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New process of goji fermented wine: effect of goji residue degradation to generate norisoprenoid aroma compounds

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Abstract

In the present study, we compared the color, flavor, and sensory attributes of wines prepared from undegraded (GW1), and physically (GW2) and biologically (GW3) degraded goji residue. Change in wine color showed a similar trend during the aging period; the color gradually became darker, with an increasing red and yellow hue. The wine obtained using the biological method was more clarified, transparent, and lustrous after 30 days of aging. Headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography–mass spectrometry (GC-MS) detected 7 norisopreniod aroma compounds in GW1 and 9 additional compounds including dihydro-β-ionone, dihydrojasmone, safranal, α-cyclocitral, β-cyclocitral, octanoic acid, 2-octenal, β-damascenon, and geraniol propionate in GW2 and GW3. However, the content of GW3 compounds was higher than GW2 and GW1 compounds. Compared to GW1 and GW2, GW3 compounds showed a more intense floral and fruity aroma with typical wolfberry notes. Sensory evaluation revealed that panelists preferred GW3, followed by GW1 and GW2. Altogether, biodegradation of goji residue produced goji wine with the best color, flavor, and organoleptic qualities. We believe this study will contribute to a better understanding of the brewing and aging process of goji wine and improve its quality.

Keywords: goji wine; carotenoids; degradation; norisoprenoids; fermentation.

Practical Application: The practical application of our study is to investigate the impact of different degradation methods of carotenoids on the flavor of goji berry wine. The findings of this research can be useful for wine producers to optimize the production process and improve the sensory properties of their products.

1 Introduction

Goji berries are widely present in Asia, particularly Ningxia, which is located in northwestern China. Apart from food, the berries of Goji are also used as medicines. The daily consumption of goji significantly increased certain measures of immunological function without any adverse reactions and may lower long-term cardiovascular disease risk (Han et al., 2022; Toh et al., 2021). Goji berries can be prepared into goji wine via a process similar to other fruit wines with low alcohol content. The wine is primarily of two types, steeped and fermented wines (Niu et al., 2017). Fermenting goji berry wine is a process of biological fermentation by adding yeast. It has a rich aroma and an agreeable taste (Liu et al., 2022). Although China has a long history of goji berry cultivation, the goji berry deep processing industry is still very new. In November 2022, China released the national standards for goji wine (China Standardization Administration Committee, 2022), which not only provides processing standards to enterprises but also protects the interests of consumers. Thus, goji wine has broad development prospects.

Carotenoids have been an interesting research topic among a wide range of disciplines. It is an umbrella term for naturally occurring pigments found widely in plants and microorganisms in nature (Torres-Montilla & Rodriguez-Concepcion, 2021). They are characterized by a specific basic chemical structure consisting of 8 isoprene units that constitute 40 carbon atoms. This structure bestows them the coloring property (Santos et al., 2021). To date, more than 700 kinds of naturally occurring carotenoids have been discovered. Goji berries are rich in carotenoids, mainly in the form of β-carotene, zeaxanthin, and zeaxanthin dipalmitate (Lerfall, 2016). Goji-fermented wine retains the water-soluble nutrients present in goji berries. Carotenoids, in contrast, are lipid-soluble and water-insoluble compounds (Kim et al., 2018). The residue is filtered out during the brewing of goji wine and is a waste. Currently, methods for carotenoid degradation fall mainly into three categories, physical, chemical, and biological (Liang et al., 2021). Oxidatively and light-generated free radicals act on these conjugated double bonds causing their degradation (Syamila et al., 2019b). All carotenoids degraded under the combined influence of photolysis and OH scavenging and the major degradation products were apo-aldehydes and apo-ketones (Semitsoglou-Tsiapou et al., 2022). Cheng et al. (2021) reported that the accumulation of norisoprenoid volatiles is regulated by carotenoid cleavage oxygenases (CCDs). In addition, these carotenoid-derived volatile compounds have floral, fruity, fatty, and sweet-like flavors, which greatly enhance the quality of the aroma of goji wine (Simkin, 2021). Valerio et al. (2021) reported that both β-carotene and zeaxanthin degraded at a faster rate at higher temperatures. Rodriguez & Rodriguez-

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Amaya (2007) identified the oxidation products with $KMnO_4$ as β-apo-8-carotenal, β-apo-10-carotenal, β-apo-12-carotenal, β-apo-14-carotenal, and β-apo-15-carotenal, along with semi-β-carotene and monohydroxy-β-carotene-5,8-epoxide. However, thermal and chemical degradation, are not conducive to practical applications because of the negative effects of elevated temperature and chemical reactants during processing. In view of these shortcomings of chemical and thermal degradation, the advantages of biodegradation include high yield, mild catalytic conditions, high specificity, and high efficiency of degradation (Nagai et al., 2015). Cheng et al. (2021) reported the overexpression of *SlCCD1A* indicated that it could cleave lycopene, α-carotene, and β-carotene to produce 6-methyl-5-hepten-2-one, geranylacetone, α-ionone, and β-ionone, increasing the floral, fruity, fatty, and sweet-like aromas of tomato fruits. Long et al. (2021) suggested that biological enzymes function like free oxygen on the C-C double bonds in carotenoids and cleave them to produce fragrance. Although studies on the degradation law of carotenoids have been reported, there are as yet few reports on the application of the microbial degradation of carotenoids, in particular goji wine.

In previous experiments, they screened a strain of Kurthia, named *NXU-GQ 15*, was capable of degrading carotenoids (Lee et al., 2017). In the present study, carotenoids in the Goji residue were degraded using a biological method involving inoculation with the *NXU-GQ 15* enzyme and a physical method of autoclaving. Then Goji wine was inoculated with *Saccharomyces cerevisiae* fermentation in goji juice. The degraded goji residue was then returned to the goji wine and macerated. These following indicators were analyzed: (1) physicochemical properties; (2) color parameters; (3) volatile compounds; and (4) sensory evaluation. To detect the types and relative contents of norisoprenoid, headspace solid phase extraction gas chromatography-mass spectrometry (HS-SPME-GC-MS) was used. We believe this study will significantly contribute to improving the quality of goji wine and its brewing process.

