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Prevention of browning during the saccharification of juicy peach syrup and storage of peach wine

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ABSTRACT

Peach fruit is rich in nutrients and its fermented fruit wine is popular. However, peach fruit pulp is susceptible to browning during saccharification and wine is also prone to browning during storage, which hinders its further development. In this study, different anti-browning agents (ethylenediamine tetraacetic acid (EDTA), ascorbic acid (AA) and potassium metabisulfite (PM)) were used in the process of fruit pulp saccharification and wine storage to evaluate their anti-browning effect. Firstly, the browning inhibition ratio (BIR) was determined by single-factor experiments to select the appropriate variable concentrations. Furthermore, orthogonal experiments were designed to analyse the optimal combination of anti-browning agents. The results showed that the combination of 0.40 g/L EDTA, 0.20 g/L AA, and 0.10 g/L PM had a better anti-browning effect in fruit pulp saccharification. During the storage of peach wine, 0.15 g/L PM only had a better anti-browning effect than EDTA and AA. This study provides a reference for the anti-browning technique of peach fruit wine and further contributes to improving the quality of fruit wine.

1. INTRODUCTION

Peach (*Amygdalus persica*) belongs to the genus Prunus of Rosaceae. The flower can be ornamental and the fruit is juicy. Peach fruit can be eaten raw or made into preserved peaches and canned goods. Peach is rich in nutrition [1]. For example, the protein content of peach is twice as high as apple and grape, seven times higher than pear. In addition, the iron content of peach is three times higher than apple and five times

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higher than pear [2]. Peach is also rich in a variety of vitamins, of which vitamin C content is the highest [3]. In addition, peaches also have positive health effects, such as improved function of the lungs, expectorant, skin beauty, stomach clearing and so on [4]. Peach is generally made of fresh food, and there are also many products made of peach as raw materials in the market, such as canned peaches, peach paste, dried and preserved peaches, peach juice and peach wine.

Fruit wine is an alcoholic beverage made from fruit by crushing, fermentation or soaking, with a low alcohol content of not more than 18% [5]. Due to the different flavours of different raw materials, fruit wine not only contains the unique flavour of raw materials, but also inherits the original nutrients of the fruit, such as vitamins, organic acids, minerals, amino acids and sugars [6]. Fruit wine is reported to have the function of anti-oxidation, anti-ageing, cholesterol reduction and other health benefits [7]. Therefore, fruit wine is a healthy and low-alcohol beverage in-coupled with its flavour that is valued by consumers [8].

Peach wine is a low-alcohol fruit wine produced with peach pulp fermented by yeast, which could well retain the nutritional components of peach fruit. However, peach is susceptible to oxidative browning during processing, which seriously affects the colour of peach wine and even leads to deterioration [9]. The main cause of browning in many fruits and vegetables is enzymatic browning [10]. Since polyphenol oxidase plays an important role in the browning process of fruits and vegetables, it is important to inhibit the enzymatic reaction of polyphenol oxidase [11]. There are many kinds of polyphenol oxidase inhibitors, such as benzoic acid and its derivatives, ascorbic acid (AA), cysteine, citric acid, sulfite, ethylenediaminetetraacetic acid (EDTA) and thiourea etc [12-15].

Polyphenol oxidase (PPO) is the key enzyme involved in enzymatic browning [16]. Other studies have shown that browning leads to a significant loss of fruits and vegetables during processing [17]. The characteristics of PPO are various in different fruits. At present, there is a vast report on the characteristics and activity inhibition of polyphenol oxidase in fruits and vegetables, such as apple, pear, yam, lotus root, potato and so on [18]. There is also research on polyphenol oxidase in peach, which mainly focuses on extraction and enzymatic properties, but there are few reports on the inhibition of polyphenol oxidase in peach processing [19]. Studies have investigated the inhibitory effects of AA, citric acid, EDTA, and NaCl solution on polyphenol oxidase in *Aurora nectarine* and *Danmo nectarine* [20].

In recent years, since the application of a single browning inhibitor has the defects of using high concentration and not being able to maintain the colour stability of fruits for a long period. Therefore, many researchers have used mixed inhibitors to control the browning of fruits and vegetables, and good browning control effects have been achieved, such as in apple, pear, potato, and lettuce [21-23]. However, only few studies have been conducted on the browning inhibition of peach wine by using complex inhibitors [24].

This study investigates the effects of several commonly used anti-browning agents including EDTA, AA and potassium metabisulfite (PM) in the saccharification of fruit pulp and during wine storage period. Also, single factor and orthogonal experiments were conducted to investigate the effects of different anti-browning agents on the fermentation and storage of peach fruit wine, aiming to find a more suitable reagent combination and higher browning inhibition ratio (BIR). This experiment provides a reference for the anti-browning technique of peach fruit wine, and further improves the quality of fruit wine.

