/L pyrogallol solution were added into a test tube, followed by an addition of 970 μL of luminol phosphate buffer to initiate luminescence. The absorbance of the mixture was recorded at 320 nm by an ultraviolet spectrophotometer (UV-1100B, Shanghai MAPADA Instruments Co. Ltd., Shanghai, China). The calculation was performed as:

$$O_2^- \text{ scavenging rate} = \frac{A_0 - A_i}{A_0} \times 100\%$$

where, A_0 was the absorbance of the control group (Fru/Ser), A_i was the absorbance of model system.

2.11. Determination of hydrogen peroxide (H_2O_2)

The yield of hydrogen peroxide (H_2O_2) was determined using the FOX method based on the procedure described by Li et al. (2017) with some modifications. An aliquot of 50 μL was diluted with 500 μL of water and stood in the dark at room temperature for 15 min. Subsequently, 20 μL of the sample was mixed with 180 μL of FOX solution prepared by dissolving 0.45 mmol/L xylenol orange and 0.45 mmol/L Fe $(NH_4)_2(SO_4)_2$ in 0.11 mol/L $HClO_4$. After incubated for 30 min, the absorbance was measured at 550 nm using an ultraviolet spectropho-tometer (UV-1100B, Shanghai MAPADA Instruments Co. Ltd., Shanghai, China). A blank was prepared using 60 μL of water. Finally, the content of H_2O_2 was calculated based on a standard curve.
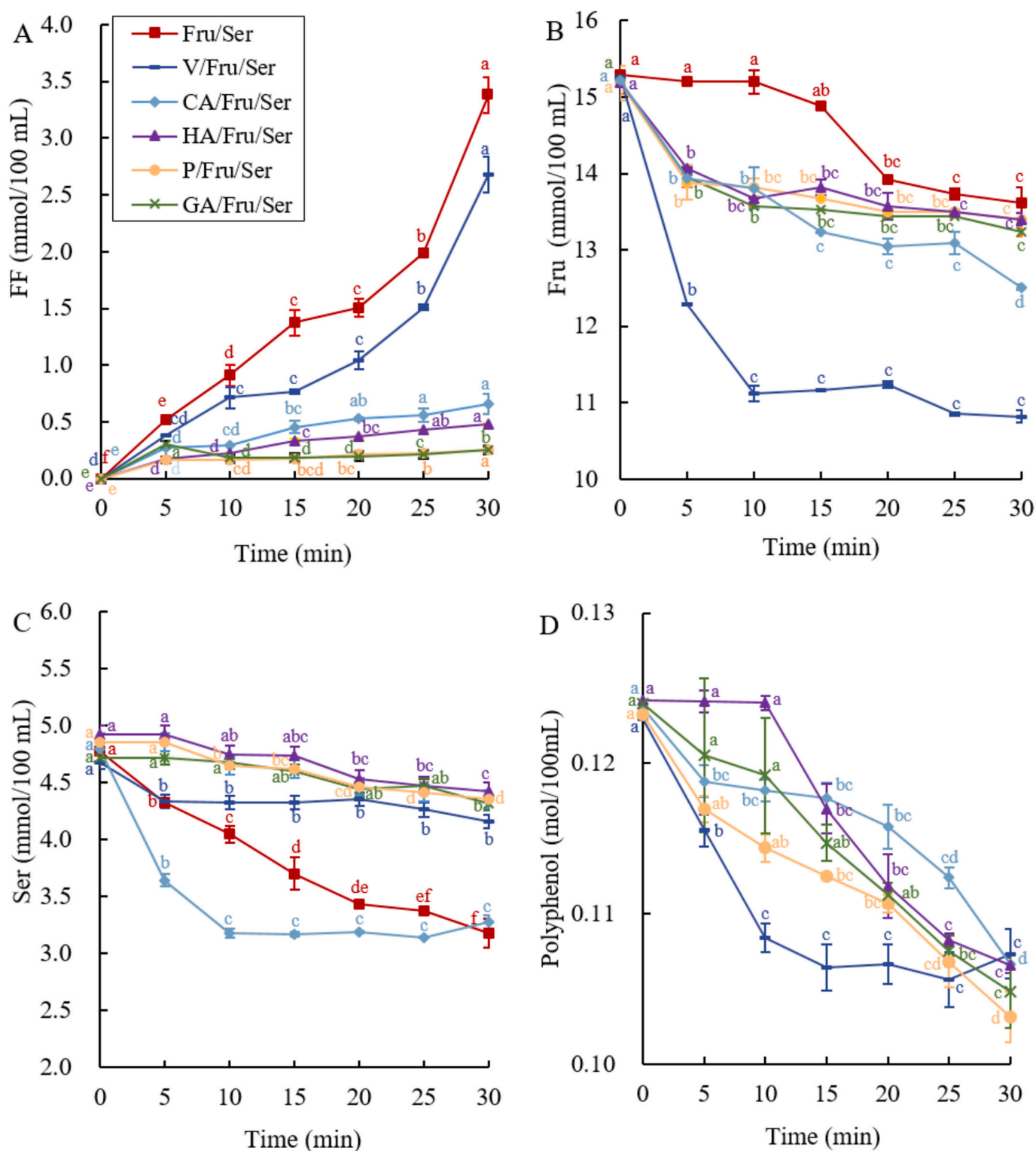
Fig. 2. Changes of FF, precursors and intermediates in model systems. A, changes of FF; B, changes of Fru; C, changes of Ser;, D, changes of polyphenols; E, changes of 3-DG; F, changes of FS.

