Research Article

Dynamic Changes of Active Components in Greengage *(Prunus mume)* Wine During Fermentation

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Abstract
Greengage wine is abundant in functional active ingredients, such as phenolics, which interact with proteins to induce turbidity. Therefore, understanding the dynamic changes during fermentation is essential for controlling the quality of greengage wine. In this study, the production process of four different greengage wines was designed by cross-fermentation of two yeasts and two enzyme preparations. The results indicated that variations in alcohol content, reducing sugars, pH, and soluble solids were primarily associated with the selected fermentation microorganisms. However, no significant differences were observed among the four processes. Notably, protein content varied significantly, reaching up to 142.82 ± 20.90 mg·L^{-1}. The total phenol content exhibited a downward trend and ultimately stabilized at approximately 388.92 ± 2.39 mg/L. Conversely, total flavonoid content initially increased before experiencing a slight decline, thus indicating that the fermentation process had a substantial impact on its levels. Tannin content remained relatively stable throughout fermentation. Analysis of monomeric phenols revealed that greengage wine contained 14 common monomeric phenols, with chlorogenic acid being present at the highest concentration. Antioxidant activity in greengage wine continued to rise during the early stage of fermentation, peaking on day six before subsequently declining thereafter. These findings provide valuable insights for the further development of greengage wine.

Keywords: Active components, Dynamic changes, Greengage wine, Phenolics, Turbidity

1 Introduction

In contemporary society, low-alcohol, healthy-oriented and trendy fruit wines have gained popularity among young consumers [1]. Fruit wine is a good source of phenolic compounds in beverages [2], [3] Greengage, a variety of *Prunus mume*, is extensively cultivated in Northern Thailand, China, Japan, and Australia [4]. It contains a diverse array of nutrients, including various organic acids, sugars, phenolics, vitamins and minerals [5]. Greengage is commonly processed into juice, fruit vinegar, fruit wine, jelly, preserved fruit or health food [6] primarily due to its high content of acidic compounds, such as amygdalin and relatively low sugar levels. Consequently, it has a sour and astringent taste when consumed directly [7].

Greengage wine is characterized by its refreshing taste, ease of consumption, and relatively affordable price, which contributes to its promising development prospects in the market [8]. It exhibits effects such as

refreshing properties, anti-fatigue benefits, anti-tumor activity, lipid-lowering effects, antibacterial properties, and memory enhancement [9]. The quality of greengage wine is closely associated with its active ingredients, among which phenolic compounds represent a significant component. These compounds not only exhibit strong antioxidant activity but also demonstrate effects such as free radical scavenging, immune enhancement and aging delay. [10].

Greengage contain many kinds of organic acids in extremely high content [11]. According to the research of Lin *et al*., citric acid is the organic acid with the highest content in greengage, followed by malic acid and oxalic acid [12]. In addition, there are tartaric acid, succinic acid, acetic acid, lactic acid, etc., [13]. Among the raw plum components, epicatechin, neochlorogenic acid, and proanthocyanidins are the most abundant free phenols [14], [15]. Chlorogenic acid and neochlorogenic acid are the main antioxidant compounds in plums [16]. Besides, there are many kinds of volatile substances in greengage. Jiang Wei *et al*., found that the phenolic substances in greengage were at a high level. A total of 81 volatile substances were detected in the eight greengage varieties studied. The main aldehydes were n-hexanal and 2-hexenal, and the main ester was butyl acetate [17].

Following fermentation, greengage wine exhibits an increased concentration of volatile components. Concurrently, microorganisms synthesize bioactive compounds and unique flavor compounds during their metabolic processes [18]. This leads to fermented greengage wine not only retaining most of the nutrients present in greengages but also incorporating additional nutrients generated during fermentation [19]. Xin *et al*., investigated the influence of carbon source, inoculum size, pH value, and temperature on amino acid content in greengage wine. The results indicated that total amino acids and essential amino acids increased with a higher glucose ratio, lower yeast inoculation levels, elevated temperatures and increased initial pH [20]. The effect of fermentation time on active substances during the brewing process of blueberry fruit wine was examined using HPLC [21]. Non-yeast species were co-fermented with brewing yeast and found that this approach enhanced the concentration of volatile organic compounds in apple cider, enriched its aromatic profile, balanced acidity, and improved overall quality [22]. Another study revealed that over 40 distinct phenolic compounds were identified in wines produced from white bilberry fermented with eight different non-yeast strains; notably, concentrations of phenolic acids, flavonol glycosides, and flavan-3-ols significantly increased following fermentation [23].