2. MATERIALS AND METHODS

2.1 Peach pre-treatment

Juicy peach was harvested from Fenghua District, Ningbo City, Zhejiang Province, China. Well-ripened peaches were selected and washed. Initially, the samples were de-watered in the refrigerator and retained the bioconjugate water. Subsequently, the peach kernels were removed, and then cut into small

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pieces with a knife. Then, 350 g of peach pulp samples were put into each 500 mL brown jar. Finally, pectinase was added with the dosage for each 0.15 g/350 g peach pulp.

2.2 Anti-browning treatment method

After bottling the pulp of pre-treated peach fruit, different percentages of EDTA (0.10, 0.20, 0.30, 0.40, 0.50 g/L), AA (0.10, 0.20, 0.30, 0.40, 0.50 g/L), PM (0.05, 0.10, 0.15, 0.20, 0.25 g/L), were added respectively. Samples without antioxidants were used as controls. All samples were prepared in triplicates. On the third day, the peach pulp was filtered through two layers of gauze, followed by four additional layers of gauze, and then put into a clean container. A 5 mL aliquot of filtered peach juice was removed using the pipette and centrifuged at 8000 g for 10 min. The absorbance was measured with a spectrophotometer at a wavelength of 420 nm, repeated three times, and the experimental data were recorded.

2.3 Orthogonal experiment

In general, mixed anti-browning agent treatments inhibit browning better than one-factor browning agents. Therefore, a three-factor, three-level orthogonal experiment was designed by combining the three anti-browning agents (EDTA, AA, and PM) in order to find a better combination to inhibit browning. Based on the suitable concentration of anti-browning agent in the previous single-factor experiment, the orthogonal experiment was designed and the anti-browning treatment during saccharification was developed according to the orthogonal experiment protocol [25].

2.4 Anti-browning treatment during storage of peach wine

85 g sucrose, 100 ml water and 0.025 g lactase were added to a 500 mL brown bottle, followed by 220 ml of prepared peach juice. Then, activated yeast (*Saccharomyces cerevisiae*, Angel BV818) was added and the fermentation vessel was placed at 25 ℃ for fermentation. The sugar level was monitored daily by an Erma Handheld Refractive Brix Meter. The fermentation was completed by pressing and filtration to obtain clarified wine samples. Subsequently, various concentrations of browning inhibitors (EDTA, AA, and PM) were added to the samples, with samples without any antioxidants serving as the control. Each treatment contained three biological replicates. Following one month of storage, the absorbance at a wavelength of 420 nm was measured for all wine samples, with each measurement repeated three times.

2.5 Measurement of BIR

The coloured substance produced by the browning reaction has a spectrophotometry absorption peak at 420 nm [5]. The degree of browning was quantified by measuring the absorbance at a wavelength of 420 nm, using distilled water as the baseline, where a higher BIR indicated a more pronounced inhibition of browning (Eq. 1).

$$\mathrm{BIR} = \frac{(\text{Control sample} - \text{Treated sample})}{\text{Control sample}} \times 100\% \quad (1)$$

2.6 Statistical analysis

Each experiment included at least three biological replicates. Data were expressed as mean with standard deviation (SD). Statistical analyses to determine significant differences were performed using SPSS 26.0 software, employing Duncan's and LSD tests.

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3. RESULTS AND DISCUSSION

3.1 One-factor anti-browning during syrup saccharification

Peach fruit is prone to browning, which not only affects the appearance of food but also reduces the nutritional value [26]. In the same way, browning has a significant impact on the storage and development of peach fruit and its fermented wine. Therefore, anti-browning is an important measure to ensure quality for the processing and storage of peach wine.

Effect of EDTA on browning degree during syrup saccharification

EDTA, has lone pair electrons on the 2 nitrogen and 4 carboxyl groups, which can fill the empty orbitals of metal ions to form complexes [27]. Therefore, EDTA has a strong coordination ability and can react with metal ions, playing a role in inhibiting browning. From Table 1, it was shown that the BIR of peach pulp saccharification increased generally with the increase of EDTA concentration. When EDTA concentration was ≥0.20 g/L, there was a good and stable inhibition effect (BIR>40%).

Table 1. Effect of EDTA on browning degree during syrup saccharification

EDTA g/L	**0**	**0.10**	**0.20**	**0.30**	**0.40**	**0.50**
A420	0.550	0.351	0.305	0.309	0.313	0.282
BIR %	/	36.18	44.55	43.82	43.09	48.73

Effect of PM on browning degree during syrup saccharification

AA is a new type of biological antioxidant, anticorrosion, freshness preservation and colouring aid in the food industry [28]. It can maintain the colour, natural flavor and extend the shelf life of food [29]. AA has also been used to prevent enzymatic browning, as it not only lowers the pH of the reaction system, but also acts as a reducing agent which reduces the quinones and their derivatives in the system to phenols and decreases the oxygen content of the system by oxidizing itself [15]. As shown in Table 2, there was no obvious regular correlation between BIR and AA concentration. When AA was added at a concentration of 0.10 g/L, it had the best inhibition effect.