2 Materials and methods

2.1 Wines and sample preparation

Fruit material

Fresh goji berries (*Ningqi No.7*; residual sugars, 17.0 g/L) were obtained from a goji berry farm in Zhongning, Ningxia Province, western of China on July 1. 2022. Harvesting was performed at the optimum ripening stage with good sanitation. After collection, goji berries (10 kg) were immediately transported to the laboratory.

Juice sample preparation

Fruit juice was prepared according to the method described by Donghui & Yan (2010). The production of fruit juice and subsequent goji wine (GW) was carried out at the Ningxia Food Microbiology Application Technology and Safety Control Laboratory. 300 g goji berries were juiced using a high-speed blender (L18-P132, Jiuyang, Shandong, China). Put 1 mL/L of a 6% sulfurous acid and 40 mL/L pectinase into the filtered juice. After that, adjusting the sugar to 25.0 g/L. Adjusted the pH to 4.2 with citric acid and then inoculated activated dry yeast (Excellence XR, LAMOTHE-ABIRT, France, 0.2 g/L) in the juice at a constant temperature of 28 °C. The fermentation ended until the residual sugar dropped to 4.0 g/L. Alcohol content after fermentation ranged from 13 to 14%.

Goji residue preparation

Residues obtained after juicing goji berries were divided into three equal groups. The first group was no treatment group. In the second group, the residue was autoclaved (0.15 MPa, 121 °C, 20 min) and degraded. Third, the Kurthia (*NXU-GQ 15*) culture stored at 37 °C was expanded to yield bacterial solutions, and after 4 days of incubation, the supernatant solution was centrifuged at 4 °C, 10000 r/min for 10 min using the ultra-low freeze dryer to pellet the *NXU-GQ 15* powder. Afterward, it was added to the residue for enzymatic hydrolysis.

Goji wine samples

The three degraded goji residue groups were added back to the wine under standard temperature conditions for 10 min and filtered. In the first group, goji wine 1 (GW1) served as the control. GW2 consisted of goji wine obtained by physical degradation of the residue. GW3 comprised goji wine obtained by biodegrading the residue. The clarified goji wine was transferred to a closed glass bottle, stored at 15 °C, and protected from light for 30 days to complete the aging process (Donghui & Yan., 2010) (Figure 1).

2.2 Basic chemical characteristics

The pH, sugar, volatile acid, and alcohol were measured after 30 days of aging. The pH was measured by a multi-parameter analyzer (Leici DZS-708L, Shanghai Jingke, China). The residual sugar concentration was determined using a handheld refractometer (MyBrix, METTLER TOLEDO, China). The content of volatile acid and alcohol was measured as per the National Standard of the People's Republic of China (GB/T 15038-2006) (Standardization Administration of China, 2006)

*2.3 Spectrophotometric L*a*b* measurements*

Color analysis was performed on a wedge-colorimeter (WSF, INESA, China) with a 3.0 cm length glass cell; the wavelength scanned was between 400 and 700 nm. The CIELAB is a color space defined in 1976 (ISO 11664-4: 2008) (International Organization for Standardization, 2008). In this system, the L^* represents the brightness of the wine sample that ranges from 0 (black) to 100 (white). The a^* and b^* represent chromaticity. The a^* showed positive values for red colors and negative values for green colors. The b* showed positive values for yellow colors and negative values for blue colors. With GJ as the control group, the difference of color is expressed as ΔE^*, which reads (Equation 1):

$$\Delta E^* = \left[\left(\Delta L^*\right)^2 + \left(\Delta a^*\right)^2 + \left(\Delta b^*\right)^2\right]^{1/2} \tag{1}$$

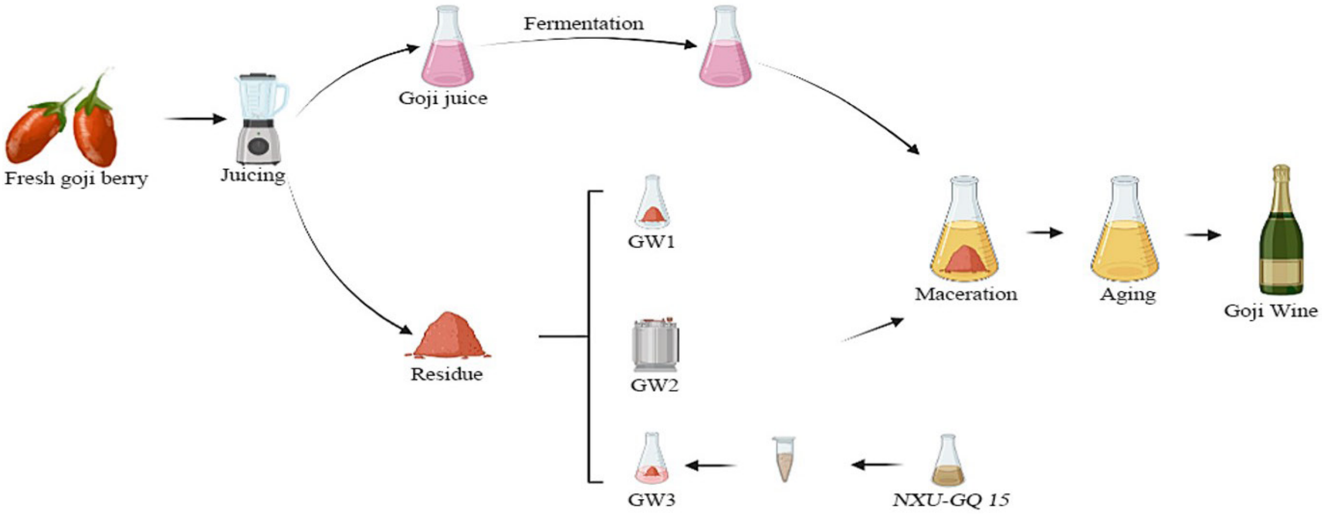

Figure 1. Production of goji wine using different maceration strategies.

2.4 Carotenoid and norisoprenoid compounds analysis

Preparation of carotenoid solution and standard curve

A 0.005 g β-carotenoid, zeaxanthin, or zeaxanthin dipalmitate standard sample was mixed with 10 mL dichloromethane in the dark. After complete dissolution of each standard, 1 g Tween-80 was added to emulsify. The mixture was diluted to 10 mL with distilled water, and the stock solution of each standard was stored at 4 °C. The solution was diluted into different mass concentration gradients as needed before the measurement. A linear regression equation was established with the mass concentration as the x-axis and the peak area as the y-axis (Table 1).

