**ORIGINAL PAPER** 



# Effects of maceration with phenolic additives on the physicochemical properties and antioxidant activity of blackened jujube (*Ziziphus jujuba* Mill.)

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# Abstract

*Ziziphus jujuba* cv. *Goutouzao* (GTZ) was pretreated by macerating with phenolic additives (green tea powder, tea polyphenols, EGCG and pomegranate peel ellagic acid) before the blackening process to improve the functional quality. The antioxidant capacity and physicochemical properties during the process of different treatment groups were evaluated by high-performance liquid chromatography (HPLC) and spectrophotometric assays. The results showed that the total phenolic content and antioxidant capacity were higher with each tested additive than the blank control group. Among them, the EGCG group had the highest total phenolic content and the strongest antioxidant capacity, whereby the total phenolic content of the blackened jujube was 24.59 times higher than the control group at the end of blackening (84 h). The scavenging of DPPH radicals, scavenging of ABTS radicals and total reducing capacity were 26.36, 5.51 and 4.58 times higher than those of the control group, respectively. The 5-hydroxymethylfurfural (5-HMF) content of blackened jujube in the EGCG and tea polyphenol groups was significantly reduced, and was 72.36 and 36.47% lower than the control group, respectively. Correlation analysis showed a highly significant negative correlation between total phenolic content and 5-HMF content (p < 0.01). The heatmap showed that the EGCG-macerated blackened jujube had the best comprehensive medicinal profile, with low 5-HMF, high residual cAMP, and increases total phenolic content, resulting in high antioxidant activity.

Keywords Jujube  $\cdot$  Blackening process  $\cdot$  Phenolic additives  $\cdot$  5-HMF  $\cdot$  cAMP  $\cdot$  Antioxidant

# Introduction

Jujube (*Ziziphus jujuba* Mill.), as a traditional dietary and medicinal food mainly distributed in Europe, southern and eastern Asia, and Australia, is recognized as the primary fruit production species in the buckthorn family Rhamnaceae as well as a good source of bioactivity ingredients

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<sup>2</sup> Department of Food Science and Formulation, Gembloux Agri-Bio Tech, Université de Liège, Gembloux, Belgium [1], such as phenolic substances [2], polysaccharides [3], and triterpenoid acids [4], especially the content of cyclic adenosine monophosphate (cAMP) could reach 160  $\mu$ g/g, which is the highest among more than 180 kinds of natural plants [5, 6]. Also, a large number of previous studies reported that jujube fruit has important effects on the human body such as anti-inflammatory, anti-tumor, antioxidant, etc. [1, 7–9]. However, in the jujube processing industry, the main products are still traditional jujube sauce, jujube powder, candied jujube, fermented beverages, etc. [10]. The research and development of functional jujube products are still in their infancy [11]. Functionality, bio-accessibility and the High-value-added product of novel jujube products are required for further studies.

Blackening is a type of fermentation of Maillard reaction in which amino compounds in food react with carbonyl compounds to produce nitrogenous polymers and a large number of small molecules under high temperature and humidity [12]. In recent years, blackening has become an emerging technology that attracts more attention in the fruit and vegetable processing industry [13-15]. And its excellent applicability to jujube fruit has made the blackened jujube a research hotspot [4, 16]. During the blackening process, in addition to the apparent color change, the functional components contained have also undergone significant changes, such as the content of sucrose decreased, reducing sugar (glucose and fructose), total phenolic, and triterpenoid acids increased, besides, the increased 2-acetyl furan, furan and its derivatives provided the caramel flavor to the blackened jujube [6, 17]. However, the cAMP, as a second messenger widely used in health care products and pharmaceuticals and played important roles in cell growth, differentiation also has significant effects on immunomodulatory, anticancer, antioxidant and neuroprotection [18], significantly degraded by 65.85% in the blackening process [19]. In addition, 5-hydroxymethylfurfural (5-HMF), as an intermediate product of the Maillard reaction, accumulated to 3.52 g/kg in blackened jujube [19, 20], which can cause colon aberrant crypt foci in rats, skin papillomas and hepatocellular adenomas in mice [21]. Additionally, the main pathways for the formation of 5-HMF include the 1,2-enolization reaction under acidic conditions, followed by the formation of 1,2-enaminol and 3-deoxy-1,2-dicarbonyl compounds, which were finally converted into carbonyl methyl furfural or furfural [22]. It has been found that 5-HMF was easily converted into the toxic 5-sulfinylmethylfurfural (5-SMF), and its potential danger cannot be ignored [23]. Therefore, it's of great necessity to eliminate the potentially harmful substance while maintaining the nutritional components during the blackening process [24].