Fig. 2 including 6 line charts displayed the changes of FF, Fru, Ser, polyphenols, 3-DG and FS in simplified model systems along with heating time.

Note: FF, furfural; Fru, fructose; Ser, serine; 3-DG, 3-deoxyglucosone; FS, N-(1-Deoxy-D-fructos-1-yl)-L-serine; GA,gallic acid; P, 3,4 p-hydroxybenzoate acid; HA, p-hydroxybenzoate acid; CA, chlorogenic acid; V, vanillin. Different lowercase letters indicate a significant difference ($P < 0.05$) among model systems heated for different time as determined by Fisher's LSD.
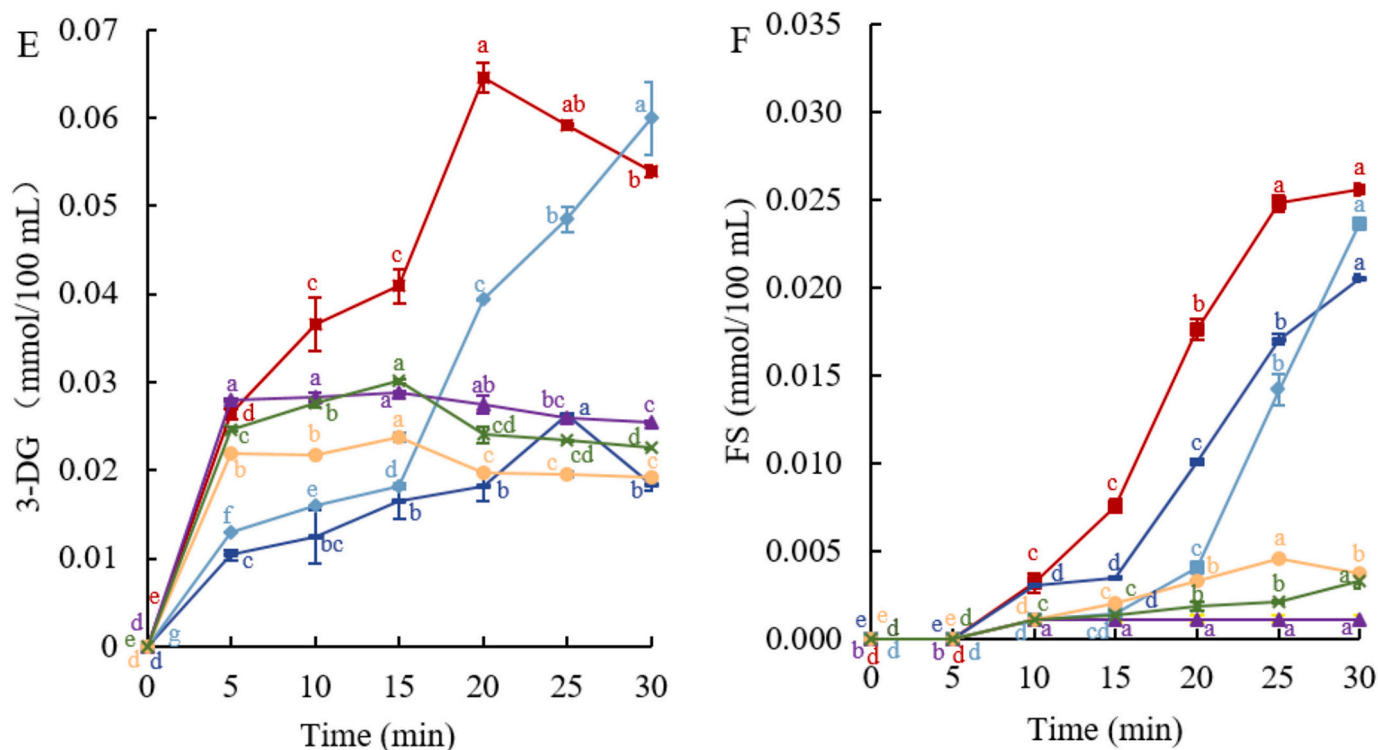
Fig. 2. (*continued*).

2.12. Statistical analysis

All samples were measured in triplicate and results were expressed as mean ± standard deviation. Data were subjected to statistical significance analysis by Fisher's least significant difference (LSD) ($p < 0.05$) using JMP 10.0 software (SAS Institute Company, Cary, NC, USA). The heatmap of correlation analysis was generated through Hiplot Pro (htt ps://hiplot.com.cn/).

3. Results and discussion

3.1. The inhibition effects of phenolic on FF in sugarcane juice

The levels of FF in thermal treated sugarcane juice with polyphenols were exhibited in Fig. 1. As the concentration of FF in fresh juice was higher than that with polyphenolics obviously, a comparative study was performed among the other 5 samples to classify the results obtained with each phenolic compounds. After heating for 20 min, the inhibition rate of all these 5 polyphenols on the FF formation could reach over 90 % in comparison with the sugarcane juice without polyphenols. The inhibitory effect of V on FF was the best, followed by P and GA. As reported in previous study (Zheng et al., 2024), the inhibition ratio of cathine could reach up to 100 %, which was superior to the results obtained in this work.