Table 2. Effect of AA on browning degree during syrup saccharification

AA g/L	**0**	**0.10**	**0.20**	**0.30**	**0.40**	**0.50**
A420	0.550	0.288	0.294	0.332	0.305	0.302
BIR %	/	47.74	46.50	39.73	44.66	45.26

Effect of PM on browning degree during syrup saccharification

PM acts as a food bleaching agent, preservative and antioxidant [30]. It can be used as a bleaching agent as a food additive, which can destroy or inhibit colouring factors in food, hence colouring or food is free from browning. [31]. The colour of an organic compound is produced by the chromophore contained in its molecule [32]. Chromophores contain unsaturated bonds, and the release of hydrogen atoms from reducing bleach can cause the unsaturated bonds contained in the chromophore to become single bonds, resulting in the loss of colour in organic compounds [33]. The browning of some foods is caused by the presence of trivalent ions, adding reducing bleach can change trivalent iron ions into divalent iron ions to prevent browning of food [34]. The experimental results in Table 3 showed that when the concentration of PM was equal or greater than 0.10 g/L, the browning of the syrup during saccharification was significantly inhibited (BIR>50%).

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Table 3. Effect of PM on browning degree during syrup saccharification

PM g/L	0	0.05	0.10	0.15	0.20	0.25
A420	0.550	0.299	0.261	0.234	0.237	0.258
BIR %	/	45.70	52.54	57.46	57.05	53.18

In summary, different anti-browning agent additions had different inhibiting effects on browning during fruit pulp saccharification in peach. All three anti-browning agents in this study, EDTA, AA, and PM, all had a pronounced inhibitory effect during the saccharification of peach pulp. PM had the most significant effect on anti-browning, but the amount of PM should not be too high in production, otherwise it will have adverse effects on human health [35]. Therefore, considering the above analysis comprehensively, the most appropriate single inhibitor for preventing the saccharification browning was PM at 0.10-0.15 g/L, where BIR was between 52% and 57%.

3.2 Orthogonal experiment on anti-browning during saccharification process

Orthogonal experiment is a design method to study multifactorial multilevel experiments [36]. According to the orthogonality, some representative points are selected from all the experimental protocols. These representative points have the characteristics of uniform dispersion and comparability, which not only reduce the number of experiments, but obtain the experimental results efficiently, rapidly and accurately. Based on the results of the above one-factor experiments, the appropriate level of variables was selected (Table 4) and a three-factor three-level orthogonal experiment was conducted (Table 5).

From the experimental data (Table 6), it can be seen that different combinations of browning inhibitors exhibit varying degrees of effectiveness in suppressing browning, proving to be more effective than the addition of individual inhibitors alone. In orthogonal experiments, a larger value of R indicates a more significant effect of this factor on the results [37]. Based on the extreme difference R, the order of the browning inhibition effect on browning inhibition during the saccharification process in the mixed browning agent was as follows: EDTA(R=17.60) > AA(R=5.34) > PM(R=1.46). Besides, the treatment of test 5, 0.40 g/L EDTA, 0.20 g/L AA, and 0.10 g/L PM, was the most effective in inhibiting browning during the process of syrup saccharification of peach fruit pulp. In addition, the treatments of test 1 (0.3 g/L EDTA, 0.1 g/L AA, and 0.10 g/L PM) and test 3 (0.3 g/L EDTA, 0.3 g/L AA, and 0.15 g/L PM) also had a pronounced inhibitory effect on browning during saccharification. Comprehensively, according to the optimal choice, the treatment of test 5 was the most effective in inhibiting browning (BIR=57.34%) during the saccharification of peach pulp.

Table 4. Factor levels for orthogonal experiments

Horizontal factors	EDTA g/L	AA g/L	PM g/L
1	0.30	0.10	0.10
2	0.40	0.20	0.15
3	0.50	0.30	0.20

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Table 5. Orthogonal experimental design

Test number	EDTA	AA	PM
0	0	0	0
1	1	1	1
2	1	2	3
3	1	3	2
4	2	1	3
5	2	2	1
6	2	3	2
7	3	1	2
8	3	2	3
9	3	3	1

Table 6. Results of orthogonal experiments on anti-browning during saccharification

Test number	EDTA	AA	PM	BIR/%
0	0	0	0	/
1	1	1	1	54.65
2	1	2	3	48.37
3	1	3	2	50.42
4	2	1	3	44.06
5	2	2	1	57.34
6	2	3	2	47.24
7	3	1	2	26.75
8	3	2	3	29.93
9	3	3	1	43.83
K1	51.17	41.80	43.92	
K2	49.51	45.15	45.37	
K3	33.54	47.13	44.80	
R	17.60	5.34	1.46	