To our best knowledge, There are methods to reduce 5-HMF production, such as yeast fermentation, ultra-highpressure homogenization and formulation adjustment, which can achieve a 60% reduction of the 5-HMF content [22]. However, there is still no method that can reduce the 5-HMF while increasing the cAMP content in the blackening process of jujube. In this study, four common edible phenols were selected as additives to decrease oxidation during the maceration of jujube. (1) Tea polyphenol is the general name for the total polyphenols in tea, while EGCG is a catechin-like monomer isolated from tea leaves with a strong antioxidant effect [25-27]. (2) Green tea powder is a finely milled preparation of green tea, with a good antioxidant effect with a phenolic content of  $23.60 \pm 5.00$  mmol/L [28–30]. (3) Pomegranate peel ellagic acid is a polyphenol that significantly contributes to the total phenolic content of pomegranate peel, reaching 1313.08–1700.07 mg/L [31]. (4) Pomegranate peel ellagic acid was found in a variety of plants and fruits, and it can be used in the cosmetic industry [32] or as a natural food additive [33]. With macerating

by phenolic additives, the physicochemical properties and antioxidant activity of blackened jujube were investigated.

# **Materials and methods**

### **Chemicals and materials**

DPPH (2,2'-diphenyl-1-picrylhydrazyl) was obtained from Hualan Chemical Technology Co. LTD (Shanghai, China), ABTS (2,2'-azinobis (3-ethylbenzothia zone-6-sulfonic acid) diammonium salt) was from Hefei Bomei Biological Technology Co. LTD (Anhui, China). Ascorbic acid (VC), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), epigallocatechin gallate (EGCG) (purity > 98%), and standard 3',5'-cyclic AMP (purity > 98%) were from Shanghai Yuanye Bio-Technology Co., LTD. (Shanghai, China). Tea polyphenols and green tea powder were purchased from Hebei Bio-Technology Co., LTD. (Hebei, China), pomegranate peel ellagic acid was purchased from Xi'an Shengqing Bio-Technology Co., LTD. (Shanghai, China), standard 5-HMF (purity > 98%) was purchased from Shanghai Ruiyong Biological Technology Co. LTD (Shanghai, China). Methanol was HPLC grade and was purchased from Shandong Yu Wang He Tianxia New Material Co. LTD. (Yucheng, China). Unless otherwise specified, all other chemicals were from commercial sources and were of analytical grade.

Goutouzao (GTZ, jujube fruit; the moisture content of about 21%) was purchased from Yanchuan County, Shaanxi (GPS E110° 05' N36° 50') China on November 19, 2019. The samples were stored at -10 °C before the experiments.

## **Blackening process**

The pitted jujubes were pretreated by soaking in deionized water (control), 10% (w/v) green tea power solution, 10% (w/v) tea polyphenol solution, 10% (w/v) EGCG solution, or 10% (w/v) pomegranate peel ellagic acid solution at 25 °C for 40 min. Then blackened according to the method of Gao et al. [17] Briefly, The soaked jujubes were blackened at 75 °C and 80% relative humidity for 84 h, with sampling every 12 h. The blackened jujube pulp was lyophilized (ALPHA 2–4, Henan Brother Instrument Equipment Co., China) and powdered for analysis.

#### Extraction

The extraction was referred to Zhang et al. [34] with some modifications. Briefly, jujube powder  $(1.00 \pm 0.05 \text{ g})$  was homogenized with 16 mL of 60% aqueous ethanol and extracted by ultrasonication (KQ2200DE, Jiangsu Kunshan Co., China) at 600 W and 60 °C for 30 min. The supernatant

was obtained by centrifugation at 3000r/min for 8 min. The process was repeated twice for each sample and the extracts were combined before analyzing the total phenolic content and antioxidant activity.

