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Distillers' Grains Peptides Scavenge Free Radicals and Influence Volatile Compounds of Chi-Aroma Baijiu

Renjie Zhang [1,†], Xi Hu [1,†], You Luo [2], Siqin Zheng [1,3] and Zhenqiang Wu [1,*]

[1] School of Biology and Biological Engineering, South China University of Technology, Guangzhou 510006, China
[2] School of Liquor and Food Engineering, Guizhou University, Guiyang 550025, China; you.luo@uq.edu.au
[3] Pan Asia (Jiangmen) Institute of Biological Engineering and Health, Jiangmen 529080, China
* Correspondence: btzhqwu@scut.edu.cn; Tel.: +86-20-3938-0663
† These authors contributed equally to this work.

Abstract: The production of Chi-aroma Baijiu generates free radicals, which may pose potential health risks. This study aimed to scavenge free radicals in Chi-aroma Baijiu and assess the impact on the composition of volatile compounds (VOCs) by using distillers' grains-derived homologous peptides. Five peptides—Pro-Ala (PA), Pro-Val (PV), Gly-Leu (GL), Asp-Phe (DF), and Pro-Leu (PL)—were isolated from the distillers' grain filtrate. These peptides are present in trace amounts in Chi-aroma Baijiu. By supplementing five peptides to Chi-aroma Baijiu, they could completely scavenge hydroxyl radicals. The rate of scavenging alkoxy radicals ranged from 10.84% to 35.66%. Furthermore, these peptides had an impact on ethyl nonanoate, diethyl acetal, and octanal, which were recognized as characteristic compounds. Ultraviolet spectroscopy simulation revealed that the highest absorption peaks of certain peptides shift when they interact with various esters. Those peptides interact with ethyl acetate and ethyl lactate via hydrogen bonding and Van der Waals forces, demonstrating their influence on VOCs. These findings offer new insights into free radical scavenging, flavor compound regulation, and the valorization of Baijiu by-products in Chi-aroma Baijiu production.

Keywords: distillers' grains peptides; by-products; free radicals scavenging; volatile organic components; chi-aroma Baijiu

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1. Introduction

Baijiu, one of the six globally distilled spirits, has a 2000-year history and a rich cultural heritage in China [1]. The yearly output surpasses 4.6 million kiloliters, generating a market profit of 1560 billion RMB (statistics from the China Alcoholic Drinks Association) [2]. Baijiu mostly features 12 distinct flavors. Chi-aroma Baijiu, also known as Yubingshao, is considered a distinctive Baijiu from the Pearl River Delta area of China. It is prevalent in Southeast Asian nations because its distinctive Chi-aroma is derived from a unique aging process including the infusion of old pork fat [2,3]. After soaking in fat meat, the Baijiu becomes clear and transparent, mellow and smooth in taste, and has a unique Chi flavor [4]. Nevertheless, the fatty meat undergoes significant oxidation while being soaked, leading to a cascade of free radical reactions. Alkoxy radicals are produced by oleic and linoleic acids, while hydroxyl radicals are somewhat produced by unsaturated fatty acids in fatty meat [5]. The oxidation of fatty meat generates free radicals, which are then transferred to Baijiu, potentially posing a risk to human health. Free radicals induce oxidative stress, leading to cellular malfunction [6]. Overproduction of free radicals has a close relationship with increased incidences of cancer [7]. However, previous research has overlooked the scavenging of these free radicals within Chi-aroma Baijiu [5]. Therefore, how to scavenge free radicals in Chi-aroma Baijiu and improve the health of Chi-aroma Baijiu has become an urgent problem to be solved.

Peptides serve as intermediate products of proteolysis and possess protein-like activity [8]. Peptides have been demonstrated to scavenge free radicals, enhancing the body's capacity to mitigate damage [9]. Currently, free radical scavenging capacity is commonly assessed using indicators, including 2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-Azinobis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS), oxygen radical absorbance capacity (ORAC), and superoxide and hydroxyl radicals [10]. Furthermore, peptides, as non-volatile components of Baijiu, can interact with VOCs, enhancing their flavor characteristic [11]. Zhang's research found that endogenous peptides within Chi-aroma promoted or inhibited the volatilization of VOCs [12].

However, peptides are commonly obtained from animals and plants, resulting in significant financial expenses. Consequently, there is an increasing interest in finding inexpensive sources for extracting peptides. Distillers' grain filtrate residues are the largest by-product in the Baijiu production process [13] and contain several proteins and peptides [14]. Previous studies addressed the extraction of proteins from solid distillers using protease hydrolysis. Jiang [15] identified two peptides from Jiupei: Val-Asn-Pro (VNP) and Tyr-Gly-Asp (YGD). They demonstrate an antioxidant impact on the nonenzymatic antioxidant system (GSH, GSSG, and MDA) as well as the enzymatic antioxidant system (SOD, CAT, and GSH-Px). Wu [16] hydrolyzed distilled waste grains of Baijiu and then purified a strong ACE inhibitory peptide (Gln-Gly-Val-Pro), which exhibited possible antihypertensive effects. A significant quantity of peptides exists in the distillers' grain filtrate; Liao [17] identified 32 types of dipeptides and cyclic dipeptides from the distillers' grain, indicating that free radicals preferentially associate with oligopeptides composed of hydrophobic amino acid residues such as Leu, Val, Phe, and Pro, thereby facilitating antioxidant activity. In reality, the distillers' grain filtrate is disposed of straightaway, leading to environmental pollution and inadequate resource utilization, resulting in waste [18]. Furthermore, distillers' grain peptides had anti-inflammatory, antioxidant, and hypotensive effects. Wei [19] hydrolyzes to isolate angiotensin-I-converting enzyme (ACE) inhibitory peptides, which effectively inhibit ACE and have considerable antihypertensive properties. Thus, isolating peptides from wine by-products and reintroducing peptides with antioxidant properties into Baijiu serves as an optimal strategy to improve its functional quality [20] and improve the value and utility of distiller's grains, while also modifying the volatile compound composition. This study aimed to investigate the impact of peptides on volatile compounds as well as scavenge free radicals using distillers' grain filtrate peptides.