However, current research provides limited information on the dynamic changes of active ingredients, such as phenolic compounds, during the fermentation of greengage wine. Additionally, significant variations exist in the fermentation processes employed by different wine production companies and are reported in various literature sources. Therefore, we conducted an experiment involving greengage wine fermented through four distinct processes utilizing cross-fermentation with two yeast strains and enzyme preparations. The content of various physical and chemical components, along with phenolic substances and their antioxidant activity throughout the fermentation process, was analyzed to compare the dynamic changes of active ingredients across different greengage wine fermentation methods. The objective is to investigate both the fermentation process and the dynamic alterations of active ingredients during this period, identify characteristic phenols in greengage wine, and provide a theoretical foundation for subsequent basic research on the post-turbidity mechanism associated with greengage wine.

2 Materials and Methods

2.1 *Raw materials and chemicals*

The raw materials used in the experiment were fresh greengage from Sichuan, China. SY yeast and K1 yeast were provided by Sichuan Yibin Wuliangye Xianlin Ecological Wine Co., Ltd. Sugar, pectinase, cellulase, potassium citrate, potassium bicarbonate, baking soda, citric acid, potassium metabisulfite were all food grade and produced in China, and the other reagents used were of analytical grade and produced by Shanghai Macklin Biochemical Co., Ltd.

2.2 *Main solution preparation*

Preparing gallic acid stock solution: A 200 $mg \cdot L^{-1}$ of gallic acid stock solution was prepared by dissolving 20.0 mg of gallic acid standards in a small amount of distilled water in a beaker, bringing the volume up to 100 mL, and storing it at 4 ℃ away from light for future use.

Preparing rutin stock solution: A 200 $mg \cdot L^{-1}$ of rutin stock solution was prepared by dissolving 20.0 mg of rutin standards in a beaker with a small amount of methanol, followed by dilution with distilled water to

a final volume of 100 mL. The solution was then stored at 4 ℃ away from light for future use.

Preparing tannic acid standard stock solution: A 1 $g \cdot L^{-1}$ of tannic acid standard stock solution was prepared by dissolving 100.0 mg of tannic acid standards in a small amount of distilled water in a beaker, adjusting the volume to 100 mL, and storing it at 4 ℃ away from light for future use.

Preparing vitamin C (VC) stock solution: A 0.5 $mg \cdot mL^{-1}$ of VC stock solution was prepared by dissolving 50.0 mg of VC standards in a small amount of distilled water in a beaker, adjusting the volume to 100 mL, and storing it at 4 ℃ away from light for future use.

2.3 *Fermentation process route*

Four distinct fermentation processes resulted in the production of four types of greengage wines, as illustrated in Figure 1.

Pretreatment: The greengage that is 90% ripe and free of pests and diseases should be carefully selected. After cleaning with water and removing the pits, greengage was put in a juicer and broken to get the puree. An appropriate amount of VC solution should also be added to prevent browning. The puree was transferred to a centrifuge tube and subjected to centrifugation at 3000 rpm for 10 min in order to obtain juice. Potassium metabisulfite, with its antioxidant and color-protective properties, as well as its inhibitory effects on undesirable microorganisms, was added to the juice at a concentration of 120 mg/L. The mixture was thoroughly stirred and allowed to stand for 0.5 hours.

Pasteurization: The juice was placed in a water bath with a constant temperature of 60 ℃ for a duration of 30 minutes.