# Measurement of the color change during jujube blackening

The L (brightness), a (redness) and b (yellowness) values of jujube peels were determined using a colorimeter (WSC-2B, Shanghai Optical Instrument Co., Ltd., China). Each sample was analyzed 9 times. The formula for calculating the color difference ( $\Delta E$ ) was as follows [35]:

$$\Delta E = \sqrt{(L - L_0)^2 + (a - a_0)^2 + (b - b_0)^2}$$

where  $L_0$ ,  $a_0$ , and  $b_0$  are the *L*, *a*, and *b* values of jujube; *L*, *a*, and *b* are the *L*, *a*, and *b* values of blackened jujube, respectively.

# **Measurement of the 5-HMF content**

The 5-HMF content was measured as reported by Sun et al. [19], with minor modifications as follows: 0.5 g jujube powder was dispersed in 25 mL of 50% methanol and extracted by ultrasonication at 600 W for 30 min at room temperature. Then, the solution was filtered through a 0.45  $\mu$ m pore-size PTFE membrane. The content of 5-HMF was determined using a Shimadzu HPLC system with a UV–visible detector (Kyoto, Japan). A methanol–water mixture (5:95, v/v) was used as the mobile phase and eluted through an Inertsil ODS-3 column (250 mm × 4.6 mm, 5 um) at a flow rate of 1 mL/min. The detection temperature was 35 °C, the detection wavelength was 282 nm, and the injection volume was 10  $\mu$ L.

#### Measurement of the total phenolic content

The total phenolic content was measured as reported by Kou et al. [36]. Briefly, 0.5 mL of the jujube extract described above was mixed with 0.5 mL Folin-Ciocalteu reagent (0.50 N) and 2.5 mL 10% w/v Na<sub>2</sub>CO<sub>3</sub>. Then, the volume was adjusted to 10 mL with distilled water. The absorbance was measured at 760 nm after 60 min of reaction in the dark and gallic acid was used as a standard (mg GAE/g DW).

#### Measurement of the cAMP content

The cAMP content was measured using a method reported by Chen et al. [37], as follows: 0.5 g jujube powder was dispersed in 25 mL of distilled water and extracted by ultrasonication at 600 W for 30 min at room temperature. Then, the solution was filtered through a 0.45  $\mu$ m pore-size PTFE membrane. The content of cAMP was determined using a Shimadzu HPLC system with a UV–visible detector (Kyoto, Japan). The mobile phase was a methanol-potassium dihydrogen phosphate (0.05 mol/L) mixture (10:90, v/v) eluted on an Inertsil ODS-3 column ( $250 \times 4.6$  mm ID, 5 µm; Waters, flow rate 1 mL/min, detection temperature 30 °C, detection wavelength 282 nm, injection volume 10 µL.

#### **DPPH scavenging activity**

The DPPH scavenging activity was measured as described by Wang et al. [18]. Briefly, 0.1 mL jujube extract was mixed with 5 mL DPPH (0.1 mM). The absorbance was measured at 517 nm after 30 min of reaction in the dark, and ascorbic acid was used as a standard (mg VC/g DW).

# **ABTS scavenging activity**

The ABTS scavenging activity was measured according to Re et al. [38]. After mixing equal volumes of 2.45 mmol/L  $K_2S_2O_8$  and 7.00 mM ABTS, the mixture was left in the dark at room temperature for 12–16 h. The working solution was obtained by dilution with 80% ethanol solution to an absorbance of  $0.700 \pm 0.050$  at 734 nm. Then, 0.2 mL of jujube extract was mixed with 5 mL of the working solution. The absorbance was measured at 734 nm after 8 min of reaction in the dark, and Trolox was used as a standard (mg TE/g DW).

# **Reducing activity**

The reducing activity was measured as reported by Feng et al. [39]. Briefly, 0.6 mL of jujube extract was mixed with 2.5 mL 0.20 mol/L phosphate buffer and 2.5 mL 1% W/V potassium ferricyanide solution. The mixture was incubated at 50 °C for 20 min. Then, the solution was mixed with 2.5 mL 10% W/V trichloroacetic acid. Finally, 2.5 mL of the supernatant was mixed with 2.5 mL distilled water and 2.5 mL 0.1% W/V ferric chloride solution. The absorbance was measured at 700 nm after 10 min of reaction in the dark and Trolox was used as a standard (mg TE/g DW).