2. Materials and Methods

2.1. Experimental Materials

Distillers' grain filtrate and a 3-year-old fat-soaked Chi-aroma Baijiu were provided by Guangdong Jiujiang Distillery, (Guangdong Jiujiang Distillery Co., Ltd., Foshan, China) refrigerated for preservation. Ethanol, acetic acid, formic acid, acetonitrile, ethyl acetate, ethyl lactate (chromatographic grade, Yuanye Bio-Technology Co., Ltd., Shanghai, China), glutathione (GSH), DPPH, ABTS, 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), pyrogallol, potassium persulfate, and ferrous sulfate were purchased from Aladdin Bio-Chem Technology Co., Ltd. (Shanghai, China). Peptides, including PA, PV, GL, DF, and PL (Shanghai Taopu Biotechnology Co., Ltd., Shanghai, China).

2.2. Preparation of Distillers' Grain Filtrate Peptides

The distillers' grain filtrate was centrifuged at 8000 rpm using a high-speed centrifuge for 10 min. Subsequently, the distillers' grain filtrate was filtered through a 0.22 μm filter membrane and passed through an ultrafiltration centrifuge tube with a 1 kDa cutoff to separate components with a molecular weight below 1 kDa. The resulting solution was put onto a column filled with 1–2% volume of dextran gel LH-25 for distillers' grain filtrate, the detection was conducted at an ultraviolet wavelength of 220 nm, and the peptide of each peak was collected multiple times, followed by freeze-drying.

2.3. Preparation of Chi-Aroma Baijiu Peptides

The Chi-aroma Baijiu peptides were prepared using Wu's approach, involving a direct concentration method combined with mass spectrometry to analyze the peptide content in Baijiu [21]. A 1000 mL sample of Chi-aroma Baijiu was concentrated to 10 mL at 45 °C using a rotary evaporator under low vacuum (0.09 MPa). The residue was extracted three times with 30 mL of freshly distilled ethyl acetate, collected repeatedly, and subsequently freeze-dried. The solution was diluted with 1 mL of distilled water and stored for future use.

2.4. Peptide Structure Identification

The peptides were identified by HPLC-Q-TOF-MS/MS (Agilent Technologies, Santa Clara, CA, USA) equipped with a Waters Venusilxbp C_{18} column (4.6 × 250 mm, 300 Å, 5 μm). The identification technique followed the previously reported method with some modifications [22]: a flow rate of 1 mL/min, a column temperature set at 28 °C, and a detection wavelength of 220 nm. Data was collected and analyzed using the Agilent Masshunter B 06.00 software. The peptide concentration was determined using peak area ratios, with GSH serving as the internal standard. The alignment of the database, identification of the peptide structure, and confirmation of the structure were achieved through the use of synthetic standards.

2.5. Antioxidant Activity of Distillers' Grain Filtrate Peptides

The DPPH and ABTS free radical scavenging activity was analyzed by the method described by Luo [23], the oxygen radical absorbance capacity and hydroxyl radical was measured using the procedure described by Jin [24]. The GSH served as the positive control. The final result measured in GSH equivalent, GSH mg/peptide mg. The free radical scavenging rate was calculated using the following formula:

$$\text{Free radical scavenging rate} = [(\text{Control} - \text{Sample})/\text{Control}] * 100\% \quad (1)$$

2.6. Determination of Free Radicals in Chi-Aroma Baijiu

The peptides PA, PV, GL, DF, and PL were dissolved in the Baijiu solution at a concentration of 0.1 g/L. The procedure outlined in our previous method was followed [5]. The electron paramagnetic resonance (EPR) detection parameters were as follows: microwave frequency of 9841.84 MHz, microwave power of 2.00 mW, modulation frequency of 100 kHz, modulation amplitude of 1 G, central field strength of 3506 G, field width of 71 G, resolution of 2048 points, and a total of 3 scans. GSH was used as the positive control to measure the scavenging activity of free radicals, determined by EPR detection of total spin.

$$\text{Free radical scavenging rate} = [(\text{Control} - \text{Sample})/\text{Control}] * 100 \quad (2)$$

2.7. Determination of VOCs in Chi-Aroma Baijiu

The peptides PV, PA, GL, DF, and PL were added into the baijiu samples at a concentration of 0.1 g/L, GSH was used as the control. Subsequently, 2 mL of Baijiu sample was transferred into a headspace bottle, followed by the addition of 3 g of sodium chloride and 100 μL of the internal standard sec-octanol (2.58 g/L in 60% (*v*/*v*) methanol as solvent). The HS-SPME-GC-MS analysis was conducted following the protocol described by Shen [25]. The VOCs were identified using the National Institute of Standards and Technology (NIST) Mass Spectral Library and the Wiley Registry of the Mass Spectral Database.

The relative concentration of each compound was calculated via area normalization. The data of the VOCs was acquired with the formula:

$$C_i = \frac{A_i}{A_j} * C_j \quad (3)$$

where C_i represents the relative concentration of VOCs, A_i represents the peak area of VOCs, A_j represents the peak area of VOCs, and C_j represents the concentration of the internal standard.

2.8. Simulated Interaction between Peptides and Ester Compounds

2.8.1. Ultraviolet Spectroscopy Analysis

The study employed ultraviolet spectroscopy to simulate the interaction between peptides, ethyl acetate, and ethyl lactate. The aim was to analyze the influence of peptides on volatile organic compounds (VOCs) in Chi-aroma Baijiu. A solution of peptide with a concentration of 0.1 g/L was prepared. Ethyl lactate and ethyl acetate were added to the solution in a specified concentration ratio of 1:5 [26]. A 45% ethanol solution served as the blank, and baseline correction was conducted using the ultraviolet spectrophotometer. Subsequently, the prepared peptide solution, the mixed peptide, ethyl acetate, and ethyl lactate solution were scanned at full wavelengths within the range of 190–260 nm, with each group scanned three times. The interaction between peptides and esters was determined by monitoring changes in the maximum absorption peak and wavelength.