Enzymatic Hydrolysis: 0.12% (w/w) of composite enzyme 1 (pectinase: cellulase = 1:1) or 0.01% (w/w) of composite enzyme 2 (pectinase: protease = 5:1) was added to the juice.

Yeast Inoculation: Yeast was introduced into the juice in a 1:20 (g:mL) ratio within a water bath maintained at 38 °C for 30 min. In a sterile environment, the juice was transferred to sterilized 1 L Erlenmeyer flasks, ensuring that the volume of fermentation liquid in each flask did not exceed 70%. Subsequently, the juice was inoculated with either 0.5% Angel yeast SY (incubated at 28 °C) or 0.2% K1 yeast (incubated at 22 °C).

Fermentation: The triangular bottles containing the fermentation liquid were transferred to a constant temperature shaker for fermentation for 12 days.

Clarification: On days 0, 1, 3, 6, 9, and 12, 35 mL of fermentation broth was taken out under the ultra-clean bench, and then transferred to centrifuge tubes for centrifugation at 10,000 rpm for 30 min. Subsequently, it was filtered using a 0.22 μm nylon membrane and bottled, and stored at 4 °C in the dark for future use.

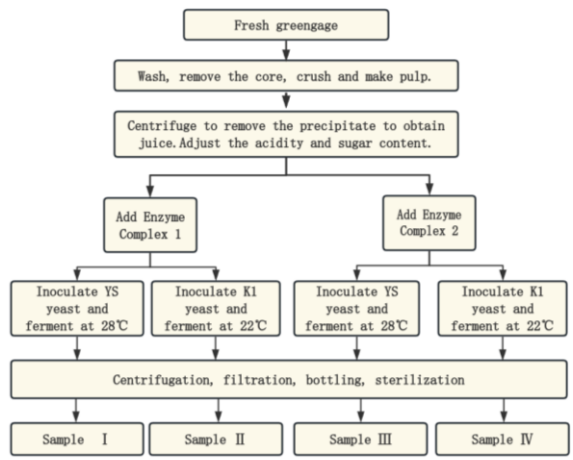

Figure 1: Flowchart of the fermentation process of greengage wine.

2.4 *Determination methods of physical and chemical components*

The alcohol content was determined using Gas Chromatography in accordance with the Chinese national standard GB/T15038-2006, "General Analytical Method for Grape and Fruit Wine" [24]. The pH value was measured using a pH meter, soluble solids were assessed with an Abbe refractometer, and protein content was determined by the Kjeldahl method [25]. The total phenol content was quantified using the Folin-phenol method with gallic acid as a marker [26]. Flavonoid content was assessed using rutin as a reference [27]. Tannin content was determined according to the method described by Zhang *et. al.*, [28]. The concentrations of 14 monomeric phenols were analyzed via High Performance Liquid Chromatography (HPLC).

10 mL of the sample was taken out and placed in 50 mL centrifuge tubes, and the pH was adjusted to 7.0, followed by the addition of 10 mL of ethyl acetate to extract the organic phase, and the extraction process

was repeated three times. Subsequently, the pH of the remaining aqueous phase was adjusted to 2.0, and another 10 mL of ethyl acetate was added to extract the organic phase containing acidic phenolic compounds, with this extraction also repeated three times [29]. The combined neutral and acidic phenolic extracts were transferred to a rotary evaporator and evaporated at 35 °C until no mobile phase remained, and then 2.5 mL of methanol were added for re-dissolution, after which it was filtered through a 0.22 μm nylon membrane. The filtrate was collected and analyzed using equipment in accordance with Nan *et al*., method [30], then stored at –80 °C.

2.5 *Antioxidant activity assay*

2.5.1 Determination of DPPH free radical scavenging ability

The determination method for DPPH free radical scavenging ability was adapted from Chen [31] with minor modifications. Prior to analysis, the wine sample was diluted 100-fold. The standard curve is represented by the equation y = 0.0363x + 0.0881, with an R^2 value of 0.9993 and a linear range of 0 to 10 μg·mL^{-1}.