Table 1. Regression equation of carotenoids.

Compound	Standard curve	Correlation coefficient (R^2)
β-carotenoid	$Y = 53.478X - 3.669$	0.9996
zeaxanthin	$Y = 465.74X - 58.517$	0.9987
zeaxanthin dipalmitate	$Y = 1045.32X - 371.482$	0.9997
Total carotenoids	$Y = 0.025X + 0.015$	0.9998

Measurement of carotenoids

The contents of g β-carotenoid, or zeaxanthin, were determined by Agilent 1100 HPLC (Agilent Technologies, CA, USA). Isocratic elution was used with V(methanol):V(acetonitrile):V(n-hexane):V(dichloromethane) = 15:40:20:20 as mobile phase. The flow rate was 1 mL/min. The column temperature was at 25 °C, and the detection wavelength was at 450 nm.

Measurement of total carotenoids

A stock solution with a mass concentration of 0.4 mg/mL was prepared by diluting 4 mg of β-carotene to 10 mL with petroleum ether. The absorbance was measured at 454 nm, and the total amount of carotenoids was determined by plotting the standard curve (Table 1) with the mass concentration of the solution as the horizontal coordinate and the absorbance as the vertical coordinate.

Extraction of norisoprenoid compounds

Analysis of norisoprenoid compounds was carried out following the method of Wang et al. (2016) with slight modifications. The method was as follows.Aroma enrichment by headspace solid phase microextraction (HS-SPME): In brief, 8 mL of wine was added to a 20 mL glass headspace vial containing NaCl (1.5 g, Tianjin Chemical Technology, Tianjin, China). Next, an internal standard (2-Octanol, 8 µL, 400 mg/L in ethanol) was added to obtain a final concentration of 2.01 mg/L. The mixture was agitated on an orbital shaker at 250 r/min and 40 °C for 3 min. The SPME fiber (50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), length 1 cm Supelco, Bellefonte, PA, USA) was aged at 250 °C for 10 min, cooled, and subsequently added to the sample vial and agitated at 40 °C for an additional 30 min.

GC-MS analysis

The GC-MS analysis was performed using an Agilent 7890B gas chromatograph (Agilent, Santa Clara, CA, USA), fitted with a Gerstel PAL RSI 85 autosampler (CTC Analytics AG, Zwingen, Switzerland) and an Agilent 7000D mass-selective detector. Thermal desorption of analytes from the SPME fiber was performed via a split-less injection (straight glass liner, 0.75 mm i.d.) at 240 °C for 10 min. The column oven temperature program was as follows: initial temperature of 40 °C, held for 3 min, increased to 120 °C at 5 °C/min, then to 230 °C at 8 °C/min, held for 10 min. The MS detector was operated in the scan mode (mass range 40-300 m/z), and the transfer line to the MS system was maintained at 250 °C. Helium was used with a linear velocity of 1.0 mL/min (constant flow). The mass selective detection was performed in the scan mode (20-450 amu, EI (70 eV), interface temperature of 250 °C, and an ion source temperature of 200 °C).

Identification and quantification of norisoprenoid compounds: A total of 16 norisoprenoid compounds were identified in goji wines using experimentally obtained Kovats retention indices (RI). The mass spectrometry information of each substance obtained by GC-MS analysis was automatically retrieved and compared with the standard in the NIST MS library, and the compound structure characteristic matching index was more than 80% as the substance identification standard. RI was used as a criterion was used to identify the polar compounds as a criterion. The investigated compounds were quantified by comparing the peak areas obtained from total ion chromatograms after the deconvolution process against those of the internal standard 2-octanol using a response factor of one for each compound.

Evaluation method of key volatile substances

The relative odor activity value (ROAV) is a combination of the relative content and threshold of aroma substances to describe the contribution of each component compound to the overall aroma (Zhu et al., 2020). When the components of ROAV ≥ 1 were the key flavor compounds of the analyzed sample, the components of 0.1 ≤ ROAV < 1 had an important modifying effect on the overall flavor of the sample (Equation 2).

$$\boldsymbol{ROAV} = 100 \times \frac{\boldsymbol{C_A}}{\boldsymbol{C_{Max}}} \times \frac{\boldsymbol{T_{Max}}}{\boldsymbol{T_A}} \qquad (2)$$

Where C_A (%) represented the relative content of the compound; C_{Max} (%) represented the relative content of the component that contributes the most to the overall flavor of the sample; T_{Max} represented the threshold of the maximum component for the overall flavor contribution of the sample; T_A represented the threshold of the compound.

2.5 Sensory analysis

For sensory assessment, a trained group was used. The group consists of 12 professional tasters (7 women, 5 men, aged 19-26), all with more than 3 years of wine tasting experience and familiar with goji wine. Choosing a well-lit, clean and odourfree venue, using ISO international standard tasting glasses fitted with a lid. Panellists were allowed to open the lid only when assessing the sample. Marked the number at the bottom of the wine glass, randomly group and number the wine samples, and scored the goji berry wine from four aspects: appearance, aroma, taste, and typicality. During scoring of different samples, subjects were instructed to rinse their mouth with purified water to eliminate bad breath. The scoring rules were shown in Table 1. The scoring rules were shown in Table 2 (Yuan et al., 2016).

2.6 Statistical analysis

All the experiments were performed in triplicate. Means and standard deviations were calculated using Excel (Microsoft, Redmond, WA, USA). The statistical data analysis was conducted using the analysis of variance (ANOVA) by Spss 26.0 (Chicago, IL, USA). Turkey's HSD and Duncan's test were used as a comparison test when samples were significantly different after ANOVA ($p < 0.05$) for chemical and color analyses.

3 Results

3.1 Effect of degradation mode on the physical and chemical properties of goji wine

The characteristics of the goji juice (GJ) and the final product were shown in Table 3. ANOVA was used to explore differences in the pH, residual sugar, volatile acid, and alcohol value of wines obtained using different degradation methods. There were significant differences between goji juice and wine in these four properties. In addition, significant differences were observed in sugars, pH, and alcoholic strength of the wolfberry wine, except for no significant differences in volatile acids. This meant that differences in the degradation method of the residue also affected the winemaking parameters of the goji wine.

During the fermentation and aging process, residual sugars (25.00 g/L) and pH (4.20) of GJ reduced, and the content of

Table 2. Sensory rating table of goji wine.