2.8.2. Thermodynamic Data Determination

Based on the method of Zhang [27] with slight modifications, peptide dissolved in ester solutions at a concentration ratio of 1:5. These solutions were each subjected to reactions at temperatures of 25 °C, 35 °C, and 45 °C, respectively. The mixed solution was scanned three times at a full wavelength range of 190 nm to 260 nm. The thermodynamic equilibrium constant (K), Gibbs free energy (ΔG°), enthalpy (ΔH°), and entropy (ΔS°) values were calculated using the following formulas:

$$\ln \frac{\Delta A}{A_0} = n\ln C + \ln K \quad (4)$$

$$\ln K = \frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (5)$$

$$\Delta G^0 = -RT\ln K = \Delta H^0 - T\Delta S^0 \quad (6)$$

where ΔA is the change in absorption value of the mixed solution before and after adding esters, A_0 is the absorption value of the peptide, and R is the gas constant.

2.9. Statistical Analysis

The experiments were performed in triplicate, and the data were presented as mean ± standard deviation (SD). Origin 2023 software (OriginLab, Northampton, MA, USA) was used for plotting, SIMCA 14.1 for partial least squares discriminant analysis, and SPSS software (version 19.0; IBM Corp., Armonk, NY, USA) for discriminant analysis. Additionally, Spearman's correlation analysis was performed. Volatile compounds were identified using Qualitative Navigator B.08.00. Duncan's multiple range test was conducted, with significant differences ($p < 0.05$) indicated using different lowercase letters (a, b, c, d, e).

3. Results and Discussion

3.1. Amino Acid Composition Analysis

Peptide identification typically involves mass spectrometry and database alignment to determine the composition and arrangement of an amino acid [28]. Figure 1 illustrates the secondary mass spectra of five peptides obtained from the HPLC-Q-TOF-MS/MS analysis. The secondary mass spectra revealed peptide ion fragments with a mass-to-charge ratio (M/Z) of 187.09. It was determined that the ion fragments at M/Z = 116.07 could be Pro+H+ and those at M/Z = 187.09 could be Pro-Ala+H+. This observation indicated the presence of the amino acid sequence prolyl-alanine (Pro-Ala or PA), determined as shown in Figure 1A.

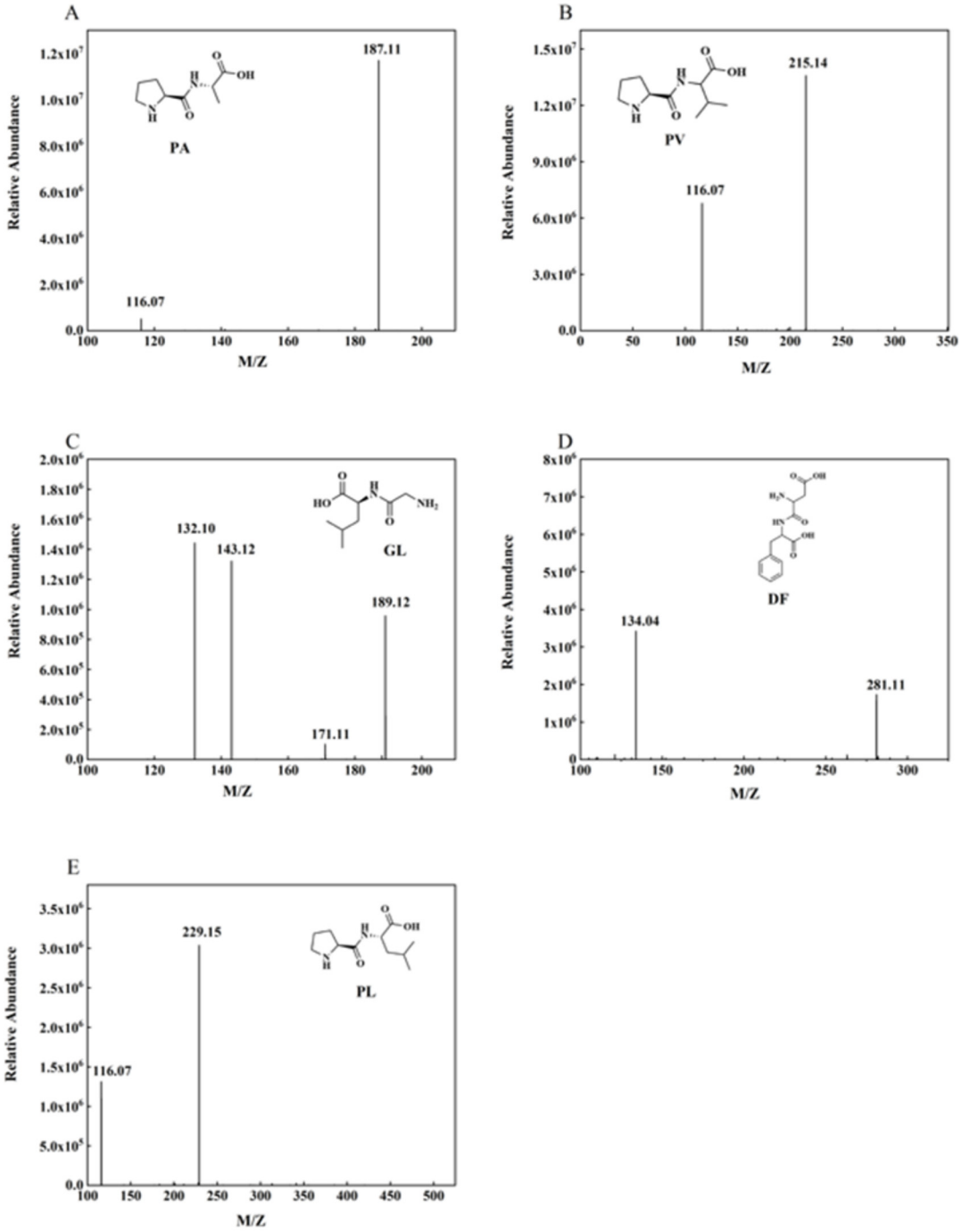

Figure 1. MS/MS spectrum of five peptides fractions. (**A**–**E**) represents the peptides Pro-Ala (PA), Pro-Val (PV), Gly-Leu (GL), Asp-Phe (DF), and Pro-Leu (PL) MS/MS spectrum respectively.