2.5.2 Determination of ABTS free radical scavenging ability

The method for assessing ABTS free radical scavenging ability was adapted from Bu [32] and Zhang [33], with minor modifications. Prior to analysis, the wine sample was diluted 20-fold. The standard curve is represented by the equation y = 15.28x + 0.0101, with an R^2 value of 0.9934 and a linear range of 0 to 50 μg·mL^{-1}.

2.5.3 Determination of Fe^{2+} chelating ability

The method for determining iron ion-reducing ability (FRAP method) was adapted from Yang [34] with minor modifications. Prior to measurement, the wine sample was diluted 20-fold. The standard curve is represented by the equation y = 0.577x + 0.0026, with an R^2 value of 0.9960 and a linear range of 0 to 1.2 mM.

2.6 *Data processing*

All experiments were conducted in triplicate. Graphs were generated using Origin software, and differences among the experimental groups were analyzed employing the Duncan multiple comparison test within IBM SPSS Statistics 20 (p-value < 0.05).

3 Results and Discussion

3.1 *Dynamic changes of physicochemical components during fermentation of four greengage wines*

3.1.1 Alcohol content determination

Figure 2 (a) illustrates the variations in alcohol content during the fermentation of four types of greengage wine. The data indicate that the alcohol content of all four fermentation samples initially increased before gradually stabilizing, with a final alcohol concentration of approximately 9% vol, which aligns with theoretical expectations based on the initial sugar content of the fermentation system. However, different yeast strains exhibit varying capacities and rates for metabolizing sugars and producing alcohol; notably, SY yeast demonstrates a faster fermentation rate than K1 yeast in the early stages. Ultimately, the final alcohol content across all four wines remained at 9% vol.

3.1.2 Reducing sugar content determination

Sugar serves as a crucial carbon source for yeast during the fermentation process. Yeast metabolizes sugar to produce ethanol and carbon dioxide, thereby converting the sugars present in wine into alcohol. Consequently, changes in sugar content can reflect both the rate at which yeast utilizes sugar and the extent of fermentation occurring in greengage wine. Additionally, it is an important indicator for rapidly assessing whether the fermentation process is proceeding normally [35].

Figure 2(b) illustrates that the reducing sugar content in the system remained relatively stable on the first day. However, from day 1 to day 6, significant differences were observed in sugar content changes between the two yeast treatments. K1 yeast initially converted polysaccharides into monosaccharides, leading to an increase in total reducing sugar content before subsequently decomposing them. In contrast, SY yeast directly decomposed the reducing sugars; however, its decomposition efficiency was more rapid during the first two days and slowed down in the subsequent days. After day 0, the sugar content stabilized at approximately zero, indicating that fermentation was complete.

3.1.3 pH measurement

During the fermentation process, pH value significantly influences the composition and activity of the enzyme system within yeast, thereby regulating its metabolic processes [36]. As illustrated in Figure 2(c), the pH of the four wines gradually increased throughout fermentation. Due to elevated environmental pressure, yeast underwent autolysis. This autolysis resulted in the dissolution of numerous alkaline substances, consequently raising the pH [37]. The pH rose from an initial value of 3.50 to 3.74 ± 0.01.

3.1.4 Soluble solids determination results

Figure 2(d) illustrates the changes in soluble solids content during the fermentation process of the wines. As fermentation progressed, the soluble solids content of the fermentation liquid decreased significantly from days 0 to 6, subsequently stabilizing. This decline can be attributed to yeast rapidly adapting to the fermentation environment and consuming substantial amounts of sugar in the early stages of fruit wine fermentation. Additionally, polyphenols may also bind with sugars during this process, further contributing to the reduction in soluble solids content [38].

3.1.5 Protein Content Determination

Figure 2(e) illustrates that protein content initially decreases and then increases during the fermentation process. In the early stages of fermentation, the primary reason for the reduction in protein content is that yeast utilizes proteins as a nitrogen source [39]. In later stages, some high molecular weight proteins combine with other substances to form precipitates. These precipitates accumulate in the fermentation liquid, leading to an apparent increase in measured protein content [40]. However, this observation reflects proteins existing in an insoluble form rather than a true increase in total protein content [41]. The total protein content of fermented wine sample II was the highest, reaching 142.82 ± 20.90 mg/L^{-1}.