	Scoring Standards	Score
Appearance (30)	Clear, transparent, shiny and attractively, with a vivid light orange color.	28-30
	Clear, transparent, with the color of this product, light orange.	25-28
	Clear, without inclusions, poor color, orange-yellow or yellow.	22-25
	Slightly mixed, matte, artificially colored	20-22
Aroma (25)	The flavor is pleasant and rich, with a complex aroma that highlights the aroma of the raw material of goji berries and fermented wine.	22-25
	The flavor is pleasant and soft, with a variety of aromas	20-22
	The aroma is light, and the characteristic aroma is not prominent.	18-20
	Uncomfortable smell.	15-18
Texture (25)	The palate is harmonious and balanced, refreshing on the palate, full bodied and flavorful.	22-25
	The taste is not refreshing, and the body is pretty full.	20-22
	The taste is not refreshing, and the flavor is average	18-20
	The taste is poor and incongruous, and the flavor is lack.	15-18
Typicality (20)	It has a pleasant style and unique typicality of goji fermented wine.	18-20
	Pretty typical and well-styled.	15-18
	Unclearly Typica, the style is average.	13-15
	Not typical.	10-13

Table 3. Residual sugar content, pH, volatile acidity and alcohol content of different residue treatment in the samples.

Name	GJ	GW1	GW2	GW3
Residual sugar (g/L)	20.00 ± 0.02 ^{a}	10.62 ± 0.02 ^{c}	11.10 ± 0.1 ^{c}	11.54 ± 0.06 ^{b}
pH	4.20 ± 0.01 ^{a}	3.93 ± 0.01 ^{b}	3.88 ± 0.01 ^{c}	3.84 ± 0.02 ^{d}
Volatile acidity (g/L)	0.52 ± 0.01 ^{b}	0.67 ± 0.01 ^{a}	0.66 ± 0.01 ^{a}	0.66 ± 0.01 ^{a}
Alcohol content (%,v/v)	0 ^{a}	13.75 ± 0.06 ^{c}	13.00 ± 0.04 ^{b}	13.50 ± 0.04 ^{c}

GJ was goji juice; GW1 was the control group; GW2 was the high-temperature treatment group. GW3 was the enzyme *of NXU-GQ 15* treatment group. Data presented as average value ± standard deviation; ANOVA to compare data; different letters indicate significant differences between the samples of all treatments (Tukey's test. $P < 0.05$).

volatile acids (0.55 g/L) and alcohols (0.00%) increased. This is ascribed to the fact that during the production of goji wine, sugar is metabolized to produce alcohol, and polyphenols get oxidized when they come in contact with air, resulting in elevated content of volatile a consequent change in pH (Shisong et al., 2018). In addition, certain changes were introduced to goji wine. Among the three samples, GW1 showed the highest pH (3.93). The degradation of goji residue reduced the pH value of the wine sample. The pH value of GW2 (3.88) was higher than that of GW3 (3.84). GW3 had a maximum residual sugar of 11.54 g/L, with a significant difference between GW2 (1.10 g/L) and GW3 (11.54 g/L). All three samples were dry goji wines based on their residual sugars being less than 12.0 g/L. No significant differences were noted in the volatile acidity. The highest alcohol content was found in GW1 (13.75%), followed by GW3(13.50%), and the lowest alcohol content was observed in GW2 (13.00%). These differences were significant.

3.2 Effect of degradation method on goji wine color

With aging, all three samples gradually became darker in color with an increasing red and yellow hue (Figure 2). For the first 10 days, the color changed significantly, and then the change was slow. Carotenoids in goji wine underwent a change in color. Next, the polyphenols in the wine were oxidized and condensed during the entry of oxygen (Ya et al., 2017), and the color was due to non-enzymatic browning. Regarding the a* and b* values, GW1 exhibited more red and yellow hues. Therefore, the red and yellow hue should be faded after the degradation of carotenoids. Chakraborty et al. (2015) detected an increase in the browning indices of pineapple puree with increasing temperature. The color of GW2 changed due to the presence of the numerous of reducing sugars and proteins in the goji residues, suggesting that at higher temperatures, discoloration occurs due to non-enzymatic browning through several biochemical reactions including, but not limited to, Maillard condensation, pigment destruction and caramelization of sugars.

Table 4 shows the color parameters of the GJ and the final product after aging 30 days of aging. The color of GJ is an important indicator to evaluate its quality. The change in the color value was caused by a change in the main components inside the juice, which directly affected the sensory quality of the juice. Compared with GJ, the wine had a higher *L** value and was more yellow in color, but lighter in red. Goji juice contains numerous undegraded carotenoids. However, during the winemaking process, the juice undergoes browning and deepens in color. There were significant differences in the *L**, *a**, and *b** values of GW1, GW2, and GW3. The *L** values were significantly brighter in GW3 (94.58) than in GW1 (93.72) and GW2 (90.65), implying that the enzymatic degradation of carotenoids in the residue by the *NXU-15* resulted in a more clarified, transparent and lustrous wine. GJ (-22.36) showed the lowest *a**, followed by GW2 (-13.77), GW3 (-13.58), GW1 (-12.71). Interestingly, on the blue-yellow hue, GJ (82.59) had the highest