Further, the precursor fragments at M/Z = 215.14, M/Z = 189.12, M/Z = 281.11, and M/Z = 229.15 were identified as prolyl-valine (Pro-Val or PV), glycyl-leucine (Gly-Leu or GL), aspartyl-phenylalanine (Asp-Phe or DF), and proline-leucine (Pro-Leu or PL), respectively, using the same analytical approach (Figure 1B–E). The five peptide standards were analyzed using HPLC-Q-TOF-MS/MS under the same conditions. The retention durations of the precursor fragments matched the previously observed ion fragment cleavage peaks. In a previous study, Wei [19] utilized the UPLC-Q-FTP-MS technique to forecast the structure of the peptide. The ion fragments detected had a mass-to-charge ratio (M/Z) of 272.1654, with M/Z = 175.1185 corresponding to the y_1 ion, perhaps indicating the presence of Arginine with an additional hydrogen ion (H+). The M/Z value of 98.0343 is

believed to correspond to the b_1 ion, whereas the parent ion with an M/Z value of 272.1654 is identified as the peptide PR.

3.2. Concentration of the Five Peptides in Distillers' Grain Filtrate and Chi-Aroma Baijiu

A concentration of the five peptides was also detected in Chi-aroma Baijiu. These peptides were then compared with distillers' grain filtrate, as illustrated in Figure 2. The results indicated that distillers' grain filtrate had a comparatively high peptide content, whereas Baijiu had considerably lower levels of these peptides. Based on the continuous cooking and fermentation procedures involved in the production, it is reasonable to assume that the peptides in question come from rice [16,29]. This process causes gluten to be broken down into peptides, amino acids, and other nitrogen-containing compounds and small molecules [26]. A minor proportion of these peptides, in conjunction with ethanol and water, was distilled into the Baijiu, with further dilution occurring during the Baijiu blending process. The majority of intact proteins from the raw materials remain in the fermentation residue [30]. Du [31] previously measured the levels of 39 short-chain peptides in Baijiu, ranging in concentration from 0.16 to 279.33 μg/L. Zhang [32] reported that the levels of lichenysin found in Chinese liquor varied between 0.01 and 111.74 μg/L.

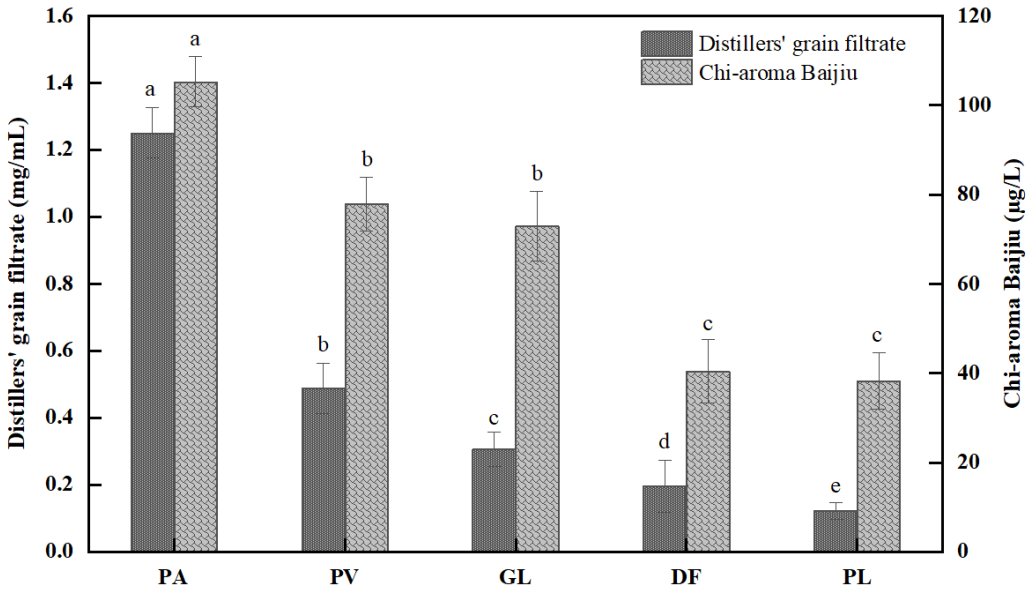

Figure 2. The content of peptides Pro-Ala (PA), Pro-Val (PV), Gly-Leu (GL), Asp-Phe (DF), and Pro-Leu (PL) in distillers' grain filtrate and Chi-aroma Baijiu. Different letters represent statistically different values at $p < 0.05$.

3.3. Antioxidant Capacity of Five Peptides

The five peptides exhibited different degrees of in vitro antioxidant activity, as evaluated by chemical antioxidant tests (Figure 3). The scavenging ability of peptides PA, PV, and PL was superior to that of GHS in DPPH and ABTS free radical experiments. The scavenging effect of peptide PV was the most effective. The scavenging ability of peptides PL and GL was approximately 1.2 times greater than that of GSH in the superoxide free radical experiment. Similarly, the scavenging ability of five peptides was superior to that of GSH in the hydroxyl free radical scavenging experiment. As it turns out, all five peptides demonstrated antioxidant capability.