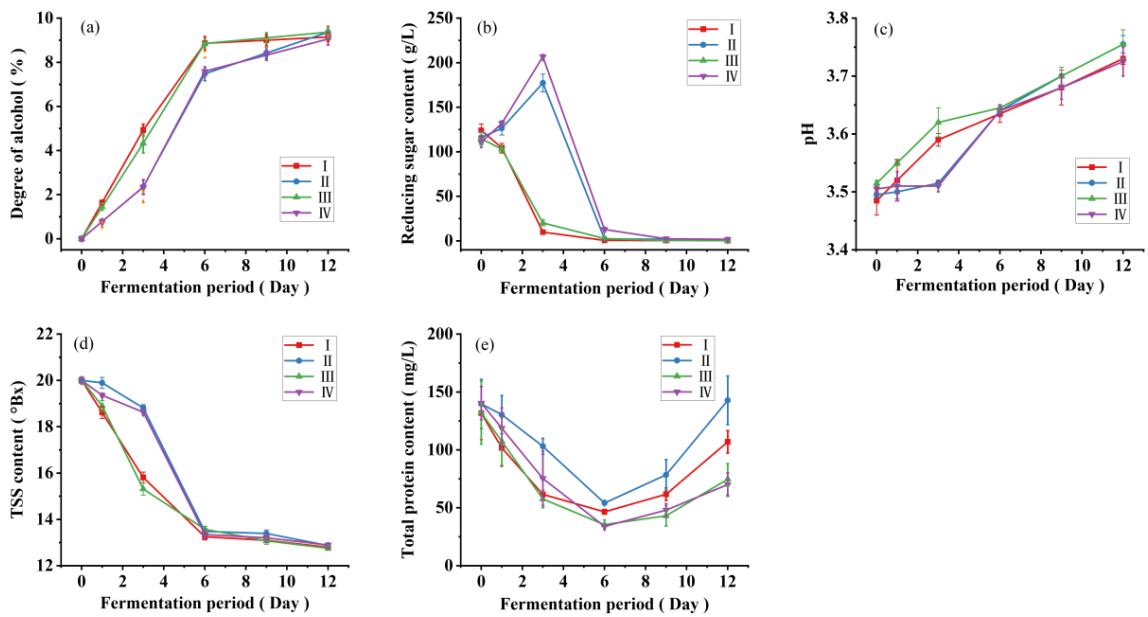

Figure 2: Dynamic changes of (a) alcoholic content, (b) reducing sugar, (c) pH value, (d) TSS, and (e) total protein during the fermentation of four types of greengage wine.

3.2 *Dynamic changes of phenolic compounds during the fermentation of four greengage wines*

The dynamic changes of total phenolic, total flavonoid, and Tannin during the fermentation of four types of greengage wine are shown in Table 1.

3.2.1 Total phenol content determination

Phenolic compounds and their derivatives in greengage wine are the primary antioxidant components, with total phenol content closely linked to wine quality [42]. The data indicate that the total

phenol content of the four wines exhibited an overall downward trend during fermentation. Specifically, the total phenol content decreased from 461.22 mg·L^{-1} to 388.92 mg·L^{-1}, suggesting that the changes in the total phenol content had no direct relationship with the fermentation strain and enzyme preparation. The reduction in phenolic substances may be attributed to oxidation or precipitation during fermentation, as well as interactions with proteins, polysaccharides, and other compounds leading to their loss [43].