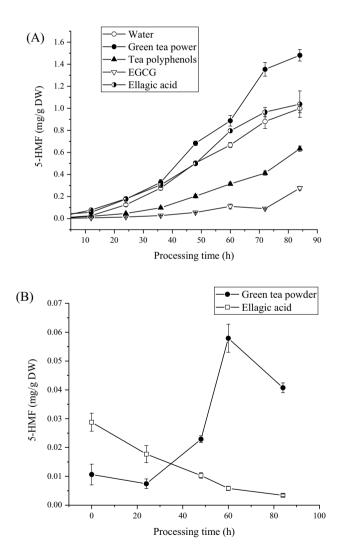
#### **Statistical analysis**

The results were expressed as averages  $\pm$  standard deviations. SPSS Statistics Software (version 25; IBM, New York, USA) was used for data analysis. Duncan's multiple range test was used to determine the significance of differences, with a threshold of p < 0.05 or p < 0.01. Correlations were analyzed using the standard Pearson correlation method. The best treatment was deduced via heatmap analysis.

# **Results and discussion**

# Changes in the 5-HMF content of jujube during blackening

The 5-HMF content of the blackened jujube macerated with the four tested polyphenolics or water as a negative control is shown in Fig. 1A. The 5-HMF contents gradually increased as the blackening proceeded, which was similar to a report by Park et al. [40]. However, it can be seen that the 5-HMF content of jujube macerated with EGCG and tea polyphenols was significantly lower than that of the control at the end of blackening. The corresponding contents were 0.28 and 0.63 mg/g DW, representing decreases by 72.36 and 36.47% compared with the control group, respectively. The result



**Fig. 1** A 5-HMF content of jujube macerated with water, green tea powder, tea polyphenols, EGCG and pomegranate peel ellagic acid during blackening, respectively; **B** 5-HMF content of green tea powder and pomegranate peel ellagic acid during blackening process

indicated that tea polyphenols and EGCG could reduce the formation of 5-HMF in blackened jujube, which was similar to a report by Qi et al. [33].

Combined with previous studies, there were two main reasons that EGCG inhibited the formation of HMF. One would be the reaction between EGCG and 3-deoxyglucosone (3-DG) to form a dimer adduct, and the 3-DG is a sugar fragment carbonyl compound during the Maillard reaction as well as the precursor of the formation of 5-HMF [41], thereby inhibiting the formation of 5-HMF. Secondly, researchers have also found that EGCG also directly forms dimer adducts with 5-HMF to reduce its content [42]. In addition, HMF is also an intermediate in the pathway to form acrylamide, which is neurotoxic and genotoxic, and the combination of EGCG and HMF can also reduce the formation of acrylamide to improve food safety [42, 43].

By contrast, the 5-HMF content of jujube macerated with green tea powder and ellagic acid was higher than that of the control group, reaching 1.48 and 1.04 mg/g DW at the end of blackening, representing 48.11 and 3.82% increases compared with the control group, respectively. To further investigate this phenomenon, green tea powder and pomegranate peel ellagic acid were individually blackened under constant temperature and humidity (70 °C, 80% RH), and the 5-HMF content was determined as shown in Fig. 1B. It can be seen that green tea powder and pomegranate peel ellagic acid itself contained little amount of 5-HMF at 0.03 and 0.01 mg/g DW, respectively, and obviously changed during the same blackening process, but both remained at low levels compared to the additional increase in green tea powder group and pomegranate peel ellagic acid group, which means that the 5-HMF in the two groups were not only the sums of the contents produced in blackened jujube and the additives individually. This may be due to the involvement of components in green tea powder and pomegranate peel ellagic acid in 5-HMF synthesis. Besides, the previous studies had proved that 5-HMF can also be detected in green tea during the roasting or fermentation process [44, 45], therefore, it can be speculated that the phenolic content it possesses cannot completely inhibit the formation of its own 5-HMF. The mechanism of the phenomenon needs to be further studied.