3.2. Changes of FF, precursors and intermediates in model systems

To further investigate the inhibitory mechanisms of polyphenols on FF, simplified model systems were established based on the components of sugarcane juice. The inhibitory effects of different polyphenols on FF were shown in Fig. 2A. In consisted with previous study (Huang et al., 2023), the amount of FF in Fru/Ser system was increased along with the increasing of heating time. All polyphenols added into model systems exerted a certain inhibitory effect on FF since 5 min. The changes in FF over time in the CA, HA, P, and GA systems were not as substantial as those in the V and control group.

Seen from the chemical structure of these polyphenols, V is a polyphenol containing aldehyde groups and the other 4 polyphenols are phenolic acids. Throughout the entire thermal treatment process, the concentrations of FF in these 4 phenolic acid systems were maintained below 1.0 mmol/100 mL. It was evident that those 4 polyphenols could achieve a favorable inhibitory effect in the initial stage of thermal treatment. The differences in the inhibition rates of polyphenols on FF might be related to their antioxidant properties, the ability to capture carbonyl compounds, and the molecular structure of the polyphenols. The phenolic hydroxyl groups have the ability to quench the free radicals generated in Maillard reaction through the hydrogen transfer mechanism. For the structurally similar polyphenols, more phenolic hydroxyl groups could result in a stronger antioxidant capacity (Chen et al., 2020; Wong et al., 2022). GA, HA and P each contain 7 carbon atoms, while the numbers of phenolic hydroxyl groups in GA, HA and P is 3, 2, and 1 respectively. The highest inhibition rates of FF was also observed in GA/Fru/Ser system, likely due to the function of its 3 phenolic hydroxyl groups. CA is an ester compound formed by caffeic acid and quinic acid. The large molecular structure may produce a steric hindrance effect, which hinders the approach of phenolic hydroxyl groups to the intermediates or free radicals in the Maillard reaction. Although CA contains 2 phenolic hydroxyl groups, its inhibition rate of FF was not as good as the other 3 phenolic acids. In the case of V/Fru/Ser system, the content of FF was increased along with the heating time, with a slight decrease relative to Fru/Ser. The thermal decomposition of V might diminish its inhibitory effect on FF (Wu et al., 2022).

However, the inhibitory effects of polyphenols in model systems were quite different from the results of sugarcane juice. This might result from the complex composition of sugarcane juice. It has been confirmed that vitamin C could regenerate oxidized polyphenols to restore the bio functions of polyphenols (Pallavi & Rajashekaraiah, 2023). The total antioxidant activity of some flavonoids was significantly higher than the sum of the antioxidant activities of individual compounds. Polyphenols including apigenin, luteolin, tricin derivatives, hydroxycinnamic, caffeic, sinapic acids, vanillin and chlorogenic acid have been detected in sugarcane juice previously (Maurício Duarte-Almeida et al., 2006; Wang

Table 1
UPLC-MS/MS performances of the adducts.

Analyte	Chemical formula	Molecular ion	Quantitative m/z
V-Fru	$C_{14}H_{20}O_9$	333	153、181
CA-Fru	$C_{22}H_{30}O_{15}$	543	400、181
HA-Fru	$C_{13}H_{18}O_9$	319	138、181
P-Fru	$C_{13}H_{18}O_{10}$	335	154、181
GA-Fru	$C_{13}H_{18}O_{11}$	351	169、181

Note: GA-Fru, gallic acid-fructose adduct; HA-Fru, 3,4-dihydroxybenzoic acid -fructose adduct; P-Fru, P-hydroxybenzoic-fructose adduct acid; CA-Fru, Chlorogenic acid-fructose adduct acid; V-Fru, vanillin-fructose adduct acid.

et al., 2020). Thus, other components in sugarcane juice might have a promoting or competitive effect on the inhibitory action of polyphenols on FF during the thermal treatment.

As shown in Fig. 2B, the content of Fru decreased obviously with the increase of heating time ($P < 0.05$). The consumptions of Fru in the polyphenol systems were greater than that in Fru/Ser. It could be speculated that the consumptions of Fru in polyphenol systems might be involved in chemical reactions unrelated to FF formation. Ding et al. (2021) also found the potential mechanism of chlorogenic acid and epicatechin in inhibiting the formation of heterocyclic amines in roasted lamb meats might be attributed to the competition of polyphenols for crucial precursors like glucose and fructose. The level of Fru in V/Fru/Ser was consistently lower than that in the other polyphenol systems, even though the inhibition effect of V was not particularly prominent. The concentration of Fru in polyphenol systems began to change gently since 10 min. Thus, the reactions between polyphenols and Fru might occur at the initial stage of thermal treatment.