3.2.2 Total flavonoid determination

As presented in Table 1, the total flavonoid content in greengage wine was found to be higher than that of total phenols. During the fermentation process, the total flavonoid content generally exhibited a trend of initially increasing followed by a gradual decrease. As yeast fermentation progressed, flavonoids from greengage were released into the wine. Additionally, certain enzymes or microorganisms produced during fermentation may also enhance flavonoid production, leading to an increase in their content during the initial stages [44]. In the late fermentation period, yeast released secondary metabolites such as pyruvic acid and acetaldehyde, which react with flavonoids to form macromolecular derivatives, resulting in a decrease in the concentration of dissolved flavonoids. Additionally, the decomposition and oxidation of polyphenols can also contribute to a reduction in flavonoid content. These factors collectively led to a decline in flavonoid levels during the later stages of fermentation [45]. The observed differences in flavonoid content among the four wine samples suggest variations in the efficacy of complex enzyme 1 and complex enzyme 2 for decomposing large molecules into smaller flavonoids. Ultimately, the total flavonoid content reached 616.95 ± 20.41 mg/L^{-1};

3.2.3 Tannin content determination

Tannin is the main primary contributor to the astringency of fruit wine [46]. The tannins in greengage wine primarily originate from the peel and seeds [47]. As illustrated in Table 1, the tannin content of the four wines exhibited a slight increase during the fermentation process. Among the samples, the tannin content of wine IV exhibited a significant difference before and after fermentation (p-value < 0.05), increasing by 17.94 mg/L^{-1}. This variation may be attributed to the specific fermentation method employed for the fruit wine.

Table 1: Dynamic changes of total phenolic, total flavonoid, and Tannin during the fermentation of four types of greengage wine.

Days	Total phenolic (mg/L^{-1})				Total flavonoid (mg/L^{-1})				Tannin (mg/L^{-1})			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
0	451.94 ±7.07	455.9± 17.12	465.52 ±4.27	471.54 ±7.12	408.06 ±2.08	388.06 ±7.59	417.5± 17.73	405.56 ±20.46	226.27 ±3.34	224.57 ±5.06	223.55 ±3.46	222.69 ±2.53
1	445.7± 6.52	444.26 ±15.18	450.91 ±4.47	462.47 ±6.59	431.57 ±2.84	433.52 ±5.92	506.2± 19.29	479.35 ±18.88	226.59 ±2.94	224.75 ±4.63	224.93 ±2.59	222.34 ±2.97
3	433.23 ±17.15	420.98 ±11.31	421.7± 4.89	444.32 ±5.54	478.61 ±4.37	504.44 ±2.58	683.61 ±22.41	626.94 ±23.73	227.21 ±2.15	225.12 ±3.76	227.68 ±0.85	221.64 ±3.85
6	399.99 ±12.42	397.5± 9.83	407.13 ±3.43	402.87 ±17.3	436.39 ±11.83	436.39 ±11	643.89 ±9.53	601.39 ±11.91	227.3± 3.08	233.88 ±4.93	223.86 ±5.92	226.29 ±5.96
9	390.81 ±9.24	393.64 ±8.46	399.85 ±2.71	394.5± 10.22	412.92 ±7.66	427.08 ±20.29	630.42 ±12.47	551.39 ±19.52	228.02 ±3.59	232.64 ±4.87	228.82 ±5.16	233.46 ±4.86
12	387.63 ±6.52	389.36 ±11.2	392.56 ±1.98	386.13 ±7.13	389.44 ±3.49	417.78 ±19.58	616.94 ±20.41	501.39 ±20.13	228.74 ±4.11	231.4± 4.81	233.79 ±4.4	240.63 ±3.75

3.2.4 Determination of monomeric phenol content

Figure 3 illustrates that 14 colorless monomeric phenols were detected in the greengage wine polyphenols using HPLC, with chlorogenic acid exhibiting the highest concentration. The next most abundant compounds were syringic acid, epicatechin, and catechin; these four polyphenols are characteristic of greengage wine. Chlorogenic acid decreased sharply during fermentation, potentially participating in specific chemical reactions or being utilized by microorganisms [48]. Despite a significant reduction in its content after fermentation, chlorogenic acid remains substantially higher than the other 13 phenolic compounds.