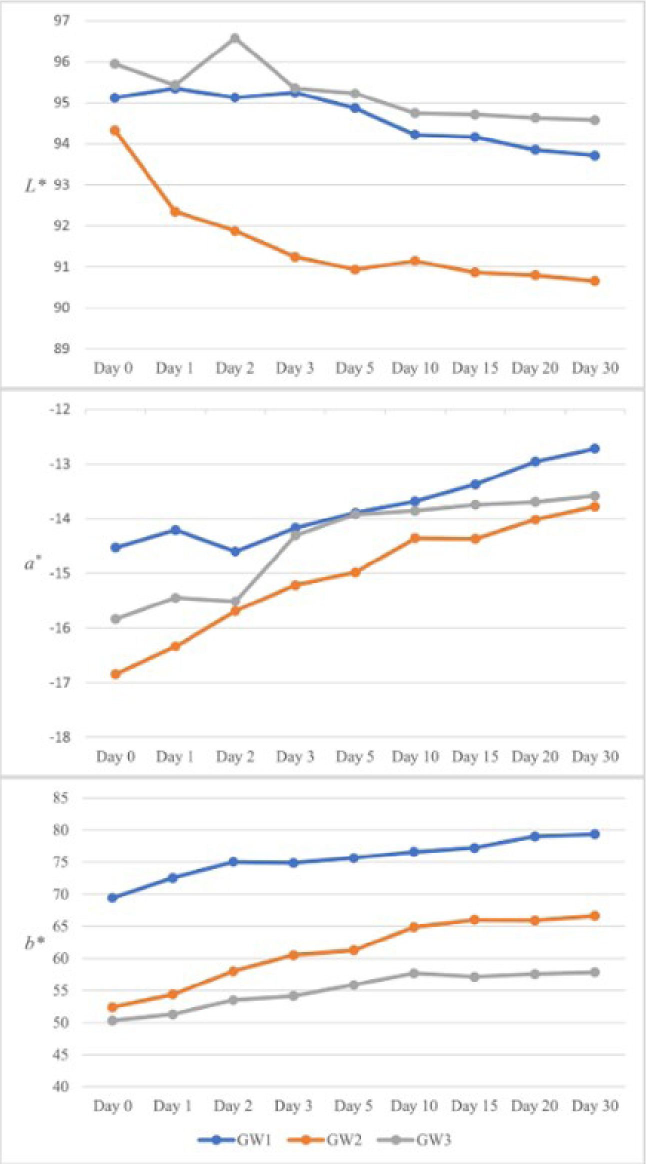

Figure 2. The value of L*, a*, b* in goji wine GW1 to GW3 with 30 days of aging.

*b**, followed by GW1 (79.32), GW2 (66.59), and the lowest was GW3 (57.83). The changes in these two parameters showed a deepening of the red color and a weakening of the yellow color from goji juice to wine. As per sample categorization described by Cserhalmi et al. (2006), ΔE was considered as 'greatly noticeable' when it crossed the corresponding lower limit of 6. The color of these 4 samples was visually different. This study concluded that the biological degradation of goji residue positively affected the quality of goji wine.

3.3 Effect of degradation treatment on volatile compounds of goji wines

Table 5 shows the contents and degradation rate of carotenoids in the GJ and GW. By measuring the carotenoid content in 4 samples, the highest degradation rate was found for GW3 (84.73%), followed by GW2 (56.77%), and GW1 (27.25%). It is interesting that β-carotene, zeaxanthin, and zeaxanthin dipalmitate showed the same degradation trend in the wine, which meant that biological methods, especially *NXU-GQ 15* enzyme, were more efficient in the degradation of carotenoids. Inbaraj et al. (2008) studied that zeaxanthin bipalmitate has the highest percentage of carotenoids in goji berries. In comparison to β-carotene and zeaxanthin, we found that zeaxanthin bipalmitate were more completely degraded. The degradation rates from GJ to GW3 were 29.88%, 69.09%, 84.23%. Zeaxanthin dipalmitate is characterized by many double bonds and methyl groups, which lead to oxidation and substitution reactions, and the double bonds are easily broken. Some small molecule compounds are generated associated with C_6-C_7, C_7-C_8 or C_{12}-C_{13} bonds breaking, such as 2,2,6-trimethyl-cyclohexanone, limonene, 2-octenal, etc (Geng et al., 2021). A qualitative and quantitative analysis of the aroma substances of goji wine obtained by GC-MS revealed the presence of 16 norisoprenoid compounds (Table 6, Figure 3). In this study, three norisoprenoids were found in GJ (β-ionone, geranylacetone, and 2,2,6-trimethyl-cyclohexanone) and seven in GW1 (2-ethylbutyl methacrylate, 2,4-nonadienal, β-ionone, geranylacetone, 2,2,6-trimethyl-vyclohexanone, 2-ethylbutyl methacrylate, dihydroactinidiolide). During the fermentation process, the content of the three norisoprenoids increased significantly and 4 new compounds were generated. The presence of norisoprenoids in the juice were due to the degradation of carotenoids by crushing, light, oxygen, temperature, and other conditions during the preparation of GJ. The degradation of carotenoids in the wine was attributed to the pre-treatment of raw materials before the development of juice (pectinase, pH, etc.) and other metabolites such as ethanol after the alcoholic fermentation by yeast. Significant differences were detected in the types and contents of norisoprenoid compounds in GW1, GW2, and GW3. A total of 16 norisoprenoids were detected in GW2 and GW3, and the content of the compound GW3 was higher than that of GW2. It was shown that the degradation of carotenoids

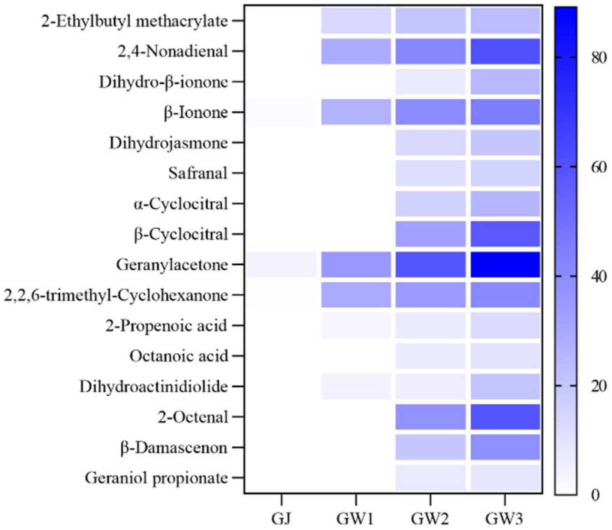

Figure 3. Differences of norisoprenoid compounds in the goji juice and final product.

Table 4. Color parameters of the goji juice and final product.

Name	GJ	GW1	GW2	GW3
L*	53.24 ± 0.01 ᵈ	93.72 ± 0.02 ᵇ	90.65 ± 0.02 ᶜ	94.58 ± 0.01 ᵃ
a*	-22.36 ± 0.02 ᵈ	-12.71 ± 0.01 ᵃ	-13.77 ± 0.01 ᶜ	-13.58 ± 0.01 ᵇ
b*	82.59 ± 0.01 ᵈ	79.32 ± 0.02 ᶜ	66.59 ± 0.03 ᵇ	57.83 ± 0.02 ᵃ
ΔE	44.03 ± 0.02 ᵃ	0 ᵈ	13.14 ± 0.02 ᶜ	21.52 ± 0.01 ᵇ

Data presented as average value ± standard deviation; ANOVA to compare data; different letters indicate significant differences between the samples of all treatments (Tukey's test. P < 0.05).