As shown in Fig. 2C, only the content of Ser in the system of CA/Fru/Ser was lower than that of Fru/Ser, while it was higher in the other 4 polyphenol systems. The substantial loss of Fru might block the reaction
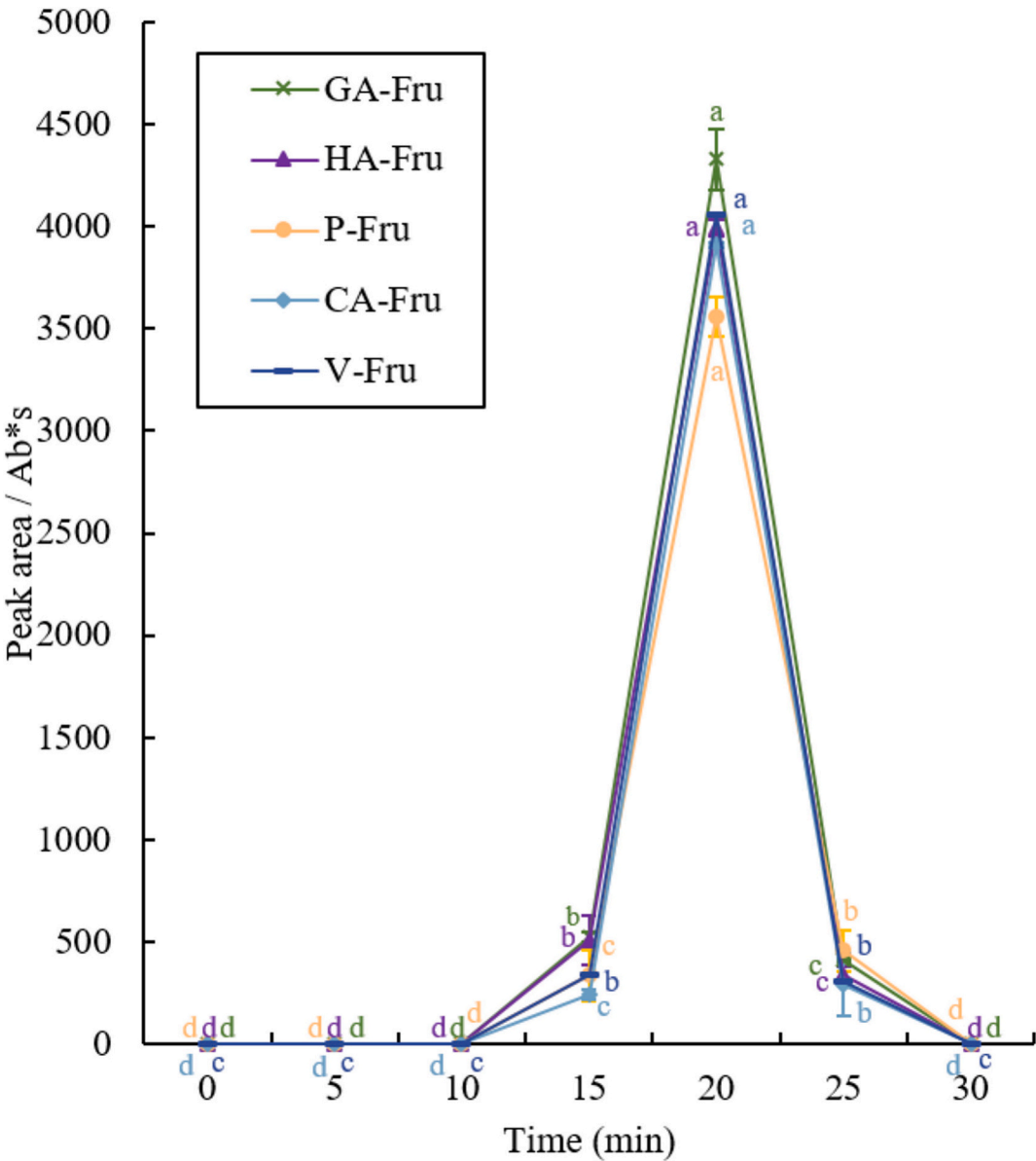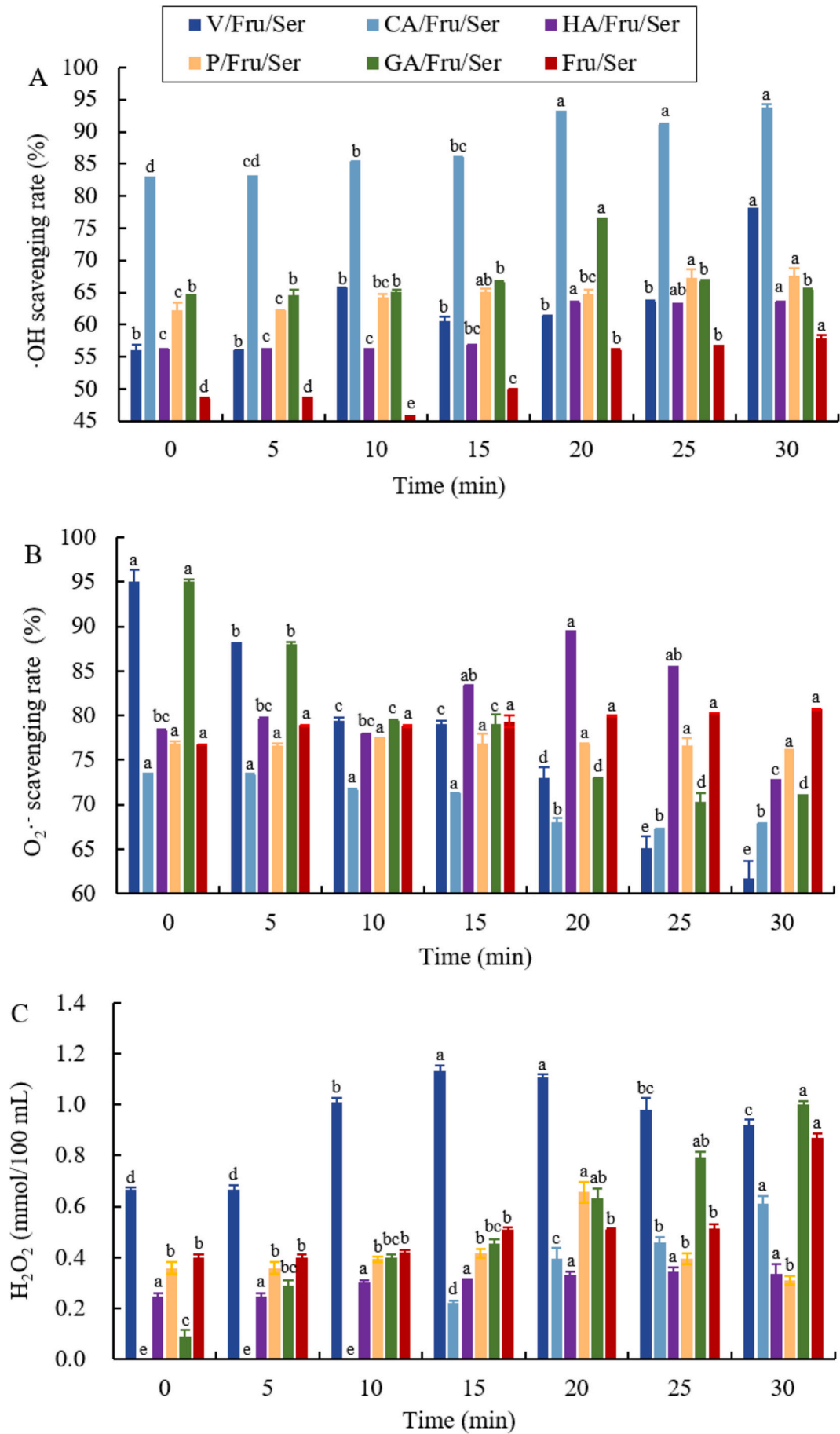