3.3 Anti-browning during storage of peach wine

The BIR of the peach wine during storage was determined after the peach wines had been treated using anti-browning agent and left to stand for one month. EDTA is widely used to prevent browning. It is well documented that chelates have an anti-browning effect, such as control the trend of enzymatic browning of fruit and vegetable products [21]. As shown in Figure 1(a), the best browning inhibition effect in EDTA group was achieved at the concentration of 0.50 g/L EDTA. In AA treated wines (Figure 1(b)), the best browning inhibition effect was achieved at the concentration of 0.10 g/L AA. Related literature suggested that the anti-browning function of AA can be attributed to the reduction of enzymatically formed o-quinones to their precursor diphenols [21]. However, the anti-browning properties of AA may not be strong when applied to peach wine. After PM treatment, BIR fluctuated with increasing PM concentration, and a concentration of 0.15 g/L was the most effective in inhibiting browning (Fig.1(c)).

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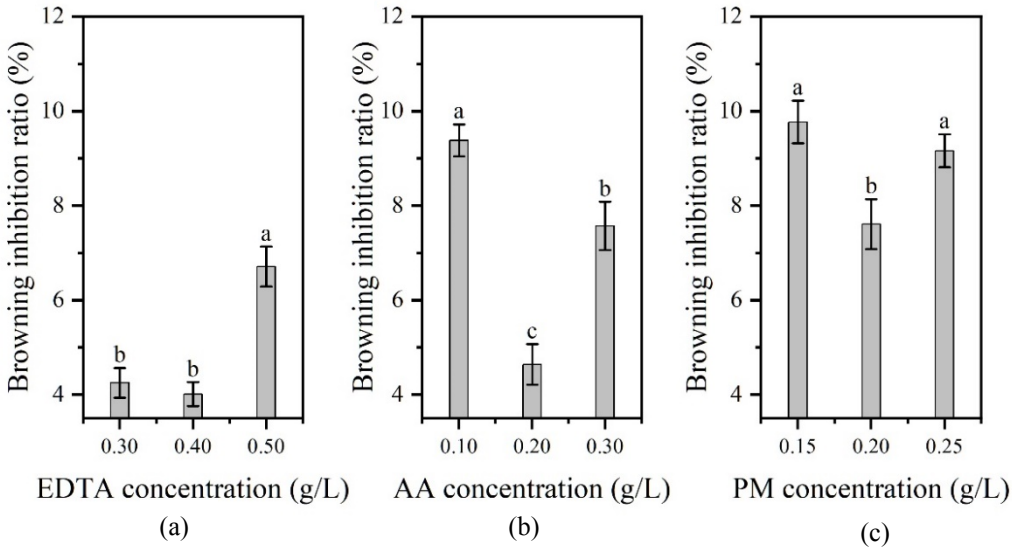

Fig. 1. Effect of EDTA (a), AA (b), and PM (c) on inhibiting browning during wine storage. Different lowercase letters on bar charts represent significant differences at p = 0.05 level

In brief, the addition of different anti-browning agents could give different inhibition effects on the browning of peach wine. However, compared to their inhibiting effect during saccharification, the effect of these three anti-browning agents was not as remarkable during wine storage.

4. CONCLUSION

This experiment was carried out to investigate the browning inhibition during peach fruit pulp saccharification and wine storage by using different anti-browning agents. The results showed that the addition of anti-browning agents with different concentrations had different effects on preventing browning. Using single factor and orthogonal design, EDTA, AA, and PM were investigated for their effectiveness in preventing browning during the saccharification of peach fruit pulp and peach wine storage. In the single factor experiment, PM had the best anti-browning effect (BIR=57%), followed by AA (BIR=47%). Considering that the addition amount of EDTA should not be too high, the anti-browning effect of EDTA was the least obvious in comparison (BIR=43%). It was presented that the combined treatment of 0.40 g/L EDTA, 0.20 g/L AA, and 0.10 g/L PM could have the best inhibitory effect (BIR=57.34%) during syrup saccharification, while the browning inhibition effects of EDTA, AA, and PM during wine storage were not remarkable, all below 10%.

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6. CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts and declare the absence of conflicting interests with the funders.

7. AUTHORS' CONTRIBUTIONS

Xueyuan Han: Conceptualisation, methodology, writing-original draft and funding acquisition; **Yuxin Liu**: Formal analysis, investigation and writing-original draft; **Fangfang He**: Methodology, formal analysis and interpretation of the data; **Jiandi Zhou, Lingkang Chen**: Supervision, writing- review and validation; **Guochang Sun, Jian Sun**: Supervision, resources and writing- review.

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