Table 5. Contents and degradation rate of carotenoid in the goji juice and final product.

carotenoids	GJ	GW1		GW2		GW3	
	Content (mg/L)	Content (mg/L)	Degradation rate (%)	Content (mg/L)	Degradation rate (%)	Content (mg/L)	Degradation rate (%)
β-carotenoid	236.56 ± 0.79 ᵈ	197.57 ± 0.84 ᶜ	16.48	115.40 ± 0.69 ᵇ	51.22	52.98 ± 0.36 ᵃ	77.60
zeaxanthin	249.04 ± 0.27 ᵈ	208.87 ± 0.55 ᶜ	16.13	95.04 ± 0.48 ᵇ	61.84	55.64 ± 0.12 ᵃ	77.66
zeaxanthin dipalmitate	506.21 ± 0.74 ᵈ	354.97 ± 0.11 ᶜ	29.88	156.64 ± 0.09 ᵇ	69.06	79.83 ± 0.22 ᵃ	84.23
Total carotenoids	1255.36 ± 3.32 ᵈ	913.24 ± 0.53 ᶜ	27.25	542.63 ± 0.43 ᵇ	56.77	191.68 ± 0.02 ᵃ	84.73

Data presented as average value ± standard deviation; the different letters indicated significant difference between the samples of all treatments (Tukey's test. P < 0.05).

in goji residue by biological method was more effective than that of physical methods using high temperature. The specific and efficient catalytic properties of biological enzymes greatly reduce the activation energy required for the reaction and the reaction is accelerated. NXU-GQ 15 exogenous enzymes were extracted directly for fermentation, which shortened the proliferative and metabolic timescale of the bacterium, and rescued the nutrients required for bacterial activity. The concentration of enzymes isolated and crude extracted by direct inoculation was significantly higher than that secreted by NXU-GQ 15 inoculation. The enzyme concentration was a positive function of the reaction rate when the substrate concentration was sufficient and constant. However, goji wine, a kind of fruit wine, is a heat-sensitive material. High temperature and pressure can further decompose and degrade a portion of the norisoprenoids, resulting in volatile substances with lower molecular weight but no aroma (Fanlai et al., 2013). This could be the reason for the low content of norisoprenoids in GW2 than in GW1.

The relative odor activity value (ROAV) is a combination of the relative content and threshold of aroma substances that reflects the contribution of each component compound to the overall aroma (Table 7). No ROAVs were calculated for GJ because the odor thresholds of its compounds were only found for β-ionone and geranylacetone. They had about the same relative content in GJ, however, the odor threshold of β-ionone (0.09 μg/L) is much lower than that of geranylacetone (60 μg/L). This indicated that β-ionone played an important role in the aroma of GJ. Using 2,4-nonadienal as a standard, a total of 11 norisoprenoid

Table 6. Contents of norisoprenoid compounds in the goji juice and final product.

Compound	Formula	RI	Content (mg/L)				Descriptor
			GJ	GW1	GW2	GW3	
2-Ethylbutyl methacrylate	$C_8H_{14}O$	982	ND	13.72 ± 0.54 ^{a}	20.10 ± 0.73 ^{b}	23.28 ± 0.92 ^{c}	Lemon, grass
2,4-Nonadienal	$C_9H_{14}O$	1210	ND	28.85 ± 0.39 ^{a}	41.64 ± 0.76 ^{b}	61.02 ± 0.83 ^{c}	Flower, fusel, fatty
Dihydro-β-ionone	$C_{13}H_{22}O$	1435	ND	ND	6.72 ± 0.35	24.78 ± 0.78	Wood, fruity, flower
β-Ionone	$C_{13}H_{20}O$	1483	1.36 ± 0.02 ^{a}	26.73 ± 0.45 ^{b}	40.38 ± 0.52 ^{c}	45.24 ± 0.85 ^{d}	Violet, wood
Dihydrojasmone	$C_{11}H_{18}O$	1214	ND	ND	13.50 ± 0.68 ^{a}	19.98 ± 0.62 ^{b}	Jasmine, fruity
Safranal	$C_{10}H_{14}O$	1196	ND	ND	11.34 ± 0.92 ^{a}	15.15 ± 0.98 ^{b}	Wood, spicy, medicinal, pollen
α-Cyclocitral	$C_{10}H_{16}O$	1121	ND	ND	15.96 ± 1.32 ^{a}	25.26 ± 0.98 ^{b}	Fruity
β-Cyclocitral	$C_{10}H_{16}O$	1217	ND	ND	32.82 ± 0.63 ^{a}	57.84 ± 0.82 ^{b}	Fruity
Geranylacetone	$C_{13}H_{22}O$	1450	3.59 ± 0.03 ^{a}	35.72 ± 1.05 ^{b}	59.04 ± 0.43 ^{c}	89.16 ± 1.12 ^{d}	Green, grass, wax, wood
2,2,6-trimethyl-Cyclohexanone	$C_9H_{16}O$	1029	0.61 ± 0.01 ^{a}	29.14 ± 0.88 ^{b}	34.38 ± 1.23 ^{c}	40.62 ± 1.53 ^{d}	Fruity
Limonene	$C_{10}H_{16}$	1031	ND	3.26 ± 0.15 ^{a}	6.66 ± 0.36 ^{b}	12.36 ± 1.06 ^{c}	Lemon, orange
Octanoic acid	$C_8H_{16}O_2$	1013	ND	ND	6.38 ± 0.25 ^{a}	9.82 ± 0.36 ^{b}	Sweat
Dihydroactinidiolide	$C_{11}H_{16}O_2$	1525	ND	4.31 ± 0.24 ^{a}	6.12 ± 0.32 ^{b}	20.52 ± 0.62 ^{c}	Wood, toast, tea, peach
2-Octenal	$C_8H_{14}O$	1053	ND	ND	38.28 ± 0.92 ^{a}	58.92 ± 1.03 ^{b}	Fatty, meat
β-Damascenon	$C_{13}H_{20}O$	1378	ND	ND	19.92 ± 0.86 ^{a}	38.88 ± 0.53 ^{b}	Peach, apple
Geraniol propionate	$C_{13}H_{22}O_2$	1708	ND	ND	7.50 ± 0.51 ^{a}	8.52 ± 0.54 ^{b}	Fruity, flowery

"ND" indicated "not detected". Data presented as average value ± standard deviation; the different letters a, b, c, d indicated significant difference between the samples of all treatments (Tukey's test. $P < 0.05$).