Fig. 3. Changes of peak areas of adducts.
This chart showed the alterations in the peak areas of the characteristic ions of the polyphenol-Fru adducts as a function of heating time.
Note: GA-Fru, gallic acid-fructose adduct; HA-Fru, 3,4-dihydroxybenzoic acid -fructose adduct; P-Fru, P-hydroxybenzoic-fructose adduct acid; CA-Fru, Chlorogenic acid-fructose adduct acid; V-Fru, vanillin-fructose adduct acid. Different lowercase letters indicate a significant difference ($P < 0.05$) among model systems heated for different time as determined by Fisher's LSD.

(caption on next page)

between Fru and Ser, thereby leading to a maintenance of Ser. Totlani and Peterson (2006) also reported that the interaction between tea polyphenols and sugar fragments could prevent the binding of sugar fragments with amino acids. However, the reasons for the depletion of Ser in CA/Fru/Ser still need further investigation.

The content of polyphenols in each model system decreased with the extension of heating time. After thermal treated for 30 min, the consumption of polyphenols in descending order was as follow: P (0.020 mmol/100 mL) > GA (0.019 mmol/100 mL) > HP (0.018 mmol/100 mL) > CA (0.017 mmol/100 mL) > V (0.016 mmol/100 mL). Similar results were also found in glucose-glycine and asparagine-fructose systems by Oral et al. (2014). The formation of furan and acrylamide were inhibited effectively after thermal treatment, with a 10–15 % reduction in polyphenols. The levels of V reached to the lowest (0.018 mmol/100 mL) at 15 min, which might be attributed to the degradation of V under heating condition (Mourtzinos et al., 2009).

The changes of 3-DG in model systems were illustrated in Fig. 2E. After heated for 10 min, the contents of 3-DG in polyphenol systems were significantly lower than that in Fru/Ser. Only the content of 3-DG in CA/Fru/Ser increased along with the increase of heating time. There was no significant change in 3-DG in the other 4 polyphenol systems since 5 min. At the end of thermal treatment, the 3-DG content was ranked from lowest to highest as follows: V/Fru/Ser < P/Fru/Ser < GA/Fru/Ser < HA/Fru/Ser < Fru/Ser < CA/Fru/Ser. As an intermediate product of both caramelization and Maillard reactions (Huang et al., 2023), the reduction of 3-DG indicated that the formation of FF was inhibited by impeding these 2 reactions. The ability of polyphenols to capture carbonyl compounds has been reported previously (Akıllıoğlu et al., 2022; Syeunda & Awika, 2024). Catechin could reduce the yield of glyceraldehyde in a maltose-lysine system by 40 % (Chen et al., 2019). As reported by Totlani and Peterson (2006), carbonyl compounds were captured by epicatechin through electrophilic aromatic substitution reactions. Moreover, the identification of the adducts formed from polyphenols and 3-DG need further exploration.