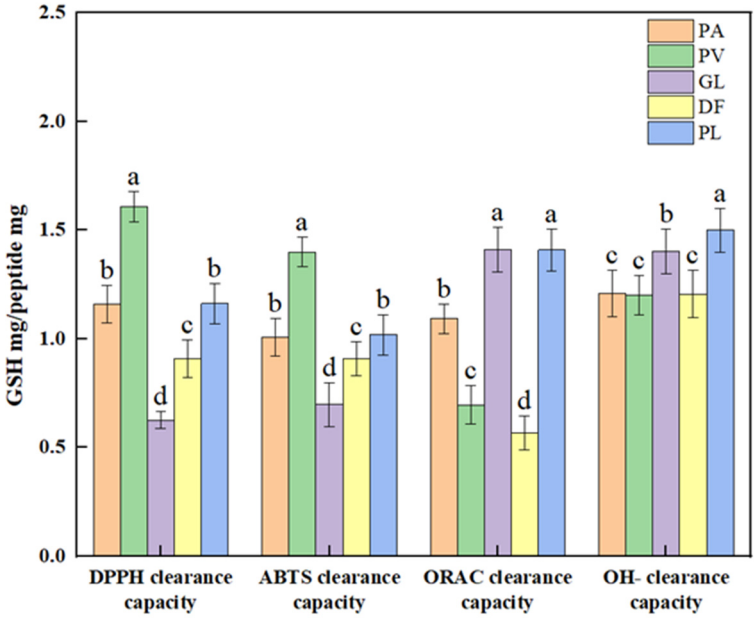

Figure 3. Free radical scavenging capacity of five peptides Pro-Ala (PA), Pro-Val (PV), Gly-Leu (GL), Asp-Phe (DF), and Pro-Leu (PL). Different letters represent statistically different values at $p < 0.05$.

The free radical scavenging capability of peptides has been shown to be considerably influenced by their amino acid content, sequence organization, and molecular weight [33,34]. In in vitro chemical assays, the antioxidant capacity of peptides may be associated with the presence of aromatic and hydrophobic amino acids at either the C- or N-terminus of the peptide [35]. Antioxidant oligopeptides typically consist of 0–20 amino acids. Their sequences, which include Pro, Gly, Ala, and Val, have the potential to exhibit antioxidant action. Oligopeptides containing aromatic rings (Tyr, Trp, Phe, and His) and sulfur groups (Met and Cys) directly neutralize free radicals [36]. Among the five peptides, DF contained aromatic amino acids such as phenylalanine, whereas PV, PA, GL, and PL contained hydrophobic amino acids such as tryptophan, phenylalanine, valine, leucine, and proline. This provides a potential approach to applying functional components from distillers' grain filtrate for by-product utilization.

3.4. Free Radical Scavenging Activity upon the Addition of Five Peptides in Chi-Aroma Baijiu

Following a three-year soaking period and three months of aging, DMPO was employed as a trap to capture free radicals. These free radicals combine with $^{\alpha}$C of $^{\alpha}$C=N groups on DMPO molecules to form free radical adducts [37,38]. The identification of free radical types was contingent upon the g value and ultrafine cleavage of these adducts. Two types of free radical compounds were identified: hydroxyl radical adducts (DMPO-OH, g = 2.0038, ^{a}N = 15.81 G, ^{a}H = 15.6 G) and alkyl radical adducts (DMPO-R, g = 2.0038, ^{a}N = 15.88 G, ^{a}H = 22.5 G), as shown in Figure 4A. Jiang [39] also identified the presence of free radicals in red wine using electron paramagnetic resonance (EPR). Upon the addition of a 0.1 mg/mL peptide solution, the total spin of free radicals in Chi-aroma Baijiu was changed (Table 1). Depending on the total amount of spin, the hydroxyl radical clearance rate was almost 100%. The alkoxy radical scavenging rates of peptides PV, PA, GL, DF, PL, and GSH were 10.84%, 11.54%, 36.37%, 27.27%, 35.66%, and 34.27%, respectively, as shown in Figure 4B.

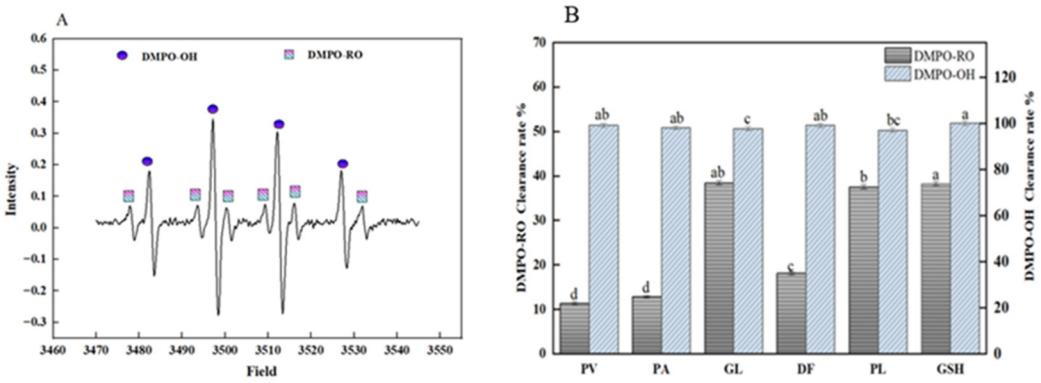

Figure 4. The changes of free radicals in Chi-aroma Baijiu. (**A**) EPR spectrum of free radical, (**B**) Pro-Ala (PA), Pro-Val (PV), Gly-Leu (GL), Asp-Phe (DF), Pro-Leu (PL) free radical scavenging rate in Chi-aroma Baijiu. Different letters represent statistically different values at $p < 0.05$.

Table 1. Total free radical spin in Chi-aroma Baijiu after peptide supplementation.

Baijiu Sample	DMPO-RO (Spins)	DMPO-OH (Spins)
CK	2.86×10^{15}	1.19×10^{15}
PV	2.55×10^{15}	1.07×10^{13}
PA	2.53×10^{15}	2.38×10^{13}
GL	1.82×10^{15}	2.79×10^{13}
DF	2.08×10^{15}	1.13×10^{13}
PL	1.84×10^{15}	3.57×10^{13}

Where CK indicates a sample of Chi-aroma Baijiu without peptides.

The total quenching of hydroxyl radicals may be due to peptides containing acidic functional groups that react with OH- to yield H_2O [40]. Hydroxyl radicals can target both the amino acid side chains and the peptide chain backbone, resulting in the generation of several peptide radical derivatives [41]. The sulfhydryl (-SH) group of GSH provides hydrogen and free-radical-pairing electrons, thereby contributing to a strong free radical scavenging effect [33]. Moreover, they can react with peptide side chains containing amino acid residues such as valine (Val) and leucine (Leu) to form 3- and 4-hydroperoxyvaline or 4- and 5-hydroperoxyleucine, thus leading to the complete quenching of hydroxyl radicals in Baijiu [42]. Therefore, the five peptides obtained from distillers' grain filtrate exhibit potential as effective radical scavengers.