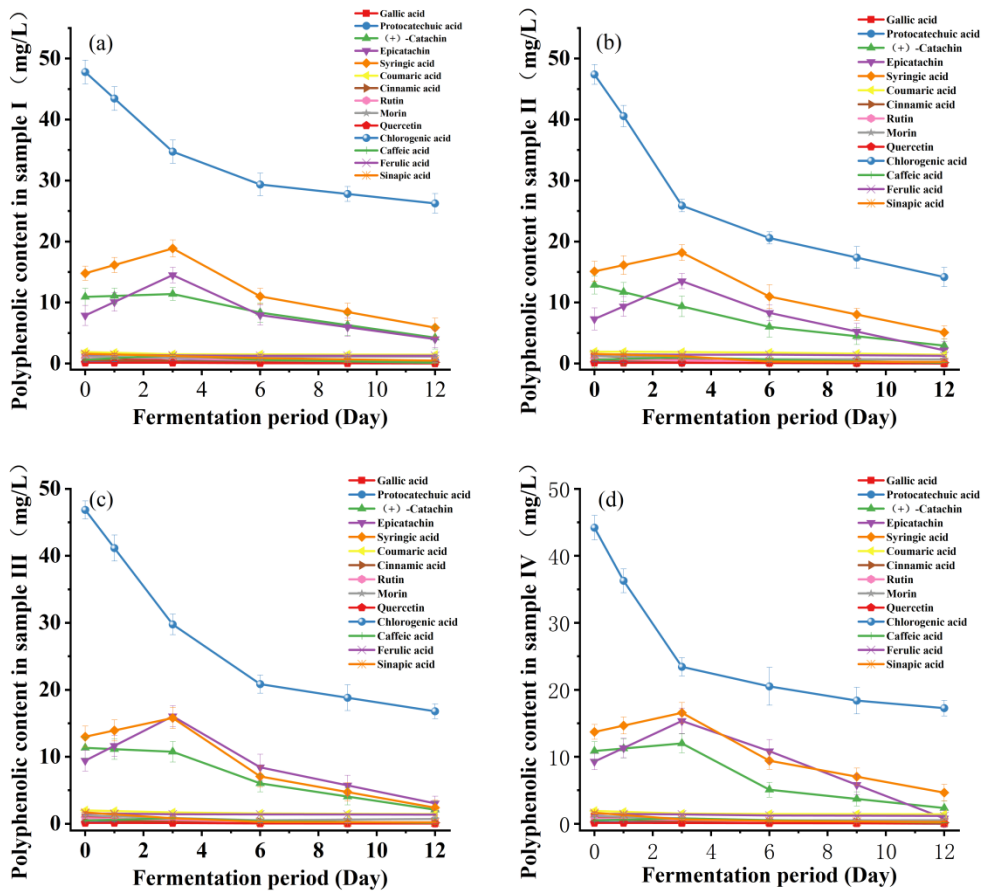

Figure 3: Dynamic changes of monomeric phenol in wine (a) sample I, (b) sample II, (c)sample III, and (d) sample IV.

3.3 *Dynamic changes of antioxidant activity of four greengage wines during fermentation*

Three methods were employed to assess the *in vitro* antioxidant activity of fermented greengage wine [44]. Figure 4 illustrates the dynamic changes in *in vitro* antioxidant activity during the fermentation stage of greengage fruit wine. The antioxidant activity of the four wines increased continuously during the early stages of fermentation, peaking on the sixth day before subsequently declining. This initial increase in antioxidant activity can be attributed to the rapid accumulation of active compounds such as total phenols, flavonoids, and tannins precipitated at this stage. Concurrently, the rise in alcohol content enhanced the solubility of these active substances. In the late stage of fermentation, the concentration of active substances increased slowly, while protein content also rose. The proteins formed complexes with the active substances, thereby reducing their availability in solution and resulting in a decrease in antioxidant activity. The results from the three determination methods indicate that wine II exhibited the strongest antioxidant activity among the four wines. Its final DPPH free radical scavenging capacity was 0.38 ± 0.02 g VCE/L, its ABTS free radical scavenging capacity was 0.79 ± 0.02 g VCE/L, and its Fe^{2+} chelation capacity was 6.87 ± 0.47 mM Fe^{2+}.