Table 7. Odor threshold, relative content and ROAVs of norisoprenoid compounds in the goji juice and final product.

Compound	Odor threshold (μg/L)	Relative content (%)			ROAV		
		GW1	GW2	GW3	GW1	GW2	GW3
2-Ethylbutyl methacrylate	100 ^{D}	2.89 ± 0.01 ^{a}	3.35 ± 0.01 ^{b}	3.88 ± 0.01 ^{c}	0.036	0.029	0.023
2,4-Nonadienal	0.06 ^{F}	4.81 ± 0.01 ^{a}	6.94 ± 0.12 ^{b}	10.17 ± 0.02 ^{c}	100	100	100
Dihydro-β-ionone	461 ^{A}	ND	1.12 ± 0.00 ^{a}	4.13 ± 0.01 ^{b}	-	0.002	0.005
β-Ionone	0.09 ^{B}	4.46 ± 0.02 ^{b}	6.73 ± 0.02 ^{c}	7.54 ± 0.10 ^{d}	61.816	64.649	49.426
β-Cyclocitral	5 ^{C}	ND	5.47 ± 0.01 ^{a}	9.64 ± 0.03 ^{b}	-	0.946	1.137
Geranylacetone	60 ^{C}	5.93 ± 0.05	9.84 ± 0.03 ^{a}	14.86 ± 0.69 ^{b}	0.123	0.142	0.146
Limonene	10 ^{C}	0.54 ± 0.01 ^{a}	1.11 ± 0.00^{b}	2.06 ± 0.01 ^{c}	0.067	0.096	0.122
Octanoic acid	500 ^{B}	ND	1.06 ± 0.00 ^{a}	1.64 ± 0.01 ^{b}	-	0.002	0.002
Dihydroactinidiolide	3800 ^{A}	0.72 ± 0.00 ^{a}	1.02 ± 0.00 ^{b}	3.42 ± 0.02 ^{c}	0.000	0.000	0.001
2-Octenal	500 ^{F}	ND	6.38 ± 0.01 ^{a}	9.82 ± 0.49 ^{b}	-	0.011	0.012
β-Damascenon	0.0514 ^{B}	ND	3.32 ± 0.01 ^{a}	6.48 ± 0.70 b	-	55.843	74.378

Data presented as average value ± standard deviation; the different letters a, b, c, d indicated significant difference between the samples of all treatments (Tukey's test. $P < 0.05$). Odor thresholds obtained from references: A (Liu & Tang, 2015), B (Ferreira et al., 2000), (Jinhua et al., 2022), C (Xiaohui et al., 2022), D (Libiao et al., 2022), E (Milo & Grosch, 1996), F (Brown et al., 1973).

compounds were screened according to Equation 1. GW1 had two key flavor compounds (ROAV ≥ 1) and one flavor-altering compound from goji wine (0.1 ≤ ROAV < 1). The use of physical and biological methods for degradation of goji residue resulted in GW2 and GW3 with floral and fruity aromas and containing more key and modifying compounds. GW2 had three key flavor compounds and two modifier compounds, whereas GW3 contained four key flavor compounds and one modifier compound. The ROAV of 2,4-nonadienal was the maximum among all compounds. 2,4-Nonadienal filled the wine with floral and oily aromas, and its ROAV was the largest of all compounds. The second was β-ionone, which provided a violet floral and woody fragrance. β-Damascenon was a key aroma compound for GW2 and GW3. It added a fresh aroma-like peach and apple flavor to goji wine. β-Cyclocitral resulted in a purple, woody, and raspberry aroma to goji wine (Bolton et al., 2019), which was a key flavor compound in GW3, whereas in GW2 it was a modifying compound. This compound resulted in the goji wine being more mellow and plumb. Geranylacetone was found to have a modifying effect on all three wines. It imparted a fresh floral and sweet aroma to goji wine. Limonene had a slightly citrus aroma and was the modifier compound for GW3 (Cheng et al., 2021). Thus, these compounds imparted goji wine a fresh floral and fruity aroma in general, accompanied by woody aromas and had the typical characteristics of wine. The ROAV of the other compounds was small. However, these compounds enrich the flavor of goji berry wine. For example, 2-ethylbutyl methacrylate and dihydro-β-ionone making contributed to the fruity flavor of goji wine (Xu et al., 2019). 2-Octenal gave a sweet taste to goji wine. Dihydroactinidiolide imparted aromatic qualities to goji wine, plucking the aroma from it and masking the acrid odor in the wine (Caja et al., 2009). Some compounds could not be analyzed because the threshold was not found, in which the α-cyclocitral was structurally similar to the β-cyclocitral protein, and the threshold should be similar as well, which should contribute to the lemony aroma and fragrance of the goji wine product.

In the case of PC1, β-cyclocitral, α-cyclocitral and geranylacetone were the most important indicators (Figure 4). In the case of PC2, dihydroactinidiolide and dihydro-β-ionone were the primary indicators. In this case, GW1 was located in the negative direction of PC1, where GJ, GW2 and GW3 were located in the positive direction of PC1, indicating that the aroma substances in the degraded and non-degraded goji residues differed. A more pronounced floral and fruity aroma was present in the degraded goji wine. GJ and GW2 was located in the negative direction of PC2 and GW3 was located in the positive direction of it, indicating that dihydroactinidiolide and dihydro-β-ionone exerted the greatest influence on the aroma of goji wine obtained by physical and biological degradation of carotenoids. Therefore, the goji wine obtained with *NXU-GQ 15* exhibited a hint of fruitiness and floral aromas as well as a woody and toasty aroma that was well balanced.