FS, as another important intermediate product of FF formation, was also detected in this study (Fig. 2F). The contents of FS in polyphenol systems were lower than that in Fru/Ser during thermal treatment. In Fru/Ser, V/Fru/Ser and CA/Fru/Ser systems, the contents of FS were increased with the extension of heating time. In the other 3 systems, the FS content maintained below 0.03 mmol/100 mL. After thermal treatment, the lowest production of FS was observed in HA/Fru/Ser. Jiang et al. (2019) also achieved a 6.7 % reduction in the content of the Amadori rearrangement product fructoselysine in model systems by adding 1 mM epigallocatechin. Since FS could be converted into 3-DG (Huang et al., 2023), the reduction of FS in polyphenol systems would also lead to a decrease in 3-DG.

3.3. Identification of adducts

In order to elucidate the inhibitory mechanisms of these 5 polyphenols on FF, a UPLC-MS/MS analysis in positive ionization mode was employed to preliminarily identify the potential adducts formed in model system. The speculative molecular formulas of identified adducts were presented in Table 1. Previous study has demonstrated that catechin could combine with Fru, Ser, 3-DG and FS to form adduct products (Zheng et al., 2024). Therefore, the targeted fragments of polyphenols, the specific precursors and intermediates were explored in this study. However, only the adducts of polyphenols and Fru were identified.

In V/Fru/Ser, the molecular formula of the adduct with an *m/z* of 333 might be $C_{14}H_{20}O_9$. The ion fragments of V (m/z 153) and Fru (m/z

181) observed in the mass spectrum suggested that this compound might be an adduct (V-Fru) resulting from the interaction between V and Fru during heating process. Similar with V/Fru/Ser, the adduct formed from polyphenol and Fru were also found in the systems of HA/Fru/Ser, P/Fru/Ser and GA/Fru/Ser. The ion fragments of corresponding polyphenol and Fru observed in the mass spectrums could support these inference.

The adduct identified in CA/Fru/Ser might be $C_{22}H_{30}O_{15}$, with an m/z of 534. CA tended to be oxidized into quinone with an m/z of 400 under heating condition (Muñoz et al., 2007; Saito et al., 2003). As the ion fragments with m/z 181 and m/z 400 were detected in the mass spectrum, it could be proposed that this compound was also generated from CA and Fru.

Consistent with the findings of this study, Totlani and Peterson (2007) found adducts between epicatechin and sugar fragments in a glucose-glycine system using isotope labeling techniques. Predictably, FF was inhibited by the interaction of polyphenols with Fru in all model systems.

3.4. Changes of adducts

The alterations in the peak areas of the characteristic ions of the adducts as a function of heating time were shown in Fig. 3. In all of the 5 model systems, the formation of the polyphenol-Fru adducts began at 10 min, peaked at 20 min, and then declined rapidly to 0 at 30 min. This result indicated that these 5 polyphenols might primarily inhibit the production of FF by competitively binding with Fru during the period of 10–20 min. However, the Fru in polyphenol systems was mainly consumed in the first 10 min. The formation of adducts might be delayed relative to the consumptions of precursors. There were evidences indicating that the activity of phenolic acids declined during thermal process (Zhao et al., 2018). Furthermore, adducts might also be decomposed subsequently. Thus, the yields of adducts exhibited a trend of increasing first and then decreasing in this study.

3.5. Changes of hydroxyl radical, superoxide anion and hydrogen peroxide

It has been demonstrated that reactive oxide species including ·OH, O_2^- and H_2O_2 could promote the Maillard reaction (Zhang et al., 2023). Therefore, the scavenging rates of ·OH and O_2^- and the yields of H_2O_2 in different models were compared in Fig. 4. Throughout the entire thermal process, the addition of polyphenol led to a higher scavenging rate of ·OH than that in Fru/Ser. Despite the absence of polyphenols in Fru/Ser, there was still an observable scavenging effect on ·OH. That might be attributed to the capacity of amino acid to inactive the free radicals (Guidea et al., 2020). The phenolic hydroxyl groups in polyphenols could be oxidized into quinone structure, which exhibited strong scavenging capabilities against reactive oxygen species. At the end of thermal treatment, the scavenging rate for ·OH in CA/Fru/Ser was the highest, reaching 93 %. CA is a kind of antioxidant phenolic acid rich in R-OH groups, which are effective in eliminating ·OH.