#### Color change of jujube peel during blackening

As shown in Fig. 2, the color change ( $\Delta E$ ) showed a gradual increase as the blackening progressed, with a large change in the first 36 h, which was due to the thermal cleavage of fructans in the jujube into fructose and glucose, which then reacted with amino acids to form Maillard adducts as the blackening progressed [12]. The color change of jujube macerated with tea polyphenols and EGCG solution did not decrease due to the reduction of

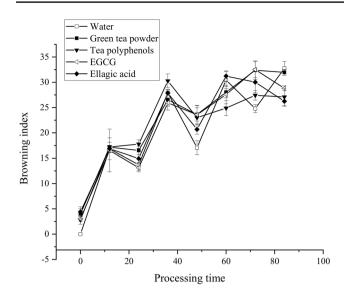


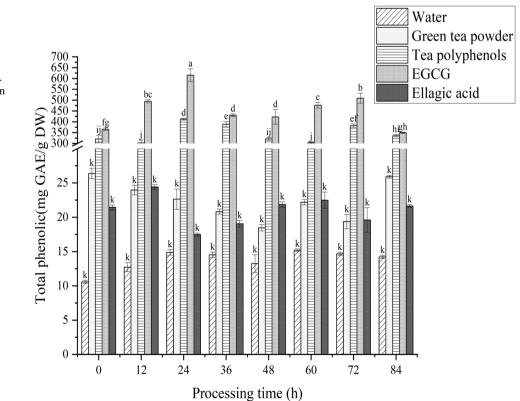
Fig. 2 The color difference value ( $\Delta E$ ) during blackening of jujube impregnated with water, green tea powder, tea polyphenols, EGCG and pomegranate peel ellagic acid, respectively

5-HMF, indicating that the reduction of 5-HMF did not significantly affect the change in color and did not prevent the proceeding of the Maillard reaction, which was similar to a report by Lee et al. [35].

# Changes of the total phenolic content of jujube during blackening

As shown in Fig. 3, the total phenolic content of the control group showed an overall trend of gradual increase as the blackening progressed. The results of this study were similar to those of Kim et al. who reported that the total phenolic content of blackened jujube increased constantly during the blackening process [46]. The total phenolic content of the control jujube before blackening was 10.58 mg GAE/g DW (Fig. 3), which was similar to a report by Aneta et al. who studied four Spanish jujube cultivars, with the total phenolic content of 14.42 mg/g DW of the cultivar '*Dátil*' [47]. In the end of the blackening process, the total phenolic content of the control group was 14.22 mg/g DW, which was 34.38% higher than that of the beginning.

The total phenolic content of the jujube macerated with green tea powder, tea polyphenols, EGCG and pomegranate peel ellagic acid was significantly higher than that of the control group at the end of blackening. This showed that the addition of polyphenols during the maceration treatment can effectively increase the phenolic content of blackened jujube. Among them, the total phenolic content of the jujube macerated with EGCG at the end of blackening was the highest at 349.76 mg GAE/g DW, which was 24.59 times higher than in the control group.



**Fig. 3** Total phenolic content of jujube macerated with water, green tea powder, tea polyphenols, EGCG and pomegranate peel ellagic acid during blackening, respectively. [Different letters in each column indicate significant differences (p < 0.05)]

# Changes of the cAMP content of jujube during blackening

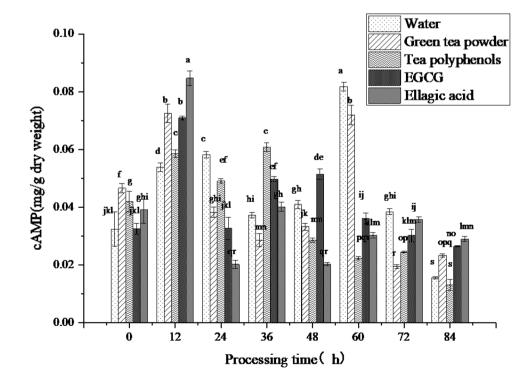
As shown in Fig. 4, the cAMP content of jujube was 0.03 mg/g DW before blackening, which was within the wide range reported by Xue et al., who found that the cAMP content in 204 red jujube cultivars was 0.008-0.181 mg/g DW [48]. After 12 h of blackening, the cAMP content of the samples macerated with water, green tea powder, tea polyphenols, EGCG, and pomegranate peel ellagic acid increased by 34.77, 8.80, 31.82, and 57.38% compared with the red jujube, respectively. At the end of the blackening process, all groups' cAMP contents were significantly lower than the red jujube (p < 0.05). These may be caused by the high temperature during blackening which led to the degradation of cAMP, and the results were highly consistent with the research of Sun et al. who reported that cAMP content decreased by 63% at 84 h of the blackening process [19]. Also, the thermal treatment of jujube such as air-drying, sun-drying and microwave-drying can significantly cause the degradation of cAMP and cGMP in jujube [49, 50]. The cAMP content of blackened jujube macerated with green tea powder, EGCG and pomegranate peel ellagic acid was higher than that of the control group, reaching 0.023, 0.026, and 0.029 mg/g DW respectively, which was 50, 70, and 87% higher than in the control group. Among them, the blackened jujube macerated with pomegranate peel ellagic acid had the highest cAMP content. The results therefore demonstrate that phenolic treatment can reduce cAMP depletion, with pomegranate peel ellagic acid performing the best. Although the mechanism is not clear and needs further study, the inhibitory effect of phenolic additives on cAMP degradation is of important implications for the development of blackened jujube products.