3.5. Changes in VOCs upon the Addition of Five Peptides in Chi-Aroma Baijiu

3.5.1. Changes in VOCs Content

The overall flavor of Baijiu can be assessed by analyzing VOCs. In Chi-aroma Baijiu, 37 major VOCs were identified and quantified, as illustrated in Supplementary Table S1. At five peptide concentrations of 0.1 g/L (peptides as additives conform to Chinese National Standards GB 2760-2014), the concentrations of VOCs were changed (Figure 5A). And PV, PA, GL, and PL demonstrated an inhibitory effect on total acid levels, decreasing the content by 8% to 19.8%. Conversely, DF and GSH promoted the total acid content, increasing levels by 3.1% to 12.5%. The addition of PV, PA, GL, and GSH resulted in a reduction in total esters by 2.7% to 8.6%, while the addition of DF and PL led to an increase in total esters by 2.7% to 3.8% (Figure 5B). Zhang [12] found that peptides in Baijiu would affect the content of total acid and total alcohol; the content of peptides was negatively correlated with total ester and total alcohol and positively correlated with total acid. Scalone [43] verified the effect of whey protein hydrolysate on the generation of flavor volatiles; oligopeptides derived from whey protein hydrolysate played a major role in the generation of pyrazines. This may be due to the different amino acid types and arrangements of peptides [12], so peptides have different effects on the content of VOCs. And VOCs and macromolecular compounds

can interact, such as peptides and proteins. These findings provide potential insights into the application of peptides in regulating the flavor of Baijiu. It is essential to explore the sensory changes in Baijiu resulting from the influence of peptides on characteristic volatile substances through sensory evaluation in future studies.

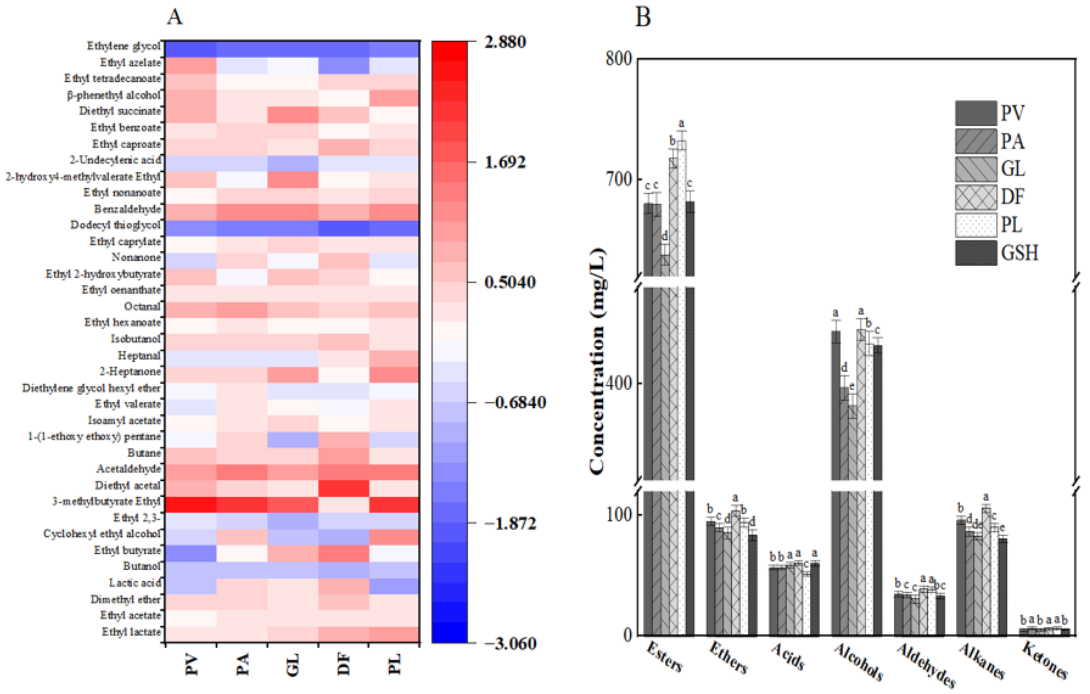

Figure 5. The changes of VOCs in Chi-aroma Baijiu after adding peptides. (**A**) Heat map of VOCs, (**B**) Concentration of VOCs. Different letters represent statistically different values at $p < 0.05$.

3.5.2. Changes of VOC Characteristics

In order to identify the characteristic substances that allow peptides to exert a significant influence on the VOCs in Baijiu, we used partial least squares analysis (PLS-DA) to assess the influence of different peptides on Baijiu's VOCs [44], presented in Figure 6A,B. The results demonstrated clear distinctions among the Baijiu samples, indicating variations in volatile components resulting from different peptide additions. Baijiu samples containing peptides DF, PL, PV, and PA were positioned closer to each other, suggesting that these four peptides exert a similar effect on Baijiu's VOCs. In contrast, peptides GSH and GL were distinctly separated. Hierarchical cluster analysis (HCA) was also conducted. The HCA results were similar to those of the PLS-DA; all samples were separated into two groups (Figure 6C). The above results indicate that the Baijiu presented different volatile flavor compounds after supplementing peptides.

Additionally, based on the analysis model, we assessed the importance of each VOC using VIP values [44], identifying 15 VOCs with VIP > 1. Spearman correlation analysis yielded correlation coefficients between the addition of different peptides and volatile compounds, selecting 19 VOCs with $p < 0.05$ (Supplementary Table S2), of which 14 compounds exhibited a positive correlation, while five exhibited a negative correlation. Three VOCs with positive correlations, VIP values > 1, and $p < 0.05$ were screened out: ethyl nonanoate, diethyl acetal, and octanal. These three substances were identified as the characteristic substances of the influence of peptides on volatile components. Yue [45] used the criteria of VIP > 1 and $p < 0.05$ to identify the components that significantly contribute to distinguishing the three varieties of Hehong tea. Yang's [46] research revealed that 26 volatile compounds, which were analyzed using PLS-DA, had a substantial contribution to the flavor of marinated tofu. These chemicals were determined to have a statistical significance ($p < 0.05$) and VIP > 1.