The scavenging rates of O_2^- in all model systems were fluctuant with heating time (Fig. 4B). From 0 to 10 min, V/Fru/Ser and GA/Fru/Ser had stronger scavenging abilities than Fru/Ser. However, HA/Fru/Ser started to surpass all the other systems from 15 min and sustained to 25 min. By the end of thermal treatment, the scavenging rates of Fru/Ser was the highest. As shown in Fig. 4C, the yields of H_2O_2 in V/Fru/Ser kept higher than Fru/Ser. Nevertheless, the content of H_2O_2 in GA/Fru/Ser exhibited an increasing trend with the extension of heating time
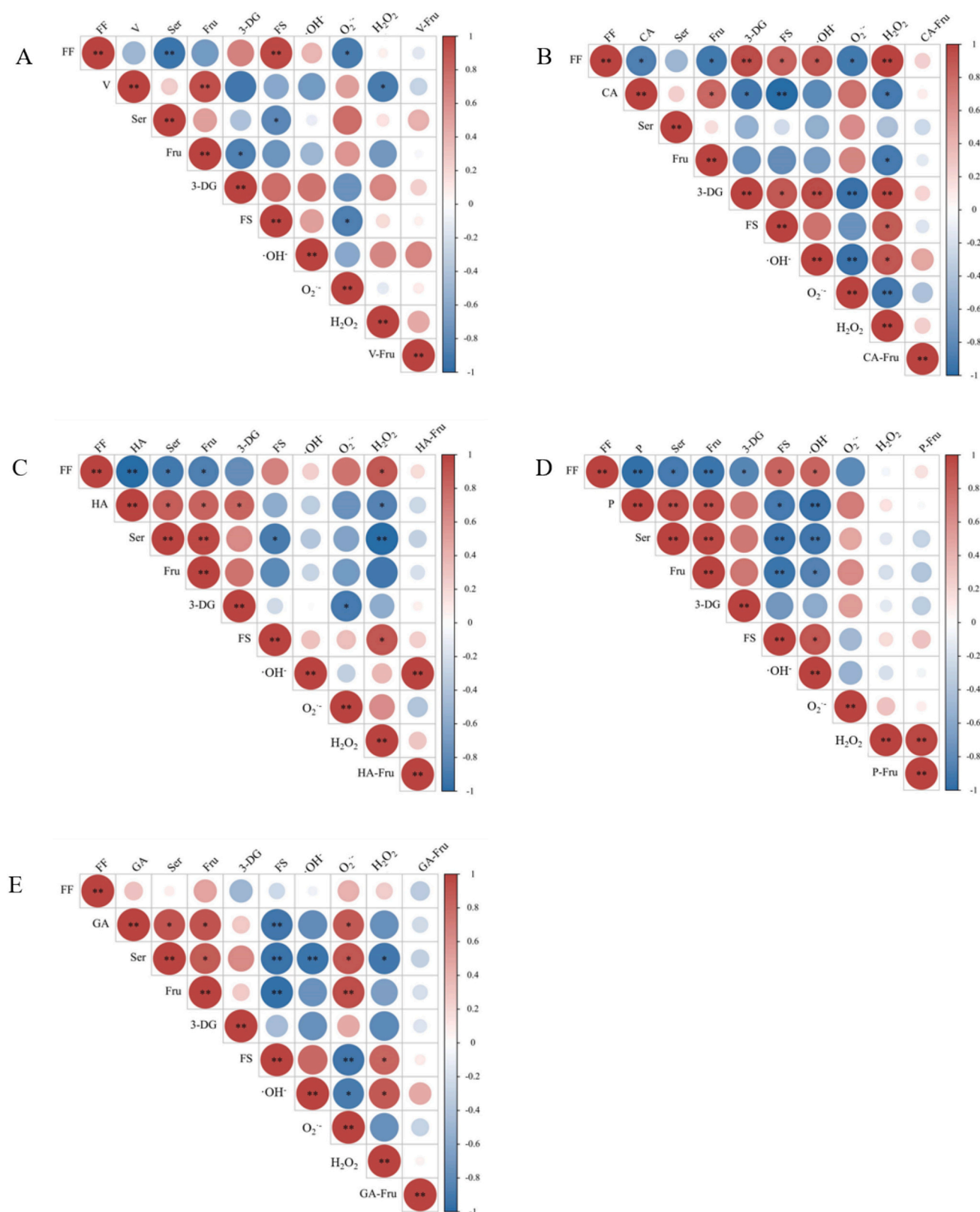
Fig. 5. Correlation analysis of substances and radicals in different model systems during thermal treatment. A, V/Fru/Ser system; B, CA/Fru/Ser system; C, HA/Fru/Ser system; D, P/Fru/Ser system; E, GA/Fru/Ser system.
Fig. 5 was consisted of 5 graphs that displayed the correlation analysis results among the substances and radicals in different model systems.
Note: Red circle represented positive correlation and blue circle was negative correlation. The deeper red or blue represented higher correlation values. *$P < 0.05$, **$P < 0.01$. FF, furfural; V, vanillin; CA, chlorogenic acid; HA, 4-Hydroxybenzoic acid; P, 3, 4-dihydroxybenzoic acid; GA, gallic acid; Fru, fructose; Ser, serine; 3-DG, 3-deoxyglucosone; FS, N-(1-Deoxy-D-fructos-1-yl)-ʟ-serine; V-Fru, vanillin-fructose adduct acid; CA-Fru, Chlorogenic acid-fructose adduct acid; GA-Fru, gallic acid-fructose adduct; HA-Fru, 3,4-dihydroxybenzoic acid -fructose adduct; P-Fru, P-hydroxybenzoic-fructose adduct acid. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

ultimately becoming the highest at 30 min among all the systems.