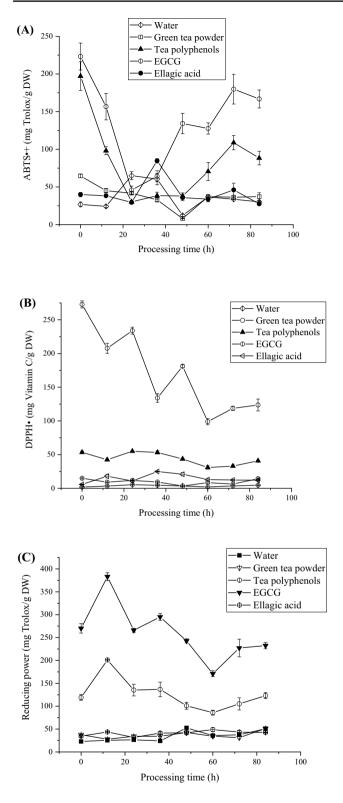
# Changes of the antioxidant capacity of jujube during blackening

As shown in Fig. 5, the antioxidant capacity showed a trend of first decreasing and then increasing with the progression of blackening. This can be explained by the fact that tea polyphenols, EGCG, green tea powder and pomegranate peel ellagic acid were oxidized with the progression of blackening, and the antioxidant capacity was reduced. However, with the progress of blackening, the scavenging capacity of DPPH and total reducing power of each group showed an increasing trend, which may be due to the increase of the late product of the Maillard reaction that had high antioxidant capacity [13, 14].

It can be seen from Fig. 5 that the scavenging capacity of DPPH free radicals, ABTS free radicals and total reducing power of control jujubes were enhanced by 146.84%, 12.91%, and 120.51% to 4.69 mg VC/g DW, 30.28 mg Trolox/g DW and 50.70 mg Trolox/g DW at the end of blackening compared with red jujube, respectively. These results indicated that blackening could enhance the antioxidant capacity of jujube, which was similar to a report by Yu et al. who showed that the blackened garlic extract had higher FRAP values, ORAC values and ABTS scavenging activity [51].

**Fig. 4** cAMP content of jujube macerated with water, green tea powder, tea polyphenols, EGCG and pomegranate peel ellagic acid during blackening, respectively. [Different letters in each column indicate significant differences (p < 0.05)]





**Fig.5** A ABTS•+ scavenging activity; **B** DPPH• scavenging activity; **C** Reducing power of jujube macerated with water, green tea powder, tea polyphenols, EGCG and pomegranate peel ellagic acid at the during blackening, respectively

Among them, the antioxidant capacity of jujube in the EGCG group was the strongest. Its scavenging ability of DPPH free radicals, ABTS free radicals and the total reducing power were respectively 26.36, 5.51 and 4.58 times higher than in the control group, reaching 123.62 mg VC/g DW, 166.78 mg Trolox/g DW, and 232.43 mg Trolox/g DW at the end of the blackening process.

For tea polyphenol group, the antioxidant capacities were all the second highest in terms of DPPH, ABTS and the total reducing power and respectively increased by 770.08%, 191.88%, and 142.60% compared with the control group, reaching 40.84 mg VC/g DW, 88.38 mg Trolox/g DW, and 123.00 mg Trolox/g DW at the end of the blackening process. It can be seen that the treatment with tea polyphenols is also a good choice for obtaining highly antioxidant blackened jujube.