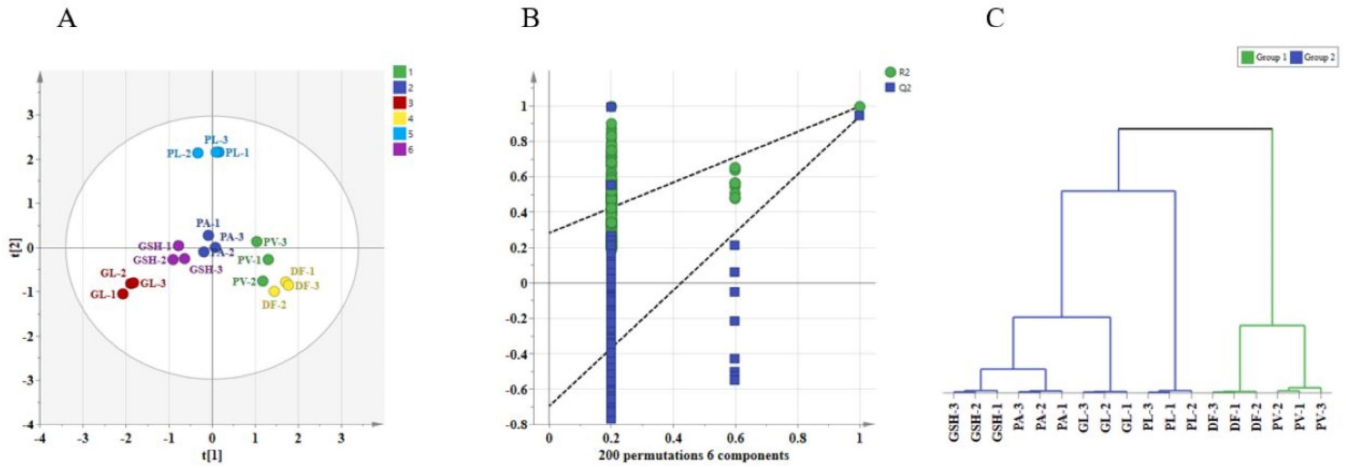

Figure 6. PLS-DA analysis. (**A**) Score graph, (**B**) Model validation, (**C**) Hierarchical clustering analysis.

3.6. Simulation of the Interaction between Five Peptides and Ester Compounds in Chi-Aroma Baijiu

To investigate the interaction between peptides and VOCs, ethyl lactate and ethyl acetate were selected, which were key volatile components in Chi-aroma Baijiu (Figure 7A–F). A UV spectrophotometer was used to assess alterations in absorption. Peptide PA exhibited a peak absorption at 213 nm, reaching a maximum absorbance value of 0.538.

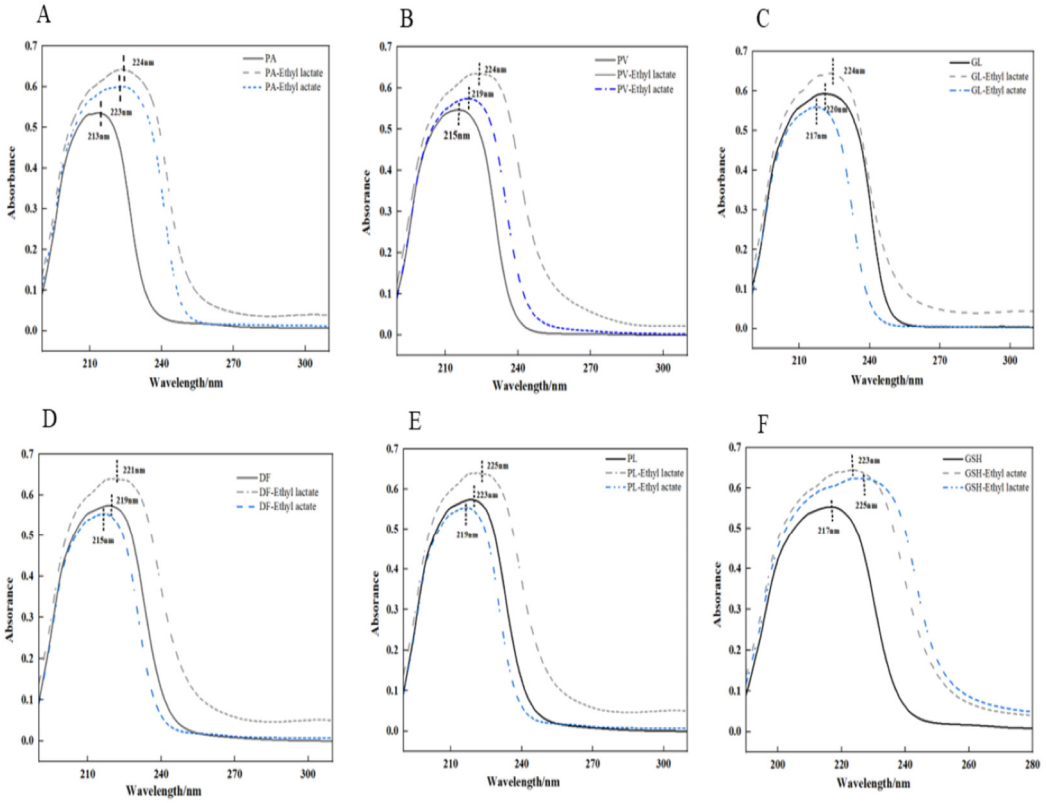

Figure 7. Ultraviolet absorption spectrum of mixed peptides and esters. (**A**–**F**) represents the peptides Pro-Ala (PA), Pro-Val (PV), Gly-Leu (GL), Asp-Phe (DF), Pro-Leu (PL), and glutathione (GSH) mixed with ethyl acetate and ethyl lactate, respectively.