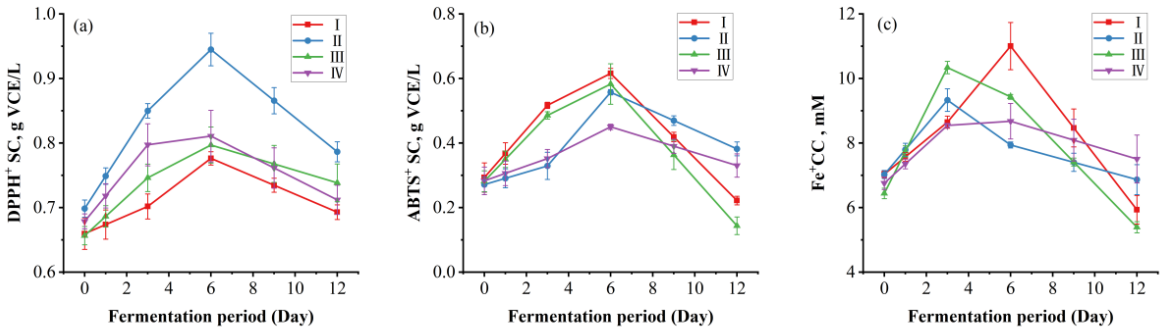

Figure 4: Changes in the antioxidant capacity of four greengage wines during fermentation: (a) DPPH + SC, (b) ABTS + SC, (c) Fe^{2+} CC.

4 Conclusions

In conclusion, this study utilized greengage as a raw material to investigate the dynamic changes of bioactive compounds in greengage wine produced through four different fermentation processes. The results indicated that the alcohol content of greengage wine gradually increased during fermentation, ultimately reaching approximately 9% vol. The pH level also rose steadily to about 3.74, while total sugar and solids exhibited a gradual decline. Total protein content initially decreased before increasing again, with wine sample II exhibiting the highest protein concentration at 142.82 ± 20.90 mg/L^{-1}. Overall, total phenol content demonstrated a downward trend, culminating in a final concentration of 388.92 ± 2.39 mg/L^{-1}. Total flavonoid content showed an initial increase followed by a slight decrease; notably, wine sample III had the highest flavonoid concentration at 616.95 ± 20.41 mg/L^{-1}. Tannin content remained relatively stable throughout fermentation. Analysis of monomeric phenols revealed that chlorogenic acid was present in the highest concentration among the polyphenols identified in greengage wine, followed by syringic acid, epicatechin, and catechin—these four being characteristic phenolic compounds of greengage wine.

The dynamic changes in the *in vitro* antioxidant activity of greengage wine during fermentation indicated that, in the early stages, the antioxidant activity of all four wines consistently increased throughout the process, peaking on the sixth day before subsequently declining. Combining results from the three determination methods revealed that wine II exhibited the strongest antioxidant activity among the samples. In practical production settings for greengage wine, turbidity often develops after storage, which significantly hinders product sales. Notably, wine sample II displayed the most pronounced turbidity among all four samples; this may be attributed to its highest protein content reacting with polyphenols and other compounds.

The post-haze phenomenon that occurs during the transportation and storage of fruit wines directly impacts shelf life and consumer sensory experience, thereby becoming a critical issue affecting product quality. Existing research indicates that the primary cause of this post-haze phenomenon in fruit wines is the interaction between polyphenols and proteins. This study examines the dynamic changes in active components throughout the fermentation process of four types of Qingmei fruit wine and identifies four characteristic phenolic compounds present in these wines, providing valuable insights for further investigation and control of post-haze phenomena.

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Author Contributions

Y.W.: investigation, methodology, writing an original draft; Y.Z.: research design, data analysis; P.C.: literature research, experimental operation; P.C.: conceptualization, data curation; A.T.: conceptualization, investigation, reviewing and editing; X.C.: reviewing and editing, funding acquisition, project administration. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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