The scavenging ability of DPPH free radicals of blackened jujube macerated with pomegranate peel ellagic acid increased by 157.20% compared with the control group, reaching 12.07 mg Trolox/g DW. The observed trend was similar to the results of Pan et al. who found that the antioxidant capacity of yogurt was enhanced by the addition of pomegranate juice in a concentration-dependent manner [52]. Sun et al. showed that ellagic acid had a high capacity to scavenge DPPH free radicals and superoxide anions, inhibit membrane lipid peroxidation and increase total reducing activity. The total antioxidant capacity of the intestine, as well as the superoxide dismutase activity of the liver and intestinal cells treated with ellagic acid was significantly increased (p < 0.05) [53].

The antioxidant capacity of jujube in the green tea powder group was also improved, and the ability of jujube to scavenge DPPH and ABTS radicals, as well as total reducing power respectively increased by 205.97%, 22.56% and 1.02% compared with the control group, reaching 14.35 mg VC/g DW, 37.11 mg Trolox/g DW and 51.22 mg Trolox/g DW at the end of blackening.

#### **Correlation analysis**

Table 1 shows the correlation coefficients and significant correlations between the active ingredients and antioxidant capacity during the blackening process. It can be seen that the total phenolic content of jujube showed a highly significant positive correlation with the scavenging ability of DPPH free radicals, ABTS free radicals and the total reducing power, while it showed a significant negative correlation with 5-HMF content (p < 0.01 in both cases). In addition, the cAMP content showed a significant negative correlation with the 5-HMF content (p < 0.01). These results indicated that the total phenolics were the main antioxidant substances in jujube fruit, and the addition of phenolic compounds can reduce the production of 5-HMF during the blackening

Table 1	Correlation coefficients (r) between antioxidant activity and			
physicochemical components during the blackening of jujube				

	TPC	cAMP	ΔΕ
5-HMF	- 0.457**	- 0.344**	0.611**
DPPH•	0.794**		
ABTS∙+	0.626**		
Reducing power	0.879**		

Correlations among the data from a standard Pearson analysis. \*p < 0.05, \*\*p < 0.01

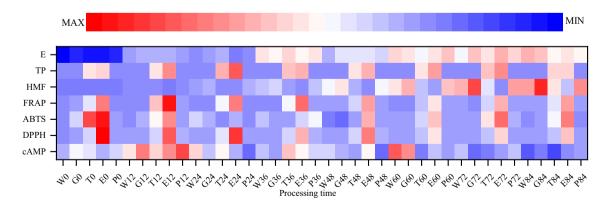
process, which in turn maintained the content of cAMP. A highly significant correlation between total phenolics and antioxidant properties in jujube has also been found in a previous study [36].

## **Heatmap analysis**

In the heatmap shown in Fig. 6, red indicates higher values of the indicated physicochemical properties and antioxidant capacity, while blue indicates lower values. It can be seen that after the blackening process, the antioxidant capacity and total phenolic content of jujube were the strongest in the EGCG group, followed by the tea polyphenol group. In addition, the 5-HMF content of jujube was the lowest in the EGCG group, followed by the tea polyphenol group. The cAMP content of jujube was the highest in the ellagic acid group, followed by the EGCG group. In conclusion, the addition of EGCG during maceration had the best comprehensive effect on the antioxidant capacity and content of bioactive compounds in blackened jujube.

# Conclusions

The maceration with the addition of EGCG or tea polyphenols could significantly reduce the formation of 5-HMF during the blackening process, while the content of harmful 5-HMF at the end of blackening was reduced by 72.36 and 36.47%, respectively. Green tea powder, EGCG and pomegranate peel ellagic acid could significantly increase the content of bioactive cAMP remaining at the end of the blackening process, which increased by 50%, 73% and 87%, respectively. In addition, this method also resulted in jujube with high antioxidant activity. The 5-HMF content was significantly negatively correlated with the total phenolic content, indicating that phenolic additives could effectively inhibit the production of 5-HMF (p < 0.01). Heatmap analysis revealed that EGCG had the best comprehensive effect on blackened jujube. Moreover, the phenolic maceration method not only improved the safety but also increased the biological activity of jujube, which provides a theoretical basis for the development of the jujube industry.



**Fig.6** Heat map of the blackening process of jujube macerated in five solutions. W represents water; G represents green tea powder; T represents tea polyphenols; E represents EGCG; P represents pomegranate peel ellagic acid. Numbers represent blackening time (h)

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# Declarations

**Conflict of interest** The authors declare that they have no known conflict of interest associated with the publication of this manuscript.

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