After the introduction of ethyl acetate, the absorption peak moved to a wavelength of 224 nm, and the absorbance value was measured to be 0.642. The addition of ethyl lactate to PA caused a shift in the absorption peak to 223 nm, reaching a maximum absorbance

value of 0.601. As for the peptides PV, GL, DF, PL, and GSH, it was observed that their maximum absorption peaks shifted to approximately 224 nm following their combination with ethyl acetate. All peptides showed a rise in absorbance values, except for PL.

Upon mixing with ethyl lactate, the maximum absorption peaks shifted to 219 nm, 217 nm, 215 nm, 223 nm, and 223 nm, respectively. These findings indicate that the peptides and the two esters underwent polymerization, resulting in the creation of novel molecules, according to Luang Boer's rule [47].The peptide bonds exhibit their highest level of absorption at around 210 nm [48], but the chromogenic groups and the amino acid residues of peptides can undergo alterations in various situations [49]. It is possible to ascertain the reaction between a small molecule drug and a peptide by analyzing the spectrum changes seen prior to and following their combination [50].

3.7. Interaction Mechanism between Peptides and Esters

In order to gain a deeper understanding of how peptide–esters and peptide–alcohol complexes interact in simulated solutions, it is imperative to investigate the interaction between peptides and esters or alcohols by determining the equilibrium constant and thermodynamic parameters [51]. As illustrated in Table 2, the binding abilities of different peptides with ethyl acetate and ethyl lactate vary. The binding ability between the peptide and ethyl acetate was as follows: PA > PV > GSH > DF > PL > GL. Similarly, the binding ability between the peptide and ethyl lactate was as follows: GSH > PA > GL > PL > PV > DF. This indicates that the peptide PA exhibited the strongest binding affinity for ethyl acetate, whereas the peptide GL exhibited the weakest binding affinity for ethyl acetate. The peptide GSH exhibited the strongest binding affinity for ethyl lactate, and the peptide DF exhibited the weakest binding affinity for ethyl lactate.

Table 2. Thermodynamic parameters of the interaction between peptides and esters.

	PA	PV	GL	DF	PL	GSH
K_1 (mol/L)	820.73	783.37	136.03	230.44	180.71	250.38
K_2 (mol/L)	621.37	237.25	303.89	178.39	284.24	1087.33
$\Delta G_1{}^0$ (KJ/mol)	−16.62	−16.51	−12.17	−13.48	−12.87	−13.68
$\Delta G_2{}^0$ (KJ/mol)	−15.93	−13.54	−14.15	−12.84	−13.99	−17.32
$\Delta H_1{}^0$ (KJ/mol)	−35.52	−24.02	5.36	5.85	−25.22	19.8
$\Delta H_2{}^0$ (KJ/mol)	−26.67	−48.75	−24.84	−26.01	−32.03	6.35
$\Delta S_1{}^0$ (J/mol)	−63.42	−7.51	58.8	64.86	−41.44	112.3
$\Delta S_2{}^0$ (J/mol)	−36.04	−35.21	−35.87	−44.16	−60.04	79.42

Where K_1, $\Delta G_1{}^0$, $\Delta H_1{}^0$, $\Delta S_1{}^0$ represents the thermodynamic parameters of peptide and ethyl acetate, K_2, $\Delta G_2{}^0$, $\Delta H_2{}^0$, $\Delta S_2{}^0$ represents the thermodynamic parameters of peptide and ethyl lactate.

Meanwhile, the equilibrium constants of the same peptide for various esters were compared. For instance, the equilibrium constants for peptide PA, ethyl acetate, and ethyl lactate were 82.0, 73 mol/L, and 621.37 mol/L, respectively. This suggests that the effect of the same peptide on various ester compounds varied depending on the mechanism of action. By determining the enthalpy and entropy changes of peptides at various temperatures, we were able to gain a better understanding of the specific sorts of contact forces involved. Huang [26] evaluated the thermodynamic characteristics of the interaction between peptides and volatile compounds in order to ascertain their mechanism of interaction. The interactions between PA, PV, and PL with ethyl acetate and ethyl lactate can be ascribed to van der Waals forces or hydrogen bonds. In the same way, the connections between GL and DF with ethyl acetate are influenced by hydrophobic contacts, but the connections between GL and DF with ethyl lactate are influenced by van der Waals forces or hydrogen bonding.

Furthermore, the interactions involving GSH with ethyl acetate and ethyl lactate are likewise characterized by hydrophobicity. The interactions between small-molecule compounds and peptides mostly include weak intermolecular forces, such as hydrogen

bonding, Van der Waals forces, electrostatic forces, and hydrophobic forces [51,52]. This is similar to the result of Zhang [32], who demonstrated that a cyclic peptide largely interacts with 4-ethylguaiacol via hydrogen bonding. Specifically, the C=O group can attach to OH-, and the amino acid residues in the peptide may create hydrogen bonds with the hydroxyl group. Huang [26] discovered a tetrapeptide (DRAR) in sesame flavor-type Baijiu. The stability of the DRAR-esters and DRAR-alcohols complexes was mostly maintained by hydrogen bonds and van der Waals forces. Our results establish that peptides interact with ethyl lactate and ethyl acetate, thereby influencing the content and properties of these esters. This interaction may play a crucial role in shaping the overall flavor profile of Baijiu.

4. Conclusions

In conclusion, five peptides isolated from distillers' grain filtrate showed strong antioxidant activities. These peptides could scavenge hydroxyl radicals and alkoxy radicals, and selectively influence the VOCs in Chi-aroma Baijiu. Notably, ethyl nonanoate, diethyl acetal, and octanal were identified as characteristic substances affected by the peptides. Additionally, ultraviolet spectroscopy revealed that the peptide interacts with specific ester compounds via hydrogen bonding or Van der Waals force and hydrophobicity. This study sought to offer valuable insights into the scavenging free radicals of Chi-aroma Baijiu and understanding the interaction processes between aroma compounds and peptides. Future research should also investigate the impact of peptides on different elements of its sensory characteristics.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app14209326/s1, Table S1: Volatile organic compounds in Chi-aroma Baijiu; Table S2: Statistical analysis between peptides and volatile substances.

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