3.4 Effect of degradation on sensory analysis of goji wines

As shown in Figure 5, GW3 comprehensive score was up to 96 points, which was better than that of GW1 and GW2 in terms of appearance, aroma, taste, and typicality. The degradation of NXU-GQ 15 caused the breakdown of the macromolecular carotenoid chain. Carotenoids impart color to goji wine, such that the orange color of goji wine became paler and clearer after carotenoids are degraded. Carotenoid degradation was followed by the generation of norisoprenoid compounds with very low thresholds, imparting goji wine a distinct floral and fruity aroma, increasing its fragrance, and making it more balanced and typical than other goji wines. GW1 was orange in color and had a lighter aroma due to insufficient carotenoid degradation. The lowest GW2 score was due to color and aroma because of the unstable products produced by high-temperature carotenoid cleavage. High temperature promoted the browning reaction of norisoprenoid compounds of goji residue, thereby deepening the color of goji wine obtained after maceration.

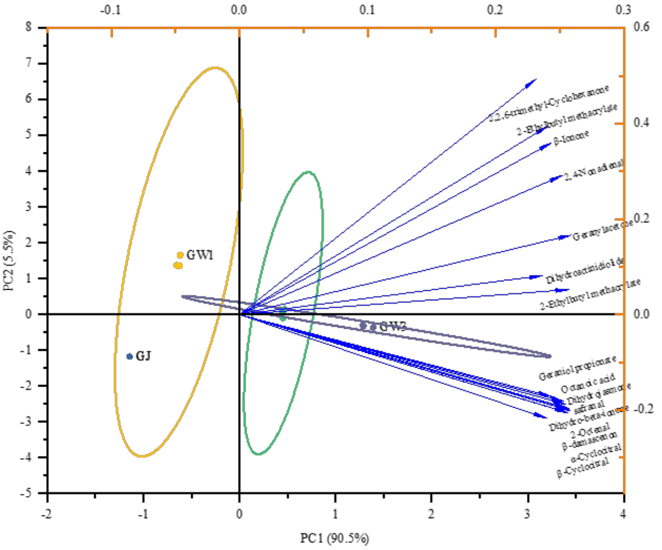

Figure 4. PCA (principle components analysis) plot based on norisoprenoid compounds of the goji juice and final product.

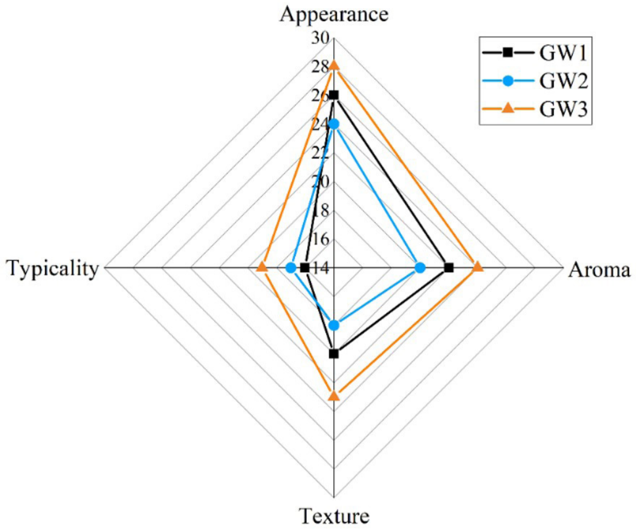

Figure 5. Sensory evaluation of goji wine.

In addition, the goji residue inevitably produced a steamy taste after high-temperature processing, affecting the quality of goji wine. Thus, the use of *NXU-GQ 15* extraction enzyme-assisted fermentation method resulted in high-quality goji berry wine.

4 Discussion

We determined the effect of physical and chemical methods on the degradation of goji residue on the color and aroma of goji wine. We demonstrated that the introduction of degraded goji residue during maceration enhanced the flavor of goji wine. In addition, we showed that the use of *NXU-GQ 15* enzyme to degrade goji residue, on the one hand, improved the degradation rate of carotenoids, thereby producing more norisoprenoid aroma components. On the other hand, the biological enzymatic reaction temperature was low and the reaction conditions were mild (Mahendran et al., 2020), which inhibited the adverse flavor of goji wine due to high-temperature heating. The flavor of goji wine largely depends on norisoprenoid compounds. During the brewing of goji wine, a part of the carotenoids is degraded due to light and oxygen (Syamila et al., 2019a). However, numerous carotenoids are not utilized in goji berry residue. To improve the utilization of goji residue and increase the aroma of wine, the residue was added during maceration. We found 3, 7 and 16 norisoprenoid compounds in GJ, undegraded wine and degraded wine, respectively. Carotenoids, especially zeaxanthin dipalmitate, constituted the major lipid-soluble substances in goji berries (Gong et al., 2020). Geng et al. (2021) reported that the double bonds of zeaxanthin dipalmitate were destroyed to produce certain norisoprenoid compounds with characteristic aromas, such as 2,2,6-trimethyl-cyclohexanone, 2-ethylbutyl methacrylate, 2-octenal, geraniol propionate, and dihydroactinidiolide. The norisoprenoids were more in GW2 and GW3 than in GW1. Both physical and biological degradation of carotenoids significantly increased the content of norisoprenoid aroma compounds in goji wine. Wang (2015) reported that certain norisoprenoid compounds, such as β-ionone and geranylacetone, in goji wine were detected in goji berries that impart the characteristic aromas to goji berries. In goji fermented wine, newly formed norisoprenoid compounds, such as dihydroactinidiolide, are detected, whose thresholds are low, and most of them have a characteristic floral or fruity aroma, which can greatly improve the aroma quality of goji berry wine and significantly contribute to the aroma of goji wine. In this study, a total of 16 isoprene-reducing compounds were produced by the three methods of degrading carotenoids. These C_{13}-norisoprenoid compounds provide a complex aroma to goji wine. Therefore, in the process of brewing goji wine, goji residue can be degraded by *Kurthia* enzyme and can be moderately autoclaved to improve the brewing process of wolfberry wine and improve the aroma of wolfberry wine. This study should, therefore, be of value to practitioners wishing to reduce waste and improve the fragrance of goji wine.

Recently, the functional role of goji berries has been increasingly recognized by consumers. However, winemakers and oenologists have been committed to improving the quality of grapes and wines, and goji berry wine has largely been ignored. Degradation of carotenoids is more often found in bacteria than in yeast with fermentation capacity. Commercial yeasts used to ferment wine may not be well suited to goji berry wine. In this study, a bacterial strain was used to degrade the residue of Goji that can generate fragrance; unfortunately, it does not metabolize sugar to produce alcohol. In conclusion, screening a yeast that can both degrade carotenoids and make wine will be the next research direction.

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