3.6. Correlation analysis

In order to explore the inhibitory mechanisms of polyphenols on FF, the correlation analysis among the substances and radicals in different model systems was conducted in this study (Fig. 5). CA, HA and P exerted negative effects on the yield of FF. The potent inhibitory effects of these 3 polyphenols were also supported by these results. In the system of V/Fru/Ser (Fig. 5A), the content of FF was positive correlated with FS content and negative with Ser and O_2^- scavenging rate significantly ($P < 0.05$). The loss of Ser might contribute to the inhibition of FF to some extent, even though the V-Ser adduct was not observed. Besides, O_2^- scavenging rate also had an obvious negative effect on FS ($P < 0.05$). Polyphenols could inhibit Maillard reaction through its free radical scavenging ability (Sakač et al., 2018). This result implies that the elimination of O_2^- induced by V could potentially slow down the formation of FF by inhibiting the conversion of FS into FF. Even though Amadori rearrangement products could also be captured by inhibitors (Cui et al., 2021), the adduct of polyphenol-FS was not detected in this study. According to the results of Wang et al. (Wang, Cui, et al., 2022), the inhibition effect on pyrazine relayed on the addition time of ellagic acid. Ellagic acid reacted exclusively with Amadori rearrangement products during the accumulation period of Amadori rearrangement products in the Maillard reaction. Since the polyphenols were added at the initial stages of Maillard reaction in this work, the generation of FS might be prevented via the reaction between the polyphenols and Fru. Besides, both polyphenols and Amadori rearrangement products had strong antioxidative capacity (Yang et al., 2024). The changes of radicals in model systems during thermal treatment might be related to both the addition of polyphenols and the elimination of FS.

In the case of CA/Fru/Ser (Fig. 5B), ·OH and H_2O_2 played positive roles in the accumulation of FF, while O_2^- had a negative impact. Both of the 2 intermediate products were positive associated with the level of FF significantly ($P < 0.05$). The inverse correlation between Fru and FF indicated that the reduction of FF might be due to the loss of Fru. In the systems of HA/Fru/Ser (Fig. 5C) and P/Fru/Ser (Fig. 5D), the precursors including Ser and Fru presented negative associations with the formation of FF ($P < 0.05$). Apart from precursors, the intermediate product, 3-DG might also be responsible for the decrease of FF in P/Fru/Ser. The free radicals contributed to the depletion of FF was H_2O_2 yield in HA/Fru/Ser, whereas ·OH scavenging rate in P/Fru/Ser. It has been reported that ·OH could facilitate the generation of glyoxal and methylglyoxal (Fujioka & Shibamoto, 2004). Thus, scavenging free radicals can effectively reduce the formation of carbonyl compounds during the Maillard reaction, thereby inhibiting the generation of FF. However, no significant factors associated with the inhibition of FF was obtained in GA/Fru/Ser (Fig. 5E). Although there was a negative correlation between GA and FS, the interaction between GA and FS still required further investigation.

According to the results of adducts identification and correlation analysis, it could be concluded that the inhibitory effect of polyphenols on FF primarily occurred through 2 ways. Firstly, polyphenols might inhibit the formation of FF by a competitive binding mechanism with the precursors. For example, each of these 5 polyphenols could interact with Fru to form polyphenol-Fru adducts. Secondly, the conversion of FS and 3-DG to FF might be prevented due to the free radical instigated by polyphenols. In accordance with previous study (Wong et al., 2022), diverse polyphenol exhibited different antioxidative ability. O_2^- functioned mainly in the systems involving V and CA, H_2O_2 played important role in HA/Fru/Ser, and ·OH was highlighted in P/Fru/Ser.

4. Conclusions

The inhibitory effects of 5 polyphenols including V, CA, HA, P and GA on FF in simplified sugarcane juice model systems were compared

and the related inhibition mechanisms were investigated in this study. The inhibition rate of all these 5 polyphenols on the FF formation could reach over 90 % in comparison with the sugarcane juice without polyphenols. The contents of Fru and 3-DG in polyphenol system were lower than those in Fru/Ser. The content of Ser in the system of CA/Fru/Ser was lower than that of Fru/Ser, while it was higher in the other 4 polyphenol systems. The content of polyphenols in each model system decreased with the extension of heating time. The adducts formed from polyphenol and Fru were found in all systems and exhibited a trend of increasing first and then decreasing. The addition of polyphenol led to a higher scavenging rate of ·OH than that in Fru/Ser during thermal process. The results of correlation analysis indicated CA, HA and P exerted negative effects on the yield of FF. Polyphenols inhibited the generation of FF probably by 2 ways: competitive binding mechanism with the precursors and preventing the conversion of FS and 3-DG to FF by scavenging the free radicals. The results of study will provide basis for improving the quality and safety of sugarcane juice and the application of polyphenol in food industry. However, the interaction between polyphenols and intermediates still need further investigation.

CRediT authorship contribution statement

Weikun Zou: Writing – original draft, Data curation. **Huimei Huang:** Data curation, Conceptualization. **Jiebo Chen:** Writing – review & editing, Project administration. **Lu Wang:** Writing – review & editing, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The 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.

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Data availability

Data will be made available on request.

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